

***In vivo- und In vitro-Untersuchungen zum
Einfluss der Magenentleerung auf das
Zerfalls- und Freisetzungerverhalten
schnell freisetzender Arzneiformen
nach nüchterner Applikation***

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Abkürzungsverzeichnis

ACC	N-Acetylcystein
BCS	Biopharmaceutics Classification System
DOFTA	Dynamic Open Flow-Through Test Apparatus
EMA	European Medicines Agency
FDA	Food and Drug Administration
GI	Gastrointestinal
HPLC/MS	High Pressure Liquid Chromatography/ Mass Spectrometry
HPMC	Hydroxypropylmethylcellulose
STT	Salivary Tracer Technique
MRT	Magnetresonanztomographie/ Magnetresonanztomograph
MMC	Migrerender Motorischer Komplex
PBPK	Physiologie-basierte Pharmakokinetik
TDM	Therapeutisches Drug Monitoring
VE	Voll entsalzt

1 Einleitung und Zielstellung

1.1 Einleitung

Für zahlreiche Arzneimittel wird die Anwendung auf nüchternen Magen empfohlen. Die Rationale für diese Einnahmeempfehlung kann ganz unterschiedlich und unter anderem auf formulierungsbedingte Aspekte zurückzuführen sein. Um die Gefahr einer frühzeitigen Auflösung zu minimieren, wird etwa für magensaftresistente Arzneiformen die Einnahme auf leeren Magen empfohlen. Interessanterweise findet man diesen Hinweis in der Vergangenheit auch vermehrt bei oralen Onkologika.

Die Einnahmeempfehlung oraler Onkologika ist vielfach auf nüchternen Magen, obwohl diese Substanzen teilweise eine erhöhte Bioverfügbarkeit nach Nahrungsaufnahme (positiver *Food Effect*) zeigen. Solch eine Empfehlung widerspricht dem gängigen Prinzip einer möglichst hohen Bioverfügbarkeit bei der oralen Pharmakotherapie und wird in dieser Form nur bei wenigen Indikationsgebieten von Zulassungsbehörden wie der amerikanischen Food and Drug Authority (FDA) empfohlen.¹ Onkologen wie Kang und Ratain argumentieren dagegen, dass bei postprandialer Einnahme oraler Onkologika mit positivem *Food Effect* die benötigte Dosis reduziert werden könnte, was zu ökonomischen Einsparungen führen würde und zudem die Chance böte, den Anteil an nicht resorbiertem Arzneistoff im Organismus zu reduzieren.² Im Fall von Abirateron, einem Steroidhormon zur Behandlung des kastrationsresistenten Prostatakrebses, widerspricht der Hersteller dieser Argumentation.³ Todd und Kollegen begründen die Empfehlung auf nüchternen Magen mit dem ausgeprägten *Food Effect* der Substanz (10-fach höherer Plasmaspiegel nach Einnahme fettreiche Mahlzeit), welcher bei mangelnder Adhärenz zu schweren Nebenwirkungen infolge der hohen Variabilität führen kann. Weiterhin argumentieren Todd und Kollegen, dass die nüchterne Einnahme von Arzneimitteln grundsätzlich zu reproduzierbaren Plasmaspiegeln führe und dementsprechend genauer kalkulierbar sei als die postprandiale. Aber ist das tatsächlich der Fall?

Die physiologische Variabilität verschiedener gastrointestinaler Parameter und deren Einfluss auf das Zerfalls- und Freisetzungerverhalten bei der nüchternen Einnahme schnell freisetzender Arzneiformen wird allgemein als gering erachtet. Beispielsweise werden im Rahmen klinischer Phase-1-Studien häufig Pilotstudien mit verschiedenen Formulierungen desselben Arzneistoffes durchgeführt. Dabei wird zumeist die Dosis variiert, um die optimale Wirkstärke zu finden. Es ist aber ebenso möglich, dass die Formulierung weiter optimiert wird, um etwa die Bioverfügbarkeit zu erhöhen oder die Einnahme des Arzneimittels zu erleichtern. Dies kann zum Beispiel durch den Wechsel von einer Tablette zu einer Kapsel oder von einer Hartgelatinekapsel zu einer Hydroxypropylmethylcellulose (HPMC)-basierten Kapsel geschehen. Im Rahmen der Formulierungsentwicklung kann jedoch nicht jede Änderung der Formulierung direkt in klinischen Studien auf ihre Auswirkungen hin überprüft werden. Zu diesem Zweck kommen zumeist *In vitro*- oder Tiermodelle zum Einsatz. Es stellt sich dabei allerdings die Frage, inwieweit die Daten aus solchen Modellen auf den Menschen übertragbar sind und ob diese Modelle überhaupt in der Lage sind, mögliche Veränderungen im *In vivo*-Freisetzungerverhalten vorherzusagen.

Die vorliegende Arbeit wird sich im Folgenden mit der Beleuchtung der Frage beschäftigen, wie sich schnell freisetzende Arzneiformen im nüchternen Magen verhalten und wie sich dies, vor der Durchführung klinischer

Studien, vorhersagen lässt. Für die Beantwortung dieser Frage ist ein detailliertes Verständnis der Physiologie des oberen Gastrointestinal (GI)-Traktes von essentieller Bedeutung. Insbesondere die physiologischen Vorgänge im Magen, dem wahrscheinlichen Zerfalls- und Freisetzungsort, für nüchtern eingenommene, schnell freisetzende Arzneimittel sind in dieser Hinsicht hervorzuheben. Im folgenden Abschnitt wird daher kurz die Anatomie und Physiologie des Magens beschrieben, um relevante Einflussfaktoren für die nüchterne Applikation von Arzneimitteln zu identifizieren.

Anatomie und Physiologie des Magens

Wie in Abbildung 1 ersichtlich ist, lässt sich der Magen, makroskopisch betrachtet, in die folgenden Abschnitte einteilen: Mageneingang (*Cardia*), Magengrund (*Fundus*), Magenkörper (*Korpus*), Erweiterung vor dem Magenausgang (*Antrum*) und den Magenpfortner (*Pylorus*). Die Muskulatur des Magens besteht aus Muskelfasern, welche ringförmig, entlang und diagonal zu distalen Abschnitten des Magens verlaufen können. Im Bereich des Pylorus ist die Dicke der Ringmuskulatur verstärkt, während die Längsmuskulatur dies im Bereich der Kurvaturen ist.

Die physiologischen Aufgaben des Magens bestehen in der temporären Speicherung, Prozessierung und Weitergabe aufgenommener Flüssigkeiten und Mahlzeiten. Der Magen ist in der Lage, in proximalen Abschnitten zu relaxieren, um große Volumina an Flüssigkeiten und Mahlzeiten aufnehmen zu können.⁴ Die Aufnahme kalorischer Nahrungsbestandteile führt über verschiedene Feedback-Mechanismen zudem zur Kontraktion des Pylorus. Damit wird sichergestellt, dass der Speisebrei so lange im Magen verbleibt, bis dieser ausreichend verdaut worden ist. Da die Kontraktion des Pylorus hauptsächlich auf Feedback-Mechanismen zurückzuführen ist, die durch enthaltene Nährstoffe wie etwa Glucose oder Fettsäuren im Dünndarm ausgelöst werden, können akalorische Flüssigkeiten den Magen in der Regel schneller passieren.⁵ Zudem ist zu beachten, dass es einen physiologischen Mechanismus gibt, der es erlaubt, dass selbst nach Aufnahme hochkalorischer und fettreicher Mahlzeiten akalorische Flüssigkeiten den Speisebrei umfließen können. Dementsprechend können diese ähnlich schnell wie im nüchternen Zustand entleert werden.⁶ Dieses Phänomen wird als „Magenstraße“ bezeichnet und wurde bereits Anfang des 20. Jahrhunderts bei Hunden nachgewiesen und in den letzten Jahren mittels Magnetresonanztomographie (MRT) ebenso im Menschen gezeigt.^{6,7}

Als treibende Kraft für die Magenentleerung von akalorischen Flüssigkeiten nach nüchtern Gabe wird im Allgemeinen die Druckdifferenz zwischen dem Duodenum und dem proximalen Magen angesehen.^{8,9} Zusätzlich wird diskutiert, ob der Migrerende Motorische Komplex (MMC, auch myoelektrischer Motorkomplex) ebenso einen Einfluss auf die Flüssigkeitenentleerung aus dem nüchternen Magen hat.¹⁰

Der MMC setzt sich aus mehreren Phasen propulsiver motorischer Aktivität der Magen- und Dünndarmwand zusammen. Diese Aktivitäten laufen zwischen den Mahlzeiten (interdigestiv) grundsätzlich in drei Phasen ab

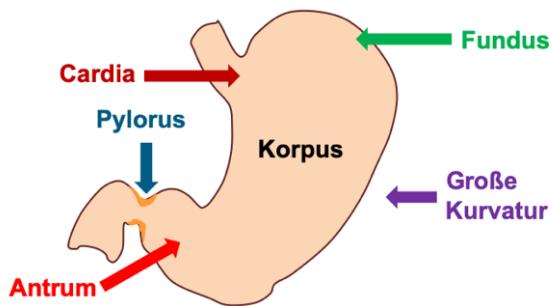


Abbildung 1 Anatomie des Magens.

und dienen vor allem der Säuberung des Magens von unverdaulichen Nahrungsbestandteilen. Die Aufnahme von Nahrung oder kalorischen Flüssigkeiten führt üblicherweise zur Unterbrechung des MMC.¹¹ Die Phasen des MMC unterteilen sich in Zeiträume von unterschiedlicher Dauer mit geringer (Phase 1), mittlerer (Phase 2) und hoher peristaltischer Aktivität (Phase 3). Die Kontraktionswellen in Phase 3 werden auch *Housekeeper Waves* genannt.^{12,13} Üblicherweise dauert die Phase 1 etwa 40 – 60 min, Phase 2 etwa 30 – 45 min und die Phase 3 nur etwa 10 – 15 min.^{14,15} Das Auftreten der *Housekeeper Waves* wird als Abschluss eines MMC Zyklus betrachtet und resultiert üblicherweise in einer vollständigen Entleerung des Magens.^{16,17}

Der Magen sezerniert fortlaufend 2 – 3 L Magensaft pro Tag.¹⁸ Dieser Magensaft besteht im Wesentlichen aus Salzsäure, Muzinen, Pepsinogenen, Intrinsic-Faktor und Hydrogencarbonat. Dabei variiert die Zusammensetzung in Abhängigkeit der Stimulation der Magensaftsekretion. Unstimuliert wird hauptsächlich Hydrogencarbonat und Muzin mit einer Sekretionsrate von 1 - 2 mL/min gebildet.¹⁹ Nach Nahrungsaufnahme bilden die Belegzellen des Magens hingegen verstärkt Salzsäure, die unter anderem die Verdauung der aufgenommenen Mahlzeit unterstützt.^{20–22}

Nach längerem Fasten ist der Magen in der Regel kollabiert und in seinen Dimensionen gegenüber dem postprandialen Magen deutlich reduziert. Der noch vorhandene Inhalt bildet eine Flüssigkeitsansammlung im Magen, die als Nüchternvolumen bezeichnet wird. Dieses Volumen beträgt im Mittel 10 – 50 mL, unterliegt aber erheblichen intra- und interindividuellen Schwankungen.^{23–25} Bei der Einnahme eines Arzneimittels auf nüchternen Magen bildet dieses Nüchternvolumen zusammen mit der zur Arzneimittelgabe eingenommenen Flüssigkeit das Volumen, welches dem Arzneimittel zum Zerfall und zur Freisetzung zur Verfügung steht. Somit sind das Volumen und die Zusammensetzung sowohl der Magenflüssigkeit als auch der eingenommenen Flüssigkeit für die orale Arzneimitteltherapie relevant.

Physiologische Aspekte bei der Einnahme oraler Arzneiformen

International anerkannte Richtlinien zur Durchführung von Arzneimittelstudien werden, unter anderem von der Europäischen Arzneimittelagentur (EMA) und ihrem amerikanischen Pendant, der FDA, erstellt. Die EMA schreibt im Rahmen einer klinischen Prüfung eine begleitende Wassergabe zur Arzneimitteleinnahme in einer Größenordnung von 150-240 mL vor.^{26,27} Die FDA wird an dieser Stelle konkreter und empfiehlt ein Volumen von 240 mL.²⁸ Aus diesem Grund werden die meisten Studien zur Bioverfügbarkeit und Bioäquivalenz oral applizierter Arzneimittel mit einem Volumen von 240 mL durchgeführt. Unter Berücksichtigung des Nüchternvolumens, das nach einer Fastenzeit von 10 h im Magen vorliegt, ergibt sich somit ein initiales Volumen an Flüssigkeit von etwa 250 – 290 mL. Allerdings kommt es direkt nach Flüssigkeitseinnahme bereits zur Entleerung von Flüssigkeit aus dem Magen. Verschiedene Arbeitsgruppen konnten zeigen, dass die Magenentleerung einer akalorischen Flüssigkeit ebenfalls ein stark variabler Prozess ist. Mit Hilfe bildgebender Verfahren wie der MRT kann die gastrale Flüssigkeitseentleerung hochauflöst dargestellt und das luminal vorliegende Volumen berechnet werden.^{6,25,29} In der Abbildung 2 sind die mittels MRT bestimmten Flüssigkeitsvolumina über die Zeit im Magen von 12 Probanden nach nüchternner Einnahme von 240 mL Wasser dargestellt.³⁰ Es zeigt sich, dass das eingenommene Wasser innerhalb von 5 – 60 min entleert wird, während ein Proband nach der initialen Entleerung von etwa 50-100 mL kaum noch eine Entleerung im

Beobachtungszeitraum zeigt. Im Mittel kann aber konstatiert werden, dass die eingenommenen 240 mL Wasser durchschnittlich nach circa 45 min aus dem Magen entleert sind.

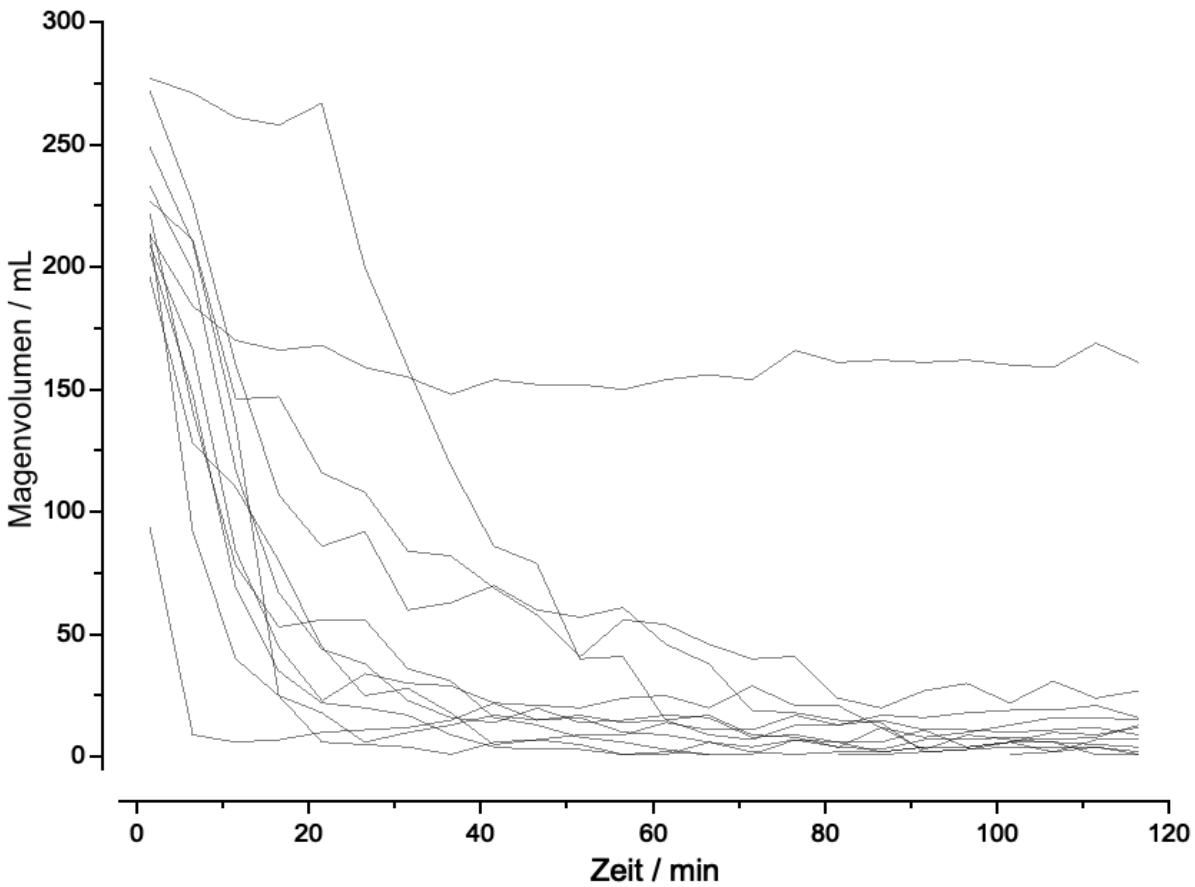


Abbildung 2 Flüssigkeitsvolumina über die Zeit im Magen von 12 Probanden nach nüchternner Einnahme von 240 mL Wasser. Modifiziert nach Paulick et al.³⁰

Die im Magen vorliegende Flüssigkeit stellt für eine oral applizierte Arzneiform üblicherweise das Freisetzungsmittel dar. Kommt es demnach zu einem späten Zerfall der Arzneiform, ist ein Großteil des parallel zugeführten Wassers bereits entleert und steht dementsprechend nicht mehr als Freisetzungsmittel zur Verfügung. Die Magenentleerung kann daher einen relevanten Einfluss auf die Pharmakokinetik einer schnell freisetzenden Arzneiform haben. Besonders drastisch ist dieser Einfluss bei gut permeablen und gut wasserlöslichen Arzneistoffen, deren Pharmakokinetik lediglich durch die Freisetzung aus der Arzneiform sowie die Magenentleerung beeinflusst wird.

Neben der Magenentleerung und dem vorliegenden Volumen des Mageninhaltes gibt es noch weitere gastrale Parameter, die für die Freisetzung von oral applizierten Arzneiformen relevant sind. Zu diesen zählen etwa die Motilität inklusive luminal auftretender Drücke sowie Temperaturen und pH-Werte.³¹ Zur Charakterisierung dieser Parameter im humanen GI-Trakt hat sich insbesondere der Einsatz telemetrischer Kapseln in der Vergangenheit als nützlich erwiesen.³² Diese können mittels miniaturisierter Messtechnik Parameter wie pH, Temperatur und Druck in relativ hoher zeitlicher Auflösung aufnehmen. Die aufgezeichneten Messwerte werden nach Einnahme der Kapsel durch einen Probanden oder Patienten an einen mobilen Datenschreiber

gesendet. Systeme wie die Heidelberg pH-Kapsel und die Intellicap® sind in der Lage, die Temperatur und den pH-Wert hochauflöst zu messen.^{33–35} Zusätzlich zu diesen Parametern können mit der SmartPill® ferner Druckmessungen vorgenommen werden.^{36–38} Aus diesem Grund kann die SmartPill® klinisch zur Aufzeichnung und zur Diagnose von Motilitätsstörungen wie der Obstipation oder der Gastroparese angewandt werden.^{39,40}

In der Biopharmazie werden telemetrische Kapseln hauptsächlich dazu genutzt, um neben den genannten Parametern zusätzliche Informationen über den Transit eines unverdaulichen, monolithischen Objekts durch den GI-Trakt zu erhalten. Es wird davon ausgegangen, dass sich diese Erkenntnisse auf bestimmte Arzneiformen mit ähnlichen Eigenschaften, wie beispielsweise Matrixtabletten oder magensaftresistente Tabletten, übertragen lassen. Der Aufenthaltsort der Kapsel wird in der Regel über ein charakteristisches pH-Profil bestimmt.³³ Während im Magen eher saure pH-Werte im Bereich von pH 1 – 3 vorherrschen, steigt der pH Wert vom proximalen bis zum distalen Dünndarm von pH 6 – 6,5 im Duodenum kontinuierlich auf etwa pH 8 – 8,5 im terminalen Ileum an.^{41,42} Speziell der Übergang der Kapsel vom Magen zum Dünndarm kann eindeutig ermittelt werden, da hier ein ausgeprägter pH-Sprung vom stark Sauren zum annähernd Neutralen stattfindet. Im Falle der SmartPill® kann der Transit vom Magen zum Dünndarm auch anhand charakteristischer Druckereignisse erkannt werden. Vor der Passage der Kapsel durch den Pylorus ins Duodenum kommt es häufig zu Drücken von über 300 mbar.^{38,43} Diese werden wahrscheinlich dadurch erzeugt, dass die peristaltischen Wellen des MMC die Kapsel gegen den geschlossenen Pylorus drücken.³⁸ Nur wenn die Koordination zwischen Pylorusöffnung und peristaltischer Welle gegeben ist, kann die Magenentleerung eines solch großen Objektes erfolgen. Da die SmartPill® pH-Werte und Drücke simultan misst, kann durch die Kombination beider Profile die Aussagekraft der Transitsdaten erhöht werden.

In vivo-Methoden zur Charakterisierung des Verhaltens oraler Arzneiformen im humanen Gastrointestinaltrakt

Der Einsatz von *In vivo*-Methoden wie der MRT oder telemetrischen Kapseln führte zu einem besseren Verständnis der physiologischen Prozesse, die die Arzneimitteltherapie beeinflussen.⁴⁴ Idealerweise werden diese Methoden mit pharmakokinetischen Studien kombiniert, um das *In vivo*-Verhalten einer neuen Formulierung detailliert beschreiben zu können. Die derzeit immer noch gängigste Methode zur Evaluierung neuer Formulierungen ist aber deren Anwendung in klinischen Studien am Menschen.

Erste klinische Testungen von Arzneimitteln erfolgen in Phase-1-Studien („*first in human*“) am Menschen. Dabei wird gesunden Probanden eine definierte Dosis des Arzneimittels verabreicht und anschließend üblicherweise die Arzneistoffkonzentration im Blutplasma bestimmt. Die resultierenden Plasmakonzentrations-Zeit-Profile, auch Plasmaspiegel genannt, können neben dem entsprechenden Einblick in die Pharmakokinetik des Arzneistoffes ebenso wertvolle Informationen über das Verhalten einer Formulierung im humanen GI-Trakt liefern. Zum Beispiel kann bei oraler Gabe einer Kapselformulierung eines gut löslichen und gut permeablen Arzneistoffes (Biopharmaceutics Classification System (BCS)-Klasse 1), der Plasmaspiegel Informationen über die Zerfallszeit der Kapselhülle liefern.⁴⁵

Wenn die Pharmakokinetik eines Arzneistoffes bekannt ist, kann die Applikation eines solchen Arzneistoffes auch dazu genutzt werden, um einen tieferen Einblick in die individuelle Physiologie zu erlangen. So wird beispielsweise die Paracetamol-Absorptions-Technik dazu genutzt, die Magenentleerung zu charakterisieren.⁴⁶

In diversen Studien konnte gezeigt werden, dass bei oraler Administration in Form einer Lösung das Anfluten des Paracetamols im Blutplasma hauptsächlich durch die Magenentleerung bestimmt wird.^{46–48} Ein Nachteil dieser Methode ist jedoch, dass solche Studien in aller Regel unter das Arzneimittelgesetz fallen und aus diesem Grund mit einem erhöhten regulatorischen Aufwand verbunden sind. Zusätzlich bedarf diese Technik der Abnahme von Blutproben, was einerseits invasiv für den Probanden ist und andererseits die Anwesenheit von medizinisch geschultem Personal erfordert.

Um die Blutabnahme im Falle der Paracetamol-Absorptions-Technik zu umgehen, haben Sanaka und Kollegen in einer vergangenen Arbeit eine Alternative zum Nachweis von Paracetamol im Plasma präsentiert. Ihre Idee war es, Paracetamol im Speichel nachzuweisen. Zu diesem Zweck gaben sie den Probanden eine orale Paracetamol-Lösung und verglichen anschließend die Speichelprofile mit gleichzeitig bestimmten Blutplasmaprofilen.⁴⁹ Trotz intensiven Spülens gelang es aber nicht, eine Kontamination des Mund- und Rachenraumes mit Paracetamol zu verhindern. Aus diesem Grund konnten die Paracetamol-Konzentrationen in Speichel und Blut in den ersten 45 min des Experiments nicht miteinander korreliert werden. In diesem Zeitraum findet, wie eingangs festgestellt, allerdings größtenteils die Magenentleerung statt. Nichtsdestotrotz ist der Nachweis von Arzneistoffen im Speichel ein sehr vielversprechender Ansatz, um nicht invasiv klinische Daten über Pharmakokinetik, physiologische Prozesse, oder auch über bestimmte Darreichungsformen zu gewinnen. Bereits in den sechziger Jahren konnte gezeigt werden, dass Arzneistoffe, wie zum Beispiel Sulfonamide, im menschlichen Speichel nachweisbar sind. Dementsprechend stellt Speichel eine interessante Matrix für das Therapeutische Drug Monitoring (TDM) dar.⁵⁰ Für die Nutzung von Speichel im TDM ist es jedoch notwendig, dass eine robuste und reproduzierbare Korrelation zwischen der Arzneistoffkonzentration im Speichel und der Blutplasmakonzentration an freiem Arzneistoff besteht.⁵¹

In vitro-Methoden zur Charakterisierung der Wirkstofffreisetzung

In der Formulierungsentwicklung neuer Arzneimittel ist eine klinische Prüfung nicht in jeder Phase immer möglich und sinnvoll. Es bedarf daher aussagekräftiger *In vitro*-Methoden, die geeignet sind, im Vorfeld Aussagen über das *In vivo*-Verhalten neuer Formulierungen zu treffen. Auf diese Weise lässt sich die Belastung der Probanden und der Einsatz von Ressourcen auf ein akzeptables Maß reduzieren. Diese Methoden sollten jedoch allgemein in der Lage sein, frühzeitig formulierungsbedingte Probleme zu erkennen, um so das Scheitern einer klinischen Prüfung zu verhindern.

Die verwendeten *In vitro*-Methoden sollten in der Lage sein, bestimmte physiologische Bedingungen und Prozesse in geeigneter Weise nachzubilden.⁵² Diesbezüglich existiert bereits eine Vielzahl unterschiedlicher Testsysteme, die sich vor allem im Grad ihrer Komplexität unterscheiden. In der Arbeit wird grob nach zwei Methoden unterteilt, in vereinfachte und komplexe. Modelle die den Anspruch haben, ausgewählte Parameter zu simulieren, sind unter anderem der *Rotating Beaker*, das *Artifical Stomach Duodenal Model*, der *Gastro-intestinal Simulator* und das *Dynamic Gastric Model*.^{53–57} Die genannten Modelle orientieren sich in ihrem Aufbau mitunter an den kompendialen Freisetzungsmethoden, versuchen dabei aber physiologisch relevantere Bedingungen, wie zum Beispiel kleinere Testvolumina oder eine intensivere mechanische Belastung, zu simulieren.

Ein Modell mit einem sehr hohen Grad an Komplexität ist das TNO TIM-1 (Zeist, Niederlande).^{58,59} Dieses Modell kommt ursprünglich aus der Lebensmittelforschung und kann neben Verdauungsprozessen ebenso die Bedingungen in verschiedenen Abschnitten des GI-Traktes simulieren. In diesem Modell wird nicht ein spezifischer Parameter isoliert betrachtet, sondern es wird eine möglichst umfassende und simultane Simulation mehrerer Faktoren angestrebt.

Ein Mittelweg zwischen diesen beiden Ansätzen ist die von Garbacz und Weitschies entwickelte Stresstest-Apparatur. Mithilfe dieser Apparatur ist es möglich, physikalische Parameter wie Hydrodynamik, Bewegung und Druck zu simulieren und ihren Einfluss auf die Freisetzung von Arzneiformen näher zu untersuchen.^{60,61} Elementarer Bestandteil dieses Modells ist ein Ballon, mit dem es möglich ist, definierte Drücke auf eine zu testende Arzneiform auszuüben. Garbacz und Kollegen konnten zeigen, dass biorelevante Druckereignisse, welche zum Beispiel während der Magenentleerung einer Arzneiform auftreten, zum schlagartigen Freisetzen (*dose dumping*) des Wirkstoffes aus einer Matrix-Tablette führen können.⁶² In weiterführenden Arbeiten nutzten Koziolek und Kollegen diesen Ansatz, um eine dynamischere Variante der Stresstest-Apparatur zu entwickeln, die den gefüllten Magen simulieren kann und als *Fed Stomach Model* bezeichnet wurde.⁶³ Diese Vorarbeiten bildeten ebenfalls die Basis der vorliegenden Arbeit.

1.2 Zielstellung

Das grundlegende Ziel der vorliegenden Arbeit war es, dass Freisetzungsvorverhalten von schnell freisetzenden Arzneiformen im nüchternen Magen umfangreicher zu verstehen. Grundsätzlich sollten zu diesem Zweck zwei experimentelle Lösungsansätze parallel verfolgt werden. Der eine Ansatz sollte sich mit der Entwicklung und Anwendung von *In vivo*-Methoden zur Untersuchung des Zerfalls- und Freisetzungsvorverhaltens schnell freisetzender Arzneiformen beschäftigen, während der andere Ansatz die Optimierung und Anwendung eines biorelevanten Freisetzungsvormodells zum Ziel hatte.

Zunächst sollten im Rahmen einer Literaturrecherche und basierend auf Vorarbeiten im Arbeitskreis, relevante Einflussfaktoren für das Freisetzungsvorverhalten von schnell freisetzenden Arzneiformen identifiziert und in einem vorhandenen Freisetzungsvormodell, dem *Dynamic Open Flow-Through Test Apparatus*, abgebildet werden. Dieses Modell sollte im Rahmen eines Kooperationsprojektes mit einem Industriepartner für biorelevante Freisetzungsvoruntersuchungen zweier N-Acetylcystein-Formulierungen genutzt werden. Die Ergebnisse sollten anschließend mit den *In vivo*-Daten der Formulierung verglichen werden, die im Rahmen einer Bioäquivalenzstudie erhoben wurden. Die in dieser Studie gewonnenen Erkenntnisse sollte dazu genutzt werden, um das Modell entsprechend zu erweitern und zu optimieren.

Parallel zur Optimierung des *In vitro*-Modells, sollte eine *In vivo*-Methode entwickelt werden, die es erlaubt, sowohl die Magenentleerung als auch das Zerfallsverhalten von Arzneiformen unter Nutzung von Speichelkonzentrationen in gesunden Probanden zu charakterisieren. Zu diesem Zweck sollte ein Marker gefunden werden, der grundsätzlich unbedenklich ist und aus rechtlicher Sicht den Status eines Lebensmittels oder eines Nahrungsergänzungsmittels hat, um die Durchführung von Studien am Menschen zu erleichtern.

Im letzten Teil der Arbeit sollten die beiden Untersuchungsansätze zusammengeführt werden. Dazu sollten zunächst, unter Nutzung des *In vitro*-Modells, Freisetzungsvoruntersuchungen an schnell freisetzenden Arzneiformen durchgeführt werden. Anschließend sollten diese Arzneiformen in einer klinischen Studie unter Verwendung der Speichelmarker-Methode untersucht werden. Der Vergleich der *In vitro*- und *In vivo*-Daten sollte die Validierung und Optimierung des Freisetzungsvormodells ermöglichen.

2 Diskussion der Ergebnisse

Das Verständnis der physiologischen Begebenheiten, die im Zuge der gastrointestinalen Passage einer oral applizierten Arzneiform auftreten, ist für die Entwicklung neuer Formulierungen von essentieller Bedeutung. Die aktuellen Kenntnisse zur Bedeutung der gastrointestinalen Physiologie bei der oralen Pharmakotherapie wurden von uns in einem Übersichtsartikel zusammengefasst (Publikation 1).

Bei neuen Arzneimitteln wird typischerweise versucht, über die Formulierung ein pharmakokinetisches Profil zu erzeugen, das an die therapeutischen Bedürfnisse des Patienten angepasst ist. Zudem wird versucht, eine möglichst hohe orale Bioverfügbarkeit des Wirkstoffs sicherzustellen. Bei der Entwicklung eines Generikums ist man hingegen bestrebt, ein pharmakokinetisches Profil zu erzeugen, das dem des Originators möglichst ähnlich ist. Das Ziel ist hier der Nachweis der Bioäquivalenz. Dabei kann sich durch bestimmte Änderungen der Formulierung sogar die Einnahmeempfehlung des Generikums vom Originator unterscheiden. Das kann etwa zur Verbesserung der Compliance genutzt werden und bietet dem Generikahersteller einen möglicherweise entscheidenden Vorteil auf dem Markt.

