Etablierung patientenspezifischer In-vitro-Freisetzungsmodelle für perorale Darreichungsformen unter Berücksichtigung gastrointestinaler Besonderheiten

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Für meine Eltern

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Abkürzungsverzeichnis

AUC	*Area under the curve*, Fläche unter der Plasmaspiegelkurve
bzw.	beziehungsweise
c_{max}	maximale Plasmakonzentration
COMT	Catechol-*O*-Methyltransferase
DDI	Dopa-Decarboxylase-Inhibitor
d. h.	das heißt
dpm	Dips pro Minute
et al.	*et alii*, und andere
EMA	*European Medicines Agency*, Europäische Arzneimittel-Agentur
FDA	*Food and Drug Administration*, US-amerikanische Lebensmittel- und Arzneimittelüberwachungsbehörde
h	Stunde (Einheit)
HPLC	*High Performance Liquid Chromatography*, Hochleistungsflüssigkeits-chromatographie
min	Minute (Einheit)
mL	Milliliter (Einheit)
mm	Millimeter (Einheit)
MMC	*Migrating Motor Complex*, Migrierender Motilitätskomplex
PBPK	*Physiologically Based Pharmacokinetics*, Physiologie-basierte Pharmakokinetik
Ph. Eur.	*Pharmacopoea Europaea*, Europäisches Arzneibuch
SGF	*Simulated Gastric Fluid*, künstlicher Magensaft
t_{max}	Zeitpunkt des Erreichens der maximalen Plasmakonzentrationen
u. a.	unter anderem
USP	*United States Pharmacopeia*, US-amerikanisches Arzneibuch
z. B.	zum Beispiel

1 Einleitung und Zielstellung

1.1 Einleitung

Die perorale Verabreichung von Arzneimitteln ist die bevorzugte und am weitesten verbreitete Applikationsart [1] und bietet gegenüber anderen Applikationswegen eine Vielzahl von Vorteilen, z. B. eine einfache Anwendung und hohe Akzeptanz durch den Patienten, die Eignung für die lokale und systemische Therapie und eine gute Dosierbarkeit [2, 3].

Nach peroraler Einnahme einer festen Darreichungsform erfolgt die Freisetzung des Wirkstoffs aus der Formulierung ins Lumen des Gastrointestinaltrakts, die Auflösung in den luminalen Flüssigkeiten und meist die anschließende gastrointestinale Resorption. Diese Prozesse sind sehr komplex und können maßgeblich durch die physiologischen Bedingungen im Gastrointestinaltrakt des Patienten, die pharmazeutische Formulierung, die physikochemischen Eigenschaften des Wirkstoffs oder durch die Einnahmebedingungen bestimmt werden (Tabelle 1).

Tabelle 1: Faktoren, die die Freisetzung eines Wirkstoffs aus einer peroralen Darreichungsform, dessen Löslichkeit/Auflösung und Resorption beeinflussen können [4].

Arzneistoffbezogene Faktoren	Formulierungsbezogene Faktoren	Patientenbezogene Faktoren
Molekulargewicht	Art der Darreichungsform	Alter
Chemische Struktur	Freisetzungsprofil	Erkrankung
Wasserlöslichkeit	Partikelgröße	Geschlecht
Säure-/Baseneigenschaften	Zustand (kristallin/amorph)	Ethnie
Lipophilie (logP-Wert)	Hilfsstoffe	Genom
Stabilität		Mikrobiom
Polymorphie		Einnahmebedingungen
		Co-Medikation

Der Gastrointestinaltrakt gliedert sich in Mundhöhle, Speiseröhre, Magen, Dünndarm, Dickdarm und die damit assoziierten Organen Leber, Gallenblase und Pankreas. Die einzelnen Segmente des Verdauungstrakts sind durch individuelle luminale Gegebenheiten, z. B. unterschiedliche pH-Werte [5-8], Volumina [9-11] und physikochemische Eigenschaften [12-16] der luminalen Flüssigkeiten sowie Motilitätsmuster [17-20] und Druckverhältnisse [6, 8, 18, 19, 21] gekennzeichnet.

Die Verweildauer einer festen Darreichungsform im Mund-Rachen-Raum ist nach peroraler Einnahme in der Regel relativ kurz. Nach Abschluss der ösophagealen Passage gelangt die Arzneiform in den Magen. Der Magen ist der erste Abschnitt des Gastrointestinaltrakts, in dem ein intensiver und länger anhaltender Kontakt zwischen der Darreichungsform und den luminalen Flüssigkeiten stattfindet. Je nach Formulierungstyp kann das In-vivo-Verhalten einer peroral applizierten Darreichungsform, d. h. der Beginn und das Ausmaß der Wirkstofffreisetzung, in

unterschiedlichem Ausmaß von den vorherrschenden luminalen Bedingungen im Magen beeinflusst werden.

Die luminalen Verhältnisse im Magen werden vor allem vom prandialen Zustand (nüchtern/präprandial oder postprandial) bestimmt. Die Zusammensetzung des Mageninhalts kann insbesondere für die Löslichkeit eines Wirkstoffs und das Ausmaß der In-vivo-Freisetzung einer peroral applizierten Darreichungsform von essenzieller Bedeutung sein. Der Magen von gesunden Erwachsenen ist im nüchternen Zustand beispielsweise durch ein saures Flüssigkeitsmilieu gekennzeichnet (Tabelle 2), welches infolgedessen für basische Arzneistoffe besonders gute Auflösebedingungen darstellt [22, 23]. Der niedrige pH-Wert der Magenflüssigkeit kann jedoch für die Stabilität mancher Wirkstoffe problematisch sein [24]. Im postprandialen Zustand werden die luminalen pH-Bedingungen im Magen je nach Zusammensetzung und Menge der eingenommenen Flüssigkeiten bzw. Nahrung verändert [13]. Der Magen spielt jedoch hinsichtlich der Wirkstoffresorption, mit Ausnahme von einzelnen kleinen sauren Molekülen, keine Rolle [25].

Tabelle 2: Überblick über die luminalen Gegebenheiten im Magen und Dünndarm gesunder Erwachsener. Angegeben sind/ist der Bereich der berichtete(n) Mittel- und Medianwerte.

	Parameter	Präprandial	Postprandial
Magen	pH-Wert	1,4 - 3,3 [13, 26]	* [13]
	Pufferkapazität (mmol/L/ΔpH)	14 [27]	14 - 30 * [28, 29]
	Oberflächenspannung (mN/m)	34 - 35 [16, 27]	30 - 31 * [28]
	Osmolalität (mOsm/kg)	190 - 221 [16, 27, 30]	559 * [28]
	Gallensalzkonzentration (mM)	0,2 - 0,8 [16, 30]	0,022 * [31]
	Flüssigkeitsvolumen (mL)	31 - 45 [9, 10, 32]	* [13]
Dünndarm	pH-Wert		
	Duodenum	5,7 - 6,6 [5-7, 33, 34]	5,6 - 6,6 * [6, 28, 35]
	Jejunum	6,1 - 7,0 [7, 33, 34, 36]	
	Ileum	6,8 - 7,8 [5-7, 33, 34]	7,2 [6]
	Pufferkapazität (mmol/L/ΔpH)		
	Duodenum	5,6 [28]	18 - 30 * [28]
	Oberflächenspannung (mN/m)		
	Duodenum	32 [28]	28 [28]
	Jejunum	34 [16]	
	Osmolalität (mOsm/kg)		
	Duodenum	178 [28]	271 - 420 * [28]
	Jejunum	271 - 278 [16, 30, 36]	
	Gallensalzkonzentration (mM)		
	Duodenum	1,6 - 7 [28, 35, 37, 38]	3,7 - 13,4 * [35]
	Jejunum	1,52 - 2,9 [16, 30, 36]	
	Flüssigkeitsvolumen (mL)	43 - 105 [9-11]	54 [9]

* Abhängig von Menge/Zusammensetzung der Mahlzeit und/oder Zeitpunkt der Probennahme

Ein weiterer wichtiger Faktor für das Auflöse- und Freisetzungsverhalten eines Wirkstoffs kann das im Magen zur Verfügung stehende Flüssigkeitsvolumen sein. Bei der nüchternen Einnahme eines Arzneimittels setzt sich dieses Volumen aus dem physiologischen Flüssigkeitsvolumen des Magens (Tabelle 2), welches erheblichen intra- und interindividuellen Schwankungen unterliegen kann [39], und dem zur Arzneimitteleinnahme applizierten Flüssigkeitsvolumen zusammen.

Die Motilität des nüchternen des Magens wird hauptsächlich durch den migrierenden Motilitätskomplex (*Migrating Motor Complex*, MMC) bestimmt [20, 40]. Dieser Komplex besteht bei gesunden Erwachsenen aus drei aufeinander folgenden Phasen mit unterschiedlicher motorischer Aktivität der Magenwand. Phase I ist durch die weitestgehende Abwesenheit peristaltischer Aktivität gekennzeichnet. Das Einsetzen von unregelmäßigen Kontraktionen mit steigender Frequenz markiert den Beginn der zweiten Phase des MMC. Die sich daran anschließende Phase III dauert nur etwa 2 - 10 min, ist jedoch die Phase mit den stärksten Kontraktionen und der höchsten Frequenz [13, 19]. Während dieser Phase werden vor allem nicht verdauliche Nahrungsbestandteile und andere Objekte, wie z. B. monolithische Darreichungsformen, aus dem Magen in den vorderen Dünndarm entleert [41]. Der MMC wiederholt sich im Durchschnitt ungefähr alle 80 - 175 min mit einer hohen intra- und interindividuellen Variabilität, was auch für die jeweiligen Anteile der einzelnen Phasen an der Gesamtdauer des MMC gilt [19, 42, 43]. Bei Aufnahme von Nahrung oder kalorischen Flüssigkeiten wird der MMC durch ein postprandiales Aktivitätsmuster unterbrochen, welches der Phase II des MMC ähnelt [13]. Im präprandialen Zustand kann sich die jeweilige, während der Einnahme einer peroralen Darreichungsform vorherrschende, motorische Phase des MMC maßgeblich auf die Dauer der Magenverweilzeit auswirken [19]. Darüber hinaus ist die Magenverweilzeit einer Arzneiform von einer Vielzahl an weiteren Faktoren abhängig, z. B. von der Art und Größe der Darreichungsform, der physiologischen intra- und interindividuellen Variabilität luminaler Parameter sowie dem prandialen Zustand [44, 45]. Multipartikuläre Arzneiformen werden im Vergleich zu monolithischen Arzneiformen in der Regel schneller und kontinuierlicher aus dem Magen in den Dünndarm entleert [44, 46].

Der Dünndarm stellt den Hauptresorptionsort für die meisten Arzneistoffe dar [47]. Die intestinalen Verhältnisse werden ebenfalls durch die Aufnahme von Flüssigkeiten bzw. Nahrung aus dem Magen sowie durch sekretorische Prozesse beeinflusst. Im nüchternen Zustand ist das Milieu des Dünndarms von gesunden Erwachsenen in der Regel durch einen kontinuierlich ansteigenden pH-Gradienten charakterisiert (Tabelle 2). Für das Auflöseverhalten von lipophilen Wirkstoffen ist vor allem die bei Nahrungsstimuli in den Dünndarm sezernierte Gallenflüssigkeit von großer Bedeutung [48]. Die Gallenflüssigkeit wird in der Leber produziert, in der Gallenblase gespeichert und besteht hauptsächlich aus Gallensalzen und Phospholipiden. Durch Ausbildung von Mischmizellen können schwer lösliche, nicht-ionisierbare Arzneistoffe solubilisiert und anschließend resorbiert werden.

Das intestinale Flüssigkeitsvolumen von gesunden Erwachsenen ist nicht kontinuierlich entlang des Dünndarms verteilt, sondern diskontinuierlich in Form von Flüssigkeitstaschen mit variablen Volumina [9]. Mudie *et al.* berichteten von 8 ± 1 Flüssigkeitstaschen mit einem Volumen von jeweils 4 ± 1 mL [10]. Die wenigen, neueren Studien berichten von hochvariablen intestinalen

Gesamtflüssigkeitsvolumina in gesunden, nüchternen Erwachsenen (Tabelle 2). Im postprandialen Zustand ist das intestinale Flüssigkeitsvolumen hingegen verringert [9], während die Anzahl der Flüssigkeitstaschen zunimmt [9, 10]. Aufgrund des relativ geringen intestinalen Flüssigkeitsvolumens und deren inhomogenen Verteilung im Dünndarm steht eine modifiziert freisetzende Darreichungsform während der intestinalen Passage nur sporadisch in Kontakt mit den luminalen Flüssigkeiten. Diese Umstände können in der Folge zu einem variablen Freisetzungsverhalten und einer variablen Resorption führen [9].

Die bisher charakterisierten gastrointestinalen Verhältnisse von gesunden Erwachsenen bilden die Grundlage für die bis dato etablierten In-vitro-Freisetzungsmodelle. Bei Betrachtung des klinischen Alltags stellen gesunde Erwachsene allein jedoch selten die Zielgruppe einer medikamentösen Therapie dar. Patientenspezifische Faktoren, wie z. B. Alter, Erkrankungen und Geschlecht, können die morphologischen und physiologischen Gegebenheiten des Gastrointestinaltrakts und folglich auch das In-vivo-Verhalten einer peroral applizierten Darreichungsform sowie die Pharmakokinetik eines Wirkstoffs stark beeinflussen. Darüber hinaus können diese Unterschiede zu einer großen Variabilität der Wirkstoffexposition führen, was in der Folge zu einer verminderten Wirksamkeit und/oder einer höheren Inzidenz von Nebenwirkungen und somit einer beeinträchtigten Adhärenz während der Anwendung durch den Patienten führen kann. Mögliche Wechselwirkungen zwischen gemeinsam verabreichten Arzneimitteln, zwischen dem Wirkstoff und den Hilfsstoffen einer Formulierung sowie ein arzneistoffvermittelter Effekt auf die gastrointestinale Physiologie verstärken die Komplexität der oralen Arzneimitteltherapie noch weiter. Wie bereits erwähnt, wurden in der Vergangenheit bei der Entwicklung von neuartigen Formulierungen sowie von In-vitro-Modellen vorwiegend die luminalen Gegebenheiten von gesunden Erwachsenen simuliert. Für eine sichere und effektive Arzneimitteltherapie ist es jedoch in sehr vielen Fällen wichtig, dass patientenspezifische Besonderheiten sowie individuelle Unterschiede der gastrointestinalen Physiologie berücksichtigt werden. Der Einfluss diverser Faktoren und deren Bedeutsamkeit auf die gastrointestinalen Verhältnisse wurden bereits in einer Reihe von Übersichtsartikeln (Alter ([49-52], Geschlecht [52, 53], Erkrankungen [52, 54-56] und Ernährungszustand [52, 57, 58]) diskutiert.

Das Alter eines Patienten kann wesentlich die vorherrschenden luminalen Gegebenheiten innerhalb des Gastrointestinaltrakts bestimmen. Insbesondere in der pädiatrischen Patientenpopulation kommt es zu deutlichen anatomischen und physiologischen Veränderungen in der (früh)kindlichen Entwicklungsphase [59]. In einer detaillierten Literaturrecherche wurde deutlich, dass mit zunehmenden Alter die Speichelsekretionsraten, gastrale Flüssigkeitsvolumina sowie Magensäuresekretionsraten ansteigen [26]. Außerdem wurde ein Entwicklungsprozess der gastrointestinalen Motilität, der exokrinen Pankreasfunktion und der duodenalen Sekretion von Gallensalzen beschrieben. Folglich überrascht es nicht, dass sowohl die Geschwindigkeit als auch das Ausmaß der Resorption von Arzneistoffen bei Kindern im Vergleich zum Erwachsenen verändert ist, wobei die größten Unterschiede bei Neugeborenen zu beobachten sind [60]. Auch im späteren Lebensabschnitt erfährt der Gastrointestinaltrakt mit fortschreitendem Alter eine Vielzahl von morphologischen und funktionellen Veränderungen. In zahlreichen Studien konnte gezeigt werden, dass altersbedingte Effekte auf die luminalen Bedingungen des Gastrointestinal-

trakts nicht nur in der pädiatrischen, sondern auch in der geriatrischen Population auftreten. So wurde beispielsweise für geriatrische Patienten im Vergleich zu jüngeren Erwachsenen eine altersspezifische Erniedrigung der Speichelsekretion [61-65], ein verringertes nüchternes Flüssigkeitsvolumen im Magen [66], eine verlangsamte Magenentleerung von telemetrischen Kapseln [67, 68] und eine verlangsamte Dickdarmpassage [69, 70] berichtet.

Neben altersspezifischen Veränderungen kann auch das Geschlecht eines Patienten Auswirkungen auf das gastrointestinale Milieu haben [52]. So wurden z. B. eine niedrigere Speichelproduktion bei Frauen [62, 64] und, bedingt durch eine geringere Magensäuresekretion [53, 71, 72], höhere gastrale pH-Werte bei weiblichen Probandinnen beobachtet [53, 73]. Weiterhin wurde bei Männern eine schnellere Magenentleerung einer telemetrischen Kapsel berichtet [67] und es wurden unterschiedliche pH-Werte und Gallensalzkonzentrationen der intestinalen Flüssigkeiten zwischen beiden Geschlechtern beobachtet [53].

Wie bereits anfangs dargestellt sind die gastrointestinalen Gegebenheiten von gesunden Erwachsenen durch eine hohe intra- und interindividuelle Variabilität gekennzeichnet [12]. Diese Variabilität kann beispielsweise bei Patienten mit gastrointestinalen oder systemischen Erkrankungen durch pathologische Veränderungen noch weiter verstärkt werden. Unterschiede in der gastrointestinalen Motilität und der Hydrodynamik wirken sich z. B. auf den Transit einer Darreichungsform entlang des Gastrointestinaltrakts aus. Gleichermaßen können Veränderungen in der Zusammensetzung und den physikochemischen Eigenschaften der luminalen Flüssigkeiten die Freisetzung und die Solubilisierung eines Wirkstoffs beeinflussen.

Eine Vielzahl von Erkrankungen kann die physiologischen Verhältnisse im Gastrointestinaltrakt und somit auch die Freisetzung, Auflösung und Resorption eines peroral applizierten Wirkstoffs beeinflussen [74-76]. In den letzten Jahren wurden viele Initiativen gestartet, das Verständnis der gastrointestinalen Physiologie und deren funktionelle Veränderungen in Folge einer gastrointestinalen oder systemischen Erkrankung zu verbessern [52, 54-56]. Das Vorhandensein einer Erkrankung sowie deren Schweregrad sind zentrale Faktoren für die häufig beobachtete intra- und interindividuelle Variabilität des In-vivo-Verhaltens einer peroralen Darreichungsform sowie der daraus resultierenden Arzneistoffdisposition [12]. Für einige gastrointestinale Erkrankungen konnte gezeigt werden, dass sie das Milieu des Verdauungstrakts in unterschiedlichem Ausmaß beeinflussen [52, 54]. Patienten, die an den chronischen Darmerkrankungen *Morbus Crohn* oder *Colitis ulcerosa* leiden, weisen neben einer chronischen Entzündung von Dünn- oder Dickdarmschleimhaut beispielsweise auch eine gestörte, hochvariable gastrointestinale Motilität auf [77-83]. Eine gestörte Motilität ist gleichermaßen bei dem häufig vorkommenden Reizdarmsyndrom präsent [84]. Nahrungsmittelunverträglichkeiten wie Glutenintoleranz (Zöliakie), die auch durch eine chronische Entzündung der Dünndarmschleimhaut gekennzeichnet ist, gehen ebenfalls mit veränderten gastrointestinalen Freisetzungs- und Resorptionsbedingungen einher [85-90]. Aber auch systemische Erkrankungen, die den Körper in seiner Gesamtheit betreffen und nicht unmittelbar mit dem Gastrointestinaltrakt in Verbindung stehen, können sich auf die luminalen Verhältnisse des Verdauungstrakts, die Wirkstoffresorption und das In-vivo-Verhalten einer Formulierung auswirken. Veränderte gastrointestinale Bedingungen wurden für Stoffwechselstörungen, z. B. Mukoviszidose [91-93]

und *Diabetes mellitus* [94-96], psychische Erkrankungen, z. B. Angststörungen und Depressionen [97] sowie neurodegenerative Erkrankungen, z. B. *Morbus Parkinson* [98] berichtet.

Morbus Parkinson ist eine progressive neurodegenerative Erkrankung, resultierend aus einem kontinuierlichen Verlust dopaminerger Neuronen in der *Substantia nigra*, und ist gekennzeichnet durch die primär motorischen Symptome Tremor, Bradykinesie, Rigidität und posturale Instabilität. Es ist weltweit die zweithäufigste neurodegenerative Erkrankung und betrifft etwa 0,1 % der europäischen Bevölkerung [99]. Die Parkinson-Krankheit wird zusätzlich von mehreren sekundär motorischen Symptomen, wie z. B. neuro-ophthalmologischen Anomalien und respiratorischen Funktionsstörungen, sowie von nicht-motorischen Symptomen begleitet, u. a. autonome Störungen (z. B. Blasenfunktionsstörungen, orthostatische Hypotonie), kognitive Symptome und gastrointestinale Störungen [100, 101]. Die Prävalenz von gastrointestinalen Symptomen bei Parkinson-Patienten ist sehr hoch. Es sind alle Abschnitte des Gastrointestinaltrakts betroffen und die Symptome sind in allen Stadien der Erkrankung präsent [98]. In den letzten Jahren wurde deutlich, dass gastrointestinale Dysfunktionen schon in frühen Krankheitsstadien vorkommen, sogar oft bevor andere Symptome überhaupt in Erscheinung treten [98, 102]. In mehreren klinischen Studien wurde bereits der Einfluss der Parkinson-Krankheit auf bestimmte gastrointestinale Gegebenheiten, wie beispielsweise die Dauer der Magenentleerung, im Vergleich zum gesunden Patienten untersucht. Jedoch erfolgten bisher keine zusammenfassende Darstellung und Auswertung der aus diesen Studien gewonnenen Ergebnisse bzw. Erkenntnisse. Für die Entwicklung von Parkinson-spezifischen biorelevanten In-vitro-Freisetzungsmodellen für peroral verabreichte Darreichungsformen sind diese Erkenntnisse jedoch unverzichtbar.

In-vitro-Freisetzungsmodelle, die ursprünglich zur Chargen-Qualitätskontrolle und für Stabilitätsprüfungen in der pharmazeutischen Industrie entwickelt wurden, finden heutzutage zunehmend in unterschiedlicher Komplexität und Biorelevanz Anwendung im Rahmen der Entwicklung und des Screenings neuartiger pharmazeutischer Formulierungen, zur Etablierung von In-vitro-/In-vivo-Korrelationen und zur Vorhersage des In-vivo-Verhaltens einer applizierten Darreichungsform. In den letzten Jahrzehnten wurden eine Reihe von biorelevanten Freisetzungsmethoden zur umfangreichen Untersuchung der In-vitro-Wirkstofffreisetzung aus oralen Darreichungsformen mit unterschiedlichsten Freisetzungscharakteristika eingeführt und erfolgreich angewendet, um die In-vivo-Wirkstofffreisetzung bei gesunden Erwachsenen vorherzusagen [103-113]. Biorelevante In-vitro-Testmodelle zielen u. a. darauf ab, Vorhersagen zur Bioverfügbarkeit eines Arzneimittels zu treffen und den Umfang von zeit- und kostenintensiven Tier- oder Humanstudien zu reduzieren. Um möglichst gute Vorhersagen zum In-vivo-Freisetzungsverhalten einer peroral applizierten Darreichungsform treffen zu können, ist es essenziell, die relevanten gastrointestinalen Parameter sowie wahrscheinliche bzw. die vorgesehenen Einnahmebedingungen in der Entwicklung biorelevanter In-vitro-Testmethoden hinreichend zu berücksichtigen. Dazu gehören vor allem wichtige luminale Parameter wie Volumina, Zusammensetzung und physikochemische Eigenschaften der gastrointestinalen Flüssigkeiten sowie Hydrodynamik, Motilität und Verweil- bzw. Passagezeiten. Wie bereits erwähnt, wird die Zusammensetzung der luminalen Flüssigkeiten stark von den Einnahme-

bedingungen beeinflusst. Es ist daher weiterhin wichtig zu unterscheiden, ob die Darreichungsform nüchtern oder zusammen mit Nahrung bzw. einem Applikationsvehikel eingenommen wird.

Die Entwicklung biorelevanter In-vitro-Freisetzungsmethoden erfordert eine geeignete In-vitro-Freisetzungsapparatur, biorelevante Freisetzungsmedien und ein geeignetes Testprotokoll, welches die gastrointestinalen Bedingungen der adressierten Patientenpopulation und relevante Einnahmebedingungen hinreichend berücksichtigt. Eine Reihe von etablierten Freisetzungsapparaturen zur Untersuchung des In-vitro-Freisetzungsverhaltens von festen oralen Darreichungsformen sind bereits in verschiedenen Arzneibüchern beschrieben. Das Europäische Arzneibuch (*Pharmacopoea Europaea*, Ph. Eur., Version 10) führt diesbezüglich vier unterschiedliche Testapparaturen auf: Apparatur 1 - Drehkörbchen (*Basket apparatus*), Apparatur 2 - Blattrührer (*Paddle apparatus*), Apparatur 3 - Eintauchender Zylinder (*Reciprocating cylinder*) und Apparatur 4 - Durchflusszelle (*Flow-through cell*) [114] (Abbildung 1). Neben der jeweiligen Beschreibung des apparativen Aufbaus werden in der Monographie 2.9.3. auch die jeweiligen Testbedingungen für Darreichungsformen mit unterschiedlichen Freisetzungscharakteristika angegeben. Zur Vorhersage des In-vivo-Freisetzungsverhaltens von modifiziert freisetzenden Formulierungen bietet sich insbesondere die Apparatur des Eintauchenden Zylinders an. Bei dieser Apparatur können die einzelnen Abschnitte der gastrointestinalen Passage einer peroral verabreichten Darreichungsform unter Verwendung unterschiedlicher Freisetzungsmedien simuliert werden. Die Eintauchender-Zylinder-Apparatur wird ebenfalls im US-amerikanischen Arzneibuch (*United States Pharmacopeia*, USP) unter der Bezeichnung „Apparatus 3“ (*Reciprocating cylinder*) beschrieben. Im weiteren Verlauf der vorliegenden Arbeit wird für diese Apparatur die Bezeichnung „USP-3-Apparatur“ verwendet.

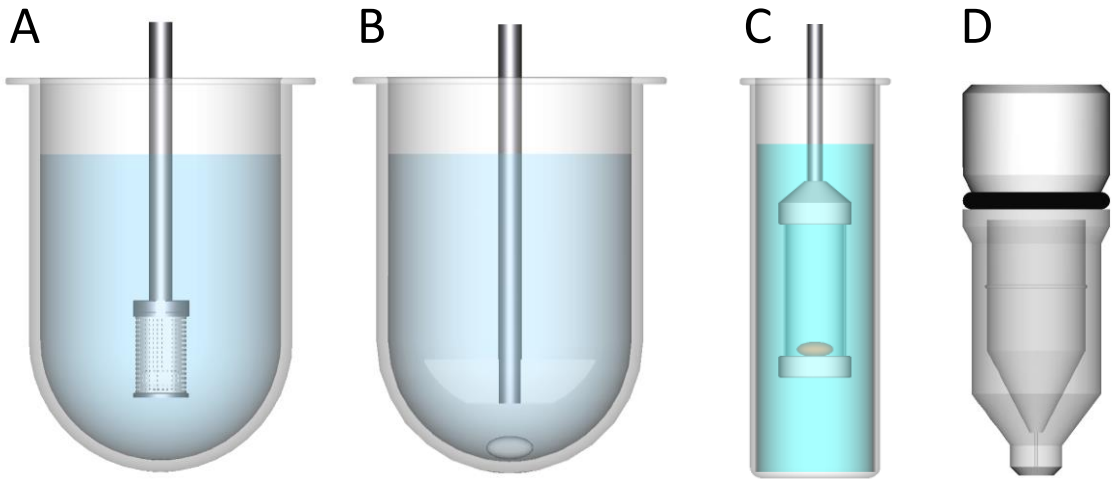

Abbildung 1: **Im Europäischen Arzneibuch aufgeführte In-vitro-Freisetzungsapparaturen zur Untersuchung des Freisetzungsverhaltens von festen oralen Darreichungsformen: A) Apparatur 1 - Drehkörbchen (*Basket apparatus*), B) Apparatur 2 - Blattrührer (*Paddle apparatus*), C) Apparatur 3 - Eintauchender Zylinder (*Reciprocating cylinder*), D) Apparatur 4 - Durchflusszelle (*Flow-through cell*).**

Neben der Freisetzungsapparatur sind auch die verwendeten Freisetzungsmedien integrale Bestandteile von In-vitro-Freisetzungsmethoden. Biorelevante Medien wurden entwickelt, um die luminalen Flüssigkeiten des Gastrointestinaltrakts vor und nach einer Mahlzeit möglichst präzise simulieren zu können [22, 115]. Diese Medien basieren auf In-vivo-Daten zur Zusammensetzung und den physikochemischen Eigenschaften der luminalen Flüssigkeiten gesunder Erwachsener, wohingegen kompendiale Medien in der Regel nur typische durchschnittliche pH-Bedingungen im Magen, Dünn- bzw. Dickdarm widerspiegeln. Dieser Umstand ist besonders bei lipophilen Arzneistoffen problematisch, da der Fettgehalt und die Gallensalzkonzentration sich als die wichtigsten Faktoren für deren Löslichkeit erwiesen haben [48]. In den letzten Jahren gab es daher immer mehr Bestrebungen, patientenspezifische biorelevante Medien zu entwickeln und zu etablieren [74-76, 116].

Mehrere biorelevante In-vitro-Freisetzungsmethoden zur Simulation der gastrointestinalen Passage in einem durchschnittlichen gesunden Erwachsenen wurden von Klein *et al.* auf Grundlage der USP-3-Apparatur und unter Berücksichtigung physiologischer Gegebenheiten des Gastrointestinaltrakts entwickelt, um das In-vivo-Freisetzungsverhalten von peroralen Arzneiformen mit modifizierter Freisetzung vorherzusagen [109, 110, 117, 118]. In diesen Modellen wurden erstmals, abweichend von dem bis dahin oft verwendeten Drei-Stufen-Design (Magen-Dünndarm-Dickdarm) [119], die simulierten Darmabschnitte hinsichtlich der Verweilzeiten und pH-Bedingungen weiter differenziert. So war es möglich, den Verlauf und den Ort der Wirkstofffreisetzung präziser zu charakterisieren und darüber hinaus mit klinischen In-vivo-Daten korrelieren zu können [109]. In einer weiteren Studie wurden unter Einbeziehung von Literaturdaten zu gastrointestinalen Transitzeiten und pH-Werten eine Reihe von In-vitro-Testmodellen zur Simulation der gastrointestinalen Passage von verschiedenen Mesalazin-Formulierungen in individuellen Probanden entwickelt [120]. Das in der Studie verwendete Testdesign wurde dahingehend modifiziert, dass die Verweilzeiten in den unterschiedlichen Abschnitten des Gastrointestinaltrakts und die dazugehörigen pH-Werte an die In-vivo-Daten von Probanden angepasst wurden. In diesen Studien konnte gezeigt werden, dass die USP-3-Apparatur in Kombination mit physiologischen In-vivo-Daten und biorelevanten Testmedien eine vielversprechende, prädiktive In-vitro-Testmethode für Darreichungsformen mit modifizierter Wirkstofffreisetzung darstellt. Zusammen mit den entsprechenden Daten zur gastrointestinalen Physiologie wäre diese Testmethodik auch zur Vorhersage des In-vivo-Verhaltens von modifiziert freisetzenden Arzneiformen in individuellen Patienten bzw. spezifischen Patientenpopulationen vielversprechend. Die von Klein *et al.* etablierten Methoden wurden auf Basis einer kompendialen Apparatur unter Anwendung einfacher und biorelevanter Methoden entwickelt. In den letzten Jahren wurde eine Reihe komplexer Methoden unter Einsatz nicht-kompendialer Geräte zunächst mit dem Ziel entwickelt, klinische Studienbedingungen in durchschnittlichen Probanden zu simulieren. Heutzutage liegt der Fokus aber auch zunehmend darauf, individuelle Patienten bzw. spezifische Patientengruppen besser abzubilden. Beispiele hierfür sind das Antrum-Modell [121], GastroDuo [122, 123], Fed Stomach Model [124], Stresstest-Apparatur [125], Dynamic Gastric Model (DGM) [126-128] und TNO TIM-1- [129, 130] und TIM-2-System [131].

Biorelevante In-vitro-Freisetzungsmodelle werden darüber hinaus immer häufiger in Verbindung mit Physiologie-basierten pharmakokinetischen (*Physiologically Based Pharmacokinetic*, PBPK) In-silico-Modellen verwendet, um bessere Vorhersagen zum In-vivo-Verhalten eines Arzneimittels treffen zu können. PBPK-Modelle sind mathematische Mehrkompartiment-Modelle zur Beschreibung und Vorhersage der Resorption, Verteilung, Metabolisierung und Elimination von Arzneistoffen im menschlichen oder tierischen Körper. Ursprünglich entwickelt zur Bewertung der Toxikologie von Arzneistoffen, haben sich PBPK-Modelle in den letzten Jahren als wichtiges biopharmazeutisches Werkzeug zur Vorhersage der Pharmakokinetik und Bioverfügbarkeit von Wirkstoffen etabliert. PBPK-Modelle berücksichtigen Daten zur Anatomie und Physiologie der zu simulierenden Spezies, physikochemische und biologische Eigenschaften des Wirkstoffs bzw. der Wirkstoffe, Informationen zum Studiendesign, Dosierung und Einnahmebedingungen, klinische In-vivo-Daten sowie In-vitro-Freisetzungsdaten der getesteten Arzneiform (Abbildung 2). Heutzutage werden PBPK-Modelle routinemäßig in der pharmazeutischen Industrie verwendet und sowohl die *European Medicines Agency* (EMA, [132]) als auch die *Food and Drug Administration* (FDA, [133]) haben bereits Leitlinien zur Verwendung von PBPK-Modellen veröffentlicht. Prädiktive PBPK-Modelle finden in unterschiedlichsten Bereichen Anwendung, z. B. für die Rationalisierung der Arzneimittelentwicklung, in der effizienten Planung von präklinischen und klinischen Studien sowie der präklinischen Dosisfindung [134-136].

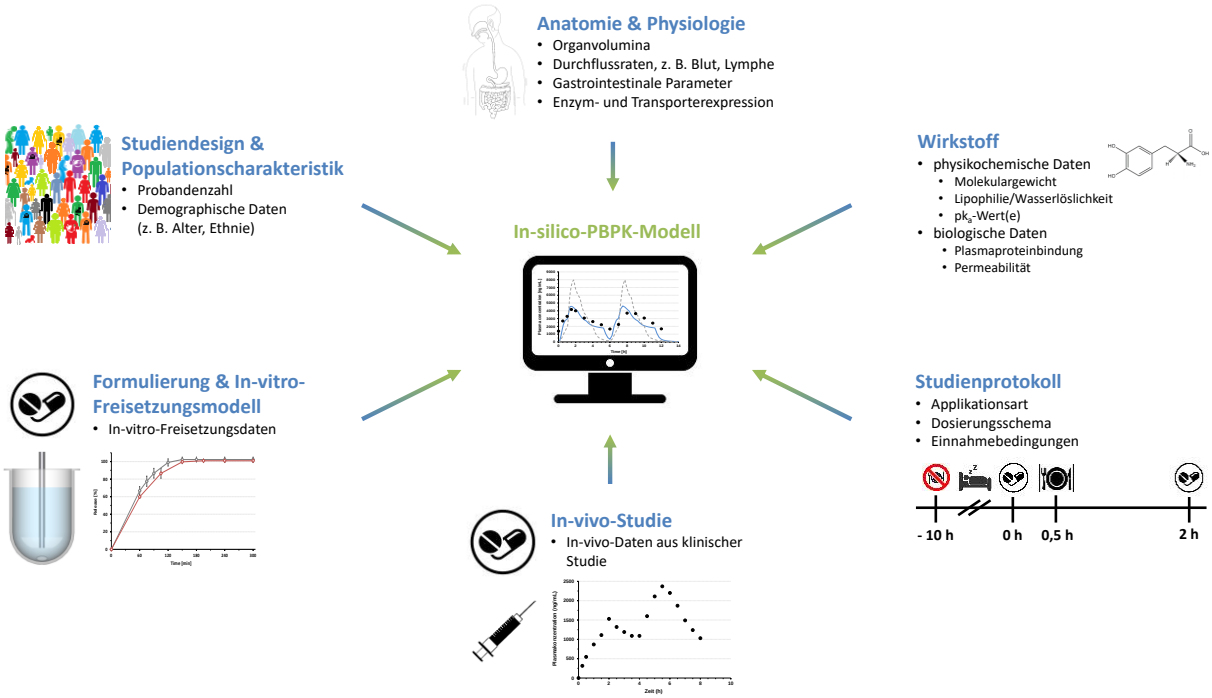

Abbildung 2: Informationseinheiten, die bei der Erstellung eines In-silico-PBPK-Modells Berücksichtigung finden (müssen).

Eine robuste und vorhersagbare Wirkstofffreisetzung im Gastrointestinaltrakt eines Patienten ist eine wesentliche Voraussetzung für eine wirksame, zuverlässige und sichere perorale Arzneimitteltherapie. Freisetzung und Auflösung eines Wirkstoffs sind entscheidende Schritte im gastrointestinalen Resorptionsprozess aus einer peroral verabreichten Darreichungsform. Folglich

sollten im Verlauf der Formulierungsentwicklung Prototypen mit biorelevanten und prädiktiven In-vitro- und In-silico-Modellen evaluiert werden, um eine bestmögliche Vorhersage zum In-vivo-Verhalten einer peroralen Darreichungsform für die Gesamtbeurteilung der Wirksamkeit und des Sicherheitsprofils zu ermöglichen. Im besten Fall lassen sich so auch zeit- und kostenintensive Tier- oder Humanstudien gemäß dem 3R-Prinzip (*Replacement, Reduction, Refinement*; Vermeidung, Verringerung, Verbesserung) reduzieren. Biorelevante In-vitro- und In-silico-Modelle können darüber hinaus Vorhersagen bzw. Auswertungen von klinischen In-vivo-Daten aus pharmakokinetischen Studien unterstützen. Für die Modellentwicklung ist es ebenfalls von essenzieller Bedeutung zu wissen, an welche Patientenpopulation verabreicht werden soll und unter welchen Bedingungen die Einnahme erfolgen wird. Bis dato werden relevante Applikationsbedingungen jedoch für viele Patientengruppen in klinischen Studiendesigns noch nicht hinreichend berücksichtigt. Daher ist es notwendig, In-vitro-Freisetzungsmodelle und In-silico-PBPK-Modelle, die die gastrointestinalen Gegebenheiten individueller Patienten bzw. besonderer Patientenpopulationen sowie patientenrelevante Einnahmeszenarien so präzise wie möglich wiedergeben, zu entwickeln, um geeignete Formulierungsansätze für die anvisierte Patientenpopulation zu selektieren und prädiktive bzw. erklärende Aussagen zum In-vivo-Verhalten einer oral applizierten Arzneiform zu ermöglichen.

1.2 Zielstellung

Das grundlegende Ziel der vorliegenden Arbeit war die Entwicklung und Etablierung von biorelevanten, patientenspezifischen In-vitro-Freisetzungsmodellen für peroral applizierte Darreichungsformen unter Berücksichtigung gastrointestinaler Gegebenheiten und patientenrelevanter Einnahmebedingungen zweier unterschiedlicher Patientenpopulationen: pädiatrische Patienten und Parkinson-Patienten.

Ziel des ersten Teils der Arbeit war die Etablierung eines Physiologie-basierten, pädiatrischen In-vitro-Freisetzungsmodells, welches die Bedingungen im Magen nach oraler Einnahme einer neuartigen Hydrocortison-Darreichungsform und Co-Verabreichung mit typischen pädiatrischen Applikationsvehikeln simulieren sollte. Mit Hilfe des entwickelten In-vitro-Modells sollte die Kompatibilität der Darreichungsform mit den untersuchten Applikationsvehikeln bewertet werden und der Einfluss der Verabreichungsbedingungen auf die In-vivo-Freisetzung von Hydrocortison abgeschätzt werden.

Das Ziel des zweiten Teils der Arbeit war die Etablierung eines patientenspezifischen In-vitro-Freisetzungsmodells unter Berücksichtigung der gastrointestinalen Gegebenheiten in Parkinson-Patienten. Mit Hilfe des In-vitro-Modells sollte die In-vivo-Freisetzung von Levodopa aus ausgewählten, modifiziert freisetzenden Handelspräparaten und Formulierungsprototypen evaluiert werden. Die zu verwendende In-vitro-Plattform sollte die Simulation von patientenspezfischen, individualisierten In-vitro-Testszenarien ermöglichen. Abschließend sollte die Aussagekraft des entwickelten Freisetzungsmodells im Hinblick auf die In-vivo-Wirkstofffreisetzung im Gastrointestinaltrakt von Parkinson-Patienten unter Verwendung eines geeigneten In-silico-PBPK-Modells bewertet werden.

2 Diskussion

In den letzten Jahren haben potenzielle Auswirkungen von patientenspezifischen Faktoren auf das In-vivo-Verhalten von oral verabreichten Darreichungsformen und folglich auch auf den Erfolg der oralen Arzneimitteltherapie deutlich mehr Aufmerksamkeit erhalten [52]. Ein umfangreiches Verständnis der physiologischen Verhältnisse im Gastrointestinaltrakt eines Patienten bzw. einer Patientenpopulation sowie der patientenspezifischen Einnahmebedingungen sind für die Entwicklung von bioprädiktiven In-vitro- und In-silico-Modellen von essenzieller Bedeutung. Der erste Teil der Arbeit fokussierte auf die Entwicklung von In-vitro-Modellen zur Untersuchung des Freisetzungsverhalten einer neuartigen pädiatrischen Darreichungsform mit alterstypischen Applikationsvehikeln unter Berücksichtigung der pädiatrischen gastralen Physiologie.

2.1 Entwicklung von In-vitro-Modellen zur Simulation der Wirkstofffreisetzung unter besonderen Verabreichungsbedingungen in pädiatrischen Patienten

Eine wichtige Voraussetzung für eine sichere und wirksame perorale Arzneimitteltherapie bei pädiatrischen Patienten sind adäquate, altersgerechte Darreichungsformen sowie eine geeignete Verabreichungspraxis. Peroral applizierte Arzneimittel werden pädiatrischen Patienten häufig zusammen mit Applikationsvehikeln verabreicht, um die Einnahme der Arzneimittel zu erleichtern, z. B. durch eine Verbesserung der Schluckbarkeit, des Mundgefühls und/oder des Geschmacks [137]. Typische Applikationsvehikel sind Flüssigkeiten (z. B. Milch, Säfte) oder halbfeste Nahrungsmittel (z. B. Joghurt, Apfelmus). Bei einer solchen Anwendungspraxis muss jedoch sichergestellt werden, dass die eingenommene Arzneiform mit dem jeweiligen Applikationsvehikel kompatibel ist, d. h., dass die Qualität und das In-vivo-Verhalten des Arzneimittels nicht nachteilig beeinträchtigt werden [137]. Die Beurteilung der Kompatibilität einer Arzneiform mit einem oder mehreren Applikationsvehikeln kann z. B. in klinischen In-vivo-Studien evaluiert werden. Jedoch ist es aufgrund ethischer Bedenken sehr schwierig oder gar unmöglich, solche Studien auch an gesunden Kindern durchzuführen.

Moderne, aussagekräftige In-vitro-Methoden könnten eine zukünftige Alternative zu klinischen In-vivo-Studien sein. Mit Hilfe solcher Modelle könnte evaluiert bzw. vorhergesagt werden, ob die Art der Verabreichung eines Arzneimittels dessen Stabilität und In-vivo-Freisetzungsverhalten beeinträchtigt. Die Frage nach einer geeigneten In-vitro-Methode zur Abschätzung des Einflusses verschiedener Verabreichungsmethoden stellte sich auch im Rahmen der Entwicklung und Zulassung von Alkindi® (Diurnal Limited, Cardiff, Großbritannien). Alkindi® ist eine multipartikuläre, geschmacksmaskierte und schnell freisetzende Formulierung von Hydrocortison für die perorale Applikation in Dosen von 0,5 - 5,0 mg. Alkindi® wurde speziell zur pädiatrischen Anwendung für die adäquate Behandlung der Nebenniereninsuffizienz entwickelt, da bis dato keine altersgerechten Fertigarzneimittel für die Therapie dieser Erkrankung verfügbar

waren [138, 139]. Die Mehrheit der pädiatrischen Patienten mit Nebenniereninsuffizienz wurde bisher mit individualisierten, in der Apotheke hergestellten Arzneimitteln für Erwachsene behandelt. In einer Studie von Neumann *et al.* wurde jedoch aufgezeigt, dass diese Arzneimittel aufgrund eines stark schwankenden Wirkstoffgehalts zur dauerhaften Substitutionstherapie ungeeignet sind [139]. Insbesondere bei sehr jungen Patienten hätte dies eine ungenügende Therapiekontrolle zur Folge und könnte beispielsweise bei einer Unterdosierung zu einer potenziell lebensbedrohlichen Nebennierenkrise führen [139]. Es bestand daher ein großes Interesse an einer geeigneten, altersgerechten Hydrocortison-Formulierung für die Substitutionstherapie der Nebenniereninsuffizienz von pädiatrischen Patienten, woraufhin die Entwicklung von Alkindi® initiiert wurde. Die vorgesehene Art der Verabreichung von Alkindi® war es, den Kapselinhalt in Form von Pellets entweder auf einen trockenen Löffel bzw. direkt auf die Zunge aufzustreuen und anschließend zusammen mit etwas Flüssigkeit, wie z. B. Wasser oder Milch, einzunehmen oder alternativ direkt auf ein halbfestes Applikationsvehikel, wie z. B. Joghurt, aufzustreuen.

Im ersten Teil der vorliegenden Arbeit wurden pädiatrische In-vitro-Freisetzungsmodelle entwickelt, um zu evaluieren, ob die Stabilität und das In-vivo-Freisetzungsverhalten der neuartigen Alkindi®-Formulierung durch Co-Verabreichung mit alterstypischen Applikationsvehikeln beeinträchtigt werden. Die gewonnenen In-vitro-Daten sollten auch in Hinblick auf die Erteilung der Marktzulassung durch die europäische und US-amerikanische Arzneimittelbehörde EMA bzw. FDA Anwendung finden. Für die In-vitro-Untersuchungen wurden zwei separate In-vitro-Studien konzipiert und durchgeführt. Der Fokus der Modellentwicklung lag insbesondere auf der Simulation der Mageninhalte von pädiatrischen Patienten und weniger auf einer umfangreichen Darstellung der gastrointestinalen Physiologie.

Die erste In-vitro-Studie galt der Untersuchung der Freisetzungs- und Kompatibilitätseigenschaften von Alkindi® nach simulierter Gabe mit typischen Applikationsvehikeln für Kinder unter 6 Jahren. Um eine genauere Abstufung innerhalb dieser Patientenpopulation zu erreichen, wurden die Dosierungsbedingungen für drei verschiedene Altersgruppen, d. h. Neugeborene, Säuglinge/Kleinkinder und Vorschulkinder, simuliert. Im Rahmen des europäischen Zulassungsverfahrens von Alkindi® wurde durch die EMA eine Auswahl an zu untersuchenden Applikationsvehikeln, d. h. Wasser, Muttermilch, Formulamilch und Vollmilch, vorgegeben. Die In-vitro-Wirkstofffreisetzung von Alkindi® wurde unter typischen Dosierungsbedingungen für jede der genannten Altersgruppen untersucht, d. h. für die Altersgruppen der Neugeborenen und Säuglinge/Kleinkinder mit Muttermilch und Formulamilch und für die Altersgruppe der Vorschulkinder mit Vollmilch. Für alle Altersgruppen wurde zudem die Verabreichung mit Wasser simuliert. Die in der Studie verwendete Muttermilch entstammte der Muttermilchbank des Universitätsklinikums Greifswald. Die In-vitro-Untersuchungen wurden mit Muttermilch aus zwei verschiedenen Quellen durchgeführt, um die natürliche Variabilität in der Zusammensetzung von Muttermilch zu berücksichtigen. Weiterhin wurden zwei verschiedene Arten von Formulamilch verwendet, um typische Dosierungsbedingungen bei Neugeborenen und Säuglingen/Kleinkindern zu simulieren.

Auf der Basis von Literaturdaten wurden die in den In-vitro-Freisetzungsuntersuchungen verwendeten Testvolumina für die jeweilige pädiatrische Altersgruppe an die direkt nach der Einnahme eines Arzneimittels mit Flüssigkeit verfügbaren Magenvolumina angepasst [26]. Diese umfassten die nüchternen Magensaftvolumina von Neugeborenen, Säuglingen/Kleinkindern oder Vorschulkindern plus die zusammen mit der Arzneiform eingenommenen Flüssigkeitsvolumina. Das Versuchsdesign sollte die physiologischen Verhältnisse adäquat wiedergeben und die Freisetzungsuntersuchungen sollten nach Möglichkeit mit einer arzneibuchkonformen Apparatur durchgeführt werden. Allerdings erlaubten die meisten der in der In-vitro-Studie simulierten Magenflüssigkeitsvolumina nicht die Verwendung von standardisierten Freisetzungsapparaturen. Die In-vitro-Freisetzungsuntersuchungen wurden daher mit der Mini-Paddle-Apparatur (Erweka GmbH, Langen, Deutschland) durchgeführt, welche eine miniaturisierte Version der arzneibuchkonformen Blattrührer- bzw. Paddle-Apparatur darstellt [140, 141]. Die Verwendung der Mini-Paddle-Apparatur ermöglichte die Anwendung von kleineren Testvolumina im Vergleich zur arzneibuchkonformen Paddle-Apparatur [141].

In allen In-vitro-Testszenarien wurde eine schnelle und vollständige Hydrocortison-Freisetzung der Alkindi®-Formulierung beobachtet, unabhängig vom eingesetzten Applikationsvehikel oder den Dosierungsbedingungen. Die Ergebnisse der In-vitro-Studie bestätigten die Kompatibilität von Alkindi® mit ausgewählten pädiatrischen Applikationsvehikeln und deuten darauf hin, dass die In-vivo-Wirkstofffreisetzung und die Bioverfügbarkeit von Alkindi® durch die Zusammensetzung und Menge der untersuchten Flüssigkeiten nicht beeinträchtigt werden.

Die Ergebnisse der ersten Studie zum In-vitro-Freisetzungsverhalten der neuartigen Alkindi®-Formulierung mit pädiatrischen Applikationsvehikeln für Kinder unter 6 Jahren wurden 2018 unter dem Titel „*Biorelevant in vitro assessment of dissolution and compatibility properties of a novel paediatric hydrocortisone drug product following exposure of the drug product to child-appropriate administration fluids*" im Journal „*European Journal of Pharmaceutics and Biopharmaceutics*" veröffentlicht (siehe Kapitel 5.1).

In der zweiten In-vitro-Studie wurde die Freisetzung der Alkindi®-Formulierung in flüssigen und halbfesten Applikationsvehikeln untersucht. Darüber hinaus wurde die Kompatibilität der Arzneiform mit diesen Vehikeln getestet. Im Fokus der Untersuchung standen pädiatrische Patienten, die neben Milch auch andere flüssige Vehikel sowie halbfeste Nahrung zu sich nehmen können. Aufgrund dessen wurde der Altersbereich der adressierten Patienten-population auf 2 - 16 Jahre verändert. Im Rahmen des US-amerikanischen Zulassungsverfahrens von Alkindi® wurde durch die FDA eine Auswahl an zu untersuchenden Applikationsvehikeln, d. h. Wasser, Apfelsaft, Orangensaft, Tomatensaft, Apfelmus und Joghurt, vorgegeben, ohne jedoch genauere Vorgaben hinsichtlich spezieller Produkte der Flüssigkeiten bzw. halbfesten Nahrungsmittel festzulegen. Jedoch gibt es eine Vielzahl an unterschiedlichen Produkten der jeweiligen Matrices, die sich in ihrer Zusammensetzung und ihren physikochemischen Eigenschaften teilweise deutlich voneinander unterscheiden [142-144]. Um die Variabilität der physikochemischen Eigenschaften innerhalb einer Produktgruppe besser abschätzen und anschließend jeweils ein geeignetes Produkt für die In-vitro-Studie auswählen zu können, wurden

für jede Gruppe der flüssigen bzw. halbfesten Matrices (mit Ausnahme von Wasser) drei verschiedene Produkte ausgewählt, wovon möglichst ein Produkt US-amerikanischer Herkunft sein sollte.

Nach eingehender physikochemischer Charakterisierung aller Produkte und der Auswahl von Produkten für die In-vitro-Studie wurde das pädiatrische In-vitro-Testmodell entwickelt. Dies simulierte die Magenbedingungen unmittelbar nach der Einnahme von Alkindi® zusammen mit einem flüssigen bzw. halbfesten Applikationsvehikel unter Verwendung der Mini-Paddle-Apparatur und auf Basis physiologischer Daten, die einem eigenen Übersichtartikel entstammten [26]. Zu Beginn der Freisetzungsuntersuchungen wurde das nüchterne gastrale Flüssigkeitsvolumen unter Verwendung von simulierter Magenflüssigkeit (*Simulated Gastric Fluid*, SGF) pH 1,8 bzw. 4,0 im Vessel vorgelegt, als nächstes erfolgte die Zugabe der Alkindi®-Pellets zusammen mit dem jeweiligen Applikationsvehikel und anschließend die Zugabe einer Flüssigkeit, die die Aufnahme eines definierten Flüssigkeitsvolumens unmittelbar mit/nach Einnahme der Arzneiform simulierte (Abbildung 3). Die Volumina/Mengen der einzelnen Komponenten waren abhängig von der jeweils simulierten pädiatrischen Altersgruppe.

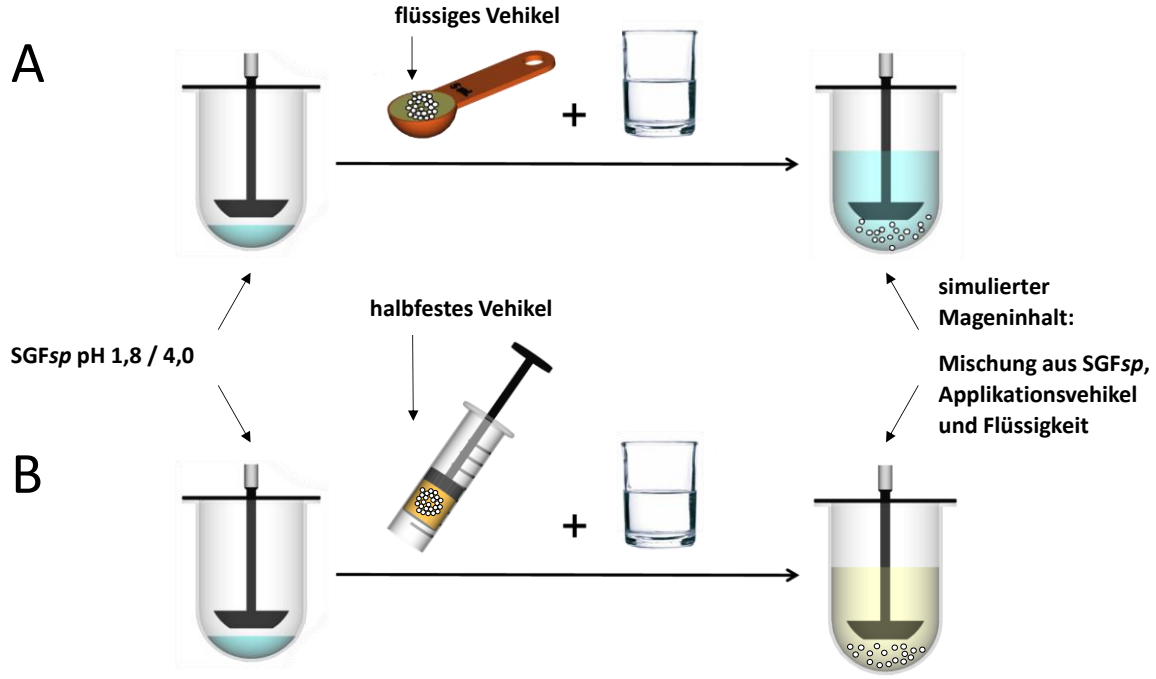

Abbildung 3: Schematische Abbildung des In-vitro-Modells zur Simulation der Wirkstofffreisetzung von Alkindi® bei gleichzeitiger simulierter Verabreichung mit einem (A) flüssigen bzw. (B) halbfesten Applikationsvehikel und zusätzlicher Flüssigkeit.

Die Ergebnisse der zweiten In-vitro-Studie bestätigten ebenfalls die Kompatibilität von Alkindi® mit den ausgewählten pädiatrischen Applikationsvehikeln. In allen Testszenarien wurde eine schnelle und vollständige Wirkstofffreisetzung der Alkindi®-Formulierung aufgezeigt und es wurde keine Wirkstoffpräzipitation oder -degradation über den Untersuchungszeitraum beobachtet. In Übereinstimmung mit der ersten Studie deuten auch diese Ergebnisse darauf hin, dass die In-vivo-Freisetzung und die Bioverfügbarkeit der untersuchten Arzneiform nicht durch

die untersuchten Applikationsvehikel beeinflusst werden und diese folglich zur gemeinsamen Einnahme mit der Alkindi®-Formulierung geeignet sind.

Diese Beobachtungen sind in guter Übereinstimmung mit den Ergebnissen einer publizierten In-vivo-Studie von Daniel *et al*. [145]. In dieser randomisierten Cross-over-Studie wurde Alkindi® gesunden Erwachsenen unter Anwendung von drei unterschiedlichen Dosierungsszenarien verabreicht. Die Alkindi®-Pellets wurden den Probanden entweder direkt auf die Zunge gegeben oder auf 5 mL Apfelmus sowie auf 5 mL Joghurt aufgestreut, und zusammen mit jeweils 240 mL Wasser eingenommen. Anschließend wurden die Plasmakonzentrations-Zeit-Profile von Hydrocortison bestimmt und die Bioverfügbarkeit anhand pharmakokinetischer Parameter evaluiert. Es konnte gezeigt werden, dass zwischen den unterschiedlichen Verabreichungsszenarien keine signifikanten Unterschiede zu erkennen waren.

Die Ergebnisse der zweiten Studie zum In-vitro-Freisetzungsverhalten der neuartigen Alkindi®-Formulierung mit pädiatrischen Applikationsvehikeln für Kinder zwischen 2 - 16 Jahren wurden 2020 unter dem Titel „*A biopredictive in vitro approach for assessing compatibility of a novel pediatric hydrocortisone drug product within common pediatric dosing vehicles*" im Journal „*Pharmaceutical Research*" veröffentlicht (siehe Kapitel 5.2).

2.2 Entwicklung von In-vitro- und In-silico-Modellen zur Simulation der Wirkstofffreisetzung in Parkinson-Patienten

In den letzten Jahrzehnten wurde zunehmend erkannt, dass neben den bereits bekannten motorischen Symptomen der Parkinson-Krankheit, wie z. B. Rigor oder Tremor, eine ganze Reihe weiterer, nicht-motorischer Krankheitssymptome existieren [146]. Vor allem die bis dato wenig beachteten gastrointestinalen Symptome können durchaus einen entscheidenden Einfluss auf die orale Arzneimitteltherapie in Parkinson-Patienten nehmen [98, 146] und sollten daher in der Entwicklung von In-vitro- und In-silico-Modellen Berücksichtigung finden.

Der erste Teil der vorliegenden Arbeit, der sich auf die Entwicklung eines pädiatrischen In-vitro-Freisetzungsmodells konzentrierte, beschränkte sich auf einen relevanten Aspekt, nämlich die Simulation der Mageninhalte nach oraler Einnahme einer Arzneiform mit alterstypischen Applikationsvehikeln. Der zweite Teil der Arbeit befasste sich hingegen weitaus detaillierter mit der Simulation der physiologischen Bedingungen im Gastrointestinaltrakt von Parkinson-Patienten, insbesondere auf der nüchternen gastrointestinalen Physiologie, und dementsprechend mit der Entwicklung eines neuartigen, Parkinson-spezifischen In-vitro-Freisetzungsmodells sowie zusätzlich mit der Entwicklung eines Parkinson-spezifischen PBPK-Modells.

Für ein besseres Verständnis der Pathophysiologie und dessen Einfluss auf den Gastrointestinaltrakt von Parkinson-Patienten wurde in den letzten Jahren eine Vielzahl von klinischen In-vivo-Studien mit verschiedensten Studiendesigns, Patientenkollektiven und Untersuchungstechniken durchgeführt. Im ersten Abschnitt dieses Teils der Arbeit wurde der aktuelle Wissensstand zu den gastrointestinalen Gegebenheiten in Parkinson-Patienten recherchiert und in Form eines Übersichtsartikels zusammengefasst. Das Ziel war die Erstellung

eines zuverlässigen und aktuellen Datensatzes bezüglich gastrointestinaler Parameter und deren Variabilität in dieser Patientengruppe, der die Entwicklung von neuartigen, biorelevanten und Parkinson-spezifischen In-vitro- und In-silico-Modellen zur Simulation der Wirkstofffreisetzung von peroralen Darreichungsformen ermöglicht. Nach umfangreicher Literaturrecherche wurden insgesamt 150 Publikationen in den Übersichtsartikel eingeschlossen [147]. Es wurden Studien von Parkinson-Patienten aller Krankheitsstufen berücksichtigt, um ein möglichst vollständiges Bild der gastrointestinalen Verhältnisse und deren Abhängigkeit vom Schweregrad der Erkrankung zu erhalten. Die Daten aus den inkludierten Parkinson-Studien wurden mit Referenzdaten aus Studien mit gesunden Erwachsenen bzw. Senioren verglichen und ausgewertet.

Bereits in der Mundhöhle von Parkinson-Patienten werden krankheitsbedingte Veränderungen ersichtlich. Sowohl die basale als auch die stimulierte Speichelproduktion ist vermindert [148-152]. Die bei Parkinson-Patienten paradoxerweise häufig beobachtete Sialorrhö, d. h. die übermäßige Speichelansammlung in der Mundhöhle, ist hingegen nicht das Resultat einer vermehrten Speichelproduktion, sondern vielmehr das Ergebnis eines ineffizienten und unregelmäßigen Schluckvorgangs [146]. Darüber hinaus scheint die Zusammensetzung des Speichels pathologisch verändert zu sein [148]. Das markanteste gastrointestinale Krankheitsmerkmal ist die gestörte Motilität des gesamten Gastrointestinaltrakts (Abbildung 4). Schluckstörungen, auch bezeichnet als oropharyngeale Dysphagie, sind ein häufiges nichtmotorisches Symptom von Parkinson-Patienten [146, 153]. Die Dysphagie-Problematik äußert sich beispielsweise in einem reduzierten Bolusvolumen und einer verringerten Schluckgeschwindigkeit [154-156]. Probleme beim Schlucken betreffen nicht nur die Einnahme von Nahrung und Flüssigkeiten, sondern auch die Einnahme von peroralen Darreichungsformen [157]. Die beeinträchtigte Motilität der Speiseröhre verstärkt die Dysphagieproblematik nur noch weiter oder ist vielleicht sogar der auslösende Faktor [158, 159]. In Studien konnte gezeigt werden, dass die gestörte ösophageale Motilität von Parkinson-Patienten eine verlängerte Passagezeit von Flüssigkeiten durch die Speiseröhre verursacht [154, 160]. Ebenfalls wurden eine gestörte ösophageale Peristaltik und verminderte Druckverhältnisse des oberen und unteren Speiseröhrensphinkters berichtet [161, 162].

Die beeinträchtigte Magenmotilität führt zu einer Verzögerung der Magenentleerung, die als Gastroparese bezeichnet wird [98, 146, 158]. Studien deuten darauf hin, dass Störungen der Magenmotilität sowohl in frühen als auch in fortgeschrittenen Krankheitsstadien mit einer Prävalenz von bis zu 100 % auftreten [98, 102]. Die Anzahl an klinischen Studien zur Untersuchung der Magenentleerungszeit bei Patienten mit *Morbus Parkinson* hat in den letzten Jahren deutlich zugenommen. Zur genaueren Beurteilung der Magenentleerung von Flüssigkeiten und fester Nahrung bei Parkinson-Patienten wurden in der Vergangenheit vor allem Szintigraphie und der ^{13}C-Atemtest eingesetzt. Zwar werden die aktuell verfügbaren Literaturdaten u. a. durch das jeweilige Studiendesign einschließlich der Untersuchungsmethode, den verabreichten Testmahlzeiten und -flüssigkeiten sowie den Charakteristika der Patienten und deren Medikation beeinflusst, doch zeigen die Ergebnisse aller Studien, wenn auch nicht immer signifikant, dass die Magenverweilzeit bei Parkinson-Patienten im Vergleich zu gesunden Kontrollgruppen erhöht war [163] (Abbildung 4). Ergebnisse aus Studien zur Untersuchung der intestinalen Motilität und

Passagezeit zeigen darüber hinaus, dass auch diese Parameter krankheitsbedingt beeinträchtigt sind, z. B. wurde über einen verzögerten Transit durch den Dünn- und Dickdarm berichtet [98, 164, 165]. Insgesamt stellen aktuell verfügbare Literaturdaten nur einige Momentaufnahmen aus diversen Studien dar, die in eher kleinen Studienkohorten und mit unterschiedlichen Messtechniken durchgeführt wurden, sie zeigen jedoch eindeutig eine gestörte Motilität und veränderte Passagezeiten auf. Die beschriebenen gastrointestinalen Symptome haben nicht nur schwerwiegende Auswirkungen auf die Lebensqualität der Patienten, sondern können auch die Pharmakokinetik von peroral verabreichten Arzneimitteln, insbesondere solcher, die zur symptomatischen Behandlung der Parkinson-Krankheit eingesetzt werden, ernsthaft beeinträchtigen [163]. So konnte bereits in mehreren Studien gezeigt werden, dass eine verzögerte Magenentleerung von Levodopa die Unvorhersagbarkeit von motorischen Fluktuationen erhöht, verursacht durch eine verzögerte und variable Resorption der Levodopa-Dosis in den vorderen Dünndarmabschnitten [166-168].

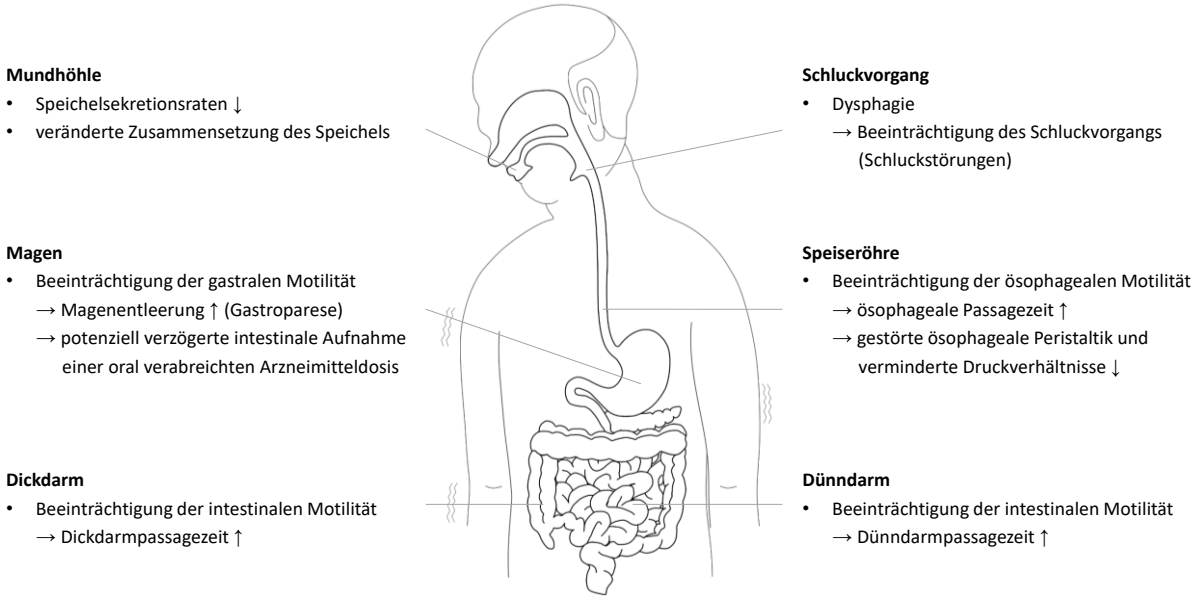

Abbildung 4: Zusammenstellung der gastrointestinalen Beeinträchtigungen von Parkinson-Patienten. Alle Bereiche des Gastrointestinaltrakts sind durch die Parkinson-Krankheit in unterschiedlichem Ausmaß und Ausprägung betroffen.

Die im Übersichtsartikel diskutierte Datenlage macht deutlich, dass es für Parkinson-Patienten noch immer einen großen Mangel an In-vivo-Daten hinsichtlich kontraktiler Motilitätsmuster und Drücke im gesamten Gastrointestinaltrakt sowie von Dünn- und Dickdarmpassagezeiten gibt. Auch existieren kaum robuste In-vivo-Daten bezüglich der Zusammensetzung und physikochemischen Eigenschaften luminaler Flüssigkeiten, die jedoch für die Etablierung eines biorelevanten In-vitro-Testmodells unabdingbar sind. Der Einsatz von nicht-invasiven Messtechniken, wie z. B. telemetrischen Kapseln oder Magnetresonanztomographie, und die Probengewinnung von gastrointestinalen Flüssigkeiten mit anschließender biochemischer Analyse könnten für ein besseres Verständnis der Parkinson-Krankheit sowie der Aufklärung wesentlicher gastrointestinaler Parameter in der betroffenen Patientengruppe beitragen. Die

Implementierung dieser Informationen in Parkinson-spezifische In-vitro- und In-silico-Modelle würde auch deren Prädiktivität hinsichtlich des In-vivo-Verhaltens von peroral applizierten Arzneimitteln in dieser Patientenpopulation erhöhen.

Die Ergebnisse der Literaturrecherche zu den luminalen Gegebenheiten im Gastrointestinaltrakt von Parkinson-Patienten wurden 2017 in einem Übersichtsartikel unter dem Titel „*A review of patient-specific gastrointestinal parameters as a platform for developing in vitro models for predicting the in vivo performance of oral dosage forms in patients with Parkinson's disease*" im Journal „*International Journal of Pharmaceutics*" veröffentlicht (siehe Kapitel 5.3).

Im nächsten Abschnitt der Arbeit lag der Fokus auf: i) der Konzeption von Parkinson-spezifischen In-vitro-Freisetzungsmodellen unter Anwendung des erhobenen Literaturdatensatzes bezüglich der gastrointestinalen Physiologie von Parkinson-Patienten, ii) der Zusammenstellung der Modellanforderungen an die zu verwendende In-vitro-Testplattform in Form eines Lastenhefts, iii) der Durchführung von In-vitro-Freisetzungsuntersuchungen unter Anwendung von Parkinson-spezifischen- sowie „standardmäßigen" Testmodellen, und iv) der Validierung der Parkinson-spezifischen Testmodelle mit Hilfe von In-silico-PBPK-Modellen.

Die grundlegenden Arbeiten dieses Teils der Arbeit erfolgten im Rahmen der Laufzeit des EUROSTARS-Projekts *Improved Treatment OF Morning Akinesia* (ITOFMA; FKZ: 01QE1504B), welches die Entwicklung einer neuartigen Levodopa-haltigen Darreichungsform zur Behandlung von Parkinson-Patienten in einem fortgeschrittenen Krankheitsstadium mit ausgeprägter Morgenakinese zum Ziel hatte. Die Wirkstofffreisetzung der entwickelten Prototypformulierungen sowie ausgewählter modifiziert freisetzender Levodopa-Handelspräparate sollte unter Verwendung von Parkinson-spezifischen In-vitro-Freisetzungsmodellen charakterisiert werden, um vielversprechende Formulierungsansätze zu erkennen und Strategien zu entwickeln, wie man diese Ansätze weiter verbessern kann. Für die vorliegende Arbeit spielen die entwickelten Formulierungsprototypen jedoch keine Rolle und werden im Folgenden nicht weiter thematisiert.

Die USP-3-Apparatur wurde als geeignete In-vitro-Testplattform ausgewählt, da sich diese Apparatur insbesondere zur Untersuchung von Darreichungsformen mit modifizierter Wirkstofffreisetzung eignet, bereits in einer Vielzahl von analytischen Laboren etabliert ist und auch für andere Testzwecke, z. B. für Freisetzungsuntersuchungen im Rahmen der Qualitätskontrolle, verwendet werden kann. Wie bereits einleitend erwähnt, wurden in „standardmäßig" verwendeten In-vitro-Freisetzungsmodellen für feste, perorale Darreichungsformen bisher vorwiegend die gastrointestinalen Gegebenheiten von gesunden, durchschnittlichen Erwachsenen („Standardpatient") simuliert, patientenspezifische Einflüsse jedoch kaum berücksichtigt. Es sollten daher bioprädiktive In-vitro-Freisetzungsmodelle entwickelt werden, die die physiologischen Verhältnisse im Gastrointestinaltrakt von Parkinson-Patienten adäquat simulieren und In-vitro-Freisetzungsdaten mit möglichst hoher Aussagekraft generieren. Im ersten Schritt sollten die In-vitro-Freisetzungsprofile der ausgewählten Levodopa-Handelspräparate unter Verwendung der arzneibuchkonformen USP-3-Apparatur und bereits etablierten Testsetups zur Simulation einer gastrointestinalen Passage im gesunden Erwachsenen untersucht werden, um einen Vergleichsdatensatz zu generieren. Im weiteren Verlauf der

vorliegenden Arbeit werden diese Testmethoden vereinfacht als „standardmäßig“ bezeichnet. Im zweiten Schritt sollte die Charakterisierung der Wirkstofffreisetzung mit dem zu etablierenden Parkinson-spezifischen In-vitro-Freisetzungsmodellen erfolgen. Anschließend sollten die generierten Datensätze aus den jeweiligen In-vitro-Modellen miteinander verglichen werden, um die Einflüsse der veränderten Testparameter auf die Wirkstofffreisetzung der untersuchten Handelspräparate näher zu untersuchen. Mit Hilfe von PBPK-Modellen sollte abschließend die Prädiktivität der neuartigen Parkinson-spezifischen Freisetzungsmodelle gegenüber den standardmäßigen Testmodellen in Bezug auf die In-vivo-Wirkstofffreisetzung der untersuchten Levodopa-Präparate in Parkinson-Patienten evaluiert werden.

Die In-vitro-Freisetzungsuntersuchungen unter Standardbedingungen wurden mit der arzneibuchkonformen Ausführung der USP-3-Apparatur (RRT 10, Erweka GmbH, Langen, Deutschland) durchgeführt [114, 169]. Die Apparatur besteht aus einem temperierbaren Wasserbad und enthält acht Reihen mit je sechs zylindrischen Glasgefäßen (Vessel), in die in der Regel jeweils ein standardmäßiges Testvolumen von 200 - 250 mL eingefüllt wird. Oberhalb der Vessel sind sechs Glaszylinder in vertikaler Position angebracht, in die jeweils die zu untersuchende Arzneiform eingebracht wird. Siebe mit definierter Maschenweite am oberen und unteren Ende ermöglichen den Ein- und Austritt des Freisetzungsmediums und stellen gleichzeitig sicher, dass die Darreichungsform in den Glaszylindern zurückgehalten wird. Während einer Freisetzungsuntersuchung werden die Glaszylinder mit der davon umschlossenen Darreichungsform in den temperierten Medien mit einer fixen Geschwindigkeit von 5 - 40 Dips pro Minute (dpm) und einer definierten Dipstrecke von 100 mm auf und ab bewegt. Zu bestimmten Zeitpunkten werden die Glaszylinder mit der darin enthaltenen Arzneiform von einer Vesselreihe zur nächsten transferiert. Die Apparatur ist weiterhin durch eine fixe Start- und Stoppposition der Glaszylinder und begrenzte Einstellmöglichkeiten von nur zwei Aktionen pro Vesselreihe, d. h. fixe Dipgeschwindigkeit und -zeit, gekennzeichnet. Infolgedessen ist die untersuchte Darreichungsform während der gesamten Versuchsdauer (mit Ausnahme des Reihenwechsels) in der Regel vollständig vom jeweiligen Freisetzungsmedium umgeben. Aufgrund der geringen Variationsmöglichkeiten lässt das Standardsystem keine Simulation typischer Motilitätsmuster im humanen Gastrointestinaltrakt zu und noch weniger die Simulation von individuellen, patientenspezifischen Motilitätsprofilen.

Um diese technischen Limitationen zu überwinden, wurde für die Weiterentwicklung des arzneibuchkonformen Modells (RRT 10) ein Lastenheft erstellt. Das Lastenheft enthielt detaillierte Anforderungen und Angaben für die Entwicklung und Produktion der neuen Testapparatur und wurde mit Hilfe eines Projektpartners (Erweka GmbH) umgesetzt. Das apparative Grunddesign des modifizierten Modells (RRT 10i, Erweka GmbH, Langen, Deutschland) entspricht der arzneibuchkonformen Ausführung der USP-3-Apparatur. Die neue Freisetzungsapparatur zeichnet sich jedoch zusätzlich durch eine größere Gesamtdipstrecke sowie frei konfigurierbare Dipstrecken aufgrund variabler Start- und Stopppositionen des Glaszylinders aus, z. B. zur Simulation geringen oder intensiven Flüssigkeitskontakts einer Darreichungsform mit den Freisetzungsmedien (Abbildung 5). Darüber hinaus kann die Arzneiform auch zeitweise oberhalb des Mediums bewegt werden, wo kein oder nur sporadischer Kontakt mit dem Freisetzungs-

medium besteht. Weiterhin wird auch die Konfiguration verschiedener Dippausen sowie deren Dauer und Position innerhalb oder außerhalb der Testmedien ermöglicht. Diese Einstellmöglichkeit kann u. a. Anwendung bei der Simulation einer gastrointestinalen Passage finden, bei der sich eine Arzneiform abwechselnd in flüssigkeitsreichen gastralen bzw. intestinalen Arealen und in eher „trockenen" bzw. flüssigkeitsarmen Zonen befindet. Wie bereits beschrieben, ist die Flüssigkeit entlang des Gastrointestinaltrakts nicht homogen verteilt, es findet viel mehr ein Wechsel zwischen flüssigkeitsreichen und -armen Bereichen statt, der in der Regel zu einer intermittierenden Passage und variierendem Flüssigkeitskontakt einer Arzneiform führt [9, 10]. Die Erweiterung des Dipgeschwindigkeitsbereichs auf 2 bis zu 70 dpm ermöglicht die Simulation von Testszenarien mit Phasen niedriger und hoher gastrointestinaler Motilität. Zuletzt erforderte die Konfiguration von detaillierten patientenspezifischen Motilitätsprofilen eine Erweiterung auf mehrere Aktionen pro Vesselreihe. Mit der modifizierten Testplattform kann nun eine zu untersuchende Darreichungsform in jedem Bereich des Vessels mit beliebiger Amplitude und einem großen Geschwindigkeitsspektrum bewegt werden. Insgesamt bietet der modifizierte apparative und operative Ansatz des RRT 10i zehn Variationsmöglichkeiten in puncto Flüssigkeitsvolumina, Motilitätsprofilen und Kontaktzeit einer Darreichungsform mit dem Freisetzungsmedium pro Vesselreihe und ist sowohl für arzneibuchkonforme, als auch für individualisierte Freisetzungsuntersuchungen einsetzbar. Mit dieser neuen technischen Plattform, in Kombination mit physiologisch relevanten Medien und Volumina, lassen sich patientenspezifische In-vitro-Testszenarien hinsichtlich der gastrointestinalen Motilität und Passage einer festen peroralen Darreichungsform in verschiedenen Parkinson-Patienten simulieren, sie bietet jedoch auch eine Fülle von weiteren Möglichkeiten und Anknüpfungspunkten im Bereich der patientenspezifischen In-vitro-Freisetzungsuntersuchung.

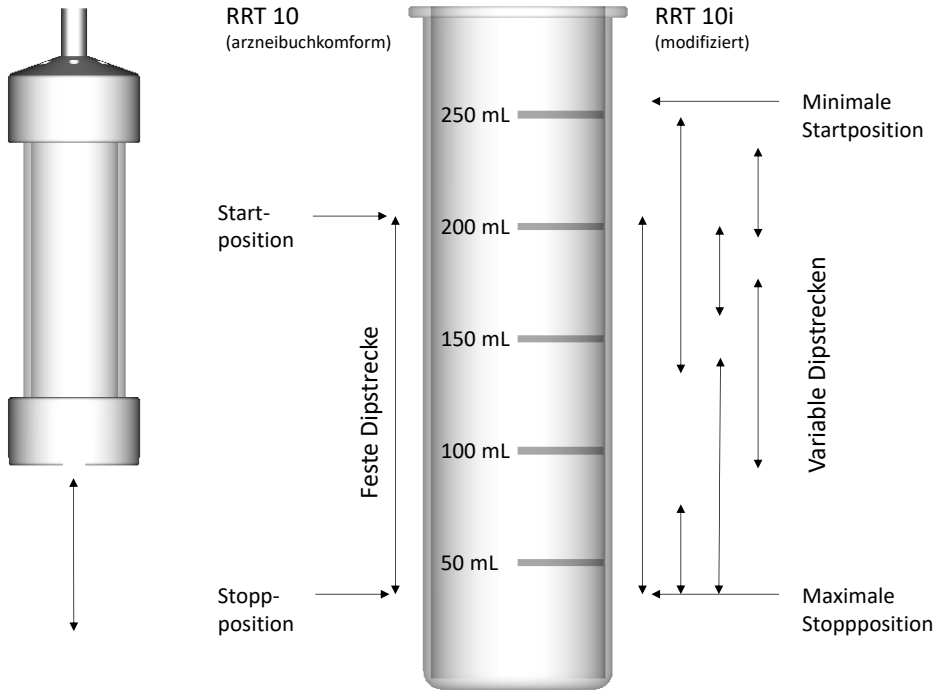

Abbildung 5: **Die arzneibuchkonforme (RRT 10, links) und modifizierte (RRT 10i, rechts) USP-3-Apparatur mit Start- und Stopppositionen. Für die modifizierte Apparatur sind Beispiele für variable Dipstrecken gegeben.**

Im ersten Teil der In-vitro-Studie wurde die Freisetzung von ausgewählten, modifiziert freisetzenden Levodopa-Fertigarzneimitteln im gesunden, durchschnittlichen Erwachsenen („Standardpatient“) unter Verwendung der arzneibuchkonformen USP-3-Apparatur simuliert. Der Fokus der In-vitro-Untersuchungen lag auf der simulierten Passage durch den oberen Gastrointestinaltrakt einer Arzneiform nach nüchterner Einnahme mit etwas Wasser, da Levodopa typischerweise ohne Nahrung eingenommen wird. In den verwendeten standardmäßigen In-vitro-Testmodellen wurden zwei typische Passagemuster einer festen Darreichungsform im Gastrointestinaltrakt gesunder Erwachsener unter Anwendung bereits etablierter In-vitro-Testsetups simuliert [170]. Multipartikuläre Darreichungsformen sind gekennzeichnet durch eine kontinuierliche Dünndarmpassage, während monolithische Formulierungen eher einen diskontinuierlichen Transit durch den Dünndarm aufweisen [171]. In den Untersuchungen wurde eine Vielzahl biorelevanter, unterschiedlich komplexer Freisetzungsmedien verwendet, die die wechselnden luminalen Verhältnisse während der Passage durch den nüchternen Magen, Dünndarm und des oberen Dickdarms hinsichtlich pH-Wert und Zusammensetzung der gastrointestinalen Flüssigkeiten von gesunden, durchschnittlichen Erwachsenen simulierten. Typische Testparameter waren 200 mL Freisetzungsmedium pro Vessel und eine Agitation von 10 dpm über die arzneibuchkonforme Dipstrecke von 10 cm (siehe Abbildung 5), die Gesamtdauer der Untersuchungen betrug jeweils 5 h. Die so generierten In-vitro-Daten sollten einen (ersten) Eindruck über das Freisetzungsverhalten der ausgewählten Arzneiformen unter standardmäßigen Testbedingungen vermitteln, Aussagen über den Einfluss von biorelevanten Freisetzungsmedien unterschiedlicher Komplexität auf das Freisetzungsverhalten der Arzneiformen liefern sowie als Vergleichsdatensatz in der nachfolgenden In-silico-Studie dienen.

Im zweiten Teil der In-vitro-Studie wurde die Freisetzung der untersuchten Levodopa-Fertigarzneimittel mit neuartigen Parkinson-spezifischen In-vitro-Testmodellen charakterisiert, die eine Vielzahl von verschiedenen gastrointestinalen Motilitätsszenarien simulierten. Diese Parkinson-spezifischen In-vitro-Freisetzungsmodelle wurden unter besonderer Berücksichtigung von Motilität, Passagezeiten und Flüssigkeitsvolumina auf Grundlage des beschriebenen Übersichtsartikels und unter Anwendung der modifizierten USP-3-Apparatur entwickelt. Im Vergleich zu den standardmäßigen In-vitro-Testmodellen wurden die Parkinson-spezifischen Untersuchungen mit einem Medienvolumen von 50 mL durchgeführt. Die Kontaktzeit der Darreichungsform mit den Freisetzungsmedien wurde, je nach Testsetup, verkürzt oder verlängert, um die intermittierende Flüssigkeitsverteilung und -kontaktzeiten innerhalb des Gastrointestinaltrakts zu simulieren. Eine reduzierte Agitation der Darreichungsform, umgesetzt durch (außerordentlich) niedrige Dipgeschwindigkeiten, simulierte die pathologisch verringerte gastrointestinale Motilität in Kombination mit kurzen Phasen hochintensiver Bewegung, z. B. während der simulierten Magenentleerung. Die aus der geringeren Motilität resultierende Verlängerung des gastrointestinalen Transits wurde ebenfalls berücksichtigt, indem die Magenverweil- sowie Dünndarmpassagezeiten (teils) deutlich verlängert wurden. Eine Ausnahme bildeten Testsetups, die einen Parkinson-Patienten mit noch relativ gesunder bzw. hoher Motilität simulierten. Der Transit durch den Dickdarm spielte hingegen keine Rolle, da das Resorptionsfenster von Levodopa im proximalen Dünndarm lokalisiert ist und folglich eine

mögliche Levodopa-Freisetzung im Bereich des Dickdarms für die Resorption/Exposition nicht mehr relevant ist [3]. Daher ist die vollständige Wirkstofffreisetzung im oberen Gastrointestinaltrakt eine essenzielle Voraussetzung für therapeutische Plasmakonzentrationen und eine erfolgreiche Levodopa-Therapie. Darüber hinaus wurde zu Beginn der In-vitro-Testsetups ein sehr kurzer, aber intensiver (30 dpm) oro-ösophagealer Transit der Darreichungsform simuliert. In den Parkinson-spezifischen Freisetzungsuntersuchungen wurden ebenfalls biorelevante Freisetzungsmedien verwendet, die die luminalen Flüssigkeiten von gesunden Erwachsenen im präprandialen Zustand simulierten. Die Entwicklung von Parkinson-spezifischen Freisetzungsmedien war nicht vorgesehen bzw. nicht möglich, da es bis dato kaum Literaturdaten zu den physikochemischen Eigenschaften der luminalen Flüssigkeiten von Parkinson-Patienten gab.

Die individuelle Variabilität der gastrointestinalen Motilität von Parkinson-Patienten wurde durch die Etablierung von drei unterschiedlichen motilitätsspezifischen In-vitro-Freisetzungsmodellen berücksichtigt. In diesen Testszenarien wurden Patienten mit einer auf den durchschnittlichen Parkinson-Patienten bezogenen hohen, moderaten bzw. niedrigen gastrointestinalen Motilität und einer daraus resultierenden schnellen, moderaten bzw. langsamen Passagezeit simuliert (Abbildung 6). Die drei Motilitätsmuster wurden jeweils weiter differenziert in Testsetups, die einen gastrointestinalen Transit einer Darreichungsform mit kontinuierlichem Flüssigkeitskontakt (PD 1, 4 & 9), mit intermittierenden Flüssigkeitskontakt (PD 6 & 8) oder Kombinationen aus beiden simulierten (PD 2, 3, 5, 7, 10 & 11). Insgesamt kamen 11 verschiedene patientenspezifische PD-Testszenarien (PD 1-11) zur Anwendung.

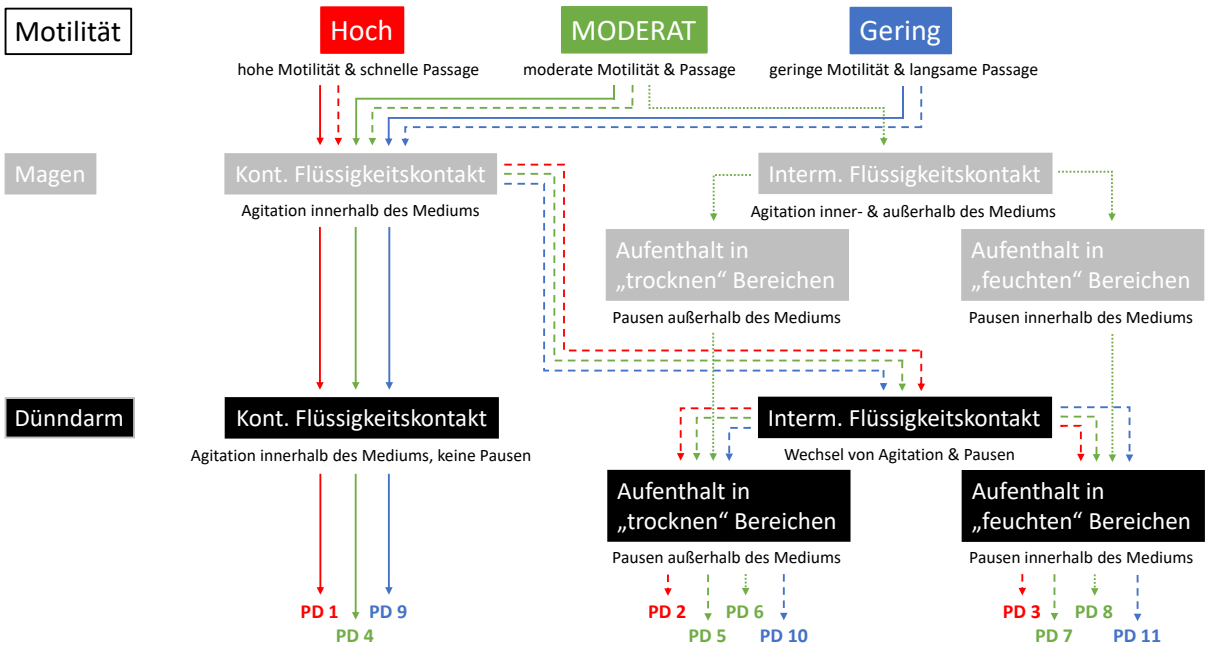

Abbildung 6: **Übersicht über die verwendeten Parkinson-spezifischen In-vitro-Freisetzungsmodelle (PD 1-11). Die drei Motilitätsmuster wurden jeweils weiter differenziert in Testsetups, die einen gastrointestinalen Transit einer Arzneiform mit kontinuierlichem Flüssigkeitskontakt, mit intermittierenden Flüssigkeitskontakt oder Kombinationen aus beiden simulierten.**

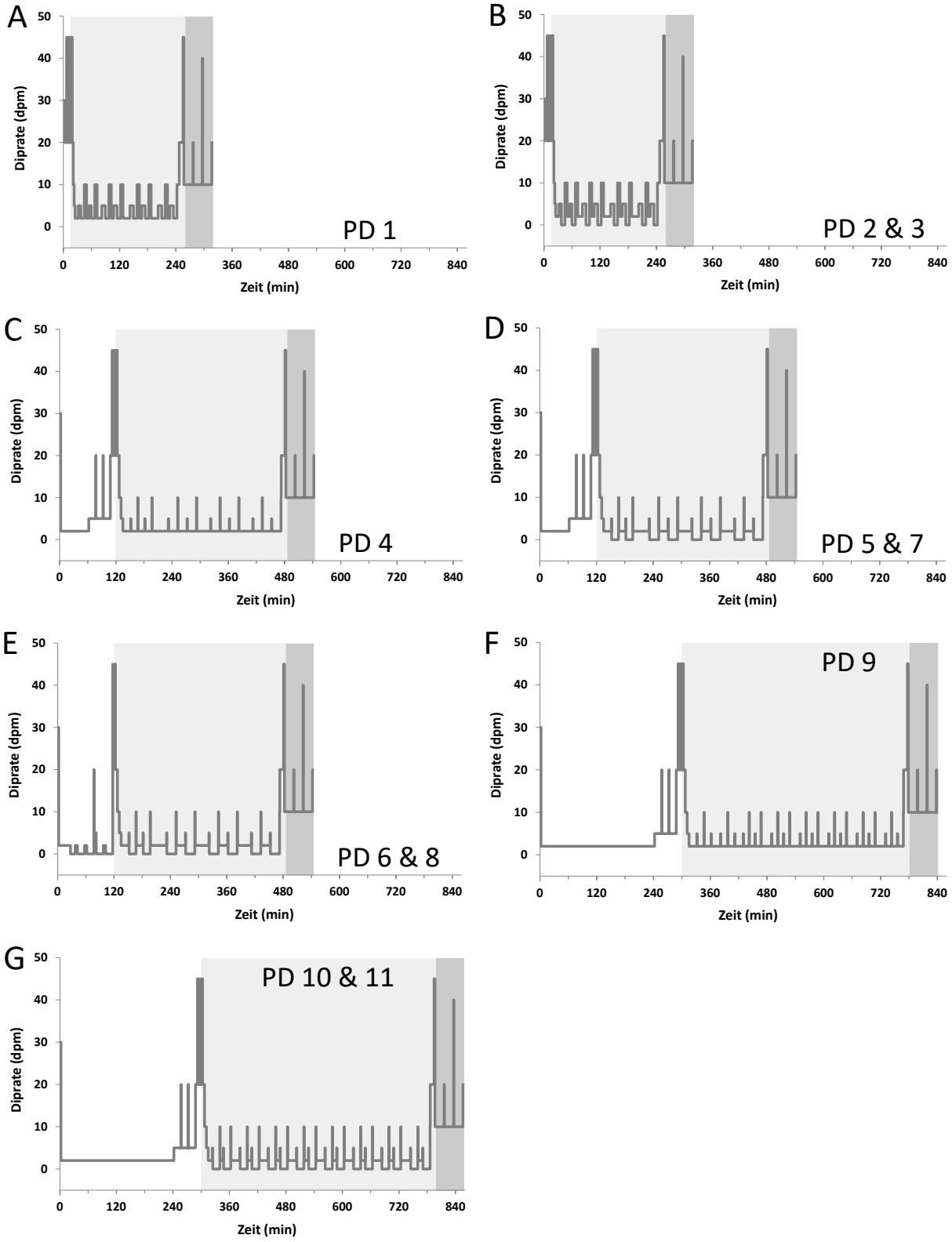

Abbildung 7: **Überblick über die verwendeten Motilitätsprofile, die Parkinson-Patienten mit hoher (A, B), moderater (C, D, E) und geringer (F, G) gastrointestinaler Motilität simulierten, dargestellt als Diprate pro Minute über die simulierte gastrointestinale Passage hinweg. Der nicht-schattierte Bereich zeigt den oro-ösophagealen und gastralen Abschnitt, die schattierten Bereiche illustrieren die Verweilzeiten im Dünndarm (hellgrau) und Dickdarm (dunkelgrau).**

Die kontinuierlichen In-vitro-Freisetzungstestsetups waren gekennzeichnet durch eine über die gesamte Versuchsdauer hinweg konstante Agitation der zu untersuchenden Darreichungsform (PD 1, 4 & 9). Die Kontaktzeit der Arzneiform mit den Freisetzungsmedien war abhängig vom jeweiligen simulierten Motilitätsprofil. Im Gegensatz dazu wurde in den intermittierenden Testsetups eine gastrointestinale Passage simuliert, die durch längere statische Phasen in Verbindung mit kurzen dynamischen Intervallen charakterisiert war (PD 6 & 8). Umgesetzt wurde dies durch Ruhepausen der Arzneiform innerhalb oder außerhalb der Testmedien, womit der Aufenthalt in flüssigkeitsreichen oder „trockenen" gastrointestinalen Bereichen simuliert werden sollte, und zu stark variierendem Flüssigkeitskontakt der Arzneiform führte. Darüber hinaus wurde auch ein kontinuierlicher gastraler Transit, d. h. konstante Agitation der Arzneiform während der simulierten Magenpassage, mit einer intermittierenden Dünndarmpassage, d. h. statische Agitationspausen der Arzneiform während des intestinalen Transits, kombiniert (PD 2, 3, 5, 7, 10 & 11). In Abbildung 7 C-E sind die detaillierten Motilitätsprofile, die die gastrointestinale Passage einer Darreichungsform in Parkinson-Patienten mit moderater gastrointestinaler Motilität simulierten, als Diprate pro Minute über die simulierte gastrointestinale Passage hinweg dargestellt. In-vitro-Testmodelle, die Parkinson-Patienten mit hoher gastrointestinaler Motilität simulierten, waren charakterisiert durch deutlich reduzierte Passagezeiten in den oberen Abschnitten des Gastrointestinaltrakts und einer erhöhten Agitation der Darreichungsform (Abbildung 7 A-B), während diese Parameter in Testsetups für Parkinson-Patienten mit geringer Motilität verlängert bzw. erniedrigt waren (Abbildung 7 F-G). Die jeweilige Gesamtdauer der In-vitro-Freisetzungsuntersuchungen betrug 5,3 h, 9 h bzw. 14 h.

Die In-vitro-Freisetzungsuntersuchungen wurden mit drei modifiziert freisetzenden Levodopa-Handelspräparaten, die durch jeweils unterschiedliche Formulierungsstrategien gekennzeichnet waren, durchgeführt (Tabelle 3). Nacom® Retardtabletten (MSD Sharp & Dohme GmbH, Haar, Deutschland; international bekannt als Sinemet® CR) stellen eine Tablettenformulierung mit verlängerter Wirkstofffreisetzung von Levodopa und Carbidopa (Dopa-Decarboxylase-Inhibitor, DDI) dar, in welche die Wirkstoffe in einer degradierenden Polymermatrix aus Hydroxypropylcellulose inkorporiert sind [172]. In-vivo-Studien haben gezeigt, dass Levodopa nach Einnahme der Darreichungsform kontinuierlich über einen Zeitraum von 4 - 5 h resorbiert wird [173]. In den Freisetzungsuntersuchungen wurde eine Dosisstärke von 200 mg Levodopa und 50 mg Carbidopa verwendet. Rytary® (Amneal Pharmaceuticals Inc., Bridgewater, USA) ist eine multipartikuläre Formulierung von Levodopa und Carbidopa mit dualen Freisetzungseigenschaften [174]. Unmittelbar nach der Einnahme erfolgt eine schnelle Freisetzung eines Teils der Dosis, die restliche Dosis wird anschließend pH-abhängig freigesetzt, da ein Teil der Pellets mit einem funktionellen Überzug aus Methacrylsäure-Methylmethacrylat-Copolymer im Verhältnis 1:1 bzw. 1:2 in Kombination mit Ethylcellulose versehen ist. In den In-vitro-Versuchen wurde Rytary® mit einer Dosisstärke von 195 mg Levodopa und 48,75 mg Carbidopa untersucht. Madopar® Depot (Roche Pharma AG, Basel, Schweiz; international bekannt als Madopar® HBS) ist eine Retard-Kapselformulierung von Levodopa (100 mg) und Benserazid (DDI, 25 mg). Die auch als „Schwimmkapsel" bezeichnete gastroretentive Arzneiform zeichnet sich durch ein Formulierungsprinzip, welches als „*hydrodynamically balanced system*" bekannt ist,

aus [175]. Nach Kontakt mit der Flüssigkeit im Magen und anschließender Auflösung der Gelatinehülle soll der Kapselinhalt einen hydrophoben, retardiert freisetzenden Körper mit einer Dichte unter 1 g/cm^3 bilden. Aufgrund der geringeren Dichte soll dieser auf dem Mageninhalt aufschwimmen und die beiden Wirkstoffe über einen langen Zeitraum im Milieu des Magens freisetzen [175].

Tabelle 3: Modifiziert freisetzende Levodopa-Fertigarzneimittel, die Bestandteil der In-vitro- und In-silico-Studien waren.

Handelspräparat	Darreichungsform	Wirkstoffe (Dosierung)	Hilfsstoffe
Nacom® Retardtabletten [176]	Retardtablette	Levodopa (200 mg), Carbidopa (50 mg)	Hyprolose, Poly(E)-but-2-ensäure-co-vinylacetat (0,05:1), Magnesium-stearat
Rytary® [177]	Hartkapsel mit schnell- und modifiziert frei-setzenden Pellets	Levodopa (195 mg), Carbidopa (48,75 mg)	Weinsäure, Mikrokristalline Cellulose, Mannitol, Ethylcellulose, Hypromellose, Natrium-stärkeglykolat, Natriumlaurylsulfat, Povidon, Talkum, Methacrylsäure-Copolymere (1:1 & 1:2), Triethylcitrat, Croscarmellose-Natrium, Magnesiumstearat (Hartkapsel: Gelatine, Titandioxid)
Madopar® Depot [178]	Hartkapsel mit retardierendem Inhalt	Levodopa (100 mg), Benserazid (25 mg)	Calciumhydrogenphosphat, Hypromellose, hydriertes Pflanzenöl, Povidon K 30, Magnesiumstearat, Mannitol, Talkum (Hartkapsel: Gelatine, Titandioxid)

Die Durchführung von experimentellen In-vitro-Untersuchungen erforderte die vorherige Etablierung einer geeigneten analytischen Methode. Aufgrund der ähnlichen physikochemischen Eigenschaften von Levodopa, Carbidopa und Benserazid sowie der unterschiedlichen Komplexität der verwendeten Freisetzungsmedien wurde die Hochleistungsflüssigkeitschromatographie (*High Performance Liquid Chromatography*, HPLC) als geeignete analytische Plattform ausgewählt. Zusätzlich sollte die Methode auch für den Catechol-*O*-Methyltransferase (COMT)-Inhibitoren Entacapon aufgrund zukünftiger Studien, die auch Formulierungen mit diesem Wirkstoff umfassen sollten, geeignet sein. In der bis dato aktuellen wissenschaftlichen Literatur wurde jedoch keine geeignete Methode für eine simultane Analyse der vier Analyten veröffentlicht. Unter Verwendung der HPLC wurde daraufhin eine entsprechende Methode entwickelt und nach offiziellen Leitlinien validiert. Die analytische Methode wurde 2017 unter dem Titel „*Development and Validation of a Robust and Efficient HPLC Method for the Simultaneous Quantification of Levodopa, Carbidopa, Benserazide and Entacapone in Complex Matrices*" im Journal „*Journal of Pharmacy & Pharmaceutical Sciences*" veröffentlicht (siehe Kapitel 5.4).

In den In-vitro-Testsetups zur Simulation der gastrointestinalen Passage in einem durchschnittlichen Erwachsenen zeigten die untersuchten Arzneiformen keine relevanten Unterschiede in ihrem jeweiligen Freisetzungsverhalten. Dies deutete darauf hin, dass die Zusammensetzung der verwendeten Freisetzungsmedien, beispielsweise mit oder ohne Zusatz von oberflächenaktiven Gallensalzen, keinen Einfluss auf die Freisetzungsprofile der ausgewählten Arzneimittel hatte. In den eingesetzten Parkinson-spezifischen In-vitro-Freisetzungsmodellen wiesen die untersuchten modifiziert freisetzenden Darreichungsformen hingegen eine unterschiedliche Sensitivität gegenüber den veränderten Testparametern auf.

Im Vergleich zu den standardmäßigen In-vitro-Freisetzungsmodellen wurde für Nacom® Retardtabletten in den Parkinson-spezifischen Freisetzungsmodellen eine deutlich verlangsamte Wirkstofffreisetzung beobachtet. Die verkürzte Kontaktzeit mit den simulierten gastrointestinalen Flüssigkeiten und die geringe Agitation der Arzneiform führten zu einer verringerten hydrodynamischen Beanspruchung der Formulierung und in der Folge zu einer verlangsamten Quellung und Erosion der Matrix, was vermutlich ursächlich für die beobachtete reduzierte Freisetzungsgeschwindigkeit war. Darüber hinaus erklärt dieser Umstand auch die langsamere Wirkstofffreisetzung in den Parkinson-Testmodellen mit sehr geringer Agitation sowie geringer Kontaktzeit mit den Freisetzungsmedien, z. B. bei der Simulation von „trockenen" Bereichen während der intermittierenden gastrointestinalen Passage. Im Gegensatz dazu machten die aus den Parkinson-spezifischen Testszenarien gewonnenen Freisetzungsprofile der multipartikulären Rytary®-Formulierung deutlich, dass die Wirkstofffreisetzung aus dieser Darreichungsform weder durch die unterschiedlichen Agitationsprofile, die variablen Medienkontaktzeiten, noch die verringerten Medienvolumina beeinflusst wurde. Eine signifikante Auswirkung auf das Freisetzungsverhalten der Rytary®-Formulierung zeigten jedoch die unterschiedlichen Verweilzeiten in den simulierten gastrointestinalen Abschnitten und den vorherrschenden pH-Bedingungen. Sowohl für Nacom® Retardtabletten, als auch für Rytary® deuten die mit Hilfe der Parkinson-spezifischen In-vitro-Testmodellen generierten Freisetzungsdaten auf eine größtenteils im Magen und Dünndarm stattfindende Freisetzung hin. Mehr als 80 % der untersuchten Dosen wurden vor bzw. innerhalb des Resorptionsfensters von Levodopa freigesetzt.

Für Madopar® Depot konnte in allen verwendeten In-vitro-Testmodellen eine langsame, aber konstante Freisetzung von Levodopa beobachtet werden. In Bezug auf die Freisetzungsergebnisse aus den standardmäßigen In-vitro-Testmodellen wurden in der Literatur ähnliche Freisetzungsprofile von Erni und Held [175] sowie von Schneider *et al.* [179] berichtetet. In den Parkinson-spezifischen Testmodellen wurde eine starke Verlangsamung der Freisetzung von Levodopa beobachtet, welche auf die Reduzierung der Agitation sowie Reduktion der Kontaktzeit der Darreichungsform mit den Freisetzungsmedien zurückzuführen war und folglich zu einer langsameren Quellung der Formulierung führte. Die verschiedenen Motilitätsszenarien der Parkinson-spezifischen In-vitro-Testmodelle schienen hingegen keinen Einfluss auf das Freisetzungsprofil von Levodopa zu haben. Dies deutet darauf hin, dass die Wirkstofffreisetzung unabhängig vom pH-Wert der Freisetzungsmedien und den verschiedenen Agitationsprofilen war.

Madopar® Depot wird den gastroretentiven Arzneiformen zugeordnet, jedoch werden die gastroretentiven Eigenschaften der Formulierung stark angezweifelt [179]. In einer In-vitro-Studie von Schneider *et al.* wurde gezeigt, dass Madopar® Depot sensitiv gegenüber physiologischen Druckbelastungen ist, wie sie z. B. im Magen während der Verdauung oder bei der Pyloruspassage auftreten [179]. Die Autoren berichteten, dass bereits geringe Drücke die Integrität der Arzneiform stark beeinträchtigten und in der Folge zur schnellen und vollständigen In-vitro-Wirkstofffreisetzung führten. Betrachtet man hingegen In-vivo-Daten aus klinischen Studien, so sind zumindest retardierende Freisetzungseigenschaften von Madopar® Depot nicht von der Hand zu weisen [180, 181]. Aufgrund der in Vorversuchen beobachteten pH- und agitationsunabhängigen Wirkstofffreisetzung der Formulierung wurde jedoch in den entwickelten Parkinson-spezifischen In-vitro-Testmodellen eine durch das Formulierungsprinzip bedingte verlängerte Magenverweilzeit nicht berücksichtigt. In der weiterentwickelten Version der USP-3-Apparatur war es noch nicht möglich, mechanische Druckbelastungen in den In-vitro-Untersuchungen zu simulieren. In der nächsten Entwicklungsstufe sollte dies jedoch umgesetzt werden, da physiologische Druckbelastungen, wie bereits erwähnt, die Integrität und folglich das Freisetzungsverhalten einer Arzneiform stark beeinträchtigen können. Ein weiteres denkbares Extrem wäre die rasche Entleerung der intakten Madopar® Depot-Formulierung aus dem Magen, wodurch aufgrund der langsamen Wirkstofffreisetzung nur ein geringer Teil der Dosis innerhalb des Resorptionsfensters von Levodopa freigesetzt werden würde, was in der Folge zu einem Wirkstoffverlust und subtherapeutischen Plasmakonzentrationen führen würde.

Die beobachteten Anfälligkeiten der untersuchten Levodopa-Handelspräparate gegenüber den unterschiedlichen (patho)physiologischen gastrointestinalen Bedingungen von Parkinson-Patienten sowie eine teils unvollständige Wirkstofffreisetzung innerhalb des Resorptionsfensters von Levodopa könnten ursächlich für die in klinischen Studien häufig beobachteten hochvariablen Plasmakonzentrationen und unvorhersehbaren Bioverfügbarkeiten gewesen sein [166, 182-195].