Mit einem solchen Beispiel haben wir uns im Rahmen eines Projektes mit der Firma HEXAL AG beschäftigt (Publikation 2). In diesem Fall sollte die alte Formulierung, eine Tablette mit dem Wirkstoff N-Acetylcystein (ACC), die mit 240 mL Wasser eingenommen werden soll, durch ein Granulat ersetzt werden, dass trocken geschluckt werden kann. Im Rahmen einer Bioäquivalenzstudie wurde dementsprechend das Generikum mit dem Originator (FLUIMUCIL) verglichen. Dazu erhielten 11 gesunde Probanden nüchtern die zwei verschiedenen ACC-Formulierungen. Die Studienarme 1 und 2 sahen die Einnahme der Tablette und des Granulates mit jeweils 150 mL Wasser vor, während der dritte Studienarm die Einnahme des Granulates ohne Wasser vorsah. Die Plasmaprofile der drei Studienarme bestätigten die Bioäquivalenz der Granulat-Formulierung. Überraschenderweise stellte es sich heraus, dass die ACC-Plasmaspiegel im Falle des Granulates unabhängig von der Wassereinnahme waren (Abbildung 3). Aufgrund der guten Wasserlöslichkeit des ACC von ca. 200 mg/mL war bei der Applikation mit 150 mL Wasser ein schnellerer Transport des Wirkstoffes in den Dünndarm erwartet worden.^{64,65} Da ACC zudem noch über eine gute Permeabilität verfügt, ist es in die BCS-Klasse 1 einzurordnen. Es wäre demnach ein schnelleres Anfluten des Arzneistoffes im Blut zu erwarten

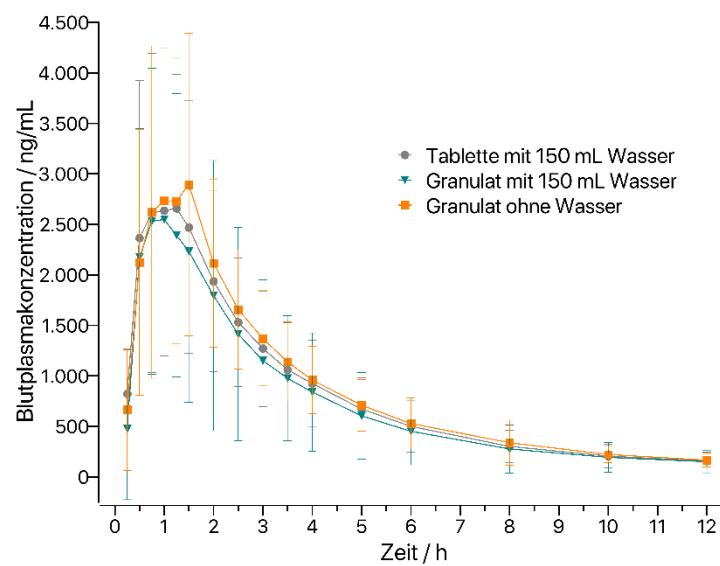


Abbildung 3 Blutplasmakonzentration von N-Acetylcystein über die Zeit nach Einnahme einer Dosis von 600 mg entweder als schnell freisetzende Tablette mit 150 mL Wasser oder als Granulat mit und ohne 150 mL Wasser (Mittelwert \pm SD).

gewesen, da im Falle der Einnahme ohne Wasser mit einem längeren Verbleib im Magen gerechnet werden muss. Die Gründe für diese in der Bioäquivalenzstudie beobachteten Effekte konnten mit kompendialen Freisetzungsmethoden nicht beschrieben werden. In diesen Experimenten zeigten sich deutliche Unterschiede zwischen den Formulierungen, die vielmehr darauf hingedeutet hätten, dass die Formulierungen nicht bioäquivalent sind. Aus diesem Grund haben wir die Freisetzung aus der Tablette und dem Granulat in dem von Garbacz und Weitschies entwickelten *Dynamic Open Flow-Through Test Apparatus* (DOFTA) noch einmal unter biorelevanten Bedingungen untersucht (Abbildung 4). Insbesondere die Möglichkeit zur Simulation verschiedener Magenentleerungskinetiken war für diese Fragestellung von großer Bedeutung. Da es sich beim DOFTA um ein Durchflusszellensystem handelt, war es ebenso möglich, die verschiedenen Einnahmeempfehlungen *in vitro* nachzustellen. Des Weiteren wurde der Einfluss von mechanischen Ereignissen wie Druck oder Bewegung auf die Freisetzung des Wirkstoffes untersucht.

Die Ergebnisse dieser *In vitro*-Experimente offenbarten, dass unter biorelevanten Bedingungen weder ein Einfluss der Magenentleerungskinetik noch des parallel zugeführten Wassers festgestellt werden konnte. Die

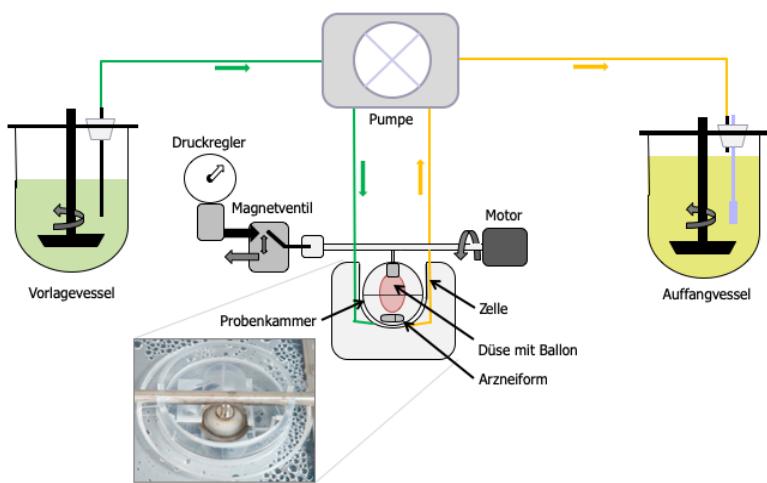


Abbildung 4 Schematische Darstellung der dynamischen Durchflusszelle nach Garbacz et. al. 2008.

simulierten mechanischen Ereignisse hatten ebenso keinen Einfluss auf das Freisetzungsverhalten. Diese Ergebnisse waren sehr interessant, da in den kompendialen Freisetzungsuntersuchungen Unterschiede zwischen den Arzneiformen festgestellt wurden. Häufig beobachtet man jedoch gegenteilige Effekte bei der Anwendung biorelevanter

Typischerweise werden im Zuge der Entwicklung von Generika neue Formulierungen mit kompendialen Methoden untersucht und das Freisetzungsprofil an das des Originators angepasst. Werden in verschiedenen Medien vergleichbare Freisetzungsprofile erzeugt, wird eine Bioäquivalenzstudie initiiert. Scheitert eine solche Studie, kann es sinnvoll sein, die Arzneiformen retrospektiv mit biorelevanten Freisetzungsapparaten zu untersuchen, um die Studienergebnisse besser verstehen zu können.⁶⁷ Häufig kommen dann Unterschiede im Freisetzungsverhalten zwischen den Formulierungen zutage, die zuvor nicht erfasst wurden. In unserem Fall war es jedoch so, dass das Freisetzungsverhalten des Granulates kaum von der gleichzeitigen Wasserzufluss beeinflusst wurde. Die Ergebnisse der biorelevanten Freisetzungsuntersuchungen legten vielmehr nahe, dass die langsame Auflösungsgeschwindigkeit des ACC ursächlich für die *in vivo* beobachteten Effekte waren. Infolge der zügigen Magenentleerung des parallel zugeführten Wassers, verblieb ein Großteil des Arzneistoffes in Form

ungelöster Partikel im Magen. Der Übertritt in den Dünndarm war daher stark abhängig vom Auftreten der eingangs beschriebenen Phase-3-Aktivität des MMC.

Diese Studie zeigte noch einmal, dass die Magenentleerung großen Einfluss auf das pharmakokinetische Profil oral applizierter Arzneimittel haben kann. Um diesen Effekt tatsächlich quantifizieren zu können, müssten pharmakokinetische Studien mit *In vivo*-Methoden kombiniert werden, die es erlauben, die Magenentleerung näher zu untersuchen. Zu diesen zählen etwa die MRT oder Szintigraphie. Häufig bedürfen diese Methoden jedoch kostenintensives Equipments sowie entsprechend geschultes Personal.³¹ Pharmakokinetische Ansätze zur Bestimmung der Magenentleerung, wie zum Beispiel die Paracetamol-Absorptions-Technik bedürfen hingegen der Entnahme von Blutproben und sind somit invasiv. Unser Ziel war es daher, eine simple und damit breit anwendbare *In vivo*-Methode zu entwickeln, mit der sich die Magenentleerung von Flüssigkeiten und Arzneiformen auf nicht invasive Weise und ohne medizinisch geschultes Personal ermitteln lässt.

Aufgrund der Einfachheit der Probengewinnung haben wir uns dazu entschieden, eine Methode zu entwickeln, die auf der Verteilung eines Markers in den Speichel beruht. Dieser Marker musste dazu die in der Zielstellung genannten Voraussetzungen erfüllen. Im Rahmen eines Screenings wurden dazu mehr als 30 Substanzen in Pilotversuchen getestet.

Damit eine Substanz im Mundspeichel nachweisbar ist, muss diese ungebunden im Blut vorliegen und von dort die lipophile Zellmembran der Azini-Zellen in den Speicheldrüsen passieren.⁶⁸ Somit gelangen die meisten Substanzen über den Weg der passiven Diffusion in den Mundspeichel.⁶⁹ Aus diesem Grund spielt die Lipophilie und der pK_a-Wert eine entscheidende Rolle im Hinblick auf die Verteilung zwischen Blutplasma und Speichel. Diverse Arbeitsgruppen konnten zeigen, dass unter physiologischen Bedingungen ungeladene kleine Moleküle ein robusteres Konzentrationsverhältnis zwischen Speichel und Blut haben, beziehungsweise überhaupt im Speichel nachweisbar sind.^{50,70,71} Obwohl die passive Diffusion als häufigster Transportweg beschrieben ist, wurden in der Vergangenheit auch Fallbeispiele publiziert, denen ein aktiver Transportmechanismus zugrunde liegt. Ein gut untersuchtes Beispiel hierfür ist die Sekretion von Lithium über einen aktiven Transporter in den Speichel.^{72,73} Der häufigste extrazelluläre Transportweg ist die Ultrafiltration, welcher über die Schlussleisten (*tight junctions*) zwischen den Zellen abläuft.^{74,75} Abseits der unmittelbaren Anwendungsgebiete, wie dem TDM oder der Schnelltests für Drogenabusus, ist Speichel ebenfalls als Plattform für die klinische Diagnose von bestimmten Erkrankungen interessant. Jost und Kollegen demonstrierten, dass Koffein eine robuste und enge Korrelation zwischen Speichel- und Blutplasmakonzentration zeigt. Sie nutzen diese Korrelation, um nach oraler Gabe von 280 mg Koffein die Halbwertszeit von Koffein im Speichel zu bestimmen. Da Koffein hauptsächlich in der Leber metabolisiert wird, weisen Patienten mit einer Lebererkrankung signifikant längere Koffeinhalbwertszeiten auf.⁷⁶ Somit war es möglich mithilfe einer einfachen und nicht invasiven Speichelmethode, Aussagen über die metabolische Variabilität eines Patientenkollektives zu treffen.

Koffein erwies sich in dem eingangs erwähnten Screening exogener Marker ebenfalls als hervorragend geeignet für unsere Zwecke. Koffein wird nach oraler Applikation schnell und vollständig im Dünndarm resorbiert und gelangt anschließend über den systemischen Kreislauf zu den Speicheldrüsen, um dort in den

Speichel zu diffundieren. Wie von Jost und Kollegen beschrieben, korreliert die Koffeinkonzentration im Speichel sehr eng mit der Konzentration im Blut.

In den nachfolgenden Versuchen galt es nun, die Hypothese zu bestätigen, dass nach Gabe einer oralen Lösung die Kinetik der Koffeinabsorption ausschließlich von der Kinetik der Magenentleerung bestimmt wird. Als Problem stellte sich jedoch die Kontamination der Mundhöhle bei der Applikation der Lösung heraus. Selbst durch mehrmaliges Spülen mit hohen Volumina an Wasser, waren die initialen Koffein-Konzentrationen im Speichel noch stark erhöht. Dieses Problem konnte durch eine Eiskapsel (Abbildung 5), bestehend aus reinem VE-Wasser, in die der Marker gefüllt wurde, erfolgreich gelöst werden. Die Eiskapsel verhindert eine Kontamination des Mundraumes, schmilzt im Magen aber innerhalb kürzester Zeit und gibt das Koffein im Magen somit umgehend frei.

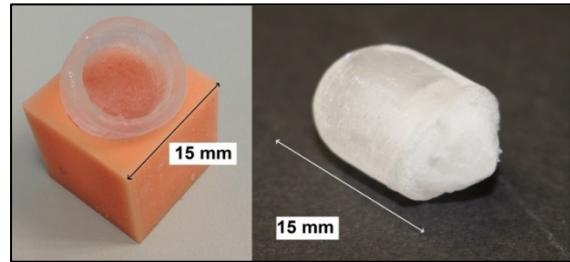


Abbildung 5 Links: Ungefüllte Eiskapsel in Silikonnegativ. Rechts: Gefüllte Eiskapsel.

Im Rahmen einer Pilotstudie untersuchten wir zunächst, ob es möglich ist, die Magenentleerung von 240 mL Wasser sowohl nüchtern als auch postprandial mit Hilfe dieser als *Salivary Tracer Technique* (STT) bezeichneten Methode zu bestimmen (Publikation 3). In dieser Studie wurde das Konzentrationsprofil des Koffeins im Speichel mit den mittels MRT bestimmten Entleerungskinetiken des Wassers korreliert. Die MRT ist aufgrund ihrer geringen Invasivität und den guten Erfahrungen der letzten Jahre mittlerweile ein breit akzeptiertes Verfahren zur *In vivo*-Bestimmung der Entleerung von Flüssigkeiten aus dem Magen. Nach Einnahme der Eiskapsel zusammen mit 240 mL Wasser, erfolgte im Anschluss die Probennahme durch selbstständige Abgabe von Speichel in 2 mL-Vials durch die Probanden. Die Aufarbeitung der Speichelproben bestand aus einem Proteinfällungsschritt mit Acetonitril und anschließender Verdünnung mit Wasser, welches einen internen Standard enthielt. Die so aufgearbeiteten Proben wurden mittels einer validierten HPLC/MS-MS-Methode analysiert. Anschließend wurden die Koffeinkinetiken im Speichel bestimmt, auf C_{max} normiert und mit den Entleerungsdaten der MRT korreliert.

Es zeigte sich, dass die STT zur Entleerungskinetik von Flüssigkeiten sowohl aus dem nüchternen als auch aus dem postprandialen Magen geeignet ist. Der Vergleich der mittleren Entleerungsprofile zwischen den beiden Applikationsbedingungen konnte wiederum die Existenz der Magenstraße belegen. Die Korrelation der Entleerungsprofile zwischen MRT und STT war nüchtern und postprandial sehr eng. Eine Limitation der STT postprandial ist, dass die Halbwertszeit des Koffeins signifikant höher ist als nüchtern. Eine mögliche Erklärung könnte eine teilweise Verteilung des Koffeins in den Speisebrei sein, was in der Folge zur langsamen, sukzessiven Entleerung des Koffeins, zusammen mit dem Speisebrei, führt. In einer vorangegangenen Arbeit wurde gezeigt, dass die vollständige Entleerung des Magens mehrere Stunden dauert.⁷⁷ Das bedeutet, dass in dieser Zeit weiterhin Koffein absorbiert wird und somit die Halbwertszeit nicht sauber bestimmt werden kann. Für diese Hypothese spricht zudem das erniedrigte mittlere C_{max} des Koffeins im *fed state*. Grimm und Kollegen konnten zeigen, dass die Entleerung von Wasser entlang der Magenstraße ähnlich schnell ist wie nach nüchtern Wassergabe.⁶ Das bedeutet, dass das erniedrigte C_{max} nicht auf eine langsamere Magenentleerung, sondern

wahrscheinlich auf die Verteilung eines gewissen Anteils des Koffeins in den Speisebrei zurückzuführen ist. Für die initiale und damit für die Bestimmung der Flüssigkeitsentleerung relevante, Absorptionsphase spielte diese Einschränkung infolge der Normierung auf C_{max} jedoch nur eine untergeordnete Rolle.

Die Entwicklung der STT basierte auf der Kombination verschiedener wissenschaftlicher Vorarbeiten. Es war bereits bekannt, dass Koffein im Speichel bestimbar ist und dass schnell resorbierbare Marker, wie zum Beispiel Paracetamol, geeignet sind, die Magenentleerung von Flüssigkeiten zu bestimmen.^{46,78} Im Gegensatz zu älteren Studien zur Bestimmung von Koffein im Speichel, konnten wir aufgrund der hohen Sensitivität der HPLC-MS/MS-Methode die zu applizierende Dosis deutlich reduzieren. Prinzipiell sind 25 mg Koffein ausreichend, um die Magenentleerung von Flüssigkeiten aus dem Magen zu beschreiben. Das entspricht in etwa der Menge an Koffein, die in einem einfachen Espresso enthalten ist.

Die Anwendung von Speichel als Testmatrix erlaubt es uns, gänzlich auf Blutproben zu verzichten. Das Tracking von Arzneiformen mittels des Koffeinnachweises im Speichel wurde bereits im Jahr 1998 von japanischen Wissenschaftlern beschrieben. Muraoka und Kollegen entwickelten eine drucksensitive Arzneiform, die ihren Wirkstoff erst bei Erreichen des Kolons abgeben sollte.⁷⁹ Als Modellarzneistoff wurde Koffein ausgewählt, da es nach drucksensitiver Abgabe aus der experimentellen Arzneiform einfach im Speichel bestimmt werden konnte. Somit zeigten die japanischen Kollegen damals bereits, dass der Koffeinnachweis im Speichel prinzipiell geeignet ist, den Zerfall von Arzneiformen im Menschen zu untersuchen.

Für die Absorption eines oral applizierten Arzneistoffes spielt neben den beschriebenen dynamischen Transport- und Entleerungsvorgängen im Gastrointestinaltrakt der Zerfall der Arzneiform ebenfalls eine entscheidende Rolle. Der Zerfall ist in der Regel die Voraussetzung dafür, dass sich ein Arzneistoff in den luminalen Flüssigkeiten quantitativ auflösen kann. Für die Charakterisierung des *In vivo*-Zerfallsverhalten von Arzneiformen stellt aktuell die Szintigraphie noch den Goldstandard dar, wenngleich die MRT heutzutage ebenfalls für diesen Zweck eingesetzt werden kann.^{31,80} Die Anwendung der MRT hat den Vorteil, dass auf die Gabe radioaktiver Marker verzichtet werden kann, und das weitere Information über die individuelle Anatomie und Physiologie des Probanden oder Patienten ermittelt werden können. Aus diesem Grund haben wir in einer weiteren Studie, in der die Bestimmung des *In vivo*-Zerfalls von schnell freisetzenden Arzneiformen mittels STT erfolgen sollte, die MRT wiederum als Referenzmethode genutzt.

In einer kontrollierten, offenen klinischen Studie erhielten acht Probanden, zusammen mit 240 mL Wasser, eine Hartgelatinekapsel die mit 50 mg Koffein und 5 mg Eisenoxid gefüllt war (Publikation 4). Unmittelbar nach Applikation der Kapsel wurden die Probanden im MRT platziert und Messungen in kurzen Zeitabständen durchgeführt. Im Anschluss an jede Messung mussten die Probanden im Liegen eine Speichelprobe abgeben. Das Eisenoxid in den Kapseln induzierte einen starken Signalverlust in den verwendeten T2-gewichteten MRT-Sequenzen, was zu schwarzen Suszeptibilitätsartefakten im MRT Bild führte (Abbildung 6).^{81,82} Das Eisenoxid diente somit als nicht-resorbierbarer Marker für das *In vivo*-Tracking der Kapsel nach Applikation. Das Auftreten von mehr als einem isolierten Artefakt wurde in dieser Studie als Indikator für den Zerfall der Kapsel herangezogen. Auf diese Weise konnte der Messzeitpunkt, bei dem mehrere Artefakte im MRT-Bild identifizierbar waren, als Zerfallszeitpunkt bestimmt werden.

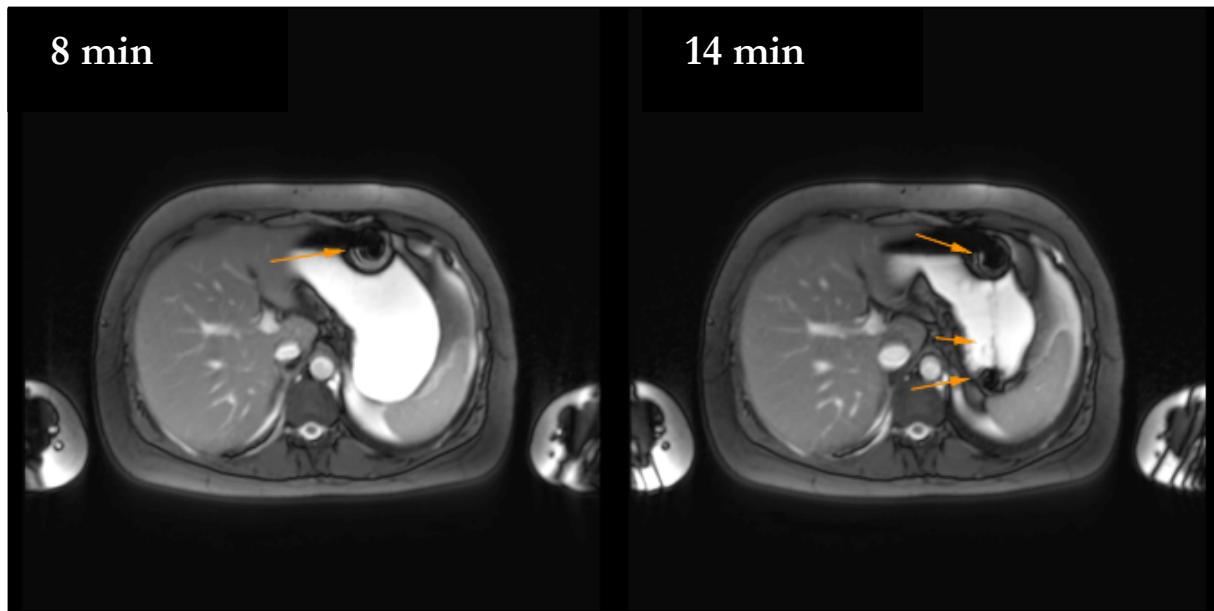


Abbildung 6 Transversale MRT-Aufnahmen des Abdomens eines Probanden. Die Aufnahmen erfolgten 8 min (links) beziehungsweise 14 min (rechts) nachdem eine Hartgelatinekapsel, die 50 mg Koffein und 5 mg Eisenoxid enthielt, zusammen mit 240 mL Wasser eingenommen wurde. *Links:* Der orange Pfeil markiert die intakte Kapsel, die an einem isolierten Artefakt erkennbar ist. *Rechts:* Die orangenen Pfeile zeigen auf multiple Artefakte, die durch dorsal sedimentierendes Eisenoxid hervorgerufen werden und den Zerfall der Kapsel anzeigen.

Bei der STT erfolgte die Bestimmung des Zerfallszeitpunkts auf Grundlage des Koffeinnachweises mittels HPLC/MS-MS in den parallel zu den MRT-Messungen genommenen Speichelproben. Der Zeitpunkt, bei dem eine Koffeinkonzentration von 15 ng/mL, die dem dreifachen Wert der Bestimmungsgrenze der analytischen Methode von 5 ng/mL entsprach, erreicht wurde, konnte als Zerfallszeitpunkt der Kapsel gewertet werden.

Zu jedem einzelnen Experiment wurden im Anschluss die Zerfallszeitpunkte der beiden Methoden verglichen. Generell waren die mit der STT ermittelten Zerfallszeitpunkte, verglichen zur MRT, im Mittel 4 min verzögert. Dieser Versatz der beiden Methoden erscheint plausibel, da mittels MRT der Zerfall der Kapsel auf visuelle Weise direkt im Magen bestimmt werden kann, während im Falle der STT bis zum Zeitpunkt der Detektierbarkeit des Koffeins im Speichel noch mehrere Zwischenritte ablaufen. So muss das Koffein nach dem Zerfall der Kapsel in Lösung gehen, aus dem Magen entleert werden und nach erfolgter Absorption aus dem Dünndarm über die Speicheldrüsen in den Mundspeichel diffundieren. Die Dauer des Zusammenspiels dieser Faktoren führt zum Versatz der STT in Relation zur MRT.

In der Arbeit konnten zudem Anhaltspunkte gefunden werden, dass der Versatz zwischen den Methoden ebenso vom Ort des Zerfalls abhängt. So nahm die Differenz der Zerfallszeitpunkte der Methoden vom Fundus über das Antrum bis hin zum Dünndarm ab. Eine mögliche Erklärung für diese Beobachtung ist die erhöhte Motilität im Bereich des Antrums verglichen zum Fundus. Diese führte im Antrum zu einer stärkeren Durchmischung des Mageninhalts und damit letztendlich zum schnelleren Anfluten des Koffeins. Im Falle der einen Kapsel, welche erst im oberen Dünndarm zerfiel, betrug die Differenz jedoch nur eine Minute. Aufgrund der Speichelabgabe nach der MRT-Messung entspricht diese Minute dem systematischen Methodenversatz. Diese Arbeit bestätigte, dass die STT prinzipiell für die Bestimmung des Zerfalls fester oraler Arzneiformen

geeignet ist. Im Falle von Arzneiformen, die erst im Darm zerfallen, könnte die STT sogar sensitiver sein als die MRT.

Gegenüber den etablierten Methoden, im Speziellen gegenüber den bildgebenden Verfahren, bietet die STT erhebliche Vorteile, insbesondere im Hinblick auf die Kosten und den Grad der Invasivität. Die Methode hat jedoch auch Limitationen. So muss bei der Interpretation von Zerfallszeiten, die mit der STT bestimmt wurden, immer berücksichtigt werden, dass die Magenentleerung die Bestimmung des Zerfallszeitpunktes beeinflusst. Dieser Umstand erschwert den Vergleich zu existierenden Literaturdaten, beispielsweise für die per Szintigraphie ermittelten Zerfallszeitpunkte.^{83–85} Nichtsdestotrotz ist die STT eine einfache und kostengünstige Methode, die es im *Cross-over*-Design erlaubt, relative Aussagen zum Zerfallsverhalten mehrerer Arzneiformen untereinander zu treffen. Eben jener Ansatz wurde im Folgenden genutzt, um die Biorelevanz der Aussagen, die mit den eingangs vorgestellten *In vitro*-Methoden generiert werden, zu überprüfen.

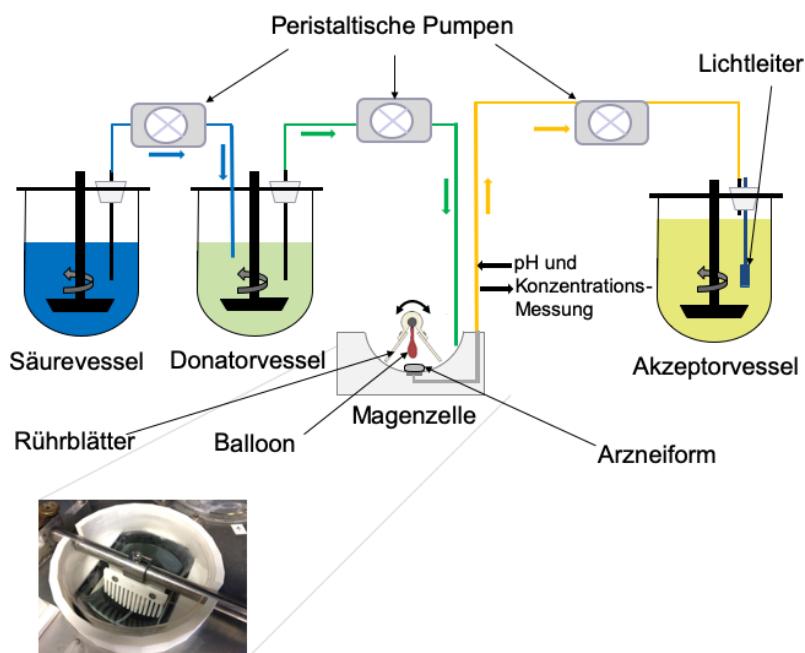


Abbildung 7 Schematische Darstellung des GastroDuo mit Photographie der Magenzelle.

Das GastroDuo stellt die Weiterentwicklung des in der ACC-Studie verwendeten DOFTA sowie des *Fed Stomach Model* dar (Publikation 5). Im Gegensatz zu den genannten Modellen, die sich auf bestimmte prandiale Zustände und Arzneiformen beschränken, wurde das GastroDuo so konzipiert, dass es die Testung schnell und modifiziert freisetzender Arzneiformen sowie die Simulation des nüchternen und postprandialen Magens erlaubt. Die Simulation unterschiedlicher prandialer Zustände erfolgt über die Variation verschiedener Parameter. Der Aufbau des GastroDuo ist in Abbildung 7 schematisch dargestellt. Es ist erkennbar, dass die Magenzelle das zentrale Element darstellt. In diese wird die Arzneiform eingebracht und definierten, an die Physiologie angepassten, Parametern ausgesetzt. Die Magenzelle stellt eine Durchflusszelle im offenen System dar. Peristaltische Pumpen sorgen dafür, dass mit bestimmten Raten das Donormedium aus dem Donorgefäß in die Magenzelle gelangt und aus dieser in das Akzeptorgefäß gepumpt wird. Auf diese Weise lässt sich das Zusammenspiel von Sekretion und Entleerung simulieren. Durch Nutzung eines separaten steuerbaren

Säurebehälters sowie vorgekühlter Medien, können zudem verschiedene pH- und Temperatur-Profile in der Magenzelle simuliert werden. Mechanischer Stress lässt sich über einen aufblasbaren Ballon, der direkt über der Arzneiform positioniert ist, sowie die Rührblätter ausüben. Hydrodynamische Belastungen werden hingegen über die Variation der Flussraten durch die Magenzellen simuliert. Alle Parameter sowie die Magentransitzeit sind über eine Software frei einstellbar.

Basierend auf verschiedenen *In vivo*-Daten, wurden Testprogramme erstellt, die die Bedingungen nach nüchterner Einnahme in ihrer Variabilität wiedergeben sollen.^{33,41} Zu diesem Zweck wurden sechs verschiedene Testprogramme (A-F) definiert (Tabelle 1). Das Programm A stellt das Standardprogramm auf Grundlage der Mittelwerte der verschiedenen Parameter dar. Die anderen Programme spiegeln hingegen physiologische Extrema wieder. Dies umfasst unter anderem eine kürzere Magenverweilzeit mit erhöhter Hydrodynamik (Programm C) und eine längere Magenverweilzeit mit reduzierter Hydrodynamik (Programm D).

Der Vorteil einer sequenziellen Veränderung und Kontrolle der Testbedingungen erlaubt es, eine Aussage darüber treffen zu können, welcher Parameter für die Freisetzung der Arzneiform von Relevanz ist. Somit ist es möglich, verschiedene Formulierungen gezielt und kontrolliert unterschiedlichen physiologischen Bedingungen auszusetzen, um eventuell Rückschlüsse auf ihr späteres *In vivo*-Verhalten zu ziehen. Ein solches Vorgehen ist für die simulierten Parameter mit den kompendialen Methoden nicht möglich.

Um ein möglichst umfassendes Verständnis von den dynamischen Zerfalls- und Freisetzungsprozessen schnell freisetzender Arzneiformen im GastroDuo zu erlangen, wird die Arzneistoffkonzentration direkt am Ausfluss der Magenzelle sowie im Akzeptorvessel erfasst. Die Messungen erfolgen photometrisch mittels eines Lichtleitersystems in Intervallen von einer Minute. Während die Messung direkt am Ausfluss Informationen über die den Magen verlassende Arzneistoffkonzentration gibt, ist es möglich, über die Konzentration und das Volumen im Akzeptorvessel die Massebilanz des Versuches zu ermitteln.

Tabelle 1 Testprogramme des GastroDuo mit Simulationsparameter der Magenzelle.

Testprogramm	Magenverweilzeit	Druck Ereignisse	Hydrodynamik	pH	Temperatur
A	30 min	100 mbar 300 mbar	Standard	Profil	Profil
B	30 min	300 mbar	Standard	Profil	Profil
C	15 min	100 mbar 300 mbar	erhöht	Profil	Profil
D	45 min	100 mbar 300 mbar	reduziert	Profil	Profil
E	30 min	100 mbar 300 mbar	Standard	pH 1.2	Profil
F	30 min	100 mbar 300 mbar	Standard	Profil	37°C

Im Vergleich zu anderen, in der Vergangenheit entwickelten biorelevanten Freisetzungsmodellen, bildet das GastroDuo einen Kompromiss zwischen einer möglichst umfassenden Simulation aller relevanten Parameter und der genauen Kontrolle dieser. Wie eingangs beschrieben, hat zum Beispiel das TIM (TNO, Niederlande) ein deutlich komplexeres Magenmodell mit weit mehr Freiheitsgraden. Die generierten Daten sind aber, aufgrund ihrer Komplexität, schwerer zu interpretieren. Weiterhin gibt es weniger komplexe Freisetzungsmodelle, wie zum Beispiel der *Rotating Beaker*, welcher einerseits sehr reproduzierbare und

kontrollierbare Bedingungen darstellen kann, anderseits aber auch relevante Parameter, wie Druck, nicht in Betracht zieht. Alle beschriebenen Modelle haben ihre Vor- und Nachteile. Somit muss ihre Anwendbarkeit je nach Fragestellung evaluiert werden.

Zur Überprüfung des GastroDuo hinsichtlich seiner Eignung als biorelevantes Freisetzungsmodell wurden Versuche mit vier schnell freisetzenden Arzneiformen durchgeführt, die allesamt Koffein als Modellarzneistoff enthielten. Bei diesen Formulierungen handelte es sich einerseits um drei Hartkapsel-Formulierungen auf Basis von Hartgelatine (Coni Snap®, Capsugel) und HPMC (Vcaps® plus, Capsugel sowie Quali-V®, Qualicaps), die mit einer Pulvermischung aus Laktose-Monohydrat, Croscarmellose und 25 mg Koffein gefüllt wurden. Aus derselben Pulvermischung wurde zudem mit Polyvinylpyrrolidon ein Klebstoffgranulat hergestellt und zu oblongen Tabletten verpresst. Im Anschluss wurden diese bis zu einem Massezuwachs von 5% (w/w) mit HPMC im Trommelcoater überzogen. Die drei verwendeten Typen an Hartkapseln sind bereits am Markt etabliert und waren bereits Gegenstand verschiedener *In vitro*-Untersuchungen.^{86,87}

Das Zerfalls- und Freisetzungsverhalten der vier genannten Formulierungen wurde einzeln in allen sechs Testprogrammen im GastroDuo untersucht. Es konnte dabei gezeigt werden, dass die verschiedenen simulierten Parameter einen relevanten und für die jeweiligen Arzneiformen unterschiedlichen Einfluss auf die *In vitro*-Ergebnisse hatten. Hervorzuheben ist hierbei insbesondere die Peristaltik, die einen starken Einfluss auf die Freisetzung aus schnell freisetzenden Arzneiformen zu haben scheint. Ähnliche Effekte konnten bereits von van den Abeele und Braeckmanns gezeigt werden.^{88,89} Sie konnten mittels hochauflösender Manometrie und Konzentrationsmessung eine Korrelation zwischen gastrischer Aktivität und Arzneistoffverteilung darstellen. Zusammenfassend zeigten die *In vitro*-Ergebnisse, dass das GastroDuo in der Lage war, zwischen diesen vier einfachen Formulierungen zu diskriminieren.

Die Überprüfung der auf Basis von *In vitro*-Versuchen getroffenen Aussagen erfolgte mit Hilfe der STT. Es wurden daher dieselben vier Arzneiformen verwendet, die bereits im GastroDuo untersucht wurden. Zu diesem Zweck wurde eine randomisierte, klinische Studie im *Cross-over*-Design durchgeführt. Die 14 Probanden nahmen nach einer Fastenzeit von mindestens 10 h und einer Koffeinabstinenz von mindestens 48 h, am Morgen eines Studientages, eine der zu testenden Arzneiformen zusammen mit 240 mL Wasser auf nüchternen Magen ein. Anschließend wurden über einen Zeitraum für 60 min engmaschig Speichelproben gesammelt, während in den darauffolgenden 3 h nur noch alle 15 min eine Probe abgegeben wurde. Schlussendlich musste jeder Proband an vier Studientagen teilnehmen und erhielt jede der vier Arzneiformen genau einmal.

Wie bereits in der zuvor beschriebenen Studie, wurden die initialen Zerfallszeitpunkte der einzelnen Arzneiformen anhand der Konzentrationsprofile des Koffeins (= Koffeinkonzentration > 3x Bestimmungsgrenze) im Speichel bestimmt. Zusätzlich wurden zudem die Differenzen zwischen dem Zeitpunkt der maximalen Koffeinkonzentration im Speichel (T_{max}) und dem initialen Zerfallszeitpunkt berechnet. Diese Differenz (ΔT_{max}) wurde als Maß für die Geschwindigkeit des *In vivo*-Zerfalls betrachtet. Der Ansatz einer differenzierten Betrachtung des *In vivo*-Zerfalls wurde bereits in szintigraphischen Studien verfolgt, in denen anhand der Szintigraphie-Bilder zwischen initialem und vollständigem Zerfall der Arzneiform unterschieden wurde.⁹⁰ Teilweise wurden für die in dieser Studie verwendeten verschiedenen Kapselhüllen schon in Studien

anderer Arbeitsgruppen die *In vivo*-Zerfallszeitpunkte mittels Szintigraphie bestimmt.^{83,90,91} Beispielsweise untersuchten Tuleu *et al.* die verwendeten HPMC-Kapselhüllen mit dem Geliermittel Carrageenan (Quali-V®, Firma Qualicaps).⁸⁴ Aufgrund des bereits diskutierten Versatzes der Zerfallszeitpunkte, der bei Nutzung der STT unumgänglich ist, ist ein direkter Vergleich mit den Ergebnissen von bildgebenden Verfahren nicht möglich.

Die in dieser Studie gewonnenen *In vivo*-Zerfallsdaten wurden genutzt, um relative Aussagen innerhalb der vier untersuchten Arzneiformen treffen zu können. So zeigte sich, dass die beiden HPMC-Kapselhüllen im Vergleich zur Hartgelatinekapsel und Filmtablette *in vivo* signifikant längere initiale Zerfallszeitpunkte aufwiesen. Dies wurde im Fall der Filmtablette mit dem schnellen Einsetzen der Erosion der Formulierung erklärt. Im Falle der Hartgelatinekapsel wurde aufgrund der *In vitro*-Daten und des im Magen vorliegenden Temperaturprofils mit einer längeren Verzögerung bis zum Einsetzen des Zerfalls gerechnet. Scheinbar waren hier jedoch die mechanischen Bedingungen limitierend. Im Falle von HPMC-Kapselhüllen kommt es zur Quellung und nicht zu einem Gel-Sol-Übergang, wie bei der Gelatine. Aus diesem Grund sind für solche Kapselhüllen leichte Drücke erforderlich, die im nüchternen Magen allerdings nur in bestimmten Phasen des MMC auftreten. Dies erklärt womöglich auch die höhere Variabilität der Daten.

Bei der Betrachtung der Kinetik des Zerfalls (ΔT_{\max}) ergab sich ein anderes Bild. Hier zeigte sich, dass die Filmtablette und die HPMC/Carrageenan-Kapsel (Quali-V®) signifikant langsamer zerfielen als die Hartgelatinekapsel, während die reine HPMC-Kapsel (Vcaps® plus) sich zwischen diesen beiden Gruppen einordnete.

Der Vergleich der *In vivo*-Daten mit den zuvor gewonnenen *In vitro*-Daten bedurfte, aufgrund der Fülle der Daten, einer differenzierten Betrachtung und Interpretation. Es zeigte sich, dass im Falle der Hartgelatinekapsel, ausgehend von den *In vitro*-Daten, *in vivo* mit einer höheren Variabilität des Zerfalls gerechnet wurde. Tatsächlich zeigten die Hartgelatinekapseln *in vivo* jedoch die geringste Variabilität in Kinetik und initialem Zerfall. Ein Grund hierfür ist vermutlich die noch fehlende Gewichtung der sechs Testprogramme. So muss bei der Interpretation der *In vitro*-Daten berücksichtigt werden, dass das Testprogramm A des GastroDuo statistisch gesehen wesentlich repräsentativer für die *In vivo*-Situation ist als das Testprogramm E, welches eine konstante Temperatur der Magenzelle von 37°C aufweist. Dadurch weisen die *In vitro*-Ergebnisse eine hohe Variabilität auf, welche *in vivo* so nicht beobachtet werden konnte. Nichtsdestotrotz ist die *In vitro*-Simulation von Extrema wichtig, um die Freisetzungsmechanismen der Arzneiform zu verstehen und mögliche Risiken bei der Anwendung durch viele verschiedene Patienten zu evaluieren.

Die Untersuchungen im GastroDuo sind generell hilfreich, um den Zerfall und die Freisetzung aus schnell freisetzenden Arzneiformen unter physiologisch relevanten Bedingungen zu charakterisieren. Dieses *In vitro*-Tool ist in der Lage, Unterschiede zwischen den Arzneiformen zu zeigen, die einerseits *in vivo* beobachtet werden können, aber anderseits in kompendialen Freisetzungstests nicht abgebildet werden.

Abseits der schnell freisetzen Formulierungen konnte bereits in einer ersten MRT-Validierungsstudie gezeigt werden, dass die STT auch geeignet ist, magensaftresistente Arzneiformen zu untersuchen (Publikation 6). Mit Hilfe der STT war es möglich den Zerfall einer mit 50 mg Koffein und 5 mg Eisenoxid gefüllten magensaftresistenten

Kapselhülle (enTRinsic™ DDT, Capsugel) zu bestimmen. Hierbei wurden die Zerfallszeitpunkte, welche mit der STT bestimmt wurden, mithilfe einer etablierten

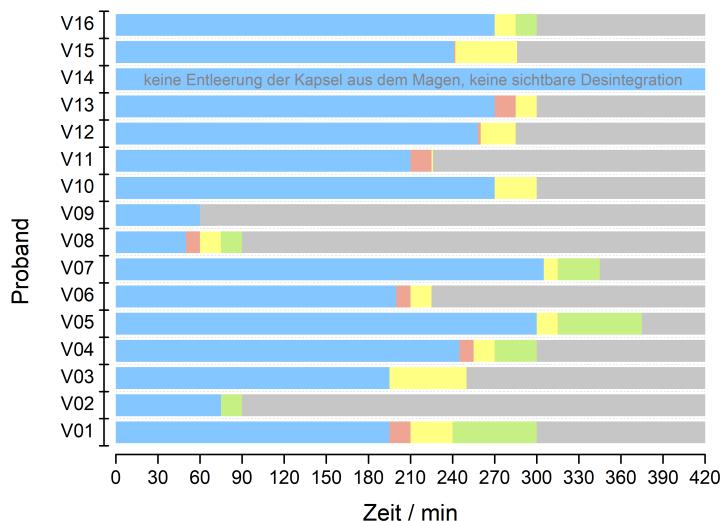


Abbildung 8 Gastrointestinaler Transit durch die verschiedenen Kompartimente (Magen: Blau, Duodenum: Rot, Jejunum: Gelb, Ileum: Grün, Zerfallen: Beginn Grau) bis zum Zerfall von enTRinsic™ DDT Kapseln gegeben nach Nahrungsaufnahme (500 kcal).

MRT- Methode verifiziert. Die Kombination der MRT-Lokalisationsdaten mit den Zerfallszeiten (Abbildung 8) der STT ergab einen deutlichen Informationsgewinn. Während des Zerfalls der Arzneiform in Dünndarm konnte eine schnelle Detektierbarkeit des Koffeins im Speichel beobachtet werden. Dies ermöglichte eine sehr empfindliche Bestimmung von Rupturen in den Kapselhüllen, welche teilweise in den MRT-Bildern nicht zu erkennen waren (siehe Abbildung 7, Proband V14). Zusätzlich konnte anhand der Speicheldaten ein Rückschluss auf die *In vivo*-Freisetzung geschlossen werden, da die Daten nicht durch Magenentleerungsprozesse beeinflusst wurden. Nichtsdestotrotz ist die MRT ein elementarer Bestandteil der durchgeführten Studie, da sie Informationen über den physiologischen Hintergrund und die Lokalisation der Arzneiform liefert. Zukünftige Studien sollten somit idealerweise die beiden Methoden kombinieren, um ein Maximum an Informationen aus der klinischen Studie zu gewinnen.

3 Zusammenfassung

Die meisten der auf dem Markt befindlichen Arzneimittel sind so konzipiert, dass sie ihren Wirkstoff schnell und reproduzierbar im oberen Gastrointestinaltrakt abgeben, um die Absorption aus dem Dünndarm zu erlauben. Für das Zerfalls- und Freisetzungsverhalten dieser Arzneiformen spielt vor allem die gastrale Physiologie eine entscheidende Rolle. Das Zusammenspiel der Formulierung mit bestimmten Parametern des Magens, insbesondere der Magenentleerung, ist aber noch nicht völlig verstanden. Im Rahmen dieser Arbeit wurde deshalb mit Hilfe von *In vivo*- und *In vitro*-Methoden untersucht, wie das Freisetzungsverhalten fester, oraler Arzneiformen nach nüchterner Applikation von der gastrointestinalen Physiologie beeinflusst wird.

Im ersten Teil der Arbeit beschäftigten wir uns mit den Ergebnissen einer Bioäquivalenzstudie, in der von einem Industriepartner zwei schnell freisetzende N-Acetylcystein-Formulierungen untersucht wurden. Dabei wurde der Originator, eine schnell freisetzende Tablette eingenommen mit Wasser, mit einem Granulat verglichen, welches einmal mit Wasser und einmal trocken eingenommen wurde. In beiden Studienarmen der Granulat-Formulierung konnte die Bioäquivalenz zum Originator gezeigt werden. Darüber hinaus wurden die Bioäquivalenzkriterien ebenso für die beiden Granulat-Studienarme erfüllt. Aufgrund der guten Permeabilität und der hohen Wasserlöslichkeit des N-Acetylcysteins war ein Einfluss der begleitenden Wassergabe auf die Pharmakokinetik erwartet worden. Die Ursachen für das Abweichen von dieser Erwartung wurden mittels *In vitro*-Freisetzungsmethoden weiter untersucht. Um die komplexen Bedingungen des Magens *In vitro* simulieren zu können, wurde das von Garbacz und Weitschies entwickelte biorelevante Freisetzungsmodell *Dynamic Open Flow-Through Test Apparatus* zur Untersuchung der beiden Formulierungen angewendet. Dabei konnte durch Simulation von biorelevanten Magenentleerungsprofilen gezeigt werden, dass die Wassergabe einen geringen Beitrag zum Transport des Arzneistoffes in den Dünndarm hat. Aufgrund dieser *In vitro*-Versuche konnten die Ergebnisse der Bioäquivalenzstudie umfassender erklärt werden.

Um die physiologischen Begebenheiten, welche für den Zerfall schnell freisetzender Arzneiformen kritisch sind, *In vivo* untersuchen zu können, wurde die *Salivary Tracer Technique* entwickelt. Diese Technik beruht auf der Einnahme einer geringen Dosis Koffein zusammen mit Wasser und anschließender Koffeinkonzentrationsmessung im Speichel. In einer MRT-Humanstudie konnten wir zeigen, dass die Entleerungskinetiken des eingenommenen Wassers aus dem Magen eng mit den Koffeinprofilen im Speichel korrelieren. Je nach Kinetik der Magenentleerung, erreichte gelöstes Koffein den Dünndarm und wurde dort schnell resorbiert und über das Blut in die Speicheldrüsen transportiert. Damit war die Magenentleerung der geschwindigkeitsbestimmende Schritt für das Anfluten von Koffein im Speichel. Somit war es möglich, indirekt, einfach und wenig kostenintensiv, die Magenentleerung einer akalorischen Flüssigkeit zu bestimmen, ohne auf die MRT mit entsprechend geschultem Personal zurückgreifen zu müssen.

In einem nächsten Schritt wurde die *Salivary Tracer Technique* genutzt, um die *In vivo*-Zerfallszeiten von Hartgelatinekapseln zu bestimmen. Als Referenz- und Goldstandardmethode diente wiederum die MRT, da es durch die Zugabe von Eisenoxid im MRT möglich war, die Hartgelatinekapseln sichtbar zu machen und deren Integrität zu beurteilen. Um parallel den Zerfall mit der *Salivary Tracer Technique* zu bestimmen, wurden die Kapseln zusätzlich mit 50 mg Koffein gefüllt. In einer Humanstudie wurden die mit den beiden

Markersubstanzen beladenen Kapseln auf nüchternen Magen appliziert. Direkt im Anschluss wurden die Probanden im MRT platziert und Messungen in kurzen Zeitintervallen durchgeführt. Nach jeder Messung mussten die Probanden selbstständig eine Speichelprobe von geringem Volumen abgeben. Sobald eine Koffeinkonzentration, die größer als die dreifache Bestimmungsgrenze der analytischen Methode war, in einer Speichelprobe nachgewiesen werden konnte, wurde dieser Zeitpunkt als Zerfallszeitpunkt bestimmt. Der statistische Vergleich der Zerfallszeitpunkte der beiden Methoden zeigte eine robuste Korrelation der Werte. Ein geringer Versatz der Zerfallszeitpunkte bei der *Salivary Tracer Technique* ließ sich dadurch erklären, dass mittels MRT der Zerfall direkt im Magen bestimmt wurde, während für den Koffeinnachweis im Speichel noch das Verteilen, das Lösen und/oder die Entleerung des Koffeins aus dem Magen mit anschließender Resorption im Dünndarm erfolgen muss. Trotz des Versatzes der beiden Methoden von circa 4 min konnte die Studie belegen, dass die *Salivary Tracer Technique* geeignet ist, mit einer robusten Korrelation zu etablierten Methoden und mit moderatem Studienaufwand den Zerfall schnell freisetzender Arzneiformen zu bestimmen. Besonders der relative Vergleich mehrerer verschiedener Formulierungen in einem *Cross-over*-Design erscheint mit der *Salivary Tracer Technique* sinnvoll, da hierbei der Einfluss der Magenentleerung auf die Zerfallszeiten der verschiedenen Formulierungen gleichbleibend sein sollte.

Da eine *In vivo*-Testung von (Entwicklungs-)Formulierungen, trotz der vereinfachten Methodik, nicht immer möglich und angemessen ist, wurde das *Fed Stomach Model* als *In vitro*-Freisetzungsmodell im Rahmen dieser Arbeit weiterentwickelt. Die wesentlichen Modifizierungen des GastroDuo gegenüber dem *Fed Stomach Model* sind die dünnwandige, transparente Magenzelle, welche geringe Volumina von bis zu 25 mL simulieren kann sowie biorelevante Temperaturprofile des eingebrachten Mediums. Weiterhin wurde die Möglichkeit geschaffen, diese Medien anzusäubern und so verschiedene pH-Profile zu simulieren. Eine weitere Neuheit des GastroDuo ist die Fähigkeit der kontinuierlichen Konzentrationsmessung direkt am Ausgang über ein UV-Lichtleitersystem. Das System eignet sich somit dazu, Druck, Bewegung, pH-Wert, Temperatur und Hydrodynamik in physiologisch relevanter Weise zu simulieren. Diese Parameter werden in sechs Testprogrammen, welche sowohl die mittleren Bedingungen im Magen simulieren als auch die physiologischen Extrema berücksichtigen, in unterschiedlicher Weise abgebildet.

In einer ersten Studie wurde das GastroDuo genutzt, um das Freisetzungerverhalten von vier schnell freisetzenden Arzneiformen genauer zu untersuchen. Weiterhin wurden vergleichende Freisetzungsdaten mittels kompendialer Methoden erhoben. In diesem Screening wurde der Zerfall und die Freisetzung von Koffein aus einer Hartgelatinekapsel, zwei verschiedenen HPMC-Kapseln sowie einer Filmtablette verglichen. Die Auswertung der Freisetzunguntersuchungen zeigte, dass manche Formulierungen empfindlich gegenüber bestimmten Parametern waren, während eine andere Formulierung dies für diesen Parameter nicht war. Im erstellten Datensatz ergab sich so ein sehr heterogenes Bild der Freisetzungsdaten, das abhängig vom Testprogramm und der jeweiligen Formulierung war.

Im Folgenden sollte untersucht werden, ob die *in vitro* generierten Daten prädiktiv für das Verhalten der Arzneiformen im Menschen waren. Dafür wurden dieselben vier Formulierungen in einer „4-Wege cross-over“-Humanstudie, mit Hilfe der *Salivary Tracer Technique*, auf ihr *In vivo*-Verhalten untersucht. Es ließ sich feststellen, dass das GastroDuo in der Lage war zwischen den Arzneiformen zu unterscheiden, die auch *in vivo* Unterschiede

aufweisen. Dies war mit kompendialen Methoden nicht immer möglich. Der signifikante Vorteil des GastroDuo gegenüber anderen Systemen ist die Möglichkeit, physiologische Extrema getrennt voneinander darzustellen und ihren Einfluss zu quantifizieren. Daher bietet das GastroDuo ein wertvolles Werkzeug, um den Zerfall und die Freisetzung von schnell freisetzenden Arzneiformen besser zu verstehen.

Abschließend lässt sich sagen, dass es uns in der vorliegenden Arbeit gelungen ist, wichtige Hilfsmittel für das Verständnis des Zerfalls- und Freisetzungsverhalten von schnell freisetzender Arzneiformen im nüchternen Magen weiterzuentwickeln. Es ist uns gelungen, bestehende *In vitro*-Modelle technisch zu verbessern, eine neue *In vivo*-Methode zu entwickeln und zu validieren, welche eine sinnvolle Ergänzung zu bereits bestehenden *In vivo*-Methoden darstellt.

4 Ausblick

Das GastroDuo soll zukünftig dazu angewandt werden, das Zerfalls- und Freisetzungsverhalten neuer Formulierungen unter physiologisch relevanten Bedingungen zu untersuchen. Ein tieferes Verständnis der Mechanismen, die das Zerfalls- und Freisetzungsverhalten wesentlich beeinflussen, kann dazu beitragen neue Formulierungen gezielt zu entwickeln.

Derzeit stellen insbesondere die oralen Onkologika ein besonders spannendes Feld dar, da die orale Krebstherapie zunehmend an Bedeutung gewinnt, die dabei eingesetzten Wirkstoffe aber zugleich viele biopharmazeutische und pharmazeutisch-technologische Unwägbarkeiten bergen.⁹² Zu diesen zählen etwa die begrenzte Löslichkeit in Wasser, die häufig eine niedrige orale Bioverfügbarkeit mit zugleich hohem positivem *Food Effect* bedingt. Infolgedessen werden oft sogenannte *Enabling Formulations* eingesetzt, deren Arzneistoffbeladung jedoch in vielen Fällen begrenzt ist. Aus diesem Grund sind Dosierempfehlungen von mehreren Kapseln oder Tabletten in der oralen Krebstherapie durchaus üblich. Dementsprechend gibt es Bestrebungen neue Formulierungen zu entwickeln, welche die Compliance der Patienten verbessern können, in dem die Einmaldosis in einer einzigen schnell freisetzenden Arzneiform formuliert wird. Vor diesem Hintergrund könnte das GastroDuo ein hilfreiches Tool sein, um frühzeitig formulierungsbedingte Probleme zu erkennen und so die Anzahl klinischer Prüfungen zu reduzieren. Der nächste logische Schritt wäre, die GastroDuo Daten als Grundlage für Physiologie-basierte Pharmakokinetik (PBPK) *In Silico*-Simulationen zu verwenden. Somit ließen sich die GastroDuo-Daten eingehender verstehen und diese würden eine bessere Datenbasis für die Simulationen bieten als kompendiale Freisetzungsdaten. Das Ziel dieser *In Silico*-Simulationen ist die Pharmakokinetik einer Arzneiform vorauszusagen. Durch die Kombination von biorelevanten Freisetzungsuntersuchungen und PBPK-Simulationen ist es in der Zukunft potentiell möglich die Anzahl an klinischen Tier- und Humanstudien zu reduzieren.

Weitere Studien konnten bereits zeigen, dass die Salivary Tracer Technique noch Potential für Verbesserungen und Weiterentwicklungen hat. Der Einsatz stabiler ¹³C-Isotope des Koffeins als Marker hat sich bereits in ersten Studien als sehr vielversprechend erwiesen. Durch die Verwendung von Koffein-Isotopen unterschiedlicher Massezahlen stehen multiple Marker zur Verfügung, um den Zerfall von mehreren Arzneiformen gleichzeitig untersuchen zu können. Weiterhin kann durch die Verwendung eines ¹³C-Isotopes auf eine Koffein-Abstinenz der Probanden verzichtet werden. Dies könnte die Compliance und die Robustheit der STT erhöhen. Zukünftige Forschung könnte sich, durch den Einsatz der Isotope, mit der Charakterisierung gastrointestinaler Parameter von geriatrischen und pädiatrischen Populationen beschäftigen. Zu diesem Zweck muss die Dosis des Koffeins weiter reduziert werden, um die Methode auch bei diesen sensibleren Populationen anwenden zu können. Durch die Verwendung stabiler Isotope und eine Weiterentwicklung der analytischen Methoden zur Detektion des Koffeins im Speichel, sollte eine solche Dosisreduktion mit moderatem Aufwand möglich sein und die STT einem breiteren Anwendungsspektrum zugänglich machen.

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6 Publikationen

6.1 Navigating the human gastrointestinal tract for oral drug delivery: Uncharted waters and new frontiers (*Advanced Drug Delivery Reviews* 2016, 101, 75–88)

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Navigating the human gastrointestinal tract for oral drug delivery: Uncharted waters and new frontiers☆



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ABSTRACT

Many concepts of oral drug delivery are based on our comprehension of human gastrointestinal physiology. Unfortunately, we tend to oversimplify the complex interplay between the various physiological factors in the human gut and, in particular, the dynamics of these transit conditions to which oral dosage forms are exposed. Recent advances in spatial and temporal resolution of medical instrumentation as well as improved access to these technologies have facilitated clinical trials to characterize the dynamic processes within the human gastrointestinal tract. These studies have shown that highly relevant parameters such as fluid volumes, dosage form movement, and pH values in the lumen of the upper GI tract are very dynamic. As a result of these new insights into the human gastrointestinal environment, some common concepts and ideas of oral drug delivery are no longer valid and have to be reviewed in order to ensure efficacy and safety of oral drug therapy.

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1. Introduction

The oral administration is the most obvious, the most convenient, and, as a consequence, the prevalent route for drug therapy. Unfortunately, it is not as simple as it seems. The extent and rate of drug absorption from the gastrointestinal (GI) tract depends on different factors that are either related with the drug itself, the formulation or the patient (Table 1) [1–10]. It is typically not known which of the numerous confounding influences effectively affects drug absorption from the GI tract. Despite manifold attempts using animal models, *in vitro*, tissue or cell culture test systems as well as *in silico* calculations, reliable predictions of the extent and rate of drug absorption in man – as well as in other species – are extremely difficult and often deficient. Obviously, there are still many “unknowns” that are determining drug absorption from the GI tract. Thus, the determination of oral bioavailability and the identification of parameters that might interfere with drug absorption, e.g., metabolizing enzymes, uptake, and efflux transporters or concomitant food, intake, are still a matter of human drug absorption studies.

In the vast majority, the available absorption characteristics have been obtained in clinical studies on pharmacokinetics or bioequivalence with healthy volunteers under the strongly standardized phase I conditions that do not reflect the real life situation. Unfortunately, only a very few information is available for drug absorption under “real life” conditions or in patients with certain diseases. However, to understand the physiological rationale behind drug absorption from the GI tract, it is essential to characterize the conditions under which pharmacokinetic data are gathered.

The present article was written to point out the often neglected importance of the dynamics of the gastrointestinal conditions for the *in vivo* performance of orally administered medications in both fasted and fed subjects. We will provide examples for dynamic processes in the human gut as recently explored using modern medical measurement technologies and explain how these processes may influence oral drug absorption. Particular attention is paid to the gastrointestinal conditions arising in clinical trials as they are the background of the pharmacokinetic data published in literature. Moreover, we will discuss adapted dissolution test methods capable of simulating critical dynamic conditions arising during the GI passage of oral dosage forms, as well as possible contributions of gastrointestinal dynamics to the variability in drug absorption of small and large molecules. It must be noted that this article expresses the authors' opinions and that it is specifically focused on selected physiological parameters in stomach and small intestine rather than on comprehensively reviewing the transit conditions in the human GI tract or the biopharmaceutical tools used to predict oral drug absorption. The interested reader is referred to reviews published by the European Innovative Medicines Initiative (IMI) on *Oral Biopharmaceutics Tools (OrBiTo)* and others [11–16].

2. Gastrointestinal (hydro)dynamics

The recent progress in non-invasive medical measurement techniques such as magnetic resonance imaging (MRI), magnetic marker

monitoring (MMM) or telemetric capsules, and the rapidly advancing capabilities of medical imaging devices provided new fascinating insights into gastrointestinal physiology, but also into the fate of orally administered drugs and drug delivery systems within the human GI tract [17–19]. Furthermore, some groups even re-evaluated old knowledge on gastrointestinal physiology and came up with surprising results. For instance, Helander and Fändriks revealed that the surface of the human gut mucosa is not in the order of a tennis court ($250\text{--}300\text{ m}^2$), but approximately half the size of a badminton court (approximately 32 m^2) [20].

Apart from major improvements in image quality and spatial resolution, modern medical measurement techniques provide essentially higher temporal resolution. The reduction of measurement duration and movement artifacts led to a turn of the acquired information from rather static to dynamic. As a result, a number of studies were conducted in recent years, which aimed at the characterization of the dynamics of the gastrointestinal transit conditions. These studies showed that the common idea of a more or less continuous transport of drug delivery systems and drug substances through the GI tract was a misconception. Indeed, the opposite holds true as the gastrointestinal transport of solid oral dosage forms was found to be extremely discontinuous. In all major segments of the GI tract, i.e., stomach, small intestine, and colon, gastrointestinal transport is characterized by phases of rest, slow propagation, and events of rapid transport of variable duration and range.

In the following chapters, we will present the results of recent studies, in which the dynamics in the upper GI tract were investigated with the aid of modern medical measurement technologies in both fasted and fed state. We will focus on luminal fluid volumes, pH values, and GI motility, and we will discuss how these parameters may affect the gastrointestinal transit behavior of solid oral dosage forms.

2.1. Luminal fluid volumes

2.1.1. Fasted state

In the current guidelines for the determination of oral bioavailability or bioequivalence, investigations in fasted state are recommended after a fasting period of at least 8 h (EMA) or 10 h (FDA) prior to drug administration together with at least 150 mL (EMA) or 240 mL of water (FDA) [21–23]. After such long overnight fasting period, the stomach is regarded as almost empty. However, a small volume of gastric content is always present in the gastric lumen. Recent MRI investigations that considered the conditions of the guidelines revealed that the fasted state volume of the stomach is typically below 50 mL but can vary considerably (Table 2). These data are in good accordance with published data for fasted state gastric content volumes determined with other tools under varying conditions [24,25].

The (hydro)dynamic conditions in the fasted human upper GI tract mainly result from the distinct cyclic pattern of propagating myoelectric activation that typically starts in the stomach and ends in the distal small intestine, the so-called interdigestive migrating motor complex (IMMC) [32]. As can be seen in Fig. 1, the IMMC consists of three phases of different duration. Phase I is a longer phase of rest, whereas phases II and III are characterized by strong motility. In particular, during phase

Table 1
Different factors known to influence drug absorption from the gastrointestinal tract [1–10].

Drug-related factors	Formulation-related factors	Patient-related factors
Molecular weight	Drug release profile	Intake condition
Water solubility	Excipients	Disease
Partition coefficient		Age
Stability toward gastrointestinal conditions, including digestive enzymes and pH values in the physiological range of pH 1–8		Ethnic group
		Genetic polymorphisms
		Gender
		Lifestyle and eating habit
		Co-medications

Table 2
Gastric content volumes after a fasting period of at least 8 h as determined by MRI (n/a=unreported data).

MRI study	No. of subjects	Gastric content volumes (mL)			
		Min	Max	Median	Mean \pm SD
Schiller et al. [26]	n = 12	13	72	47	45 \pm 18
Goetze et al. [27]	n = 12	n/a	n/a	n/a	65 \pm 22
Goetze et al. [28]	n = 12	n/a	n/a	n/a	40 \pm 27
Babaei et al. [29]	n = 10	n/a	n/a	n/a	85 \pm 29
Koziolek et al. [30]	n = 12	4	64	28	31 \pm 19
Mudie et al. [31]	n = 12	n/a	n/a	n/a	35 \pm 7
Data on file	n = 72	1	95	17	21 \pm 17

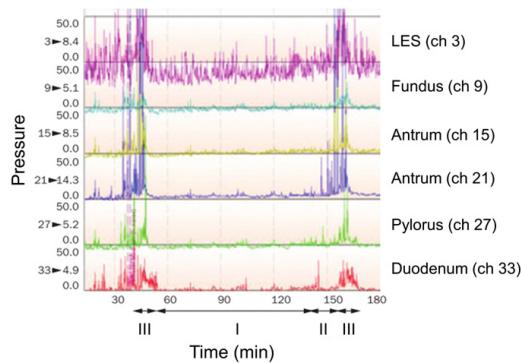


Fig. 1. Pressure-time profiles obtained by high-resolution manometry (36 pressure channels) illustrating the different phases (I–III) of the interdigestive migrating motor complex. LES—lower esophageal sphincter. Adapted by permission from Macmillan Publishers Ltd: Nat. Rev. Gastroenterol. Hepatol. (Deloose et al.), copyright (2012) [32].

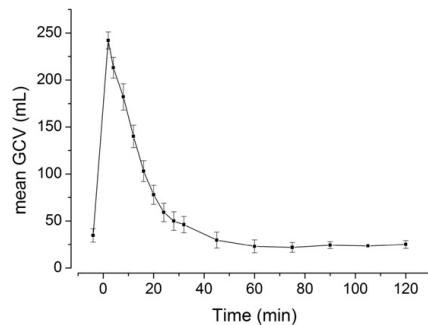


Fig. 2. Mean gastric content volumes after administration of 240 mL of water in fasted state investigated by MRI over a period of 120 min, $n = 12$. Reprinted from Mudie et al. [31]. Copyright (2014) American Chemical Society.

III, strong peristaltic waves of variable amplitudes are generated in the stomach and propagate toward the terminal ileum (Fig. 1).

It is very likely that the stomach is completely empty immediately after the occurrence of the strong peristaltic contractions of IMMC phase III (housekeeping waves). Therefore, to our understanding, the

observed variability in residual gastric content volumes reflects the time that has passed since the last housekeeping wave. During the subsequent quiescent phase I of the IMMC, oral and gastric secretions are gathered within the lumen of the stomach, but not emptied into the small intestine. Assuming a typical basal gastric secretion rate of about 1 mL/min and a saliva flow rate of 0.1–1 mL/min, about 75 mL of gastric juice may accumulate within 1 h after the last IMMC phase III [33].

Under fasting conditions, the 240 mL of water co-ingested with the medication according to the guidelines is emptied from the stomach typically within 15–30 min, as shown by Mudie and co-workers (Fig. 2) [31]. It is believed that non-caloric liquids such as water are emptied from the stomach mainly by contractions of the distended stomach wall. Even though the volunteers are in supine position during the MRI investigations, gastric water emptying in fasted state is almost complete. Fig. 3 illustrates nicely how MRI can be used to visualize the process of gastric emptying of water.

The small intestine is mostly empty in fasted subjects and, unlike the gas filled colon, the small intestinal walls are collapsed. Non-absorbed small intestinal fluid is segregated in a few “fluid pockets” of variable volume [26]. After overnight fasting, a mean total volume of about 50 to 100 mL of fluid is present in the small intestine (Table 3).

The largest pocket is typically found in the terminal ileum (see Fig. 4), where also non-absorbable material gathers. By contrast, free water is rarely observed in the colon [26], although the typical filling volumes of the colon are high [35].

The 240 mL of water swallowed for drug administration obviously undergo rapid absorption from the small intestine. Thus, the total fluid volume in the small intestine remains nearly unchanged [31]. Water reaching the small intestine is immediately scattered over the jejunum and absorbed as illustrated in Fig. 5. The common idea of a water front traveling rather slowly down the small intestine is not supported by MRI data undertaken with high sampling rates.

2.1.2. Fed state

Food intake leads to numerous physiological changes in the upper GI tract and, therefore, can exert significant effects on drug absorption [33,36]. A classification of how food intake can influence drug absorption from the human GI tract is given in Fig. 6.