Im letzten Schritt wurde eine umfangreiche In-silico-Studie durchgeführt, um die Aussagekraft der entwickelten Parkinson-spezifischen- im Vergleich zu den standardmäßigen In-vitro-Testmodellen hinsichtlich des vorhergesagten In-vivo-Verhaltens der untersuchten Fertigarzneimittel einschätzen zu können. In dieser Studie wurden für jede der untersuchten Arzneiformen individuelle Plasmakonzentrations-Zeit-Profile von Levodopa auf Basis der individuellen In-vitro-Freisetzungsdatensätze simuliert und mit den jeweiligen klinischen In-vivo-Daten unter Anwendung eines pharmakokinetischen Simulationsprogramms korreliert. Die In-silico-Simulationen wurden mit der Software PK-Sim® Version 9.1 (Bayer Technology Services GmbH, Leverkusen, Germany) durchgeführt. In PK-Sim® ist ein Ganzkörper-PBPK-Modell implementiert, welches zur Beschreibung der komplexen pharmakokinetischen Vorgänge eines applizierten Arzneistoffs, z. B. nach peroraler Einnahme, verwendet wird. Für die Durchführung von In-silico-Simulationen in PK-Sim® sind eine Vielzahl verschiedener Datensätze notwendig, z. B. Anatomie und Physiologie des Patienten/der Population, physikochemische Eigenschaften des Arzneistoffs und In-vitro-Freisetzungsdaten der applizierten Darreichungsform [136]. Optimalerweise sollten PBPK-Modelle alle pathophysiologischen Veränderungen einbeziehen, die für die Wirkstofffreisetzung und -disposition bei Patienten mit gastrointestinalen oder

systemischen Erkrankungen im Vergleich zu gesunden Probanden relevant sind. In der aktuellen Literatur finden sich bislang nur wenige detaillierte PBPK-Modelle für besondere Patientenpopulationen. Bis dato wurden noch keine PBPK-Modelle für Patienten mit gastrointestinalen Erkrankungen publiziert, einzig Darwich *et al.* veröffentlichten ein In-silico-Modell für Patienten nach bariatrischen Eingriffen, z. B. Post-Roux-en-Y-Magenbypass [196]. Auch für systemische Erkrankungen existieren kaum publizierte In-silico-Modelle. Bislang wurden nur krankheitsbezogene PBPK-Modelle für Patienten mit Leber- und Nierenfunktionsstörungen veröffentlicht, jedoch enthalten diese Modelle wenig bis keine detaillierten Informationen über populationsspezifische Resorptionsunterschiede [52]. Ein Parkinson-spezifisches PBPK-Modell wurde bisher in der Literatur noch nicht beschrieben. Aufgrund der begrenzten Datenlage bezüglich der pathophysiologischen Veränderungen von Parkinson-Patienten wurden die Standardeinstellungen zur Anatomie und Physiologie in PK-Sim® nicht verändert. Es wurden lediglich Daten bezüglich der Speichelflussrate und des gastrointestinalen Transits aus dem beschriebenen Übersichtsartikel implementiert. Allgemeine altersbedingte anatomische und physiologische Veränderungen waren bereits in der PK-Sim®-Software integriert. Nach der Etablierung eines geeigneten PBPK-Modells wurden eine Reihe von Simulationen durchgeführt und anschließend pharmakokinetische Parameter (*Area under the curve* (AUC), maximale Plasmakonzentration (c_{max}), Zeitpunkt des Erreichens der maximalen Plasmakonzentrationen (t_{max})) und der dynamische Verlauf der simulierten Plasmakonzentrations-Zeit-Profile mit den klinischen In-vivo-Daten verglichen [136].

Die Auswertung der In-silico-Studie zeigte, dass für alle untersuchten Levodopa-Präparate Simulationen mit integrierten Freisetzungsdaten aus Parkinson-spezifischen In-vitro-Testmodellen jeweils deutlich größere Übereinstimmungen mit klinischen und pharmakokinetischen In-vivo-Daten aufwiesen als Simulationen, die Freisetzungsdaten aus den Standardtestmodellen enthielten. In-silico-PBPK-Modelle mit integrierten In-vitro-Daten aus standardmäßigen Freisetzungsmodellen waren gekennzeichnet durch starke Fluktuationen der simulierten Plasmakonzentrations-Zeit-Profile im Vergleich zu den In-vivo-Daten mit dem Ergebnis deutlich erhöhter AUC- und c_{max}-Werte.

Zwei klinische Studien (LeWitt *et al.* [187] und Bowes *et al.* [197]) mit Nacom® Retardtabletten wurden mit PBPK-Modellen repliziert. Die In-vivo-Daten der LeWitt-Studie wurden am besten repliziert durch In-silico-Simulationen mit integrierten Freisetzungsdaten aus In-vitro-Testmodellen, die eine gastrointestinale Passage mit kontinuierlichem Flüssigkeitskontakt bei einem Parkinson-Patienten mit moderater Motilität (PD 4) simulierten. Die größte Übereinstimmung zwischen den klinischen Daten der Bowes-Studie und den simulierten Daten wurde mit PBPK-Modellen erzielt, die In-vitro-Freisetzungsdaten aus Parkinson-spezifischen Freisetzungsuntersuchungen enthielten, die eine Passage mit kontinuierlichem Flüssigkeitskontakt bei einem Parkinson-Patienten mit hoher gastrointestinaler Motilität (PD 1) simulierten.

Die größte Übereinstimmung der replizierten In-vivo-Studie (Hauser *et al.* [198]) der Rytary®-Formulierung wurde mit einem PBPK-Modell erhalten, welches einen Parkinson-Patienten mit hoher gastrointestinaler Motilität und eine Passage mit intermittierendem Flüssigkeitskontakt in den intestinalen Abschnitten (PD 2) simulierte. Diese Beobachtung stimmt

mit der Tatsache überein, dass der In-vivo-Transit von multipartikulären Darreichungsformen durch eine eher kurze Verweildauer im Magen gekennzeichnet ist [44, 46].

Für die beiden replizierten In-vivo-Studien von Madopar® Depot (Malcolm *et al.* [180] und Stocchi *et al.* [181]) wurde die beste Vorhersage der Plasmakonzentrations-Zeit-Profile mit Simulationen erreicht, die Freisetzungsdaten von Parkinson-Testmodellen mit langsamer (PD 10) bzw. moderater (PD 5) simulierter Passagezeit und wenig Flüssigkeitskontakt in den intestinalen Abschnitten enthielten. Dies lässt zumindest darauf schließen, dass die Arzneiform für längere Zeit im oberen Gastrointestinaltrakt verweilt und Levodopa freisetzt.

Die klinischen Vergleichsstudien der untersuchten Fertigarzneimittel berichteten nur durchschnittliche In-vivo-Plasmakonzentrations-Zeit-Profile der jeweiligen Studienpopulation, individuelle Wirkstoffprofile mit dazugehörigen patientenbezogenen Informationen wurden nicht publiziert. Eine Ausnahme bildete die Studie von Stocchi *et al.*, in der u. a. ein individuelles pharmakokinetisches Profil eines Studienteilnehmers nach Einnahme von Madopar® Depot präsentiert wurde, jedoch ohne essenzielle patientenrelevante Informationen, z. B. Schweregrad der Erkrankung, anzugeben. Allgemein lässt sich festhalten, dass wichtige patientenbezogene Daten in veröffentlichten In-vivo-Studien häufig nur oberflächlich beschrieben werden oder gar gänzlich fehlen. Aus diesem Grund war eine vollständige Validierung der neuartigen In-vitro- und In-silico-Modelle nicht möglich. Eine Modellvalidierung soll sicherstellen, dass die In-vivo-Freisetzung einer untersuchten Arzneiform bei einzelnen Patienten anhand eines patienten-spezifischen In-vitro-Modells möglichst genau vorhergesagt werden kann. Eine ausführliche Validierung der Parkinson-spezifischen In-vitro- und In-silico-Modelle würde den individuellen Vergleich der In-vivo- und In-silico-Plasmakonzentrations-Zeit-Profile für jede der untersuchten Darreichungsformen beinhalten, vorausgesetzt: i) die Formulierungen wurden Parkinson-Patienten ii) mit bekanntem Krankheitsstatus verabreicht, iii) individuelle Patienteninformationen für die Implementierung in die In-vitro- und In-silico-Modelle sind bekannt, und iv) individuelle In-vivo-Plasmakonzentrations-Zeit-Profile für eine Korrelation stehen zur Verfügung. Ein weiterer Schritt wäre die Korrelation zwischen dem Krankheitszustand eines Patienten, dem In-vitro-Freisetzungsprofil (generiert aus einem patientenspezifischen In-vitro-Modell mit Berücksichtigung des entsprechenden Krankheitszustands), und dem individuellen In-vivo-Plasmakonzentrations-Zeit-Profil, welches nach Applikation der untersuchten Darreichungsform an diesen Patienten erzielt wurde. Wie bereits erwähnt, waren jedoch für die drei untersuchten Levodopa-Formulierungen keine individuellen Plasmakonzentrations-Zeit-Profile in der bis dato aktuellen Literatur verfügbar, daher wurden die simulierten Plasmakonzentrations-Zeit-Profile mit den beobachteten durchschnittlichen In-vivo-Plasmaprofilen verglichen. Dementsprechend kann dieser Modellierungsansatz sicherlich keine vollständige Validierung der entwickelten Modelle liefern, kann aber als ein erster wichtiger Schritt in diese Richtung betrachtet werden.

Patientenspezifische In-vitro-Freisetzungsmodelle stellen ein vielversprechendes Instrument zur Vorhersage der In-vivo-Wirkstofffreisetzung dar und können u. a. hilfreich in der Auswahl von geeigneten, neuartigen Formulierungen für bestimmte Patientengruppen sein. In Verbindung mit prädiktiven PBPK-Modellen lassen sich z. B. die Auswirkungen unterschiedlicher Formulierungsansätze oder der Variabilität der gastrointestinalen Physiologie auf In-vivo-

Plasmaspiegel untersuchen, ohne dass umfangreiche zusätzliche In-vivo- oder In-vitro-Studien erforderlich sind. Im vorliegenden Fall wurde eine neuartige USP-3-Apparatur speziell für die Simulation individueller gastrointestinaler Bedingungen von besonderen Patientenpopulationen entwickelt und erstmalig in Kombination mit neuen patientenspezifischen Testprotokollen verwendet. Es konnte deutlich gezeigt werden, dass die entwickelten In-vitro-Freisetzungsmodelle ein vielversprechendes Instrument zur Vorhersage der Wirkstofffreisetzung in Parkinson-Patienten darstellen. Weiterhin sollte bei der Konzeption von patientenspezifischen In-vitro-Freisetzungsmodellen neben den physiologischen Gegebenheiten auch das zu erwartende In-vitro-Freisetzungsverhalten der Arzneiform sowie der Formulierungstyp selbst (z. B. multipartikulär, monolithisch) sorgfältig berücksichtigt werden. In einem nächsten Schritt der Modellentwicklung sollte die Simulation des postprandialen Status anvisiert werden, da der prandiale Status einen starken Einfluss auf die Wirkstofffreisetzung haben kann. Für Levodopa, welches unter nüchternen Bedingungen eingenommen werden soll, wäre dies zwar nicht zielführend, jedoch werden auch andere Arzneistoffe von Parkinson-Patienten eingenommen, für die eine postprandiale Einnahme unter Umständen relevant sein kann. Der diskutierte methodische Ansatz der vorliegenden Studie könnte zukünftig zu einer verbesserten Arzneimitteltherapie für Parkinson-Patienten, aber auch für andere spezifische Patientengruppen beitragen.

Die Entwicklung der modifizierten USP-3-Apparatur und der Parkinson-spezifischen In-vitro-Freisetzungsmodelle sowie die Ergebnisse der In-vitro-Freisetzungsuntersuchungen und des PBPK-Modellings wurden 2022 unter dem Titel „*Patient-specific in vitro drug release testing coupled with in silico PBPK modeling to forecast the in vivo performance of oral extended-release levodopa formulations in Parkinson's disease patients*" im Journal „*European Journal of Pharmaceutics and Biopharmaceutics*" veröffentlicht (siehe Kapitel 5.5).

3 Zusammenfassung

Biorelevante In-vitro-Freisetzungsmodelle werden u. a. für das Screening neuartiger Formulierungen, zur Etablierung von In-vitro-/In-vivo-Korrelationen und zur Vorhersage des In-vivo-Verhaltens einer applizierten Darreichungsform angewendet. Die Entwicklung von In-vitro-Freisetzungsmodellen für peroral verabreichte Arzneiformen fokussierte bisher vorwiegend auf die Abbildung der gastrointestinalen Physiologie eines gesunden, „durchschnittlichen" Erwachsenen. Patientenspezifische Faktoren, wie z. B. das Alter, Erkrankungen oder Geschlecht sowie individuelle Unterschiede, die die gastrointestinalen Verhältnisse und folglich auch das Freisetzungsverhalten einer peroral applizierten Arzneiform beeinflussen können, wurden bisher kaum berücksichtigt. Der Fokus dieser Arbeit lag auf der Entwicklung und Etablierung von patientenspezifischen, bioprädiktiven In-vitro-Freisetzungsmodellen für perorale Darreichungsformen unter Berücksichtigung der gastrointestinalen Gegebenheiten zweier unterschiedlicher Patientenpopulationen: pädiatrische Patienten und Parkinson-Patienten.

Eine wichtige Voraussetzung für eine sichere und wirksame perorale Arzneimitteltherapie bei pädiatrischen Patienten sind altersgerechte Darreichungsformen sowie eine geeignete Einnahmepraxis. Peroral applizierte Arzneimittel werden pädiatrischen Patienten häufig zusammen mit Applikationsvehikeln verabreicht, um die Einnahme der Arzneimittel zu erleichtern. Es muss jedoch bei einer solchen Anwendungspraxis sichergestellt werden, dass die eingenommene Arzneiform mit dem jeweiligen Applikationsvehikel kompatibel ist. Die Beurteilung der Kompatibilität ist anhand klinischer In-vivo-Studien an gesunden Kindern jedoch aufgrund ethischer Bedenken kaum möglich. Zur Evaluierung der Kompatibilität könnten In-vitro-Freisetzungsmethoden als eine mögliche Alternative eingesetzt werden. Im ersten Teil der vorliegenden Arbeit wurden pädiatrische In-vitro-Freisetzungsmodelle entwickelt, um zu evaluieren, ob die Stabilität und das In-vivo-Freisetzungsverhalten der neuartigen Alkindi®-Formulierung durch Co-Verabreichung mit alterstypischen Applikationsvehikeln beeinträchtigt werden. Zur Beantwortung dieser Fragestellung wurden im Anschluss an eine intensive Literaturrecherche Physiologie-basierte In-vitro-Modelle auf Basis der Mini-Paddle-Apparatur entwickelt. In der ersten Studie wurde die In-vitro-Wirkstofffreisetzung nach simulierter Applikation der Alkindi®-Formulierung mit typischen Applikationsvehikeln für Kinder unter 6 Jahren, d. h. Muttermilch, Formulamilch und Vollmilch, untersucht. In der zweiten In-vitro-Studie wurde der Altersbereich der adressierten Patientenpopulation auf 2 - 16 Jahre verändert und eine Reihe weiterer flüssiger sowie halbfester Applikationsvehikel, wie z. B. Orangensaft und Joghurt, verwendet. In beiden Studien konnte deutlich gezeigt werden, dass die Alkindi®-Formulierung ein robustes Freisetzungsverhalten aufwies und kompatibel mit den untersuchten Matrices war. Auf Grundlage der Ergebnisse der In-vitro-Untersuchungen wurde geschlussfolgert, dass die In-vivo-Freisetzung und die Bioverfügbarkeit der untersuchten Arzneiform nicht durch die untersuchten Applikationsvehikel beeinflusst werden und folglich diese Vehikel zur gemeinsamen Einnahme mit der Alkindi®-Formulierung geeignet sind. Diese Beobachtungen wurden darüber hinaus durch publizierte Ergebnisse einer korrespondierenden In-vivo-Studie in Erwachsenen bestätigt.

Der zweite Teil der Arbeit befasste sich mit der Entwicklung eines neuartigen, Parkinson-spezifischen und Physiologie-basierten In-vitro-Freisetzungsmodells. Für die Entwicklung von biorelevanten In-vitro-Modellen zur Simulation der luminalen Bedingungen im Gastrointestinaltrakt einer spezifischen Patientenpopulation sind umfangreiche Kenntnisse über die jeweiligen gastrointestinalen In-vivo-Bedingungen und deren Variabilität unerlässlich. Im Rahmen einer Literaturrecherche wurde der aktuelle Wissensstand zu den gastrointestinalen Gegebenheiten in Parkinson-Patienten recherchiert, ausgewertet und zusammengefasst. Die Ergebnisse der Literaturstudie machen deutlich, dass sich die gastrointestinalen Bedingungen von Parkinson-Patienten teilweise erheblich von gesunden Erwachsenen unterscheiden. Das bedeutendste gastrointestinale Merkmal von Parkinson-Patienten ist die beeinträchtigte Motilität des Gastrointestinaltrakts, was sich u. a. in einer Verlangsamung der Magenentleerung sowie der intestinalen Passage äußert. Demgegenüber steht jedoch ein großer Mangel an Daten für eine Reihe von gastrointestinalen Parametern. Dies betrifft z. B. die Zusammensetzung und physikochemischen Eigenschaften der luminalen Flüssigkeiten des Gastrointestinaltrakts.

Als geeignete In-vitro-Testplattform wurde die USP-3-Apparatur – auch als Eintauchender Zylinder (Europäisches Arzneibuch, Ph. Eur.) und *Reciprocating cylinder* (Ph. Eur. und US-amerikanisches Arzneibuch, USP) bezeichnet – ausgewählt, da sich diese Testplattform insbesondere zur Untersuchung von Darreichungsformen mit modifizierter Wirkstofffreisetzung eignet und bereits in einer Vielzahl von analytischen Laboren etabliert ist. Die Nutzung der kompendialen USP-3-Apparatur ließ aufgrund der geringen Variationsmöglichkeiten keine Simulation typischer Motilitätsmuster im humanen Gastrointestinaltrakt zu und eignete sich noch weniger für die Entwicklung und Etablierung von individuellen, patientenspezifischen Motilitätsprofilen. Um diese technischen Limitationen zu überwinden, wurde für die Weiterentwicklung des arzneibuchkonformen Modells ein Lastenheft erstellt, welches detaillierte Anforderungen für die Entwicklung der neuen Testapparatur enthielt. Auf Grundlage des beschriebenen Übersichtsartikels und unter Anwendung einer auf Basis des Lastenheftes modifizierten USP-3-Apparatur wurden unter besonderer Berücksichtigung von Motilität, Passagezeiten und Flüssigkeitsvolumina Parkinson-spezifische In-vitro-Freisetzungsmodelle entwickelt. Für ausgewählte modifiziert freisetzende Levodopa-Fertigarzneimittel wurde anschließend eine vergleichende Serie von In-vitro-Freisetzungsuntersuchungen unter Anwendung von Parkinson-spezifischen- oder „standardmäßigen“ Testmodellen durchgeführt, wobei letztere die gastrointestinalen Gegebenheiten eines „durchschnittlichen“, gesunden Erwachsenen simulierten. Für eine Beurteilung der Aussagekraft der entwickelten Parkinson-spezifischen Testmodelle wurden die generierten In-vitro-Freisetzungsdaten aus den Parkinson-spezifischen- und den „standardmäßigen“ Freisetzungsuntersuchungen in ein In-silico-PBPK-Modell implementiert und die jeweiligen simulierten Plasmakonzentrations-Zeit-Profile von Levodopa anschließend mit klinischen, durchschnittlichen In-vivo-Daten korreliert. Für PBPK-Modelle mit integrierten Parkinson-spezifischen In-vitro-Freisetzungsdaten wurde eine höhere Prädiktivität des In-vivo-Verhaltens der untersuchten Levodopa-Darreichungsformen beobachtet. Es konnte gezeigt werden, dass die entwickelten Parkinson-spezifischen In-vitro-Modelle ein vielversprechendes und prädiktives Instrument zur Vorhersage der In-vivo-Wirkstofffreisetzung

von modifiziert freisetzenden Levodopa-Darreichungsformen darstellen. Der diskutierte methodische Ansatz der vorliegenden Studie könnte zukünftig das Screening neuartiger Formulierungen deutlich optimieren und somit zu einer verbesserten Arzneimitteltherapie für Parkinson-Patienten, aber auch für andere spezifische Patientengruppen beitragen.

4 Literatur

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5 Veröffentlichungen

5.1 Biorelevant *in vitro* assessment of dissolution and compatibility properties of a novel paediatric hydrocortisone drug product following exposure of the drug product to child-appropriate administration fluids

E. Wollmer, G. Neal, M. J. Whitaker, D. Margetson, S. Klein

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Diese Veröffentlichung beschreibt die erste von zwei Studien zur Untersuchung der In-vitro-Freisetzungs- und Kompatibilitätseigenschaften der speziell zur pädiatrischen Anwendung entwickelten Darreichungsform Alkindi® nach simulierter Co-Verabreichung mit alterstypischen Applikationsvehikeln. Alkindi® ist eine multipartikuläre, geschmacksmaskierte und schnell freisetzende Formulierung von Hydrocortison zur peroralen Applikation, indiziert zur Substitutionstherapie bei Nebenniereninsuffizienz bei Neugeborenen, Kindern und Jugendlichen. Peroral applizierte Arzneimittel werden pädiatrischen Patienten sehr häufig mit Hilfe von Applikationsvehikeln verabreicht, um die Einnahme der Arzneimittel zu erleichtern, wie z. B. durch eine Verbesserung der Schluckbarkeit und/oder des Geschmacks.

In dieser Studie wurde ein In-vitro-Modell zur Simulation der Freisetzung von Hydrocortison nach simulierter Applikation der Alkindi®-Formulierung mit typischen Applikationsvehikeln für Kinder unter 6 Jahren, d. h. Wasser, Muttermilch, Formulamilch und Vollmilch, sowie eine geeignete analytische Methodik und Probenaufbereitung entwickelt. Der Fokus der Modellentwicklung lag insbesondere auf der Simulation der Mageninhalte der pädiatrischen Patienten und weniger auf einer umfangreichen Darstellung der gastrointestinalen Physiologie. Um eine genauere Abstufung dieser Patientenpopulation zu erreichen, wurden die Dosierungsbedingungen jeweils für die drei verschiedenen Altersgruppen Neugeborene, Säuglinge/Kleinkinder und Vorschulkinder simuliert. In der Studie konnte deutlich gezeigt werden, dass die Alkindi®-Formulierung ein robustes Freisetzungsverhalten aufwies und die In-vivo-Freisetzung und Bioverfügbarkeit der Formulierung sehr wahrscheinlich nicht durch die Zusammensetzung der mitverabreichten Vehikel beeinträchtigt werden.

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Eigene Leistungen:
Planung und Durchführung der In-vitro-Untersuchungen inklusive Analytik, Entwicklung von Probenaufbereitungsmethoden, Erstellung des Manuskripts

Dr. Greg Neal:
Erarbeitung der Fragestellung, Mitarbeit bei der Diskussion und Korrektur des Manuskripts

Prof. Dr. Martin J. Whitaker:
Erarbeitung der Fragestellung, Mitarbeit bei der Diskussion und Korrektur des Manuskripts

Dr. Daniel Margetson:
Erarbeitung der Fragestellung, Mitarbeit bei der Diskussion und Korrektur des Manuskripts

Prof. Dr. Sandra Klein:
Entwicklung des Testkonzeptes, Diskussion der In-vitro-Untersuchungsergebnisse, Diskussion und Korrektur des Manuskripts

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Erik Wollmer	Prof. Dr. Sandra Klein

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Research paper

Biorelevant *in vitro* assessment of dissolution and compatibility properties of a novel paediatric hydrocortisone drug product following exposure of the drug product to child-appropriate administration fluids

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Compatibility

ABSTRACT

Alkindi® is a novel oral multi-particulate taste-masked formulation of hydrocortisone for use in children with the rare disease adrenal insufficiency. The objective of the present work was to study the biorelevant dissolution and compatibility properties of Alkindi® granules following exposure to administration fluids including breast milk, artificial milk, whole milk and water. To provide *in vitro* data for a representative patient collective, dosing conditions in neonates, infants and pre-school children were assessed. Experiments included the physicochemical characterisation of the different administration fluids, hydrocortisone solubility experiments and, dissolution experiments in which initial gastric conditions after the administration of a 0.5 or 5 mg dose with 50–200 mL fluid were simulated. The total duration of the dissolution experiments was 240 min to screen both dissolution and compatibility with the different fluids. Dissolution of the 0.5 mg dose was fast and complete in all scenarios, i.e. ≥80% of the dose was released in the neonate and the infant scenario and ≥75% in the pre-school children setup within 30 min. Results for the 5 mg dose were ~5–10% lower in all simulated patient scenarios. The results obtained in the present study confirm the compatibility and in-use chemical stability of Alkindi® with all studied dosing matrices and that *in vivo* dissolution and bioavailability of the product will not be affected by the composition of the co-administered fluids studied.

1. Introduction

Adrenal insufficiency is a rare endocrine disorder that occurs when the adrenal glands do not produce enough steroid hormones. Adrenal insufficiency can be primary or secondary. In primary adrenal insufficiency, also known as Addison's disease, the glands of the adrenal cortex are damaged and do not produce sufficient amounts of cortisol and aldosterone. Secondary adrenal insufficiency is a condition in which a lack of pituitary adrenocorticotropic hormone (ACTH) prevents the body from producing enough cortisol. Paediatric adrenal insufficiency is most commonly caused by the rare genetic condition congenital adrenal hyperplasia, or can occasionally be caused by autoimmune disease or pituitary conditions, but overall is a life-long and life-threatening condition that can present with a variety of symptoms that before diagnosis often cannot be distinguished from other health conditions. Appropriate replacement doses of glucocorticoids are important to determine in primary and secondary adrenal insufficiency in children, both to avoid risks of hypoglycaemia and adrenal crisis associated with undertreatment, and to avoid growth suppression and reduced final height potential associated with steroid overdosing [1]. Hydrocortisone represents the glucocorticoid of choice for treating adrenal insufficiency in children [2]. It is administered orally in two to four divided doses per day, where the single doses should be adjusted to the individual need [2]. Since adrenal insufficiency can present at birth and usually requires life-long and patient-tailored oral glucocorticoid medication, oral dosage forms that ensure acceptability in children of different age groups are an important prerequisite for safe, reliable and effective glucocorticoid substitution. However, optimisation of hydrocortisone replacement therapy in children is challenging as currently no licensed oral dose-appropriate paediatric formulation of hydrocortisone is available for children under 6 years of age [3]. Consequently, the majority of children with adrenal insufficiency are often treated with individualised pharmacy-compounded adult medication, a procedure which demonstrably bears risks of inaccurate dosing, poor disease

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control and potential adrenal crisis, particularly in very young children such as newborns and infants [3]. Therefore, there is a strong need for developing licensed oral paediatric formulations approved by regulatory authorities such as the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) [3].

When developing oral paediatric formulations, one should consider that the acceptability of paediatric pharmaceutical products to patients and their caregivers can have a profound impact on the resulting therapeutic outcome [4]. Major factors that can affect patient adherence are the swallowability and the palatability, including smell, taste, texture, and dose volume of an oral dosage form [5]. Consequently, besides ensuring that safe and effective hydrocortisone doses can be administered, acceptability in children of different age groups is an essential characteristic to consider when developing an oral hydrocortisone formulation for paediatric use.

Alkindi® (development programme name Infacort®) is an oral multiparticulate (granule) taste-masked formulation of hydrocortisone that was initially developed for use in children aged from birth to 6 years as replacement therapy in adrenal insufficiency. The formulation is available in unit doses of 0.5 mg, 1 mg, 2 mg and 5 mg. The granules represent an immediate release (IR) dosage form intended for oral delivery. They are coated with taste-masking excipients (ethyl cellulose and hypromellose), that are acceptable for paediatric use and eliminate the bitter taste of hydrocortisone to increase acceptability. The granules are contained in size 00el HPMC hard capsules which are used as a carrier and not for consumption. Alkindi® is intended to be dosed to paediatric patients by administering the capsule contents directly onto the child's tongue. Alternatively the capsule contents can be placed on a dry spoon and administered into the patient's mouth. Immediately after administering the granules, they should be washed down with fluid such as water or milk.

The EMA agreed a Paediatric Investigation Plan (PIP – EMEA 001283-PIP01-12) for the Alkindi® product, which included confirmation of its compatibility with commonly used dosing matrices for children from birth to 6 years of age, i.e. drinking water, artificial milk, whole milk and breast milk, using an *in vitro* assessment. Results from the requested experiments should provide proof that *in vivo* hydrocortisone exposure in neonates, infants and pre-school children will not be affected by the composition of milk co-administered with the multiparticulates.

The objective of the present work was to study biorelevant dissolution and compatibility properties of Alkindi® following exposure of the drug product to administration fluids including breast milk, artificial (formula) milk and water by using physiologically-based paediatric dissolution models.

2. Materials and methods

2.1. Materials

Alkindi® capsules containing granules corresponding to a 0.5 mg (batch # 0624/2014-P), 1 mg (batch # 0625/2014-P), 2 mg (batch # 0626/2014-P) or 5 mg (batch # 0629/2014-P) hydrocortisone dose, were obtained from Glatt GmbH, Binzen, Germany. Hydrocortisone standard material, batch # W006644 (Pfizer batch # H31823) was also obtained from Glatt GmbH. All other chemicals for media preparation and sample analysis were of analytical or gradient grade, and purchased commercially. Water, formula milk and whole milk (Table 1) were purchased from a local supermarket or drugstore, respectively.

2.2. In vitro study design

2.2.1. Age groups to be addressed and dosage strengths tested

Alkindi® is intended to be administered to children from birth. As it initially was developed for use in children up to 6 years, dosing conditions in three different age groups, i.e. newborns/neonates (< 28 days old), infants and toddlers (> 28 days to 23 months) and pre-school children (2–6 years old) were simulated. In children of these age groups the proposed hydrocortisone dose per unit is 0.5 mg, 1 mg, 2 mg or 5 mg. Since the same type of formulation is to be used in all age groups and the dose is just varied by the amount of granules given to the paediatric patients, with the aim of getting an estimate of the *in vivo* performance of Alkindi®, it was regarded to be sufficient to focus on the highest and lowest single dose units administered to patients. Therefore single doses of 0.5 mg and 5 mg were used in the study.

2.2.2. Administration fluids

The administration fluids (dissolution media) applied in the study were water for all age groups, breast milk and artificial milk (hereinafter referred to as formula milk) for newborns and the infants and toddlers group, and whole milk for the pre-school children. Experiments in breast milk from two different sources were performed to address the variability in composition of breast milk. Two different formula milk types were used to address typical dosing conditions in neonates, infants and toddlers. The products used to simulate different dosing conditions in neonates, infants and pre-school children are listed in Table 1.

A commercial water for the preparation of baby food (Humana® Babywasser) was used to simulate co-administration of the dosage form with drinking water and also to prepare the formula milk for the dissolution experiments. Two different formula milks, i.e. formula for newborns (formula milk 1) and formula for infants > 10 months (formula milk 3) were used to simulate alternative baby feeds. A globally marketed brand was used to be representative for formula milks that is used in many countries of the world. Regular whole milk (UHT, 3.5% fat) was used to simulate dosing conditions in pre-school children. Finally, breast milk from 2 different mothers and two different phases in the lactation period (day 1–8 and week 7–8 after birth) was used in the experiments (see Table 1 for more details). The breast milk was sourced in bottles with filling volumes of 50–250 mL and was obtained from the breast milk bank at the Department of Neonatology and Paediatric Intensive Care, Hospital for Paediatrics, University of Greifswald. All breast milk samples were obtained in a frozen state and stored in a freezer at −20 °C. Before dissolution and solubility testing, the required number of bottles was taken from the freezer and placed in a pre-heated water bath of 37 °C for 30 min. After checking the bottles for completion of the thawing process and equilibration to 37 °C, breast milk was immediately used for the respective experiment.

2.2.3. Physicochemical characterisation of the administration fluids

To estimate how the different administration fluids might affect solubility and stability of hydrocortisone and dissolution of the granules, in a first set of experiments, the physicochemical properties of the fluids were characterised as described in [6]. Physicochemical characterisation included the following parameters: pH value and buffer capacity, osmolality, surface tension and viscosity. With the exception of osmolality parameters were recorded at two temperatures, i.e. 25 °C and 37 °C. All experiments were run in sextuplicate and results expressed as mean (± S.D.). The pH value was measured with a pH-meter (HI 99161, HANNA instruments, Woonsocket RI, USA). The buffer capacity was quantified by potentiometric titration with 0.1 N or 0.01 N hydrochloric acid, respectively. Osmolality was measured via the freezing point depression method using a semi-micro osmometer (K-7400, Knauer, Berlin, Germany). The surface tension was determined with a ring tensiometer (K11, Krüss GmbH, Hamburg, Germany) and the viscosity was determined with different types of Ubbelohde viscometers (type 0c, $K = 0.002692\,mm^2/s^2$, DIN 51562, SI Analytics, Mainz, Germany; type I, $K = 0.01008\,mm^2/s^2$ and type II, $K = 0.09939\,mm^2/s^2$, both from LaborTherm, Jena, Germany).

2.2.4. Solubility experiments

The European and the United States Pharmacopoeia [7,8] describe

Table 1
Products used to simulate different dosing conditions in neonates, infants and pre-school children.

Fluid type	(Commercial) Product	Manufacturer/Source	Batch #
Water	Humana® Babywasser	Humana GmbH, Herford, Germany	47159351
Formula milk 1 (baby formula)	Beba® PRO® 1 (Anfangsmilch)*	Nestlé Nutrition GmbH, Frankfurt, Germany	3343080623
Formula milk 3 (infant formula)	Beba® PRO® 3 (Folgemilch)**	Nestlé Nutrition GmbH, Frankfurt, Germany	3300080622
Whole milk (3.5% fat) heat treated	Weihenstephan Vollmilch 3.5% Fett	Molkerei Weihenstephan GmbH & Co. KG, Freising, Germany	03L3136X00077056
Breast milk 1	Milk from a mother of twins born in week 31 of pregnancy	not heat-treated	week 7–8 after birth
Breast milk 2	Milk from a mother of a single child born in week 42 of pregnancy	not heat-treated	day 1–8 after birth

* Powder product: Beba® PRO® 1 baby formula was prepared by suspending 1 measuring spoon (5.1 g) in 30 mL Humana Babywasser resulting in 33.33 mL finished product.

** Powder product: Beba® PRO® 3 infant formula was prepared by suspending 1 measuring spoon (4.7 g) in 30 mL Humana® Babywasser resulting in 33.33 mL finished product.

hydrocortisone as practically insoluble in water. However, hydrocortisone solubility in different kinds of administration fluids was never studied. Thus, a series of (kinetic) solubility experiments of hydrocortisone was performed in each of the administration fluids to assess the maximum amount of hydrocortisone that could dissolve in the aqueous phase of a physiological gastric volume after co-administration of a single Alkindi® dose to children of different age groups. All solubility experiments were performed in triplicate at 37.0 ± 0.5 °C using the shake-flask method. Experiments were performed as follows: An excess of drug was added to a 5 mL flask containing 4 mL of the respective fluid and the resulting suspension was stirred with a magnetic stirring bar at 220 rpm. During the mixing period, the flasks were inspected to ensure that each flask contained undissolved, excess drug. Drug was added as necessary to maintain an excess. Samples were taken at 2, 4, 8 and 24 h after the start of the experiment. This sampling schedule is somewhat different from a typical sampling schedule applied in solubility experiments, where usually just one sample is taken when an equilibrium concentration of the compound in the test medium is reached after 24 or 48 h. However, since the stability of the different administration fluids over such long test durations was not known and since it was likely that some of the media might not be stable over such a long time period, samples were also taken at earlier time points. It should be kept in mind that the solubility results from sampling at typical sampling time points (e.g. 24 h) might thus indicate media stability rather than being representative for the saturation solubility in the original medium. Nevertheless, it was thought that the sampling schedule might be helpful in estimating the fate of hydrocortisone in the different media over time.

To get an estimate of fluid stability and to simulate realistic administration conditions, where a pH-adjustment would also not happen, in all solubility experiments, in contrast to a standard solubility test protocol, the media pH was not adjusted to the original value (media pH at the start of the experiment) in case of any pH change. However, to get an idea of the reason for any pH change (as a result of drug dissolution or media instability), in parallel to each solubility experiment and, over the same time range, the pH of the respective plain medium was monitored. For this purpose, 4 mL of test media was placed in a 5 mL flask and stirred with a magnetic stirring bar at 220 rpm and a temperature of 37 °C. The pH was measured hourly for the first 8 h and again after 24 h. Each experiment was performed in triplicate.

2.2.5. Dissolution experiments – fluid volumes

The fluid volumes to be used for a dissolution experiment with a single Alkindi® dose (Table 2) were adapted to volumes estimated to be available in the stomach of children of different age categories after administering the dose with sufficient fluid. The estimated available fluid volumes comprise the resting gastric fluid volumes in neonates [9], infants [10] or pre-school children [11,12] plus the volume of co-ingested fluid.

2.2.6. Dissolution experiments – test setup and sampling procedure

Most of the gastric fluid volumes to be simulated in the study did not allow the use of standardised dissolution equipment. However, the objective of the series of experiments was to compare the impact of fluid composition and volume on *in vitro* dissolution of a given dose in a predictive and reproducible manner, i.e. the test design should be physiologically relevant, but the experiments should be performed with standardised (official) equipment. Consequently, dissolution experiments were performed with the Mini-Paddle apparatus (DT 600, Erweka, Heusenstamm, Germany) using a media volume of 200 mL [13,14]. Where necessary both the dose and the respective test volume were up-scaled proportionally (see Table 2). All experiments were conducted at 37.0 ± 0.5 °C. Before running the first set of experiments, some preliminary tests were performed to determine what would be an appropriate paddle speed and also an appropriate means of adding the test dose to the media. During these screening experiments pronounced coning of the granules in the dissolution vessel was observed at 75 rpm. The coning was as a result of the larger amount of granules that had to be added to the vessel in comparison to the media volume and was particularly relevant when administration of the 5.0 mg dose (about 0.77 g granules per 5 mg dose, resulting in 3.08 g granules in 200 mL media when simulating dosing conditions in neonates) was simulated. A paddle speed of 75 rpm was then not sufficient to freely disperse the entire sample in the lower part of the vessel. For this reason, in all experiments the paddle speed was set to 100 rpm. This paddle speed did still not guarantee free dispersion of the granules, but ensured that the granules were not sticking together. The paddle speed was not increased further in order for the method to apply sufficient discriminative power. Moreover, an excessive paddle speed (> 100 rpm) could have resulted in "abusing" the rotating paddle as a high speed mixer that could speed up disintegration and dissolution of the granules by mechanical forces. In the present case, a paddle speed of 100 rpm guaranteed reliable results and sufficient discriminatory power.

When performing the first screening experiments to determine how best to add the granules to the dissolution media, when sprinkling the granules into the vessel, they did not immediately sink to the bottom of the vessel but floated on the media surface. A sprinkle method was thus regarded as not being optimal for this purpose. Therefore, several alternative methods were screened before the final method described below was developed. The granules were filled into a straw which had been sealed with a piece of Parafilm M® laboratory film (Bemis, Neenah, WI, USA) at its lower end. With the paddle rotating at 100 rpm, the filled straw was lowered into the dissolution medium. A small amount of air pressure was used to displace the sample from the straw, resulting in release of the granules followed by immediate dispersion under the paddle (see Fig. 1).

The total duration of the dissolution experiments was 240 min. Samples of 3 mL were removed at predetermined time points, i.e. 5, 10, 15, 30, 45, 60, 90, 120, 180 and 240 min. A test duration of 240 min does not necessarily represent typical gastric residence times in

Table 2
Estimated typical gastric fluid volumes available after dose administration in children of different age groups and upscaled dose:volume ratios used in the dissolution test experiments.

Age group	Test medium	Lowest dose	Highest dose	Available volume	Lowest dose upscaled	Highest dose upscaled	Available volume upscaled
Neonates	Water Breast milk 1 Breast milk 3 Formula milk 1	0.5 mg	5.0 mg	50.0 mL	2.0 mg	20.0 mg	200.0 mL
Infants	Water Breast milk 1 Breast milk 3 Formula milk 3	0.5 mg	5.0 mg	100.0 mL	1.0 mg	10.0 mg	200.0 mL
Pre-school children	Water Whole milk	0.5 mg	5.0 mg	200.0 mL	0.5 mg	5.0 mg	200.0 mL

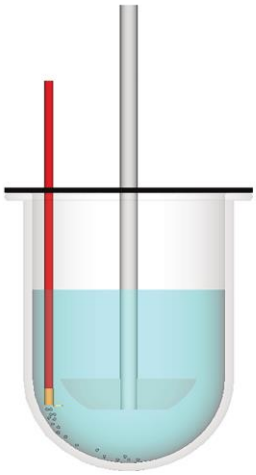

Fig. 1. Setup applied for adding the granules to the dissolution medium.

children (particularly not when the dosage form will be administered with plain water). However, since to date there is not that much known on gastric emptying times in children and it has been reported that particularly within the first days of life, gastric emptying might be much slower than with increasing age of the child [15,16], it might happen that in such patients the formulation and the co-administered fluid will rest in the stomach for a time period significantly longer than half an hour or an hour. Therefore, it was decided to run the test for a longer duration. In addition, such procedure also allowed the stability of dissolved drug and media to be studied over a longer time frame. Data obtained from later sampling time points were thus regarded as indicators of hydrocortisone stability in the test medium (precipitation, degradation), the medium itself (pH, colour, phase separation, agglomeration) or both. In all experiments the sample volume was replaced by fresh medium and all experiments were run in triplicate.

2.2.7. Sample preparation and analysis

Aqueous samples from dissolution and solubility testing in water were filtered via a 0.45 µm PVDF filter (diameter 13 mm, Whatman Schleicher & Schuell, Dassel, Germany) directly after sampling. To adapt the sample composition to that of the mobile phase (80% water and 20% tetrahydrofuran v/v) 0.2 mL tetrahydrofuran was added to 0.8 mL of the sample. Subsequently, samples were analysed by High Performance Liquid Chromatography (HPLC).

Samples from dissolution and solubility testing, which were performed in biorelevant media (breast milk, formula milk and whole milk) required a special sample preparation procedure: Samples were first transferred into 1.5 mL polypropylene SafeSeal tubes (Sarstedt, Nuembrecht, Germany) and centrifuged for 30 min at a rotation speed of 13,000 rpm resulting in phase separation. In the next step, 1 mL of the aqueous phase was removed and transferred into a new cap. Subsequently, 0.5 mL of acetonitrile was added and the samples were again centrifuged as described before. In the last step, 1 mL of the supernatant was removed and filtered via a 0.45 µm PVDF filter. Again, to adapt the samples to the composition of the mobile phase, 0.2 mL tetrahydrofuran was added to 0.8 mL of the filtrate and (as particularly when using formula milk, this resulted in slight precipitation) centrifuged for a third time to ensure complete protein/particle removal. Finally, 0.8 mL of the supernatant was transferred to HPLC vials, as necessary diluted with mobile phase and analysed by HPLC.

To ensure that due to these various separation and dilution steps all the drug that had dissolved in the aqueous phase prior to analysis could be properly quantified, for each of the experiments, the same separation and dilution steps were performed using the respective media containing known amounts of dissolved hydrocortisone. For this purpose, the amount of drug that would freely dissolve in the aqueous phase of the respective medium was calculated based on the results of the solubility experiments and used to prepare standard samples with known concentrations of dissolved hydrocortisone. After all separation and dilution steps the recovery for these standard samples was calculated. As necessary, the concentration of hydrocortisone released in the different test media was then corrected on the basis of the calculated recovery factor for the standard solution. All correction factors used for the respective calculations were between 1.00 and 1.13.

After appropriate sample preparation, samples were analysed by HPLC (Waters system consisting of an 2707 autosampler, a 1525 binary pump, a 2998 photodiode array detector and the Breeze 2 software, Waters GmbH, Eschborn, Germany) using a Waters Symmetry C18, 3.5 µm, 75 × 4.6 mm column, equipped with a Waters Symmetry C18 Sentry Guard, 5 µm, 20 × 3.9 mm precolumn equilibrated at 45 °C, under isocratic conditions using a tetrahydrofuran/water (20:80 v/v) mobile phase at a flow rate of 1.5 mL/min. Hydrocortisone was detected with UV detection at 254 nm. The method had been adapted from Glatt GmbH and was partly re-validated before use: The linearity was screened for concentration ranges of 0.001–0.050 mg/mL and 0.01–0.40 mg/mL, respectively, in both cases R^2 was 1.000. Moreover, with dilution sets in the same concentration ranges, the accuracy of the mean as well as the precision was checked. Both parameters were in the limits of ± 5%. Therefore, the method was regarded as appropriate for the intended use. The injection volume was 20 µL for samples from the solubility experiments and dissolution experiments in water. For dissolution samples in all other media, the injection volume was increased to 50 µL.

Table 3
Mean values (± S.D.) of the different physicochemical parameters of the administration fluids, (n = 6 per measurement).

Parameter	Temp.	Water	Breast milk 1	Breast milk 2	Formula milk 1	Formula milk 3	Whole milk (3.5% fat)
pH value	25 °C	7.31	6.76	6.89	6.78	6.61	6.63
		(0.01)	*(0.08)*	*(0.02)*	*(0.03)*	*(0.01)*	*(0.01)*
	37 °C	7.89	6.82	6.74	6.73	6.59	6.50
		(0.01)	*(0.03)*	*(0.04)*	*(0.02)*	*(0.02)*	*(0.06)*
Buffer capacity [mEq/pH/L]	25 °C	0.11	6.80	7.30	7.08	8.70	16.83
		(0.00)	*(0.21)*	*(0.13)*	*(0.16)*	*(0.28)*	*(0.16)*
	37 °C	0.06	6.67	7.23	7.13	8.40	19.05
		(0.00)	*(0.12)*	*(0.08)*	*(0.22)*	*(0.11)*	*(0.60)*
Osmolality [mOsmol/kg]		4	293	280	271	218	266
		(1)	*(6)*	*(10)*	*(6)*	*(8)*	*(3)*
Surface tension [mN/m]	25 °C	70.2	27.65	29.92	43.93	44.99	42.49
		(0.36)	*(0.96)*	*(2.40)*	*(0.15)*	*(0.18)*	*(0.67)*
	37 °C	68.74	25.89	30.80	40.08	43.96	41.79
		(0.46)	*(1.00)*	*(0.90)*	*(0.30)*	*(0.24)*	*(0.73)*
Viscosity [mPa * s]	25 °C	0.91	1.80	1.67	5.24	9.24	1.75
		(0.00)	*(0.00)*	*(0.01)*	*(0.05)*	*(0.08)*	*(0.10)*
	37 °C	0.72	1.16	1.33	3.87	7.67	1.31
		(0.00)	*(0.00)*	*(0.01)*	*(0.03)*	*(0.11)*	*(0.01)*

3. Results and discussion

3.1. Physicochemical properties of the administration fluids

All administration fluids had pH values in the neutral pH range. However, whereas water has no buffer capacity, the buffer capacity of the two breast milks, the formula milks and whole (cows) milk was markedly higher. Most of the milks had osmolalities in the (blood-) isotonic range, only formula milk 3 was slightly hypotonic. The most significant differences in fluid properties could be observed for the surface tensions. Surface tensions of all milks were significantly lower than that of water. The lowest surface tension was measured for the two breast milks. Surface tension of the two formula milks and whole milk was similar, but higher than that of breast milk. The viscosity of the two breast milks was slightly higher than that of water and similar to that of whole milk. The highest viscosity was observed for formula milk 3 (Table 3).

3.2. Hydrocortisone solubility in the different administration fluids

Table 4 displays the results obtained in the solubility experiments after 4 and 24 h and for comparison purposes also the pH of the different fluids without (w/o) the active pharmaceutical ingredient (API) added at corresponding time points.

Results from the solubility experiments were in the range of ~400–700 µg/mL hydrocortisone dissolved after 4 h and ~400–800 µg/mL dissolved after 24 h. Expectedly, the lowest solubility was observed in water, where as a result of hydrocortisone (pKa = 12.58, source: http://www.drugbank.ca/drugs/DB00741) dissolution, the media pH increased over the duration of the experiment. In this medium, an equilibrium concentration of dissolved hydrocortisone was therefore reached as early as after 2 h (Fig. 2A). In all other media, equilibrium was not reached within the same time frame. Particularly in the two formula milk types, the concentration of drug dissolved increased significantly over the first 4 h of the experiment (shaded area in Fig. 2A) and (slightly) further increased within the next 4 h to finally reach a plateau. However, it also became obvious that the stability of these two media seems to be limited. Independent of hydrocortisone being present (Table 4) or not (Fig. 2B), at the end of the experiment the pH of these media had dropped by ≥1 pH unit in the case of baby formula (formula milk 1) and ≥3 pH units in the case of infant formula (formula milk 3).

Slightly different solubility kinetics could be observed in the two different breast milks. This was most likely a result of differences in their composition, because they were from two different sources. However, after 24 h about the same concentration of drug dissolved could be measured both in breast milks 1 and 2, but at that time point the pH of these two media had also markedly dropped (Table 4).

Heat-treated whole milk which, over the time of the experiment, had an almost constant pH was the most stable medium. However, although within a time range of 24 h, the pH of whole milk did not change, an increase in the amount of dissolved drug could also be observed when comparing the amount of hydrocortisone dissolved after 4 and 24 h.

The solubility experiments were run over a total of 24 h to get an estimate of the saturation solubility; however, particularly by observing the pH of the different test media over time, it was obvious that a sufficient stability of some of the media, particularly formula milk, is not given for such a long time range. However, a duration of 24 h would also not be representative for a typical gastric residence time, neither in adults, nor in children. Rather than focusing on the equilibrium solubility after 24 h, the amount of drug dissolved at earlier time points such as 2 or 4 h was regarded as more important since these time ranges could possibly reflect gastric residence times in the fed state. With the

Table 4
Results obtained in the hydrocortisone solubility experiments in water and the different milk types at 37 °C and corresponding media pH (mean of n = 3 ± S.D.).

Medium	pH – start	Amount of hydrocortisone dissolved – 4 h [µg/mL]	Amount of hydrocortisone dissolved – 24 h [µg/mL]	pH – 24 h (with API)	pH – 24 h (w/o API)
Water	6.83	415.8 ± 132.1	395.40 ± 12.2	7.40	6.85
Breast milk 1	6.93	525.5 ± 2.5	632.40 ± 16.8	5.66	5.87
Breast milk 2	7.08	547.9 ± 19.0	596.20 ± 17.2	4.82	4.59
Formula milk 1	6.87	583.4 ± 37.9	693.60 ± 73.4	5.99	5.59
Formula milk 3	6.89	677.2 ± 62.1	817.40 ± 31.8	3.55	3.28
Whole milk	6.56	521.3 ± 200.5	671.40 ± 29.6	6.68	6.48

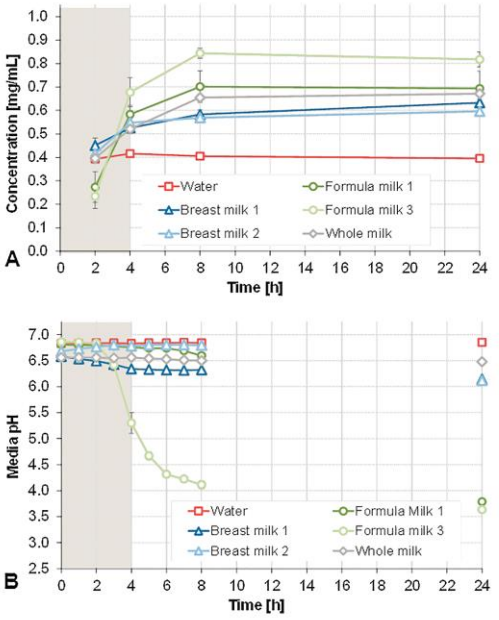

Fig. 2. Amount of hydrocortisone dissolved at different time points in water and the different milk types (A) and corresponding pH profile of the different media without hydrocortisone added (B) at 37 °C (mean of n = 3 ± S.D.).

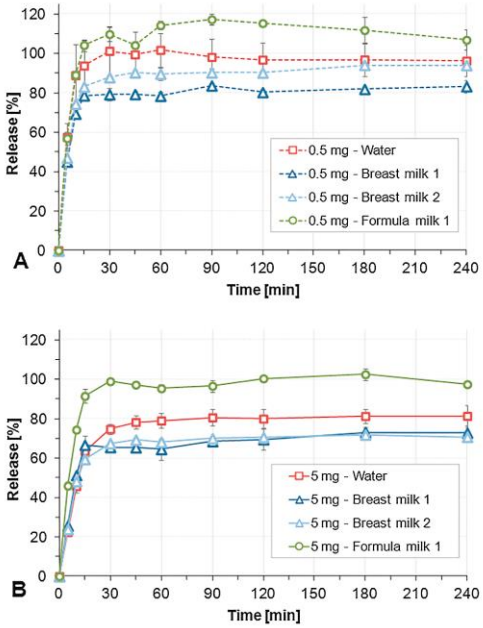

Fig. 3. Dissolution of Alkindi® 0.5 mg (A) and 5 mg (B) when simulating co-administration the formulation with water, breast milk and formula milk 1 (baby formula) to neonates (mean of n = 3 ± S.D.).

exception of formula milk 3 for which a significant drop in pH could already be observed after 4 h, the stability of the different media should not be significantly impaired after 4 h at 37 °C.

3.3. Alkindi® dissolution

Figs. 3–5 display the dissolution results obtained when simulating administration of Alkindi® 0.5 mg or 5 mg granules together with water or different age-appropriate milk types to neonates, infants or pre-school children.

In all media dissolution was fast and no drug precipitation or degradation could be observed over the entire test duration of 240 min. Results obtained in the different test scenarios (slightly) differed in the total amount of hydrocortisone. Even though in the solubility experiments the lowest hydrocortisone solubility was observed in water, dissolution in water was slightly better than in the two breast milk types. This was true for both the 0.5 mg and the 5 mg dose when simulating initial gastric conditions after co-administration of Alkindi® with the different fluids in neonates and infants. Dissolution of the 5.0 mg dose in the pre-school children setup was better in water than in whole milk. For both dosage strengths the best dissolution results were obtained in the experiments performed in baby formula (formula milk 1 – neonates).

Overall, dissolution of the 0.5 mg dose in media and volumes intended to simulate initial gastric conditions after dose administration was fast and complete in all scenarios, i.e. > 80% of the dose was released in the neonate and the infant scenario and > 75% in the pre-school children setup within 30 min. Results for the total amount of hydrocortisone dissolved when simulating administration of the 5 mg dose were slightly lower in all simulated patient scenarios. Based on the information obtained in the solubility experiments, the hydrocortisone doses applied in the present study should be soluble in the fluid

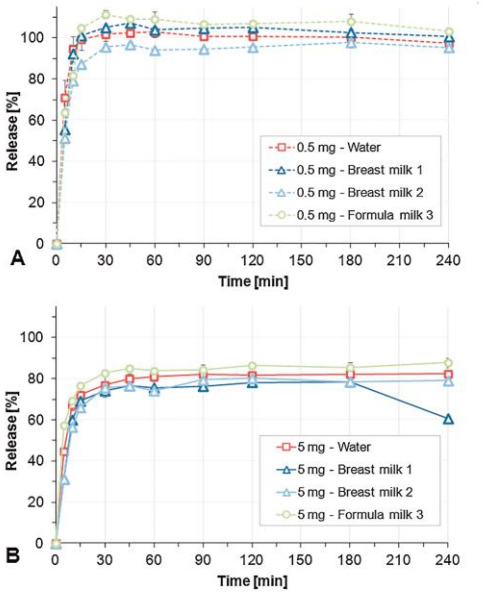

Fig. 4. Dissolution of Alkindi® 0.5 mg (A) and 5 mg (B) when simulating co-administration the formulation with water, breast milk and formula milk 3 (infant formula) to infants (mean of n = 3 ± S.D.).