In order to investigate the impact of food on drug absorption, most food effect studies are performed according to the guidelines of FDA and EMA [21–23]. These studies follow a study protocol, which is equivalent to the one used for fasted state investigations, with the only difference that the subjects receive a high-caloric (800–1000 kcal), high-fat (50% of the calories derived from fat) breakfast 30 min prior to drug intake with 240 mL of water. This meal shall provoke a drastic effect on gastrointestinal physiology and, thus, on drug absorption. In a footnote of the FDA guideline, an example for a typical test meal is given,

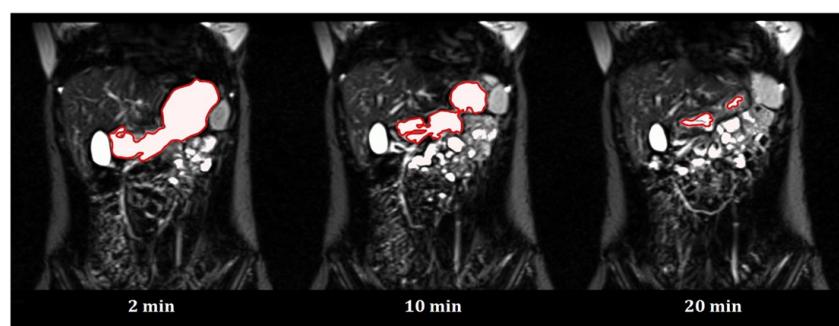


Fig. 3. MRI sequences demonstrating rapid gastric emptying of 240 mL water administered in the fasted state. Water inside the stomach is delineated by the red line. Left: 2 min—177 mL; middle: 10 min—120 mL; right: 20 min—4 mL.

Table 3
Comparison of total small intestinal water volumes observed by MRI in healthy volunteers after an overnight fast.

MRI study	No. of subjects	Small intestinal fluid volume (mL)			
		Min	Max	Median	Mean \pm SD
Schiller et al. [26]	n = 12	45	319	83	105 \pm 72
Marciani et al. [34] (calculations based on digitized data)	n = 16	12	253	81	91 \pm 68
Mudie et al. [31]	n = 12	5	159	—	43 \pm 14
Data on file	n = 48	7	230	52	65 \pm 51

consisting of two toasts with butter, two eggs fried in butter, two strips of bacon, hash brown potatoes, and 240 mL milk. Thirty minutes after start of meal intake, i.e., typically 10–20 min after finishing the meal, the medication to be tested is administered together with 240 mL of water. After drug administration, water intake is prohibited for 1 h and food intake for at least 4 h, respectively.

Food intake has a marked effect on the motility of the GI tract as the ingestion of caloric food or liquids causes an interruption of the IMMC and induces the digestive myoelectric motor activity [37,38]. The motility pattern of the fed stomach is completely different from fasted state and characterized by constantly propagating antral contraction waves with a frequency of three waves per minute that are continued in the small intestine with an increased frequency of 12 waves per minute [37]. The gastric emptying rate is reduced in fed state. Thus, large volumes can gather in the gastric lumen, which serve as the dissolution medium of solid oral dosage forms administered after food intake. In Fig. 7, individual gastric content volumes over time are provided as measured in a recent MRI study that was performed considering the above mentioned FDA recommendations for fed state bioavailability/bioequivalence studies [30].

After eating the fat-rich FDA meal and ingestion of 240 mL water, the mean gastric content amounted to about 580 mL. This volume was higher than the volume of the eaten meal, suggesting that there is additional oral and/or gastric secretion. The initial peak in volume was followed by a plateau phase, during which secretion and gastric

emptying seemed to be more or less equally. Just 60–90 min after swallowing 240 mL water, gastric volume decreased with a rate of ~1.7 mL/min. Even after more than 6 h, the gastric content exceeded the volumes assessed in fasting healthy subjects. These observations are in good accordance with emptying rates of 2–4 kcal/min reported in literature [39–41].

In that study, moreover, it was also seen that swallowing of 240 mL water for virtual drug administration was not the reason behind persistent increase in gastric volume. Actually, water was rapidly emptied within 15–35 min (Fig. 8). This finding confirms old experience that there is a gastric route for rapid emptying of liquids from the postprandial stomach known as *Magenstrasse* (stomach road) or *canalis gastricus* [42,43]. In the original concept of the *Magenstrasse*, it was assumed that the pathway follows the lower curvature of the stomach. However, the present MRI data suggest that the water flows around the chyme in the lumen along the entire stomach wall. Similar observations were made in dogs by Scheunert and co-workers already in 1912 [44]. The authors demonstrated that fluids ingested after a meal can flow around the stomach contents and thereby, reach the small intestine rapidly. It was also observed in these dog experiments that, depending on the texture of the food mass, certain amounts of the fluid can even flow through the matrix. In our understanding, it seems likely that the volume-induced relaxation of the fundus of the stomach creates a small gap between the viscous food mass and the fundus wall. Fluid that goes this way around the food mass gathers in the antrum of the stomach and is emptied from there into the duodenum within a few minutes (Fig. 8).

Similar observations were made by Malagelada and co-workers [45,46]. In their studies, the water taken together with solid meals is emptied rapidly from the stomach. By contrast, the solid food is retained in the stomach, which enables sufficient time for digestive processes. As opposed to the rapid flow of water around the food mass in the fundus, the mass itself is astonishingly static. As already described in 1923 by Goedel, food is segmented in different layers inside the stomach [47,48]. This has been confirmed for different meals by MRI [28,49]. For instance, Wilson and co-workers demonstrated the formation of a dough ball in the stomach, which was surrounded by fluids [50]. These data show that the mixing properties of the stomach for drugs taken after a solid meal are rather marginal. In a study by Faas and colleagues, this has been strikingly demonstrated for a liposomal preparation of an MRI contrast agent (Fig. 9) [51].

Due to the poor mixing properties of the fundus, non-disintegrating solid dosage forms like extended release (ER) tablets can stay for several hours on top or within the gastric content [52,53]. In case of ER tablets, the static residence of the tablets in the food bolus may result in a local accumulation of the released drug substance. If such a bolus of accumulated drug is suddenly emptied into the small intestine, e.g., by postural changes, this may create a sharp rise in the drug plasma concentration. Notably, these plasma peaks can also be misinterpreted as dose dumping caused by failure of the drug delivery system [52].

In the small intestine, food intake triggers the gastro-ileocecal reflex and, thus, causes the emptying of contents from the terminal ileum into the caecum [54,55]. Thereby, the small intestinal fluid volume decreases initially. Schiller et al. showed that the fluid volume decreases from 105 \pm 72 mL in fasted state to 54 \pm 41 mL in fed state (1 h after a meal of 803 kcal) [26]. Interestingly, the number of fluid pockets

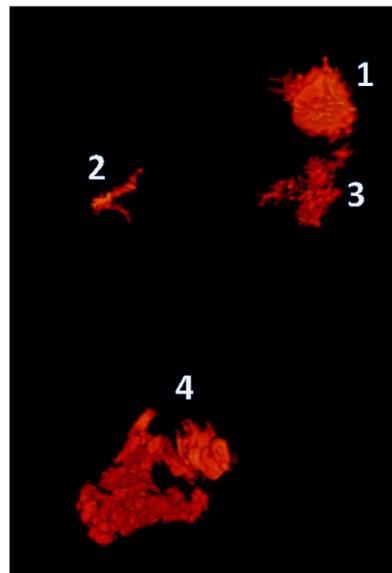


Fig. 4. Distribution of water (red) in the gastrointestinal tract of a healthy volunteer after overnight fasting as observed by MRI (frontal view). In this example, water is present in the stomach (1: 27 mL) as well as in three “fluid pockets” located in duodenum (2: 3 mL), proximal jejunum (3: 11 mL), and terminal ileum (4: 74 mL).

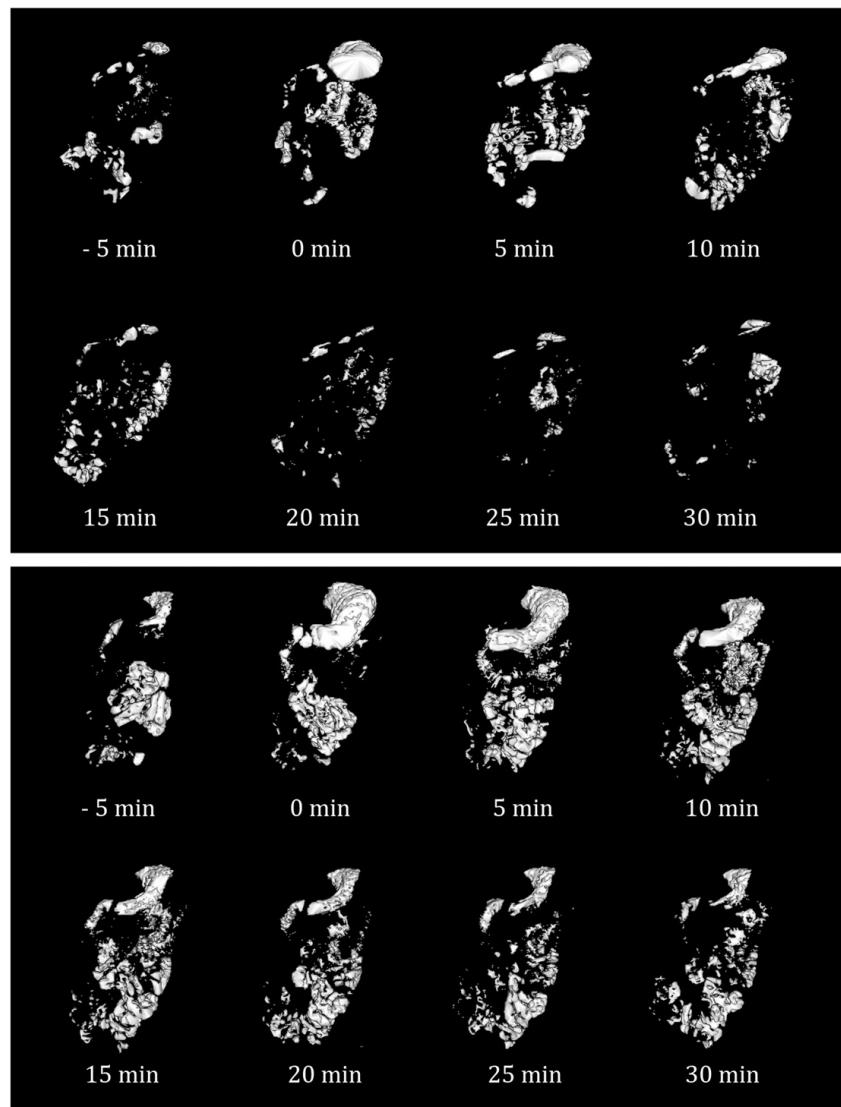


Fig. 5. Two examples of gastrointestinal water distribution directly before (-5 min) and after ingestion of 240 mL under fasting conditions. In these T2-weighted MR images only water is shown.

increased. In a recent study, Marciani and colleagues investigated the dynamics of the small intestinal fluid volume after ingestion of low-caloric test meals over a period of more than 8 h [34]. They showed that the initial decrease of the small intestinal fluid volume is followed by a short plateau phase. Around 90 min after meal intake, the fluid volume begins to rise again but reaches the original level not before 2–3 h post-meal. These data demonstrate that even after higher volumes of food, which typically cause considerable volumes of gastrointestinal secretions, the small intestine is not filled with large amounts of fluid and, thus, does not provide particularly favorable conditions for drug dissolution. However, to the best of our knowledge, the small intestinal

fluid dynamics after the FDA standard breakfast were not investigated so far.

2.2. Transit of dosage forms through the GI tract

2.2.1. Fasted state

The physiological phenomenon of the IMMC is essential for the cleansing of the stomach from non-digestible material, but it has also particular importance for the gastric emptying of non-disintegrating dosage forms like enteric coated tablets or certain extended release (ER) tablets. Such big objects are emptied from the stomach mainly

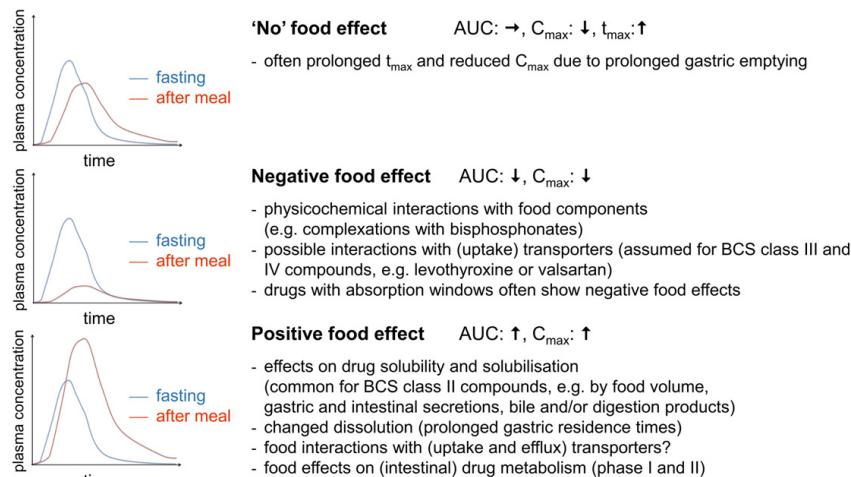


Fig. 6. Classification of food effects on oral drug absorption.

during phase III of the IMMC, in which the strongest contraction waves – the so-called housekeeping waves – sweep along the antral region. Therefore, the gastric residence times of large non-disintegrating solid oral dosage forms that are administered in the fasted state most likely depend on the IMMC phase present at the time of ingestion as well as the time until recurrence of the housekeeping waves. The mean cycle length was found to be about 120 min. This value marks the theoretically maximum possible gastric residence time under fasting conditions. However, it could be seen that for some enteric coated and ER dosage forms, gastric residence times of almost 180 min are reported [17,56]. These values are most likely the result of the high inter- and intraindividual variability of the IMMC duration. Some authors described values for the length of the IMMC of more than 200 min [57,58]. Furthermore, prolonged residence times in fasted state can also be the result of IMMC phase III contractions that arise more distal in the human GI tract (i.e. proximal small intestine) or of insufficient emptying by fasted state contractions. [32,59].

In fasted state, non-disintegrating dosage forms pass the proximal small intestine typically very fast. We observed duodenal transit times of a few seconds to less than 5 min for large non-disintegrating tablets

and capsules as well as for small particles with a diameter of 1 mm [60,61]. The time needed to reach the terminal ileum is typically in the range of 1–3 h. At this region, non-digestible materials like non-disintegrating dosage forms gather until the transfer into the colon occurs. As already mentioned, a common trigger for the transport of material from the small intestine into the large intestine is meal intake. This mechanism is known as the gastro-ileocecal reflex [62]. In pharmacokinetic studies with subjects having fasted overnight, the next meal is typically served 4 h after drug administration. Therefore, the colon arrival of non-disintegrating dosage forms is usually identical to the scheduled time of meal, i.e., 2–4 h after gastric emptying [63,64].

2.2.2. Fed state

In the fed stomach, the situation is more complex as larger objects are retained by the process of gastric sieving [48]. There is contradictory information in literature about the maximum size of objects that still experience gastric emptying under fed conditions [65]. In general, if a drug is not formulated in the form of small particles like pellets, disintegration within the stomach is necessary for gastric emptying together with food. Large non-digestible dosage forms such as most ER tablets are typically only emptied in fasted state by the action of the IMMC. Therefore, the time until recurrence of the IMMC, and thus, the duration of the IMMC interruption by food, determines the gastric emptying time of such objects. Cassilly and colleagues showed that at least 90% of a meal has to be emptied until the IMMC returns [66]. After the high-caloric, high-fat meal, it takes at least 5 h until the gastric content volumes return to baseline levels [30]. However, it must be kept in mind that in food effect BA/BE studies, a next meal will already be served after 4–5 h according to the guidelines. It is therefore likely that during food effect studies the subjects are not at all or only for a short time period before dinner under fasting conditions during the first study day. This conclusion is supported by the observation that in food effect studies, which were performed with the high-fat breakfast, gastric emptying of non-disintegrating tablets is delayed for many hours [67–69].

In a magnetic marker monitoring study, we revealed that even after a medium-fat breakfast of about 600 kcal, felodipine hydrogel matrix ER tablets are retained in the stomach for at least 3 h [52]. It could also be shown in this study that the plasma concentration time profiles of felodipine were strongly influenced by the gastric localization patterns. The specific shear and mixing conditions in fundus and antrum are

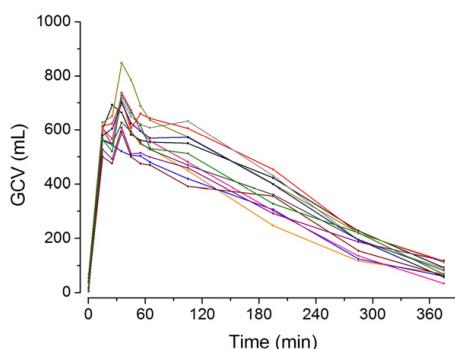


Fig. 7. Individual gastric content volume (GCV) profiles after intake of the high-caloric, high-fat standard breakfast over 375 min, $n = 12$. Immediately after $t = 0$ min, the subjects began with the intake of the breakfast, at $t = 30$ min they received 240 mL of water [30].

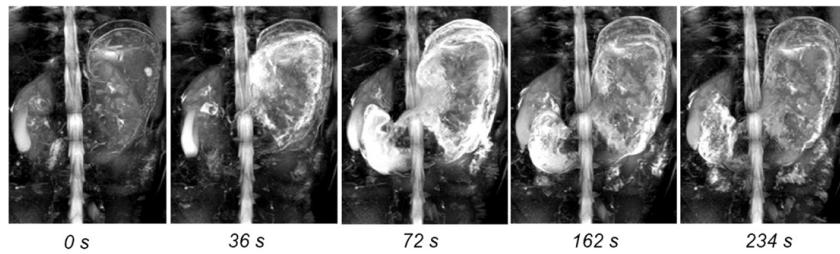


Fig. 8. Visualization of the Magenstrasse by use of maximum intensity projections (MIP) generated by MRI. In the underlying T2-weighted MR images it can be observed that water (bright areas) is flowing around the food mass in both, coronary and transversal view [30]. Copyright (2014) American Chemical Society.

obviously the rationale behind different drug release profiles from the ER tablets [33]. Particularly in distal parts, solid oral dosage forms are exposed to high shear stresses, which can change the drug release behavior. These forces are the result of the gastrointestinal motility of the fed stomach. In a worst-case scenario, this may lead to a burst release of the drug also known as dose dumping [70]. Therefore, further research is needed to characterize the mechanical forces arising during gastrointestinal transit of solid oral dosage forms.

In a recent study performed with the telemetric SmartPill® system, gastrointestinal pressures, pH, and temperature profiles were investigated under fed state intake conditions that strictly followed the FDA guidance for food effect BA/BE [67]. The SmartPill® is a telemetric motility capsule (TMC) able to measure pH, temperature and pressure in high temporal resolution over a period of at least 5 days. As shape and dimensions of this TMC (13×26 mm) resemble those of large solid oral dosage forms, this system enables the characterization of the physiological conditions, to which non-disintegrating oral drug delivery systems are exposed in the lumen of the human GI tract. The pressure profiles showed that the telemetric capsules experience only low pressures below 100 mbar during the first hours after intake. Assuming initial capsule deposition in the fundus, this confirms the hypothesis that little agitation is present in the proximal stomach. With ongoing gastric emptying, events of higher pressures of up to 200 mbar can be observed. As such high pressures can only be generated by antral contractions, it is likely that the TMC is located within distal parts of the stomach. The probability of capsule deposition in the antrum increases with the decrease of the gastric content volume. However, the maximum pressures during the GI transit of the TMC are typically observed during or shortly before gastric emptying of the capsules. Thus, it can be assumed that these high pressures with a magnitude of 200–400 mbar are caused by strong antral contractions that only occur during phase II or phase III of the IMMC. Due to its size, the TMC is most likely retained in the

stomach until recurrence of the IMMC, and thus, the measured pressures represent the maximum pressures arising under fasted state conditions. Interestingly, in a follow-up study, in which the fasted state was characterized with SmartPill®, only pressures of less than 100 mbar were recorded in subjects with very short gastric transit time. Therefore, we assume that maximum pressures occur when the capsule is pushed by strong peristaltic waves against the closed pylorus. If the pylorus is open, the capsule is pushed into the duodenum without resistance and exposed only to slight mechanical forces. Contrary to the situation in the antro-pyloric region, the pyloric transit leads to our understanding not necessarily to high mechanical stresses acting on solid dosage forms.

The small intestinal transport of dosage forms in the fed state is described to be unchanged compared to fasted state with a mean transit time of 3 ± 1 h [64]. As it is known that the residence time in the terminal ileum is influenced by the timing of further meals due to the gastroileocecal reflex, this 3 ± 1 h is characteristic for the situation in clinical studies with well-controlled meal intake but may be quite different in real life [26,63]. In contrast to the stomach, the pressures arising in the small intestine are relatively low [67].

2.3. Luminal pH values

2.3.1. Fasted state

In healthy adults, the pH values of the gastric content are in the range of pH 2–3 under fasting conditions [71]. The gastric pH values, which are present when a dosage form is taken with a glass of water after an overnight fast, have been investigated using data derived from studies with the radio-telemetric IntelliCap® system [72]. Due to dilution of the gastric content by the co-swallowed water at the time of drug intake as well as by ongoing basal gastric acid secretion and

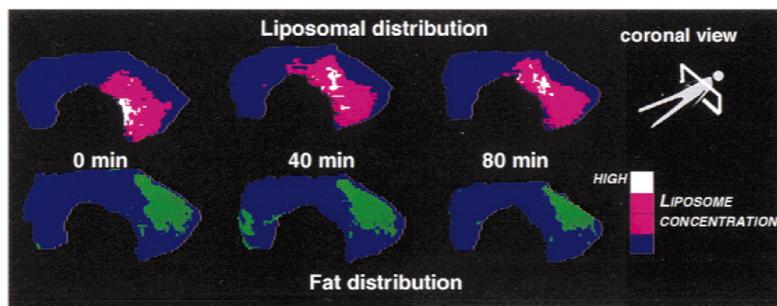


Fig. 9. Maximum intensity projections obtained by MRI indicating poor gastric mixing of a liposomal formulation after a solid meal. Reprinted with permission of Springer [51].

permanent outflow by gastric emptying, the pH profiles in the stomach are drifting and highly variable as shown in Fig. 10.

In particular for drugs with pH dependent solubility, the variation of gastric pH values can lead to either precipitation or supersaturation within the stomach. It was shown by van den Abeele and co-workers that even the initial pH values vary greatly after ingestion of 240 mL of an aqueous diclofenac-potassium solution [73]. Typically, the drug precipitated in the acidic environment of the stomach. However, precipitation did not in subjects with a gastric pH above the pK_a of the free acid. This study convincingly demonstrated that oral absorption after apparently "simple" fasted state administration can be strongly influenced by the highly dynamic physiological conditions even during the rather short residence in the stomach.

Gastric emptying is generally characterized by a sharp pH shift from the strongly acidic conditions within the stomach to the almost neutral conditions (pH 6–6.5) in the duodenum, which is caused by the secretion of alkaline bicarbonate. Often more than one of these abrupt pH shifts can be observed as shown in the examples in Fig. 10. This reflects to our understanding the transport of duodenal contents, predominantly the content of the duodenal bulb, into the stomach. This phenomenon is known as retroperistalsis and occurs predominantly during phase III contractions of the IMMC [74]. As the residual gastric content volume at the time point of phase III contractions is very small, the entry of even small volumes of almost neutral duodenal fluid into the stomach can significantly affect the gastric pH value. This hypothesis is supported by the observation that nocturnal antral pH rises are also associated with retroperistalsis [75]. Nonetheless, it cannot be ruled out that the telemetric capsule itself passes the pylorus during such a pH spike and immediately re-enters the stomach by retroperistalsis. However, based on our experiences with MMM [18], the retropulsion of large solids from the duodenum back into the stomach happens extremely rarely. In all MMM studies of the last two decades, we have

unequivocally observed the movement of a non-disintegrating tablet (an oblong ER matrix tablet with a diameter of 7 mm) from the duodenum back into the stomach in only one single case. By contrast, the retrograde transport of non-disintegrating tablets within the duodenum, but without passing through the pylorus, can be observed quite frequently, in particular immediately after gastric emptying. It is also likely that the process of retroperistalsis causes the transfer of bile into the stomach [76]. However, in contrast to other species (e.g., dogs), only very small amounts of bile are present in the human stomach as the co-ordinative mechanism of retroperistalsis in humans during phase III of the IMMC prevents the transport of larger amounts of bile into the stomach [74].

In the small intestine, a characteristic pH profile is present under fasting conditions as was shown recently [72]. Whereas the pH values in the duodenum amount to pH 6–6.5, significantly higher pH values of pH 7–8 can be measured (Fig. 11). This pH increase is probably the consequence of water absorption, which causes the concentration of bicarbonate in the luminal fluids. Interestingly, the pH values within the small intestine are less variable compared to the stomach, which could be explained by the buffering effect of bicarbonate. This also indicates that the intestinal pH in distal regions is less variable than the gastric content. By contrast, high fluctuations of the pH values were measured in the proximal small intestine, which were comparable to the fluctuations as observed in the stomach shortly before gastric emptying of the telemetric capsules (Fig. 10). The pH spikes reflect to our interpretation small amounts of gastric fluid reaching proximal parts of the small intestine during highly intense housekeeping waves. Such spikes disappear with increasing intestinal transit time and the pH reaches more stable levels in the distal small intestine [67,72]. However, apart from luminal pH values, the acidic microclimate in the unstirred water layer (UWL) has to be considered. It was shown in rats that the pH values in the UWL were slightly acidic

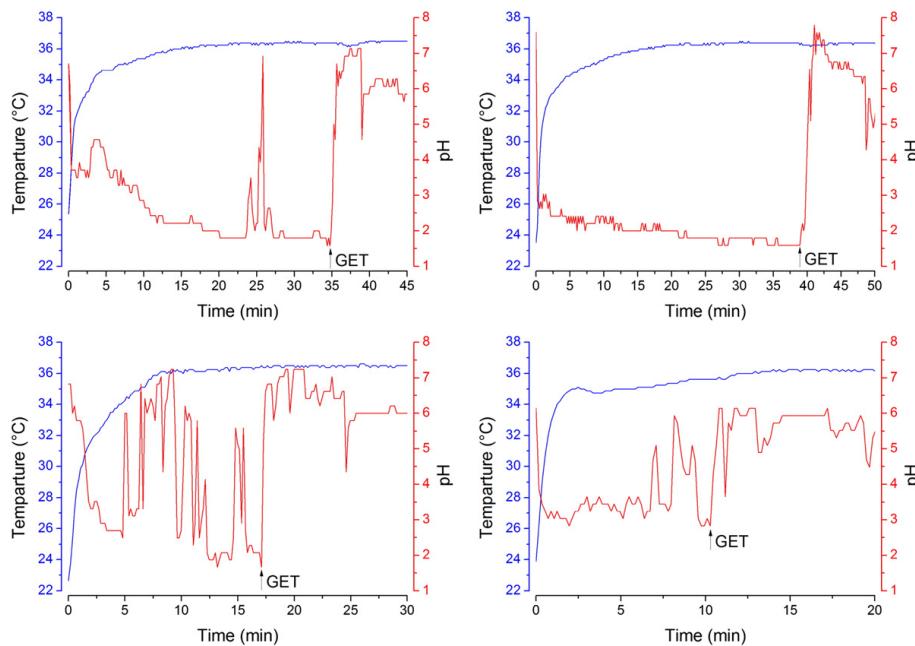


Fig. 10. Exemplary pH and temperature profiles determined using IntelliCap® during gastric residence and the first 10 min of small intestinal transit in healthy volunteers after intake together of the IntelliCap® together with 240 mL of water after overnight fasting. GET—gastric emptying time [72].

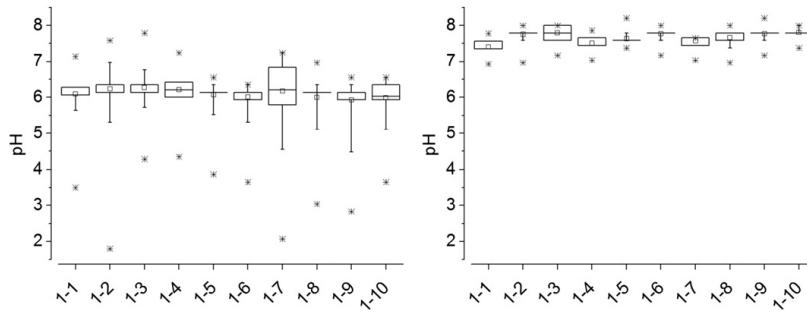


Fig. 11. Variation of the pH values in the proximal small intestine (left) and the terminal ileum (right) measured with the IntelliCap® system. Box: 50%; whisker: 5%–95%; square: mean; asterisks: max/min; $n = 10$. Reprinted with permission from Elsevier [72].

(pH 5–6) and thus lower compared with luminal pH values [77,78]. As far as we know, the pH of the UWL was not measured in humans yet.

2.3.2. Fed state

Owing to increased gastric transit times caused by decreased gastric emptying rates, the luminal pH values in the fed stomach are highly relevant for the drug release profiles, in particular for poorly water soluble drugs. Gastric pH changes are therefore a possible explanation for positive food effects. Whereas the intragastric solubility of weak acids can be increased, weak bases may experience decreased solubility in fed state compared to fasted state. As described above, the gastric pH values after the FDA standard breakfast were investigated in high temporal resolution with the aid of the SmartPill® in 19 healthy subjects. Interestingly, the median of the initial 5 min period is highly variable among the subjects and ranges between pH 3.3 and pH 5.3. These values are much lower than expected from the pH value of the shredded meal (pH 6.6) and indicate high gastric secretion rates [67]. Weinstein and co-workers determined peak acid output rates of 42 ± 22 mmol/h, which is ten times higher than the basal acid output rate of 4.2 mmol/h [79]. Thus, the stomach is able to adapt its secretion rate and typically dilutes incoming food quickly. As can be seen from Fig. 12, the pH value decreases relatively constantly over a time course of 4 h until reaching a baseline level of around pH 1. However, it can also be noted that the gastric pH value shows high variability, which in our understanding is caused by the localization of the telemetric capsule. As only gentle mixing occurs within the fundus, this part of the stomach will also experience higher variability in terms of luminal pH values. We assume that low pH values arise in areas close to the secretory gastric mucosa, whereas higher pH values are present in the middle of the chyme. Several authors also described the presence of a so-called acid pocket in the cardia, in which unbuffered hydrochloric acid gathers [80]. This phenomenon is probably the result of poor mixing in proximal parts of the stomach. It was demonstrated that the acid pocket is formed around 15 min after food intake [81]. Beaumont and colleagues showed that after a solid meal of 510 kcal, the acid pocket reaches its maximum size after 60 min and persists for at least 120 min [82]. In the antrum, the constant peristaltic activity causes intense mixing ("antral mill"), and thus, it is likely that pH values are less variable. After around 4 h, the gastric content is highly acidic, and the meal is more or less completely diluted. The intake of the next meal, i.e., lunch, causes again a rise in pH [67].

As the SmartPill® was emptied most probably in fasted state as could be seen by the low pH values prior to gastric emptying, the measured pH values were similar to the values determined in the fasted state IntelliCap® studies [72]. Owing to the large dimensions of the telemetric capsules available for gastrointestinal pH measurement, fed state small intestinal pH values can only be measured with the aid of pH catheters. Recent publications revealed that the small intestine is able to

keep the pH value relatively constant at a level of around pH 6–6.5 in proximal parts [83,84], which is necessary for optimum enzyme activity. Hence, acidic contents are typically neutralized quickly with alkaline bicarbonate. Along with secretion of bile salts, marked effects on drug solubility can be expected at this point. Whereas weakly basic drug may precipitate, weakly acidic drugs may be of higher solubility. However, the matter is not fully understood at the moment.

3. Implications for dissolution testing

With respect to the complex *in vivo* situation, several attempts were currently made in order to develop and establish *in vitro* test devices and protocols aiming at the simulation of the conditions that are present along the GI tract [85]. In order to achieve this goal, two main strategies are followed.

In the first strategy, novel devices are designed, which are intended to simulate the functionality of the intestinal organs as close to the physiological situation as possible and as complete as required. The most popular and most advanced systems are the TNO TIM-1 system for the simulation of the upper GI tract [86,87], the TNO TIM-2 system for the simulation of the colon [88], and the Dynamic Gastric Model (DGM) that mimics the stomach [89,90]. The advantage of this strategy

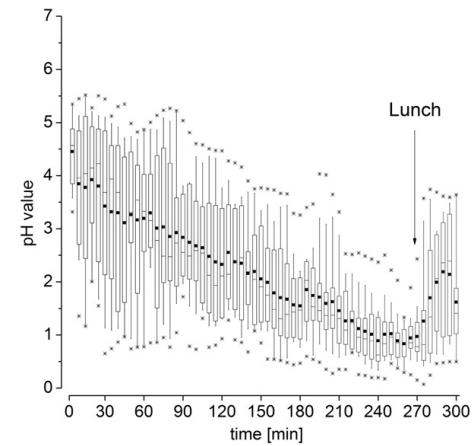


Fig. 12. Gastric pH profiles revealed by the administration of SmartPill® together with 240 mL of water 30 min after the FDA breakfast. Each box represents a 5 min interval. Box: 50%; whisker: 10–90%; square: mean; asterisks: max/min; $n = 16$. Reprinted with permission from Elsevier [67].

is that such devices have, in principle, the potential to generate predictive data on the pharmaceutical availability during all stages of drug development—from the compound to the final formulation. The disadvantage is their high complexity that necessarily results in extensive and time-consuming experiments.

The second strategy follows the creation of test systems and test protocols, which enable the simulation of segregated bio-relevant test conditions. This can be achieved in many different ways and has the advantage of higher simplicity [91]. By applying this strategy, the consecutive simulation of different bio-relevant factors that are acting on oral drug delivery systems during the GI passage is enabled. It also allows investigating the impact of selected physiological factors of bio-relevant intensity on drug delivery processes. In such a way, the critical parameters for the tested formulation can be identified in a combination of relatively basic experiments. However, the test apparatuses and protocols used in the second strategy are typically not suitable for the prediction of drug bioavailability (systemic exposure), but for the prediction of the pharmaceutical availability depending on the drug delivery system. Accordingly, they have to be seen as formulation test tools.

At the moment, we are following the second strategy. Our first approach was the development of the dissolution stress test device, a dynamic bio-relevant test device that maintains major elements of the compendial dissolution test setups (USP apparatuses 1 and 2) but adds the functionality of applying pressure of bio-relevant intensity as well as enforcing dosage form movement (transport) patterns of physiological frequency and velocity [70,92,93]. In the meantime, we extended the dissolution stress test device with two different accessories that allow the characterization of drug release from solid oral dosage forms in the stomach with respect to the discussed dynamic gastric conditions present after drug administration in either fasted or fed state. The “dynamic open flow through test apparatus” enables the investigation of the disintegration and drug release properties of immediate release dosage forms under bio-relevant dynamic conditions of drug intake in the fasted state. In this case, the primary target is the investigation of the implications of realistic gastric dynamic conditions concerning fluid volumes, temperature, pH values, gastric emptying patterns (hydrodynamics), and gastric motility (different phases of IMMC) on dosage form disintegration and drug release [94]. The second extension of the dissolution stress test device is the fed stomach model (FSM). In this approach, we consider that disintegration and drug release of solid oral dosage forms can vary depending on the intragastric location by simulating the conditions specific for antrum and fundus [95].

4. Consequences and assumptions

As shown in previous sections, various common conceptions of the physiological (hydro)dynamic conditions that oral drug delivery systems meet in the human gut are no longer scientifically justified. This applies to certain conceptions on drug delivery systems as well as to a number of ideas about the physiological conditions for absorption of small and large molecules along the GI tract. In this chapter, we will discuss commonly applied concepts that, at least, have to be questioned in the light of recent findings on human GI physiology.

4.1. Are enteric coatings made for the dissolution tester?

It is claimed that small differences in the dissolution pH of enteric coatings allow targeting of drug release to specific regions of the gastrointestinal tract. For instance, coatings with dissolution at pH 5.5 like Eudragit® L 30 D-55 and Eudragit® L 100-55 shall allow the targeting to the duodenum, while dissolution at pH 6.0 aims for drug release to the jejunum [96]. This concept is obviously based on the idea of a small intestine that is, on the one hand, continuously filled with fluid and, on the other hand, shows slow and continuous flow of material through the lumen. However, dosage forms passing the stomach

under fasting conditions experience a small intestine that is for the most part empty. Thus, the essential precondition for dissolution of the enteric coating is the contact of the coated dosage form to an intestinal fluid pocket with enough fluid volume and sufficient capacity to neutralize the acidic groups of the coating. Considering the typically rapid movement of solids through the duodenum under fasting conditions, in most cases this will not happen in the duodenum.

Moreover, due to the process of gastric sieving, enteric-coated tablets are typically emptied from the stomach only under fasting conditions. Due to the intense peristalsis, which causes rapid transfer of big objects through the proximal small intestine, duodenal or even jejunal targeting of enteric-coated tablets is impossible in our understanding. By contrast, small particles like pellets can pass the stomach under postprandial conditions, and therefore, the pH-dependent regional targeting using enteric coatings within the small intestine is essentially possible. However, this concept still needs to be confirmed *in vivo*.

As large objects like many extended release tablets can pass the stomach only in fasted state, the dissolution testing of non-disintegrating dosage forms applying dissolution media, which simulate the postprandial small intestinal conditions like FESSIF [97] does not seem adequate. Furthermore, in conventional dissolution test methods, the dissolution rate of acids like enteric polymers is usually overestimated as dissolution media for the situation in the small intestine are typically based on phosphates as buffer rather than on carbonates as the physiologically relevant buffer species [98–101]. Taken together, we assume that the small intestinal dissolution of enteric-coated dosage forms is rather erratic than targeted. However, the probability of earlier dissolution is higher for coatings that dissolve at lower pH values. This understanding is corroborated by the data that are available from imaging studies and is illustrated in Fig. 13 [53,102–108].

4.2. Drug absorption from the small intestine—do we assume the right luminal concentrations?

The findings of uptake and efflux transporter interaction studies depend on the applied drug concentrations. However, do we have the right assumptions about luminal concentrations present in the small intestine? The data presented above on the water dynamics in the gastrointestinal tract show that the common approach to use the recommended co-administered water volume of 250 mL for the assumption of realistic small intestinal drug concentrations in cell culture assays does not seem justified [109]. Due to the rapid absorption of water, realistic drug concentrations in the intestines can be far above the calculated values. On the other hand, the water accessing the small intestine is typically widely distributed over the jejunum while being absorbed (see Fig. 5). The larger this “wetted” area becomes, the

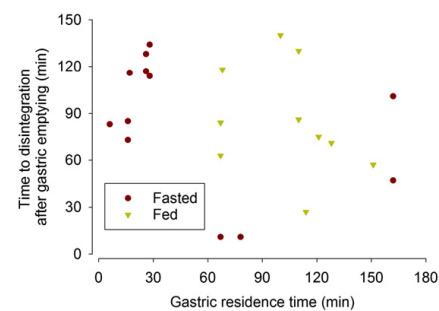


Fig. 13. Disintegration times of enteric coated tablets in the small intestine after gastric emptying, $n = 28$. Every data point represents the disintegration time of a single tablet. In this scintigraphy study, seven healthy male subjects received each two tablets in fasted and fed state (i.e., together with a light breakfast). Reproduced from [103].

more important is the contribution of the water bound in the mucus. The resulting drug concentrations at the intestinal wall and in the unstirred water layer are currently unknown but could also be far below the concentration calculated by dose divided by a volume of 250 mL water. Interestingly, it seems very likely that drug uptake into the enterocyte occurs frequently even from an empty, i.e., collapsed, small intestine. Thus, the resulting variability in effective drug concentrations at the site of drug absorption might be one major contributor to the often observed high variability in drug absorption.

A class of drugs, for which a high concentration dependency of absorption is very likely, are large molecules like peptides and proteins. In this case, we assume that the dilution of the applied dose in the gastrointestinal tract might be an important hurdle for absorption and a source of the high variability seen in clinical trials. This understanding is corroborated by the observation that the oral bioavailability of calcitonin in humans increases with decreasing volume of co-administered water [110], by its negative food effect [111] and its very short effective absorption time [112]. This indicates that absorption takes only place while a high concentration gradient is present. The common idea to use enteric coatings for such poorly absorbable compounds in order to protect them against the acidic conditions in the stomach might also act as a major contributor to the typically very high variability as – like discussed above – enteric coatings require large fluid pockets. However, these are typically located in more distal parts of the small intestine. Therefore, it might be reasonable to develop drug delivery systems for drugs that require high local drug concentration gradients like proteins and peptides. These should ensure high drug concentrations in the proximal small intestine and avoid the dilution and spreading by local small intestinal fluid pockets. We recently developed insoluble capsules that shall deliver dissolved or suspended drug directly into the proximal duodenum. These capsules are cracking at mechanical stresses that are generated by housekeeping waves during gastric emptying [113,114].

In our understanding, the *Magenstrasse* is another underestimated factor that can contribute to high variability of drug absorption in the fed state. By evaluating the individual plasma concentration profiles obtained after administration of immediate release tablets, it can often be differentiated between three more or less typical absorption profiles (Fig. 14). We assume that apart from the disintegration and dissolution behavior of the dosage form, this classification is also based on the initial localization of the dosage form within the stomach. If rapid disintegration or dissolution occurs close the *Magenstrasse*, the drug can follow this shortcut for fluids along the stomach wall and consequently, get emptied into the duodenum within few minutes. Such a scenario results in rapid onset of plasma levels directly after drug intake (Type I, Fig. 14). By contrast, if the dosage form is deposited in the fundus of the stomach and floating on top of the gastric contents, it is likely that it stays there for a long time without experiencing mechanical stresses

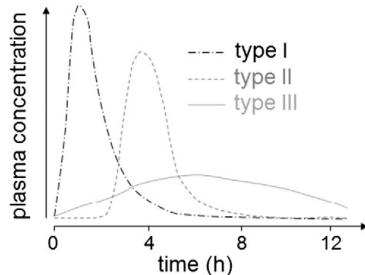


Fig. 14. Proposed schematic effects of the *Magenstrasse* on plasma concentration profiles obtained after ingestion of immediate release dosage forms. Type I: rapid onset of drug plasma concentrations, type II: rapid onset of drug plasma concentration after a lag time, type III: slow onset of drug plasma concentrations over several hours.

as was shown in recent Magnetic Marker Monitoring studies [52,53]. In such a case, the dosage form can stay there either non-disintegrated and undissolved at all or dissolved, but insufficiently mixed with gastric contents. It is possible that during the next fluid intake this dosage form might be “captured” by the ingested fluid and then follows the *Magenstrasse*. Moreover, postural changes can also cause the emptying of this drug bolus. In either case, such a behavior would result in a certain lag time that is followed by the rapid onset of drug plasma concentrations. The duration of the lag time is defined by the next fluid or meal intake (Type II, Fig. 14). However, it is also possible that the dosage form gets directly into the gastric content, where it disintegrates and subsequently, is distributed slowly and more or less homogeneously throughout the chyme. This scenario results in a drug absorption profile that follows the calorie-controlled emptying pattern of the ingested meal. Thus, a slow onset of plasma levels over several hours, i.e., over the time needed for complete gastric emptying of the co-ingested food, would result (Type III, Fig. 14).

A comparable explanation can be considered for events of sudden and rapid increases in blood concentration profiles observed after administration of ER dosage forms—events typically classified as “dose dumping”. A type II-like behavior, i.e., a lag time preceding the dose dumping event could also be indicative for a phase of accumulation of drug substance that is continuously released from the ER dosage form during residence in the stomach, but not emptied into the duodenum. Later fluid or food intake or postural changes could drive the gastric emptying of accumulated dissolved drug in form of a bolus. We could doubtlessly identify such a behavior for two dose dumping like events in an MMM study performed with an ER formulation of felodipine (Fig. 15) [52]. According to our interpretation, it is also very likely that this effect is responsible for the dose dumping observed after administration of felodipine ER tablets together with red wine [115] (Fig. 15) or nifedipine ER tablets with food [116]. We recommend classifying such events as “gastric dumping” instead of “dose dumping” as a malfunction of the drug delivery system in such cases is unlikely.

4.3. The common intake advice—are patients really fasted 2 h after a meal?

Another conclusion derived from the increasing knowledge on gastrointestinal conditions of drug release and uptake is that we have to carefully reconsider the design of food effect studies and the conclusions drawn from these studies with respect to the intake instructions for patients. It is obvious that the composition of a meal influences its gastrointestinal processing and, thus, the gastrointestinal conditions to which solid oral dosage forms are exposed. The recommended high-fat breakfast is in our opinion a helpful reference. However, it should be noted that even small deviations of the composition could lead to

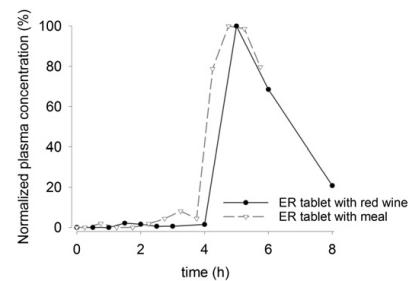


Fig. 15. Comparison of “dose dumping” effects observed for felodipine ER tablets in healthy volunteers. Gray line with open symbols: plasma profile observed for subject no. 4 after intake of a magnetically marked felodipine ER tablet after a meal [52]. Black line with closed symbols: plasma profile observed for subject no. 3 after intake of a felodipine ER tablet together with 240 mL of red wine [115]. In both cases, a meal was served 4 h after drug administration.

changes of oral, gastric and intestinal secretion as well as of gastric mixing and emptying. In consequence, we may obtain different pharmacokinetic outcomes. With respect to the *Magenstrasse*, it is absolutely necessary to control and document any fluid intake including water volume and temperature until the next meal is served. On the other hand, it should be clear that the highly standardized intake conditions of bioavailability and bioequivalence testing in fed and fasting state are not descriptive for real life conditions. This fact seems particularly critical for drugs with positive food effects. As modern drugs are often poorly water soluble, positive food effects are increasingly common. However, these are often labeled to be taken under fasting conditions [117]. This is artificial as essentially nobody is under fasting conditions as defined by the guidelines: overnight fast of at least 8 h plus another 4–5 h without food intake after drug administration. Therefore, it should be advised to take drugs with positive food effects with food in order to avoid the situation that patients, who take their medication under non-recommended conditions, are overdosed [118]. The assumption in the FDA Guidance on Food-Effect Bioavailability and Fed Bioequivalence Studies [21] that patients are under fasting conditions already 2 h after a meal is certainly wrong as was demonstrated in previous studies [30,67]. Even the gastric emptying of the radio-labeled, low-fat 255 kcal egg-white test meal used for the scintigraphic determination of gastric emptying takes more than 2 h [119]. If we do not change the common intake advice, we keep on putting many patients at risk although we would know better.

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6.2 Effect of Co-administered Water on the In Vivo Performance of Oral Formulations Containing N-Acetylcysteine: An In Vitro Approach Using the Dynamic Open Flow-Through Test Apparatus (*Molecular Pharmaceutics* 2017, 14 (12), 4272-4280)

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¹ Effect of Coadministered Water on the *In Vivo* Performance of Oral Formulations Containing N-Acetylcysteine: An *In Vitro* Approach Using the Dynamic Open Flow-Through Test Apparatus

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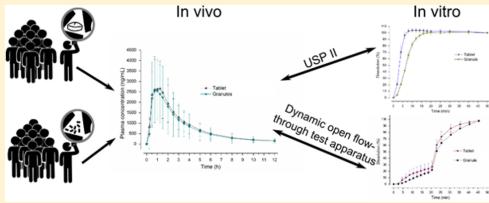
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ABSTRACT: The drug plasma profile after oral administration of immediate release dosage forms can be affected by the human gastrointestinal physiology, the formulation, and the drug itself. In this work, we investigated the *in vivo* and *in vitro* performance of two formulations (granules vs. tablet) containing the highly soluble drug N-acetylcysteine (BCS class I). Thereby, special attention was paid to the effect of the dosage form and the coadministration of water on drug release. Interestingly, the *in vivo* results from a pharmacokinetic study with 11 healthy volunteers indicated that the drug plasma concentrations were comparable for the tablet given with water as well as for the granules given with and without water. In order to mechanistically understand this outcome, we used a biorelevant dissolution test device, the dynamic open flow-through test apparatus. With the aid of this test apparatus, we were able to simulate biorelevant parameters, such as gastric emptying, hydrodynamic flow as well as physical stress. By this, it was possible to mimic the intake conditions of the clinical trial (i.e., drug intake with and without water). Whereas the experiments in the USP paddle apparatus revealed differences between the two formulations, we could not observe significant differences in the release profiles of the two formulations by using the dynamic open flow-through test apparatus. Even by considering the different intake conditions, drug release was slow and amounted to around 30% until simulated gastric emptying. These results suggest that dissolution was irrespective of coadministered water and the formulation. Despite the high aqueous solubility of N-acetylcysteine, the limiting factor for drug release was the slow dissolution rate in relation to the gastric emptying rate under simulated gastric conditions. Thus, in case of administration together with water, large amounts of the drug are still present in the stomach even after complete gastric emptying of the water. Consequently, the absorption of the drug is largely controlled by the nature of gastric emptying of the remaining drug. The data of this study indicated that the water emptying kinetics are only determining drug absorption if drug release is rapid enough. If this is not the case, physiological mechanisms, such as the migrating motor complex, play an important role for oral drug delivery.

KEYWORDS: immediate release, fasted state, dynamic open flow-through test apparatus, oral dosage forms, pharmacokinetic study, gastric model



INTRODUCTION

Immediate release (IR) dosage forms are typically administered together with a certain volume of water. In order to understand and predict the *in vivo* performance of solid oral dosage forms, fluid kinetics in the stomach have to be considered. In bioequivalence studies, the dosage form to be tested shall be administered together with a glass of table water (at least 150 mL based on EMA and 240 mL based on FDA guidelines). Further water intake is not allowed until 1 h after drug administration. Subsequent water intake is not limited in terms of timing and volume. The application of such a standardized protocol shall lead to comparable gastrointestinal (GI) conditions regarding fluid volume, gastric emptying, and gastric motility as these parameters are regarded as highly critical for the *in vivo* performance of IR products.

In fasted state, significant amounts of fluid can be emptied from the stomach within minutes. The gastric emptying of 240 mL water, which is the volume of the coadministered water recommended by FDA for bioequivalence studies, was studied by Mudie and colleagues.¹ By using magnetic resonance imaging (MRI), they found this volume to be emptied in fasted state following a first order kinetic within roughly 30 min. With respect to the fasted state administration of solid oral

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58 dosage forms, the time point of dosage form disintegration, and
59 drug dissolution may be highly relevant for drug absorption. In
60 case of delayed drug release, large parts of the water may be
61 emptied before dosage form disintegration has finished and
62 thus, this water is not available for dissolution and transport.
63 This interplay between dosage form disintegration and
64 dissolution on the one hand as well as the dynamics of gastric
65 emptying on the other hand cannot be considered by the use of
66 compendial test methods. For a mechanistic understanding of
67 the behavior of dosage forms in the human GI tract, several
68 complex (e.g., dynamic gastric model or TNO TIM-1) or
69 simple (e.g., artificial stomach duodenal model, rotating beaker
70 apparatus) biorelevant dissolution test devices can be used.^{2–5}
71 Within the past years, several research groups developed
72 different biorelevant *in vitro* dissolution tools for the
73 investigation of drug release under physiologically relevant GI
74 conditions. Depending on the dosage form (e.g., immediate vs
75 extended release) and the simulated parameters (e.g., pH,
76 hydrodynamics), these models vary from rather simple models
77 aiming to simulate certain aspects of GI transit to highly
78 complex models of GI processing.^{6,7} Apart from a mechanistic
79 understanding, the early application of such models can aid to
80 save time and costs during the development of new
81 formulations and also supports the selection of the most
82 promising formulation for *in vivo* studies.⁸

83 In order to adequately simulate gastric emptying and gastric
84 motility in dissolution testing of IR dosage forms, Garbacz and
85 co-workers developed the dynamic open flow-through test
86 apparatus.⁹ With the aid of this biorelevant dissolution test
87 model, it is possible to mimic the pressures resulting from
88 gastric peristalsis as well as dosage form movement under
89 realistic hydrodynamic conditions. The strengths of this model
90 were demonstrated in a recent study by Garbacz and colleagues,
91 in which the model was used to characterize drug release from
92 different capsule shells.⁹

93 The basis for the present work were data from a fasted state
94 pharmacokinetic study, in which the blood plasma profiles of a
95 granule and a tablet formulation containing N-acetylcysteine
96 (NAC) were investigated. To confirm the possibility of dry
97 intake, the granule formulation was administered with and to
98 without water. NAC is widely used as a mucolytic agent due to
99 its efficacy in the treatment of chronic obstructive pulmonary
100 diseases.¹⁰ At the doses used in this study, NAC is regarded as
101 highly soluble and absorbed almost completely from the human
102 intestine. Thus, it is classified as a biopharmaceutics
103 classification system (BCS) I drug. After absorption, NAC
104 has a short plasma half-life time of 1 h only and experiences a
105 strong first-pass effect in the liver. The volume of distribution is
106 0.33 L/kg and the total body clearance amounts to 0.211/h/kg.
107 An amount of around 22–30% of the drug is eliminated via the
108 urine.¹¹

109 The aim of the present work was to explain and understand
110 the unexpected plasma profiles of two NAC formulations
111 (tablets versus granules) that were determined in a
112 pharmacokinetic study, in which also different intake conditions
113 (with or without water) were applied. For this purpose, the
114 dynamic open flow-through test apparatus was used to
115 investigate the effect of motility and gastric emptying on drug
116 release. Moreover, the intake conditions of the pharmacokinetic
117 study (i.e., with or without water) were considered in dedicated
118 experiments.

■ EXPERIMENTAL SECTION

119

Materials. N-Acetylcysteine 600 mg tablets (FLUIMUCIL, 120
Zambon Nederland B.V., Netherlands), N-acetylcysteine 600 121
mg dry granule (pilot formulation, Hexal, Germany), potassium 122
dihydrogen phosphate KH₂PO₄ (Merck, Darmstadt Germany, 123
Lot: A0135473009), acetonitrile HPLC Optigrade (Braun, 124
Melsungen Germany, Lot: 140416), hydrochloric acid 37% 125
pure (neoLab, Heidelberg Germany, Lot: 3E003177), sodium 126
chloride (Caelo, Hilden Germany, Lot: 09241023), N- 127
acetylcysteine powder (Caelo, Hilden Germany, Ch.-B.: 128
10230712), and Volvic table water (Lidl Germany). 129

Pharmacokinetic Study. Study Design. The pharmacokinetic 130
study was completed by 11 healthy volunteers in an open, 131
single-dose, randomized, three-armed, crossover design with 132
the aim to compare the pharmacokinetic profiles of two NAC 133
formulations (reference vs test product). Therefore, the 134
reference formulation (FLUIMUCIL tablets) and the test 135
formulation (pilot granule formulation) were administered 136
together with 150 mL of water. In addition, the granule 137
formulation was also administered dry without water. The 138
volunteers received the formulations in an upright position, 139
which they had to maintain (by either walking, sitting or 140
standing) for at least 4 h. A wash-out period of at least 7 days 141
between each of the three study periods was maintained. All 142
subjects had to abstain from food for at least 10 h before and 143
until 4 h after drug intake. The study was conducted in 144
accordance to the Declaration of Helsinki and written informed 145
consent was obtained from all subjects. The ethical approval for 146
the presented study was given by the Ethics Committee at the 147
clinic UMHAT "Tsaritsa Yoanna-ISUL" in Sofia, Bulgaria 148
(positive statement number: 34, dated 11-Dec-2014). Demographic 149
data of the study population is given in Table 1. 150 t1

Table 1. Demographic Data of the Study Population (*n* = 12)

criteria	mean ± SD	min–max
age (years)	38.8 ± 8.8	21.0–48.0
height (cm)	166.4 ± 7.2	156.0–179.0
weight (kg)	67.9 ± 12.5	50.0–86.5
BMI	24.5 ± 3.9	19.0–29.3
male:female	5:7	

Bioanalytical Method. The determination of NAC in the 151
blood plasma samples was performed by a high performance 152
liquid chromatographic (HPLC) system coupled with a triple- 153
quad mass spectrometer detector. The validation included the 154
following parameters: Limit of quantification was 50.25 ng/mL, 155
whereas the validated concentration range was 50.25 to 3015.00 156
ng/mL. The intra-assay precision was in the range of 1.07 to 157
10.23% and the intra-assay accuracy was 102.67 to 114.49%. 158

Statistical Analysis. Statistical analysis of the major 159
pharmacokinetic parameters Area and the curve (AUC), *C*_{max} 160
and *t*_{max} was performed by using the validated SAS program 161
"NC PKP.sas" in the version SAS 9.4 (SAS Institute, Cary, 162
NC, USA). 163

**Dissolution Test Experiments. Setting and Function of 164
the Dynamic Open Flow-Through Test Apparatus.** A 165
schematic of the dynamic open flow-through test apparatus 166
(DOFTA) is given in Figure 1. The main part of the dynamic 167 f1
open flow-through test apparatus (DOFTA) is a central bar 168
connected to a stepping motor. Three probe chambers made of 169
steel wire that host the dosage form are orthogonally linked to 170
the central bar and fit exactly into the respective gastric cell. 171

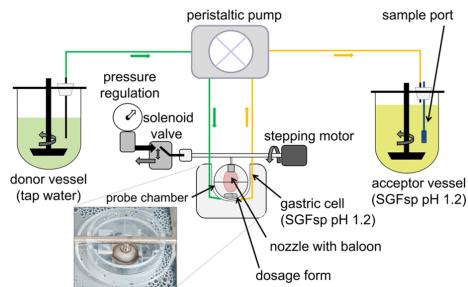


Figure 1. Schematic view of the dynamic open flow-through test apparatus (modified from Garbacz et al. 2013).

The test vessels can take up a volume of up to 50 mL representing a realistic volume for the amount of fluid in the fasted stomach. The major advantages of the DOFTA are the opportunity to exert well-defined pressures from 100 mbar up to 600 mbar (75 mmHg up to 450 mmHg) onto the dosage form located within the probe chambers and to simulate the movement of the dosage forms. The exertion of pressures is controlled by a solenoid valve that allows the inflation of a balloon, which is connected to a nozzle inside the probe chamber. By rotating the bar with the aid of the stepping motor, slight movements of the dosage form can be simulated with defined velocities. During these movements, the balloon is deflated. A thorough description of the dynamic open flow-through test apparatus is given in a recent publication by Garbacz and co-workers.⁹

With the help of a peristaltic pump, dissolution medium was pumped with defined rates from the donor to the acceptor vessel, thereby perfusing the test vessel (gastric compartment), in which the volume of 50 mL remained constant. During the experiments, the donor medium was kept at room temperature (approximately 20 °C) representing the temperature of the coadministered water, whereas the test and the acceptor medium were maintained at body temperature (37 °C). The acceptor vessel, representing the small intestine, contained 500 mL simulated gastric fluid sine pepsin (SGFsp) at the beginning of the experiment to ensure complete dissolution of the drug transported from the gastric cell into the acceptor

vessel. To ensure homogenization in the acceptor vessel, a paddle stirrer at 75 rpm was used. Samples of 1 mL were taken with a syringe (10 mL Injekt Luer Solo, B.Braun Melsungen, Germany) from the acceptor vessel every 2 min starting at 2.5 min until 25.5 min. Subsequently, the sampling frequency was 5 min until the end of the experiments (45.5 min). The samples were filtered through a 1 µm filter (Poroplast, B.Braun, Melsungen Germany) and analyzed as described in the following paragraph (*In Vitro Assay*).²⁰⁷

Test Algorithms. The two programs W1 and W3 shown in Figure 2 reflect the gastric conditions during drug intake together with a glass of water. To simulate the coadministration of water, 150 mL of table water (Volvic table water) was pumped through the gastric cell (50 mL SGFsp pH 1.2) within 20 min by applying five gradually decreasing pump rates. These ranged from 19.4 mL/min to 1.2 mL/min. Thereby, a first order water emptying kinetic was simulated. Each of these steps had a duration of 4 min. After 20 min, the occurrence of a migrating motor complex (MMC) phase 3 activity was simulated by three fast movements of the probe chamber (100 rpm) within the vessel and three pressure waves of a magnitude of 300 mbar (duration of 6 s). In addition, the pump rate was increased to 20 mL/min for 2.5 min after 20 min in all programs in order to achieve complete gastric emptying of the remaining volume of 50 mL in the test vessel. The test programs W1 and W3 differed in terms of the additional exposure of the dosage form to a single pressure event of 100 mbar and a slow movement of the probe chamber (10 rpm) after 10 min in test program W3.

As was mentioned above, the secondary aim of this study was the simulation of drug intake without water as it was also done in the *in vivo* study for the granule formulation. Therefore, a basal secretion rate of 2.5 mL/min was assumed, which represented the sum of oral and gastric fluid secretions.^{12,13} In comparison to W1/W3, the drastically reduced flow rate was the main difference of the test programs B1/B3. Analogous to test program W3, there was also an additional simulated motility event after 10 min in test program B3 (Figure 3).

Compendial Dissolution Testing. The USP paddle apparatus (PharmaTest DT 70, Hainburg, Germany) was used as a reference method. The experiments were performed at 37 °C ± 0.5 with a stirring speed of 75 rpm and a media volume of 900 mL. Simulated gastric fluid without pepsin

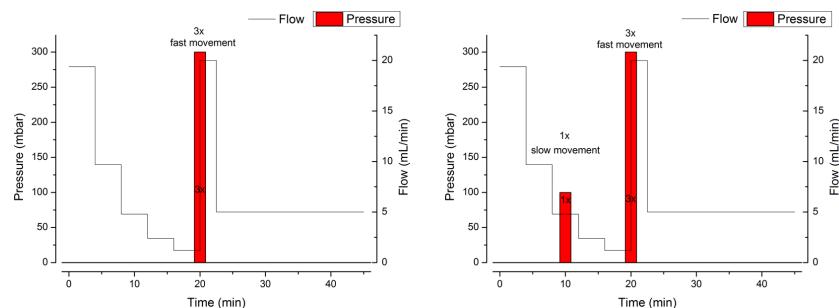


Figure 2. Schematic representation of the test programs W1 (left) and W3 (right) used for the simulation of the intake of the N-acetylcysteine formulations together with 150 mL of water in the dynamic open flow-through test apparatus. The test program W3 considers an early pressure event of 100 mbar accompanied by a slow movement of 10 rpm after 10 min. After 20 min the occurrence of MMC phase 3 activity was simulated in all programs with increased physical stress and a high flow rate to ensure complete emptying of the gastric cell.

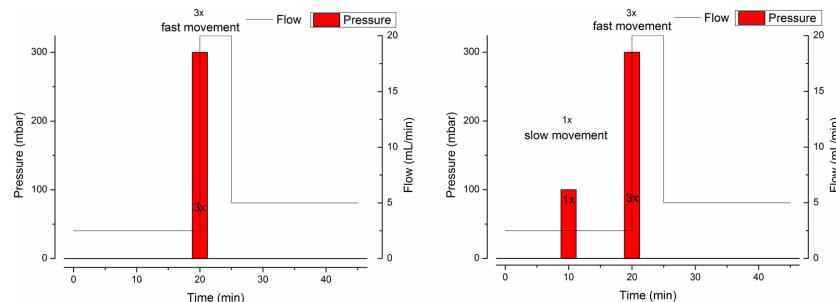


Figure 3. Schematic representation of the test programs B1 (left) and B3 (right) used for the simulation of the dry intake of the N-acetylcysteine formulations in the dynamic open flow-through test apparatus. The test program W3 considers an early pressure event of 100 mbar accompanied by a slow movement of 10 rpm after 10 min. After 20 min the occurrence of MMC phase 3 event was simulated in all programs with increased physical stress and flow rate to ensure total emptying of the gastric cell.

(SGFsp) pH 1.2 as well as table water (Volvic) were used as dissolution media. SGFsp was prepared by dissolving 2 g of sodium chloride in 1 L of purified water and by adding hydrochloric acid in order to adjust the pH to 1.2. In order to investigate the impact of dissolution media and to allow a comparison to drug release in SGFsp pH 1.2, table water (Volvic) was also used as a dissolution media. The duration of the experiments was 50 min and samples were collected every 2.5 min for the first 20 min and after this period the sampling interval changed to every 10 min. The sample preparation was the same as in DOFTA.

In Vitro Assay. The quantification of NAC in the *in vitro* experiments was done via separation of the compound by HPLC and detection with a diode array detector from Shimadzu (CBM-20A, SCL-10 Avp, Duisburg, Germany), which recorded the absorption at a single wavelength. A reversed-phase column (Kinetex, RP-C18 Phenyl; Aschaffenburg, Phenomenex Germany) with a total size 250×12 mm and a particle size of $5 \mu\text{m}$ was used. Specific parameters of the method are given in Table 2. Data analysis was performed with the corresponding software CLASS-VP (Duisburg, Shimadzu Germany).

Table 2. HPLC Parameters for the Quantification of N-Acetylcysteine

parameter	value
injection volume	$10 \mu\text{L}$
flow rate	$1 \text{ mL}/\text{min}$
wavelength	214 nm
retention time N-acetylcysteine	3.2 min
temperature column	30°C

The mobile phase consisted of a mixture of $50 \text{ mM } \text{KH}_2\text{PO}_4$ in aqueous solution and acetonitrile in a ratio of 95/5 (v/v %). Potassium dihydrogen phosphate was dissolved in water acquired from a Merck Milli-Q system (Darmstadt, Merck Germany). The mobile phase was degassed for 30 min before usage. In order to verify this HPLC method, different validation tests were done. The method was validated in respect to accuracy of the mean (95–105%), precision (method precision <2%), linearity (range: 12.5 – $350 \mu\text{g}/\text{mL}$, $R^2 > 0.995$), and limit of quantification ($12.5 \mu\text{g}/\text{mL}$). All samples were analyzed directly after acquisition.

Statistical Analysis. For comparison of the release profiles, we calculated the similarity factor (f_2) according to the formula of the FDA guideline “Dissolution Testing of Immediate Release Solid Oral Dosage Forms”.¹⁴ In contrast to the guideline, we only used six units instead of the recommended 12 units. The formula was also used for the comparison of release profiles, in which more than 85% of the drug was released within 15 min. A minimum of four data points was used for the calculations. In line with the guideline, dissolution profiles were regarded as similar when the f_2 value was above 50.

RESULTS

Clinical Study. The mean plasma concentrations of NAC after fasted state administration of the tablet and the granule formulation together with 150 mL of water as well as of the granule formulation after administration without water intake are shown in Figure 4. As can be seen from the graph, all three mean plasma profiles are comparable. A fast onset of the plasma concentration could be observed irrespective of the intake.

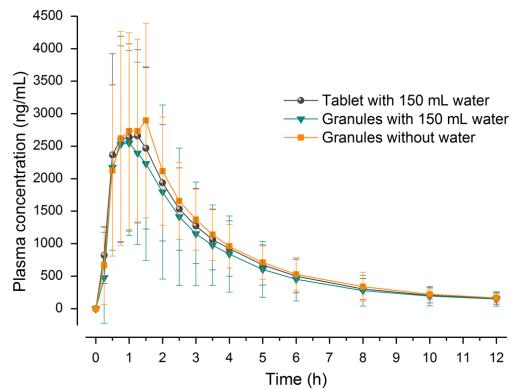


Figure 4. Blood plasma concentrations of N-acetylcysteine over time after administration of 600 mg dose either in form of an immediate release tablet administered with 150 mL water or in form of a granule formulation administered with 150 mL of water or without water (mean \pm SD, $n = 11$).