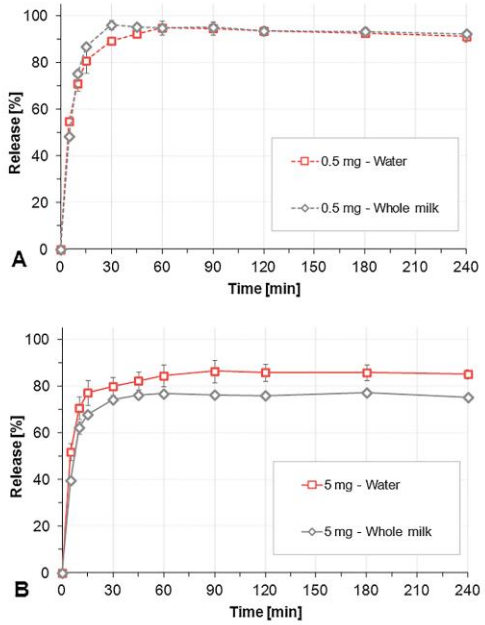

Fig. 5. Dissolution of Alkindi® 0.5 mg (A) and 5 mg (B) when simulating co-administration the formulation with water and whole milk to pre-school children (mean of n = 3 ± S.D.).

volumes that were applied to simulate the gastric fluid volumes in the different age groups. According to results from the solubility experiments, sink conditions (when defined as volume > 3 fold saturation volume) should be given. However, as discussed before, even though the agitation speed of paddle had been increased to 100 rpm, this did not result in optimal dispersion of the granules, resulting in coning and potential agglomeration of some of the granules/particles during the experiment. This observation might be the reason for some *in vitro* artifacts in the dissolution behaviour, which would be most pronounced when combining a large amount of granules and a small fluid volume, which was the case in the scenario where a 5 mg dose administration to neonates was simulated. These artifacts are unlikely to be predictive for the *in vivo* performance of the formulation since even though there might be little fluid available for gastric dissolution, the multiparticulate granules are unlikely to rest and remain in the stomach as a monolithic bulk. In contrast beside the resting fluid volume and the amount of fluid co-administered with the dosage forms, gastric secretion as well as gastric motility will facilitate digestion of the different milk types [5] as well as disintegration of the granules, drug release and solubilisation of the drug. The *in vitro* dissolution results obtained in the present study indicate that due to gastric dissolution a large amount of the administered hydrocortisone dose will be emptied into the small intestine as a solution. In the case that undissolved granules/drug will be emptied into the duodenum, bile salts in the small intestinal lumen are likely to further promote dissolution [17,18]. As a result, after administering the formulation with the given amount of fluid, bioavailability should not be an issue.

4. Conclusion

The results obtained in the present study confirm the compatibility and in-use chemical stability of Alkindi® with/in commonly used dosing matrices like water, breast milk, formula milk and milk. In most of the simulated dosing scenarios, *in vitro* dissolution was largely independent on the composition and properties of the administration fluid, rapid and complete and no precipitation was observed over a duration of 4 h which is much longer than a typical gastric residence time for an entire dose of multi-particulates [19–22]. Slight differences could be observed in the total amount of drug dissolved when comparing results of the simulated administration of the minimum and maximum hydrocortisone dose in different dosing scenarios. However, since the present test design had focused on hydrocortisone dissolution in the administration fluids, but was not supposed to address additional factors like gastric secretion over time, gastric emptying etc. that might further enhance gastric dissolution and, moreover, since the estimated typical gastric fluid volumes were very conservative (particularly in the case of neonates), it is likely that *in vivo* dissolution and bioavailability of Alkindi® will not be affected by the composition of the co-administered fluids studied. Clinically, often at times of stress (for example a fever), neonates may be administered higher doses of hydrocortisone to minimize the risk of adrenal crisis and hence a 5 mg dose of Alkindi® was simulated to neonates in this study. However, this usually does not represent a standard daily dose in neonates in chronic treatment. Therefore, this study has likely overestimated the maximum concentration of drug in the dissolution media, which again indicates that the *in vivo* dissolution and bioavailability of Alkindi® will not be affected by the composition of the co-administered fluids and that the product is compatible with child-appropriate administration fluids that are commonly used to increase acceptability of orally administered drugs in young children.

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5.2 A biopredictive *in vitro* approach for assessing compatibility of a novel pediatric hydrocortisone drug product within common pediatric dosing vehicles

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Pharmaceutical Research, 2020, 37(10): 203

Die zweite pädiatrische In-vitro-Studie, welche Inhalt dieser Veröffentlichung ist, beschäftigte sich ebenfalls mit der Entwicklung und Anwendung eines biorelevanten In-vitro-Modells zur Untersuchung der Wirkstofffreisetzung und Kompatibilität von Alkindi® nach simulierter Co-Verabreichung mit pädiatrischen Applikationsvehikeln.

In dieser Studie wurde die Freisetzung und Kompatibilität der Alkindi®-Formulierung in flüssigen und halbfesten Applikationsvehikeln untersucht. Im Fokus der Untersuchung standen pädiatrische Patienten, die neben Milch auch andere flüssige Vehikel sowie halbfeste Nahrung zu sich nehmen können. Aufgrund dessen wurde der Altersbereich der adressierten Patientenpopulation auf 2 - 16 Jahre verändert. In der Studie wurden flüssige und halbfeste Applikationsvehikel, d. h. Wasser, Apfelsaft, Orangensaft, Tomatensaft, Apfelmus und Joghurt, verwendet. Die Auswertung der In-vitro-Studie bestätigte die Kompatibilität von Alkindi® mit den ausgewählten pädiatrischen Applikationsvehikeln. In allen Testszenarien wurde eine schnelle und vollständige Wirkstofffreisetzung der Alkindi®-Formulierung beobachtet. Des Weiteren war keine Wirkstoffpräzipitation oder -degradation über den gesamten Untersuchungszeitraum erkennbar. In Übereinstimmung mit der ersten Studie deuten auch die Ergebnisse der zweiten In-vitro-Studie darauf hin, dass die In-vivo-Freisetzung und die Bioverfügbarkeit der untersuchten Arzneiform nicht durch die untersuchten Applikationsvehikel beeinflusst werden und folglich diese Vehikel zur gemeinsamen Einnahme mit der Alkindi®-Formulierung geeignet sind.

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Eigene Leistungen:
Planung und Durchführung von In-vitro-Löslichkeits- und Freisetzungsuntersuchungen sowie Kompatibilitätsstudien inklusive Analytik, Entwicklung von Probenaufbereitungsmethoden, Methodenentwicklung für Kompatibilitätsstudien, Erstellung des Manuskripts

Dr. Frank Karkossa:
Mitarbeit bei der Durchführung der In-vitro-Untersuchungen

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Mitarbeit bei der Durchführung der In-vitro-Untersuchungen

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RESEARCH PAPER

A Biopredictive *In Vitro* Approach for Assessing Compatibility of a Novel Pediatric Hydrocortisone Drug Product within Common Pediatric Dosing Vehicles

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ABSTRACT

Purpose The objective of the present work was to screen whether a novel pediatric hydrocortisone granule formulation can be co-administered with common food matrices and liquids.

Methods Pediatric hydrocortisone granules were studied using a biopredictive *in vitro* approach. Experiments included an *in situ* chemical compatibility study of active ingredient and drug product with liquid dosing vehicles and soft foods commonly ingested by infants, pre-school- and school children. Drug solubility and stability experiments in the different vehicle types and, drug release/dissolution experiments mimicking age-related pediatric gastric conditions after administering the hydrocortisone granules together with the dosing vehicles and after different exposure/mixing times were performed.

Results In the simulated dosing scenarios applied in dissolution experiments, *in vitro* dissolution in gastric conditions was rapid and complete. Results of the chemical compatibility/stability studies indicated that mixing with the different dosing vehicles studied should not be an issue regarding drug degradation products.

Conclusions A novel *in vitro* approach ensuring a proper risk assessment of the use of dosing vehicles in the administration of pediatric dosage forms was established and applied to a novel pediatric hydrocortisone drug product. The studied dosing vehicles were shown to not alter performance of the drug product and are thus considered suitable for administration with hydrocortisone granules.

KEY WORDS biorelevant dissolution · gastric media · liquids · quality assessment · soft foods

ABBREVIATIONS

API	Active pharmaceutical ingredient
AI	Adrenal insufficiency
ACTH	Adrenocorticotropic hormone
EMA	European Medicines Agency
Ph.Eur.	European Pharmacopoeia
FDA	Food and Drug Administration
GI	Gastro-intestinal
IR	Immediate release
PIP	Paediatric Investigation Plan
PK	Pharmacokinetic
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene fluoride
SGFsp	Simulated Gastric Fluid without pepsin
THF	Tetrahydrofuran
US	United States
USP	United States Pharmacopoeia

INTRODUCTION

Adrenal insufficiency (AI) is an endocrine disorder caused by impaired synthesis and release of the essential hormone cortisol and can be classified into primary or secondary AI. Primary AI is caused by destruction or dysfunction of the

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adrenal cortex and presents with impaired cortisol and aldosterone production. Secondary AI is caused by a lack of pituitary adrenocorticotropic hormone (ACTH) which prevents the body from producing enough cortisol (1).

AI remains a significant cause of morbidity and mortality in children with 1 in 200 episodes of adrenal crisis resulting in death (2). In most cases pediatric AI is caused by the rare genetic condition congenital adrenal hyperplasia. Occasionally, it can be caused by autoimmune disease (Addison's disease) or pituitary conditions. AI is a life-long and life-threatening condition and is associated with a multitude of symptoms that are often unspecific and thus make diagnosis difficult (1).

Patients diagnosed with AI require replacement therapy with glucocorticoids (steroids). To avoid risks of hypoglycemia and adrenal crisis associated with undertreatment, and to avoid growth suppression and reduced final height potential associated with steroid overdosing, appropriate replacement doses of glucocorticoids are important to determine in both primary and secondary AI in children (3). Hydrocortisone (the synthetic version of cortisol) represents the glucocorticoid of choice for treating pediatric AI patients (1,4). It is administered orally in two to four divided doses per day, where the single doses should be adjusted to the individual need based on body surface area (1,4). Since AI can present at birth and usually requires life-long and patient-tailored oral glucocorticoid medication, oral dosage forms that ensure acceptability in children of different age groups are an important prerequisite for safe, reliable and effective glucocorticoid substitution. However, to date optimization of hydrocortisone replacement therapy in children has been challenging, since until recently, no licensed oral dose-appropriate pediatric formulation of hydrocortisone was available (5). Most children with adrenal insufficiency have until now been treated with individualized pharmacy-compounded adult medication. In very young children such as newborns and infants compounding demonstrably bears risks of inaccurate dosing, poor disease control and potential adrenal crisis (5,6). Therefore, there is a strong need for licensed oral pediatric formulations approved by regulatory authorities such as the European Medicines Agency (EMA) and the United States (US) Food and Drug Administration (FDA) in the treatment of pediatric AI.

Hydrocortisone is a well-established glucocorticoid listed in the current US Pharmacopoeia (USP) and European Pharmacopoeia (Ph.Eur.). It is a neutral compound with acid-base properties (pka = 12.59) and lipophilicity (logP = 1.61) (7). In the adult setting, hydrocortisone has been reported to be a class II (high permeability and low solubility) compound (8) according to the biopharmaceutics classification system (BCS) (9). However, in the oral pediatric dose range hydrocortisone can be assigned to class I (high permeability and high solubility) where the highest marketed dose demonstrates pH-independent solubility in aqueous media across the physiologic pH range along the upper human gastro-intestinal (GI) tract (data on file). It should be noted that the current BCS refers to adult administration and that a pediatric BCS has not yet been established (10). For the physiological pH range of 1.2 to 6.8 an aqueous solubility of 0.35–0.40 mg/mL is reported (8,11). Thus, when considering an oral single dose of 5 mg in pediatric use, the dose:solubility ratio would be in the range of ~12.5–15 ml indicating that there should be no issues with regard to *in vivo* solubility, since even in very young children this is a reasonable fluid volume available in the upper GI tract (12). Alkindi® (Diurnal Europe B.V., the Netherlands) (development program name Infacort®) and in the rest of the manuscript referred to as hydrocortisone granules) is an oral multiparticulate formulation of hydrocortisone that is available in unit doses of 0.5 mg, 1 mg, 2 mg and 5 mg, that was developed for use in children from birth as an oral replacement therapy in AI. The drug product is an immediate release (IR) dosage consisting of granules that are coated with taste-masking excipients to eliminate the bitter taste of hydrocortisone to ensure acceptability in children of all age categories. The granules are contained in size 00el hypromellose hard capsules which are not intended to be administered to the children but used as a carrier.

The hydrocortisone granules are intended to be dosed to pediatric patients by sprinkling the capsule content onto the child's tongue. Alternatively, they can be placed on a dry spoon and administered into the patient's mouth. Immediately after administration, the granules should be washed down with fluid, preferably drinking water, or for younger patients, breast or artificial milk (11). However, particularly when developing oral formulations for pediatric use, besides safety and efficacy, acceptability of the drug product is an important aspect to consider (13). To increase acceptability mixing with food and beverages is a common practice when otherwise the medication would not be accepted by the child. Consequently, typical instructions that can be found in the product information leaflet of marketed formulations for pediatric use often refer to co-administration with semi-solid (soft) food matrices or liquids. To ease the administration of the hydrocortisone granules, it would be advantageous to also provide the option to administer the capsule contents with different types of soft foods or fluids. However, this would require sufficient *in situ* stability of the drug (product) in these dosing vehicles to cover the expected administration timeframe. Moreover, the type and amount of food/fluid co-administered with the dosage form should not present with a relevant food effect, i.e. not interfere with *in vivo* drug release.

According to a recently published FDA draft guidance on the "Use of Liquids and/or Soft Foods as Vehicles for Drug Administration" (14) only those liquids and/or soft foods demonstrated to have no appreciable effect on drug product performance should be proposed as a dosing vehicle. Consequently, when the aim is to propose co-administration

of the hydrocortisone granules, a study proving that the proposed dosing vehicles will not affect hydrocortisone stability and *in vivo* drug release is of upmost importance. The recent FDA guidance on information to be contained in pediatric labeling (15) does not include any details on the nature of tests to be conducted. Theoretically, compatibility and *in vivo* drug release could be assessed in an *in vivo* pharmacokinetic (PK) study. However, due to ethical concerns, it is impossible to perform such studies in children (16). The FDA guidance on exposure-response relationships (17) includes a "Pediatric Study Decision Tree" which justifies extrapolation from adult data into pediatric populations in cases where the course of the disease and effect of the drug are sufficiently similar in adults and pediatric patients. However, it is important to note that this extrapolation refers only to efficacy, but not to safety or dose adjustments. When adult data have been used to predict performance in pediatric populations there are examples of unexplained and sometimes adverse events (18–21).

A properly designed *in vitro* approach that addresses both the variability in composition and properties of different vehicles and pediatric GI physiology would be a valuable tool for assessing the compatibility and stability of pediatric drug products and common dosing vehicles.

In a previous study in Europe, under the agreed EMA Paediatric Investigation Plan (PIP – EMEA 001283-PIP01–12) the compatibility of the hydrocortisone granules with artificial milk, whole milk and breast milk was assessed. Results from this study indicated that compared to administration with drinking water, *in vivo* hydrocortisone exposure in neonates, infants and pre-school children is unlikely to be affected by co-administration with milk and the composition of milk co-administered with the hydrocortisone granules (11). To ensure the safety and efficacy of the hydrocortisone granules after co-administration with soft food and drink matrices commonly used to assist in the administration of pediatric medicines, the FDA requested proof of compatibility with soft food matrices and administration fluids that are commonly used in the US. The objective of the present work was thus to establish a biopredictive *in vitro* approach for studying i) the *in situ* chemical compatibility of the hydrocortisone granules with different dosing vehicles, ii) drug solubility and stability in the different vehicle types and iii) drug release/dissolution following a simulated co-administration of the hydrocortisone granules with these dosing vehicles after different exposure/mixing times.

MATERIALS AND METHODS

Materials

Hydrocortisone granules in unit hydrocortisone doses of 0.5 mg, 1 mg, 2 mg or 5 mg, all containing the microparticulate batches # 0627/2014-P and # 0628/2014-P (both made from manufacturer batch # 1-984-00936) and hydrocortisone standard material, batch # W005614 (manufacturer batch # 1-983-61052) were obtained from Glatt Pharmaceutical Services GmbH & Co. KG, Binzen, Germany. The granules consist of an inert cellulose core that is covered by a hydrocortisone layer followed by a hypromellose sealing layer and an outer taste masking layer containing ethylcellulose and hypromellose to permit compliant oral dosing. Both sealing layer and taste-masking layer contain a small amount of magnesium stearate as a processing aid.

The analytical standards used for the quantification of hydrocortisone and its degradation products were all obtained from LGC Limited (Teddington, Middlesex, UK) and are given in Table I. All other chemicals and solvents were of analytical or gradient grade and purchased commercially.

In Vitro Study Design

Dosage Strengths Tested

The proposed hydrocortisone doses per unit are 0.5 mg, 1 mg, 2 mg or 5 mg. Since these doses differ only in the number of multiparticulates contained in the capsule, it was regarded appropriate to focus on the most typical doses to be administered to pediatric patients in order to obtain an estimate of the *in vivo* performance of the drug product. Therefore, 2.5 mg or 5 mg representing typical doses administered to infants, pre-school children or school children, respectively, were subject of the study.

Age Groups to Be Addressed

The hydrocortisone granules are intended to be administered to children from birth. In a previous study, typical dosing conditions, i.e. co-administration of the granules with water, breast milk and formula milk for children pre-weaning have been studied (11). This study focused on age-groups that are typically weaned, i.e. infants, pre-school- and school children were simulated.

Table I Analytical Standards Used for the Quantification of Hydrocortisone and its Degradation Products

Compound	Batch #
Hydrocortisone	8.1
Cortisone	5980
Reichstein's Substance S	40844
Hydrocortisone acetate	9.1
Prednisolone	23623
Hydrocortisone for peak identification	2.0

Foods Selected for the Compatibility Study

Compatibility with the common dosing vehicles including yogurt, apple sauce, orange juice, apple juice, tomato juice and water was studied. These vehicles were selected in keeping with the FDA guidelines. Since different brands of soft foods and fluids can differ in their composition and physicochemical properties, it was regarded important to consider such potential differences in the study design. Consequently, whereas it was considered appropriate to use a single source for water, for each of the other soft foods and fluids three different brands/qualities with likely different physicochemical characteristics were used. Wherever possible, one of these brands was from the US market. All fluids and soft foods selected for the compatibility study are given in Table II.

Physicochemical Characterization of the Dosing Vehicles

Physicochemical characterization of all fluids and soft foods presented in Table II included the following parameters: pH value and buffer capacity, osmolality, surface tension and viscosity. Except for osmolality parameters were recorded at two temperatures, i.e. 25°C and 37°C as described in (22). Unless otherwise indicated, all experiments were run in sextuplicate and results expressed as mean ± S.D. Measurements were performed as follows:

The pH value was measured with a pH-meter (Five Easy Plus, Metter Toledo GmbH, Giessen, Germany). Using the same pH-meter for pH control, the buffer capacity was quantified by potentiometric titration with 0.1 M or 0.2 M hydrochloric acid, respectively.

Osmolality was measured via the freezing-point depression method using a semi-micro osmometer (K-7400, Knauer, Berlin, Germany). Most of the fluids and foods required dilution prior to the measurement. Thus, for these soft foods a set of appropriate dilutions was prepared with demineralized water. These dilutions were then first mixed for 1 min using a Vortex mixer (VWR Reagenzglasschuettler, VWR International GmbH, Darmstadt, Germany) and subsequently centrifuged for 15 min at 4000 rpm (Eppendorf Centrifuge 5702 R, Eppendorf AG, Hamburg, Germany). After centrifugation, the aqueous phase of the diluted foods was used to measure the osmolality. A linear relationship between food concentration and osmolality was observed ($R^2 > 0.995$) for the entire set of dilutions. Consequently, it was possible to extrapolate to the osmolality of the undiluted soft foods. Osmolality of some of the soft foods could not be assessed by the freezing-point depression method. For these media, i.e. MOTT's and Babylove apple sauce and Alpro yogurt, osmolality was extrapolated by preparing and measuring of a set of appropriate dilutions with a vapor pressure osmometer (No. 11.00, Knauer, Berlin, Germany).

The surface tension was determined with a ring tensiometer (K11, Kruess GmbH, Hamburg, Germany). As experienced in the osmolality measurements, the surface tension of apple sauce and yogurt could not be directly assessed. Therefore, again a set of appropriate dilutions was prepared.

Table II Fluid and Food Types and Sources

Fluid / Soft food	Commercial Product	Manufacturer / Source	Batch #
Water	Humana Babywasser	Humana GmbH, Herford, Germany	47159351
Apple juice	Tropicana apple juice	Tropicana Manufacturing Company Inc., Bradenton, USA	329500861 12
	Alosa Apfelsaft klar	Brands & Systems BSG GmbH, Hamburg, Germany	1124H
	Albi Apfelsaft klar	Albi GmbH, Berghuelen, Germany	L251113 M3B2
Orange juice	Tropicana orange juice(with pulp)	Tropicana Manufacturing Company Inc., Bradenton, USA	ZMZ K10:34
	Hohes C Orangensaft(without pulp)	Eckes-Granini Deutschland GmbH, Nieder-Olm, Germany	300 & 329
	Amecke Sanfte Saefte, Orangensaft (without pulp)	Amecke Fruchtsaft GmbH, Menden, Germany	03062SN15030
Tomato Juice	Rewe Beste Wahl Tomatensaft	Rewe Markt GmbH, Koeln, Germany	L2E2
	Tomatensaft von Sonnlaender	Sonnlaender Getraenke GmbH, Rostock, Germany	191015
	Alnatura Tomatensaft	Alnatura GmbH, Bickenbach, Germany	61049
Apple sauce	MOTT'S applesauce natural	Mott's Inc., Plano, USA	111715WA
	Babylove Apfel pur	dm-drogerie markt GmbH + Co. KG, Karlsruhe, Germany	LS272
	Oberlausitzer Apfelmus	Lausitzer Fruechteverarbeitung GmbH, Sohland, Germany	L14 16:05 LK
Yogurt	LC1 Pur Nestlé	Lactalis Nestlé Frischprodukte Deutschland GmbH, Kehl/Rhein, Germany	12/02H & 28/05 K
	Alpro Sojajoghurt	Alpro C.V.A., Wevelgem, Belgium	Y021709:16 1
	Weihenstephan Frischer Joghurt mild, 3.5% Fett	Molkerei Weihenstephan GmbH & Co. KG, Weihenstephan, Germany	BY718.150504:4902

Both at 25°C and 37°C no major change in surface activity could be observed when comparing surface tension of the different dilutions. This indicates that the surfactant concentrations were above the critical micelle concentration (CMC). The surface tension of the undiluted soft foods was thus estimated from the mean ($n = 24 \pm$ S.D. for MOTT's apple sauce, $n = 36$ for Babylove apple sauce and $n = 30$ for Alpro yogurt) surface tension of the respective sets of dilution.

Due to the very different consistencies of the samples, it was necessary to use two methods for the investigation of viscosity. The viscosity of all Newtonian fluids was determined with two different Ubbelohde viscometers (type I, K = 0.01008 mm^2/s^2 and type II, K = 0.09939 mm^2/s^2, both from LaborTherm, Jena, Germany). As expected, some of the fluids and the soft foods did not exhibit Newtonian flow characteristics. The rheological profiles of these samples, i.e. orange juice with pulp, tomato juice, apple sauce, and yogurt were obtained by measuring shear stress over a range of shear rates. Rheological profiles were recorded with rotational viscometers operating according to the Searle principle (cup and bob). These were either a proRheo R180 apparatus (proRheo GmbH, Althengstett, Germany) equipped with cup size 2 (22) or a Brookfield DV3T apparatus (Brookfield, Middleborough, MA, USA) equipped with the ULA-DIN-6Y cup and the DIN 87 spindle or the DAA-1 cup and the DIN 85 spindle, respectively.

Besides the physicochemical characterization, fluids and soft foods were also screened for energy and nutrient composition.

Dissolution and Solubility Study

Dissolution Media. Dissolution media were chosen to simulate gastric conditions immediately after dose administration. Therefore, the dissolution media applied were mixtures of physiological fasted resting volumes of simulated gastric fluids with age-appropriate pH, a typical amount of soft food or fluid co-administered with a single dose (1 teaspoon) and an age-appropriate volume of fluid that is anticipated to be ingested after administration. One brand of each soft food/fluid product, preferably a product from the US market, was selected for the dissolution studies. The products that were applied in the dissolution studies were Humana Babywasser, Tropicana apple juice, Tropicana orange juice, Rewe Beste Wahl tomato juice, MOTT'S applesauce natural and LC1 Pur Nestlé yogurt. Table III gives a detailed overview of the different dosing scenarios mimicked in the dissolution experiments.

Dissolution Test Setup and Sampling Procedure. Most of the typical gastric content volumes to be mimicked in the dissolution study did not allow the use of standardized dissolution equipment. Thus, non-pharmacopoeial test equipment would have been required for performing the experiments. However, since the objective was to run the experiments under standardized test conditions and to make the results comparable to those from the first part of the study (11), experiments were performed with the Mini-Paddle apparatus using a media volume of 200 or 195 ml, respectively. Where necessary, i.e. when mimicking gastric conditions in infants and pre-school children, hydrocortisone dose, vehicle- and additional fluid volumes were up-scaled proportionally. For infants and pre-school children scaling factors of 4 or 2 were applied when mimicking co-administration of 35 ml (setup 1) or 85 ml water (setup 2), respectively. The test design for school children did not require the use of a scaling factor. Experiments in the Mini-Paddle apparatus were performed as follows: simulated

Table III Estimated Gastric Content in Children of Different Age Categories After Co-administration of a Typical Hydrocortisone Dose

Age group	Type of soft food / fluid	Test dose	Amount of co-aministered food/fluid	Resting gastric volume	Resting gastric pH range	Volume of co-administered water	Total gastric volume
Infants & pre-school children	Water Apple juice Orange juice Tomato juice Apple sauce Yogurt	2.5 mg	1 teaspoon = 5 ml	10 ml	pH 1.8–4.0	35 ml 85 ml	50 ml 100 ml
School children	Water Apple juice Orange juice Tomato juice Apple sauce Yogurt	5.0 mg	1 teaspoon = 5 ml	25ml	pH 1.8	170 ml	200 ml

residual gastric fluid, i.e. Simulated Gastric Fluid without pepsin (SGF*sp*) pH 1.8 was placed into the vessel and preheated to 37°C. Then, at room temperature the dosing vehicle was placed in a graduated dosing spoon and the hydrocortisone granule test dose was mixed with the vehicle (sprinkling and stirring with a small plastic rod for 5 s). The resulting mixture was immediately added to the preheated gastric medium. For the experiments with soft food an additional set of experiments was performed where the soft food:granule mixture was kept at room temperature for 60 min before simulating administration/ingestion (worst case scenario assumed for a child refusing to take the medicine at the time of intended administration). When mimicking hydrocortisone granule co-administration with soft foods, water of 37°C was added to the vessel to simulate administration of some extra fluid after dosage form administration. In those cases where co-administration of the dosage form with liquids (apple juice, orange juice and tomato juice) was mimicked, we assumed that the child would drink the same type of liquid after administration and thus, instead of water, added the corresponding volumes of the respective fluid types at 37°C. The test setup is shown in Fig. 1. Over the duration of the dissolution experiment the media temperature was maintained at 37.0 ± 0.5°C and the paddle speed was set at 100 rpm (23,24). The total time of the dissolution experiments was 120 min. Samples of 3 ml were removed at predetermined time points, i.e. 5, 10, 15, 30, 45, 60, 90, and 120 min. The sample volume was replaced by fresh medium, i.e. a 1:4 mixture of SGF*sp* pH 1.8, respectively and water in all experiments. All experiments were run in triplicate.

Solubility Experiments. In addition to the dissolution experiments a small series of hydrocortisone solubility experiments was performed in selected dissolution media. The objective of these experiments was to estimate the solubility of hydrocortisone in a mixture of residual gastric fluid, vehicle and co-administered fluid. Results of the solubility experiments should be helpful in assessing the dissolution results, particularly with respect to sink conditions. As it was not possible to perform experiments in all mixtures used in the dissolution experiments, we decided to select the infant test scenario assuming an infant/pre-school child test scenario with a residual gastric fluid volume of 10 ml and a pH of 1.8 receiving the medication with a teaspoon (5 ml) of fluid or soft food and drinking 85 ml of water or the fluid used for dose administration immediately after dosing. Consequently, the test media composition for the solubility experiments was SGF*sp*:fluid 10%:90% for apple juice, orange juice and tomato juice and SGF*sp*:soft food:-water 10%:5%:85% for apple sauce and yogurt. All solubility experiments were performed in triplicate using the shake-flask method at 37.0 ± 0.5°C. Experiments were performed as follows: An excess of drug was added to a 5 ml flask containing 4 ml of each individual test medium and the resulting suspension was stirred with a magnetic stirring bar at 220 rpm. During the mixing period, the flasks were inspected to ensure that each flask contained undissolved, excess drug. Drug was added as necessary to maintain an excess. Samples were taken 24 h after the start of the experiment, i.e. when an equilibrium concentration of the compound in the test medium was reached.

Sample Preparation for High-Performance Liquid Chromatography (HPLC) Analysis. Aqueous samples from dissolution and solubility testing in baby water were filtered via a 0.22 µm polyvinylidene fluoride (PVDF) filter (diameter 25 mm, Millex-HV, Merck Millipore, Darmstadt, Germany) immediately after sampling. To adapt the sample composition to that of the mobile phase of (80% water and 20% tetrahydrofuran (THF) v/v) used for HPLC analysis, 0.8 ml of each sample was added to 0.2 ml THF. After homogenization, the resulting mixtures were analyzed by HPLC.

Samples from dissolution and solubility experiments mimicking co-administration with apple juice were first filtered through a 0.22 µm PVDF filter, then 800 µl of the filtrate were added into a 1.5 ml polypropylene SafeSeal tube (Sarstedt, Nuembrecht, Germany) filled with 200 µl of THF. The resulting mixtures were then centrifuged for 30 min at a speed of 13,000 rpm. Finally, the supernatant

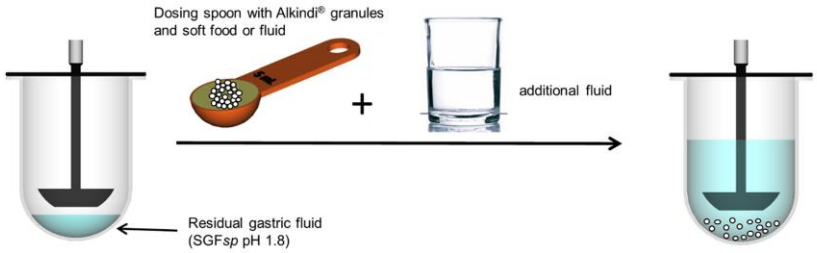

Fig. 1 General test design for the dissolution experiments.

was filtered through a 0.45 µm PVDF filter (diameter 13 mm, Whatman Schleicher & Schuell, Dassel, Germany) and transferred into HPLC vials, as necessary diluted with mobile phase and analyzed by HPLC.

Samples from dissolution and solubility testing simulating co-administration with orange juice, tomato juice and yogurt were first transferred into 1.5 ml polypropylene SafeSeal tubes (Sarstedt, Nuembrecht, Germany) and centrifuged for 1 min. The supernatant was then transferred into a new tube and again centrifuged for 30 min at a speed of 13,000 rpm resulting in phase separation. In the next step, 1 ml of the aqueous phase was removed and transferred into a new cap. Subsequently, 0.5 ml of acetonitrile was added, and the samples were again centrifuged as described before. In the last step 800 µl of the supernatant were removed and added to a new tube containing 200 µl THF. The resulting mixtures were vortexed and then filtered via a 0.45 µm PVDF filter and transferred into HPLC vials, as necessary diluted with mobile phase and analyzed by HPLC.

Samples from dissolution and solubility testing simulating co-administration with apple sauce were first filtered through a 0.22 µm PVDF filter and then transferred into 1.5 ml polypropylene SafeSeal tubes (Sarstedt, Nuembrecht, Germany). They were then centrifuged for 30 min at a speed of 13,000 rpm resulting in phase separation. In the next step, 1 ml of the aqueous phase was removed and transferred into a new cap. Subsequently, 0.5 ml of acetonitrile was added, and the samples were again centrifuged as described before. In the last step 800 µL of the supernatant were removed and added to a new tube containing 200 µL THF. The resulting mixtures were vortexed and then filtered via a 0.45 µm PVDF filter and transferred into HPLC vials, as necessary diluted with mobile phase and analyzed by HPLC.

To ensure that, due to these various separation and dilution steps all the drug that had dissolved in the aqueous phase prior to analysis could be properly quantified, for each of the experiments, the same separation and dilution steps were performed using the respective media containing known amounts of dissolved hydrocortisone. For this purpose, the amount of drug that would freely dissolve in the respective medium was calculated based on results of the solubility experiments. The recovery for these so-called standard samples was then calculated. The concentration of hydrocortisone released/dissolved in the individual test media in the dissolution experiments was then corrected by multiplication with the recovery factor obtained for the standard samples. The individual correction factors applied in the study are given in Table IV.

Table IV Correction Factors Used for Calculating Drug Concentration in the different liquid and semi-solid dosing vehicles

Medium	Correction factor
Apple juice	1.003
Orange juice	1.014
Tomato juice	0.992
Apple sauce	1.037
Yogurt	1.019

Sample Analysis. After appropriate sample preparation, samples were analyzed by HPLC (Waters system consisting of an 2707 autosampler, a 1525 binary pump, a 2998 photodiode array detector and the Breeze 2 software, Waters GmbH, Eschborn, Germany) using a Waters Symmetry C18, 3.5 µm, 75 mm × 4.6 mm column, equipped with a Waters Symmetry C18 Sensity Guard, 5 µm, 20 mm × 3.9 mm precolumn (both from Waters GmbH, Eschborn, Germany), both equilibrated at 45°C, under isocratic conditions using a tetrahydrofuran (THF)/water (20:80 v/v) mobile phase at a flow rate of 1.5 ml/min. Hydrocortisone was detected with ultraviolet (UV) detection at 254 nm. This method had been adapted from Glatt Pharmaceutical Services GmbH & Co. KG and was partly re-validated before use: The linearity was screened for concentration ranges of 0.001–0.050 mg/ml and 0.01–0.40 mg/ml, respectively, in both cases R^2 was 1.000. Moreover, with dilution sets in the same concentration ranges, the accuracy of the mean as well as the precision was checked. Both parameters were in the limits of ± 5%. Therefore, the method was regarded as appropriate for the intended use. The injection volume was 30 µl for samples from the solubility experiments and 15–30 µl for those from the dissolution experiments.

Chemical Compatibility/Stability Study

The *in situ* chemical stability profile, i.e. the compatibility of the hydrocortisone granules with soft food matrices and fluids used for mixing and administration was tested over the chosen time period to simulate a range of administration- and clinical practice scenarios. Beside chemical stability of the active pharmaceutical ingredient (API, i.e. hydrocortisone), the visual appearance and the pH of the vehicle was assessed. Experiments were performed as follows:

The contents of two capsules each containing granules with 5 mg of hydrocortisone (Glatt Pharmaceutical Services GmbH & Co. KG, batch # 0628/2014, material no. 1-984-00936) were mixed with 10 ml (corresponding to a 5 mg dose administered on a teaspoon of 5 ml) of each of the vehicles. Two different dosing scenarios simulating immediate and delayed administration after mixing were addressed. Consequently, two separate sample sets with granule resting times of 5 min and 60 min in the different vehicles were prepared. To assess the impact of the granule formulation on API stability, a corresponding set of experiments was conducted with hydrocortisone standard substance (1 mg hydrocortisone + 10 ml vehicle) (Glatt

Pharmaceutical Services GmbH & Co. KG, batch # W005614, material no. 1–983-01052) for comparison. All experiments were performed in triplicate and at room temperature (22–25°C). Prior to the experiments the content of hydrocortisone of the examined capsules had been determined as 100.2 ± 0.05% (mean of n=3 ± S.D.).

With regard to the detailed experimental procedures to be applied the six vehicles were divided into two groups, liquid vehicles (water, apple juice, orange juice and tomato juice) and semi-solid vehicles (apple sauce and yogurt).

Chemical Compatibility with Liquid Vehicles – Hydrocortisone Granules. The amount of hydrocortisone granules corresponding to a dose of 10 mg of hydrocortisone was added to a 20 ml glass vial. Then 10 ml of the respective vehicle, equilibrated to room temperature, were added into the vial and the mixtures were left standing for 5 or 60 min, respectively. Temperature and pH of all vehicles were measured before the experiment and at the end of the resting period. Samples of the vehicle matrix were taken after 5 or 60 min and immediately prepared for HPLC analysis. Due to the complexity of the vehicle compositions, vehicle-specific sample preparation methods had to be applied:

Water: Samples of 2 ml were removed, transferred into a 2 ml polypropylene SafeSeal tube (Sarstedt, Nuembrecht, Germany) and centrifuged for 1 min with a speed of 13,000 rpm to separate the vehicle matrix from the granules removed with the vehicle sample. In the next step the supernatant was filtered via a 0.45 µm PVDF filter (diameter 13 mm, Whatman Schleicher & Schuell, Dassel, Germany) into a test tube. To adapt the sample composition to the initial mobile phase composition of the media gradient used for HPLC (76% water and 24% acetonitrile v/v) and also to stop further potential degradation of hydrocortisone after sampling, 0.2 ml acetonitrile was added to 0.8 ml of the sample. Subsequently, samples were analyzed by HPLC.

Apple juice, orange juice, tomato juice: Samples of 2 ml were removed, transferred into a 2 ml polypropylene SafeSeal tube and centrifuged for 1 min with a speed of 13,000 rpm. Subsequently, 1.6 ml of the supernatant was transferred into another tube and 0.4 ml of acetonitrile was added to stop further potential degradation of hydrocortisone. The resulting mixture was then centrifuged for 30 min at 13,000 rpm. Subsequently, 0.2 ml of the supernatant was transferred into a new cap, 1.8 ml acetonitrile was added, and the samples were again centrifuged as described before. In the last step, 0.5 ml of the supernatant was transferred into a new tube and 1.5 ml of water of HPLC quality was added to adapt the sample composition to the initial mobile phase composition of the media gradient used for HPLC. The resulting mixture was filtered (0.45 µm PVDF filter) into an HPLC vial and analyzed by HPLC.

Chemical Compatibility with Liquid Vehicles – Hydrocortisone Standard. A 20 ml glass vial equipped with a 8 mm polytetrafluoroethylene (PTFE) coated magnetic stirring bar and containing 0.4 mg of hydrocortisone reference substance was placed on a magnetic stirring plate (IKA RT 15 P, IKA-Werke GmbH & Co. KG, Germany) Then, 4 ml of the respective vehicle, equilibrated to room temperature, were added. The mixture was agitated with the magnetic stir bar and temperature and pH of all vehicles were measured before the experiment and at the end of the stirring period. Samples of the vehicle matrix were removed after 5 or 60 min and immediately prepared for HPLC analysis. Again, due to the complexity of the vehicle compositions, vehicle-specific sample preparation methods had to be applied:

Water: Samples of 2 ml were removed with a glass syringe and filtered into a test tube via a 0.45 µm PVDF filter. To adapt the sample composition to the initial mobile phase composition of the media gradient used for HPLC (76% water and 24% acetonitrile v/v) and also to stop further potential degradation of hydrocortisone after sampling, 0.2 ml acetonitrile was added to 0.8 ml of the sample and the samples were analyzed by HPLC.

Apple juice, orange juice, tomato juice: Samples of 1.6 ml were removed, transferred into a 2 ml polypropylene tube and 0.4 ml of acetonitrile was added. The resulting mixture was then centrifuged for 30 min at 13,000 rpm. Following centrifugation, 0.2 ml of the supernatant was transferred into a new cap, 1.8 ml acetonitrile were added, and the samples were again centrifuged as described before. Finally, 0.5 ml of the supernatant was transferred into a new tube and 1.5 ml of water of HPLC quality was added. The resulting mixture was filtered (0.45 µm PVDF filter) into an HPLC vial and analyzed by HPLC.

Chemical Compatibility with Semi-Solid Vehicles – Hydrocortisone Granules. Due to the composition and texture of apple sauce and yogurt that did not provide direct access to an aqueous phase that could be sampled and also confronted the analyst with the task to take samples from a mixture of freely dispersed fine granules in a semi-solid vehicle, the experimental test setup was adapted as follows: at room temperature 8.84 g apple sauce (equivalent to 10 ml) or 8.64 g yogurt (equivalent to 10 ml) were spread onto the surface of a porcelain frit (pore size 2) of a Buchner (vacuum suction) funnel. Then, the amount of hydrocortisone granules corresponding to a dose of 10 mg of hydrocortisone was sprinkled on the surface of the vehicle and incorporated by gentle and brief stirring with a plastic spoon. The Buchner funnel was then placed onto a suction flask connected to a vacuum pump. After 5 or 60 min, respectively, vacuum was applied, and the filtered sample was collected into a test tube inside the suction flask.

Temperature and pH value of all vehicles were measured before the experiment and at the end of the resting period. Following filtration samples were immediately prepared for HPLC analysis as follows: 0.8 ml of the sample was transferred into a 2 ml polypropylene SafeSeal tube, 0.2 ml of acetonitrile was added, and the mixture was centrifuged for 30 min at 13,000 rpm. Then, 0.2 ml of the supernatant was transferred into a new tube, 1.8 ml acetonitrile was added, and the samples were again centrifuged as described before. Finally, 0.5 ml of the supernatant was transferred into a new tube, 1.5 ml water of HPLC quality was added. The resulting mixture was filtered through a 0.45 µm PVDF filter into a HPLC vial and analyzed by HPLC.

Chemical Compatibility with Semi-Solid Vehicles – Hydrocortisone Standard. A 20 ml glass vial equipped with a 8 mm magnetic stirring bar (PTFE) and containing 0.4 mg of hydrocortisone reference substance was placed on a magnetic stirring plate (IKA RT 15 P, IKA-Werke GmbH & Co. KG, Germany). Then, 4 ml of the respective vehicle, equilibrated to room temperature, were added. The mixture was agitated with the magnetic stir bar. Temperature and pH value of all vehicles were measured before the experiment and at the end of the resting period. Samples of the vehicle matrix were taken after 5 or 60 min and immediately prepared for HPLC as follows: 1.6 ml of the sample was transferred into a 2 ml polypropylene SafeSeal tube, 0.4 ml of acetonitrile was added and the mixture was centrifuged for 30 min at 13,000 rpm. Then, 0.2 ml of the supernatant was transferred into a new tube, 1.8 ml acetonitrile was added, and the samples were again centrifuged as described before. Finally, 0.5 ml of the supernatant was transferred into a new tube, 1.5 ml water of HPLC quality was added. The resulting mixture was filtered through a 0.45 µm PVDF filter into a HPLC vial and analyzed by HPLC.

Sample Analysis. After appropriate sample preparation, samples were analyzed by HPLC (2707 autosampler, 1525 binary pump, 2998 photodiode array detector, Breeze 2 software, all Waters) using a LiChrospher 100 RP-18, 5 µm, 250 mm × 4.6 mm column, equipped with a LiChrospher 100 RP-18, 5 µm, 4 mm × 4 mm precolumn (both from Merck, Darmstadt, Germany) equilibrated at ambient temperature, under gradient conditions (see Table V). Water and acetonitrile were used as eluents and the flow rate was set at 0.6 ml/min. Hydrocortisone and the impurities/degradation products were detected with UV detection at 254 nm.

This HPLC method had been adapted from Glatt Pharmaceutical Services GmbH & Co. KG and was partly re-validated for hydrocortisone (batch W005614) before use: The linearity was screened for the concentration range of 0.0015–0.150 mg/ml with $R^2 > 0.9999$. Moreover, with dilution sets in the same concentration range, the accuracy of the mean as well as the precision was checked. Both parameters were in the limits of ± 5%. Therefore, the method was regarded as appropriate for the intended use. The injection volume was 35 µl for samples from mixtures in water and apple juice and 95 µl for those in orange juice, tomato juice, apple sauce and yogurt. The following standards were used to screen samples for hydrocortisone content and the following related substances and impurities: Hydrocortisone, Cortisone – impurity B, Prednisolone – impurity A, Hydrocortisone Acetate – impurity C, Reichstein's substance S - impurity F, Hydrocortisone for peak identification – contains impurities D, E, G, H, I and N. Chromatograms were evaluated according to the criteria for the determination and quantification of related substances given in the hydrocortisone monograph of the Ph.Eur.

Table V HPLC Gradient Applied in the Chemical Compatibility Analysis

Time [min]	Water [%]	Acetonitrile [%]
0	76	24
18	74	26
32	55	45
48	30	70
50	76	24
60	76	24

RESULTS AND DISCUSSION

Physicochemical Properties of the Dosing Vehicles

The physicochemical characteristics of the different fluids and soft foods are given in Table VI. The viscosity profiles of the vehicles with non-Newtonian flow behavior are shown in Figs. 2, 3, 4, 5.

Results show that besides water, the pH values of all tested fluids are in a very narrow, slightly acidic pH range. Moreover, within the three selected fluids and foods of the same type (having similar nutrient properties), in most of the cases there is little variability in the physicochemical properties. Only the viscosity or the flow behavior, respectively, are quite different. Whereas water and apple juice show Newtonian flow behavior and have a rather low viscosity similar to water, the orange juices containing pulp, tomato juice and the soft foods are characterized by non-Newtonian flow behavior.

The rheological profiles of the three orange juices shown in Fig. 2 indicate that the viscosity of orange juice is affected by shear stress. However, the flow behavior is still close to that of Newtonian fluids and the viscosity of all orange juices is lower than 10 mPa*s. The somewhat

Table VI Mean Values (± S.D.) of the Different Physicochemical Parameters of Fluids and Soft Foods (n=6 per measurement)

		Fluids										Soft foods							
		Water	Apple juice			Orange juice			Tomato juice			Apple sauce			Yogurt				
Parameter	T	Humana	Tropicana	Alosa	Albi##	Tropicana	Hohes C.	Amecke##	Rewe##	Sonnlaender	Alnatura	MOTT's	babylove	Lausitzer##	LC I	Nestlé	Alpro	Weihenstephan##
pH-value	25°C	7.31 (0.01)	3.64 (0.00)	3.25 (0.01)	3.47 (0.03)	3.87 (0.00)	3.64 (0.01)	3.87 (0.04)	4.30 (0.01)	4.23 (0.02)	4.28 (0.01)	3.35 (0.01)	3.75 (0.01)	3.71 (0.02)	4.19 (0.00)	4.48 (0.01)		4.27 (0.02)
	37°C	7.89 (0.01)	3.64 (0.00)	3.24 (0.01)	3.49 (0.01)	3.87 (0.00)	3.66 (0.00)	3.85 (0.02)	4.27 (0.00)	4.21 (0.00)	4.27 (0.03)	3.35 (0.00)	3.74 (0.01)	3.70 (0.01)	4.17 (0.01)	4.45 (0.00)		4.26 (0.01)
Buffer capacity [mEq/pH/L]	25°C	0.11 (0.00)	23.4 (0.0)	31.1 (0.2)	33.4 (1.7)	49.4 (0.2)	52.2 (0.3)	48.4 (2.1)	52.6 (1.0)	41.9 (0.5)	39.1 (0.1)	32.2 (0.6)	28.1 (0.2)	26.3 (1.9)	98.3 (0.7)	72.7 (1.1)		93.8 (4.1)
	37°C	0.06 (0.0)	23.1 (0.2)	31.3 (0.3)	33.9 (0.3)	48.5 (0.3)	52.1 (0.3)	49.1 (1.1)	53.9 (0.2)	42.1 (0.2)	40.3 (0.7)	31.9 (0.4)	27.7 (0.3)	25.8 (0.4)	98.7 (0.7)	75.7 (1.6)		91.5 (0.3)
Osmolality [mOsmol/kg]		4 (1)	677 (9)	710 (10)	677 (5)	542 (22)	564 (20)	558 (8)	519 (8)	489 (4)	471 (6)	554¯ (5)	706¯ (5)	1052 (5)	488 (20)	245¯ (2)		484 (6)
Surface tension [mN/m]	25°C	70.2 (0.36)	55.32 (1.94)	60.00 (0.65)	64.17 (0.32)	45.35 (1.51)	47.02 (1.33)	42.63 (2.10)	42.12 (0.25)	38.89 (0.99)	42.79 (2.62)	52.84 (3.65)	49.32 (5.02)	45.00 (0.33)	45.70 (0.90)	50.83 (0.70)		45.23 (0.21)
	37°C	68.74 (0.46)	53.87 (2.74)	60.82 (1.46)	62.51 (0.45)	43.50 (0.71)	45.54 (0.3)	49.10 (1.10)	39.42 (0.22)	37.86 (0.48)	42.82 (1.34)	49.46 (3.49)	45.08 (4.15)	42.27 (0.22)	44.42 (0.66)	47.86 (0.83)		43.82 (0.16)
Viscosity [mPa*s]	25°C	0.91 (0.00)	1.29 (0.00)	1.27 (0.01)	1.26 (0.00)	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡		‡
	37°C	0.72 (0.00	0.96 (0.00)	0.94 (0.01)	0.96 (0.00)	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡		‡

data from Kersten E, Barry A, Klein S., Pharmazie, 2016, 71(3):122-7(16), ‡ measured with a rotational viscometer, ¯ measured with a vapor pressure osmometer

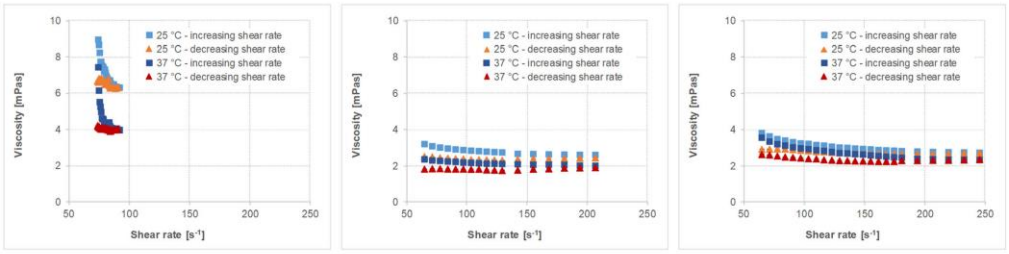

Fig. 2 Viscosity profiles of different orange juice products, i.e. Tropicana (left), Hohes C (middle) and Amecke (right panel) at 25°C and 37°C and increasing and decreasing shear rates (mean of $n=3$, S.D. not shown).

strange appearance of the flow curves of Tropicana orange juice is most likely a result of the pulp which affects the results in a rather random manner.

Viscosity of the tomato juices (Fig. 3) is somewhat higher than that observed for all other fluids used in the study, but the rheological profiles of the three tomato juices screened are very similar.

Viscosity of the soft foods (Figs. 4 and 5) is higher than that of the fluids and much stronger affected by the applied shear rate. The vehicles as such might thus have an impact on drug diffusion and consequently drug release from a co-administered dosage form.

Hydrocortisone Solubility in Different Simulated Gastric Conditions

Results from the solubility experiments (Table VII) were in the range of 0.1 mg/ml to 0.4 mg/ml hydrocortisone dissolved after 24 h. These results indicate that in the dissolution test conditions applied in the present study, 10-fold sink conditions will be provided in most of the media and that only when simulating co-administration with tomato juice followed by a small fluid volume in the infant/pre-school child test design not even 3-fold sink conditions might be achieved. However, when assessing these results, it should be noted that the objective of this initial set of solubility studies was to get a coarse estimate of the maximum amount of hydrocortisone that can dissolve in the gastric environment after co-administration with different dosing vehicles and some additional fluid. For this purpose, it was sufficient to remove just one sample from each individual glass vial at a time point where equilibrium solubility was expected to be reached.

Dissolution of Hydrocortisone Granules in Different Simulated Gastric Conditions

Figures 6 and 7 display the dissolution results obtained when simulating administration of hydrocortisone granules 2.5 mg with a teaspoon of fluid or soft food followed by ingestion of a small (35 ml) or a larger (85 ml) fluid volume to infants and pre-school children. Figure 8 displays the dissolution profiles observed when simulating administration of hydrocortisone granules 5 mg with a teaspoon of fluid or soft food followed by ingestion of a 170 ml fluid volume to school children.

When mimicking dose administration immediately after mixing with the dosing vehicle, dissolution of the 2.5 mg hydrocortisone dose in media and volumes intended to simulate initial gastric conditions after dose administration in infants and pre-school children was fast and complete, i.e. > 80% of the dose was released within 30 min (Figs. 6 and 7). This was independent of the dosing vehicle and the additional fluid

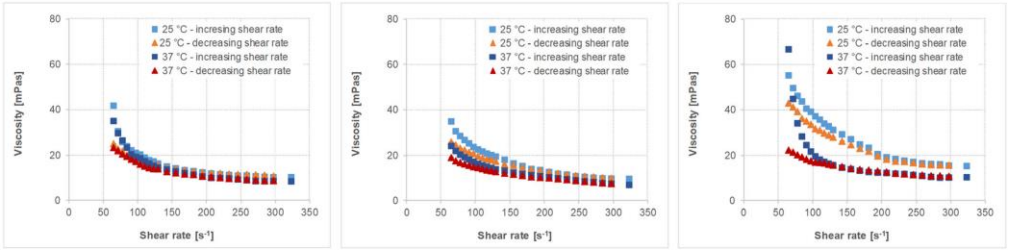

Fig. 3 Viscosity profiles of different tomato juice products, i.e. Rewe (left), Sonnlaender (middle) and Alnatura (right panel) at 25°C and 37°C and increasing and decreasing shear rates (mean of $n=3$, S.D. not shown).

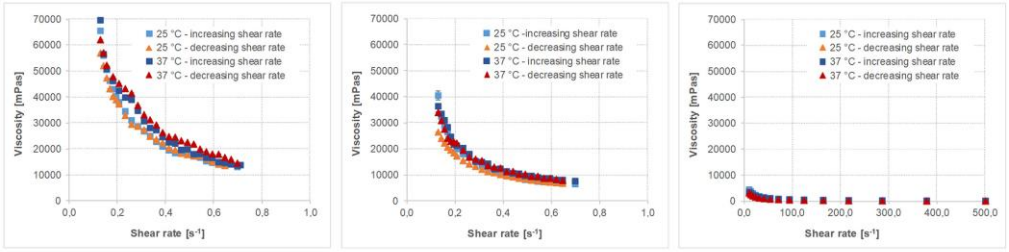

Fig. 4 Viscosity profiles of different apple sauce products, i.e. MOTT's (left), babylove (middle) and Lausitzer (adapted from data from Kersten E, Barry A, Klein S., Pharmazie, 2016, 71 (3):122–7 (16) (measured with a different cup and bob system in a different range)) (right panel) at 25°C and 37°C and increasing and decreasing shear rates (mean of $n=3$, S.D. not shown).

volume applied. Similar observations were made in the release experiments mimicking administration of a 5 mg hydrocortisone dose in school children (Fig. 8). Moreover, in all media no drug precipitation or degradation could be observed over the entire test duration of 2 h. When comparing drug release profiles shown in Figs. 6a, 7a, 8a, when simulating co-administration with water and apple juice, the release rate was slightly slower than that in tomato juice and orange juice. Based on the visual observations made during the experiments, this is likely to be an artefact resulting from the high granule mass that had to be applied in the *in vitro* experiments to simulate administration of an individual dose. Due to the very low hydrocortisone load (0.66%) of the pellets, doses of 1.5 g, 0.75 g and 0.38 g granules per 200 ml release medium were required to address the three different dosing scenarios. Whereas due to the higher viscosity of tomato juice and orange juice, the granules were freely dispersed in the dissolution medium, coning was observed in the experiments with apple juice and water. The impact of coning was most pronounced when a granule dose of 1.5 g was used. Since *in vivo* hydrodynamics are different from those in a dissolution vessel, in this case, the paddle setup is unlikely to be an exact predictor of the *in vivo* release rate and the focus should be set on the complete dissolution within a short time range. Ultimately, we do not believe that this will make a significant difference in a real-world setting.

Simulation of dose administration 60 min after mixing with apple sauce or yogurt provided similar results, i.e. > 80% of the dose released within 30 min. However, a trend towards a slightly faster drug release compared to immediate administration after mixing with the dosing vehicle became visible. This might be a result of hydration of the hydrocortisone granules which can result in dissolution of part of the hydrocortisone dose during the 60 min resting time.

The dissolution results are in good agreement with the solubility data that indicated that there should be no solubility issues within the dose range administered to infants, preschool children and school children. Overall, results from all dissolution experiments indicate that there should be no anticipated issues for the *in vivo* hydrocortisone release when the hydrocortisone granules are co-administered with common dosing vehicles such as water, apple-, orange- or tomato juice, apple sauce and yogurt. These observations are in good agreement with results from a recent clinical study where the

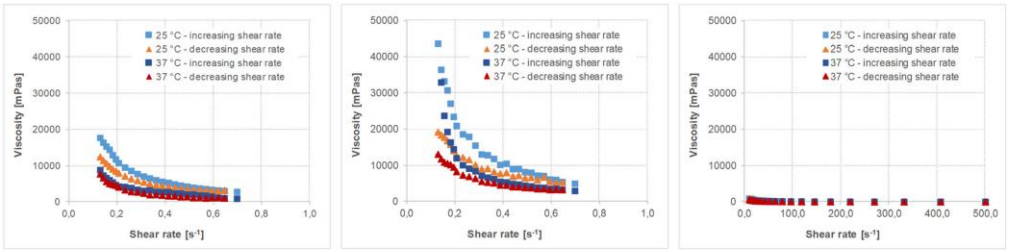

Fig. 5 Viscosity profiles of different yogurt products, i.e. LC 1 Nestlé (left), Alpro (middle) and Weihenstephan (adapted from data from Kersten E, Barry A, Klein S., Pharmazie, 2016, 71 (3):122–7 (16) (measured with a different cup and bob system in a different range)) (right panel) at 25°C and 37°C and increasing and decreasing shear rates (mean of $n=3$, S.D. not shown).

Table VII Solubility of Hydrocortisone in Different Test Media at 37°C (mean of $n=3 \pm$ S.D.) and the Corresponding Media pH at the Beginning and End of Each Experiment ($n=1$)

Medium	Solubility - 24 h c [mg/ml]	S.D.	pH - start	pH - 24 h
SGFsp pH 1.8 – Apple juice (10%/90%)	0.331	0.026	3.61	3.54
SGFsp pH 1.8 – Orange juice (10%/90%)	0.281	0.086	3.88	3.75
SGFsp pH 1.8 – Tomato juice (10%/90%)	0.101	0.071	4.12	4.06
SGFsp pH 1.8 – Apple sauce – Water (10%/5%/85%)	0.397	0.029	3.02	2.93
SGFsp pH 1.8 – Yogurt – Water (10%/5%/85%)	0.387	0.011	4.04	3.96

hydrocortisone granules administered as sprinkles onto apple sauce or yogurt were bioequivalent to those administered directly to the back of the tongue (25).

Chemical Compatibility/Stability of Hydrocortisone Granules and Dosing Vehicles

Since only those liquids and soft foods demonstrated to have no appreciable effect on drug product performance should be proposed as vehicles (14), a chemical compatibility/stability study was performed to support the observations made during the biorelevant dissolution experiments and to provide a complete *in vitro* risk assessment. Experiments were performed with both hydrocortisone standard and the hydrocortisone granules. The detailed results (Tables SII-SVII) are provided as supplementary material of this manuscript. Results of the studies demonstrated compatibility of drug and drug product with all dosing vehicles studied. After mixing the hydrocortisone granules with water or apple juice (Tables SII-SIII), very small concentrations of impurities B and G (all lower than the Ph.Eur. limit and also lower than the individual impurities limit of the USP could be observed after 60 min, but none of the other impurities were determined. In the mixtures of hydrocortisone reference with water or apple juice (Tables SII-SIII), no impurities could be detected. No impurities could be detected in samples from mixing granules or hydrocortisone reference with orange juice (Table SIV), tomato juice (Table SV), apple sauce (Table SVI) or yogurt (Table SVII) for 5 or 60 min. For some vehicles slight peak interferences (same retention times) of signals from the vehicle matrix (blank) and the peaks of some of the impurities in both the chromatograms for the hydrocortisone standard- and the hydrocortisone granule samples were observed. However, when comparing the peak areas obtained from the vehicle matrix alone with those obtained after mixing with granules or standards, it was obvious that there is no big difference, resulting in the conclusion that the peaks are caused by the vehicle matrix rather than by an impurity. This was supported by complete recovery of hydrocortisone (no change in potency) in the respective samples. Overall, results from the compatibility/stability study indicate compatibility of the proposed vehicles with the drug product.

CONCLUSION

The results obtained in the present study confirm the compatibility and in-use chemical stability of hydrocortisone granules with/in commonly used dosing vehicles such as water, apple juice, orange juice, tomato juice, apple sauce and yogurt. In

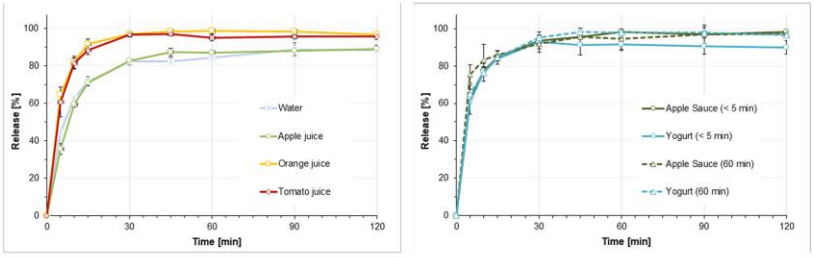

Fig. 6 (**a**) Dissolution profiles obtained when simulating co-administration of granules containing 2.5 mg hydrocortisone with 1 teaspoon (5 ml) of fluid + 35 ml fluid in infants or pre-school children with a resting gastric fluid pH of 1.8, mean of $n=3 \pm$ S.D. (**b**) Dissolution profiles obtained when simulating co-administration of granules containing 2.5 mg hydrocortisone with 1 teaspoon (5 ml) of soft food immediately after mixing (< 5 min) or after a resting time of 60 min + 35 ml fluid in infants or pre-school children with a resting gastric fluid pH of 1.8, mean of $n=3 \pm$ S.D.

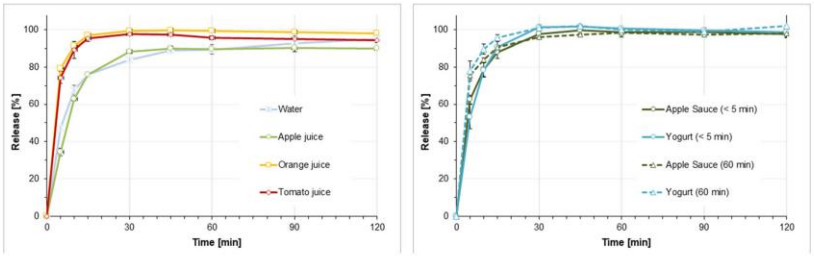

Fig. 7 (**a**) Dissolution profiles obtained when simulating co-administration of granules containing 5 mg hydrocortisone with 1 teaspoon (5 ml) of fluid + 85 ml fluid in infants or pre-school children with a resting gastric fluid pH of 1.8, mean of $n=3 \pm$ S.D. (**b**) Dissolution profiles obtained when simulating co-administration granules containing 5 mg hydrocortisone with 1 teaspoon (5 ml) of soft food immediately after mixing (< 5 min) or after a resting time of 60 min + 85 ml fluid in infants or pre-school children with a resting gastric fluid pH of 1.8, mean of $n=3 \pm$ S.D.

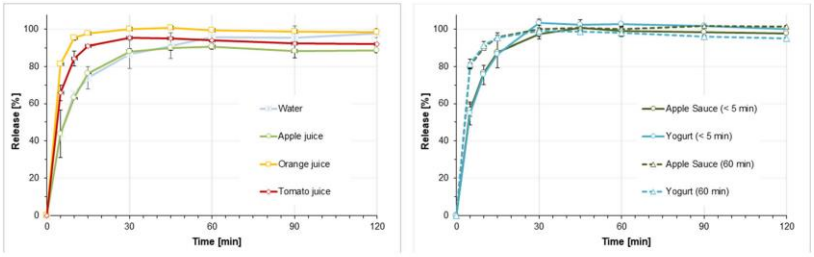

Fig. 8 (**a**) Dissolution profiles obtained when simulating co-administration of granules containing 5 mg hydrocortisone with 1 teaspoon (5 ml) of fluid + 170 ml fluid school children with a resting gastric fluid pH of 1.8, mean of $n=3 \pm$ S.D. (**b**) Dissolution profiles obtained when simulating co-administration of granules containing 5 mg hydrocortisone with 1 teaspoon (5 ml) of soft food immediately after mixing (< 5 min) or after a resting time of 60 min + 170 ml fluid school children with a resting gastric fluid pH of 1.8, mean of $n=3 \pm$ S.D.

the simulated dosing scenarios applied in the dissolution experiments, *in vitro* dissolution was fast and complete, and no precipitation was observed over a test duration of 2 h, which would represent the maximum gastric residence time. Results of the chemical compatibility/stability study indicate that mixing with the set of dosing vehicles studied should not be an issue regarding degradation products. Overall, the present study results indicate that it is likely that *in vivo* dissolution of the hydrocortisone granules will not be affected by the composition of the co-administered fluids and soft foods studied. Since the studied dosing vehicles were shown to not alter performance of the drug product, they should be considered suitable for use as vehicles with the hydrocortisone granules.

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5.3 A review of patient-specific gastrointestinal parameters as a platform for developing *in vitro* models for predicting the *in vivo* performance of oral dosage forms in patients with Parkinson's disease

E. Wollmer, S. Klein

International Journal of Pharmaceutics, 2017, 533(1): 298–314

Für die Entwicklung von bioprädiktiven In-vitro- und In-silico-Modellen zur Simulation der luminalen Bedingungen im Gastrointestinaltrakt einer spezifischen Patientenpopulation sind umfangreiche Kenntnisse über die jeweiligen gastrointestinalen In-vivo-Bedingungen unerlässlich. In dieser Übersichtsarbeit wurden die aktuellen Kenntnisse über die physiologischen Besonderheiten des Gastrointestinaltrakts von Parkinson-Patienten im Vergleich zum gesunden Erwachsenen recherchiert, ausgewertet und zusammengefasst. Während für eine Reihe von gastrointestinalen Parametern, z. B. Speichelsekretion, Speiseröhrentransit und Magenentleerung, bereits relativ robuste Datensätze in der Literatur vorhanden waren, zeigte sich hingegen ein großer Mangel an geeigneten Daten für weitere wichtige Parameter. Dies betraf beispielsweise die Zusammensetzung und physikochemischen Eigenschaften der luminalen Flüssigkeiten des Gastrointestinaltrakts. Das bedeutendste gastrointestinale Merkmal von Parkinson-Patienten ist die beeinträchtigte, d. h. verminderte, Motilität des gesamten Gastrointestinaltrakts. Dies äußert sich u. a. in einer Erhöhung der Magenverweilzeit sowie der Verlangsamung der Dünndarm- und Dickdarmpassage. Insgesamt macht die Literaturstudie deutlich, dass sich die gastrointestinalen Bedingungen von Parkinson-Patienten teilweise erheblich von gesunden Erwachsenen unterscheiden. Daher sollten die verfügbaren In-vivo-Daten in entsprechende Modelle implementiert werden, um die Aussagekraft von Parkinson-spezifischen In-vitro- und In-silico-Modellen zu erhöhen.

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Prof. Dr. Sandra Klein:
Erarbeitung der Fragestellung, Diskussion und Korrektur des Manuskripts

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Erik Wollmer	Prof. Dr. Sandra Klein

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Review article

A review of patient-specific gastrointestinal parameters as a platform for developing *in vitro* models for predicting the *in vivo* performance of oral dosage forms in patients with Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disease that presents with visible motor symptoms, but that is accompanied by several additional symptoms, including gastrointestinal symptoms that may affect pharmacokinetics of oral medications. A detailed understanding of the nature of PD-specific gastrointestinal parameters and of how they may affect drug release of orally administered dosage forms seems to be essential information for developing better oral PD medications. The availability of bio-predictive drug release models simulating PD-specific gastrointestinal parameters would also be beneficial for this purpose. The focus of the present literature review was to determine PD-specific gastrointestinal parameters that will allow for the development of a test methodology simulating the gastrointestinal passage of orally administered medications in PD patients. Whereas for some gastrointestinal segments there is quite a reasonable set of data available on fluid volumes, motility and passage times, for others there is still a big lack in information that would be required for simulating a detailed gastrointestinal passage in a PD patient. The latter is particularly true for potential disease-related changes in gastrointestinal fluid composition. However, with the availability of novel non-invasive diagnostic options there is a chance of obtaining more information in the near future.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease that presents with rigidity, bradykinesia, tremor and postural instability as the primary symptoms resulting from a continuous loss of dopaminergic neurons located in the substantia nigra. The disease affects about 0.1% of the European population (von Campenhausen et al., 2005) and is accompanied by several additional motor and non-motor symptoms including a variety of gastrointestinal (GI) symptoms (Jankovic, 2008; Chaudhuri et al., 2006). Over the last years, it became clear that the GI symptoms in PD including dysphagia, gastroparesis and constipation affect nearly all segments of the GI tract (Pfeiffer, 2003; Jost, 2010; Fasano et al., 2015). PD-related GI dysfunctions are frequent and already present in early disease stages (Fasano et al., 2015; Cersosimo et al., 2013; Reid et al., 2011). Besides affecting the patients quality of life, the GI symptoms may also seriously affect pharmacokinetics of orally administered drugs, particularly of those administered to treat typical PD symptoms (antiparkinson agents) (Fasano et al., 2015; Pfeiffer, 2011).

Oral PD drug therapy includes the administration of levodopa or dopamine agonists, monoaminooxidase (MAO) B inhibitors, catechol-O-methyltransferase (COMT) inhibitors and anticholinergic drugs (Chen and Swope, 2007). Levodopa (l-isomer of 3,4-dihydroxyphenylalanine), a dopamine precursor which was introduced almost 50 years ago, is still regarded as the "gold standard" in PD therapy and the most efficacious and best tolerated antiparkinson drug (LeWitt, 2015; Cotzias et al., 1969). Although it cannot change the course of the disease, it can markedly improve both motor and non-motor symptoms by activating central dopamine receptors. After oral absorption levodopa is rapidly converted to dopamine *via* the action of dopa-decarboxylase in the peripheral circulation. As a consequence, it is no longer able to cross the blood brain barrier and thus incapable to reach the central nervous system (CNS). To make sure that effective levodopa concentrations reach the CNS, the drug is routinely co-administered with dopa-decarboxylase inhibitors such as carbidopa and benserazide. Since before entering the CNS, levodopa may also be metabolized by the enzymes COMT and MAO-B, inhibitors of these enzymes were developed to complement oral levodopa therapy. Entacapone and tolcapone are selective, reversible inhibitors of COMT and increase the half-life and bioavailability of levodopa and consequently are part of several fixed-dose combinations used in oral PD treatment (Seeberger and Hauser, 2007). Currently used MAO-B inhibitors such as selegiline and rasagiline irreversibly bind to MAO-B (Seeberger and Hauser, 2007) and are used as a monotherapy in early stages of PD, before treatment with dopamine

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agonists or levodopa is initiated, or are part of adjunctive therapy in patients with more-advanced disease (DeMaagd, 2015). Finally, the group of dopamine receptor agonists, including bromocriptine, pramipexole or ropinirole, provides direct prolonged dopaminergic stimulation as a result of their longer biological half-life compared to levodopa (Seeberger and Hauser, 2007). The major use of dopamine receptor agonists is the monotherapy of *de novo* patients with the intention of delaying levodopa treatment (Brooks, 2000).

Even though levodopa is on the market since more than half a century and a variety of dosage form concepts for fixed-dose combinations with dopa-decarboxylase inhibitors and COMT inhibitors has been introduced over the past decades, oral PD treatment is still lacking of a dosage form providing timely, reliable and robust drug release at the main site of drug absorption. This is most likely a result of both physiological factors such as a levodopa absorption window in the small intestine (Davis, 2005), the effect of impaired GI function of PD patients on dosage form transport through the GI tract and, drug release of currently marketed dosage forms in the GI lumen of the patient. A detailed understanding of the nature of PD-specific GI parameters and of how much they may affect timing, site and extent of drug release of orally administered antiparkinson drugs seems to be one of the key factors for developing better oral PD medications. Moreover, the development of biorelevant *in vitro* drug release models taking into account these relevant PD-specific GI parameters, *i.e.* GI motility, intraluminal residence/passage times as well as composition and volumes of the intraluminal contents, would be extremely beneficial for both explaining the *in vivo* observations made with dosage forms belonging to current oral regimens and the development and screening of novel formulations.

A number of biorelevant dissolution methodologies for predicting drug release from different oral dosage form types ranging from immediate release (IR) through delayed release (DR) to extended release (ER) formulations have been introduced and successfully applied to predict *in vivo* drug release in average adults, *e.g.* (Dressman et al., 1998; Galia et al., 1998; Kostewicz et al., 2002, 2004; Klein et al., 2005, 2006, 2008; Jantratid et al., 2008, 2009; Fotaki et al., 2009; Kambayashi et al., 2014) amongst others.

Biorelevant dissolution methods have been designed to answer questions in terms of the bioavailability of oral drug formulations. For this purpose it is crucial to perform the *in vitro* dissolution experiments under conditions that closely resemble the key parameters of human gastrointestinal physiology. These include the volumes, composition and essential properties of the fluids available in the different sections of the GI tract, hydrodynamics, GI motility and passage times. During the last decades, biorelevant media have been developed to simulate intraluminal conditions in the stomach, small intestine and proximal colon of an average adult in the fasted state or after the administration of a standardized meal. These media represent the key aspects with regard to composition (*e.g.* bile compounds, enzymes, digestion products) and properties of the GI contents (pH, buffer capacity, osmolality, surface tension) relevant to *in vivo* drug release of the dosage form. Biorelevant dissolution media are of particular importance for examining IR formulations containing poorly soluble drugs. When the aim is to predict bioavailability of lipophilic drugs, fat level and bile salt concentration are usually the most pertinent factors. For ionizable drugs, buffer capacity and pH are also relevant to the dissolution rate. Biorelevant dissolution media, particularly those simulating intraluminal conditions of the (upper) small intestine, *i.e.* the main site of drug absorption for many drugs administered in IR dosage forms have been successfully applied in a number *in vitro* studies dedicated to predict bioavailability poorly soluble drug compounds (*e.g.* Kostewicz et al., 2002, 2004).

For IR formulations containing highly soluble drugs, properties and composition of GI fluids play only a subordinate role, but they can be crucial in limiting drug release and bioavailability of formulations comprising poorly soluble and/or ionizable drugs or key excipients. Therefore the rate of drug release of various DR and ER formulations can also be affected by composition and properties of the intraluminal contents. If the absorption of a compound administered in an IR formulation is limited by the solubility, but not by the permeability of the drug, a proper estimate of the *in vivo* absorption profile can often be obtained by properly simulating the conditions at the main site of drug absorption, *i.e.* the small intestine. In contrast, bio-predictive dissolution methods for DR and ER formulations require a more sophisticated test design simulating a passage through all relevant GI sections relevant to *in vivo* drug release. Beside proper simulation of the changing intraluminal environment during a passage through the relevant GI sections, simulation on motility and passage times through the different GI sections and how much these parameters vary as a result of the size of the dosage form to be administered and the dosing conditions need to be addressed in the test design. Over the last decades, bio-predictive *in vitro* drug release methods for DR and ER products for both systemic absorption and local drug delivery in the GI tract have been established (Klein et al., 2008; Fotaki et al., 2009; Jantratid et al., 2009; Kambayashi et al., 2014). However, whereas these methods were shown to be very useful in predicting *in vivo* drug release in an average adult, next to no patient-specific *in vitro* drug release models have been reported in the literature. As a starting point we have recently presented first approaches for simulating drug release in selected groups of patients and populations (Klein et al., 2013; Klein, 2015), but a PD-specific *in vitro* drug release model has not been discussed or published to date.

With the aim of developing a PD-specific biorelevant *in vitro* drug release model for orally administered medications, the focus of the present literature review was to collect a reliable set of PD-specific GI parameters that will allow for the development of a test methodology simulating the GI passage of orally administered (levodopa) formulations in early and late stage PD patients.

2. Methods

For determining relevant GI phenomena to be implemented in novel PD-specific *in vitro* drug release models, the data bases PubMed, Medline and Web of Science were screened using (combinations of) the following search terms and key words: “Parkinson’s disease”; “Parkinson”; “advanced”; “gastrointestinal”; “parameter”; “physiology”; “dysfunction”; “dysmotility”; “*esophag*”; “dysphagia”; “manometry”; “saliva*”; “stomach”; “gastric”; “gastric emptying”; “pH”; “fluid”; “conditions”; “motility”; “small intestin*”; “small bowel”; “intestinal”; “intestinal transit”; “small bacterial overgrowth”; “colon”; “constipation”; “colon* motility” and “colon* transit”. The review of these phenomena and parameters is structured according to the gastrointestinal route of an orally administered dosage form and shall provide a general and comprehensive overview of the results of studies that focusing on the investigation of GI parameters in PD.

3. GI parameters relevant to oral drug absorption and their PD-related alterations

The mouth is the site where orally administered dosage forms enter the GI tract. In the majority of subjects, residence in the oral cavity is extremely short, before the dosage form is removed by swallowing. Swallowing is a neuromuscular mechanism and the process by which food and also orally administered dosage forms are transported from the mouth to the stomach. The swallowing process itself can be divided into oral, pharyngeal, and esophageal stages, *i.e.* preparatory, transfer and transport phases that follow each other in a sequence (Matsuo and Palmer, 2008). The oral phase of swallowing a dosage form together with a certain amount of fluid, *i.e.* the preparatory phase, involves reflexes in the oral cavity that enable propagation of fluid and dosage form into the oropharynx. In the pharyngeal phase concerted reflex activities in both the mouth and pharynx ensure that the ingested material is transferred into the esophagus. The esophageal phase involves transport of the bolus through the esophagus, past the lower esophageal sphincter, and into the stomach.

3.1. Dysphagia

Dysphagia (abnormal swallowing) can result from a wide variety of diseases and disorders. Functional or structural deficits of the oral cavity,

pharynx, larynx, esophagus, or esophageal sphincters can cause dysphagia which may lead to serious complications. There is a substantial number of subjects (healthy and diseased) that has difficulties in swallowing oral dosage forms (Schiele et al., 2013). In such subjects, the dosage form might rest in the oral cavity for a certain time and might only be moved forward to finally be swallowed after several attempts. Swallowing dysfunction and impairment is a common non-motor symptom in PD (Potulska et al., 2003; Edwards et al., 1992). Numerous studies assessed the prevalence of dysphagia in PD patients. Published prevalence varies widely between 9 and 100% highly depending on the study design and the applied techniques (Cersosimo et al., 2013; Eadie and Tyrer, 1965; Edwards et al., 1993, 1991, 1994; Leopold and Kagel, 1997, 1996; Clarke et al., 1998; Fuh et al., 1997; Siddiqui et al., 2002; Verbaan et al., 2007; Kim et al., 2009; Yu et al., 2010; Muller et al., 2011; Damian et al., 2012; Picillo et al., 2013; Bushmann et al., 1989; Sung et al., 2010; Bird et al., 1994; Martinez-Martin et al., 2007; Barone et al., 2009; Park et al., 2015; Nilsson et al., 1996; Coates and Bakheit, 1997; Nagaya et al., 1998; Mamolar Andrés et al., 2017). It still remains unclear and is controversially discussed, if dysphagia is a feature of advanced-stage PD or already present in the early stages of the disease and, moreover, if there is a correlation between the severity of PD and swallowing problems (Pfeiffer, 2011; Barone et al., 2009; Ertekin, 2014; Cereda et al., 2014; Muller et al., 2001; Barichella et al., 2009; Kalf et al., 2012). Dysphagia can be present in the *on* state (PD patients treated with effective levodopa doses), as well as in the *off* state (PD patients that have not received an effective dose of levodopa) and in all three phases of swallowing (Mamolar Andrés et al., 2017; Bassotti et al., 1998; Salat-Foix et al., 2011; Warnecke et al., 2016). In severe cases dysphagia causes insufficient intake of fluids, food and medication and thus is a critical factor in antiparkinson drug therapy.

In a study published by Potulska et al. (2003) the dysphagia limit, defined as the maximum amount of water that can be swallowed at once (Ertekin, 2014), was investigated. Healthy controls were able to drink a volume of >20 mL at once, whereas the amount of water ingested by PD patients was significantly decreased (6.23 ± 3.67 mL). A similar objective was pursued by Belo et al. (2014) when estimating the dysphagia limit and average volume per swallow using different approaches (Ertekin et al., 1996; Vaiman et al., 2005, 2004). They recruited ten PD patients from stages 1–3 on the Hoehn-and-Yahr (H & Y) scale, a commonly used system for describing how the symptoms of PD progress. The H & Y scale is mainly weighted toward postural instability as the primary index of disease severity and is classifying patients into 5 different classes ranging from minimal disability in class 1 to confinement to bed or wheelchair unless aided in class 5 (Hoehn and Yahr, 1967). Dysphagia limit in the recruited PD patients was compared with that of ten age-matched healthy controls. In agreement with the previous study in the PD patients the dysphagia limit was significantly reduced (15 ± 10 mL *vs.* 33 ± 5 mL in healthy controls). Furthermore, the PD patients needed a significantly higher number of swallows and more time for drinking 100 mL of fluid than healthy controls. A similar result was obtained in a study reported by Miller et al. where 23% of a cohort of 137 PD patients was not able to drink a given volume of 150 mL water (Miller et al., 2009). Patients that could not complete 150 mL also had a significantly slower drinking rate, and were characterized by longer disease duration and higher H & Y score compared to those who could drink the 150 mL. Coriolano et al. studied swallowing in PD patients using surface electromyography (Coriolano et al., 2012). With this technique, they could also show that PD patients needed significantly more time to ingest a given volume of water and yoghurt or any other kind of semi-solid food than healthy subjects. The objective of a study of Nilsson et al. was to assess oral and pharyngeal swallowing during optimal medication in PD patients of different disease stages (Nilsson et al., 1996). For this purpose, they had recruited patients from H & Y stages 1–4 and applied the Repetitive Oral Suction Swallow (ROSS) test for their experiments (Nilsson et al., 1995) which were performed as follows: A glass with a water volume of 200 mL was placed on a balance and the patients were advised to sit in an upright position in front of the balance. They were then instructed to drink the given fluid volume in repetitive boluses of “normal” volume using a straw and sucking at their own comfortable speed. The weight of water remaining in the glass after each bolus and the suction pressure, detected by sensors attached to the side of the straw, were measured continuously. In patients of all H & Y stages, but very much pronounced in that of stage 3 (91%) and stage 4 (94%) abnormal results for oral and pharyngeal swallowing were determined, *i.e.* the bolus volume for a single swallow and the swallowing capacity (mL/s) was reduced compared to the control group. Despite of observing swallowing problems in almost all H & Y stage 3–4 patients, the authors could not find a clear correlation between the H & Y score and swallowing dysfunction. The effect of levodopa medication on swallowing in PD patients was examined by several authors (Warnecke et al., 2016; Lim et al., 2008). Lim et al. studied this effect in 10 PD patients, but without using a control group. The patients were asked to drink 150 mL of tap water as fast as possible while being *on* or *off* levodopa treatment. Several parameters such as the number of swallows needed for a given volume of fluid or the average volume per swallow were recorded. Similar to the findings reported by other authors, Lim et al. could not determine a significant levodopa effect on swallowing. However, in all studies, it became clear, that PD patients have to struggle with (severe) swallowing problems.

Swallowing issues will not only affect ingestion of food and fluids, but also the administration of oral dosage forms. When comparing administration of solid oral dosage forms to healthy subjects and PD patients, this might translate into longer residence times in the oral cavity as well as more time required for oropharyngeal and esophageal passage in PD patients. For this reason, physiology of the oral cavity and passage times and mechanical forces in the oropharynx and the esophagus might be important parameters to consider when designing novel patient-specific *in vitro* drug release test methods.

3.2. Oral cavity

Solid oral dosage forms are usually designed to be swallowed as whole and to release the drug in the stomach and/or intestines. Since in PD patients solid oral dosage forms might not be cleared from the oral cavity as fast as in healthy patients, conditions in the oral cavity might affect integrity of the dosage form and consequently might also have an impact on overall *in vivo* drug release. How much the dosage form integrity is affected by the intraoral environment will be determined by mechanical forces caused by tongue and palate as well as by the volume, pH and composition of saliva.

3.2.1. Salivary secretion & sialorrhea

Human saliva contributes to the initial stage of the digestive process in the upper part of the gastrointestinal tract (Pedersen et al., 2002). The salivary secretion rate in healthy patients ranges widely and the reported values are often highly depending on the examination method (Wang et al., 2015; Dawes, 1975; Dawes and Kubieniec, 2004; Dawes and Macpherson, 1992; Lagerlof and Dawes, 1985; Watanabe and Dawes, 1988; Engelen et al., 2003; Gaviao et al., 2004; Gittings et al., 2015; Froehlich et al., 1987; Mackie and Pangborn, 1990; Humphrey and Williamson, 2001). Excessive salivary secretion or impaired saliva clearance from the oral cavity result in exaggerated accumulation of saliva in the oropharynx which is defined as sialorrhoea or hypersalivation (Fasano et al., 2015). The prevalence of sialorrhoea or drooling in PD was evaluated in various survey studies with the result of 70–78% prevalence in PD patients compared to about 6% in controls (Pfeiffer, 2003, 2010; Bassotti et al., 1998; Chou et al., 2007). The phenomenon of observing excessive amounts of saliva in the oral cavity of PD patients is, however, not a consequence of salivary overproduction, but rather the result of inefficient and infrequent swallowing. Several studies reported that, in fact, the secretion of saliva is decreased in PD (Pfeiffer, 2003; Fasano et al., 2015; Eadie and Tyrer, 1965; Cersosimo and Benarroch, 2012; Tumilasci et al., 2006; Proulx et al., 2005; Bagheri et al., 1999; Bateson et al., 1973; Barbe et al., 2017).

In PD patients of all H & Y stages, Bagheri et al. could determine that basal salivary flow is significantly diminished in comparison to age- and sex-matched control subjects (Table 1) (Bagheri et al., 1999). Tumilasci et al. investigated basal salivary secretion as well as the effect of acid

Table 1
Summary of studies investigating basal and/or stimulated production of saliva in PD patients and healthy controls; unless otherwise stated in the respective publications, mild disease stage is defined as H & Y 1–2, H & Y 3–5 represent moderate/advanced disease stages; LD = levodopa; S.E.M. = standard error of the mean; S.D. = standard deviation; † = mean ± S.E.M. (range); ‡ = no information given; ¶ = mean (range); * = mean ± S.D. (range).

	PD patients		H & Y scores/disease duration	LD treatment discontinued?	Controls	
	n	age (years)			n	age (years)
Tumilasci et al. (2006) †	23	61.6 ± 2.4 (34–81)	8.7 years	Yes	13	63.2 ± 2.0 (44–73)
Proulx et al. (2005)¶	44	59.8 ± 11.0	H & Y: 2.47 ± 0.87	Not stated	44	59.6 ± 11.3
Bagheri et al. (1999) †	83	66.8 ± 1.0 (34–85)	H & Y 1 & 2: 28 H & Y 3 & 4: 55	No	65	63.9 ± 1.3 (36–89)
Bateson et al. (1973) ‡	dry mouth: 8 drooling: 9	67 (59–80) 67 (58–72)	Not stated	No	young: 6 inpatient: 8	26 (25–30) 66 (56–79)
Barbe et al. (2017) *	30	69.3 ± 8.0	8 ± 4.3 years	No	30	69.3 ± 7.9

Technique	Investigation period	Salivary flow rate						
		PD patients					Controls	
		basal		stimulated			basal	stimulated
Sialometry; Stimulation with citric acid	5 min (basal),	*off*	*on*	*off*	*on*	0.21 ± 0.02 mL/min	0.32 ± 0.03 mL/min	0.63 ± 0.08 mL/min
	3 min (stimulated)	0.12 ± 0.01 mL/min	0.31 ± 0.04 mL/min	0.58 ± 0.08 mL/min				
Sialometry	5 min (basal)	0.61 ± 0.53 mg/5 min		–			1.07 ± 0.73 mg/5 min	–
Weighing of saliva soaked cotton plug	2 min (basal)	535 ± 42 mg/2 min		–			834 ± 68 mg/2 min	–
Sialometry; Stimulation with acidic drop	10 min (basal),	0.23 g/min (0.05–0.59)		1.48 g/min (0.85–2.45)			0.74 g/min (0.37–1.51)	4.02 g/min (2.24–6.84)
	5 min (stimulated)	0.23 g/min (0.10–0.39)		1.66 g/min (0.22–2.31)			0.48 g/min (0.13–1.28)	0.48 g/min (0.13–1.28)
Sialometry; Stimulation by chewing on paraffin wax	5 min (stimulated)	–		0.4 mL/min			–	0.8 mL/min

stimulation and intermitted levodopa treatment, creating *on* and *off* phases, on salivary flow (Tumilasci et al., 2006). For this purpose, PD patients with a mean disease duration of 8.7 years and age-matched controls were studied using sialometry (weighing of collected saliva). Basal and stimulated salivary flow rates in PD patients were significantly decreased in the *off* state and, to a minor degree, also decreased in the *on* state. Levodopa administration resulted in a stimulation of both basal and stimulated salivary flow rate in PD, but overall, no significant correlation of saliva flow rates with disease duration or levodopa dose could be established. In another study, Proulx et al. investigated unstimulated salivary flow using sialometry in PD patients (H & Y stage 2.47 ± 0.87) and age-matched healthy controls (Proulx et al., 2005). Results of this study were in good agreement with those of previous studies, since, compared to healthy controls, the basal flow of saliva was significantly reduced in PD patients. In contrast to other studies, the authors found a correlation between the reduced saliva flow rate, levodopa dose and, when medication was not taken into account, disease duration, respectively. Bateson et al. examined both basal and stimulated salivary flow in PD patients, inpatient controls and young controls and stated a reduced flow rate in both states in PD patients and also determined a correlation with patients age (Bateson et al., 1973). Finally, in a recently published study, Barbe et al. reported a significant reduction of stimulated saliva in PD patients (Barbe et al., 2017).

Summarizing the outcomes of the studies discussed above, there is a lot of evidence that both basal and stimulated salivary secretion rate is reduced in PD patients and might to a certain extent increase in *on* phases. However, it still remains unclear if the extent of salivary flow suppression correlates with patient age or severity of the disease (Edwards et al., 1991; Tumilasci et al., 2006; Proulx et al., 2005; Bateson et al., 1973). Nevertheless, as a result of a loss of involuntary swallow reflex and pharyngeal muscle motor impairment, drooling, *i.e.* excessive pooling of saliva in the oral cavity, is a common problem in PD (Salat-Foix et al., 2011) and thus is regarded as an essential clinical symptom to be simulated in a bio-predictive *in vitro* test model for solid oral dosage forms administered to PD patients.

3.2.2. Saliva composition

When residence time in the oral cavity is intended to be implemented into a PD-specific *in vitro* model, saliva composition in PD patients will be an important point to consider. Besides investigating basal salivary secretion Tumilasci et al. assessed the composition of the collected saliva of PD patients and the control group involved in their study (Tumilasci et al., 2006). They determined abnormally high concentrations of amylase, sodium and chloride in the saliva of PD patients and found out that, in contrast to affecting basal and reflex salivary flow rate in PD patients, levodopa treatment did not affect salivary composition (Tumilasci et al., 2006). Whereas give detailed information on changes in the electrolyte composition of saliva, they do not provide any information on potential alterations in saliva pH, buffer capacity and further physicochemical parameters that might affect integrity and drug release of solid oral dosage forms. Thus, future prospective studies will be needed to fill this knowledge gap.

3.3. Oropharynx

Whereas numerous reports are available on studies screening the ability of swallowing different types and amounts of fluids and food, only very few studies focused on studying oropharyngeal transit and dynamics in PD patients. screened oropharyngeal dynamics in PD patients of different H & Y stages with and without levodopa-induced dyskinesia and a control group (Monte et al., 2005). Deglutition was assessed using a barium swallow test with videofluoroscopy. Neither the report of dysphagia nor any of the PD severity parameters correlated to the videofluoroscopic variables. Dyskinetic patients performed better in swallowing function than nondyskinetic patients, which could be a result of a greater levodopa dose. Based on these observations Monte et al. concluded that levodopa may play a role in the oral phase of deglutition and stated that dysphagia is not a good predictor of deglutition alterations in PD. With the purpose of investigating swallowing function in patients with PD with a norm-referenced, quantitative approach, Ellerston et al. measured pharyngeal transit times in PD patients using a modified barium swallow test (Ellerston et al., 2016). The majority of patients included in the study were in a stage of more severe progression of the disease. Even though reduced pharyngeal constriction was found in 30.4% of the patients, prolonged pharyngeal transit was not identified as a prominent feature. Finally, in a recently published review article, Ertekin stated that many PD patients with dysphagia and low dysphagia limits also have clinically significant oropharyngeal dysfunction represented by the accumulation of saliva and pieces of food in the mouth amongst others (Ertekin, 2014). He also stated that the clinical condition gets more pronounced with increasing disease duration.

Based on the detailed results from all reports cited above, oropharyngeal transit time itself is short and thus might not need to be considered in biorelevant *in vitro* test setup. However, the prevalence of oropharyngeal dysphagia is high (Kalf et al., 2012) and it is obvious, that oropharyngeal impairment can have a pronounced effect on both oral residence time of orally administered dosage forms and saliva volume in the oral cavity and will thus impact the test design for simulating oral residence of the dosage form.

3.4. Esophagus

Dysmotility of the esophagus contributes to swallowing dysfunction in PD or may even be the trigger for the symptoms (Pfeiffer, 2011; Sung et al., 2010; Borghammer et al., 2016; Beach et al., 2010). The reported occurrence of esophageal dysmotility does widely spread and the results seem to be highly dependent on the examination method. The prevalence of esophageal dysmotility in PD patients reported from a number of videofluoroscopic swallowing studies ranges from 5 to 86% (Leopold and Kagel, 1997; Edwards et al., 1994; Stroudley and Walsh, 1991; Blonsky et al., 1975; Gibberd et al., 1974). Studies using manometry, in contrast, indicated a more narrow prevalence of esophageal dysfunction with 61–73% of the PD patients being affected (Sung et al., 2010; Bassotti et al., 1998; Castell et al., 2001). Indicators for an esophageal dysmotility are for example a slowed esophageal transit, abnormalities in esophageal peristalsis or a reduced pressure at the lower esophageal sphincter (Nagaya et al., 1998; Bassotti et al., 1998; Blonsky et al., 1975; Gibberd et al., 1974; Castell et al., 2001; Johnston et al., 2001; Lin et al., 1994).

Potulska et al. used a scintigraphic method to investigate esophageal transit time in H & Y stage 1–3 patients (2.1 ± 0.7) for which in the first part of the study a decreased dysphagia limit (6.23 ± 3.67 mL *vs.* > 20 mL in all control subjects) had been determined and a control group (Potulska et al., 2003). The examination was performed in a supine position and patients received 5–10 mL of ^{99m}Tc-labeled water. Patients were instructed to swallow the entire bolus in one gulp. Mean transit time through the esophagus was recorded. Transit times in PD patients were significantly prolonged compared to those recorded in controls (14.46 ± 5.3 s *vs.* 7.45 ± 1.64 s). Furthermore, when comparing esophageal transit in PD patients with and without clinically diagnosed dysphagia, a significant difference in esophageal transit times was observed (17.02 ± 4.89 s *vs.* 9 ± 1.4 s). Wang et al. performed a study in which a bolus of solid gelatin (0.4 g in 4 mL of water) labeled with ^{99m}Tc had to be ingested by the patients together with 15 mL of water to imitate solid food intake (Wang et al., 1994). Transit

time of the marker was examined scintigraphically. A significant increase in transit time was observed when comparing esophageal transit in PD patients with that in age-matched controls (36.40 ± 14.14 s *vs.* 20.74 ± 9.84 s).

A few studies have been conducted to assess esophageal pressure conditions in PD patients (Bassotti et al., 1998; Higo et al., 2001; Suttrup et al., 2017). Bassotti et al. published results from a manometric study in H & Y stage 1–4 patients that indicate slight, but no significant reductions in pressure of the esophageal body and lower esophageal sphincter (Bassotti et al., 1998). Manometric abnormalities were documented in 61% of the patients, and were represented by repetitive contractions, simultaneous contractions, reduced lower esophageal sphincter pressure, and high-amplitude contractions. A specific motor pattern for PD was not identified. Only 33.3% of patients had both symptoms and manometric abnormalities. The latter observations indicate that the GI tract might be involved in PD much earlier than GI symptoms are reported by subjects. Results presented by Suttrup et al. confirm this assumption since in a comprehensive study using high-resolution manometry to detect esophageal pressure abnormalities in PD patients (Suttrup et al., 2017), they found impairments across all disease stages with a trend of more severe pathological findings especially in peristalsis (decrease) and intrabolus (increase) pressure in patients in advanced disease stages of the disease.

As stated when discussing oropharyngeal transit time, a detailed simulation of the esophageal transit might not be the focus of a biopredictive *in vitro* test design. The major point to consider when developing patient-specific models that should address the impact on dysphagia on gastrointestinal transit is certainly the excessive manipulation time in the oral cavity that is a result of swallowing problems. However, the very small dysphagia limit determined in PD patients and the decreased esophageal peristalsis give rise to think about how to secure clearance of oral dosage forms from the esophagus and how to prevent sticking and esophageal lesions caused by orally administered dosage forms.

3.5. Stomach

The stomach is typically not a site of drug absorption. However, gastric conditions and passage time are important to drug absorption as they can strongly influence the onset and extent of drug release from orally administered dosage forms. When discussing gastric effects on drug release and absorption, it is important to distinguish between administration in the fasted or fed state since both states are characterized by different motility and emptying patterns as well as by different physicochemical characteristics of gastric contents.

3.5.1. Gastric motility, gastric emptying, gastroparesis

Two distinct GI motility patterns, having a significant impact on the GI transit or disintegration of dosage forms, exist for both the stomach and small intestine: the cyclic interdigestive (fasted) pattern, referred to as the MMC (migrating motility/myoelectricity complex) and the digestive (fed) pattern. The MMC is characterized by three phases: (I) the longest phase, a resting period lasting for ~60–70 min (Englander and Greeley, 2006) in which mostly no contractions occur, (II) a phase of 20–30 min (Englander and Greeley, 2006) where muscular contractions begin to appear at increasing frequency and where intermittent electrical spikes and subsequent contractions can be measured, and (III) a phase which lasts approximately 5–10 min (Englander and Greeley, 2006) where nearly every slow wave is associated with a spike potential, resulting in forceful contractions and an intense contraction pattern. Whereas due to a high tonus the pyloric sphincter is closed to a large extent in phases I and II and can therefore only be passed by fluids and finely dispersed particles, the strong contractions in phase III cause relaxation of the pylorus which in turn results in a temporary extension of its open diameter and enables to empty all residual material (undigested food ingredients and solid dosage forms) from the stomach into the small intestine.

Upon ingesting a meal or a nutrient containing solution, the fasted MMC is interrupted and replaced with the fed state motility pattern which represents continuous contractions most similar to phase II of the interdigestive MMC. During the fed digestive motility pattern segmentive contractions are prevalent and fewer propagative contractions can be observed. The duration of the fed motor pattern is determined by the caloric content and the physicochemical properties of the food ingested. As in the early phases of the MMC, the pylorus is closed and does not allow larger particles to enter the small intestine. Only when food-related stimuli are no longer present, the motility pattern of the stomach returns to the interdigestive MMC where with the first phase III larger particles and solid dosage forms can be emptied into the small intestine.

Because of the pylorus controlling the transport of orally administered dosage form into the small intestine and since gastric retention and emptying can be affected by numerous factors (Coupe et al., 1991; Choe et al., 2001), gastric residence time seems to be the most critical parameter when discussing the GI transport of oral dosage forms (Garbacz and Klein, 2012).

Gastric motility is impaired in PD resulting in a delay in gastric emptying, also known as gastroparesis (Pfeiffer, 2003; Jost, 2010; Fasano et al., 2015). Results from various studies indicate that gastric motility disturbances occur in both early and advanced disease stages with a reported prevalence of up to 100% (Fasano et al., 2015; Cersosimo et al., 2013; Edwards et al., 1991; Barone et al., 2009; Goetze et al., 2006; Martinez-Martin, 2011). However, as in many cases it cannot be related to specific symptoms, gastric motility impairment is often undetected in routine clinical practice. Instead of relating GI problems to delayed gastric emptying, patients experience a number of nonspecific gastrointestinal symptoms such as nausea, early satiety, postprandial bloating and abdominal fullness (Edwards et al., 1991; Siddiqui et al., 2002; Hardoff et al., 2001). The pathogenesis of delayed gastric emptying is likely multifactorial with both the enteric and central nervous system involvement playing a role. Additionally, levodopa itself is known to cause delayed gastric emptying (Robertson et al., 1990; Berkowitz and McCallum, 1980). Beyond its effect on quality of life, gastroparesis poses additional challenges in PD patients by affecting the pharmacodynamics of levodopa. Studies have shown that delayed gastric emptying leads to increased unpredictability of motor fluctuations (Jost, 2010; Su et al., 2017a; Nyholm and Lennernas, 2008; Djaldetti et al., 1996a; Kurlan et al., 1988; Muller et al., 2006; Doi et al., 2012). This is likely due to delayed gastric emptying times leading to a delayed delivery of a levodopa dose that is expected to be absorbed in the small intestine.

Gastric motility is controlled by myoelectrical activity consisting of gastric slow waves (electrical control activity, ECA) and spike/s potentials (electrical response activity, ERA) (Chang, 2005; Smout et al., 1980). In healthy subjects ECA spreads with a frequency of about three cycles per minute (cpm) through the stomach and is the determinant of propagation and of gastric contraction frequency. Whereas gastric slow waves are usually not followed by muscular contractile activity, the appearance of antral contractions is directly associated with ERA that is triggered by ECA (Hinder and Kelly, 1977). Gastric slow waves can be measured noninvasively by electrogastrography (EGG) using frequency and amplitude of ECA as EGG parameters for investigating gastric motility. Several EGG studies with different study designs and investigating different key parameters were conducted to assess gastric dysmotility in PD patients (see Table 2).

Table 2
Information about subjects and study design evaluating gastric motility dysfunction; unless otherwise stated in the respective publications, mild disease stage is defined as H & Y 1–2, H & Y 3–5 represent moderate/advanced disease stages; LD = levodopa; EGG = electrogastrography; EGEG = electrogastroenterography; MRI = magnetic resonance imaging; S.D. = standard deviation; S.E.M. = standard error of the mean; cpm = cycles per minute; dB = dB; Hz = Hz; * = mean ± S.D. (range); ‡ = mean (range); † = mean ± S.E.M. (range); ° = mean, but not stated whether S.D. or S.E.M was used.

	PD patients		H & Y scores/ disease duration	Controls		LD therapy discontinued?	Technique	Investigation period	Meal	Studied parameters	Results		
											PD patients		Controls
	n	age (years)		n	age (years)						off	on	
Kaneoke et al. (1995) *	10	64 ± 8 (49–76)	H & Y: 3	10	58 ± 11 (42–71)	Yes	EGEG	10 min fasted, 40 min fed	Liquid	Dominant frequency (Hz), preprandial	0.047	–	0.050
										Mean postprandial gastric increase rate	1.19	–	2.84
Soykan et al. (1999) ‡	11	66 (46–78)	Advanced-staged PD, 9.8 years	10	38	Yes: 6	EGG	30 min fasted, 90 min fed	Solid	Dominant frequency (cpm) preprandial	3.3 ± 0.1	3.2 ± 0.01	2.88 ± 0.12
						No: 5				postprandial	3.2 ± 0.2	3.1 ± 0.1	3.05 ± 0.16
										Postprandial change of dominant power (dB)	−3.11 ± 1.01	1.17 ± 1.96	8.01 ± 1.7
										Normogastria (%)	82.6 ± 6.6	75.8 ± 13.6	88.2 ± 5.4
Lu et al. (2004) †	13	70.8 ± 1.1	11.5 ± 0.8 years	13	70.8 ± 2.4	Both	EGG	30 min fasted, 30 min fed	Solid and liquid	Dominant frequency (cpm)	2.88 ± 0.07	2.92 ± 0.10	3.00 ± 0.07
										preprandial	2.96 ± 0.07	2.97 ± 0.07	3.19 ± 0.12
										postprandial	40.4 ± 0.9	39.6 ± 1.0	40.5 ± 1.3
										Dominant power (dB) preprandial			
										postprandial	43.1 ± 1.4	45.2 ± 1.6	44.8 ± 1.2
										Normogastria (%) preprandial	49.8 ± 7.1	47.1 ± 5.2	73.9 ± 3.5
										postprandial	57.7 ± 6.6	68.9 ± 5.1	75.8 ± 4.9
										Instability coefficient of dominant frequency preprandial	0.43 ± 0.03	0.49 ± 0.04	0.31 ± 0.03
										postprandial	0.42 ± 0.03	0.30 ± 0.04	0.28 ± 0.03
Albani et al. (2011) °	23	65	H & Y: 2.3, 6.2 years	20	64	No	EGG	45 min fasted, 45 min with LD	–	Dominant frequency (cpm)	2.76 ± 0.30	2.74 ± 0.34	2.68 ± 0.24
Chen et al. (2005) ‡	20	63 (34–81)	1–10 years	11	55 (30–77)	Yes	EGG	15 min fasted, 5 min during water intake, 30 min after	Liquid	1.0- to 2.5-cpm activity (%)	44 ± 3	–	33 ± 3
										2.5- to 3.75-cpm activity (%)	33 ± 4	–	49 ± 5
										Dysrhythmia (%)	65	–	None
										Water intake until full (mL)	303 ± 45	–	627 ± 67
Naftali et al. (2005) °	36	67 ± 1.7 (48–81)	H & Y: 2.4 ± 0.9, 7.1 years (1–20)	14	71 ± 8	Yes	EGG	60 min fasted, 60 min fed	Solid and liquid	Dysrhythmia (%)	66.6	–	None
										Bradygastria (number of patients) preprandial	6	–	None
										postprandial	4		None
										both pre- and postprandial	10		None
										Tachygastria (number of patients) preprandial	1	–	None
										postprandial	1		None
										both pre- and postprandial	2		None

(continued on next page)

Table 2 (*continued*)

	PD patients		H & Y scores/ disease duration	Controls		LD therapy discontinued?	Technique	Investigation period	Meal	Studied parameters	Results		
											PD patients		Controls
	n	age (years)		n	age (years)						off	on	
Sakakibara et al. (2009) *	17	66 ± 8	H & Y: 3.2 ± 0.5, 6.1 ± 3.6	8	63 ± 8	No	EGG	24 h	Not standardized ("daily routine")	Dominant frequency (cpm) preprandial	–	2.85 ± 0.16	2.76 ± 0.21
										postprandial		2.93 ± 0.19	3.05 ± 0.26
										change		0.08 ± 0.16	0.29 ± 0.26
										Instability coefficient of dominant frequency (%) preprandial	–	11.62 ± 6.0	8.60 ± 4.53
										postprandial		13.14 ± 6.6	8.25 ± 5.03
										change		1.52 ± 4.92	−0.35 ± 4-.19
Unger et al. (2010) *	10	62 ± 11.1 (34–72)	2.7 years (0.5–7)	10	53 ± 11.0 (35–67)	Yes	MRI	Fed; 88 ± 26 min PD, 101 ± 27 min controls	Solid and liquid	Velocity of peristaltic waves (mm/s)	2.08 ± 0.63	–	2.07 ± 0.45
										Amplitude of peristaltic contractions (mm)	5.35 ± 2.85	–	8.06 ± 2.31
										Gastric motility index (mm^2/s)	11 ± 6.9	–	16.5 ± 5.1

Kaneoke et al. studied gastric myoelectrical dysfunction with electrogastroenterography (EGEG, a method to record electrical activities of the stomach and the intestine using skin electrodes) in ten moderate-staged PD patients and ten healthy controls (Kaneoke et al., 1995). Subjects were fasted overnight and first, a baseline electroenterogastrogram was recorded for 10 min. Subsequently, recording was interrupted and within 5 min the patients ingested a liquid test meal with a caloric content of 250 kcal in an upright position. 5 min after completing ingestion, data recording was continued for 40 min. Comparison of the data set obtained from healthy controls and PD patients indicated a significantly lower postprandial increase rate in generic wave frequencies in PD patients, whereas no remarkable difference between the two groups had been observed in the preprandial state. In a study investigating the pattern of gastric myoelectrical activity in patients with PD Soykan et al. screened patients with a mean disease duration of 9.8 years and a control group (Soykan et al., 1999). PD patients were stratified as "receiving (*on*) dopaminergic therapy" and "*off* therapy". Gastric myoelectrical activity was measured by means of surface EGG for 30 min before and for 90 min after a standardized meal. Both patient groups showed significant smaller increase in mean postprandial EGG amplitude compared to young controls indicating gastric dysmotility in PD patients. Another study aimed to evaluate gastric myoelectrical activity in PD patients during and after levodopa treatment was reported by Lu et al. (2004). PD patients and age-matched controls were enrolled in the study. Electrogastrography was used to record gastric myoelectrical activity in all subjects for 30 min before and 30 min after a standard meal. In PD patients, gastric myoelectrical activity was recorded during both the "*on*" (with levodopa treatment) and the "wearing-*off*" (without levodopa for at least 12 h) periods. In the wearing-*off* state, patients showed a significantly reduced frequency of slow waves in the fasted (2.88 ± 0.07 cpm *vs.* 3.00 ± 0.07 cpm in controls) and the fed state (2.96 ± 0.07 cpm *vs.* 3.19 ± 0.12 cpm). Treatment with levodopa resulted in an improvement in the fed state. Based on the results of their study, Lu et al. concluded that patients with PD have reduced slow wave rhythmicity and an impaired postprandial response in gastric myoelectrical activity and that these abnormalities may be partially corrected with levodopa treatment in the fed state.