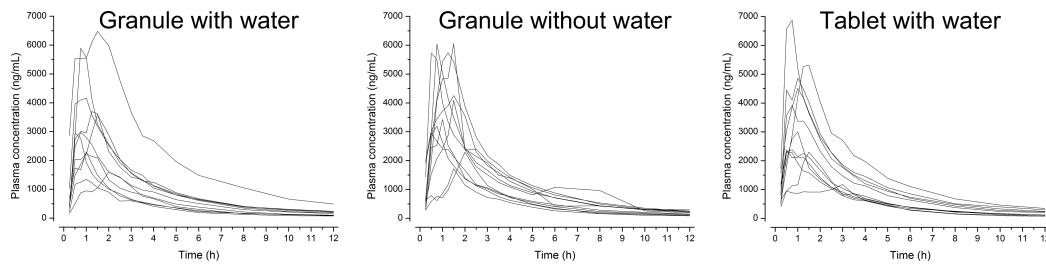


Figure 5. Individual blood plasma profiles of N-acetylcysteine for the tablet formulation administered with water as well as for the granule formulation administered with or without water ($n = 11$).

294 conditions and the formulation. In Figure 5 the individual
295 plasma profiles for all three study arms are presented in form of
296 spaghetti plots.

297 Further analysis of the major pharmacokinetic parameters is
298 given in Table 3. It can be seen that the mean value of t_{\max} is in

Table 3. Pharmacokinetic Parameters N-Acetylcysteine after Oral Administration^a

variable	statistic	granule with water	granule without water	tablet with water
AUC (0– t) (ng·h/mL)	mean	10687.19	11590.42	10983.10
	SD	6544.27	4292.06	4759.61
AUC (0– ∞) (ng ² h/mL)	mean	11633.09	12523.15	11870.37
	SD	7163.41	4617.58	5245.41
C_{\max} (ng/mL)	mean	3385.73	4085.82	3544.10
	SD	1637.87	1613.72	1694.90
t_{\max} (h)	mean	1.118	1.227	1.117
	SD	0.420	0.440	0.710

^aMean, standard deviation; SD, $n = 11$.

299 the range of 1.118 to 1.227 h for all three scenarios. The
300 granule formulation administered without water intake showed
301 with 1.227 h the highest t_{\max} . It should be noted that the t_{\max} of
302 the tablet formulation showed the highest variability, with a
303 standard deviation of 0.710 h compared to the granule
304 formulation administered with or without water (0.420 and
305 0.440 h). The granule given without water showed the highest
306 C_{\max} whereas the granule given with water exhibit the lowest
307 C_{\max} with the highest variability (relative standard deviation
308 39.5% and 48.4%).

309 **Compendial Dissolution Testing.** The dissolution experi-
310 ments with the USP paddle apparatus, as a compendial
311 dissolution test method, were able to discriminate between the
312 two investigated formulations (Figure 6). This was also
313 expressed by the similarity factors. In both set-ups, either
314 with water or SGFsp as dissolution media, the release profiles of
315 the tablets and the granules resulted in f_2 values <50 (Table 4).
316 **Dynamic Open Flow-Through Test Apparatus: Ad-**
317 **ministration with Water.** The results of the programs W1
318 and W3, which represent drug administration together with 150
319 mL of water and subsequent first order flow kinetics, are shown
320 in Figure 7. Only 15–30% of the drug dissolved until the end of
321 the assumed time point of complete gastric emptying of the
322 coadministered water (20 min). In all cases, dissolution showed
323 a short lag-time of 2.5 min. On average, the tablets tested with
324 program W1 showed the fastest dissolution, whereas the
325 granule formulation tested with program W1 exhibited the

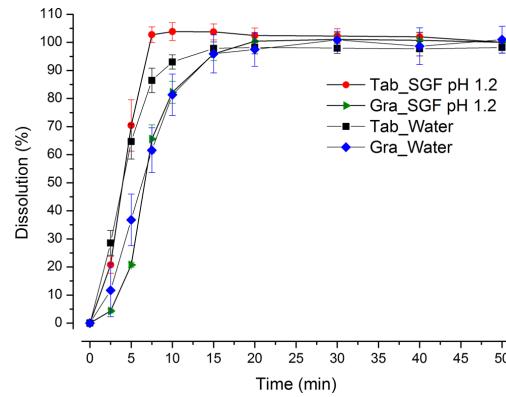


Figure 6. Dissolution profiles of N-acetylcysteine containing tablets and granules in the USP paddle apparatus with a stirrer speed of 75 rpm, vessel temperature of $37^{\circ}\text{C} \pm 0.5$, and a total volume of 900 mL in simulated gastric fluid without pepsin and table water (mean \pm SD, $n = 6$).

Table 4. F2 Values for the Comparison of the In Vitro Dissolution Profiles^a

comparison	f_2 value
tablet vs granule USP II SGFsp	25
tablet vs granule USP II water	38
tablet W1 vs tablet W3	71
granule B1 vs granule B3	80
granule W1 vs granule W3	69
tablet W3 vs granule W3	69
granule B3 vs granule W3	58

^a $n = 6$.

slowest release. The simulation of an intragastric motility event 326 by gentle agitation and pressure application after 10 min in 327 program W3 did not result in any relevant changes of the drug 328 release profiles of the tablet and the granules. This is reflected 329 by f_2 value >50 for the comparison of the release profiles of the 330 tablets (tablet W1 versus tablet W3) and for the granules 331 (granule W1 versus granule W3). However, in this program 332 standard deviations of around 8% were obtained for the tablets, 333 whereas in all other scenarios the standard deviation was below 334 1%. The calculation of the f_2 value for the comparison of the 335 release profiles of the tablets with water (tablet W3) versus the 336

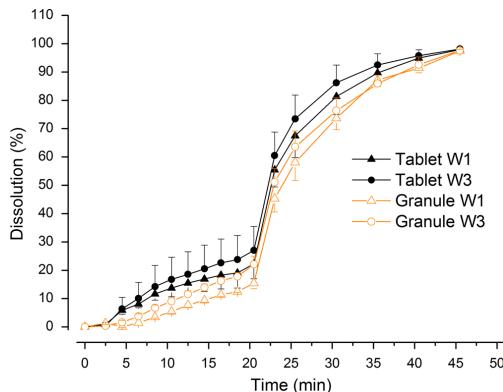


Figure 7. Dissolution profiles of the N-acetylcysteine tablet and the N-acetylcysteine granule formulation in the dynamic open flow-through test apparatus obtained after applying two test programs (W1, W3) simulating the intake of the dosage forms together with 150 mL of water (means \pm SD, $n = 6$). For both programs, complete gastric emptying was simulated after 20 min and subsequently, the vessels were flushed with medium to remove any undissolved material. For program W3, an additional event of gentle agitation and mild pressure was simulated after 10 min.

337 granules with water (granule W3) resulted f_2 values >50 , which
338 also indicated similar release profiles.

339 **Dynamic Open Flow-Through Test Apparatus: Ad-
340 ministration without Water.** The results for the test
341 programs B1 and B3 that are mimicking drug administration
342 without additional water intake are shown in Figure 8.
343 Programs B1 and B3 only considered basal secretion rate
344 and emptying rate and thus, fluid flow followed zero order
345 kinetics. It can be seen that about 30% of NAC dissolved within

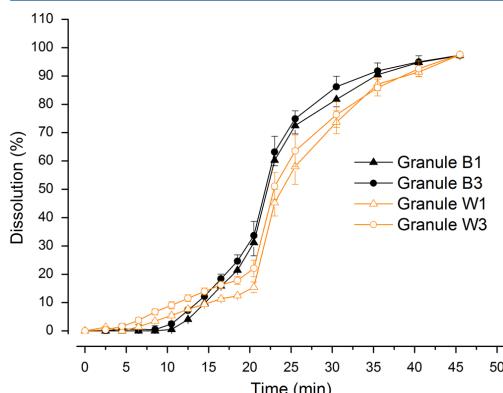


Figure 8. Dissolution profiles of the N-acetylcysteine tablet and the N-acetylcysteine granule formulation in the dynamic open flow-through test apparatus obtained after applying two test programs (B1, B3) simulating the dry intake of the dosage forms (means \pm SD, $n = 6$). For both programs, complete gastric emptying was simulated after 20 min and subsequently, the vessels were flushed with medium to remove any undissolved material. For program B3, an additional event of gentle agitation and mild pressure was simulated after 10 min.

20 min. Compared to the programs W1/W3, the lag-time until first detectable dissolution was prolonged to 8 min. After this initial delay, the slope of the graph was low for the two formulations. The gentle agitation performed in program B3 at 10 min had no relevant impact on the release profile, as the f_2 factor >50 for the comparison of program B1 versus B3 and for the comparison of the release profiles of the granules without water (granule B3) versus the granules given with water (granule W3).

■ DISCUSSION

Compendial dissolution test methods are valuable tools for quality control and early dosage form characterization, but it is obvious that they are not able to reflect the complex physiological situation, to which solid oral dosage forms are exposed in the human GI tract.¹⁵ In certain cases, the application of an unsuitable test method can lead to false conclusions. For instance, an *in vitro* experiment suggests that a novel generic product is bioequivalent to the originator product. Based on these *in vitro* observations, the formulation is chosen for clinical trials, which will reveal significant and unexpected differences in the plasma profiles of the two formulations. This hypothetical discrepancy between *in vitro* and *in vivo* data can consume time, money, and resources. Hence, powerful biorelevant dissolution test methods should be applied as early as possible, additionally to the compendial methods, in order to detect such undesired drug release behavior already *in vitro*.

In the present work, we had the less prominent case that the *in vitro* data suggested a difference in drug release for two formulations that could not be detected *in vivo*. In this study, two formulations each containing 600 mg of N-acetylcysteine that were both intended to show fast disintegration in order to provide quick drug release were investigated. By using the compendial USP paddle apparatus, it could be clearly discriminated between the tablet and the granule formulation as the tablet showed a faster dissolution. It should be noted that these differences in dissolution are not commonly regarded as crucial by authorities like the FDA, as both products show a dissolution of more than 85% in the first 15 min. However, the compendial method discriminates between the release profiles and shows at least a trend toward a faster dissolution of the tablet formulation compared to the granules. Interestingly, these differences could not be observed in the *in vivo* plasma profiles that were obtained in the pharmacokinetic study. Although the number of subjects included in this study was close to the number of 12 subjects that is requested in bioequivalence studies, a larger study population may have been beneficial for the descriptive character of this study.

In order to understand the reasons for this discrepancy between *in vitro* and *in vivo* data, we used the dynamic open flow-through test apparatus. Furthermore, in order to consider the different intake conditions (i.e., with and without water) as well as the physical stresses occurring *in vivo* in the fasted stomach, four test programs were applied in order to describe drug release under physiologically relevant conditions. Based on data from various MRI studies, a residual volume of 50 mL in the fasted stomach was assumed and used as the initial volume in the gastric cell.^{1,16,17} According to the study protocol of the pharmacokinetic study, both formulations were administered together with 150 mL of water. This fact was simulated in two test programs that simulated the administration together with water (W1/W3). Moreover, by including five steps of

408 decreasing pump rates, these test programs also considered the
409 typical first order gastric emptying kinetics of water. It was
410 shown recently by Mudie and colleagues that in the fasted state
411 the volume of fluid in the stomach decreases from 250 to 50
412 mL following first order kinetics within approximately 30 min
413 after administration of 240 mL of water.¹ The stepwise
414 reduction of the flow rate allowed us to roughly recreate the
415 gastric emptying of 150 mL of water *in vivo* according to
416 physiological data. In all four test programs, we simulated a
417 gastric residence time of 20 min, which reflected the mean
418 gastric transit time of solid oral dosage forms in previous
419 magnetic marker monitoring studies.¹⁸ After this gastric transit
420 phase, the transfer into the small intestine was simulated by
421 applying a pressure of 300 mbar and by increasing the flow rate.
422 It was shown recently with the aid of the SmartPill that
423 maximum pressures of 300 mbar or more can arise during
424 gastric emptying system.^{19–22} This can be either a result of the
425 contraction of the pyloric sphincter or of intense antral
426 contraction waves pushing larger solid objects against the
427 pylorus.²³ Recent *in vivo* data published by Schneider and
428 colleagues further suggest that beside these maximum
429 pressures, events with lower pressure can also occur in the
430 fasted stomach.²² These events are probably the result of the
431 deposition of the object in distal parts of the stomach. This
432 aspect was considered in two dedicated test programs (W3/
433 B3), which simulated the action of a single contraction wave
434 early after administration. The importance of these early
435 contraction waves for the disintegration of capsules made from
436 different shell materials was already demonstrated by Garbacz
437 and co-workers.⁶

438 With these considerations in mind, the four different test
439 programs were used to study the difference between the two
440 formulations and the effect of concomitant water admin-
441 istration. In the test programs W1/W3 first order flow kinetics
442 of water were applied to study drug release from the tablet and
443 the granule formulation given with water. Under these
444 conditions the dissolution of the tablet was slightly faster, but
445 this difference was not significant (similarity factor >50). Both
446 formulations showed a short lag phase of around 2.5 min and
447 the simulation of early pressure events had no impact on drug
448 release from the two formulations (similarity factor >50 for W1
449 versus W3 for both formulations). This observation was not
450 surprising as the tablet had already disintegrated at this time
451 point and the resulting small particles as well as the granules
452 were not sensitive to the simulation of pressures or movements.
453 Thus, the drug release profile was unaffected by the simulated
454 motility event. In the test programs that were simulating the
455 intake of the granule formulation without water (B1/B3), the
456 dissolution of the granules started with a slightly longer lag
457 phase of 8 min. Again, the simulation of early contraction waves
458 did not lead to any relevant effects. Due to the constant low
459 flow rate of 2.5 mL/min the drug was not emptied in the same
460 amount as compared to the programs simulating the
461 administration together with 150 mL of water. Moreover,
462 visual observations indicated that even undissolved particles
463 appeared at later time points in the acceptor vessel, which
464 represented the small intestine. Interestingly, the amount of
465 dissolved NAC present in the acceptor vessel after 20 min was
466 comparable in all four test programs. This can be explained by
467 the fact that the flow rate in the time frame of 15–20 min was
468 lower in W1/W3 compared to the constant flow rate of 2.5
469 mL/min in B1/B3, which caused a smoothing of the profiles.
470 Nonetheless, this flow rate used for the simulation of basal fluid

kinetics was regarded as a worst-case and based on literature values describing oral and gastric secretion rates.⁴⁷¹

The observed differences between the two formulations in the USP paddle apparatus are most likely attributable to a significantly different drug release behavior of the two formulations, since the high volume of fluid available in the USP paddle apparatus in combination with the intense stirring rates are usually leveling out potential differences between the release profiles of test and reference formulations.²⁴ However, the *in vivo* data as well as the *in vitro* data obtained with the dynamic open flow-through apparatus demonstrated that in case of the NAC formulations, it was not the solubility that controlled absorption, but it was the dissolution rate and the rate of gastric emptying. Recently, Hens et al. published similar observations, as they could show the dependence of the absorption of the BCS class 2 drug ibuprofen on gastrointestinal motility. Hens et al. could show the influence of MMC phase 3 contractions on t_{max} .²⁵ According to literature, N-acetylcysteine is freely soluble and thus, roughly 3 mL of fluid in the stomach would be enough to dissolve the full dose of 600 mg of NAC.^{26,27} Despite this high solubility, only small amounts of NAC were emptied from the gastric cell of the DOFTA either in dissolved or undissolved form within the simulated gastric residence time of 20 min. Thus, more than 70% of the drug was transferred to the acceptor vessel during the simulation of MMC phase III. The experiments clearly showed that the rate of simulated gastric emptying in the DOFTA limited the amount of drug in the acceptor vessel. Therefore, we assume that it is the rate of gastric emptying that controls drug absorption in this case. This assumption is getting supported by research from Heading et al., who reported the dependents of acetaminophen (BCS class 1) absorption on the rate of gastric emptying.²⁸ The differentiation between dissolved and undissolved drug emptied from the gastric compartment could not be addressed with the present setting of the DOFTA, as the sieve at the bottom of the gastric compartment allowed particles with a diameter of less than 1 mm to be emptied. It should be noted that special attention must be paid to the simulation of the gastric emptying of small solid particles together with the water, as this will certainly affect the results. Additionally, the dissolution rate of the drug in the small intestine could also affect the results.

The process of gastric emptying cannot be simulated with the USP paddle apparatus, but can be considered in flow-through systems, in which physiologically relevant flow rates are applicable. Thus, the differences that were observed by testing the two NAC formulations in the USP paddle apparatus had no relevance for the *in vivo* behavior of the two products. These data are in line with *in vivo* data on different NAC formulation reported by Borgström and colleagues.¹¹ In this study, NAC was given to fasted volunteers in form of five different formulations: an intravenous solution, a granule formulation, an effervescent tablet predispersed in water, an immediate release (IR) and an extended release tablet. Thereby, all oral immediate release formulations exhibited similar plasma profiles. The granules and the effervescent tablet showed a t_{max} of 0.65 and 0.67 h, respectively, and the tablet had a t_{max} of 0.75 h. Thus, Borgström et al. reported a lower t_{max} compared to our data ($t_{max} = 1.12$ h). The slightly faster onset observed by Borgström and colleagues is probably related to the predisposition of the formulations in water.

With respect to the *in vivo* situation, it must be noted that owing to the lack of coadministered water, there is no driving

534 force for gastric emptying. Thus, it is likely that the transfer of
 535 dissolved and undissolved drug into the small intestine depends
 536 on the MMC. Thus, rather than the formulation, it was the
 537 physiological parameters like the MMC that determined the
 538 plasma profile of the drug. The lack of a driving force for gastric
 539 emptying in case of the dry intake of the granule formulation
 540 may explain the slight delay in t_{max} as well as the occurrence of
 541 relatively late peaks in some individuals. The increased
 542 variability of the t_{max} of the tablet formulation compared to
 543 the granule formulation is probably a result of the disintegration
 544 behavior of the formulations. It might be explained by the lower
 545 surface area of the tablets compared to the granule formulation.
 546 Thus, it would lead to higher dependence of the tablet on the
 547 location in the stomach with regard to fluid volumes, whereas
 548 the granules can be located on several positions in the stomach.
 549 However, this effect seems to be negligible as can be seen by
 550 the plasma profiles.

551 With regard to the onset of drug plasma concentrations in
 552 case of the dry intake of oral dosage forms contradictory data
 553 can be found in literature. Observations that were similar to the
 554 ones made in this study were obtained for orodispersible tablets
 555 (ODTs) with sildenafil citrate. Damle and colleagues could
 556 show that the intake of the ODT with and without water led to
 557 superimposable plasma profiles.²⁹ On the other hand, in case of
 558 ODTs with rizatriptane (BCS class III) the intake without
 559 water clearly delayed drug absorption.³⁰ The authors
 560 hypothesized that the administration together with water
 561 facilitated gastric emptying of the drug and thus, decreased
 562 the time until the drug arrived at the site of absorption. To our
 563 understanding, there are many possible explanations for these
 564 observations. In the sildenafil study the volunteers were allowed
 565 to wet their mouth with 20 mL of water before drug intake,
 566 which was possibly enough to trigger gastric emptying.
 567 Additionally, sildenafil is getting absorbed, to a certain extent,
 568 through the oral cavity, which is not the case for rizatriptane.
 569 Thus, rizatriptane has to pass the stomach to reach the small
 570 intestine, where the coadministered water leads to a more
 571 widespread distribution of the drug on the surface of the small
 572 intestine, leading to a better and faster absorption compared to
 573 dry intake of the rizatriptane ODT. In this context, we believe
 574 that *in vitro* tools like the DOFTA will contribute to a deeper
 575 understanding of the underlying mechanisms in the near future.
 576 The results of this study demonstrated that the DOFTA can
 577 be a valuable tool in formulation development in order to
 578 simulate parameters like hydrodynamics, motility or gastric
 579 emptying. All these parameters have the potential to influence
 580 drug release from oral dosage forms. Additionally, the
 581 simulation of individual *in vivo* conditions which are not
 582 based on mean values but on individual extrema will be of great
 583 interest in future experiments, as these extrema allow a deeper
 584 understanding of the intra- and interindividual differences in
 585 terms of *in vivo* drug dissolution.

586 Despite its different advantages, the DOFTA needs further
 587 optimization and future work will include the simulation of
 588 realistic temperature and pH profiles. Hopefully, we will also be
 589 able to perform further descriptive comparisons of *in vitro* data
 590 from DOFTA with the corresponding *in vivo* data. In this way,
 591 the *in vivo* relevance of this model will be increased and a
 592 deeper understanding of the parameters crucial for drug release
 593 of certain oral dosage forms will be generated.

■ CONCLUSION

594

In a pharmacokinetic study, the plasma profiles after 595 administration of two different formulations with N-acetylcys- 596 teine (granules and tablets) were irrespective of the formulation 597 and the coadministration of water. Since this circumstance 598 could not be demonstrated by using the USP paddle apparatus, 599 the dynamic open flow-through apparatus was used to simulate 600 the effect of gastric emptying and motility in four 601 physiologically relevant test programs. Despite high aqueous 602 solubility of NAC, only small amounts of this BCS class I drug 603 were dissolved within a simulated gastric residence time of 20 604 min. The major part of the drug was transferred in form of 605 undissolved particles to the acceptor vessel simulating the 606 upper small intestine. Due to fast gastric emptying of the 607 coadministered water and the slow dissolution rate in the 608 stomach, the amount of drug that could be dissolved within the 609 simulated gastric compartment was limited. As a consequence, 610 the occurrence of MMC phase 3 activity and the later gastric 611 emptying of dissolved and undissolved drug were limiting the 612 absorption of NAC. Due to the possibility of realistically 613 simulating gastric emptying under fasting conditions as well as 614 the occurrence of antral contraction waves, the dynamic open 615 flow-through test apparatus can be a powerful *in vitro* tool to 616 investigate the dissolution behavior of solid oral dosage forms 617 under physiologically relevant conditions. 618

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Notes

625

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627

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628

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635

■ ABBREVIATIONS

636

AUC, area and the curve; BCS, biopharmaceutics classification 637 system; DOFTA, dynamic open flow-through test apparatus; 638 EMA, European Medicines Agency; FDA, US Food and Drug 639 Administration; GI, gastrointestinal; h, hour; IR, immediate 640 release; min, minutes; MMC, migrating motor complex; MRI, 641 magnetic resonance imaging; mL, milliliter; NAC, N- 642 acetylcysteine; ODT, orodispersible tablets; rpm, revolutions 643 per minute 644

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Article

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6.3 Low dose caffeine as a salivary tracer for the determination of gastric water emptying in fed and fasted state: A MRI validation study (*European Journal of Pharmaceutics and Biopharmaceutics* 2018, 127, 443–452)

Maximilian Sager, Philipp Jedamzik, Simon Merdivan, Michael Grimm, Felix Schneider, Marie-Luise Kromrey, Mahmoud Hasan, Stefan Oswald, Jens-Peter Kühn, Mirko Koziolek, Werner Weitschies

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Konzeption und Mitarbeit der Studie, Korrektur des Ethikantrags, Korrektur des Manuskriptes

Werner Weitschies

Idee des Speichelmarkers, Erarbeitung der Fragestellung, Entwurf und Korrektur des Manuskriptes



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Low dose caffeine as a salivary tracer for the determination of gastric water emptying in fed and fasted state: A MRI validation study



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ABSTRACT

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Improving our knowledge about human gastrointestinal physiology and its impact on oral drug delivery is crucial for the development of new therapies and effective drug delivery systems. The aim of this study was to develop an *in vivo* tool to determine gastric emptying of water by administration of a caffeine as a tracer substance followed by subsequent saliva caffeine analysis. For this purpose, 35 mg of caffeine were given to six healthy volunteers after a 10 h overnight together with 240 mL of tap water either on a fasted stomach or 30 min after the high-caloric, high-fat breakfast recommended for bioavailability/bioequivalence (BA/BE) studies. Caffeine was administered in form of an ice capsule in order to omit the contamination of the oral cavity with caffeine. Parallel to saliva sampling, magnetic resonance imaging (MRI) was applied in order to validate this novel approach. After administration of the ice capsule, MRI measurements were performed every 2 min for the first 20 min followed by further measurements after 25, 30, 35, 40, 50 and 60 min. Saliva samples were collected always 1 min after the MRI measurement in supine position in the MRI scanner and continued for further 240 min. The caffeine concentration in saliva was quantified after liquid-liquid extraction by a validated HPLC/MS-MS method. The obtained MRI data revealed a fast emptying of the co-administered water within 10 to 50 min in the fasted state and likewise in the fed state. Salivary caffeine kinetics showed a C_{max} from 150 to 400 ng/mL with a t_{max} from 20 to 90 min. MRI data were normalized by setting the maximum emptied volume to 100% and the salivary caffeine kinetics were normalized by setting C_{max} to 100%. In order to compare the results obtained by the MRI and the saliva method, the normalized data for each volunteer was correlated based on a linear regression. In the fasted state the mean slope for six comparisons was 0.9114 ± 0.1500 and the mean correlation coefficient was 0.912 ± 0.055 . In the fed state, a mean slope of 0.8326 ± 0.1630 and a mean correlation coefficient of 0.887 ± 0.047 were obtained. Based on these results, we could show that salivary caffeine concentrations are suitable to describe the emptying of water as a non-caloric liquid from the fasted and the fed stomach. The presented technique provides a straight-forward, inexpensive and noninvasive method to assess gastric emptying of hydrophilic liquids, which can be broadly used in oral biopharmaceutics. Possible applications are the characterization of real-life conditions, specific populations (e.g. elderly people) and the better understanding of the contribution of gastric emptying to pharmacokinetic profiles of orally administered drugs.

1. Introduction

Knowledge on human gastrointestinal (GI) physiology and its

variability is essential for modern and efficient oral drug delivery. Gastric emptying is one of the major physiological factors influencing the absorption kinetics of orally administered drugs. However, its

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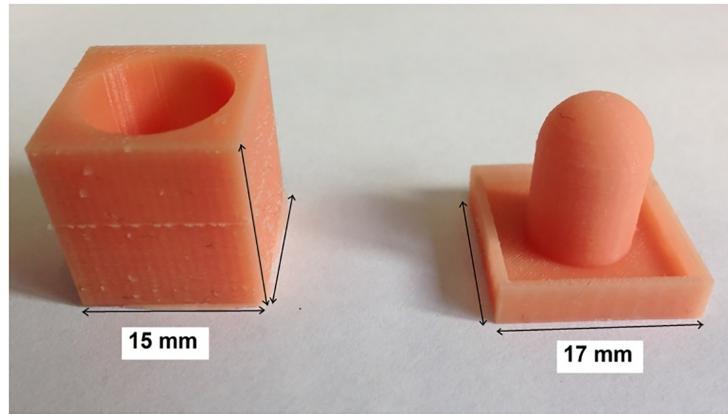


Fig. 1. Ice capsule silicon negatives (black arrows indicating scale).

reliable determination currently requires either the performance of imaging studies (e.g. by magnetic resonance imaging or scintigraphy) or the administration of validated tracer drugs like paracetamol [1–3]. For such rapidly absorbable drugs, gastric emptying is delineated from the absorption profile as gastric emptying is the rate limiting step for systemic absorption. However, this technique requires frequent blood sampling which is invasive and inconvenient. Owing to these reasons, gastric emptying is often investigated only in cost expensive clinical trials that are typically performed as phase I studies in healthy volunteers. Knowledge about the real-world variability of gastric emptying in dependence of age, sex, ethnus or disease is therefore rather limited. In order to overcome this problem, simple, reliable and non-invasive methods for the determination of gastric emptying are required. The identification and validation of such a simple method for the determination of the gastric emptying of water would offer great opportunities to the field oral biopharmaceutics.

The analysis of saliva might be a good non-invasive alternative to blood sampling. It is known for a long time that numerous substances that are either drugs or food components appear in detectable concentrations in human saliva after absorption from the gut. For instance, therapeutic drug monitoring based on salivary drug concentrations is reported for drugs such as sildenafil, melatonin, lamotrigine, moxifloxacin and various steroids [4,5]. Saliva analysis is also well-established for the investigation of drug abuse (e.g. for amphetamine, methamphetamine, cocaine and tetrahydrocannabinol) [6–8]. Koysooko, Ellis and Levy reported already in 1974 that the salivary concentrations of theophylline correlate well with the plasma concentrations [9]. Newton and colleagues showed some years later that caffeine, which is another methylxanthine, is rapidly absorbed after administration in form of an encapsulated solid and can be recovered in human saliva after oral administration with a reliable blood plasma to saliva correlation [10]. Jost and colleagues have reported that caffeine shows rapid intestinal absorption and provides a good correlation between concentration in plasma and saliva [11]. The analysis of the salivary concentrations of caffeine is also used as a simple diagnostic tool for the characterization of liver function. For this purpose, caffeine is administered in form of a solution and subsequently, the caffeine concentrations in saliva are determined [11]. As caffeine is also a substrate of the cytochrome P450 enzyme 1A2 (CYP1A2), this method can be used to quantify the activity of CYP1A2 [12]. Salivary caffeine concentrations have also been applied to study drug release from oral patches and colon-targeted drug delivery systems [13,14].

As caffeine presents ideal properties as a saliva tracer for gastric water emptying and due to its classification as a food additive by the

FDA, it was the aim of this study to validate salivary caffeine absorption kinetics as a non-invasive method for the determination of gastric emptying of liquids. The hypothesis was that the concentration profile of caffeine in saliva correlates well with the gastric emptying kinetics of water in fasted and fed state. In order to confirm this hypothesis, magnetic resonance imaging (MRI) was used as a reliable reference method for the determination of gastric emptying of 240 mL of water labeled with caffeine under fasting and fed intake conditions. Saliva was sampled simultaneously and the obtained salivary caffeine concentration profiles were compared with the fluid emptying kinetics determined by MRI.

2. Materials and methods

2.1. Materials

Caffeine was obtained from Sigma Aldrich (Steinheim, Germany). Saccharin sodium was obtained from Caelo (Hilden, Germany). Formic acid and ammonium acetate were from Merck KaA GmbH, (Darmstadt, Germany). All solvents used for LC-MS, i.e. water, acetonitrile and methanol were purchased from VWR International (Fontenay sous Bois, France) and of LC-MS grade. Silicon of food quality was purchased from Altropol GmbH (Stockelsdorf, Germany).

2.2. Preparation of the ice capsules

In order to avoid caffeine contamination in the oral cavity during intake, an ice capsule was developed, which shells were made from frozen water and which could be filled with caffeine solution (Fig. 1). The ice capsules were prepared by using negatives, which were made from food grade silicone. For this purpose, a volume of 0.65 mL of deionized water was filled into the bottom negative and displaced by the top silicon negative, which was set upside down into the bottom negative. After filling with water, the system was stored for 4 h in the freezer at -10°C to ensure complete freezing of the water. The finished ice capsule (Fig. 2) had the shape of an unsealed bottom part of a capsule. From the conus to the rim it had a height of 13 mm. The width was 12 mm and the wall thickness was about 1 mm. It was filled with 0.5 mL of a test solution containing 35 mg caffeine and 250 mg saccharine sodium. The latter was used to adjust the pH of the solution and to enable detection of unwanted contamination of the oral cavity by its sweet taste. Finally, 0.3 mL tap water was used to seal the capsule. In order to prevent melting of the capsule while filling, the test solution was pre-cooled to $2\text{--}8^{\circ}\text{C}$ and filled into the ice capsule while storing

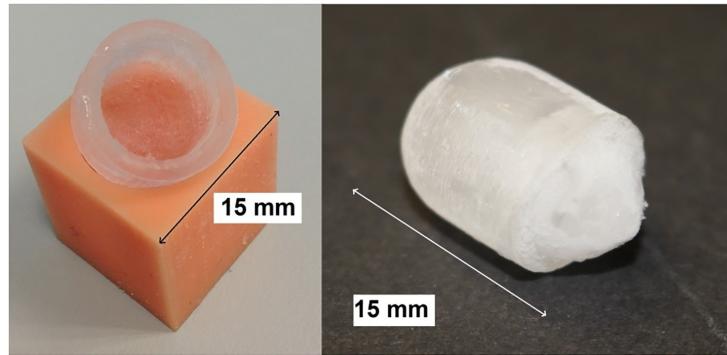


Fig. 2. Left: Ice capsule unfilled. Right: Ice capsule filled with caffeine solution and stored at -10°C .

this at -75°C . After 5 min at -75°C the capsule was stored at -10°C until administration to the subjects.

The dissolution behavior of the ice capsule was characterized in the USP paddle apparatus (Pharma Test DT 70, Erweka) by using 300 mL of simulated gastric fluid without pepsin (SGF_{sp}) pH 1.2. The experiments were performed at 25°C and a stirring speed of 25 rpm speed in order to simulate worst case conditions for dissolution. Drug concentration was measured by fiber optic UV detection (Cary 60, Agilent) at 272 nm. The experiments were performed in triplicate. They showed that dissolution of caffeine from the ice capsule was above 85% within 4 min and almost complete after 6 min.

2.3. Cross-over clinical trial

Six healthy volunteers (2 males, 4 females) aged 22–31 years and with a body mass index between 20 and 25 were included in the study. Written informed consent was obtained from all participants and the study was conducted in compliance with the Declaration of Helsinki (2013, Fortaleza, Brazil) and the “(Model) Professional Code for Physicians in Germany” (amended 2015 in Frankfurt, Germany). The study protocol was approved by the ethics committee of the University Medicine Greifswald (registration number: BB 071/17). For every participant an insurance was provided to cover commuting accidents and risks arising from participation in the study. Inclusion and exclusion criteria for subjects as well as the amount and timing of the study meals and fluids were closely adapted to EMA and FDA guidelines for bioavailability and bioequivalence studies in fasted and fed state [15,16].