In contrast to this, in a study evaluating the effects of levodopa intake on the motility of an empty stomach in PD patients with and without motor fluctuations, Albani et al. did not see a difference in frequency of gastric slow waves in the fasted state between PD patients and controls (Albani et al., 2011). The electrogastrography findings showed a normal pattern that was not influenced by levodopa intake and could not be related to plasmatic levodopa concentrations and to clinical parameters. They thus concluded that at rest gastric activity of PD patients is normal and the variability of plasma levodopa concentrations is not affected by gastric motility (Albani et al., 2011).

Chen et al. designed a study to assess the gastric myoelectrical functioning in patients with PD and in healthy controls by using EGG and the water load test and to determine the clinical utility of EGG in differentiating PD patients with or without upper gastrointestinal symptoms (Chen et al., 2005). In the study in which PD patients were stratified into subgroups without and with upper gastrointestinal symptoms gastric myoelectrical activity was assessed before and after the subjects ingested water until full. Study results were compared with those obtained in a control group of a similar size. All PD patients drank significantly less water until full as compared with the controls (303 ± 45 mL vs. 627 ± 67 mL) and overall gastric myoelectrical activity was impaired in both PD patient groups. Other EGG studies of similar design were performed by several other groups. However, whereas in some of these studies results in patients were abnormal and gastric myoelectrical dysrhythmia correlated with duration and severity of the disease and duration of levodopa treatment (Krygowska-Wajs et al., 2000; Naftali et al., 2005), even though in other studies irregular slow waves were determined in PD patients, no significant correlation could be established between this observation and other disease parameters (Sakakibara et al., 2009). Besides EGG several other techniques can be used to determine gastric motility and transit. In a pilot study by Unger et al. real-time magnetic resonance imaging (MRI) was used to evaluate gastric postprandial motility in early-staged PD patients (Unger et al., 2010). The authors observed a trend towards a decreased gastric motility in patients with PD compared with healthy controls indicated by a significant reduction in the amplitude of peristaltic contraction, but no change in the velocity of the peristaltic waves.

The number of studies assessing gastric emptying time (GET) in PD has significantly increased over the last decade. Scintigraphy with ^{99m}Tc and the $^{13}CO_2$ breath test are the major techniques applied to assess GET (Goetze et al., 2006, 2005; Hardoff et al., 2001; Djaldetti et al., 1996b; Thomaides et al., 2005; Krygowska-Wajs et al., 2009; Trahair et al., 2016; Bestetti et al., 2017; Tanaka et al., 2009, 2011; Unger et al., 2011; Epprecht et al., 2015). Recently, the wireless motility capsule (WMC) – a non-invasive and non-radioactive diagnostic tool sampling gastrointestinal pH, pressure and temperature – was introduced as an alternative tool for future studies (Su et al., 2017a; Barboza et al., 2015). An overview of gastric emptying studies in PD and the corresponding results is given in Table 3.

Djaldetti et al. published one of the first scintigraphic trials for studying gastric emptying in PD (Djaldetti et al., 1996b). They had performed a radionuclide gastric emptying study using a standard ^{99m}Tc colloid-labeled solid meal in PD patients of all disease stages, divided into patients with and without response fluctuations (RF), and a control group. Following an overnight fast, patients were given a standard meal and gastric emptying was monitored in a supine position using a gamma camera. PD patients had prolonged gastric emptying measured after 60 min compared with the normal control subjects and gastric half emptying time ($GET_{1/2}$) was significantly higher in PD patients with RFs than in those with non-RFs. A similar test design in terms of assessing technique and test meal was used by Hardoff et al. (Hardoff et al., 2001). In their study $GET_{1/2}$ in PD patients was slightly longer than in healthy controls, but no significant correlation between $GET_{1/2}$ and disease severity was found. Moreover, the frequency and amplitude of antral contractions were not significantly altered in PD patients when compared with controls. A semi-solid meal was used to assess gastric emptying in a scintigraphic study carried out by Thomaides et al., who determined a significantly shorter $GET_{1/2}$ in controls than in PD patients, but could not establish a relationship between gastric emptying and patient age or duration of disease (Thomaides et al., 2005). Gastric emptying of solid food in patients with familial and sporadic PD was evaluated in a scintigraphic work of Krygowska-Wajs et al. (2009). In 70% of patients with familial PD and in 55% of sporadic PD patients delay in gastric emptying was found across all disease stages with a tendency of becoming more pronounced in advanced disease stages. Several parameters including gastric emptying, postprandial blood pressure and blood glucose were evaluated in a comprehensive trial published by Trahair et al. (2016). Patients with mild to moderate disease and age-matched controls were advised to ingest 75 g ^{99m}Tc-labeled glucose solution and gastric emptying was scintigraphically investigated keeping the patients in an upright position for a total imaging time of 3 h. Results indicate that $GET_{1/2}$ in most of the PD patients was not essentially different to that in controls, only 14% of the patients showed an abnormal delay. Besides this study report, reports from a few additional studies where GET was not significantly delayed in PD patients can be found in the literature. Recently, Bestetti et al. published results from experiments in which gastric emptying of ^{99m}Tc-labeled acidified orange juice administered after a 12-h fasting period was assessed (Bestetti et al., 2017). They compared $GET_{1/2}$ in controls and patients with rather advanced PD using scintigraphy with the subjects placed in supine position (Bestetti et al., 2000). Mean $GET_{1/2}$ was prolonged in PD patients, but the differences were not significant when compared with $GET_{1/2}$ in control subjects. In another scintigraphic study Knudsen et al. followed gastric

emptying of a ^{99m}Tc-labeled solid meal in early-staged PD patients and healthy age-matched controls and also could not observe a significant difference between $GET_{1/2}$ in the two groups (Knudsen et al., 2017a).

As indicated before, another approach for estimating gastric emptying is the $^{13}CO_2$ breath test (Borghammer et al., 2016; Goetze et al., 2005). Briefly, a liquid or solid test meal labeled with ^{13}C-sodium acetate or −octanoate is ingested by the patient, emptied from the stomach, absorbed in the duodenum, immediately metabolized and exhaled as $^{13}CO_2$ which can then be detected and quantified (Ghoos et al., 1993; Maes et al., 1998). Gastric emptying is considered to be the rate-limiting step in the delivery of $^{13}CO_2$ to the breath. In a study performed by Goetze et al. PD patients of all disease stages and healthy age-matched controls had to ingest a solid meal labeled with ^{13}C-sodium octanoate and samples of exhaled breath were collected in 15 min intervals for a duration of 4 h (Goetze et al., 2005). $GET_{1/2}$ was significantly prolonged in PD patients and a correlation with disease severity could be established. In agreement with results from this study, a significant delay in gastric emptying was also found in a second trial of similar design conducted by the same authors (Muller et al., 2006). In another study performed by Goetze et al. gastric emptying of a liquid and a solid meal was evaluated in PD patients across all disease stages as well as in age- and sex-matched controls (Goetze et al., 2006). In PD patients a delay in gastric emptying was seen in 88% for the solid- and in 38% for the liquid meal. Overall gastric emptying time for liquids was similar for PD patients in early and advanced disease stages and for controls. For solids, however, a significant difference in $GET_{1/2}$ was found when comparing data from H & Y stage 3–5 PD patients with that for H & Y stage 1–2.5 PD patients and control subjects. Tanaka et al. also applied the $^{13}CO_2$ breath test to examine $GET_{1/2}$ of liquids in patients with early and advanced PD (Tanaka et al., 2011). They compared gastric emptying in 20 untreated early-stage PD patients, treated advanced-stage PD patients, and healthy volunteers. Subjects received a 200 kcal/200 mL liquid meal after an overnight fast of 12 h and in a sitting position expiration breath samples were collected in 10 min intervals for the next 4 h. Interestingly, $GET_{1/2}$ was similar in both patient groups, but compared to controls, a significant delay in gastric emptying could be observed. In a similar study, the same authors assessed gastric emptying for liquids in patients with and without motor fluctuations (Tanaka et al., 2009). Again, in PD patients they could observe a significant delay in $GET_{1/2}$, but results from the two patient groups did not differ from each other. Unger et al. studied GET in two groups of PD patients diagnosed with early stage PD (drug-naive and under therapy, respectively), patients with idiopathic rapid-eye-movement sleep behavior disorder (iRBD), a presumable pre-motor stage of PD, and control subjects (Unger et al., 2011). After an overnight fast, patients had to ingest a solid meal (one egg and one bread roll) labeled with ^{13}C-sodium octanoate and 250 mL of water. Breath samples were then collected and analyzed in 15 min intervals for a duration of 6 h. A significant delay in $GET_{1/2}$ could be observed in both PD patient groups when compared with $GET_{1/2}$ in controls. Patients with iRBD, in contrast, showed $GET_{1/2}$ similar to that of the control subjects. In a study investigating the relation between levodopa uptake and gastric emptying Nord et al. (2017) used the $^{13}CO_2$ breath test to assess gastric emptying of a solid meal (omelet), co-administered with a fixed dose combination of levodopa and benserazide (100/25 mg) in two patient groups with early or advanced stage PD and compared results with reference material from a control group (Dizdar et al., 1999) and observed a delay in $GET_{1/2}$ in both groups when compared with control subjects. Epprecht et al. focused on the evaluation of $GET_{1/2}$ of a solid high caloric meal (428 kcal) in patients with early-staged PD also using the $^{13}CO_2$ breath test and could not observe an overall delayed $GET_{1/2}$ in a homogenous, representative number of early motor PD patients in comparison with healthy, age-matched and younger controls (Epprecht et al., 2015). In addition, they assessed the effect of levodopa on GET in healthy aged controls and could observe a robust gastric emptying slowing as a result of levodopa intake ($GET_{1/2}$ = 132 ± 24 min *vs.*112 ± 15 min for the placebo group).

Current literature data on gastric motility and gastric emptying times strongly relate to the study design, including the method of investigation, the administered test meals and fluids, the selection of patients, the medication etc. and therefore in some cases are still contradictory. However, even though it is also still not clear, if the disease duration correlates with degree of gastroparesis (Heetun and Quigley, 2012), the studies performed to date indicate a high prevalence of delayed and disturbed gastric emptying in PD patients in all disease stages. Recently, Su et al. have published results from the first wireless motility capsule (WMC) experiments in PD patients (Su et al., 2017b, 2016). The WMC represents a promising non-invasive tool that after it is swallowed by a patient continuously samples intraluminal pH, temperature, and pressure as it moves along the GI tract (Su et al., 2017a). This technology has already successfully applied for screening the gastrointestinal environment and transit times in several prospective studies simulating typical dosing conditions in healthy volunteers (Cassilly et al., 2008; Maqbool et al., 2009; Koziolek et al., 2015a, 2015b; Schneider et al., 2016) and will hopefully contribute to a better future understanding of gastric motility and residence times in PD patients.

3.5.2. Gastric contents

Whereas over the past decades various prospective *in vivo* studies were performed to better understand volumes, composition and properties of fasted and fed state gastric contents in healthy adults, corresponding studies in PD patients are lacking.

As early as in 1967 Rivera-Calimlim et al. presented results from a study to determine levodopa absorption and metabolism by the human stomach (Rivera-Calimlim et al., 1971). Before administering the medication, they withdraw the gastric content of 8 fasted PD patients with undisclosed symptom progression and aging 59–70 years using a nasogastral tube followed by pH analysis of the aspirated fluid. 6 patients had a basal fasted gastric pH in the range from 1.2–2.1, samples from the other two patients inidicated a basal fasted gastric pH of 6.8 and 7.2, respectively. In a case study focusing on the explanation of failures of levodopa therapy in a 58 year old man diagnosed with PD, also reported by Rivera-Calimlim et al. (1970), another sample of basal gastric fluid was sampled from a PD patient using a nasogastral tube. The basal gastric fluid pH of this patient was reported as pH 1.2. Jenkins et al. assessed the secretion of gastric hydrochloric acid in 93 PD patients without providing detailed information on patient age or severity of the disease and observed achlorhydria in 31% of the patients (Jenkins et al., 1973). Finally, Yazawa et al. studied the relationship between levodopa absorption and gastric acid secretion in patients with PD who became refractory to therapy of levodopa (Yazawa et al., 1994). In this study they measured the pH and amount fasting gastric juice collected from the patients and concluded that gastric acid secretion was decreased in 58% of their patients.

As can be seen, the data set available on gastric fluid/content properties is very limited and data from most studies lack of essential details on the patient status. The data also should be handled with care, since some of the data sets are quite old and in the meantime, it is well known, that the method and site of sampling can have a massive impact on the sample composition and pH. Overall, the available data sets indicate that gastric pH and fluid volume are not seriously affected by PD. The achlorhydria observed in a significant number of subjects might be a result of the patient's age and/or co-medication burden rather than of the disease itself (Russell et al., 1993, 1994). However, it is obvious that the gastric environment of PD patients requires a much more detailed screening, *e.g.* by using the WMC to get more detailed information on gastric pH conditions, modern aspiration and imaging techniques to better understand the sampling location as well as gastric content composition and fluid volumes available in both the fasted and the fed state. The reported trends can thus only present a starting point for designing bio-predictive PD-specific *in vitro* drug release models,

Table 3
Studies assessing gastric emptying half time ($GET_{1/2}$) in PD patients and healthy controls; unless otherwise stated in the respective publications, mild disease stage is defined as H & Y 1–2, H & Y 3–5 represent moderate/advanced disease stages; LD = levodopa; m/s = moderate/severe; RFs = response fluctuations; fPD = familial Parkinson's disease; sPD = sporadic Parkinson's disease; S.D. = standard deviation; S.E.M. = standard error of the mean; * = mean ± S.D. (range); † = mean ± S.E.M. (range); # = median (range); part of this table was adapted from Heetun and Quigley (Heetun and Quigley (2012)).

	PD – mild		PD – moderate/severe		H & Y scores/ disease duration	LD therapy discontinued?	Controls		Technique	Investigation period	Meal	$GET_{1/2}$ (min)		
	n	age (years)	n	age (years)			n	age (years)				PD – mild	PD – m/s	Controls
Djaldetti et al. (2006b) *	15 non-RFs	64.8 ± 9.9	15 RFs	62 ± 9	H & Y: non-RFs: 1–4, 8.8 ± 4.6 years; H & Y: RFs: 2–4, 4.0 ± 3.3 years	Not stated	22	45 ± 3.5	Scintigraphy	1 h	Solid (overnight fast)	Non-RFs: 85 ± 31	RFs: 221 ± 202	56 (41–62)
Hardoff et al. (2001) *	29	61.5 ± 5.9	22	65.1 ± 5.6	H & Y: 1–2 (m) H & Y: 2.5–3 (m/s)	No	22	61.9 ± 6.1 (52–70)	Scintigraphy	2 h (10 min intervals)	Solid (12-h fast)	63.4 ± 28.8 (12.6–120)	54.7 ± 25.5 (12.1–121)	43.4 ± 10.8 (29.0–60.5)
Thomaides et al. (2005) †	12	57.9 ± 10.1 (45–75)	–	–	H & Y: < 3	Yes	12	58.4 ± 9.2 (44–72)	Scintigraphy	Until complete gastric emptying (15 min intervals)	Semi-solid (overnight fast)	90.6 ± 3.9	–	46.2 ± 0.7
Krygowska-Wajs et al. (2009) *	fPD: 10 sPD: 20	fPD: 59.0 ± 8.2 (49–77) sPD: 57.58 ± 10 (38–73)	fPD: – sPD: 15	fPD: – sPD: 64.3 ± 9.5 (43–77)	5 ± 3.6 years H & Y: 1–2.5, 8.4 ± 5.2 years H & Y: 2 (m), 4.5 ± 3.0 years; H & Y: 3–4 (m/s), 10.1 ± 3.6 years	No	15	59.5 ± 9.7 (48–76)	Scintigraphy	92 min	Solid (8-h fast)	fPD: 58 ± 25 (23–92) sPD: 38.4 ± 23.9 (7–68)	fPD: – sPD: 55.8 ± 24.7 (11–92)	38 (29–46)
Trahair et al. (2016) †	21	64.2 ± 1.6 (51–77)	–	–	H & Y: 1.4 ± 0.1, 6.3 ± 0.9 years	Yes: 18 No: 3	21	64.8 ± 1.8	Scintigraphy	3 h (15 min intervals)	Liquid (12-h fast)	106 ± 9.1	–	43–157
Bestetti et al. (2017) *	–	–	51	67.8 ± 9.9	H & Y: 3.35 ± 3.14, 10.7 ± 7.0 years	No	20	53 ± 13	Scintigraphy	45/60 min	Liquid (12-h fast)	–	52.3 ± 45.5	25 ± 7 (16–38)
Knudsen et al. (2017a) *	32	64.7 ± 7.1	–	–	H & Y: 2.1 ± 0.45; 4.4 ± 4 years	Yes	26	65.4 ± 6.2	Scintigraphy	3 h (30 min intervals)	Solid (overnight fast)	50.6 ± 11	–	48.2 ± 16
Goetze et al. (2005) *	21	61.6 ± 12.4	15	66.3 ± 9.0	H & Y: 0–2 (m), 5.2 ± 4.6; H & Y: 2.5–5 (m/s), 8.2 ± 5.0	Yes	22	63.6 ± 6.8 (48–71)	$^{13}CO_2$ breath test	4 h (15 min intervals)	Solid (overnight fast)	149.7 ± 28.2	196.0 ± 44.8	107.3 ± 9.9
Muller et al. (2006) *	–	–	11	58 ± 10 (40–73)	H & Y: 2.54 ± 0.35 (2–3)	No	22	63.6 ± 6.8 (48–71)	$^{13}CO_2$ breath test	4 h (10/ 20 min intervals)	Solid (overnight fast)	–	123 ± 43	107.3 ± 9.9

(continued on next page)

Table 3 (*continued*)

	PD – mild		PD – moderate/severe		H & Y scores/ disease duration	LD therapy discon-tinued?	Controls		Technique	Investigation period	Meal	$GET_{1/2}$ (min)		
	n	age (years)	n	age (years)			n	age (years)				PD – mild	PD – m/s	Controls
Goetze et al. (2006) *	44	–	36	–	H & Y: 1–2.5 (m)	Yes	40	61 (42–89)	$^{13}CO_2$ breath test	Liquid: 3 h (10 min intervals) Solid: 4 h (15 min intervals)	Liquid and solid (overnight fast)	Liquid: 140 ± 67	Liquid: 113 ± 29	Liquid: 81–131
					H & Y: 3–5 (m/s)							Solid: 149 ± 28	Solid: 191 ± 44	Solid: 87–128
Tanaka et al. (2009) #	20 non-RFs	68.0 (55–80)	20 RFs	66.5 (42–86)	H & Y: 3–4	Yes	20	69 (63–73)	$^{13}CO_2$ breath test	4 h (10 min intervals)	Liquid (12-h fast)	non-RFs: 128.4 (96–184.2)	RFs: 125.4 (103.8–198)	86.4 (78–98.4)
Tanaka et al. (2011) #	20	70.5 (54–82)	40	67.0 (42–86)	H & Y: 1–2 (m), 0.9 years (0.3–2.5); H & Y: 3–4 (m/s), 6.0 years (3.0–31.0)	m: no LD m/s: yes	20	69.0 (63–73)	$^{13}CO_2$ breath test	4 h (10 min intervals)	Liquid (12-h fast)	122.4 (78–98.4)	125.4 (96–210)	86.0 (78–98)
Epprecht et al. (2015) *	16	66.3 ± 8.5	–	–	H & Y: 2	Yes	11	61.1 ± 7.1	$^{13}CO_2$ breath test	4 h (15 min intervals)	Solid (overnight fast)	215.2 ± 96.9	–	196.2 ± 53.0
Nord et al. (2017) #	14	66.9 ± 7.5 (52–79)	12	65.8 ± 6.5 (57–78)	m: 6–16 years m/s: 7–16 years	No	50	44 (24–59)	$^{13}CO_2$ breath test	4 h (15 min intervals)	Solid	105	78	72 (47–99)
Unger et al. (2011) *	Drug-naive (D-N)		Under therapy (UT)		D-N: 1.7 ± 0.86	D-N: –	20	47.6 ± 9.3	$^{13}CO_2$ breath test	15 min before meal, following 6 h (15 min intervals)	Solid (overnight fast)	D-N: 166 ± 32.4	UT: 203 ± 46.8	123.3 ± 16.6
	21	61.4 ± 11.1	18	63.5 ± 8.7	UT: 2 ± 0.93	UT: Yes								

which then should be fine-tuned as soon as more data become available.

3.6. Small intestine

Whereas dysphagia and gastroparesis are frequently discussed issues, to date only little attention had been given to small intestinal transit in PD patients. Data on small intestinal transit times (SITT), motility as well as the composition and volumes of small intestinal (SI) contents are limited. However, since the small intestine is typically the site where maximum absorption occurs and particularly levodopa absorption is restricted to the small intestine (Gundert-Remy et al., 1983), SI dynamics are essential for both a better understanding of levodopa pharmacokinetics and the design of a bio-predictive *in vitro* model.

3.6.1. Small intestinal transit and motility

As indicated before, very recently Su et al. published results from their study using a WMC and the lactulose breath testing (LBT) to examine and evaluate gastrointestinal symptoms *e.g.* prolonged gastric emptying and SITT as well as the prevalence of small intestinal bacteria overgrowth (SIBO) (Su et al., 2017b), an often reported symptom in PD patients (Pfeiffer, 2013; Fasano et al., 2013) that in some studies has been associated with severe motor symptoms (Pellegrini et al., 2016). The Su study involved PD patients with a median H & Y score of 3 (no control group) that, after an overnight fast, ingested a light meal (260 kcal SmartBar) together with 120 mL water. Immediately following food ingestion, the patients were equipped with a data receiver and swallowed the WMC together with another 50 mL of water. Subsequently, patients were advised to fast for 6 h and then were administered a regular meal. Subsequent to that they could continue with their daily diet and routine. After excretion of the WMC, data from the receiver were analyzed. In 35% of the subjects, an increase in GET > 5 h and in 20% a prolonged SITT > 6 h was observed. The authors identified a significant relationship between the symptom of bloating and elongated SI passage. SIBO was evaluated using the LBT where subjects ingested a lactulose solution followed by analyzing the exhaled breath in 15 min intervals. 34% of PD patients exhibited SIBO, but, interestingly, no significant correlation between SIBO and intestinal dysfunction could be established. Dutkiewicz et al. studied SITT in ten PD patients with a disease duration of 6 to 12 years and ten healthy age- and sex-matched controls (Dutkiewicz et al., 2015). Following an overnight fast, subjects were advised to swallow a customized, non-disintegrating ^{99m}Tc-labeled capsule in the early morning (fluid volume not indicated). GI passage of the capsule was followed using single photon emission computed tomography (SPECT) and computed tomography (CT) and images were taken after 2, 4, 6, 8 and 24 h. During the day, the patients received regular meals. In 5 of 10 controls, the capsule had appeared in the small intestine after 2 h and in all controls had left the small intestine within 4 h. However, in only 2 of 10 PD patients the capsule could be visualized in the small intestine after 2 h and in 7 of 10 patients stayed in the small intestine for longer than 4 h. In one patient the capsule had not even left the small intestine at the end of the experiment (24 h). The authors thus concluded that oro-cecal passage, including SITT in PD patients is prolonged. PD patients (disease duration 1–16 years, H & Y stage 1–5) and a young control group were also cohorts of a study performed by Davies et al. who used the LBT to evaluate oro-cecal transit time (OCTT) in the elderly (Davies et al., 1996). They administered 20 mL of a 50% lactulose solution to fasting subjects and recorded oro-cecal passage represented by the time between ingestion of the lactulose solution and a sharp increase in breath hydrogen caused by colonic (cecal) lactulose digestion. OCTT, which, since a solution was administered to fasted patients, is most likely not much different from SITT increased with increasing age of the subjects and PD resulted in an additional delay compared to age- and sex-matched controls (10 of 15 PD patients had a OCTT of > 180 min whereas a OCTT of >180 min was observed in only 3 of 15 age- and sex-matched controls). However, the authors found no correlation between intestinal passage, disease duration and severity. A similar study design was used by Haboubi et al. who also reported an extended oro-cecal passage in PD patients (no information on the severity of the disease was provided) in comparison to an age-matched control group (194 min *vs.* 151 min) (Haboubi et al., 1988).

Similar to SITT, SI motility in PD is sparsely investigated. Dilatation of the small bowel has been reported (Lewitan et al., 1951), as have abnormalities in small-intestine motor patterns, as shown in a manometric study published by Bozeman et al. (1990). The cited study was carried out in both fasted and fed state PD patients (no information about disease severity was given) and controls. After an overnight fast and intestinal placement of a manometric tube, motility was recorded for 4–5 h. Subsequently, the subjects ingested a 500 kcal meal and were monitored for additional 2 h. In both prandial states intestinal motility was abnormal compared to healthy patients. Phenomena such as prolongation of the MMC or the occurrence of retrograde contractions could be noted. Furthermore, in an EGEG study published by Kaneoke et al. a slight, but non-significant difference in frequencies of intestinal waves when comparing fed and fasted state could be seen when screening SI motility in PD patients (H & Y stage 3) and controls (Kaneoke et al., 1995).

Current literature data on SITT and SI motility represent just a few snapshots obtained in studies performed in rather small cohorts and with different techniques. Results thus also strongly relate to the study design and can everything but provide a reliable overview of relevant physiological changes that present with different stages of PD. It seems that in PD patients, SI motility is impaired and SITT is delayed, but it is not yet clear, if these effects will become more pronounced with increasing severity of the disease. As already discussed for gastric emptying and motility the use of the WMC and modern visualization techniques in properly designed prospective studies would also be extremely helpful for getting a better understanding of SI motility and SITT in PD patients.

3.6.2. Small intestinal contents

Similar to the situation described for gastric contents, there is a lack in studies focusing on screening volumes, properties and composition of SI contents/fluids in PD patients. Since orally administered drugs and particularly levodopa are absorbed in the small intestine, one wants to ensure that the drug can freely dissolve in the small intestinal environment. Therefore future studies should focus on filling these knowledge gaps. As for the stomach, the prospective use of the WMC will help to obtain more detailed information on small intestinal pH conditions. However, it will also be necessary to properly sample fluids from fasted and fed PD patients in different stages to get an idea of how typical nutrition habits in PD affect the GI environment and also to screen, if there is a difference in the SI environment of PD patients with and without SIBO.

3.7. Colon

Constipation, caused by reduced colonic motility, is probably the most prominent gastrointestinal symptom in PD which is already present in early disease stages and often develops as the first non-motor symptom in prodromal PD (Abbott et al., 2001; Adams-Carr et al., 2016). Results from several literature reviews (Fasano et al., 2015; Knudsen et al., 2017b; Stirpe et al., 2016) reveal that the prevalence of constipation in PD patients varies widely from 4 to 70% depending on the definition and criteria of constipation, enrolled study participants and examination methods. Due to the fact that levodopa is mostly absorbed in the proximal small intestine *via* large neural amino acid (LNAA) transporters (Nyholm and Lennernas, 2008; Gundert-Remy et al., 1983; Freitas et al., 2016) whereas its colonic absorption is very limited, colonic conditions in PD are not of much interest when designing an *in vitro* dissolution test model for a modified release dosage

form containing levodopa. However, a PD patient often receives several other medications that may include MR formulations containing drugs that are absorbed in the colon. It also should be noted that very recently, a new levodopa ester-conjugate termed XP21279 (XenoPort Inc.) representing a novel concept to overcome the limitations of small intestinal absorption was introduced (Freitas et al., 2016). This prodrug is absorbed by nutritional transporters which are expressed throughout the entire gastrointestinal tract, including the colon. Furthermore, other modified release dosage forms comprising antiparkinson compounds (*e.g.* pramipexole and ropinirole) that might also be absorbed in the colon are available for PD therapy. To ensure the development and selection of safe and efficient drug formulations for PD patients, colonic conditions also need to be addressed in a bio-predictive *in vitro* test model.

3.7.1. Colonic transit time and motility

A common technique for assessing colonic transit time (CTT) is the use of radio opaque markers (ROMs) (Knudsen et al., 2017a; Sakakibara et al., 2003; Jost et al., 1994). In the respective studies subjects are advised to ingest a capsule containing a certain number of ROMs, preferably each morning following an overnight fast for several consecutive days. 24 h after the last ingestion the retained number of ingested ROMs is visualized on an abdominal x-ray and subsequently used to calculate CTT (Knudsen et al., 2017a, 2017b; Sakakibara et al., 2003). Knudsen et al. recently summarized findings from studies using this examination method in a review article (Knudsen et al., 2017b). They state for instance that Sakakibara et al. had reported a significantly prolonged CTT of 82.4 h for PD patients compared to 39.0 h in control subjects (Sakakibara et al., 2003) and that Jost et al. had also determined an increase in CTT in PD patients (Jost et al., 1994). Knudsen et al. also conducted a study in 32 PD patients (mean age 64.7 ± 7.1; H & Y 1–3) by themselves (Knudsen et al., 2017a). They investigated CTT with ROMs and reported an average transit time of 91 ± 33 h in PD patients *vs.* 48 ± 19 h in controls.

In a recently published study by Su et al. a WMC was used to examine CTT in PD patients (Su et al., 2016). The study design was consistent to that applied to evaluate SITT and which was described earlier (Su et al., 2017b). The authors had set a CTT limit of 59 h to distinguish between normal and slow transit constipation. 33 of 53 (62%) PD patients participating in the study exhibited a prolonged colonic transit (>59 h) with a median of 84.5 h, whereas 20 of 53 (38%) PD patients showed a normal transit (<59 h, median 43 h).

As already stated for gastric and small intestinal data, the current literature data on CTT is incomplete and also strongly related to the technique applied to determine colonic transit. However, the data available to date indicate that prolonged colonic transit is a typical symptom in PD patients. First experiments screening CTT with a WMC have recently been published and it is likely that this technique will contribute to a better understanding of CTT in the near future.

3.7.2. Colonic contents

Whereas next to no data are available on the composition of gastric and small intestinal contents in fasted and fed PD patients, composition and properties of (upper) colonic contents in PD patients have not been screened to date. This is not surprising, since also very few information on upper colonic content properties is available for healthy patients. Thus, it is currently not possible to discuss any trends or to even speculate about differences between healthy patients and PD patients. However even though the colon might not be a site of essential interest when discussing drug release from oral levodopa formulations and also might play a minor role for many other orally administered dosage forms, composition and properties of the contents of the upper colon can affect drug release from a variety of oral MR formulations that might be relevant for PD patients as well and thus should also get attention when developing PD-specific *in vitro* drug release models.

4. Conclusion

The aim of the present review was to collect a reliable and state of the art set of PD-specific GI parameters that will allow for the development of a PD-specific *in vitro* drug release model for orally administered medications that enables to simulate the GI passage of orally administered (levodopa) formulations in early and late stage PD patients and to predict *in vivo* drug release in these patients. GI conditions in PD have received more and more attention over the last decades. To better understand disease pathophysiology, various studies using a number of diagnostic tools to detect abnormalities of structure and function of the GI tract have been performed. However, even though there is quite a reasonable set of data available on parameters such as salivary secretion, oropharyngeal and esophageal passage and gastric emptying that can be directly implemented into a patient-specific *in vitro* test model, it became clear, that there is still a big gap in knowledge that would be required for addressing all parameters that might be relevant for drug release during a GI passage in a PD patient. The latter is particularly true for contractile motility patterns and pressures in the entire GI tract as well as for SITT. Last but not least, to date next to no attention had been given to potential disease-related changes in GI fluid composition. However a number of new or improved non-invasive diagnostic options, such as for instance the WMC and MRI offer the chance to further elucidate essential GI parameters and hold potential for better understanding the disease, but also for being able to design a bio-predictive *in vitro* drug release model for PD patients at different disease stages.

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5.4 Development and Validation of a Robust and Efficient HPLC Method for the Simultaneous Quantification of Levodopa, Carbidopa, Benserazide and Entacapone in Complex Matrices

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Die medikamentöse Therapie der Parkinson-Krankheit umfasst u. a. eine Vielzahl an unterschiedlichen Levodopa-Kombinationspräparaten. Hierzu gehören Zweifachkombinationen bestehend aus Levodopa und einem Dopa-Decarboxylase-Inhibitor (Carbidopa oder Benserazid) sowie der Dreifachkombination bestehend aus den Wirkstoffen Levodopa, Carbidopa und dem Catechol-O-Methyltransferase-Inhibitor Entacapon. Ein simultanes und vergleichendes Screening der verschiedenen Levodopa-Formulierungen, beispielsweise hinsichtlich des In-vitro-Freisetzungsverhaltens, wurde bislang durch die Abwesenheit einer geeigneten flüssigchromatographischen Methode erschwert. Diese Veröffentlichung beschreibt die Entwicklung und Validierung einer HPLC-Methode zur simultanen Quantifizierung der Wirkstoffe Levodopa, Carbidopa, Benserazid und Entacapon. Die entwickelte Methode sollte für die Quantifizierung von Proben aus In-vitro-Freisetzungsexperimenten mit Levodopa-haltigen Fertigarzneimitteln Anwendung finden, die unter kompendialen und biorelevanten Testbedingungen durchgeführt werden. Eine schnelle und robuste Methode für die Trennung und Quantifizierung der vier Arzneistoffe wurde entwickelt und nach offiziellen Leitlinien validiert. Die in dieser Arbeit vorgestellte HPLC-Methode kann sowohl in der Formulierungsentwicklung, als auch im detaillierten Freisetzungsscreening von neuartigen sowie etablierten Levodopa-Formulierungen eingesetzt werden.

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Eigene Leistungen:
Erarbeitung der Fragestellung, Planung und Durchführung der Methodenentwicklung und Validierung, Konzeption und Erstellung des Manuskripts

Prof. Dr. Sandra Klein:
Erarbeitung der Fragestellung, Diskussion der Ergebnisse, Diskussion und Korrektur des Manuskripts

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Development and Validation of a Robust and Efficient HPLC Method for the Simultaneous Quantification of Levodopa, Carbidopa, Benserazide and Entacapone in Complex Matrices

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ABSTRACT - Purpose: A variety of fixed-dose combination products is used in the therapy of Parkinson Disease. However, to date a proper analytical method applicable for comparative screening of different antiparkinson products was not available. The objective of the present work was thus to develop and validate an analytical method for the simultaneous quantification of levodopa, carbidopa, benserazide and entacapone. The method should be applicable for quantifying samples from drug release experiments with marketed products and prototype formulations performed under compendial and biorelevant test conditions. **Methods:** A fast and robust method applicable for separation and quantification of the four compounds was developed and validated according to International Conference on Harmonization guidelines. Method validation covered applicability to a wide concentration range of all compounds and peak separation in complex sample matrices such as biorelevant dissolution media. **Results:** The compounds were successfully separated by using a gradient elution method on an endcapped LiChrospher 100 RP-18 (250 x 4.6 mm, 5 µm) column coupled with a LiChrospher 100 RP-18 precolumn (4 x 4 mm, 5 µm) at a column temperature of 35.0 °C and a flow rate of 1.50 mL/min. The injection volume was 30 µL and the detection wavelengths were 280 and 210 nm, respectively. For all drug/media combinations the method was linear ($r^2 > 0.999$) for a concentration range corresponding to 1.25 - 125 % label claim (i.e. 200 mg levodopa/entacapone and 50 mg carbidopa/benserazide) released. All other validation parameters were in the specified limits over the same concentration range. **Conclusion:** The new method allows for robust and fast separation of levodopa, carbidopa, benserazide and entacapone without any interference caused by excipients or ingredients of compendial and biorelevant dissolution media and thus presents a valuable tool in both formulation development and *in vitro* drug release screening of numerous fixed-dose combinations of antiparkinson drugs.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative brain disorder in which neurons of the substantia nigra progressively degenerate. As a result, the amount of dopamine available for neurotransmission in the corpus striatum is lowered. The biochemical imbalance manifests with typical clinical symptoms that include resting tremor, rigidity and bradykinesia amongst others. PD develops gradually and while tremor may be the most well-known sign of PD, the disorder also commonly causes stiffness or slowing of movement.

PD can't be cured, but medications can markedly improve symptoms. Since almost 50 years, the most effective mode of symptomatic treatment has been the administration of the l-isomer of 3,4-dihydroxyphenylalanine (levodopa), a dopamine precursor (1). To inhibit the extracerebral decarboxylation of levodopa, allowing more levodopa to cross the blood-brain barrier to target the striatal dopamine receptors, the drug is typically administered with a peripheral dopa-decarboxylase inhibitor such as carbidopa or benserazide. Since many years the combination of levodopa and a dopa-decarboxylase inhibitor (mostly carbidopa) is thus the primary standard of PD treatment. However, since quite some time, the levodopa/carbidopa combination can be complemented by the administration of entacapone, a selective and reversible inhibitor of the catechol-o-methyl transferase (COMT) which represents another principal levodopa-metabolizing enzyme (2).

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Combination of levodopa with peripheral dopa-decarboxylase and COMT inhibitors extends the elimination half-life and plasma area under the curve of levodopa without affecting the maximal plasma concentration of levodopa (C_{max}) or the time until an oral dose of levodopa reaches its peak plasma concentration (T_{max}). Clinically, these pharmacokinetic effects permit a reduction in the levodopa dose, an increase in periods when the medication is working and symptoms are controlled (“on” time) and, a decrease in periods with reduced mobility ("off" time) in patients that had started to develop motor fluctuations as result of variations in the individual's response to levodopa. Motor benefits can also be seen in stable PD patients. COMT inhibitors are thus an alternative to increasing levodopa doses or adding dopamine agonists to reduce "off" time and enhance motor function in fluctuating PD patients (3).

Today, a variety of dosage forms comprising fixed dose combinations of levodopa/carbidopa, levodopa/benserazide or levodopa/carbidopa/entacapone is on the market. Formulation approaches for these combinations range from immediate release (IR) formulations through delayed release (DR) to extended release (ER) formulations. The variety of products allows an individualized treatment of PD patients in different disease states. Nevertheless, after several years of smooth and stable response to individualized levodopa treatment, most of the patients develop motor fluctuations manifested by “on” and “off” phases (4). Morning akinesia is the most common motor fluctuation in PD (5) since even the administration of modern fixed dose DR and ER combinations cannot prevent a dopaminergic nocturnal decline with insufficient nighttime storage or refresh the dopaminergic system during nighttime and sleep.

A novel dosage form taken at bedtime and providing drug release within the early morning hours might be of essential benefit for patients suffering from advanced PD with pronounced morning akinesia. Based on these considerations we are currently developing an oral formulation intended to provide a fixed dose combination of levodopa, a dopa-decarboxylase inhibitor and potentially also a COMT inhibitor in the early morning hours. To determine the most appropriate release pattern, prototypes of the novel formulation will be subjected to intensive *in vitro* screening applying novel patient-specific *in vitro* test models. *In vitro* drug release profiles of the prototypes will also be compared with those from currently marketed fixed dose combination products including levodopa/carbidopa (e.g. Nacom, MSD Sharp & Dohme GmbH, Germany), levodopa/benserazide (e.g. Madopar, Roche Pharma AG, Switzerland) and levodopa/carbidopa/entacapone (e.g. Stalevo, Orion Corporation, Finland).

When designing the analytical protocol for *in vitro* testing and drug quantification, we wanted to implement an analytical method allowing for the simultaneous quantification of all drugs included in the study. However, when screening the relevant literature, it quickly became clear that even though there is a multitude of valuable methods available for the simultaneous detection of levodopa and dopa-decarboxylase inhibitors, e.g. (6-8), to date no method for the simultaneous detection of levodopa, carbidopa, benserazide and entacapone has been described in the literature. A couple of methods enabling the detection of levodopa, carbidopa and entacapone in a single HPLC run have been described in the recent past (9-11) and were investigated for our purpose.

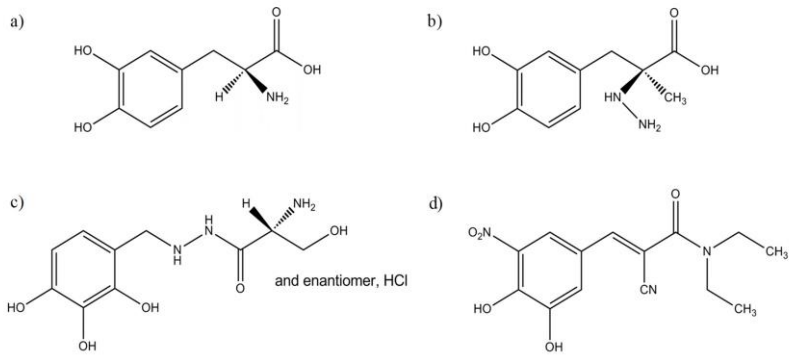

Figure 1: Chemical structures of a) levodopa, b) carbidopa, c) benserazide hydrochloride and d) entacapone

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However, it turned out that without modification the respective methods were not applicable for also properly detecting benserazide (9-10). Moreover, one of the methods turned out to not even being applicable for the detecting the drug combination given in the title of the manuscript (11).

The purpose of the present study was thus to develop and validate a robust and efficient HPLC method for the simultaneous quantification of levodopa, carbidopa, benserazide (in the form of its hydrochloride salt) and entacapone (figure 1). The method should be applicable for quantifying samples from drug release experiments performed with prototypes and marketed fixed dose combinations under compendial test conditions as well as from patient-specific *in vitro* experiments comprising a number of biorelevant dissolution media (12). The method would thus need to be robust towards both matrix effects caused by formulation excipients and ingredients of the dissolution media.

MATERIALS AND METHODS

Standards

All active pharmaceutical ingredients (API) used for method development and validation were of analytical grade. Levodopa (batch # SLBB4239V) was purchased from Sigma-Aldrich Corporation (St. Louis, USA). Carbidopa (batch # FC012961550), benserazide hydrochloride (batch # FB181711550) and entacapone (batch # FE226781551) were purchased from Carbosynth Limited (Compton, UK). All APIs were obtained with a certificate of analysis confirming that they met product specification and had a purity > 99 %. Table 1 lists the physicochemical properties of the four APIs that might affect separation. Preferably, the respective data were taken from relevant literature. However, where no or no reliable data were available, the respective structure-based properties where predicted using an online tool (13).

Drug formulations

Four different marketed dosage forms representing the different drug combinations applied in PD treatment were selected to screen method robustness towards matrix effects caused by typical formulation excipients. The selected dosage forms included Madopar Depot (Roche Pharma AG, Switzerland; batch # M2379B01), Nacom (MSD Sharp & Dohme GmbH, Germany; batch # E000146) and Stalevo (Orion Corporation, Finland; batch # 1648258) which were obtained from the hospital pharmacy of the university medicine in Greifswald. Madopar DR (Roche Pharma AG, Switzerland; batch # B4012B72) was imported from Switzerland via a local pharmacy.

Chemicals

Sodium dihydrogen phosphate dihydrate was purchased from Merck KGaA (Darmstadt, Germany), di-sodium hydrogen phosphate dihydrate was obtained from Fagron GmbH & Co. KG (Barsbüttel, Germany) and hydrochloric acid was from AppliChem GmbH (Darmstadt, Germany). HPLC gradient grade acetonitrile and orthophosphoric acid for HPLC were purchased from VWR Chemicals (Fontenay-sous-Bois, France). Water for mobile phases and sample preparation was prepared in house applying a Milli-Q reference water purification system (Merck KGaA) and filtered through a 0.22 µm polyvinylidene fluoride (PVDF) filter before use. All other compounds were of analytical grade and were purchased commercially.

Equipment

Method development and validation were performed on a Waters high performance liquid chromatography (HPLC) system (Waters Corporation, Milford, USA), consisting of a 1525 series binary pump, an 2707 automatic injector equipped with a 100 µL loop, a thermostated 1500 column compartment and an 2489 UV/Visible detector. System control, data acquisition and integration were accomplished with the Breeze™ 2 software (Waters Corporation).

Chromatographic conditions

Chromatographic separation of the analytes was achieved on an endcapped LiChrospher 100 RP-18 column (Merck KGaA, Darmstadt, Germany) with the dimensions 250 x 4.6 mm and 5 µm particle size. The column was coupled with a LiChrospher 100 RP-18 precolumn of 4 x 4 mm and 5 µm particle size (Merck KGaA). HPLC analyses were carried out by applying the gradient conditions as stated in table 2. Solvent A consisted of a 30 mM phosphate buffer pH 2.50 (20 mM sodium dihydrogen phosphate dihydrate and 10 mM di-sodium hydrogen phosphate dihydrate). The pH of the solvent was adjusted with orthophosphoric acid. Solvent B was composed of a mixture of water that prior to mixing had been adjusted to pH 2.50 with orthophosphoric acid and acetonitrile (50:50, v/v).

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Table 1: Physicochemical properties of levodopa, dopa-decarboxylase and COMT inhibitors (the corresponding literature sources are given in brackets)

	levodopa	carbidopa	benserazide	entacapone
molecular weight (g/mol)	197.2 (14)	226.2 (14)	293.7 (14)	305.3 (14)
logP	- 2.4 (15)	- 0.1 (15)	-1.90 (13)	1.63 (13)
pK_a	2.3 (15)	3.59 (13)	8.66 (13)	4.5 (15)

Table 2: HPLC gradient conditions

Time (min)	Flow rate (mL/min)	% A	% B
0	1.50	100	0
5	1.50	100	0
10	1.50	0	100
13	1.50	0	100
14	1.50	100	0
17	1.50	100	0

All eluents were filtered through a 0.22 µm polyethersulfon filter (Millipore Express plus, Merck KGaA, Darmstadt, Germany) before use. Experiments were conducted at a flow rate of 1.50 mL/min and the column temperature was maintained at 35.0 °C. The injection volume was 30 µL. Prior to starting a set of experiments the system was equilibrated with solvent A for 30 min. Levodopa, carbidopa and entacapone were detected at a wavelength of 280 nm, benserazide was monitored at 210 nm and 270 nm, respectively. Two different wavelengths were chosen for the detection of benserazide because of the fact that, whereas in some publications benserazide is detected at about 270 nm (7), in the official method of the European Pharmacopoeia benserazide is monitored at 210 nm (14). Our objective was thus to determine which wavelength is the most appropriate one for our purpose.

Preparation of stock solutions and dilutions

The decision for setting the concentration range for the standard dilutions was based on the intended use of the novel HPLC method, i.e. the quantification of antiparkinson drugs in *in vitro* drug release experiments. For the drug release experiments the following marketed dosage formulations were chosen: Madopar Depot (levodopa / benserazide 200 mg / 50 mg), Madopar DR (levodopa / benserazide 200 mg / 50 mg), Nacom (levodopa / carbidopa 200 mg / 50 mg) and Stalevo (levodopa / carbidopa / entacapone 200 mg / 50 mg / 200 mg). The dose strength of each of these dosage forms represents the highest single API dose available in fixed dose combination products, i.e. 200 mg levodopa and entacapone and 50 mg carbidopa and benserazide, respectively. According to the intended use of the method concentrations of the standard stock solutions referred to 125 % of the respective API dose in the formulation dissolved in a media volume of 900 mL which represents a typical volume of dissolution medium used in standard dissolution experiments in USP apparatus 1 or 2 (Basket or Paddle apparatus) (16). Consequently, stock solutions of levodopa, carbidopa and benserazide hydrochloride were prepared by dissolving the corresponding standards (pure APIs) in 0.1 N hydrochloric acid resulting in concentrations of 277.8 µg/mL, 69.4 µg/mL and 79.2 µg/mL (benserazide hydrochloride), respectively. Due to the limited aqueous solubility of entacapone, the entacapone stock solution was obtained by first dissolving 27.78 mg of the drug in 10 mL acetonitrile and then adding water to obtain 100.0 mL stock solution with a concentration of 277.8 µg/mL. Sets of standard dilutions covering a given "working range" for method validation were prepared by diluting the stock solutions with the corresponding solvents. All standard dilutions ("samples") were filtered through a 0.45 µm cellulose acetate membrane filter before injection.

Analytical method validation

Validation of the novel HPLC method was performed according to International Conference on Harmonization (ICH) and United States Pharmacopoeia (USP) validation guidelines (16-17). Parameters examined included linearity and range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), specificity, system suitability and robustness.

The novel HPLC method was mainly developed for quantifying drug load and drug release of ER fixed dose combinations with high drug loads. However, as possible the method should also be applicable in routine testing for instance for testing IR formulations containing

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combinations with lower doses of the same APIs. For the latter objective, the method should thus enable to appropriately quantify the APIs over a wide concentration range. Based on these considerations two separate concentration ranges were selected for the validation procedure. Accordingly, the validated concentration ranges for levodopa, carbidopa and entacapone were 1.25 to 12.5 % (0.69 to 6.94 µg/mL for carbidopa and 2.78 – 27.78 µg/mL for levodopa and entacapone) and 12.5 to 125 % (6.94 to 69.4 µg/mL for carbidopa and 27.78 – 277.8 µg/mL for levodopa and entacapone) of the maximum expected analyte concentration represented by complete dissolution of the highest single dose of each drug. Based on the significantly lower intensity of ultraviolet light absorption of benserazide at 270 nm, the validated concentration ranges for both wavelengths (210 nm and 270 nm) were 2.50 to 12.5 % (1.58 to 7.92 µg/mL) and 12.5 to 125 % (7.92 to 79.2 µg/mL) of the target concentration, respectively. The sets of dilutions for each API were examined for a linear relationship by plotting the analyte peak areas of 10 different samples versus the corresponding concentrations followed by least square linear regression and calculation of the slope, intercept and coefficient of determination. Three separate series of calibration standards for each calibration range were prepared to establish linearity.

Accuracy, expressed as mean absolute recovery and percent relative standard deviation (% RSD), for all analytes was assessed in triplicate for each concentration of the specified ranges.

The precision of the novel method expressed as repeatability (intraday) and intermediate precision (interday) was screened by preparing six individual samples of the lowest (1.25 % or 2.50 %, respectively), medium (12.50 %) and highest (125 %) concentration of the working range for each compound. To evaluate interday precision standards were prepared in the same way and analyzed on three different days. Repeatability and intermediate precision were assessed via absolute recovery and % RSD of the calculated concentrations.

The LOD is a characteristic value for the sensitivity of the method, at which the respective compound is just measurable, whereas the LOQ is the lowest concentration with acceptable linearity, accuracy and precision. The LOD was determined based on signal-to-noise ratio of 3:1, and the lowest concentration of the working range 1.25 % or 2.50 % to 12.5 % were set as the LOQ for levodopa, carbidopa, entacapone (1.25 %) and benserazide (2.50 %).

Specificity which is an essential part of method validation was assessed as follows: First, a set of standard solutions (API reference material dissolved in a simple solvent or dissolution medium) of the four analytes were prepared with the three possible API combinations available in marketed levodopa fixed-dose combination products. Different standard solutions were prepared using simple solvents (e.g. acetonitrile/water) and the compendial and biorelevant media listed in table 3. Then, the impact of excipients used in the manufacture of the selected marketed dosage forms on the proper assessment of the API peaks was screened. For this purpose, single tablets/capsules of Madopar Depot, Madopar DR, Nacom and Stalevo were placed in separate containers containing 200 mL of one of the media listed in table 3 and the fluid was slightly agitated for one hour. Following sample analysis, the obtained chromatograms were checked for peak area and interference of excipients at the API retention times.

System suitability was examined by determination of tailing factor (T_f), retention factor (k), number of theoretical plates (N), height equivalent to the theoretical plate (HETP), resolution (R_s) of the respective analytes and the reproducibility of peak areas and retention times. In addition, calibration curves of the media listed in table 3 were established for the four analytes and checked for linearity.

To determine the reliability of the proposed HPLC method robustness was evaluated by varying different method parameters. Several parameters were considered critical factors for the analysis. The self-imposed parameter limits set in for these parameters were inspired by other publications (6-7, 18) and own experience. Parameters were studied as follows: The flow of the mobile phase (± 2 %), column temperature (± 5 °C), buffer strength of the aqueous component (± 5 mM) and pH value (± 0.25) for both mobile phase A and mobile phase B were investigated by injecting a series of dilutions with three individual standards for lowest, medium and highest concentrations (of the concentration range discussed in the previous section) of levodopa, carbidopa, benserazide and entacapone in triplicate. The robustness of the method was assessed by absolute mean recovery, % RSD of recovery (precision) and r^2 of the resulting calibration curves.

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Table 3: Media used to investigate specificity and system suitability

Buffers/compendial media
Simulated Gastric Fluid *sine pepsinum* (SGF*sp*) pH 1.2
Acetate buffer pH 4.5
Simulated Intestinal Fluid (SIF) pH 6.5
Simulated Colonic Fluid (SCoF) pH 5.8
Biorelevant media
Fasted State Simulated Intestinal Fluid (FaSSIF) V-1 pH 6.5
Fed State Simulated Intestinal Fluid (FeSSIF) V-1 pH 5.0

RESULTS

Method development and optimization

The novel separation method was targeted to demonstrate acceptable chromatographic performance and to be universally applicable on standard HPLC equipment. Following an initial literature research promising HPLC methods for the targeted analytes were selected and checked for their suitability. In the early stages of the present method development, chromatographic methods with isocratic elution were tested with a Zorbax Eclipse XDB-C18 column (250 x 4.6 mm, 5 µm; Agilent Technologies, Inc., Santa Clara, USA). Subsequently, to achieve better selectivity, an endcapped LiChrospher 100 RP-18 (250 x 4.6 mm, 5 µm) was used. To protect the column from potential contamination by components of biorelevant media to be used in future dissolution experiments (e.g. bile compounds, fat droplets etc.), a precolumn was installed. All cited literature methods comprise the use of a 250 mm column. This results in long run times or the need of higher flow rates and is thus time-consuming and cost-intensive. Since the objective was to develop a robust and effective method, in the next stage of method development, promising literature methods were transferred onto a shorter column (LiChrospher 100 RP-18, 125 x 4 mm, 5 µm) and screened for their applicability in detecting the four APIs of interest. However, results from these experiments clearly indicated that with a simple switch to a shorter column with the same or stationary phase, a proper analyte separation was not possible.

After these initial set of method screening it was clear that an isocratic separation of the analytes is hardly possible within a reasonable run time. This is a result of the different physico-chemical properties of the APIs given in table 1 and can be explained as follows: Benserazide HCl was detectable with a highly polar, aqueous mobile phase without addition of any organic solvents, whereas entacapone had a stronger affinity to the stationary phase and could only be properly eluted using an eluent containing a certain amount of acetonitrile. Consequently, a gradient method had to be applied. Based on the cited observations (data not shown), theoretically, the gradient separation had to start with a polar mobile phase switching to a more apolar eluent to cover all four compounds. Based on these considerations a phosphate buffer with a pH of 2.50 was chosen as solvent A, while a mixture of water (pH adjusted to 2.50) and acetonitrile (50:50, v/v) was selected as solvent B. Furthermore, the selected eluents were optimized with regard to solvent pH and ionic strength. Suitable injection volume and column temperature were selected and the initial gradient program was also stepwise modified. The final gradient method applying the gradient shown in table 2 combined with an injection volume of 30 µL and a column temperature of 35 °C, was found to be suitable for a good chromatographic separation and selected for subsequent method validation. Figure 2 illustrates a chromatogram of the four target APIs in a mixture of standard solutions of the analytes obtained by applying the final gradient method and using a wavelength of 280 nm for simultaneous UV detection.

Linearity

The linear relationship of analyte concentrations and peak areas is expressed by the coefficient of determination (r^2). Linearity for each of the four compounds could be shown for all calibration curves over the concentration ranges stated earlier since all r^2 values were above 0.999 (see table 4).

Accuracy, precision

Results for accuracy and precision are given in table 4. For every analyte a consistent and high absolute recovery and low % RSD within the acceptance limit of ± 5 % of 100 % drug recovery were demonstrated at all concentrations. The % RSD results for repeatability and intermediate precision were inferior to 2.68 % and thus regarded as acceptable.

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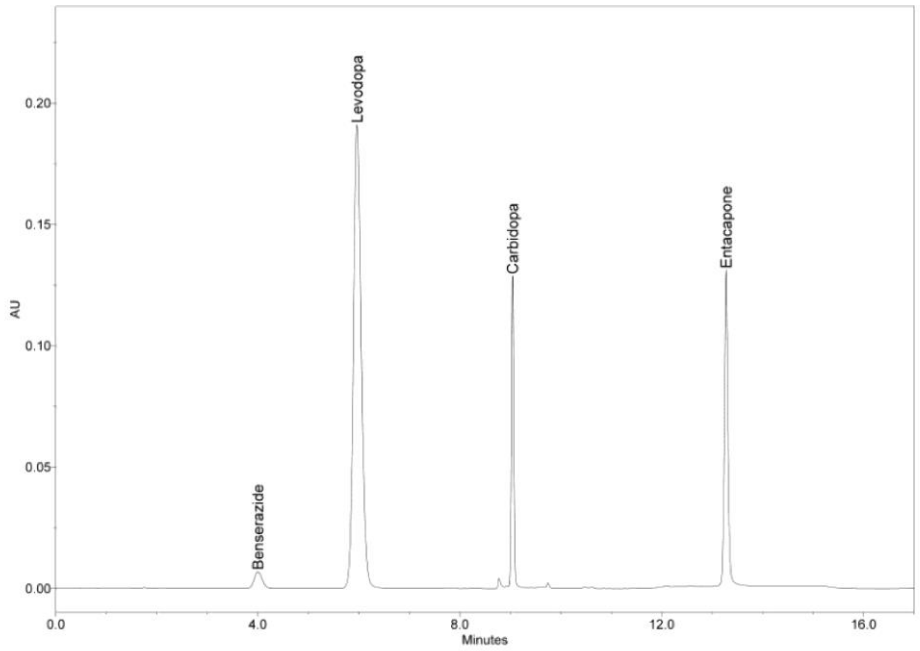

Figure 2: Chromatogram obtained by applying the gradient method given in table 2 and using a detection wavelength of 280 nm.

Specificity

The specificity of the new method was evaluated by first analyzing blank solvents/media and then samples containing a single drug or drug combinations in media of increasing complexity.

The method showed good chromatographic separation of the compounds in standard solutions (API reference material dissolved in a simple solvent), in solutions were the drugs were dissolved in more complex media (API + compendial or biorelevant dissolution medium, see table 3) as well as in samples of the disintegrated/dissolved marketed formulations (fixed dose combination product + compendial or biorelevant dissolution medium). No peak interference of the analytes with blank media, buffer components or excipients of the marketed dosage formulations was observed.

Figures 3 a) and b) show exemplary a chromatogram of a standard solution of a) levodopa and benserazide in 0.1 N HCl and of a mixture of standard solutions of b) levodopa and carbidopa in 0.1 N HCl and entacapone in water/acetonitrile, respectively. The retention times were 4.05 min for benserazide, 6.3 min for levodopa, 9.14 min for carbidopa and 13.15 min for entacapone. Resolution was above 2 in all cases. In figure 3 c) a chromatogram of a standard solution of levodopa and benserazide in FeSSIF V-1 pH 5.0, a biorelevant dissolution medium having a high osmolality and containing a large amount of bile compounds is shown. Figure 3 d) presents a chromatogram of levodopa, carbidopa and entacapone reference material in FeSSIF V-1 pH 5.0 and finally, figures 3 e-f show the chromatograms obtained from samples from a dissolution experiment of Madopar DR (figure 3 e) and Stalevo (figure 3 f) in FeSSIF V-1 pH 5.0. Chromatograms obtained from dissolution experiments of Madopar Depot and Nacom are not shown, but did also show no interferences.

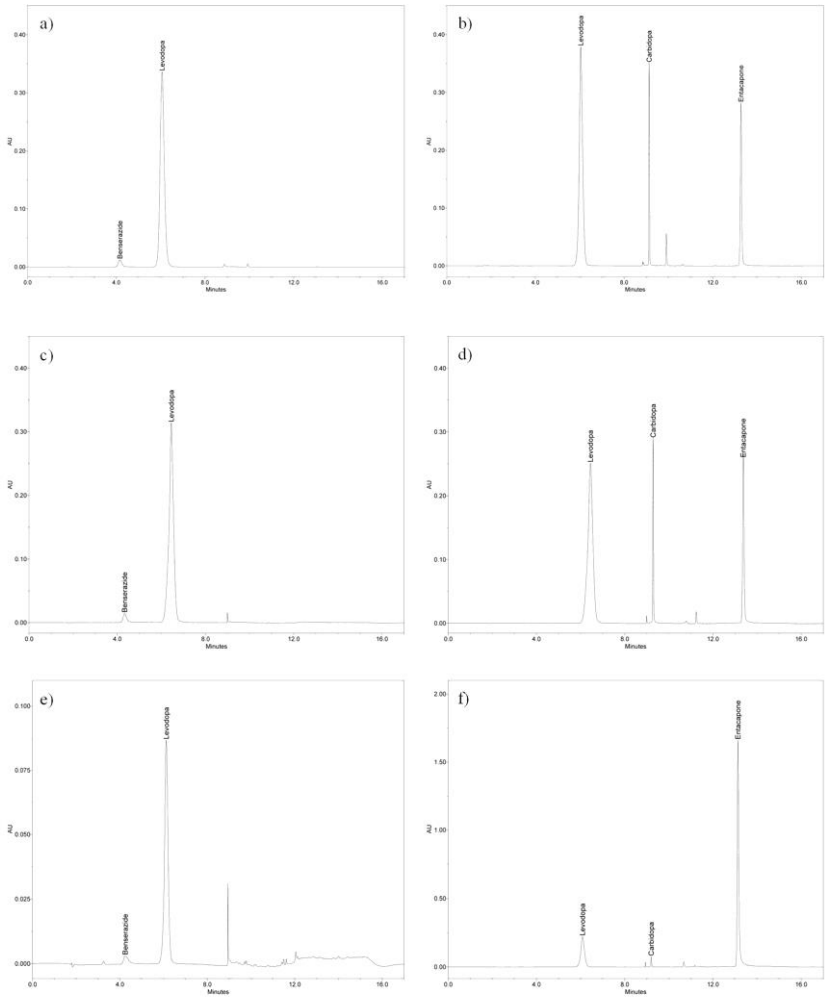

Figure 3: Chromatograms of a) standard solution of levodopa + benserazide in 0.1 N HCl, b) mixture of standard solutions of levodopa + carbidopa in 0.1 N HCl and entacapone in water/acetonitrile, c) standard solution of levodopa + benserazide in FeSSIF V-1 pH 5.0, d) mixture of standard solutions of levodopa + carbidopa and entacapone in FeSSIF V-1 pH 5.0, e) Madopar DR and f) Stalevo after immersion in FeSSIF V-1 pH 5.0 for one hour (280 nm).

System suitability

Primary parameters to evaluate system suitability such as symmetry factor, retention factor or number of theoretical plates were determined for the lowest, medium and highest concentrations for each analyte and are listed in table 4 (data only shown for the medium concentrations). Levodopa, carbidopa, benserazide and entacapone showed excellent peak symmetry. Moreover, the analyte peaks showed consistent low variability in peak areas and retention times.

Table 4: Results of HPLC method validation with the drugs ordered according to their retention times

Parameter	Values				
	Benserazide		Levodopa	Carbidopa	Entacapone
	210 nm	270 nm			
Retention time	4.05 min	4.05 min	6.3 min	9.14 min	13.15 min
Calibration ranges					
Range 1	1.58 – 7.92 µg/mL	1.58 – 7.92 µg/mL	2.78 – 27.78 µg/mL	0.69 – 6.94 µg/mL	2.78 – 27.78 µg/mL
Range 2	7.92 – 79.2 µg/mL	7.92 – 79.2 µg/mL	27.78 – 277.8 µg/mL	6.94 – 69.4 µg/mL	27.78 – 277.8 µg/mL
Linearity (r^2)					
Range 1	0.9998 / 0.9998 / 0.9996	0.9995 / 0.9998 / 0.9997	0.9996 / 0.9998 / 0.9995	0.9997 / 0.9993 / 0.9995	0.9999 / 0.9997 / 0.9999
Range 2	0.9998 / 0.9998 / 0.9990	0.9998 / 0.9997 / 0.9991	1.0000 / 0.9999 / 0.9999	0.9998 / 0.9999 / 0.9999	0.9997 / 0.9997 / 0.9996
Accuracy					
Mean absolute recovery					
Range 1	98.40 – 101.15 %	98.70 – 102.73 %	95.29 – 103.96 %	97.80 – 104.78 %	97.81 – 102.00 %
Range 2	95.84 – 102.90 %	95.76 – 103.10 %	96.12 – 100.88 %	98.59 – 103.76 %	97.85 – 101.75 %
% RSD					
Range 1	0.30 – 1.39 %	0.23 – 1.21 %	0.12 – 4.35 %	0.19 – 1.56 %	0.02 – 1.41%
Range 2	0.51 – 2.34 %	0.27 – 3.01 %	0.01 – 0.57 %	0.11 – 1.41 %	0.07 – 1.14 %
Precision					
Absolute recovery					
a) low concentration	97.43 – 102.34 %	97.39 – 103.58 %	98.26 – 100.92 %	98.38 – 101.90 %	95.12 – 102.50 %
b) medium concentration	98.41 – 101.63 %	97.68 – 101.85 %	99.47 – 100.48 %	98.81 – 101.29 %	98.26 – 101.86 %
c) high concentration	97.63 – 101.59 %	97.64 – 101.60 %	99.80 – 100.12 %	98.80 – 100.63 %	98.50 – 101.33 %
Repeatability (% RSD)					
a) low concentration	1.09 %	1.33 %	0.71 %	1.09 %	1.64 %
b) medium concentration	1.11 %	1.64 %	0.12 %	0.79 %	1.15 %
c) high concentration	0.66 %	0.66 %	0.10 %	0.25 %	0.97 %
Intermediate precision (% RSD)					
a) low concentration	1.09 % / 2.06 % / 1.29 %	1.33 % / 2.55 % / 1.76 %	0.71 % / 0.83 % / 0.96 %	1.09 % / 1.40 % / 1.06 %	1.64 % / 2.68 % / 1.92 %
b) medium concentration	1.11 % / 1.11 % / 1.01 %	1.64 % / 0.93 % / 1.02 %	0.12 % / 1.13 % / 1.06 %	0.79 % / 0.76 % / 0.83 %	1.15 % / 0.53 % / 0.95 %
c) high concentration	0.66 % / 1.55 % / 1.37 %	0.66 % / 1.55 % / 1.39 %	0.10 % / 0.13 % / 0.09 %	0.25 % / 0.68 % / 0.25 %	0.97 % / 0.76 % / 0.99 %
Limit of detection	0.03 ng/mL	3.95 ng/mL	27.8 ng/mL	13.8 ng/mL	0.01 ng/mL
Limit of quantification	1.58 µg/mL	1.58 µg/mL	2.78 µg/mL	0.69 µg/mL	2.78 µg/mL
System suitability					
Symmetry factor	1.08	1.09	0.91	1.14	1.06
Retention factor	1.39	1.39	2.73	3.96	6.82
Number of plates	3511	3553	9428	458244	219357
HETP	71.21 µm	70.36 µm	26.52 µm	0.55 µm	1.14 µm
Peak areas (% RSD)	0.91 %	1.75 %	0.20 %	0.81 %	0.28 %

Table 4. Continued…

Retention times (% RSD)	0.09 %	0.07 %	0.07 %	0.03 %	0.00 %
Robustness (abs. mean recovery)					
Flow rate					
a) 1.47 mL/min	99.10 % (1.39 % RSD)	98.95 % (0.82 % RSD)	100.00 % (0.09 % RSD)	99.07 % (1.95 % RSD)	98.97 % (0.15 % RSD)
b) 1.53 mL/min	98.96 % (0.38 % RSD)	98.42 % (0.49 % RSD)	99.76 % (0.03 % RSD)	99.20 % (1.41 % RSD)	99.15 % (0.17 % RSD)
Temperature					
a) 30 °C	98.46 % (0.85 % RSD)	98.55 % (0.94 % RSD)	99.40 % (0.04 % RSD)	101.92 % (1.60 % RSD)	100.50 % (0.44 % RSD)
b) 40 °C	99.21 % (0.48 % RSD)	99.21 % (0.30 % RSD)	100.37 % (0.23 % RSD)	99.20 % (0.61 % RSD)	100.24 % (0.40 % RSD)
Ionic strength					
a) 25 mM	99.41 % (1.17 % RSD)	100.10 % (0.63 % RSD)	100.92 % (1.01 % RSD)	101.80 % (1.55 % RSD)	99.65 % (0.88 % RSD)
b) 35 mM	99.09 % (0.50 % RSD)	99.40 % (0.37 % RSD)	99.51 % (0.73 % RSD)	100.30 % (0.72 % RSD)	99.40 % (0.94 % RSD)
pH value					
a) 2.25	101.16 % (0.92 % RSD)	101.12 % (1.91 % RSD)	100.20 % (0.62 % RSD)	100.89 % (0.63 % RSD)	98.83 % (1.89 % RSD)
b) 2.75	99.41 % (1.19 % RSD)	98.74 % (3.22 % RSD)	99.46 % (0.70 % RSD)	100.77 % (1.07 % RSD)	100.79 % (0.53 % RSD)

In all cases the coefficient of determination of the calibration curves prepared in the different media types applied in the study was above 0.999, indicating that the method was suitable for samples with simple or rather complex matrices.

Robustness

Data of the robustness study indicate that linearity, absolute mean recovery and precision of the developed method remain unaffected by small changes of critical method parameters. The corresponding results are given in table 4 (data only shown for medium concentrations). Variations of temperature, flow rate, ionic strength and pH value did not affect the recovered amount of the analytes. Absolute mean drug recovery for all compounds was within 95 – 105 % and the % RSD was below 4.32 %. The resulting calibration curves showed good linearity, i.e. the coefficients of determination were above 0.999 in all cases.

DISCUSSION

An analytical method to be applied in formulation screening, biopredictive dissolution testing and quality control of fixed-dose combination products should be robust, efficient and reliable. In the course of developing prototype formulations of fixed-dose combination products containing two or more antiparkinson drugs, it became obvious that a method fulfilling the above-mentioned criteria and that can be applied for the simultaneous quantification of levodopa, carbidopa, benserazide and entacapone had not yet been described in the literature. A detailed literature research revealed that a few robust and efficient HPLC methods for the parallel detection of levodopa and carbidopa have been published over the last decades, e.g. (6-8). These methods mainly differ in the detection method applied, but in principle would have been applicable for our purpose.

However, an initial screening of these methods in our lab indicated that the methods were not directly applicable for detecting all four antiparkinson drugs of interest. Selective and reliable HPLC methods for detecting more than two of the compounds within a single run were published by Ribeiro *et al.* (9) and Vemic *et al.* (10). However, the cited methods did not cover the complete range of APIs studied in our experiments and the overall run times of the methods were quite long. The method for the simultaneous analysis of levodopa, carbidopa and entacapone published by Vemic *et al.* (10) had for instance a total run time of 40 min, even though a relatively short 150 mm C-18 column was applied and thus was not regarded as efficient. Another published "liquid chromatographic method for the estimation of levodopa, carbidopa and entacapone in combined dosage forms" (11) reporting the application of a 250 x 4.6 mm, 5 µm C-18 column, a mobile phase consisting of a pH 4.0 phosphate buffer:methanol 60:40 (v/v), i.e., a polar eluent, a column temperature of 25 °C and a flow rate of 1.0 mL was also screened for our purpose. That method turned out to lack reproducibility due to the following reasons: The stock solutions could not be prepared in the given concentrations because of the limited solubility of the APIs and besides that, the indicated concentrations of the stock solutions were untypically high for a HPLC method. Following injection of a standard solution containing lower concentrations of levodopa, carbidopa and entacapone and exactly following the test protocol proposed by Thahaseen *et al.* (11), the dead volume of the column turned out to be much higher than the one given in the respective paper and also the retention times of the peaks were much higher. For these reasons, the cited method was not regarded as reliable or productive, respectively. Consequently, we had to develop a novel method. Using a 250 x 4.6 mm, 5 µm C-18 column with our optimized gradient separation method, we were able to obtain an excellent chromatographic separation of levodopa, carbidopa, benserazide and entacapone in the presence of complex matrices such as a variety of formulation excipients and ingredients of biorelevant dissolution media within a relatively short overall run time. The method was properly validated according to ICH guidelines and represents a robust, efficient and reliable method that can be applied in screening the uniformity of content and drug release of fixed-dose combination products containing two or more of the four antiparkinson drugs.

CONCLUSION

A RP HPLC method for an effective separation of levodopa, carbidopa, benserazide and entacapone was developed and validated. The method allows a fast and robust quantification without any interference caused by formulation excipients or ingredients of compendial and biorelevant dissolution media. The novel method thus presents a valuable tool in both formulation development and *in vitro* drug release screening of numerous fixed-dose combinations of antiparkinson drugs.

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5.5 Patient-specific *in vitro* drug release testing coupled with *in silico* PBPK modeling to forecast the *in vivo* performance of oral extended-release levodopa formulations in Parkinson's disease patients

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In dieser Arbeit wurde die Entwicklung von Parkinson-spezifischen In-vitro-Freisetzungsmodellen in Verbindung mit In-silico-PBPK-Modellen zur Vorhersage des In-vivo-Verhaltens von peroralen, modifiziert freisetzenden Levodopa-Darreichungsformen beschrieben. Zunächst wurde die arzneibuchkonforme USP-3-Apparatur auf Basis eigener Vorgaben in Form eines Lastenhefts modifiziert, da die Nutzung der arzneibuchkonformen Version aufgrund der geringen Variationsmöglichkeiten keine Simulation typischer Motilitätsmuster im humanen Gastrointestinaltrakt zuließ und sich noch weniger für die Entwicklung und Etablierung von individuellen, patientenspezifischen Motilitätsprofilen eignete. Mit dieser neuen technischen In-vitro-Plattform, in Kombination mit physiologisch relevanten Medien und Volumina, wurden patientenspezifische, individualisierte In-vitro-Testszenarien hinsichtlich der gastrointestinalen Motilität und Passage einer festen Darreichungsform in verschiedenen Parkinson-Patienten entwickelt und anschließend Parkinson-spezifische Freisetzungsuntersuchungen durchgeführt. Die parametrische Grundlage für diese Modelle bildete der aus dem zuvor beschriebenen Übersichtsartikel gewonnene Datensatz zur gastrointestinalen Physiologie von Parkinson-Patienten. Die Durchführung von In-vitro-Freisetzungsuntersuchungen unter „standardmäßigen" Testbedingungen, d. h. unter Simulation gastrointestinaler Gegebenheiten von gesunden Erwachsenen, hatte die Erstellung eines Vergleichsdatensatzes zum Ziel. Für die Beurteilung der Aussagekraft der entwickelten Testmodelle wurden die generierten In-vitro-Freisetzungsdaten aus den Parkinson-spezifischen- und den „standardmäßigen" Freisetzungsuntersuchungen in ein In-silico-PBPK-Modell implementiert und die jeweiligen simulierten Plasmakonzentrations-Zeit-Profile von Levodopa anschließend mit klinischen, durchschnittlichen In-vivo-Daten korreliert. Für PBPK-Modelle mit integrierten Parkinson-spezifischen In-vitro-Freisetzungsdaten wurde eine höhere Prädiktivität des In-vivo-Verhaltens der untersuchten Levodopa-Darreichungsformen beobachtet. Es konnte gezeigt werden, dass die entwickelten Parkinson-spezifischen Freisetzungsmodelle ein vielversprechendes und prädiktives In-vitro-Instrument zur Vorhersage der In-vivo-Wirkstofffreisetzung von modifiziert freisetzenden Levodopa-Darreichungsformen darstellen.

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Prof. Dr. Sandra Klein:
Erarbeitung der Fragestellung, Diskussion der In-vitro-/In-silico-Untersuchungsergebnisse, Diskussion und Korrektur des Manuskripts

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Erik Wollmer	Prof. Dr. Sandra Klein

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Patient-specific *in vitro* drug release testing coupled with *in silico* PBPK modeling to forecast the *in vivo* performance of oral extended-release levodopa formulations in Parkinson's disease patients

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ABSTRACT

Biorelevant *in vitro* release models are valuable analytical tools for oral drug development but often tailored to gastrointestinal conditions in 'average' healthy adults. However, predicting *in vivo* performance in individual patients whose gastrointestinal conditions do not match those of healthy adults would be of great value for optimizing oral drug therapy for such patients. This study focused on establishing patient-specific *in vitro* and *in silico* models to predict the *in vivo* performance of levodopa extended-release products in Parkinson's disease patients. Current knowledge on gastrointestinal conditions in these patients was incorporated into model development. Relevant *in vivo* pharmacokinetic data and patient-specific *in vitro* release data from a novel *in vitro* test setup were integrated into patient-specific physiologically-based pharmacokinetic models. AUC, c_{max} and t_{max} of the computed plasma profiles were calculated using PK-Sim®. For the products studied, levodopa plasma concentration–time profiles modeled using this novel approach compared far better with published average plasma profiles in Parkinson's disease patients than those derived from *in vitro* release data obtained from the 'average' healthy adult setup. Although further work is needed, results of this study highlight the importance of addressing patient-specific gastrointestinal conditions when aiming to predict drug release in such specific patient groups.

1. Introduction

Intraluminal conditions in the gastrointestinal (GI) tract, such as physicochemical properties, volume and composition of GI fluids, secretion rates, GI motility patterns and segmental transit times, can have a tremendous impact on *in vivo* drug release and absorption of orally administered drugs. GI physiology of healthy adults has already been well characterized in many clinical studies. However, other factors such as age, gender, ethnicity, nutritional and health status, which could also have a significant impact on the intraluminal environment of the GI tract and consequently directly affect the *in vivo* performance of oral dosage forms, have not been evaluated to the same extent. Since intraluminal conditions different to those in healthy adults may directly affect the kinetics of drug absorption, it is essential to consider GI conditions when estimating oral drug absorption in specific patient populations [1].

The impact of the patients' age on GI physiology is a critical factor. Rapid maturational changes can be observed in the paediatric population, particularly during the first months of life [2-4], but age-related physiological alterations also take place in geriatric patients [5]. Moreover, GI conditions can be affected by different nutritional states, i.e., malnutrition [6] and obesity [7]. Finally, GI or systemic diseases, e.g., irritable bowel syndrome [8], Crohn's disease [9-13], ulcerative colitis [9,10,12,14-17], celiac disease [18], cystic fibrosis [19-22], diabetes mellitus [23-25], depression [26], and Parkinson's disease (PD) [27], can also influence the kinetics of orally administered drugs.

PD is a progressive neurodegenerative disease, characterized by the primary motor symptoms tremor, rigidity, postural instability, and bradykinesia, resulting from a continuous loss of dopaminergic neurons located in the substantia nigra. In addition to these symptoms, the disease is accompanied by a variety of secondary motor- and other non-motor symptoms, including GI symptoms such as dysphagia, gastroparesis, and constipation. The disease affects nearly all segments of the GI tract, often even before other symptoms occur, and GI symptoms are

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evident in all disease stages. Most of the GI abnormalities associated with PD are attributable to impaired motility. At the level of the stomach, this results in delayed gastric emptying/gastroparesis. Small intestinal transit may also be affected, and the patients also often suffer from constipation. Quite frequently, symptoms differ between early and advanced stages of PD. Overall, GI symptoms do not only impact patient's quality of life, but may also seriously affect the *in vivo* performance of orally administered drugs, particularly those used for the symptomatic treatment of PD [27].

Antiparkinsonian agents for oral drug therapy include levodopa combined with a dopa-decarboxylase inhibitor (DDI), i.e., carbidopa or benserazide, dopamine agonists, monoamino oxidase (MAO) B inhibitors, catechol-O-methyltransferase (COMT) inhibitors and anticholinergic drugs [28]. Levodopa is still the most effective symptomatic PD treatment, but within years most Parkinsonian patients begin to experience motor fluctuations and dyskinesias, e.g., response failures, delayed time to 'on' where symptoms are controlled, and unpredictable periods with reduced mobility ('off' time) [29,30]. Based on these observations and given the limited options for dose adjustment, the development of oral controlled-release (CR) levodopa formulations was initiated with the aim to produce smoother, less extreme plasma levels and to prolong continuous dopaminergic stimulation combined with the advantage of a reduced frequency of daily levodopa dosing [31]. Today, a variety of different formulations comprising fixed-dose combinations (FDC) of levodopa and a DDI for the therapy of PD is available on the market.

The retardation of the drug release is associated with several pharmacokinetic alterations compared to levodopa immediate-release (IR) preparations, e.g., a prolonged time to reach maximal plasma concentrations (t_{max}), lower maximal plasma concentrations of levodopa (c_{max}), and a reduced oral bioavailability [32]. Despite the potential therapeutic benefits, clinical studies have shown that *in vivo* responses to currently marketed CR formulations of levodopa were often erratic with unpredictable, fluctuating plasma levels and pharmacokinetic properties showing a high intra- and interindividual variability [33-47]. Furthermore, the onset of effect was reported to be unpredictable. This indicates that dose failures may also occur with levodopa CR formulations. Overall, the additional therapeutic benefit of these formulations compared to IR formulations for the symptomatic treatment of PD has been critically questioned [33-47]. These observations clearly demonstrate that a robust and predictable drug release in the patient's GI tract is an essential prerequisite for effective, reliable, and safe oral levodopa therapy. The fundamental step for drug absorption after administration of an oral dosage form is the solubility of the drug in the intraluminal contents and its release before or immediately at the site of absorption. Since the physiological environment of the GI tract can significantly influence the *in vivo* behavior of an oral drug formulation, biorelevant *in vitro* release models should be used during formulation development to predict *in vivo* release and provide an overall estimate of the efficacy and safety of an oral drug product. Consequently, when developing oral drug products for special patient populations, such as PD patients, the *in vitro* dissolution/release model should mimic the patients GI conditions as closely as possible.

In the past two decades, a variety of biorelevant *in vitro* dissolution/drug release methodologies have been introduced and successfully applied for predicting the *in vivo* performance of oral drug formulations and for answering questions in terms of bioavailability and bioequivalence in average adults, e.g., [48-60]. Physiological parameters that are essential for designing biorelevant *in vitro* models for oral drug formulations comprise fluid volumes, hydrodynamics, motility, and residence times in the different GI sections. Since they can strongly affect drug release and dissolution in the GI lumen, the physicochemical properties (e.g., pH, buffer capacity, surface tension, viscosity, osmolality) and the composition (e.g., bile compounds, digestion products) of the intraluminal contents should also be properly addressed in a biorelevant test design.

To date, biorelevant *in vitro* dissolution/drug release method design has mainly focused on mimicking intraluminal GI conditions of 'average' healthy adults, i.e., the typical phase-1 clinical study population, whereas GI features of special patient populations, e.g., PD patients, have usually not been considered during formulation screening. Hence, the development of bio-predictive *in vitro* drug release models that integrate relevant PD patient-related GI parameters, might be extremely helpful in both explaining *in vivo* observations reported in the literature and guiding the development of appropriate new formulations. Biorelevant *in vitro* drug release data would also contribute to the improvement of physiologically-based pharmacokinetic (PBPK) *in silico* models, which capture anatomical and physiological features determining drug absorption, distribution, metabolism and excretion along with drug physicochemical properties, data from biopharmaceutical *in vitro* release models, and clinical *in vivo* data, to predict drug pharmacokinetics after oral administration. Well-designed PBPK models are a promising tool with many potential applications, such as streamlining of drug development, generating and validating working hypotheses, providing preclinical dosing guidance, and extrapolation of various clinical scenarios [61-63].

The aim of the present work was to develop PD-specific *in vitro* release models to predict *in vivo* drug release of levodopa CR dosage forms in PD patients. This was to be realized by implementing disease-specific GI parameters relevant to oral drug absorption into the *in vitro* test design. The obtained *in vitro* release data should then be integrated into a PD-patient-specific *in silico* PBPK model to evaluate their predictive power in terms of *in vivo* data from clinical trials in PD patients reported in the literature.

2. Materials and methods

2.1. Materials

Nacom® extended-release (ER) tablets (MSD Sharp & Dohme GmbH, Germany; batch # E000146 and # H000249) and Madopar® Depot capsules (Roche Pharma AG, Switzerland; batch # 2379B01 and # M2512B02) were obtained from a local pharmacy. Rytary® ER capsules (Amneal Pharmaceuticals Inc., Bridgewater, USA; batch # 20001950A) was imported from the USA via a local pharmacy. Levodopa (batch # FD156351550), carbidopa (batch # FC012961550) and benserazide hydrochloride (batch # FB181711501) drug substances were of analytical grade and purchased from Carbosynth Ltd. (Newbury, UK). All other chemicals for media preparation and sample analysis were of analytical- or gradient grade and were purchased commercially.

2.2. In vitro study design

2.2.1. Drug formulations

Three different oral formulation types, all ER FDC of levodopa and a DDI (carbidopa or benserazide) that are currently available on the market, were selected for the *in vitro* and *in silico* studies. Nacom® ER (=Sinemet® CR) is an ER tablet formulation containing a FDC of levodopa and carbidopa, which is embedded in a slowly degradable polymer matrix [64] ensuring a continuous levodopa absorption over 4–5 h [65]. The product is available in different dose strengths. In the *in vitro* release experiments a FDC of 200 mg levodopa and 50 mg carbidopa was tested. Rytary® is an ER multiparticulate formulation containing a FDC of levodopa and carbidopa with dual release characteristics embedded in a hard capsule [66]. Part of each dose is released immediately after ingestion, while the remaining dose is released over a prolonged period of time with pH-dependent release characteristics. In the *in vitro* release experiments a Rytary® formulation with 195 mg levodopa and 48.75 mg carbidopa was studied. Madopar® Depot (=Madopar® HBS) is an ER hard gelatin capsule formulation containing 100 mg levodopa and 25 mg benserazide (as hydrochloride). This formulation is also described as a hydrodynamically balanced system (HBS) which, when in contact with

gastric fluid and following dissolution of the capsule shell, is supposed to form a mucous body with a bulk density of less than 1 g/cm^3. This mucous body is intended to float on top of the gastric contents, and therefore, should ensure prolonged gastric residence while releasing the drugs at a desired rate [67].

2.2.2. Solubility experiments

A set of solubility measurements was performed to investigate the equilibrium solubility of levodopa in the dissolution media that were intended to be used in release studies scheduled. Results of the solubility experiments were regarded as helpful in discussing the results of the scheduled *in vitro* drug release experiments and should also provide solubility data for the development of the PD patient PBPK model. All solubility experiments were performed at 37.0 ± 0.5 °C using the shake-flask method. Briefly, experiments were performed as follows: An excess of each drug substance was added to a 5 mL flask containing 3 mL of the respective dissolution medium. The mixture was then agitated at 220 rpm using an IKA® RCT basic stirrer equipped with tempering units (IKA-Werke GmbH & Co. KG, Staufen im Breisgau, Germany). If necessary, drug was added to maintain an excess. The pH of the medium was monitored throughout the experiment and adjusted to the original value if required. Samples were taken 24 h after the start of the experiment and analyzed by High Performance Liquid Chromatography (HPLC). Each experiment was performed in triplicate.

2.2.3. Instrumental setup - compendial vs. modified Reciprocating Cylinder apparatus

Two instrumental setups were used for the *in vitro* release experiments. Experiments were either conducted with the compendial or a novel modified Reciprocating Cylinder apparatus (both from Erweka GmbH, Langen, Germany). Both apparatuses allow the use of a set of biorelevant media for mimicking the changing GI environment when a dosage form passes along the GI tract.

In the compendial apparatus (RRT10) the 'standard' test volume applied in drug release experiments is 200–250 mL. The apparatus is further characterized by a specified dipping height of 100 mm with a fixed start and stop position (Fig. 1). The dip rate can be varied between 5 and 40 dips per minute (dpm). For each individual vessel row, one dip rate and a fixed dipping time can be set. During a 'standard' experiment, the dosage form is completely immersed in the dissolution medium. Fixed agitation (dip) rates and continuous fluid contact do not necessarily compare with intraluminal conditions in the GI tract. Since the GI tract, particularly the small intestine, is not filled with a continuous fluid phase and during a GI passage there is rather a change between wet and dry areas [68], an orally administered ER formulation experiences intermittent fluid contact in the gut, which could have a significant impact on drug release. A biorelevant patient-specific *in vitro* test setup should allow such critical variables to be represented as closely as possible, but the settings applicable in the compendial Reciprocating Cylinder apparatus do not allow for consideration of patient-specific motility patterns or for mimicking passage through fluid pockets and 'dry' areas of the gut [68].

To overcome these limitations, a modified Reciprocating Cylinder apparatus offering to mimic highly variable motility patterns and residence times in 'wet' and 'dry' areas during GI passage was developed. The basic design of the novel Reciprocating Cylinder apparatus prototype (RRT10i) complies with the compendial specifications but offers some additional features that are particularly suitable for the establishment of patient-specific, individualized *in vitro* test methods. The novel (modified) device was developed based on the specifications established for the present test series. The technical configuration of the modified Reciprocating Cylinder apparatus allows to freely program and vary start- and stop positions of the cylinder, dip rate, and intervals between dipping in each vessel row. The dip rate can be varied from 2 to 70 dpm and media volumes of 50–250 mL can be applied. With the options of setting multiple actions per vessel row, moving the cylinder inside and outside the dissolution medium with different agitational speeds, and also pausing the cylinder inside or outside the fluid, the new setup allows for mimicking scenarios with different GI passage times, high and low motility as well as longer or shorter contact times of the dosage form with fluid (examples of different dipping scenarios are provided in Fig. 1). In this way, individual patient-specific GI characteristics can be mimicked in the *in vitro* release experiment.

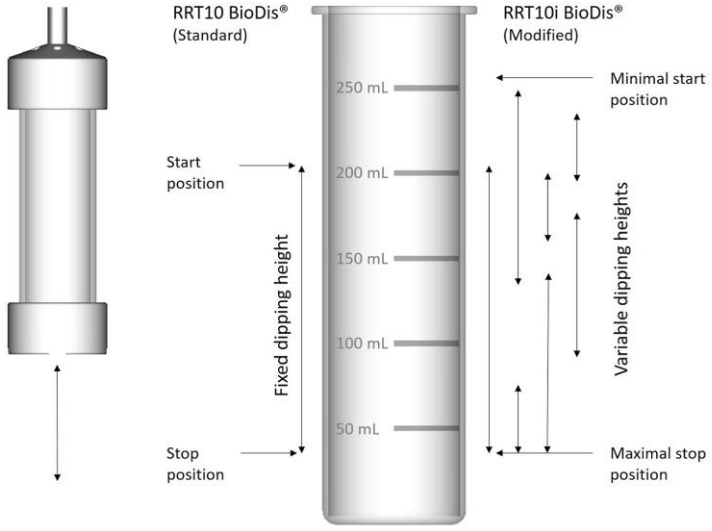

Fig. 1. The standard (left) and modified (right) Reciprocating Cylinder apparatus (Erweka GmbH, Langen, Germany) with start and stop positions of the cylinder, respectively. Examples of different dipping scenarios are given for the modified apparatus.

2.2.4. In vitro test design and sampling procedure

2.2.4.1. 'Standard' in vitro test setups mimicking a GI passage in an average healthy adult in fasted dosing conditions. Levodopa is typically administered without food. Thus, in the first set of experiments drug release of the selected marketed drug products was investigated by applying test scenarios mimicking a passage through the stomach and small intestine of an average healthy adult after fasted administration of an oral ER formulation. The variability of the residence times in different GI sections, particularly the small intestine, was addressed by mimicking either a changing transit rate (characteristic for monolithic ER dosage forms) or a constant transit rate (characteristic for multiparticulate ER formulations) through the small intestine [69]. Experiments were performed in biorelevant media, i.e., Fasted State Simulated Gastric Fluid (FaSSGF), Fasted State Simulated Small Intestinal Fluid (FaSSIF) and Simulated Colonic Fluid (SCoF) as well as in the corresponding (blank) buffers (Simulated Gastric Fluid (SGF), Blank FaSSIF) to assess the impact of added bile salts on the drug release behavior. First generation (version 1, V-1) and updated (version 2, V-2) biorelevant media were applied to also address the complexity of the composition of gastric and small intestinal contents [70]. Moreover, pH and bile salt concentrations of the FaSSIF media were adapted to average physiological values along the small intestinal lumen [71]. Tables 1-2 exemplarily show the *in vitro* test setups used for mimicking a passage through the upper fasted GI tract of an average adult with a changing- or a constant transit rate through the small intestine using V-1 media [52,72-74]. Release experiments were performed with the compendial Reciprocating Cylinder apparatus (RRT10) at 37.0 ± 0.5 °C. A media volume of 200 mL was applied in each vessel and the dip rate was 10 dpm in all experiments. The tested drug formulations were placed into the glass cylinders using top (mesh size 420 μm) and bottom (mesh size 150 μm) screens. The total duration of the drug release experiments was 5 h. Immediately after the reciprocating glass cylinder had passed the corresponding row of vessels, a sample was manually collected from each vessel using a 5 mL glass syringe connected to a stainless-steel sampling device equipped with a 10 μm poroplast cannula filter (Erweka GmbH, Langen, Germany). All experiments were run in triplicate.

2.2.4.2. PD patient-specific in vitro models mimicking a GI passage in fasted dosing conditions. PD patient-specific *in vitro* models addressing disease-specific GI features with particular respect to motility, passage times and fluid volumes were designed based on a detailed literature review [27]. The PD patient-specific *in vitro* release experiments were performed with the modified Reciprocating Cylinder apparatus (RRT10i) and all experiments were run in blank V-1 media.

To account for the GI variability of the PD patient population, three different motility patterns mimicking PD patients with high, moderate ('average') and low GI motility were applied. The different motility patterns were combined either with a GI passage with continuous fluid contact or one with intermittent fluid contact during gastric and small intestinal passage or combinations thereof. An overview of the individual *in vitro* models designed to mimic GI motility and transit in different PD patients is provided in Fig. 2. In total, 11 different PD patient-specific test scenarios (PD 1–11) were applied.

Test setups mimicking a GI passage with continuous fluid contact of the dosage form targeted to replicate constant movement of the dosage form in fluid-filled GI segments. Therefore, in the *in vitro* experiments the investigated drug formulations were agitated in the medium throughout the entire experiment (PD 1, PD 4, and PD 9) and different motility patterns were applied. Test setups mimicking a GI passage with intermittent fluid contact of the dosage form aimed to imitate a GI passage transit that is characterized by alternation of transport and pauses during which a dosage form is only temporarily surrounded by a continuous liquid phase. In the *in vitro* experiments, this was realized by implementing dipping pauses inside or outside the dissolution medium to mimic resting periods in either fluid-filled 'wet', or fluid-free 'dry' GI segments. Again, PD patients with high, moderate or low GI motility were simulated and various theoretical *in vivo* scenarios, ranging from a gastric passage with fluid contact of the dosage form, which changes into a small intestinal passage with intermittent fluid contact (PD 2, PD 3, PD 5, PD 7, PD 10, and PD 11), to an entire GI passage with intermittent fluid contact (PD 6, and PD 8) were considered. However, to avoid the number of experiments from escalating into infinity, simulation of an entire GI passage with intermittent fluid contact was limited to patients with moderate GI motility.

Table 3 shows the basic setup of the *in vitro* experiments mimicking

Table 1
In vitro test setup applied to mimic a gastrointestinal (GI) passage with a changing transit rate through the small intestine in an average healthy adult in fasted dosing conditions.

GI segments	Dissolution media			Volumes	Residence times	Dip rates
	Blank/buffer	Biorelevant	pH			
Stomach	SGF*sp*	FaSSGF	1.8	200 mL	60 min	10 dpm
Proximal jejunum	Blank FaSSIF	FaSSIF	6.5	200 mL	15 min	10 dpm
Distal jejunum	Blank FaSSIF	FaSSIF	6.8	200 mL	15 min	10 dpm
Proximal ileum	Blank FaSSIF	FaSSIF	7.2	200 mL	30 min	10 dpm
Distal ileum	Blank FaSSIF	FaSSIF	7.5	200 mL	30 min	10 dpm
Distal ileum	Blank FaSSIF	FaSSIF	7.5	200 mL	30 min	10 dpm
Distal ileum	Blank FaSSIF	FaSSIF	7.5	200 mL	60 min	10 dpm
Proximal colon	SCoF	SCoF	5.8	200 mL	60 min	10 dpm

Table 2
In vitro test setup applied to mimic a gastrointestinal (GI) passage with a constant transit rate through the small intestine in an average healthy adult in fasted dosing conditions.

GI segments	Dissolution media			Volumes	Residence times	Dip rates
	Blank/buffer	Biorelevant	pH			
Stomach	SGF*sp*	FaSSGF	1.8	200 mL	60 min	10 dpm
Proximal jejunum	Blank FaSSIF	FaSSIF	6.5	200 mL	45 min	10 dpm
Distal jejunum	Blank FaSSIF	FaSSIF	6.8	200 mL	45 min	10 dpm
Proximal ileum	Blank FaSSIF	FaSSIF	7.2	200 mL	45 min	10 dpm
Distal ileum	Blank FaSSIF	FaSSIF	7.5	200 mL	45 min	10 dpm
Proximal colon	SCoF	SCoF	5.8	200 mL	60 min	10 dpm

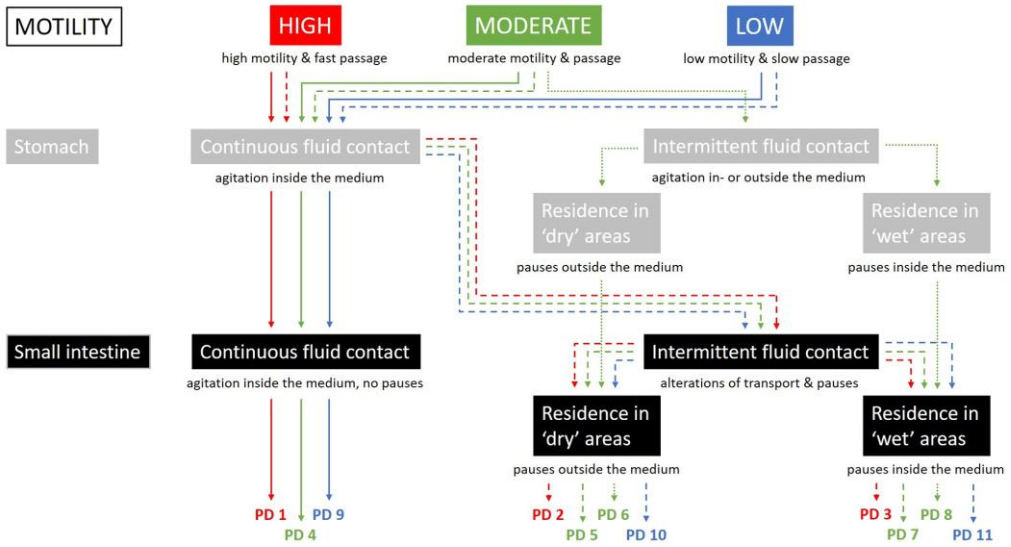

Fig. 2. Overview of the different *in vitro* release models (labeled from PD 1-11) intended to mimic the gastrointestinal (GI) transit of an oral dosage form in different Parkinson's disease (PD) patients. Three different motility patterns were combined with either a passage with continuous movement and fluid contact, or one with discontinuous movement and intermittent fluid contact or combinations thereof. Each test scenario consisted of a gastric, a small intestinal, and a short colonic stage.

Table 3
In vitro test setup used for mimicking dosage form passages with continuous fluid contact (PD 4), or intermittent fluid contact (PD 5–8) through the upper fasted gastrointestinal (GI) tract of a Parkinson's disease (PD) patient with moderate GI motility. The corresponding individual motility patterns are given in Fig. 3C-E.

GI segments	Dissolution media	pH	Volumes	Residence times	Dip rates
Mouth/esophagus	SSF pH 6.8 + water	7.1	10 + 40 mL	2 min	30 dpm
Stomach	SGF*sp*	2.5	50 mL	60 min	2 dpm
Stomach	SGF*sp*	2.5	50 mL	60 min	5–45 dpm
Duodenal passage	Blank FaSSIF	6.5	50 mL	15 min	2–45 dpm
Proximal jejunum	Blank FaSSIF	6.5	50 mL	75 min	2–10 dpm
Distal jejunum	Blank FaSSIF	6.8	50 mL	90 min	2–10 dpm
Proximal ileum	Blank FaSSIF	7.2	50 mL	90 min	2–10 dpm
Distal ileum	Blank FaSSIF	7.5	50 mL	90 min	2–45 dpm
Proximal colon	SCoF	5.8	50 mL	60 min	10–40 dpm

the passage of a dosage form through the upper fasting GI tract of a PD patient with moderate GI motility, during which continuous fluid contact (PD 4) or intermittent fluid contact (PD 5–8) occurs. While the media composition, fluid volumes, and residence times in the simulated GI segments were kept constant for all individuals (PD 4–8), dip rates and the time and frequency of fluid contact were altered to mimic different motility patterns and passage through fluid-filled segments and 'dry' regions in the gut of PD patients, respectively. At the start of the experiment, a short oro-esophageal transit of the dosage form was simulated under the assumption that this was ingested with a small amount (40 mL) of water and jointly swallowed with approximately 10 mL of saliva (represented by 10 mL of simulated salivary fluid (SSF)) (Table 3). The simulated fasted gastric fluid volume was 50 mL and contact time of the dosage form with simulated gastric fluid was decreased over time to account for rapid gastric emptying of the co-ingested fluid, leaving the dosage form in an almost empty stomach until it can be emptied into the small intestine. In the first hour of simulated gastric residence, very slight gastric motility was mimicked applying a low basal dip rate. Within the next hour, the dip rate was slightly increased and spiked with short intervals of high agitation. During the short simulated gastric emptying phase, the dip rate was further increased. Compared to the average healthy adult test designs, simulated small intestinal transit time was extended, and dip rate was low and only sporadically increased to address the overall reduced motility in PD patients. Small intestinal fluid volume was set at 50 mL. An intestinal pH gradient was applied, and, except for PD 4, intermittent contact of dosage form and fluid was mimicked. Ileocecal passage was simulated by a short phase of high agitation. The colonic phase was kept short, since levodopa is absorbed in the proximal small intestine and colonic absorption is very limited. The total test duration of these experiments was 9 h and the detailed motility profiles applied for mimicking GI transit in PD patients with moderate GI motility, visualized as dip rate per minute, are shown in Fig. 3C-E.

The basic setup of the *in vitro* experiments mimicking the passage of a dosage form through the upper fasting GI tract of a PD patient with high GI motility and continuous fluid contact (PD 1) or intermittent fluid contact (PD 2 and PD 3) is shown in Table 4. Compared to the *in vitro* settings applied to mimic GI passages in PD patients with moderate GI motility, simulated residence times in the different GI segments were reduced (Fig. 3A-B). Moreover, the duration of the intervals of high agitation was extended, whereas intervals of low agitation were shortened to address the higher GI motility. The total duration of the

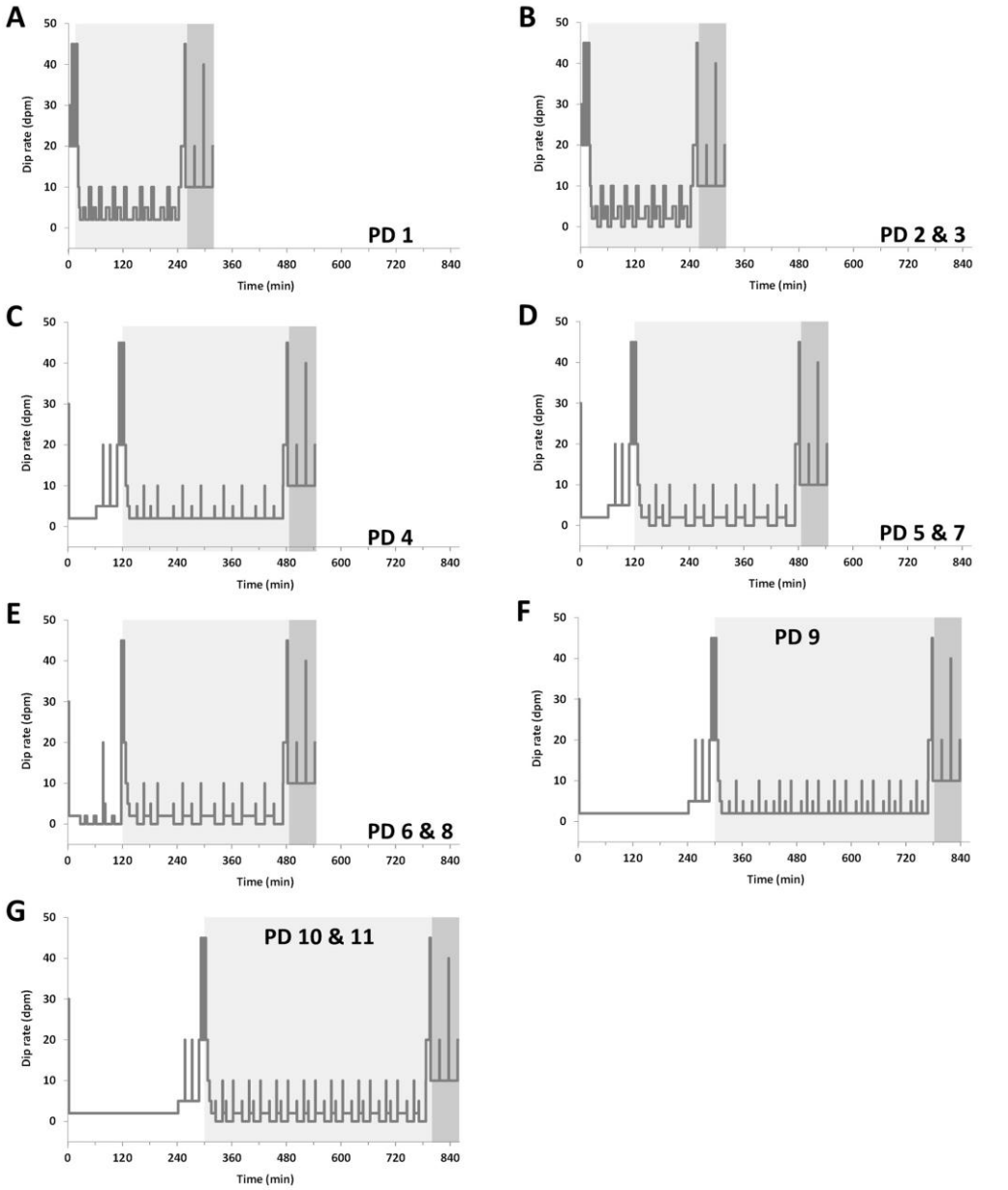

Fig. 3. Overview of the motility profiles applied for mimicking GI motility in Parkinson's disease (PD) patients with high (A, B), moderate (C, D, E) and low gastrointestinal (GI) motility (F, G). Shaded areas indicate small intestinal (bright grey) and colonic (dark grey) residence times.

experiments was 5.3 h. In contrast to this, in the corresponding test setups addressing a PD patient with low GI motility (PD 9–11), shown in Table 5, the simulated passage through the upper GI tract was characterized by longer residence times in the different GI segments, intervals of low agitation and phases without agitation (Fig. 3F-G). The total duration of these experiments was 13.9 h.

As shown in Tables 3-5, the media volume applied in the PD patient-specific *in vitro* release tests was set at 50 mL per vessel and media

Table 4
In vitro test setup used for mimicking dosage form passages with continuous fluid contact (PD 1), or intermittent fluid contact (PD 2 and PD 3) through the upper fasted gastrointestinal (GI) tract of a Parkinson's disease (PD) patient with high GI motility. The corresponding individual motility patterns are given in Fig. 3A-B.

GI segments	Dissolution media	pH	Volumes	Residence times	Dip rates
Mouth/esophagus	SSF pH 6.8 + water	7.1	10 + 40 mL	2 min	30 dpm
Stomach	SGF*sp*	2.5	50 mL	15 min	20–45 dpm
Duodenal passage	Blank FaSSIF	6.5	50 mL	10 min	2–45 dpm
Proximal jejunum	Blank FaSSIF	6.5	50 mL	50 min	2–10 dpm
Distal jejunum	Blank FaSSIF	6.8	50 mL	60 min	2–10 dpm
Proximal ileum	Blank FaSSIF	7.2	50 mL	60 min	2–10 dpm
Distal ileum	Blank FaSSIF	7.5	50 mL	60 min	2–45 dpm
Proximal colon	SCoF	5.8	50 mL	60 min	10–40 dpm

Table 5
In vitro test setup used for mimicking dosage form passages with continuous fluid contact (PD 9), or intermittent fluid contact (PD 10 and PD 11) through the upper fasted gastrointestinal (GI) tract of a Parkinson's disease (PD) patient with low GI motility. The corresponding individual motility patterns are given in Fig. 3F-G.

GI segments	Dissolution media	pH	Volumes	Residence times	Dip rates
Mouth/esophagus	SSF pH 6.8 + water	7.1	10 + 40 mL	2 min	30 dpm
Stomach	SGF*sp*	2.5	50 mL	60 min	2 dpm
	SGF*sp*	2.5	50 mL	60 min	2 dpm
	SGF*sp*	2.5	50 mL	60 min	2 dpm
	SGF*sp*	2.5	50 mL	60 min	2 dpm
	SGF*sp*	2.5	50 mL	60 min	5–45 dpm
Duodenal passage	Blank FaSSIF	6.5	50 mL	15 min	2–45 dpm
Proximal jejunum	Blank FaSSIF	6.5	50 mL	50 min	2–10 dpm
	Blank FaSSIF	6.5	50 mL	60 min	2–10 dpm
Distal jejunum	Blank FaSSIF	6.8	50 mL	60 min	2–10 dpm
	Blank FaSSIF	6.8	50 mL	60 min	2–10 dpm
Proximal ileum	Blank FaSSIF	7.2	50 mL	60 min	2–10 dpm
	Blank FaSSIF	7.2	50 mL	60 min	2–10 dpm
Distal ileum	Blank FaSSIF	7.5	50 mL	60 min	2–10 dpm
	Blank FaSSIF	7.5	50 mL	60 min	2–45 dpm
Proximal colon	SCoF	5.8	50 mL	60 min	10–40 dpm

temperature was maintained at 37.0 ± 0.5 °C. The sampling procedure was the same as described in 2.2.4.1. All *in vitro* drug release experiments were conducted in triplicate.