2.3.1. Test protocol

The study was conducted in an open cross-over design with two study arms (fasted conditions and fed conditions). In both study arms, volunteers came to the MRI in the morning after an overnight fast of at least 10 h. Prior to the study procedure, an MRI scan was performed to determine the fasted state volume. In the fasted state arm, the volunteers ingested the ice capsule directly after the first measurement together with 240 mL of tap water of room temperature in a sitting position. Under fed state conditions the volunteers received the high-caloric, high-fat standard meal 30 min prior to intake of the ice capsule. The meal was composed of two strips of bacon (Tulip Bacon, Tulip Food Company, Denmark), two slices of toast (Sammy's Super Sandwich, Harry-Brot GmbH, Germany) with butter (Meggle Alpenbutter, Meggle AG, Germany), two eggs (Freiland ei Größle L, Poseritzer EierHOF, Germany) fried with butter, 113 g of hash brown potatoes (Rösti-Ecken, Gut & Günstig EDEKA Group, Germany), and 240 mL of full-fat milk (Vollmilch 3,5% Fett Gut & Günstig EDEKA Group, Germany). Based on the product specifications, the total caloric value of the breakfast was 964 kcal. The volunteers had to abstain from the intake of caffeine

containing products like coffee, tea and chocolate for at least 3 days prior to caffeine administration in form of the ice capsule until the end of the experiments.

The standard meal had to be consumed within 15 min and saliva samples were collected directly before and after the meal to ensure that no caffeine contamination arose from meal consumption. The volunteers were placed in supine position inside the MRI immediately after intake of the standard meal to determine the postprandial gastric content volume. The specific imaging and sampling theme is given in Fig. 3.

2.3.2. Magnetic resonance imaging

Abdominal MRI measurements were performed with a 1.5 Tesla Siemens MAGNETOM Aera (Siemens Healthcare GmbH, Germany) at the Department of Radiology and Neuroradiology of the University Medicine Greifswald. Volunteers were positioned in head supine position. Four spine coils inside the MRI desk and a six-element phase array abdominal receiver coil were used for signal detection. Each image set was acquired during a single inspiration breath-hold to reduce motion artifacts. For quantification of the gastric content volume (GCV), coronary sequences with high resolution were acquired. The coronary T2-weighted HASTE sequences had a TR of 1000 ms, TE of 100 ms, a slice thickness of 6.5 mm, an interslice gap of 1.95 mm, a flip angle of 180° , a matrix of 256x256 and a resulting voxel size of 15.9 mm^3 .

2.3.3. Image analysis

The images were analyzed using OsiriX Lite v.8.5.1 32-bit (Pixmeo Sarl, Bernex, Switzerland). Automatic segmentation was not possible due to inhomogeneous shapes and signal intensities. Therefore, the regions of interest (ROI) for gastric content volumes (GCV) were tracked manually in each slice by marking the contours of gastric content. Intragastric air was excluded from volume quantification. The volumes were subsequently calculated using an integrated software tool under consideration of slice thickness and interslice gap. Air was not included in the calculations. Although the intragastric contents could be easily differentiated from surrounding structures due to their high signal intensity in the strongly T2 weighted images, the manual marking was prone to human errors even for trained investigators. Thus, two randomly chosen image sets with 34 time points were evaluated by two different observers. The inter-observer differences had to be in the range of $\pm 10\%$ for volumes above 10 mL. This value was obtained in each case. The remaining image sets were investigated by one of these two trained observers.

2.3.4. Saliva sampling and sample preparation

Saliva samples were collected by spitting directly into 2 mL SafeSeal micro tubes (Sarstedt, Nümbrecht, Germany). A minimum sample volume of 300 μL was requested, but participants were not allowed to

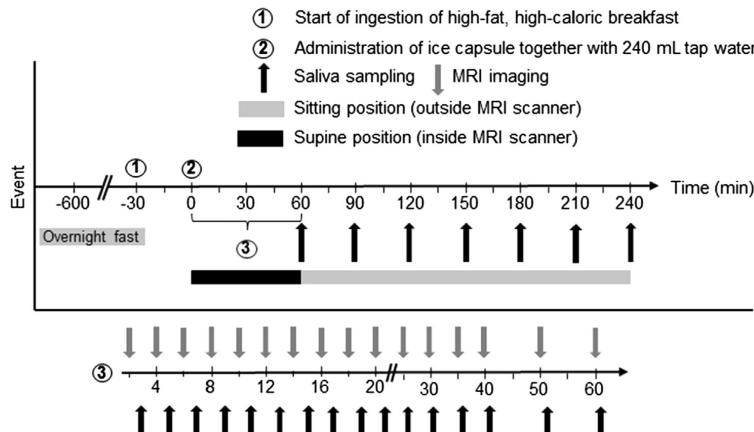


Fig. 3. Study protocol with specific MRI imaging and saliva sampling time points for both study arms. The fasted state arm did not include the meal intake.

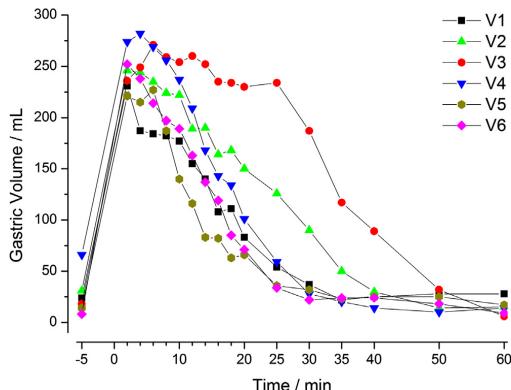


Fig. 4. Individual gastric content volumes determined by MRI over time for all six volunteers after administration of the ice capsule containing 35 mg caffeine together with 240 mL tap water after 10 h fasting.

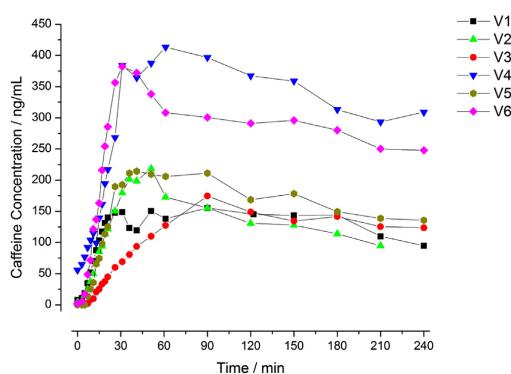


Fig. 5. Individual salivary caffeine concentrations over time for six volunteers after administration of the ice capsule containing 35 mg caffeine together with 240 mL tap water after 10 h fasting.

stimulate their saliva flow by chewing on parafilm or other common techniques. They were instructed to gather saliva in their mouth directly at the pre-determined time points and spit the saliva into the micro tube in one action, if possible. The whole process should not have taken more than 20 s. The collected saliva samples were stored at -80°C until analysis.

After thawing for 1 h, the micro tubes were centrifuged with 13000 rpm for 15 min (Biofuge pico, Heraeus, Germany). A volume of 200 μL of the supernatant was taken and mixed with 400 μL of a solution composed of acetonitrile with 6% formic acid in a 1.5 mL micro tube (Sarstedt, Nümbrecht, Germany) in order to precipitate the proteins. The resulting mixture was vortexed at maximum speed for 1 min (VORTEX 2, IKA®-Werke GmbH & CO. KG, Staufen, Germany) and afterwards centrifuged with 13000 rpm for 15 min. Subsequently, 150 μL of the supernatant was placed in 300 μL vials (ND9, PP braun, 32 x 11.6 mm, neoLab, Heidelberg, Germany) and diluted with 150 μL LC-MS grade water containing 4% of formic acid. After vortexing at maximum speed for 1 min, the samples were used for analysis.

2.3.5. Analysis of saliva samples

Determination of caffeine in the saliva samples was performed by an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to the triple quadrupole mass spectrometer API4000 QTRAP via the electrospray ionization source Turbo V™; the LC-MS/MS system was controlled with the validated Analyst 1.6 software (AB Sciex, Darmstadt, Germany).

Caffeine was separated from hydrophilic saliva components (e.g. mucines and other glycoproteins) by isocratic elution with ammonium acetate buffer (5 mM; pH 3.8) (A)/methanol (B) (50/50) as mobile phase. A flow rate of 250 $\mu\text{L}/\text{min}$ was used. An Xterra®MS reverse phase C18 column (3.5 μm , 2.1 x 100 mm; Waters, Dublin, Ireland) was used as stationary phase. The temperature of the column oven was set to 40°C . The injection volume was 20 μL .

The chromatographic flow was directed to a 0.5 μm filter device (PEEK, Supelco, Taufkirchen, Germany) to avoid particulate contamination. Ionization was done using an ESI interface (Turbo V™ ionization source) in positive ionization mode. The following gas parameters have been used: temperature, 550°C ; gas 1, 60 psi; gas 2, 60 psi (both nitrogen); voltage, 4000 V; collision-activated dissociation (CAD), 12 (arbitrary unit). The Analyst 1.6 software was applied to evaluate the chromatograms using the internal standard method and peak-area ratios for calculation (quadratic regression, 1/x weighting).

The analytical method was validated in terms of linearity, precision

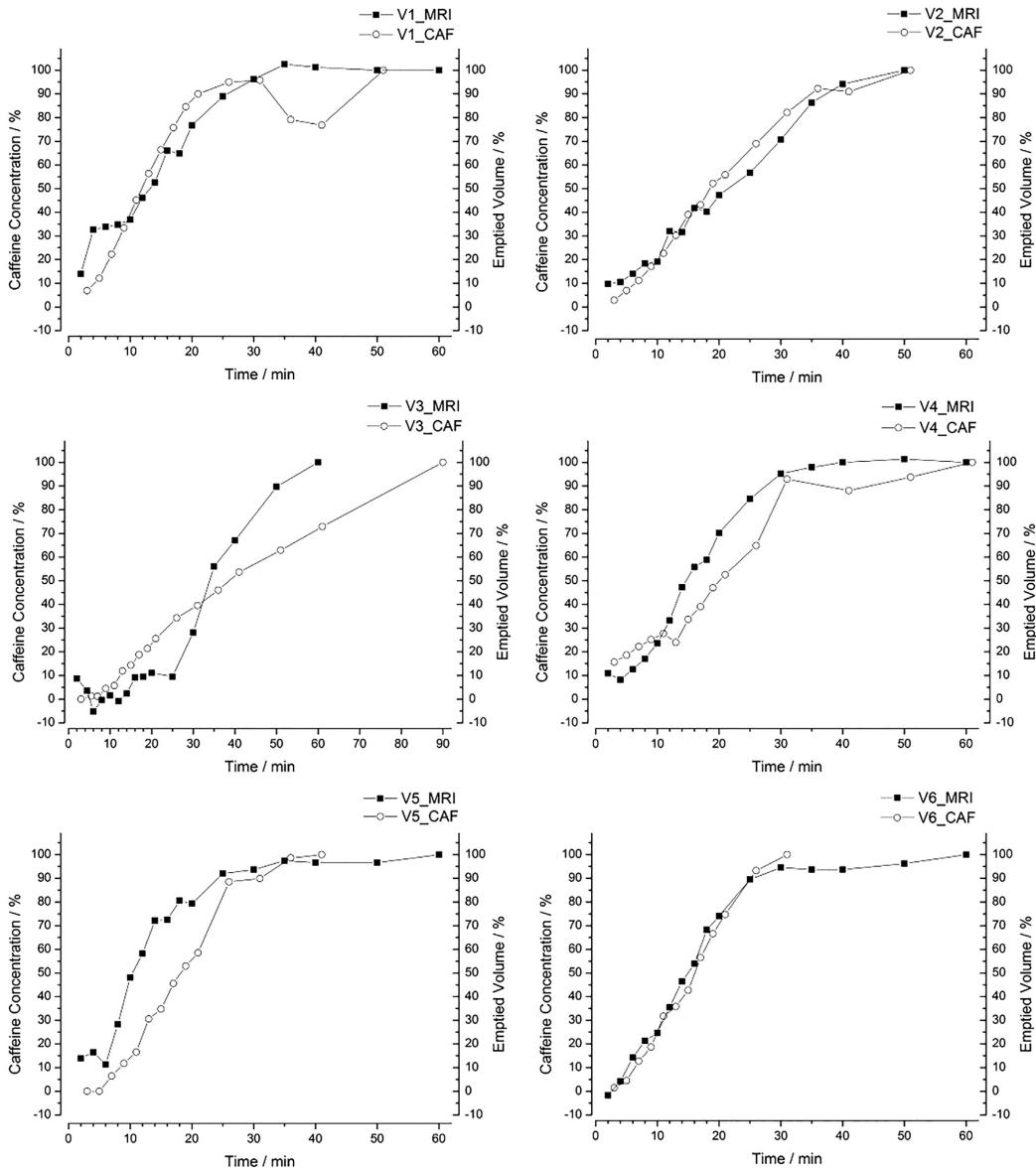


Fig. 6. Normalized MRI water emptying kinetics versus normalized salivary caffeine concentrations over time for all six volunteers after administration of the ice capsule containing 35 mg caffeine together with 240 mL tap water. Each figure represents data from a single volunteer.

(within-run and between-run), accuracy (within-run and between-run), selectivity, freeze and thaw stability and rack stability regarding the FDA Guidance for Industry “Bioanalytical Method Validation” (Issue: May 2001). All validation parameters meet the requirements of the FDA guideline. Lower limit of quantification (LLOQ) was 5 ng/mL in saliva.

2.3.6. Normalization of saliva and MRI data

Saliva data were normalized by setting C_{\max} to 100%. All graphs are displayed until C_{\max} was reached. The normalization of the MRI data

was performed in two steps. In the first step, the emptied volume (V_E) at every time point was calculated by considering the volume that was theoretically present in the stomach directly after water administration (i.e. residual fasted state volume plus 240 mL of water) and the gastric content volume (GCV) at each time point (Eq. (1)).

$$V_E(t) = (GCV_{\text{fmin}} + 240\text{mL}) - GCV(t) \quad (1)$$

The second step was different for data gathered in the fasted or the fed state arm. For normalization of fasted state data, the maximum emptied

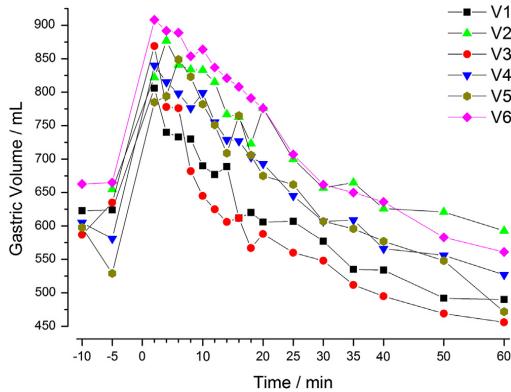


Fig. 7. Individual gastric content volumes (mL) determined by MRI over time for six volunteers after administration of the ice capsule containing 35 mg caffeine together with 240 mL tap water 30 min after beginning of the intake of the FDA standard meal.

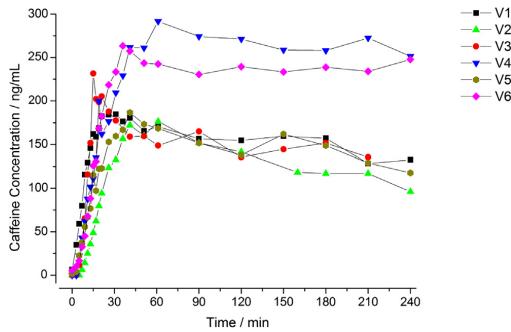


Fig. 8. Individual salivary caffeine concentrations over time for six volunteers after administration of the ice capsule containing 35 mg caffeine together with 240 mL tap water 30 min after intake of the high caloric standard meal.

volume was set to 100%. All further time points after reaching 100% of emptied volume were not considered in the analysis. In the fed state, the emptied volume of 240 mL was set to 100% and further time points after reaching 240 mL of emptied volume were not considered in the analysis. This normalization was performed as recently published by Grimm and colleagues [17].

2.4. Statistics

Data were characterized by minimums, maximums, arithmetic means, and standard deviations where appropriate. For the statistical comparison of the two *in vivo* methods for characterization of gastric emptying, the relationship of the normalized emptied volume determined by MRI and the normalized tracer concentration in saliva was calculated. With MRI data over saliva data, the PEARSON coefficient (R^2), slope and Y-intercept of the linear regression was calculated.

3. Results

3.1. Fasted state administration

3.1.1. Gastric content volumes determined by MRI

Gastric content volumes of all volunteers determined by MRI are

presented in Fig. 4. Residual gastric volumes measured 5 min before ice capsule administration varied from 8 to 66 mL. Four of six volunteers emptied the ingested water within 30 min, whereas two subjects showed a slower emptying within 40 min (V2) and 50 min (V3). Gastric fluid emptying was particularly fast in the first 20 min after water administration. However, it can be seen in Fig. 4 that V3 experienced a plateau-like phase in the first 30 min, during which only small amounts of water (around 50 mL) were emptied from the stomach. After this lag time, the residual water was also emptied rapidly.

3.1.2. Salivary caffeine concentrations

Salivary caffeine concentrations over time for six individual volunteers are presented in Fig. 5. Five out of six volunteers reached their C_{max} between 31 and 61 min, whereas the t_{max} of volunteers V3 was delayed to 90 min.

3.1.3. Comparison of normalized salivary caffeine kinetics and normalized gastric content volumes in the fasted state

Based on the results presented in Figs. 4 and 5, MRI and saliva data were normalized and compared for every volunteer separately in one graph (Fig. 6). With the exception of V3, the correlation of saliva and MRI data appeared to be high, although there was an offset of around 1 min between MRI and saliva sampling. Salivary caffeine concentrations in V3 showed a C_{max} after 90 min and a complete water emptying determined by MRI after 60 min, which was the biggest difference observed in this study between the two methods.

3.2. Fed state administration

3.2.1. Gastric content volumes determined by MRI

Gastric volumes after intake of the high-caloric FDA standard meal were determined by MRI for six volunteers and are displayed in Fig. 7. The breakfast was administered 30 min prior to ice capsule intake and the gastric content volume was measured 10 and 5 min before intake of the ice capsule. Gastric content volumes 5 min prior to caffeine administration ranged from 529 to 665 mL.

The results suggested variable gastric emptying kinetics of the water between the investigated subjects. V3 emptied 285 mL within 20 min, while V6 emptied only 126 mL during the same time period. After 60 min, all volunteers reached a gastric content volume, which was below the GCV measured 5 min before administration of the ice capsule with 240 mL of water.

3.2.2. Salivary caffeine concentrations

Salivary caffeine concentrations after administration of the FDA breakfast are shown in Fig. 8. The t_{max} ranged from 15 to 61 min with a rapid onset in concentration during the first 30 min for all volunteers. Two volunteers (V1 and V6) showed a slight increase in salivary caffeine concentration at 240 min.

3.2.3. Comparison of normalized salivary caffeine kinetics and normalized gastric content volumes in the fed state

A comparison of normalized MRI and saliva data for every volunteer is depicted in the following graphs (Fig. 9). The correlation of the MRI and saliva data is relatively high except for volunteer V5. In this case, the onset in the salivary concentration profile is slightly faster than the MRI volume profile.

3.3. Statistical analysis

In the following table the statistical parameter of the comparison of the two methods is given (Table 1). The displayed correlations are based on the normalized saliva and MRI emptying data and a linear relation between the two values was alleged.

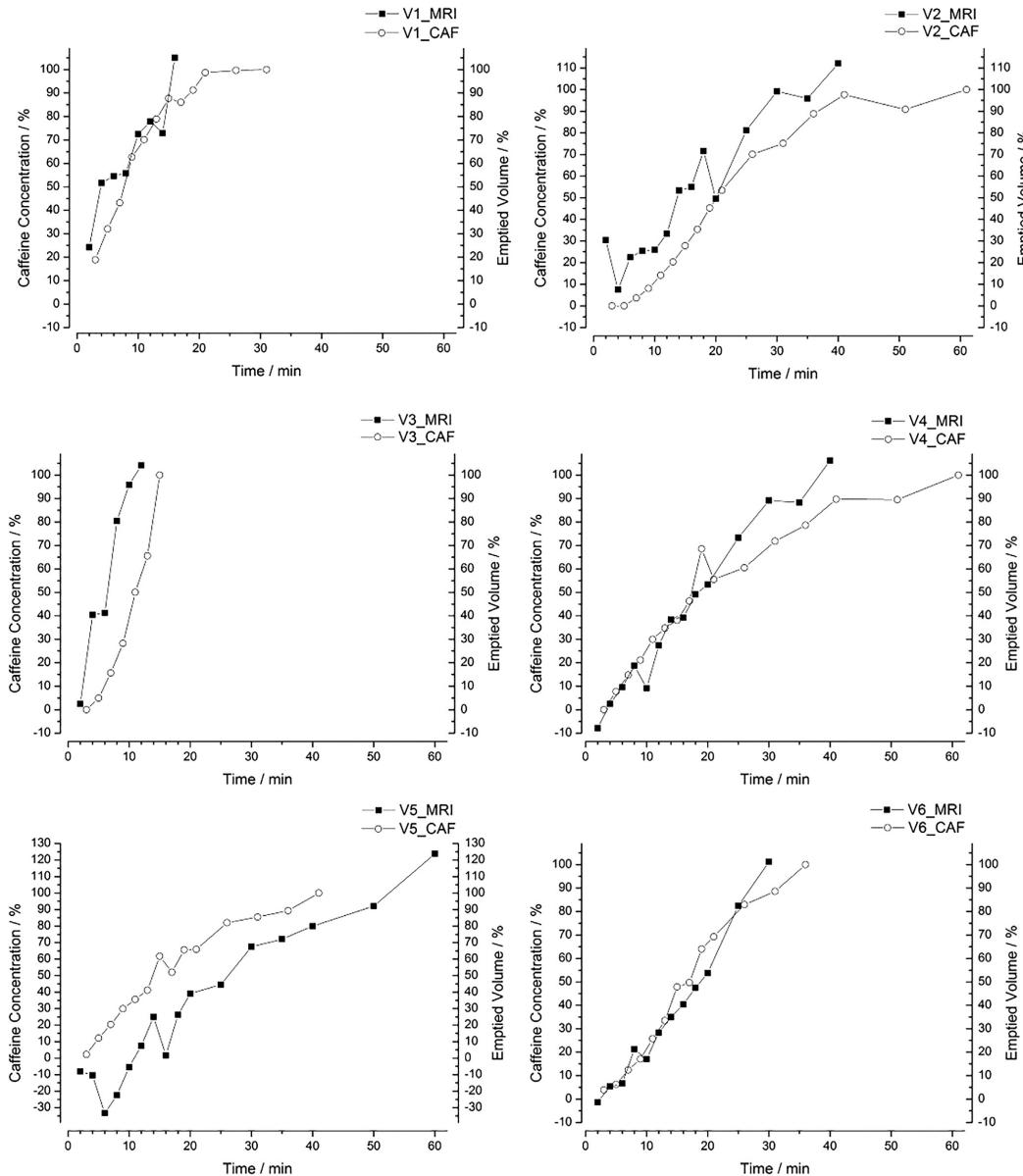


Fig. 9. Normalized MRI water emptying kinetics versus normalized salivary caffeine concentrations over time for six volunteers after administration of the ice capsule containing 35 mg caffeine together with 240 mL tap water 30 min after intake of the high caloric standard meal. Each figure represents data from a single volunteer.

3.4. Comparison of gastric water emptying under fasted and fed conditions

The mean gastric content volumes determined by MRI in fasted and fed state are provided in Fig. 10 (left). The initial volumes measured directly after water intake in fasted and fed state were 243 mL \pm 17 and 838 mL \pm 41, respectively. The variability of the fasted state

volumes was higher compared to the fed state as can be seen by the standard deviations.

After administration of the ice capsule in the fasted state, salivary caffeine concentrations reached a maximum of 235 ng/mL \pm 98 after 51 min, whereas in the fed state C_{\max} was 202 ng/mL \pm 41 and t_{\max} was 41 min (Fig. 10, right). The variability assessed in terms of the standard

Table 1

Statistical parameters for the comparison of the emptied volume determined by MRI (x-values) with the salivary caffeine concentration (y-values) based on a linear regression. Values are given for every single volunteer (V1 – V6), as well as mean, standard deviation (SD), relative standard deviation (RSD) and the minimum and maximum values (Min/Max).

	Fasted		Fed	
	R ²	slope	R ²	slope
V1	0.842	0.9576	0.871	0.9491
V2	0.971	1.0624	0.921	0.9428
V3	0.867	0.6508	0.877	0.5688
V4	0.925	0.7481	0.924	0.7727
V5	0.875	1.007	0.797	0.8204
V6	0.992	1.0225	0.934	0.9418
Mean	0.912	0.9114	0.887	0.8326
SD	0.055	0.1500	0.047	0.1360
RSD	6.01	16.46	5.27	16.33
Min/Max	0.842/0.992	0.6508/1.0624	0.797/0.934	0.5688/0.9491

deviation was again lower in fed state.

4. Discussion

The results of this study demonstrate that caffeine can be used as a salivary tracer for gastric emptying of water. Caffeine is quickly absorbed from the intestines, shows rapid distribution into human saliva and has a sufficient and largely pH independent solubility in water. Furthermore, it is classified as a food additive and therefore well acceptable in terms of regulatory aspects. In order to serve as a tracer for gastric water emptying, caffeine should be ideally administered in form of a solution. The major problem of using oral solutions of saliva tracers is the contamination of the oral cavity with the tracer itself. In pilot experiments, we experienced this problem with caffeine and even by using large amounts of water it was not possible to flush the caffeine out of the oral cavity. Even after cleaning the mouth with 100 mL water in triplicate, the initial saliva samples contained caffeine in around 1 mg/mL dimensions. Therefore, the ice capsule, which serves as a vehicle for the caffeine solution, was developed in this study. The relatively thin

capsule shell made of de-ionized water prevented contamination of the oral cavity. Dissolution experiments showed that the capsule can be expected to dissolve directly after arrival in the gastric lumen. Thus, the caffeine dissolved in the water phase within seconds to a few minutes. Such a rapid dissolution is to our understanding essential because of the rapid gastric emptying of non-caloric liquids [3,18]. The results of the salivary caffeine kinetics show that the ice capsule effectively prevents the oral cavity from caffeine contamination.

The results of the MRI measurements in fasted state are in line with our expectations and literature data with regard to residual volumes, emptying kinetics and maximum volumes [19,20]. It should be noted that the difference between the maximum volume and the residual volume determined prior to water intake was expected to be 240 mL as this was the volume of the ingested water. Due to technical reasons (e.g. time needed to move the volunteer into the MRI machine after capsule administration) it was not possible to measure directly at the time point t = 0 min. Moreover, gastric emptying already starts during ingestion of the 240 mL of water, which was allowed to take up to 60 s. Therefore, we were not able to quantify the volume of fluid, which was emptied within the first 2 min. The calculation of the water emptied from the fed stomach was slightly different from the procedure performed for fasted state data. This was due to the basic problem that by using MRI it was impossible to distinguish between ingested water and endogenous water (e.g. by gastric secretion) without the application of further contrast agents. Therefore, we normalized the emptied volume from the fed stomach by using the volume of 240 mL of the administered water as the maximum value. This normalization procedure was based on the observation that the water administered after meal intake is typically rapidly emptied from the stomach via the stomach road [2,17]. However, as the method was not able to differentiate between fluid from the meal and the water administered later, parts of the meal could have mixed with the ingested water and thus, interfered with the water emptying kinetic determined in this study. Consequently, the gastric emptying of the full 240 mL of water may have occurred slightly slower. However, recent studies have shown that it is mainly the water that is emptied during this phase. Taking into account the decrease of gastric content volumes by around 1.7 mL/min, which was determined in a former MRI study in the same setting for the same meal, the error in the calculation of the emptied volume was considered to be relatively small. Moreover, the delivery of caloric contents to the small intestine would directly cause a deceleration of gastric emptying owing to intestinal feedback mechanisms [21]. Such a behavior has not been observed in this study.

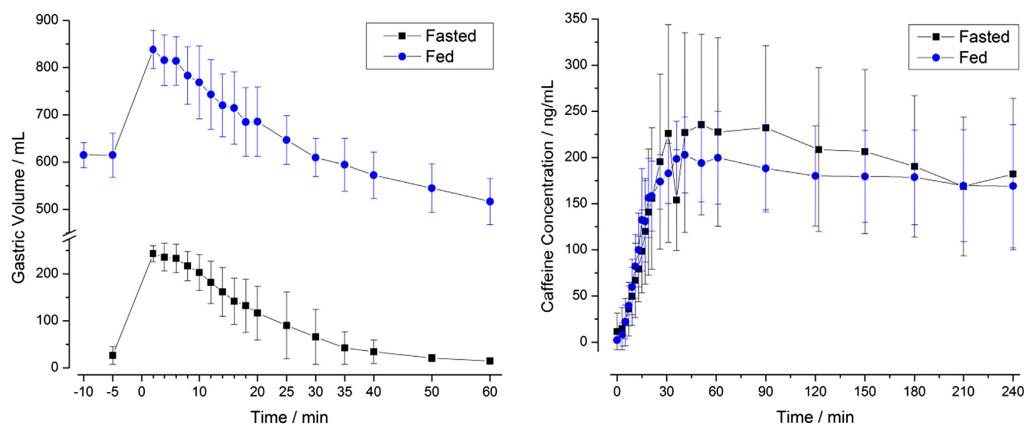


Fig. 10. Mean gastric content volumes determined by MRI (left) and mean salivary caffeine concentrations (right) after administration of 240 mL tap water in the fasted state (10 h fasting) and in the fed state, 30 min after intake of the high-caloric FDA meal (mean \pm SD, n = 6, scale halted from 275 to 550 mL).

In contrast, we have demonstrated the presence of the phenomenon called "Magenstrasse" or stomach road. This physiological effect describes the fast emptying of non-caloric liquids from the stomach. A recent study by our group has shown that the gastric emptying of water in the fed state is at least as fast as in the fasted state [17]. The observed salivary caffeine concentrations confirm the fast gastric emptying of swallowed water from the fed stomach. Interestingly, gastric emptying of water in fed state appears to be at least as fast than in fasted state (see Fig. 10).

As expected, the absorption and distribution of caffeine into the saliva appeared to be very fast. Maximum salivary caffeine concentrations were observed within 20 to 90 min after administration of the ice capsule. The calculated salivary half-life of caffeine based on the values from 90 to 240 min (six data points), was within a range of 2–8 h. This was in line with data reported in literature [22–24].

In this study, we could demonstrate that the developed caffeine ice capsule method allows an easy and non-invasive determination of gastric emptying of water in the fasted and in the fed state. Salivary caffeine concentrations correlated well with the water emptying kinetics determined by MRI. In most cases, our results just showed a small temporal offset between the two methods. This delay can be attributed to the time needed for absorption and distribution of the caffeine into the saliva. Furthermore, the time shift of 1 min between MRI measurement and saliva sampling also needs to be taken into account.

It has been claimed that caffeine affects gastric emptying, gastric secretion and esophageal sphincter pressure. This would pose a serious problem to the use of caffeine as a tracer for gastric emptying. Ideally, gastric emptying should be studied without any interference caused by changes in gastric motility. However, the applied dose of 35 mg of caffeine was relatively low. In comparison, a common cup of drip coffee contains up to 100 mg of caffeine - depending on the type of coffee beans, brew time, pressure and many other parameters [25]. Second, the claim that caffeine influences and probably delays gastric emptying could not be confirmed yet [26–28]. Lien et al. showed an accelerating effect of coffee on gastric emptying with the aid of scintigraphy by administering 4 g of common instant coffee [29]. However, the type of instant coffee is not given in the publication, which makes interpretation of the results difficult. But for instance, 4 g of Nescafe Classic Instant coffee would contain around 125 mg of caffeine, which is much higher than the 35 mg used in our study. In contrast to these data, van Nieuwenhoven and co-workers demonstrated that a carbohydrate-electrolyte solution (sports drink) with 150 mg/L caffeine does not affect gastric pH, orocaecal transit time and gastroesophageal reflux, as compared to the same drink without caffeine. However, it increases intestinal glucose uptake when it is given to healthy sporty man during exercise. Similar results were obtained by Boekema et al., and Schubert et al. who investigated the gastric emptying of a liquid meal given either with water, a caffeine solution or coffee [26,30]. They could not find significant differences in gastric emptying and orocaecal transit time between the two study arms. In accordance to this finding we could also not detect differences of the gastric water emptying in fed or fasted state between this study with caffeine and our previous study without caffeine [17]. Even the hypothesis that caffeine influences gastric acid secretion could not be proven yet. McArthur et al. (1982) conducted a clinical study where nine different beverages (e.g. cola, coffee and alcoholic drinks) were administered and there was no effect on gastric emptying and acid secretion found with correlation to the caffeine content of these beverages [31].

To sum up, we demonstrated that salivary caffeine concentrations can be used to study gastric emptying of non-caloric liquids in the fasted and in the fed state. The advantage of this method is that it does not require access to MRI or exposure of healthy volunteers to radiation as is the case for scintigraphy. Further advantages are the high temporal resolution (samples can be taken in intervals of up to 1 min) and the non-invasiveness, which makes this method convenient for the subjects and the investigators. The only intervention would be the

administration of a small dose of caffeine in form of an ice capsule. Due to the straight-forward sampling procedure, the subsequent saliva sampling can even be done by the subjects themselves. Therefore, we believe that this method can be applied routinely to identify characteristics of gastric emptying in greater study populations and also in specific sub-groups like older people or people with pre-existing conditions known to influence gastric emptying (e.g. diabetes or Parkinson's disease). Hence, this technique is particularly helpful to identify factors contributing to variability in oral drug absorption and thus, may help to improve the safety and efficacy of oral pharmacotherapy. One of the aspects that may limit broader application of this method to larger study populations could be the abstinence from caffeine for a defined period prior to the administration of the ice capsule. This can be complicated in real life as caffeine is not only part of many drinks such as coffee, black tea or cola, but is also present in various foods (e.g. chocolate). However, in this study 72 h of abstaining from caffeine were enough to assure the absence of caffeine in the saliva of the study population.

5. Conclusion

In the present work, the gastric emptying of water in fasted and fed state could be determined by using salivary caffeine concentrations. Caffeine displayed optimal properties for a tracer substance in human saliva as it is rapidly absorbed from the small intestine and shows fast distribution into the saliva. By comparing this technique with the well-established MRI determination of gastric content volumes, we were able to validate this simple, inexpensive and non-invasive method for the assessment of gastric emptying. The correlation between the fluid volume emptied from the stomach and the salivary caffeine concentration was high in all six volunteers irrespective of the intake conditions. Combinations with pharmacokinetic studies will be of great interest as this provides a better understanding of the outcome of these studies. Furthermore, the method allows the broad investigation of gastric emptying in specific populations (e.g. diabetics, elderly people).