2.2.5. Sample analysis

Samples obtained from drug release experiments and solubility testing were filtered via a 0.45 µm cellulose acetate filter (diameter 30 mm, Whatman Schleicher & Schuell, Dassel, Germany) immediately after sampling, and diluted with the respective media. Subsequently, samples were analyzed by HPLC with a previously developed and validated method [75]. The HPLC system (Waters Corporation, Milford, USA) consisted of a 2489 UV/Visible detector, a 1525 series binary pump, a 2707 autosampler and a thermostated 1500 column compartment. System control, data acquisition and integration were accomplished with the Breeze® 2 software. HPLC analyses were performed under gradient conditions with solvent A consisting of a 30 mM

Table 6
Physicochemical and pharmacokinetic properties of levodopa representing the input parameters for the physiologically-based pharmacokinetic (PBPK) model in PK-Sim®.

	Parameter	Value	Reference(s)
Physicochemical properties	Molecular weight	197.19 g/mol	[82]
	Lipophilicity (logP)	-1.8	[82]
	pKa_1 / pKa_2	1.65 (acid) / 9.06 (base)	[82]
	Solubility	5.0 mg/mL No significant pH-dependency within the gastrointestinal pH range	see 3.1.
	Fraction unbound (fu)	50%	[83]
	Binding plasma protein	Albumin	[84]
Pharmacokinetic properties	Specific intestinal permeability	3.41×10^{-4} cm/s	[77]
	Distribution calculations -	Rodgers and Rowland	[78-81]
	Partition coefficients Distribution calculations -	PK-Sim® Standard	PK-Sim®
	Cellular permeabilities Distribution calculations -	Calculated by PK-Sim®	PK-Sim®
	Specific organ permeabilities Metabolizing enzymes	Dopa-decarboxylase COMT Tyrosine aminotransferase Oxidases (e.g., MAO B)	[85]

phosphate buffer pH 2.50 and solvent B representing a mixture of water pH 2.50 and acetonitrile (50:50, v/v). The run time was 17 min, the flow rate was set at 1.5 mL/min [75] and the injection volume was 30 µL. Chromatographic separation was achieved with an endcapped LiChrospher 100 RP-18 column (Merck KGaA, Darmstadt, Germany), 5 µm, 250 mm × 4.6 mm coupled with a LiChrospher 100 RP-18 precolumn (Merck KGaA), 5 µm, 4 mm × 4 mm equilibrated at 35 °C. Levodopa was detected at 280 nm.

2.3. In silico study design

2.3.1. Model structure and parameterization

The *in silico* plasma concentration–time profiles were simulated using the PK-Sim® software version 9.1 (Bayer Technology Services GmbH, Leverkusen, Germany), which implements a whole-body PBPK model consisting of 21 organs and tissues, and fully integrates liberation, absorption, distribution, metabolism, and excretion. A PBPK model build with PK-Sim® requires information regarding the biometrics, anatomy and physiology of the simulated individual/population, physicochemical and pharmacokinetic properties of the compound, *in vitro* drug release- and solubility data, and *in vivo* plasma concentration–time profiles in accordance with dosing conditions.

The virtual populations were either of European or American background depending on the origin of the respective *in vivo* study selected for simulation. The age of the simulated population and the proportion of male and female subjects was based on the information provided from the respective *in vivo* studies, and in all cases, total population size was 100 individuals per simulation. Other anthropometric parameters, i.e., weight, height, and body mass index, were generated by database average values with respect to the populatioṅs age. Default anatomy and physiology was not altered as the data situation for PD patients is very limited. Only data with respect to salivary flow rate and GI transit in PD patients were implemented from a review article [27]. Age-mediated anatomical and physiological changes were already incorporated in the PK-Sim® software [76].

The selected pharmacokinetic *in vivo* studies provided plasma concentration–time profiles for levodopa, but neither for carbidopa nor benserazide. Consequently, only levodopa drug-related physicochemical and pharmacokinetic data used for model parameterization, which are listed in Table 6, were implemented in the PBPK model. Intestinal permeability of levodopa was taken from the literature [77] and the specific organ permeability was calculated by PK-Sim® based on the drug's lipophilicity and molecular weight. Tissue/plasma partition coefficients were predicted using the *in silico* tissue composition approach proposed by Rodgers and Rowland [78-81]. Levodopa is undergoing extensive metabolization via different metabolic pathways, and thus, the metabolizing enzymes (located in various organs and tissues, e.g., liver and GI tract) listed in Table 6 were added to the model. Finally, data from the 'standard' and PD patient-specific *in vitro* drug release experiments of the different drug formulations were implemented into the PBPK model.

2.3.2. Model optimization

In the first step of the PBPK model development process, the physicochemical and pharmacokinetic input parameters for levodopa (listed in Table 6), and pharmacokinetic data obtained from intravenous levodopa administration from elderly subjects (n = 8, 69–76 years) in a clinical study [86], were used to establish and refine a model for intravenous levodopa administration. Data were digitized with DigitizeIt® version 2.3.3, Bormisoft, Braunschweig, Germany. For model optimization, the plasma profiles obtained in the *in vivo* studies were compared with the output plasma profiles of the initial *in silico* simulations. Both simulated and observed plasma concentration–time profiles were visually checked focusing on the absolute plasma concentrations and the appropriateness of the dynamic shape of the simulated plasma profile. Subsequently, the intrinsic clearance of the levodopa-metabolizing enzymes was incrementally adjusted to minimize the error between simulated and observed plasma concentration–time profiles.

2.3.3. Model application and verification

Once the intravenous PBPK model was established, it was utilized to build a model for oral administration in order to create *in silico* plasma profiles of the levodopa drug formulations studied in the *in vitro* experiments. To verify these profiles, *in vivo* data from several clinical studies in PD patients were taken from the literature [36,39,42,66,87,88] and plasma levodopa concentrations were estimated directly from the presented *in vivo* plasma concentration–time profiles. The approach taken for each of the dosage forms in question is described in detail in the following sections.

2.3.3.1. Nacom® ER. Only a few published clinical studies investigated the *in vivo* pharmacokinetic performance of Nacom® ER tablets in PD patients. Two eligible studies were conducted by LeWitt *et al.* [39] and Bowes *et al.* [87]. Yeh *et al.* also conducted a pharmacokinetic study with Nacom® ER in PD patients, but did not provide sufficient information on study subjects and -procedures (e.g., prandial state) [65]. Another study by Müller *et al.* was not considered for *in silico* PBPK modeling because the provided levodopa plasma concentrations were conspicuously low and only about one-tenth of those reported in other *in vivo* studies, even though the same levodopa dose was administered [89]. Finally, two clinical studies provided levodopa plasma concentration–time profiles only in steady state dosing conditions [33,90].

The randomized, open-label, cross-over study performed by LeWitt *et al.* included 17 PD patients (nine males and eight females) aged 63.1 ± 9.1 years (mean ± S.D.) [39]. Most of the subjects were suffering from rather mild to moderate PD symptoms and were classified as stage 2 (11/17 subjects) and stage 2.5 (1/17 subjects) on the Hoehn-and-Yahr (H&Y) scale, a commonly used system for describing how the symptoms of PD progress [91]. The H&Y scale is mainly focused on postural instability as the primary index of disease severity and divides patients into five different classes ranging from minimal disability in class 1 to confinement to bed or wheelchair without assistance in class 5 [27]. The other five PD patients exhibited a moderate/severe grade of disease, classified to H&Y stage 3 (4/17 subjects) and 4 (1/17 subjects). At the time of enrolment, all PD patients were managed with a stable daily regimen of levodopa, and other antiparkinsonian medications (except for COMT inhibitors) were allowed, provided their use was unchanged for at least four weeks before enrolment and throughout the study [39]. The first study dose (one Nacom® ER tablet with 200 mg levodopa) was administered in the morning at 8 a.m., shortly after a low-fat and low-protein breakfast (fruit and toast) representing the first levodopa dose of the day. The second dose of Nacom® ER with the same dose strength was administered at 12 a.m., and a lunch with a low protein content was provided 45 min after dose administration. Blood sampling was performed 60 min before the first levodopa dose and then at predetermined time intervals for 8 h. The authors reported single and average levodopa plasma profiles of the 17 PD patients. However, since in the cited publication all individual plasma profiles are shown in a common graph, neither the course of all individual plasma levels could be clearly identified, nor could individual plasma profiles be assigned to a specific PD patient. Thus, the average plasma concentration–time profile of levodopa was used in the PBPK simulations.

Besides establishing *in silico* plasma profiles to replicate the average levodopa plasma concentration–time profile obtained in the clinical study performed by LeWitt *et al.*, *in silico* plasma concentration–time profiles were also established to replicate those reported from a randomized, double-blind, cross-over study by Bowes and co-workers [87]. This study included eight PD patients (two males and six females) aged 69.9 ± 6.6 years (mean ± S.D.) with a mean duration of clinical Parkinsonism of 13.9 ± 7.0 years treated with levodopa for 9.6 ± 4.6

years, respectively. On separate study occasions at 10 a.m. in the morning, a single dose of one (treatment A) or two (treatment B) tablets of Nacom® ER, each containing 200 mg levodopa, was administered to the study subjects, which received a light, low protein breakfast 90 min prior to drug administration. No other food or drink was allowed after dosing. Blood sampling was conducted before the first levodopa dose and then hourly for 6 h after administration of the study medication. For both studies (A and B) the authors provided average levodopa plasma concentration–time profiles, which were used for *in silico - in vivo* comparison.

2.3.3.2. Rytary®. So far, only one published study investigated the pharmacokinetic profile of Rytary® capsules in PD patients [92]. In a randomized, open-label, cross-over study performed by Hauser *et al.*, multiple-dose pharmacokinetics of Rytary® was investigated in 27 subjects (21 males/6 female) with advanced PD and a mean age of 62.7 years (range 48–81 years) [92]. Most of the study subjects (85%) were classified as H&Y stages 2 or 3 and were on a stable levodopa regimen for at least one month. The multiple-dose pharmacokinetic assessment was carried out over a duration of 12 h. Subjects received individual dosing regimens (levodopa doses of 245 mg, 490 mg, or 735 mg) and took two doses of Rytary® every 6 h. The first study medication was administered in the morning in fasting conditions and blood samples were obtained at multiple time points over a 12-h period. Whereas in their publication Hauser *et al.* disclose all details of their study design, for undisclosed reasons, they do not provide any plasma profiles. However, unexpectedly the average levodopa plasma concentration–time profiles obtained in this study were then published in a review article by Mittur *et al.* which was co-authored by two colleagues involved in the Hauser study [66]. Therefore, the plasma concentration–time profiles reported in this review article were used for *in silico - in vivo* comparison.

2.3.3.3. Madopar® Depot. Current information on the *in vivo* pharmacokinetic performance of Madopar® Depot in PD patients is very limited. Two clinical studies performed by Malcolm *et al.* [88] and Stocchi *et al.* [36] proved suitable for the purpose of the present study. Results from another *in vivo* study published by Descombes *et al.* was not considered for further evaluation as fed state dosing conditions were applied [42]. Finally, the clinical studies performed by Pezzoli *et al.* [93] and Rondot *et al.* [94] did not provide sufficient information with regard to both study subjects and -procedure.

Single dose pharmacokinetics of Madopar® Depot were investigated in a study carried out by Malcolm *et al.* including ten PD patients, all of which exhibited 'on-off'-fluctuations [88]. However, the authors did not provide information on age, severity of the disease or gender ratio of men and women. Prior to dosing the patients were fasted for 9 h. Then they received two capsules with 100 mg levodopa per capsule and had to refrain from eating for 6 h after dosing, whereas water was available *ad libitum*. Blood samples were withdrawn every 30 min for 6 h. The authors reported an average levodopa plasma concentration–time profile for the ten PD patients which were used for *in silico - in vivo* comparison in the present study.

In addition to establishing *in silico* plasma profiles to replicate the average levodopa plasma concentration–time profile published by Malcolm *et al.*, *in silico* plasma concentration–time profiles were also established in an attempt to replicate those reported from a cross-over study performed by Stocchi *et al.* [36]. In that study, a single morning dose of two Madopar® Depot capsules (100 mg levodopa per capsule) was administered to three PD patients (one man, two women), aged 55–58 years, which showed marked fluctuations in motor performance. The capsules were administered at 9 a.m. in fasting conditions and blood samples were obtained at several time points over a total duration of 10 h. However, although three PD patients were enrolled in the study, the authors reported only a single levodopa plasma concentration–time profile from one of the three subjects.

3. Results and discussion

3.1. Solubility of levodopa

The equilibrium solubility of levodopa was determined in a series of solubility experiments in order to verify sink conditions in both 'standard' and patient-specific *in vitro* drug release experiments. For this purpose, it was regarded sufficient to remove just one sample from each individual glass vial at a time point where equilibrium solubility was expected to be reached.

A set of solubility experiments was performed in SGF*sp* pH 1.8 and 2.5, SCoF pH 5.8 and, various blank FaSSIF V-1 media (pH 6.5, 6.8, 7.2, and 7.5) for a test duration of 24 h. The solubility of levodopa (logP = -1.8; pk_a = 1.65 [82]) was independent of the pH of the media with results in the range of 5.35 to 7.73 mg/mL levodopa dissolved. These results indicated that the solubility of levodopa should have no effect on the results of the 'standard' *in vitro* release experiments using 200 mL media volume per vessel as at least 3-fold sink conditions were achieved in all media. By contrast, in the PD patient-specific release experiments, where lower media volumes of 50 mL per vessel were employed, 3-fold sink conditions were not achieved. However, due to the sustained release nature of the selected drug formulations, only fractions of the total dose were released per vessel row (as shown in the following section), and thus, an adverse effect on the release behavior was neither anticipated nor was observed in the experiments.

3.2. In vitro drug release experiments

The discussion of the results of the release experiments focuses on the release of levodopa from the FDC tested. The release profiles for carbidopa and benserazide hydrochloride were also determined experimentally, but are not presented here since, due to the lack of *in vivo* data for these two drugs, the PBPK model was built exclusively for levodopa.

3.2.1. 'Standard' in vitro setups mimicking a GI passage in an average healthy adult in fasted dosing conditions

A series of *in vitro* experiments mimicking a fasted GI transit in an average healthy adult with either a changing- or a constant transit rate through the small intestine was performed in biorelevant media and in their corresponding (blank) buffers. Both original (V-1) and updated (V-2) buffers and biorelevant media were used. In all experimental setups, nearly identical release profiles were obtained for the individual dosage forms, indicating that neither the composition of the different buffer versions nor the addition of bile salts had a marked impact on the drug release profiles of the products studied (data not shown). Fig. 4 exemplarily displays the drug release profiles obtained when using blank V-1 media. Each of the formulations studied showed its own characteristic levodopa release pattern in both *in vitro* experimental setups, which was largely independent of whether a GI passage with changing or constant small intestinal transit rate was mimicked.

A sustained *in vitro* release of levodopa over a duration of 120 min was observed for Nacom® ER. A large part of the dose was released during the simulated passage through the stomach and the proximal part of the small intestine (Fig. 4A). The multiparticulate drug formulation Rytary® exhibited an extended *in vitro* drug release over a period of 240 min, i.e., during the simulated gastric and small intestinal passage (Fig. 4B). Drug release of the Madopar® Depot capsules was much slower and after a test duration of 240 min, which represented the end of the simulated small intestinal transit, i.e., the absorption window for levodopa [38,95], only 70% of the levodopa dose had been released (Fig. 4C).

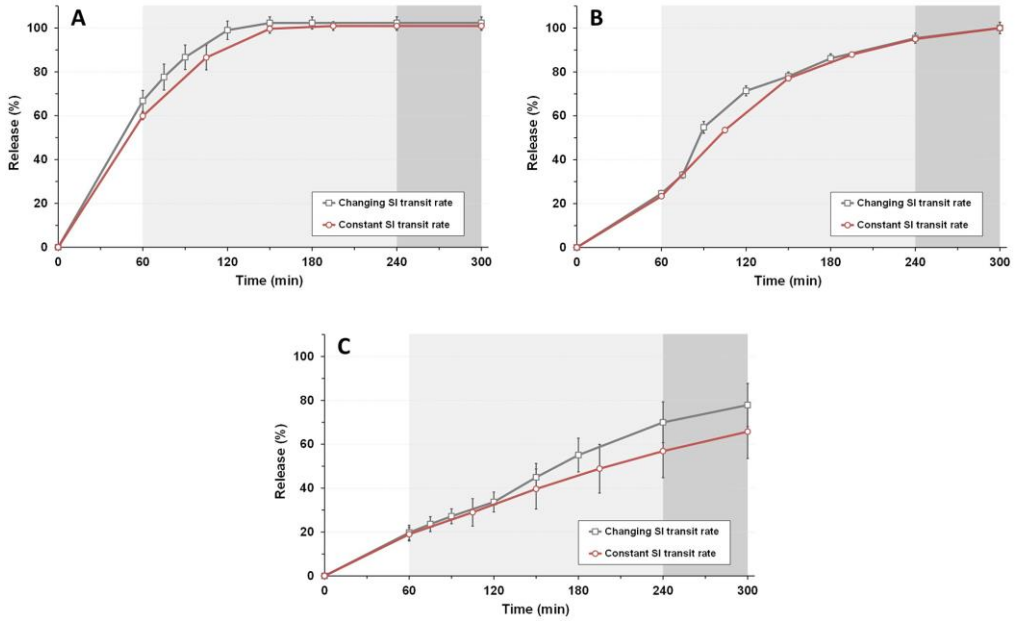

Fig. 4. Levodopa release profiles of (A) Nacom® ER, (B) Rytary®, and (C) Madopar® Depot when mimicking a fasted gastrointestinal (GI) passage with changing small intestinal transit rate and constant small intestinal (SI) transit rate in an average healthy adult (mean of n = 3 ± S.D.). Shaded areas indicate passage through stomach (white), small intestine (light grey), and proximal colon (dark grey), respectively.

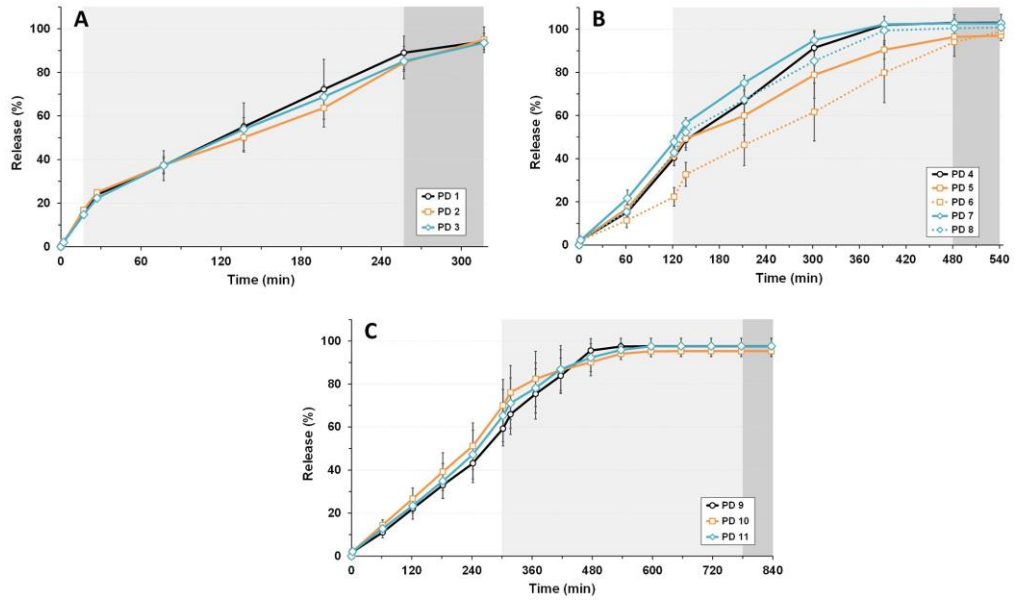

Fig. 5. Levodopa release profiles of Nacom® ER obtained when mimicking a fasted gastrointestinal (GI) passage in a Parkinson's disease (PD) patient with (A) high GI motility (PD 1–3), (B) moderate GI motility (PD 4–8), and (C) low GI motility (PD 9–11) (mean of n = 3 ± S.D.). Shaded areas indicate passage through stomach (white), small intestine (light grey), and colon (dark grey), respectively.

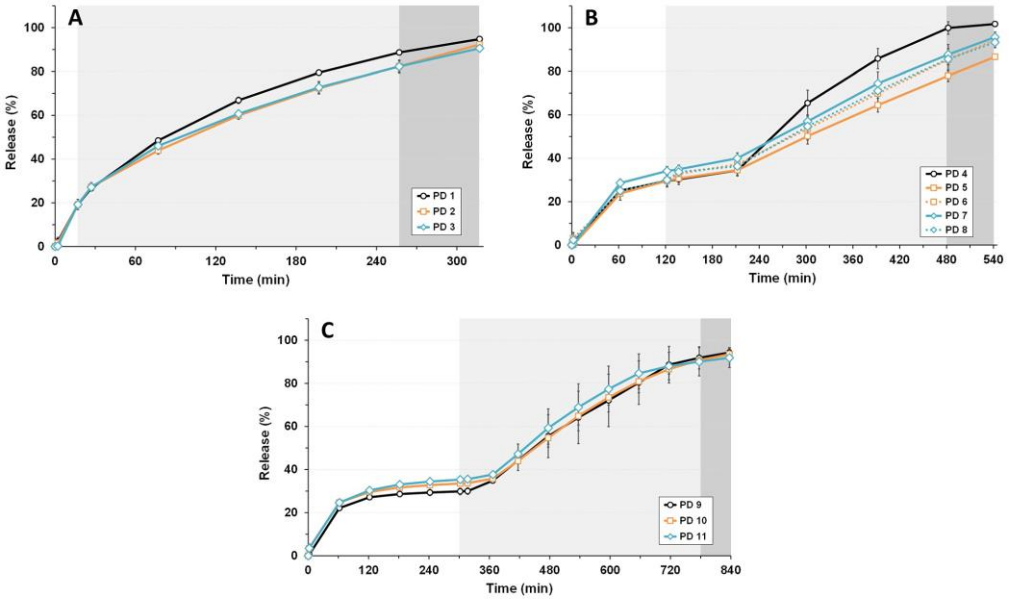

Fig. 6. Levodopa release profiles of Rytary® capsules when mimicking a fasted gastrointestinal (GI) passage in a Parkinson's disease (PD) patient with (A) high GI motility (PD 1–3), (B) moderate GI motility (PD 4–8), and (C) low GI motility (PD 9–11) (mean of n = 3 ± S.D.). Shaded areas indicate passage through stomach (white), small intestine (light grey), and colon (dark grey), respectively.

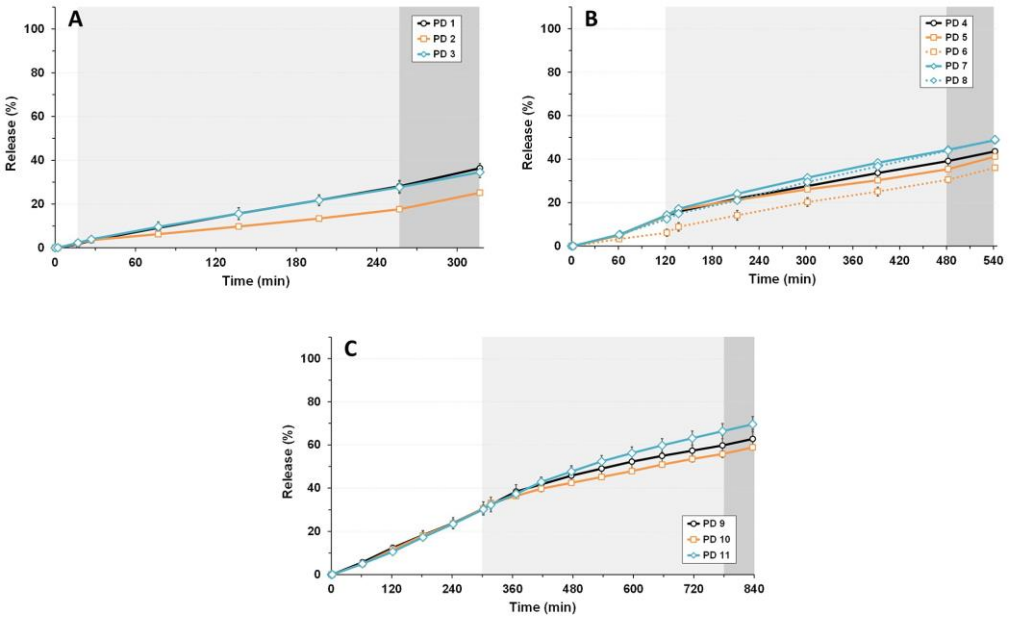

Fig. 7. Levodopa release profiles of Madopar® Depot capsules when mimicking a fasted gastrointestinal (GI) passage in a Parkinson's disease (PD) patient with (A) high GI motility (PD 1–3), (B) moderate GI motility (PD 4–8), and (C) low GI motility (PD 9–11) (mean of n = 3 ± S.D.). Shaded areas indicate passage through stomach (white), small intestine (light grey), and colon (dark grey), respectively.

3.2.2. PD patient-specific in vitro setups mimicking a GI passage in fasted dosing conditions

3.2.2.1. Nacom® ER. Fig. 5 shows the results of the PD patient-specific release experiments with Nacom® ER. Compared to the release profiles obtained with the 'standard' test setups (Fig. 4A), a prolonged drug release was observed. Lowering the media volumes from 200 mL to 50 mL together with shortening the contact time of the dosage form with the simulated GI fluids resulted in delayed swelling and erosion of the drug formulation, which was characterized by a significantly sustained levodopa release.

A closer look at the results of the PD patient-specific release experiments revealed a clear impact of the different test scenarios on the drug release profiles (Fig. 5). The fastest drug release was observed in the test scenarios mimicking a PD patient with high GI motility (PD 1–3, Fig. 5A), most likely due to increased agitation and contact time of the dosage form with the dissolution medium. Reduced agitation and fluid contact, particularly when mimicking a PD patient with low GI motility (PD 9–11, Fig. 5C), resulted in a prolongation of drug release from Nacom® ER tablets.

When mimicking PD patients with average GI motility, levodopa release was faster during a simulated GI passage with intermittent fluid contact with long residence times in 'wet' GI sections (PD 7–8, B) than in one with long residence times in 'dry' GI sections (PD 5–6, Fig. 5B).

These differences in levodopa release in patient-specific *in vitro* release models may provide at least part of the explanation for the highly variable and fluctuating plasma levodopa concentration profiles and unpredictable bioavailability observed for Nacom® ER in clinical trials in PD patients.

3.2.2.2. Rytary®. The levodopa release profiles of Rytary® obtained in the patient-specific *in vitro* release experiments are displayed Fig. 6. Drug release of this formulation was robust and not noticeably affected by reduced media volumes (Fig. 4B vs. Fig. 6A), or by varying the contact time with the dissolution medium (Fig. 6A-C). By contrast, the residence times of the drug formulation in the different simulated GI segments had a strong influence on the release behavior (Fig. 6A-C).

In comparison to results from experiments mimicking a GI passage in an average healthy adult (Fig. 4B) and in a PD patient with high GI motility (Fig. 6A), *in vitro* drug release of Rytary® was markedly different in the *in vitro* models mimicking PD patients with moderate (Fig. 6B) and low (Fig. 6C) GI motility. While under conditions of a simulated GI passage in a PD patient with high motility, a quite fast and constant drug release was observed and after 240 min about 90% of the levodopa dose was released, a more pulsatile and pH-dependent drug release profile was observed in corresponding experiments in which a GI passage in PD patients with medium or low motility was simulated. In the latter experiments after about 40% of the levodopa dose had been released under gastric conditions, the remaining amount was released when the formulation encountered media with higher pH, mimicking small intestinal conditions. This pH-dependent drug release can be attributed to the formulation design of the Rytary® drug product representing a mixture of coated pellets intended to allow for immediate, delayed, as well as sustained drug release. Due to this formulation design, the residence time of the dosage form in the different simulated GI segments had a profound effect on the drug release behavior of this formulation. However, it should be noted that the simulated gastric and small intestinal residence times in the low GI motility test setup are quite long for multiparticulates. Since the same *in vitro* models were used for all ER formulations studied, as a first approach in the present series of experiments, multiparticulates were treated as a bolus, whereas in reality, the administered dose will be emptied from the stomach in individual portions [96]. While this will not change the fact that only a portion of the levodopa is released in fasted gastric conditions, the *in vitro* models could certainly be further refined to better reflect the gastric emptying pattern of the multiparticulates.

3.2.2.3. Madopar® Depot. As in the *in vitro* release experiments mimicking a GI passage in an average healthy adult (Fig. 4C), a rather slow and constant drug release was observed in all PD patient-specific drug release experiments (Fig. 7). Interestingly, the different patient-specific motility scenarios seemed to have no influence on the levodopa rates indicating that drug release from Madopar® Depot capsules was pH-independent and not affected by different degrees of agitation (Fig. 7). However, in all patient-specific release experiments drug release from Madopar® Depot was markedly slower than in the average adult models (Fig. 4C). Only 20–37% of the total levodopa dose was released after 300 min in the *in vitro* models mimicking different patient-specific GI passage scenarios, whereas 68% and 78% of the dose were released within the same time range in the average adult test setups, respectively. This was most likely due to the smaller media volume and shorter contact time with the liquid, which resulted in less swelling of the formulation and consequently slower and prolonged levodopa release.

Madopar® Depot is categorized as a gastroretentive dosage form as it is intended to remain in the stomach for an extended period of time. In the presented patient-specific test setups, a further prolongation of gastric residence time due to the proposed gastroretentive characteristics of the dosage form was not considered, as the gastroretentive properties of Madopar® Depot are critically questioned [97]. In the *in vitro* models used in this study, increasing the gastric residence time would likely not have altered the release profiles shown in Fig. 7, as drug release from Madopar® Depot capsules was found to be independent of media pH and degree of agitation. Moreover, in an *in vitro* study performed by Schneider *et al.*, a high sensitivity of Madopar® Depot towards physiologic gastric pressures was observed [97]. The authors concluded that the dosage form, even when floating on the fasted gastric contents, will most likely be damaged or destroyed by the intense peristalsis caused during MMC phase III activities, which could result in levodopa dose dumping.

The fluctuating plasma concentrations and highly variable bioavailability of levodopa following administration of Madopar® Depot might be explained by the effects of gastric pressure events on the integrity of the formulation, which are difficult to predict. Furthermore, assuming that the gastroretentive behavior of the Madopar® Depot formulation cannot be guaranteed, the results of the *in vitro* release experiments show that probably not the entire dose administered would be available within the levodopa absorption window, which in turn could lead to subtherapeutic plasma concentrations. Overall, it can be concluded that most likely an interplay of different physiological factors contributes to the clinical observations made for Madopar® Depot. Although the formulation has been on the market for about 30 years, it may be advisable to critically scrutinize the advantages over a conventional non-floating sustained-release tablet.

3.3. In silico PBPK modeling

The outcomes of the *in silico* studies are presented in the following section. In the first step, a series of PBPK simulations using *in vitro* release data of the individual drug products from experiments mimicking different GI conditions in PD patients (3.2.2) was performed, and the models providing the best fit with *in vivo* data of the respective drug product were selected for final PBPK modeling. In the second step, the final simulations were performed with input parameters that differed only in the integrated *in vitro* release data, which came either from release experiments simulating a GI passage in average healthy adults (3.2.1) or the pre-selected PD patient-specific *in vitro* models to ensure that differences in simulated plasma concentration–time profiles were solely attributed to the input *in vitro* release data. The results of the simulations were then depicted graphically, and pharmacokinetic

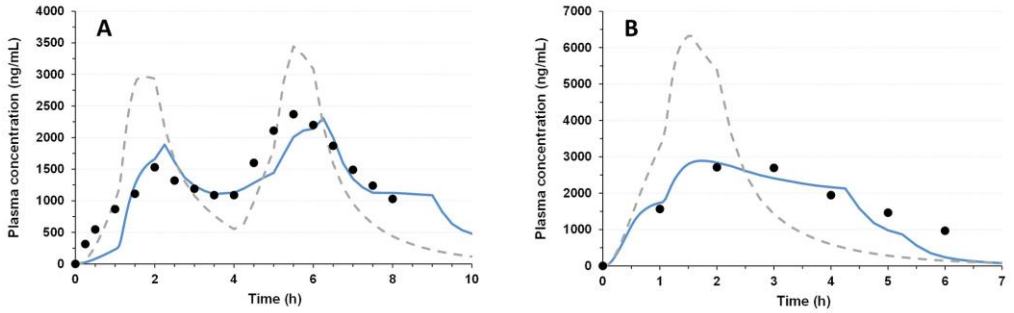

Fig. 8. Comparison of observed and predicted plasma levodopa concentration–time profiles following fasted administration of Nacom® ER in the studies of (A) LeWitt *et al.* (dose of 200 mg) and (B) Bowes *et al.* (dose of 400 mg). Single data points represent observed data, solid lines represent simulations with Parkinson's disease (PD) patient-specific dissolution data and dashed lines represent simulations with healthy adult dissolution data.

Table 7
Summary of observed and predicted pharmacokinetic data for Nacom® ER.

LeWitt *et al.* [39]	Dose strength (mg)	$c_{max\ (0\text{-}4h)}$ (ng/mL)	$t_{max\ (0\text{-}4h)}$ (min)	$c_{max\ (4\text{-}8h)}$ (ng/mL)	$t_{max\ (4\text{-}8h)}$ (min)	$AUC_{(0\text{-}8h)}$ (ng*h/mL)
Observed[1]	200	1840	144	2738	324	11,043
Predicted[3] **using PD patient-specific dissolution data**	200	1889	135	2309	375	10,524
Predicted[3] **using average healthy adult dissolution data**	200	2958	105	3445	330	11,833

Bowes *et al.* [87]	Dose strength (mg)	$c_{max\ (0\text{-}6h)}$ (ng/mL)	$t_{max\ (0\text{-}6h)}$ (min)	$AUC_{(0\text{-}6h)}$ (ng*h/mL)
Observed[2,3]	400	2710	120	10,862
Predicted[3] **using PD patient-specific dissolution data**	400	2892	105	10,653
Predicted[3] **using average healthy adult dissolution data**	400	6322	90	11,337

[1] Numerical data from literature [39].
[2] Data calculated with graphical data from literature [87].
[3] Data calculated from simulated concentration–time profiles using PK-Sim®.

parameters were tabulated for each of the three drug formulations.

3.3.1. Nacom® ER

When investigating the pharmacokinetics of Nacom® ER tablets in PD patients in different stages of the disease LeWitt *et al.* administered the first study dose (200 mg levodopa) shortly after a low fat- and low protein breakfast at 8 a.m. in the morning [39]. The second dose (200 mg levodopa) was administered at 12 a.m., i.e., four hours later and a lunch with a low protein content was provided 45 min after administering the second dose. When establishing the *in silico* PBPK model replicating the study of LeWitt *et al.*, the ingestion of a light meal (340 kcal) before the first dose was taken into account, while the intake of the second meal was not.

The PD patients participating in the study by Bowes and coworkers were given a light breakfast with low protein content 90 min prior to drug administration. After this they received a single dose of one (treatment A) or two (treatment B) tablets of Nacom® ER, each containing 200 mg of levodopa, and were not permitted to have any more food or drink [87]. Since this light breakfast was administered at a much greater time interval than in the LeWitt study, for the Bowes study the intake of a light meal was not considered in the simulation. It is also important to note that the same *in vitro* release data were incorporated when building the PBPK models to replicate the results of both study arms, i.e., treatments A (200 mg dose) and treatment B (400 mg dose), as it was assumed that the release rates of a single and two simultaneously applied Nacom® ER tablets should theoretically not differ.

Fig. 8 displays the mean plasma levodopa concentration–time profiles derived from PBPK models integrating healthy adult- and individual PD patient *in vitro* release data, respectively, in comparison with the observed average data from the two pharmacokinetic *in vivo* studies for Nacom® ER [39,87]. Values for the observed area under the curve (AUC), c_{max}, and t_{max} were available from the study by LeWitt *et al.* (Table 7). These numerical data were not provided by Bowes and co-workers. Therefore, for the Bowes study, c_{max} and t_{max} were graphically determined from the *in vivo* profiles and the corresponding AUC was calculated using PK-Sim®. Likewise, for the predicted plasma concentration–time profiles, the values for c_{max}, t_{max}, and AUC were calculated using PK-Sim®.

The PBPK models established on the basis of patient-specific *in vitro* release data allowed the best prediction of the average *in vivo* plasma concentration–time profile and pharmacokinetic parameters from both clinical trials. When aiming to replicate average plasma profiles and pharmacokinetic parameters from the LeWitt *et al.* study, *in silico* simulations using *in vitro* release data from experiments mimicking a GI passage with continuous fluid contact in a PD patient with moderate GI motility (PD 4, Fig. 8A) provided the best fit with the observed plasma profiles.

The study population enrolled in the LeWitt *et al.* study covered almost all PD disease stages, but most of the PD patients were assigned to mild/moderate PD. Thus, it could be argued that these PD patients have a rather moderate impairment of GI motility compared to average healthy adults, which would be in good agreement with the PBPK modeling results.

To replicate the results of the Bowes study, simulations with the best matches between simulated and observed data were obtained for both dosing strengths using PBPK models incorporating *in vitro* release data

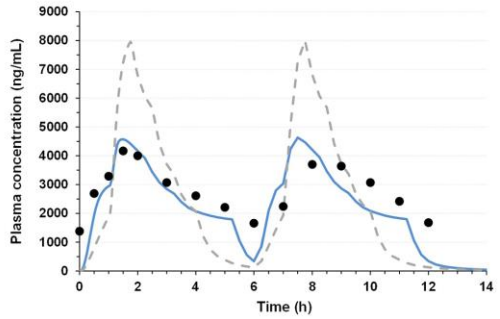

Fig. 9. Comparison of observed and predicted plasma levodopa concentration–time profiles following fasted administration of Rytary® (dose of 735 mg) in the study by Hauser *et al.* Single data points represent observed data, solid lines represent simulations with Parkinson's disease (PD) patient-specific dissolution data and dashed lines represent simulations with healthy adult dissolution data.

from experiments mimicking a GI passage with continuous fluid contact in a PD patient with high GI motility (PD 1). Fig. 8B displays the results for the 400 mg dose study. The authors of the study provided information on the duration, but unfortunately not the severity of the disease of the PD patients participating in the study. Therefore, the extent of GI impairment in the study participants can only be speculated. Since, comparing all patient-specific *in vitro* models, the parameters used to simulate GI conditions for the PD 1 patient were closest to those employed to simulate GI conditions in an average healthy adult, the results could potentially indicate that the study participants were at a PD stage that did not yet tremendously impair GI functions. However, when attempting to reproduce the results from the two Nacom® ER studies with PBPK models that incorporate *in vitro* release data obtained with the *in vitro* model(s) mimicking a GI passage in average healthy adults, plasma profiles that were characterized by wide fluctuations in levodopa plasma concentrations with elevated AUC and c_{max} values were obtained (Table 7). These results highlight the importance of considering patient-specific parameters when building predictive *in vitro* and *in silico* models.

3.3.2. *Rytary®*

The pharmacokinetics of levodopa in PD patients after multiple doses of Rytary® was described in a study performed by Hauser *et al.* [92]. In this study, subjects, most of whom were assigned to H&Y stages 2 or 3, received two doses of Rytary® according to an individualized levodopa dosing schedule (one Rytary® capsule á 245 mg, two Rytary® capsules á 490 mg, three Rytary® capsules á 735 mg). The first study dose was administered to subjects in the fasting state in the morning, and the second dose was administered six hours later. Whereas in the Hauser study dosing strengths differed among the patients, in the *in vitro* release experiments the Rytary® formulation with 195 mg levodopa was studied. The rationale for this is that one of the goals of the *in vitro* study was to allow direct comparison of the release data from Nacom® ER and Rytary®, so equal doses of levodopa should be used whenever possible. Since Rytary® is a multiparticulate formulation, this did also not pose a problem because the various commercial dosages differ only in the number of pellets in the capsules administered, not in the formulation as such. In building the PBPK model to represent the reported average levodopa plasma concentration–time profile of the *in vivo* study, it was assumed that the release profile of the multiparticulate formulation is independent of the amount of multiparticles administered. Accordingly, *in vitro* dissolution data from experiments with the 195 mg Rytary® formulation were incorporated into all PBPK models.

Fig. 9 depicts the observed and predicted plasma concentration–time profiles for Rytary® containing 735 mg levodopa administered in fasted state conditions. The pharmacokinetic parameters c_{max} and t_{max} were graphically determined from the *in vivo* profiles as these data were not reported in the literature [66]. The values for c_{max} and t_{max} of the computed profiles as well as the AUCs of the observed and predicted plasma concentration–time profiles were calculated using PK-Sim® (Table 8). Simulations with the best agreement of predicted with observed plasma concentration–time profiles were obtained with a PBPK model based on patient-specific *in vitro* release data, i.e., when mimicking a GI passage with intermittent fluid contact in a PD patient with high GI motility (PD 2). This finding is in good agreement with observations made for the *in vivo* GI transit of multiparticulate formulations. Multiparticulates are not emptied from the stomach as a bolus, but since individual pellets/granules can usually pass the pylorus unimpeded, at least part of the administered dose usually appears in the small intestine more quickly on average than after application of a monolithic dosage form. Moreover, a relatively constant small intestinal passage is often observed for multiparticulates [98]. In all dosing scenarios, the PD patient-specific simulations demonstrated a closer fit to the *in vivo* plasma concentration–time data than such obtained with *in silico* models incorporating *in vitro* release data obtained by mimicking a GI transit in average healthy adults. The latter simulations were characterized by under- and overprediction of the observed data represented by high peak-to-trough fluctuations in plasma concentrations (Fig. 9), whereas the simulated profiles obtained by integrating patient-specific *in vitro* release data were in quite good agreement with the observed c_{max} and t_{max} data (Table 8).

3.3.3. *Madopar® Depot*

With the aim of studying the pharmacokinetic *in vivo* performance of this drug product in PD patients, Malcolm *et al.* administered two Madopar® Depot capsules of 100 mg levodopa to fasting PD patients (without information on disease stage) and reported a mean plasma levodopa concentration–time profile [88].

Stocchi *et al.* also reported a mean plasma concentration–time profile of levodopa, which resulted from administration of a single morning dose of two Madopar® Depot capsules to three PD patients with marked fluctuations in motor performance in fasting conditions [36]. No further information regarding disease stage of these patients was reported.

As depicted in Fig. 10A, when aiming to replicate results of the study of Malcolm *et al.*, simulations with the best fit to the observed data were obtained with *in vitro* release data from experiments targeted to mimic a GI passage with intermittent fluid contact and long residence times in 'dry' sections of the GI tract in a PD patient with low GI motility (PD 10). Data from PD patient-specific dissolution experiments mimicking a GI

Table 8
Summary of observed and predicted pharmacokinetic data of Rytary®.

	Dose strength (mg)	$c_{max\ (0\text{-}6h)}$ (ng/mL)	$t_{max\ (0\text{-}6h)}$ (min)	$AUC_{(0\text{-}6h)}$ (ng*h/mL)	$c_{max\ (6\text{-}12h)}$ (ng/mL)	$t_{max\ (6\text{-}12h)}$ (min)	$AUC_{(6\text{-}12h)}$ (ng*h/mL)
Observed[1]	735	4170	90	17,140	3700	480	16,740
Predicted[2] **using PD patient-specific dissolution data**	735	4571	87	14,808	4633	450	15,127
Predicted[2] **using average healthy adult dissolution data**	735	7960	105	16,226	7992	465	16,429

[1] Data calculated with graphical data from literature [66].
[2] Data calculated from simulated concentration–time profiles using PK-Sim®.

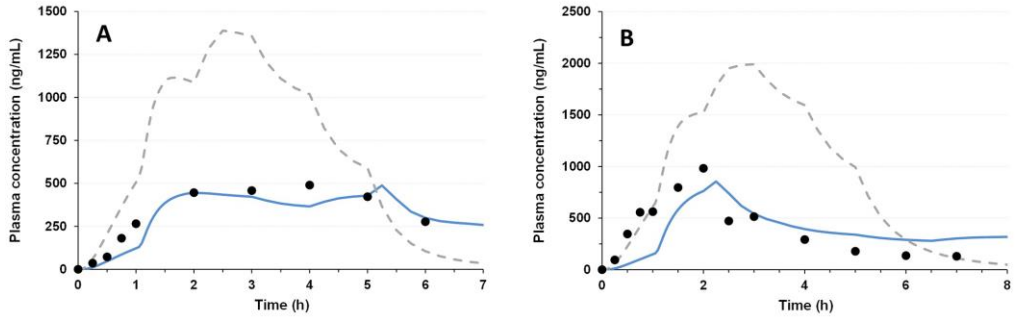

Fig. 10. Comparison of observed and predicted plasma levodopa concentration–time profiles following fasted administration of Madopar® Depot in the studies of (A) Malcolm *et al.*, and (B) Stocchi *et al.* Single data points represent observed data, solid lines represent simulations with Parkinson's disease (PD) patient-specific dissolution data and dashed lines represent simulations with healthy adult dissolution data.

Table 9
Summary of observed and predicted pharmacokinetic data for Madopar® Depot.

Malcolm *et al.* [88]	**Dose strength (mg)**	**c_{max} (ng/mL)**	**t_{max} (min)**	**$AUC_{(0\text{-}6h)}$ 8(ng*h/mL)**
Observed[1]	200	490	240	2192
Predicted[3] **using PD patient-specific dissolution data**	200	488	315	2020
Predicted[3] **using average healthy adult dissolution data**	200	1389	150	4657
Stocchi *et al.* [36]	**Dose strength (mg)**	**c_{max} (ng/mL)**	**t_{max} (min)**	**$AUC_{(0\text{-}7h)}$ (ng*h/mL)**
Observed[2]	200	982	120	2641
Predicted[3] **using PD patient-specific dissolution data**	200	855	135	2718
Predicted[3] **using average healthy adult dissolution data**	200	1990	180	7092

[1] Numerical data from literature [88].
[2] Data calculated with graphical data from literature [36].
[3] Data calculated from simulated concentration–time profiles using PK-Sim®.

passage with intermittent fluid contact and long residence times in 'dry' sections also provided the best fit when aiming to replicate results of the study of Stocchi and co-workers. However, in that case, results from the PD 5 setup, mimicking a PD patient with moderate GI motility were most suitable (Fig. 10B). Values for the AUC, c_{max}, and t_{max} of the computed concentration–time profiles were determined by using PK-Sim® for both studies and are stated in Table 9. For both *in vivo* data sets simulations with incorporated *in vitro* release data from experiments mimicking GI conditions in healthy adults resulted in plasma concentration–time profiles with poor fit and rather elevated AUC und c_{max} values (Fig. 10). Unfortunately, as the authors of both studies did not provide sufficient information on the background of the study participants, no conclusive statements can be made about a correlation between the disease state of the study participants and the *in vitro* motility profiles applied. It can, however, be argued that the PBPK modeling results show that, due to the slow drug release characteristics of the Madopar® Depot formulation and its (theoretical) gastroretentive behavior, *in vitro* drug release models mimicking a moderate/slow GI passage with prolonged residence times in the different GI segments of the PD patient provide a more realistic test scenario than those mimicking a GI passage in a healthy adult.

Interestingly, although in both clinical trials the study participants were administered two capsules of Madopar® Depot with a total dose of 200 mg of levodopa, the observed c_{max} in the published case study by Stocchi *et al.* was increased twofold compared to that observed in the Malcolm *et al.* study, whereas the t_{max} was considerably lower compared to that reported by Malcolm and colleagues. This observation supports the hypothesis that, at least in some cases, Madopar® Depot capsules may not provide reliable gastroretention associated with slow and controlled release, but rather may be emptied earlier than intended and, in some cases, fail to remain intact for the intended time.

3.3.4. Overall discussion

When evaluating the results of the *in silico* simulations, results obtained using *in vitro* data from patient-specific experiments proved to be most suitable for predicting average plasma levels in the corresponding study populations. However, depending on the dosage form investigated, the *in vivo* study design and the PD patients included in the study, an individual setup proved to be the most appropriate in each case. Overall, results of the study suggest that both the formulation type itself (e.g., multiparticulate, monolithic) and the expected *in vitro* release behavior should be carefully considered when developing patient-specific *in vitro* release experiments for novel formulations.

The modified Reciprocating Cylinder apparatus allows the simulation of various individual GI motility profiles and was specifically designed for mimicking characteristic GI conditions in special patients such as PD patients. In this study, this new test system was used for the first time in combination with new patient-specific test protocols aimed to simulate GI conditions in PD patients. Incorporation of the *in vitro* release data obtained in the patient-specific *in vitro* release experiments into patient-specific PBPK models provided promising results. However, as no individual *in vivo* plasma concentration–time profiles are available for the investigated dosage forms, the new *in vitro* and *in silico* models could not yet be fully validated.

As part of a validation of the PD-specific *in vitro* and *in silico* models presented in this study, one would want to compare *in vivo* and *in vitro* plasma concentration–time profiles for each of the investigated (or other) levodopa ER formulations, provided, that i) the formulations

were administered to PD patients ii) with known disease status, that iii) individual patient information was available for implementation in the *in vitro* and *in silico* models and that iv) individual *in vivo* plasma concentration–time profiles would be available for correlation. One of the goals of such validation is to determine whether *in vivo* release in individual patients can be predicted using a patient-specific *in vitro* model. Another objective is to establish a correlation between the disease state of the PD patient, the *in vitro* release profile obtained in a patient-specific *in vitro* model accounting for this disease state, and the individual *in vivo* levodopa plasma levels obtained after administration of the dosage form in question to this patient. However, for levodopa ER products investigated in this study only mean plasma concentration–time profiles in PD patients were available in the literature. Two studies did report individual *in vivo* profiles, but these could not be linked to individual patients and/or no further patient information such as disease severity was provided. In general, published *in vivo* studies often lack such patient-related information or provide only superficial descriptions. For this reason, predicted plasma profiles were compared with observed mean plasma profiles to determine the best matches. The use of average data certainly cannot provide a complete validation of the developed models but should be considered as a first important step in this direction. The results of our study show that it is important to consider GI motility, passage times, and, at least to some extent, varying contact with GI fluids. However, for the future, individual *in vivo* plasma profiles together with information on the physical condition of the individual PD patient are needed to optimize the predictive power of the *in vitro* drug release- and PBPK models and, based on this, to develop further models for other patient groups with altered GI physiology that can then be used in the development of patient-specific formulations.

4. Conclusions

In this study, *in vitro* release models mimicking GI conditions specific to PD patients were successfully used in conjunction with *in silico* PBPK models to predict the *in vivo* performance of three marketed levodopa ER preparations. By utilizing current knowledge of relevant intraluminal absorption conditions in PD patients in the development of novel *in vitro* release models, it was possible to generate patient-specific *in vitro* release profiles that could be used to estimate whether the specific GI conditions in PD patients influence drug release. The incorporation of patient-specific *in vitro* release profiles into PBPK models provided simulated levodopa plasma concentration–time profiles for each of the formulations tested, which provided much better agreement with published average plasma profiles in PD patients than those obtained when incorporating release data from *in vitro* models developed to simulate GI conditions in average healthy adults.

Since there are no published individual *in vivo* plasma profiles of levodopa after administration of the investigated formulations to PD patients, the predicted plasma profiles were compared with the observed mean plasma profiles in PD patients. This modeling approach can certainly only represent a first step in terms of validating the *in vitro* drug release- and PBPK models. Future correlations should certainly comprise the use of individual plasma profiles together with essential information about the disease state of the corresponding patient. Although further work is needed to validate the presented models, the results of the present study have impressively demonstrated that *in vitro* drug release models that mimic different patient-specific GI conditions, with particular attention to motility scenarios, are a promising tool for predicting drug release in PD patients. In the future, such patient-specific *in vitro* models may be of particular importance in determining the most appropriate drug release pattern of novel formulations for PD patients.

In addition, patient-specific PBPK models generally offer the possibility to investigate the effects of variations in formulation design or variability in GI physiology on *in vivo* plasma profiles without the immediate need for extensive additional *in vivo* or *in vitro* studies. The methodological approach discussed in the present study will therefore hopefully gain wider application in the future and can contribute significantly to better, effective, and safe drug therapy for PD patients but also other specific patient groups.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2022.09.015.

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E. Wollmer, S. Klein. *Patient-specific in vitro drug release testing coupled with in silico PBPK modeling to forecast the in vivo performance of oral extended-release levodopa formulations in Parkinson's disease patients.* European Journal of Pharmaceutics and Biopharmaceutics, 2022, 180: 101–118.

Begleitende Publikation

Während des Promotionsprojekts wurde die folgende Publikation ebenfalls veröffentlicht, die jedoch in der vorliegenden Arbeit nicht berücksichtigt wurde:

E. Wollmer, A.-L. Ungell, J.-M. Nicolas, S. Klein. *Review of paediatric gastrointestinal physiology relevant to the absorption of orally administered medicines.* Advanced Drug Delivery Reviews, 2021, 181: 114084.

Kongressbeiträge

Vorträge:

E. Wollmer, L. Freerks, A.-E. Hetberg, F. Karkossa, G. Neal, M. J. Whitaker, D. Margetson, J. Porter, S. Klein. *Dissolution and compatibility of hydrocortisone granules following exposure to common paediatric administration fluids and food matrices*.

9th Conference of the European Paediatric Formulation Initiative (EuPFI), September 2017, Warschau, Polen

E. Wollmer, S. Klein. *A novel patient-specific in vitro drug release model incorporating physiological features relevant to oral drug absorption in Parkinson patients*.
2nd UNGAP spring meeting, Februar 2019, Sofia, Bulgarien

Poster:

E. Wollmer, G. Neal, M. J. Whitaker, D. Margetson, S. Klein. *Biorelevant dissolution and compatibility of hydrocortisone granules following exposure to water, breast-, whole- and artificial (formula) milk.*
8th Conference of the European Paediatric Formulation Initiative (EuPFI), September 2016, Lissabon, Portugal

E. Wollmer, L. Freerks, A.-E. Hetberg, F. Karkossa, G. Neal, M. J. Whitaker, D. Margetson, S. Klein. *Dissolution and compatibility of hydrocortisone granules following exposure to common paediatric administration fluids and food matrices.*
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E. Wollmer, S. Klein. *Biorelevant drug release experiments for better understanding the in vivo performance of currently marketed levodopa products in Parkinson patients*.
11th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, März 2018, Granada, Spanien

E. Wollmer, J.-M. Nicolas, A.-L. Ungell, S. Klein. *A literature review of paediatric gastrointestinal parameters that could support the development of predictive paediatric physiologically-based pharmacokinetic (PBPK) models.*
10th Conference of the European Paediatric Formulation Initiative (EuPFI), September 2018, London, Großbritannien

E. Wollmer, S. Klein. *A novel patient-specific in vitro drug release model incorporating physiological features relevant to oral drug absorption in Parkinson patients*.
2nd UNGAP spring meeting, Februar 2019, Sofia, Bulgarien

E. Wollmer, S. Klein. *A novel patient-specific in vitro drug release model to predict in vivo drug release in Parkinson patients*.
3rd European Conference on Pharmaceutics, März 2019, Bologna, Italien

E. Wollmer, S. Klein. *A novel patient-specific in vitro drug release model that allows assessing the variability of in vivo drug release in patients suffering from Parkinson´s Disease*.
33th AAPS Annual Meeting and Exposition, November 2019, San Antonio, USA

Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

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Erik Wollmer

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