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6.4 Combined application of MRI and the salivary tracer technique to determine the *in vivo* disintegration time of immediate release formulation administered to healthy, fasted subjects (*Molecular Pharmaceutics* 2019, 16, 1782 - 1786)

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Idee des Speichelmarkers, Erarbeitung der Fragestellung, Entwurf und Korrektur des Manuskriptes

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Combined Application of MRI and the Salivary Tracer Technique to Determine the *in Vivo* Disintegration Time of Immediate Release Formulation Administered to Healthy, Fasted Subjects

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ABSTRACT: The process of disintegration is a crucial step in oral drug delivery with immediate release dosage forms. In this work, the salivary tracer technique was applied as a simple and inexpensive method for the investigation of the *in vivo* disintegration time of hard gelatin capsules filled with caffeine. The disintegration times observed with the salivary tracer technique were verified by magnetic resonance imaging (MRI). After an overnight fast of at least 10 h and caffeine abstinence of minimum 72 h, conventional hard gelatin capsules containing 50 mg caffeine and 5 mg iron oxide were administered to 8 healthy volunteers. For the period of 1 h after capsule intake, subjects were placed in supine position in the MRI scanner, and scans were performed in short time intervals. Each MRI measurement was directly followed by saliva sampling by drooling. Salivary caffeine concentrations were determined by high performance liquid chromatography followed by mass spectrometric detection (LC/MS-MS). The time point of capsule disintegration was determined by visual inspection of the MR images as well as by an increase in the salivary caffeine concentration. The results indicated that the difference in mean disintegration times of the capsules as determined by the two *in vivo* methods was around 4 min (8.8 min for MRI vs 12.5 min for saliva). All disintegration times determined by the salivary tracer technique were slightly higher. This delay could be explained by the fact that the appearance of caffeine in saliva required drug absorption in the small intestine. Because capsule disintegration happened mainly in the stomach, the exact site of disintegration as well as the processes of gastric mixing and gastric emptying contributed to the delay between the two methods. This work demonstrated the feasibility of the salivary tracer technique to investigate the *in vivo* disintegration of immediate release dosage forms in a simple and reliable manner.

KEYWORDS: salivary tracer technique, magnetic resonance imaging (MRI), *in vivo* study, fasted state, caffeine, hard gelatin capsules



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INTRODUCTION

A profound knowledge about the fate of solid dosage forms within the human gastrointestinal (GI) tract is essential for successful oral drug delivery. Despite various *in vitro* and *in silico* approaches, the bioequivalence between two different oral formulations (e.g., tablets or capsules) is still difficult to predict with a desirable degree of certainty. Even in the case of immediate release dosage forms administered in the fasted state, which probably represents one of the simplest cases of oral drug delivery, various aspects of the *in vivo* disintegration and dissolution behavior are still not fully understood. Therefore, clinical studies with healthy volunteers remain the most common source of information on the disintegration and dissolution behavior of novel or generic oral drug products. To gain a mechanistic understanding of the *in vivo* drug release

behavior of oral dosage forms, several imaging and diagnostic techniques such as magnetic resonance imaging (MRI), aspiration of luminal contents, scintigraphy, and capsule endoscopy are used by pharmaceutical scientists and clinical pharmacologists.^{1–4} In brief, all of these methods and techniques have specific advantages and disadvantages. The major restrictions are regulatory aspects related to their invasiveness (e.g., classic pharmacokinetic studies), exposure to radiation (e.g., scintigraphy), or high costs caused by expensive instruments (e.g., MRI). In an attempt to overcome

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these limitations, we developed the salivary tracer technique. In a recent publication, we demonstrated the applicability of caffeine as a salivary tracer to monitor liquid gastric emptying in fasted and fed state in healthy volunteers.⁵ This method is reliable, simple, inexpensive and noninvasive and thus could be a suitable alternative for the investigation of the *in vivo* disintegration behavior of oral dosage forms. Salivary tracer methods with acetaminophen are already described in the literature.⁶

The primary aim of this work was to apply the salivary tracer technique for the determination of disintegration times of hard gelatin capsules and to validate these disintegration times against data obtained by an established method. For this purpose, the *in vivo* disintegration of conventional hard gelatin capsules was investigated in fasted healthy volunteers by magnetic resonance imaging.^{1,7–9}

MATERIALS AND METHODS

Materials. Caffeine was obtained from Fagron GmbH & Co. KG (Barsbüttel, Germany), mannitol from Fagron GmbH & Co. KG (Barsbüttel, Germany), silicon dioxide from Fagron GmbH & Co. KG (Barsbüttel, Germany), black iron oxide E172 from Caesar & Loretz GmbH (Hilden, Germany). Formic acid and ammonium acetate were from Merck KGaA (Darmstadt, Germany). All solvents used for LC-MS, i.e. water, acetonitrile, and methanol, were purchased from VWR International (Fontenay sous Bois, France) and were of LC-MS grade.

Capsules. Conventional hard gelatin capsules Coni-Snap (size 1) were purchased from Capsugel/Lonza (Colmar/Strasbourg, France). The hard gelatin capsules were filled manually with 250 ± 5 mg of the powder mixture by using an analytical balance. The powder mixture consisted of 20% (w/w) caffeine, 2% (w/w) black iron oxide, and standardized capsule filling powder (mannitol 99.5% and 0.5% colloidal silicon dioxide). Black iron oxide was added for contrast enhancement and visibility of disintegration in the MRI because it is known to be a suitable negative contrast agent for MRI and also classified as a food colorant.

Fasted State Clinical Trial. Eight healthy volunteers (4 males, 4 females) aged 22 to 31 years and with a body mass index between 20 and 25 were included in the study. Written informed consent was obtained from all participants, and the study was conducted in compliance with the Declaration of Helsinki (2013, Fortaleza, Brazil) and the Professional Code for Physicians in Germany (amended 2015 in Frankfurt, Germany). The study protocol was approved by the local ethics committee of the University Medicine Greifswald (registration numbers: BB 132/17 and BB 132/17a). Insurance was provided for every participant to cover commuting accidents and risks arising from participation in the study. Inclusion and exclusion criteria for subjects as well as the amount and timing of the study fluids were closely adapted to the EMA and FDA guidances for bioavailability and bioequivalence studies in fasted state.^{10,11}

Test Protocol. The study was conducted in an open-label design with one study arm. The volunteers arrived at the study unit in the morning after an overnight fast of at least 10 h. Prior to the study procedure, an MRI scan was performed to determine the fasted state gastric volume. Afterward, the volunteers ingested the dosage form together with 240 mL of tap water of room temperature in a sitting position ($t = 0$ min). Subsequently, volunteers were placed inside the MRI scanner

for the whole time of the experiment. MRI was then performed at time points 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, and 60 min. Saliva samples were collected inside the MRI scanner directly after every MRI measurement.

The volunteers had to abstain from the intake of products containing caffeine such as coffee, tea, and chocolate for at least 72 h prior to the capsule administration until the end of the experiments.

Magnetic Resonance Imaging. The abdominal investigations were performed using a Siemens MAGNETOM Aera MR-scanner (Siemens Healthcare, Erlangen, Germany) with a field strength of 1.5 T, which was located at the Institute of Diagnostic Radiology and Neuroradiology of the University Medicine Greifswald. All measurements were performed in supine position.

Two different MRI sequences were applied in this study. As long as the capsules resided in the stomach, only transversal image slices were obtained. After gastric emptying of intact capsules, the imaging plane was switched to coronal for better traceability of the capsules and their disintegration behavior. For the investigation of the transit and disintegration behavior of the capsules as well as for anatomical assignment and the determination of gastric volumes, a T1/T2-weighted TRUFI (True Fast Imaging with Steady State Precession) sequence was used. Subjects were asked to hold their breath for up to 23 s for each image set to reduce motion artifacts. The coronal and transversal TRUFI had a TR of 3.55 ms, a TE of 1.48 ms, a flipangle of 65°, a slice thickness of 5 mm, an interslice gap of 0.75 mm, 29 to 33 slices (variable amount of slices due to individual anatomy), an acquisition matrix of 256 × 243, and a resulting voxel size of approximately 0.04 mL. Four spine coils inside the MRI desk and a six-element phase array abdominal receiver coil were used for signal detection.

Image Analysis. Imaging data were analyzed using the Software Horos 2.2.0 (The Horos Project). Tracking, assignment to stomach or small intestines, and evaluation of disintegration were performed manually.

The time point of detected disintegration (disintegration time) was defined as the time of spreading of the characteristically shaped susceptibility artifact in the GI tract or visible sedimentation of the capsule filling within the stomach. The disintegration did not necessarily lead to a spreading of filling and thus, a compact settled artifact on the bottom of the stomach was obtained in several cases. Thus, the first time point in which the black artifact was not floating on top of gastric contents anymore was typically determined as the time point of disintegration time.

Furthermore, the time point of disintegration was also determined by the appearance of caffeine in the saliva. Ideally, this value should be in line with MRI data and provided an additional and independent marker for capsules disintegration and impermeability.

Salivary Tracer Technique. Saliva samples were collected by spitting directly into 2 mL SafeSeal microtubes (Sarstedt, Nümbrecht, Germany). A minimum sample volume of 300 μ L was requested, but participants were not allowed to stimulate their saliva flow by chewing on parafilm or other commonly applied techniques. They were instructed to gather saliva in their mouth directly at the predetermined time points and spit the saliva into the microtube in one action, if possible. The whole process should not have taken more than 20 s. The collected saliva samples were stored at -80°C until analysis.

The analytical method was validated in terms of linearity, precision (within-run and between-run), accuracy (within-run and between-run), selectivity, freeze and thaw stability, and rack stability regarding the FDA Guidance for Industry "Bioanalytical Method Validation" (Issue: May 2018).¹² All validation parameters met the requirements of the FDA guideline. The lower limit of quantification (LLOQ) for caffeine was 5 ng/mL in saliva. The time where the salivary caffeine concentration is three times higher than LLOQ was defined as capsule disintegration time.

The sampling procedure, preparation, and analytical method were published in another article recently.⁵

Statistics. Data were characterized by minimums, maximums, arithmetic means, median, standard deviations, and relative standard deviations where appropriate. Normality of the measured values was checked with the Kolmogorov–Smirnov Test, and *p*-values were calculated for the paired *t*-test. The statistical evaluations were performed with GraphPad Prism 5.01 (GraphPad software Inc., La Jolla, United States) and OriginPro 8.5.1 (OriginLab Corp, Northampton, United States).

RESULTS

Capsule Disintegration Time Determined by MRI. In this study, the time point of capsule disintegration could be successfully determined in all subjects by using MRI. For the visualization of capsule disintegration, iron oxide was incorporated into the powder mixture filled into the capsules as it is known to cause artifacts in MR imaging. An exemplary explanation of how capsule opening was identified with the aid of MRI is given in Figure 1. The capsule disintegrated between 6 and 14 min. For one subject, disintegration was observed in the small intestine.

Salivary Caffeine Kinetics. Caffeine concentrations in saliva after administration of hard gelatin capsules are presented in Figure 2. An increase in the salivary caffeine concentration was observed between 7 and 21 min. The

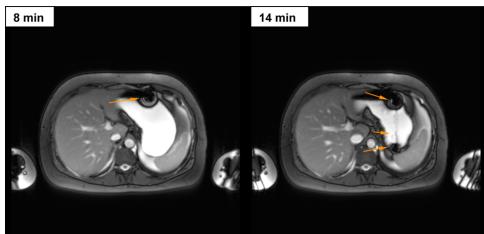


Figure 1. Transversal MR image of the abdomen taken 8 min (left) and 14 min (right) after the oral administration of a hard gelatin capsule marked with 5 mg iron oxide together with 240 mL of water. In the left image, the capsule position inside the stomach can be identified by the black artifact (orange arrow), which gives a sharp contrast against the bright gastric content. It can be seen that the capsule is floating on the top of the gastric content. In the right image, additional artifacts occurred as indicated by the orange arrows. A smaller second artifact was located at the bottom part of the stomach, which indicated the disintegration of the capsule because some parts of the incorporated iron oxide could leave the capsule. These sedimented at the bottom part of the stomach and most probably caused the additional artifact.

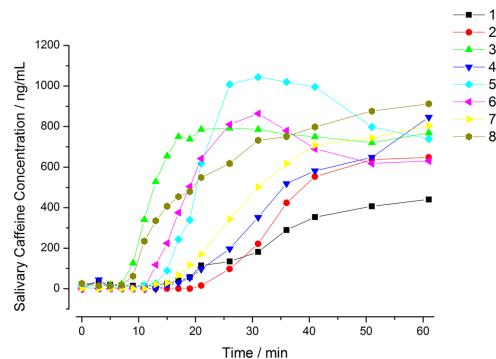


Figure 2. Individual caffeine kinetics in human saliva over time for 8 volunteers after fasted state intake of hard gelatin capsules loaded with 50 mg caffeine.

maximum concentration observed in the first hour after HGC administration ranged from 400 ng/mL to 1000 ng/mL.

Comparison of the Disintegration Times Obtained by Use of MRI and the Salivary Tracer Technique. A graphical comparison of the disintegration times of the two tracer methods is given in Figure 3. According to the results

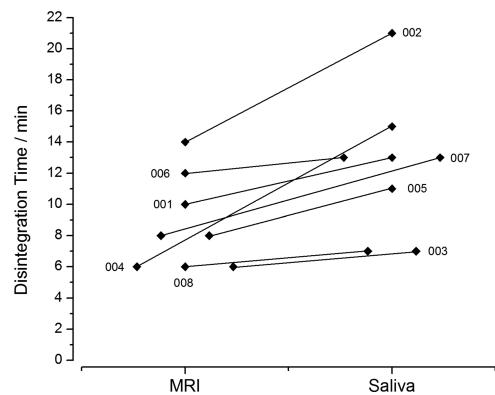


Figure 3. Individual disintegration times obtained from MRI and salivary tracer technique. Numbers indicate the volunteer.

from both techniques, the hard gelatin capsules showed a mean disintegration time of 8.8 vs 12.5 min (MRI/saliva). The disintegration times obtained by MRI were 3.7 min shorter for the hard gelatin capsules as compared to the results obtained by saliva analysis. However, this difference was statistically significant (*p* < 0.05). All values as well as the corresponding statistical parameters are presented in Table 1.

DISCUSSION

Magnetic resonance imaging is a widespread diagnostic tool in the clinical practice. Over the last years, it became more and more relevant for the exploration of physiological conditions in the human GI tract, e.g. mechanism of gastric emptying and gastric secretion, which are highly relevant for the development

Table 1. Comparison of the Disintegration Times of the Hard Gelatin Capsules Obtained by MRI and Salivary Tracer Method

	hard gelatin capsule	
	MRI/min	saliva/min
mean	8.8	12.5
median	8	13
SD	3.0	4.5
RSD	34.4%	36.0%
min/max	6/14	7/21

of oral formulations. Furthermore, MRI is used for the evaluation of the *in vivo* performance of drug delivery systems, for example disintegration times of capsules.^{13,14}

The salivary tracer technique is an elegant way to determine the *in vivo* disintegration of oral dosage forms with relatively small efforts in terms of regulatory and financial aspects. The determination of capsule disintegration by using the salivary tracer technique was based on an increase of the salivary caffeine concentrations above quantifiable concentrations. Therefore, it was important to reach a baseline concentration that is below the detection limit and to avoid contamination by the administration of the capsule. In this study, the initial salivary caffeine concentrations were low, which indicated that the manual capsule filling did not lead to contamination of the capsule shell and that the caffeine abstinenz of 72 h was suitable. Thus, the first appearance of caffeine in saliva could be successfully related with the disintegration of the dosage form.

One of the advantages of using MRI for *in vivo* disintegration studies is that it provides information about disintegration time and the location of capsule disintegration. Nevertheless, the salivary tracer technique is also able to determine disintegration times of capsules. In this study, we explored that there is an offset of around 4 min between the MRI method and the salivary tracer technique. A delay between the two methods seemed reasonable as it is possible to detect the disintegration of the capsule directly in the stomach with MRI, whereas for the salivary tracer technique, dissolution of the caffeine, gastric emptying of the fluid as well as absorption and distribution into the saliva have to be considered. After capsule disintegration within the stomach, the process of gastric emptying controls the rate by which undissolved and dissolved caffeine appears in the small intestine, where it can get absorbed. However, a previous publication has demonstrated that the time between gastric emptying of a caffeine solution and the appearance of caffeine in saliva is rapid.⁵ Therefore, we assume that it is not only the process of gastric emptying but also the dissolution rate of caffeine that is contributing to the delay between MRI and saliva data. Moreover, the dissolution of caffeine is affected by the nature of capsule disintegration. Whereas rapid mixing of the capsule content with gastric fluids would be ideal for fast dissolution, the situation may be different in reality. First, mixing with gastric contents is typically poor in the fasted stomach (except for short periods of intense peristalsis) and second, the capsule content may not be released instantaneously after rupture of the shell. The offset between the two investigated methods decreases from the fundus to the antrum of the stomach (Figure 4). This observation seems reasonable as the antrum typically is a part of the stomach with more movement and contractions, leading to a faster mixing of the gastric fluids with the salivary tracer.

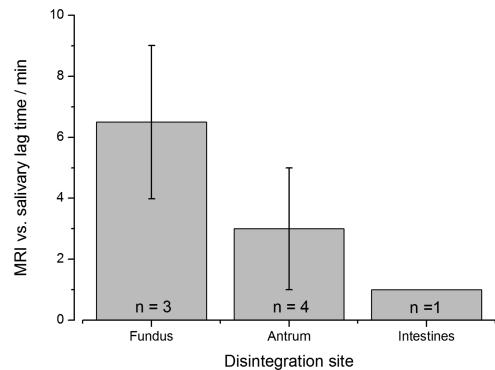


Figure 4. Offset between MRI and salivary tracer technique in dependence of disintegration site (mean \pm SD, fundus $n = 3$, antrum $n = 4$, intestine $n = 1$).

Furthermore, we also observed one case where the dosage form disintegrated inside the small intestine. In this case, the difference between MRI and saliva disintegration times was only about 1 min, which is basically the sampling offset. Thus, absorption and appearance in saliva seemed to be very fast in these cases. So, absorption and distribution are not regarded as limiting processes for the appearance of caffeine in human saliva.

Both techniques applied in this study were able to determine the disintegration times of the capsule formulation investigated. The *in vivo* disintegration times of HGC were already studied by various research groups. Brown et al. investigated the *in vivo* disintegration of hard gelatin capsules administered in the fasted state together with 200 mL of water by gamma scintigraphy.¹⁵ The mean disintegration time was found to be 8 ± 2 min ($n = 9$). In a similar setup, Digenis and colleagues also reported mean disintegration times of HGC of 7 ± 5 min ($n = 10$).¹⁶ In this study, MRI and the salivary tracer technique came to similar results. For instance, by using MRI, we obtained a mean disintegration time of 8.8 ± 3 min for HGC administered together with 240 mL of water to fasted healthy subjects. The difference in terms of the mean disintegration time obtained by MRI and the saliva tracer technique was 3.8 ± 2.8 min for the HGC.

In conclusion, the presented work demonstrated the applicability of the new salivary tracer technique to investigate the *in vivo* performance of immediate release dosage forms. The new method was validated with an established MRI method. Further work will focus on the application of both techniques to investigate the disintegration behavior of dosage forms administered in the presence of food.

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Notes

The authors declare no competing financial interest.

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6.5 Comparison of in vitro and in vivo results using the GastroDuo and the salivary tracer technique: Immediate release dosage forms under fasting conditions (*Pharmaceutics* 2019, 11(12), 659)

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Beiträge der Autoren:

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Idee des Speichelmarkers, Erarbeitung der Fragestellung, Entwurf und Korrektur des Manuskriptes

*Article*

Comparison of In Vitro and In Vivo Results Using the GastroDuo and the Salivary Tracer Technique: Immediate Release Dosage Forms under Fasting Conditions

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Abstract: The fasted state administration of immediate release (IR) dosage forms is often regarded as uncritical since physiological aspects seem to play a minor role for disintegration and drug release. However, recent in vivo studies in humans have highlighted that fasted state conditions are in fact highly dynamic. It was therefore the aim of this study to investigate the disintegration and drug release behavior of four different IR formulations of the probe drug caffeine under physiologically relevant conditions with the aid of the GastroDuo. One film-coated tablet and three different capsule formulations based on capsule shells either made from hard gelatin or hydroxypropylmethyl cellulose (HPMC) were tested in six different test programs. To evaluate the relevance of the data generated, the four IR formulations were also studied in a four-way cross-over study in 14 healthy volunteers by using the salivary tracer technique (STT). It could be shown that the IR formulations behaved differently in the in vitro test programs. Thereby, the simulated parameters affected the disintegration and dissolution behavior of the four IR formulations in different ways. Whereas drug release from the tablet started early and was barely affected by temperature, pH or motility, the different capsule formulations showed a longer lag time and were sensitive to specific parameters. However, once drug release was initiated, it typically progressed with a higher rate for the capsules compared to the tablet. Interestingly, the results obtained with the STT were not always in line with the in vitro data. This observation was due to the fact that the probability of the different test programs was not equal and that certain scenarios were rather unlikely to occur under the controlled and standardized conditions of clinical studies. Nonetheless, the in vitro data are still valuable as they allowed to discriminate between different formulations.

Keywords: dissolution methods; biorelevant in vitro model; clinical study; salivary tracer technique; GastroDuo

1. Introduction

It is generally assumed that the fasted state administration of immediate release (IR) drug products results in less variable drug plasma concentrations as compared to fed state administration. This conception is based on two main assumptions: (1) drug release from IR formulations is fast and, (2) the physiological parameters (e.g., gastric emptying, gastric pH) of the human gastrointestinal (GI) tract which are important for oral drug delivery are less variable. For this reason, drug release from IR formulations under fasted state conditions is often not assessed by use of biorelevant dissolution test methods.

After oral administration, several physiological factors such as luminal fluid volumes present in stomach and small intestine, mechanical stresses resulting from peristalsis, luminal pH values, and temperature can be relevant for drug release from IR formulations. In particular, the gastric emptying kinetics of the coadministered fluid is crucial since it controls the delivery of dissolved and undissolved drug to the small intestine—the main site of absorption. A deeper understanding of the physiological variability of these parameters under the controlled conditions of bioequivalence and bioavailability studies (BE/BA) conducted in accordance with Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidelines [1], Ref. [2] was gained in recent years through different *in vivo* investigations that aimed at characterizing the physiological conditions in the stomach and the small intestine [3–6]. These studies revealed that the conditions in the human GI tract can be highly variable and in particular, gastric emptying was shown to be largely dynamic [5]. Recently, Grimm et al. investigated the inter and intrasubject variability in gastric emptying [6].

Gastric emptying causes dynamic changes of the fluid volumes present in the fasted stomach. Since this medium represents the medium available for drug dissolution, the kinetics of disintegration and dissolution have a direct impact on intestinal drug concentrations which in turn are relevant for drug absorption (e.g., passive diffusion, capacity of uptake transporters) as well as for intestinal metabolism and/or elimination (e.g., efflux transporters). For instance, a delayed disintegration of a formulation may lead to higher drug concentrations in the stomach because of reduced gastric fluid volumes present at later time points. As a consequence, drug concentrations in the small intestine will be higher. Thus, variable drug release in the stomach can lead to variable drug concentrations in the small intestine, which potentially affect drug absorption.

In order to reduce the variability in drug release and to develop robust and reliable IR formulations, biorelevant in vitro test methods can be applied to simulate the dynamic situation in the human stomach. The application of compendial dissolution tests does typically not allow mimicking the complex luminal environment to which oral drug products are exposed in reality. In the last years, several groups have made great efforts to overcome this issue by developing biorelevant dissolution media as well as in vitro models of higher physiological relevance [7]. These range from rather simple (e.g., transfer model, BioGIT) to more complex models (e.g., Dynamic Gastric Model, TNO TIM-1) [8–10]. Depending on the focus of the dissolution experiment, each model has specific advantages and disadvantages [7,11].

With respect to gastric emptying and motility, there is a clear need for improved in vitro test systems that enable the consideration of the variability of the physiological conditions in the stomach. In the past, we have already demonstrated the importance of gastric emptying for the drug plasma concentrations of two different IR formulations containing N-acetylcysteine by use of the Dynamic Open Flow-Through Test Apparatus [12]. In this work, we describe the design and application of the GastroDuo as an optimized biorelevant in vitro tool for the investigation of drug release in the stomach. The GastroDuo was initially introduced by Schick et al. as a valuable tool to in vitro simulate crucial parameters of the human stomach [13]. The primary aim of this work was to assess

the biorelevance of the results obtained with the GastroDuo by comparing in vitro and in vivo data on drug release from four different IR formulations containing caffeine as a probe drug. For this purpose, drug release from the four IR formulations was first investigated in vitro by using compendial dissolution methods and the GastroDuo. Subsequently, we performed an in vivo study with 14 healthy volunteers, in which the salivary tracer method was used to determine the in vivo disintegration behavior of the four formulations [14]. The secondary aim of this study was to elaborate on the effect of different physiological factors on the drug release behavior of three different capsule shells and a film-coated tablet.

2. Materials and Methods

2.1. Materials

Caffeine, croscarmellose, and lactose monohydrate were supplied by Caelo (Hilden, Germany). Polyvinylpyrrolidone (PVP) 90, magnesium stearate, silica dioxide, and hydroxypropyl methylcellulose K4M (HPMC) were purchased from Fagron (Barsbüttel, Germany).

Water HiPerSolv CHROMANORM LC-MS grade (VWR international, Fontenay-sous-Bois, France), Methanol HiPerSolv CHROMANORM LC-MS grade (VWR international, Fontenay-sous-Bois, France), Formic acid (Merck KaA GmbH, Darmstadt, Germany), Acetonitrile HiPerSolv CHROMANORM LC-MS grade (VWR international, Fontenay-sous-Bois, France), ammonium acetate (Merck KaA GmbH, Darmstadt, Germany), completely desalinated water (based on tap water, Stadtwerke Greifswald, Germany, prepared by double reverse osmosis).

2.2. Dosage Forms Investigated

2.2.1. Immediate Release Tablets

Immediate release tablets were compressed from a powder mixture composed of caffeine (7% w/w), croscarmellose (12% w/w), and lactose (81% w/w), which was granulated by using a solution (4.5% v/v) of PVP-90. Magnesium stearate and silica dioxide were added directly before tableting. In the end, biconvex tablets (14 × 6 mm) with a total weight of 350 mg were prepared on a rotary tablet press (Riva Piccola, Hampshire, UK) at a compression force of approximately 6.7 kN. The tablets showed a mean crushing strength of 87 N ($n = 10$) in a range from 76 to 100 N. Subsequently, an HPMC coating of 5% (weight gain) was applied onto the tablets with the aid of a drum coater (Glatt, Germany).

2.2.2. Capsules

Three different capsule shells (size 0) were used in this study (Table 1). All capsules were directly filled with 350 mg of the nongranulated powder mixture that was also used for tableting. Hard gelatin and Vcaps® plus capsules were purchased from Capsugel (Morristown, NJ, USA). Quali-V® capsules were supplied by Qualicaps (Yamatokoriyama Nara, Japan).

Table 1. Dosage forms used in the present study.

Dosage Form	Ingredients
Immediate release tablets with 5% HPMC coating	Caffeine, croscarmellose, lactose, magnesium stearate, silica dioxide, hydroxypropyl methylcellulose
Hard gelatin capsule (size 0)	Caffeine, croscarmellose, lactose, gelatin
Quali-V® capsules (size 0)	Caffeine, croscarmellose, lactose, hydroxypropyl methylcellulose, carrageenan
Vcaps® plus capsules (size 0)	Caffeine, croscarmellose, lactose, hydroxypropyl methylcellulose

2.3. In Vitro Investigations

The in vitro investigations were performed by using the compendial USP II paddle apparatus and a novel biorelevant dissolution test device, the GastroDuo.

2.3.1. Compendial Dissolution

The USP II paddle apparatus (PharmaTest DT70, Erweka, Heusenstamm, Germany) mainly served as a reference in this work. All dosage forms were tested in 900 mL of simulated gastric fluid (SGF) pH 1.2 at a temperature of 37 °C and a stirring speed of 75 rpm. Drug concentrations inside the vessels were determined with a spectrophotometer (Cary 60, Agilent, Santa Clara, CA, USA) that was equipped with a 16-channel fiber optic system. The detection wavelength was 272 nm.

2.3.2. GastroDuo

Construction and Setting

The GastroDuo is a biorelevant dissolution test device that combines the different advantages of the different in vitro models (i.e., Dissolution StressTest device, Dynamic Open Flow-Through Test Apparatus, and Fed Stomach Model) that were previously developed in our group [12,15–17]. The GastroDuo is able to simulate certain physiological aspects of the stomach and the small intestine. This includes gastric emptying kinetics, luminal pH, and temperature profiles as well as a realistic simulation of pressures arising during GI transit due to motility. In order to enable the in vitro simulation of these factors, several modifications were made to the construction of the Fed Stomach Model [16].

As can be seen from Figure 1, the central element of the GastroDuo is the gastric cell, which represents a flow-through cell with a maximum volume of 50 mL. The gastric cell is perfused with medium from the donor vessel. The pH value of this donor medium can be further adjusted by the addition of acidic media from the acid vessel. To simulate realistic gastric emptying kinetics, the medium from the gastric cell is pumped with defined rates into the acceptor vessel, which contains a medium that allows complete drug dissolution. During the experiment, the volume inside the gastric cell is kept constant. A proper temperature control is ensured by the surrounding water bath and the low wall thickness (approximately 0.75 mm) of the gastric cells that are made from Vivak® (PET-G, Bayer, Kaiser-Wilhelm-Allee 1, 51373 Leverkusen, Germany).

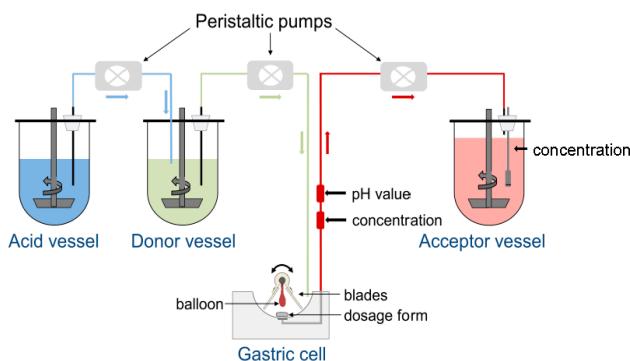


Figure 1. Schematic presentation of the experimental setup for the GastroDuo.

For the investigation of drug release, a formulation is placed in the center of the gastric cell. Two comb-like blades that are connected to a central axis enable the simulation of formulation movement in the stomach as well as mixing. A balloon located between the blades is directly connected to the central axis and can be inflated with different pressures to simulate mechanical stresses acting on the formulation. The magnitude of these stresses is based on recent *in vivo* investigations with the SmartPill® [3]. The drug concentrations are constantly measured at two positions: (1) at the outlet of the gastric cell (in-line) to assess changes of drug concentration in the fluid emptied from the simulated gastric compartment and, (2) in the acceptor vessel to determine the sum of dissolved

and undissolved drug substance emptied from the gastric cell. In addition, pH and temperature are constantly monitored in the outflow of the gastric cell with the aid of a pH electrode (InLab® Expert Pro, Mettler Toledo, Switzerland) and a temperature sensor (Board: USB-μPIO TEMP12, Abacom; Software: RealView, Electronics Software; Sensor: DS18B20). In total, the GastroDuo consists of three gastric cells so that three experiments can be conducted at the same time. In the Supplementary Material, a video can be found that shows the functional principle of the GastroDuo.

Test Programs

The GastroDuo allows the simulation of different conditions and events. In this study, the effect of different rates of gastric emptying, different pH and temperature profiles, and pressure events of physiological magnitude were studied. In order to reflect the physiological variability of these factors in the fasted stomach, six test programs (A–F) were defined. The ranges of the physiological parameters that are covered by these test programs were based on recent *in vivo* studies performed with SmartPill and MRI [3,5,6].

All test programs consisted of a gastric transit part with a defined duration and a subsequent artificial part that was used to flush out all residues of the drug from the gastric cell. The gastric transit part always ended with a simulated gastric emptying pattern that included an increased flow rate of 20 mL/min, three pressure events of 300 mbar and three fast movements of the blades. This gastric emptying event simulated the complete emptying of the stomach by a migrating motor cycle (MMC) phase III contraction wave (“housekeeping wave”). At the beginning of the experiments, the gastric cells were filled with 25 mL of pure deionized water. The first order like gastric emptying of the coadministered water was simulated by six decreasing flow rate “steps”. In each experiment, a cumulative volume of 240 mL was perfused through the gastric cell, which was equal to the volume of water given in the *in vivo* study. The poor mixing of the stomach was simulated by a slow movement of the blades which was performed every 3 min.

Program A was designed to represent the average conditions in the stomach after administration of 240 mL of tap water and was based on mean values in terms of gastric emptying, motility, pH, and temperature. All other programs (B–F) simulated the extremes of these particular parameters. Test program A simulated a gastric transit time (GTT) of 30 min and a small stress event after 10 min, whereas test program B had no such stress event after 10 min. Test programs C and D were designed to simulate short (15 min, C) and long (45 min, D) GTT and, therefore, different hydrodynamic stresses. Thus, the test programs A–D simulated the variability of gastric emptying and gastric peristalsis (Figure 2). In all these programs, dynamic temperature and pH profiles were simulated. The simulated profiles were based on recent *in vivo* data, which were obtained by administering telemetric capsules in the fasted state together with 240 mL of tap water [3]. These measurements clearly showed that both parameters, pH and temperature, follow a distinct profile in the fasted stomach. As can be seen from Figure 3, the GastroDuo enabled the simulation of specific pH and temperature profiles. Whereas the pH value of the simulated gastric medium was increased for a short time, the temperature fell to values below 20 °C.

The test programs E and F served as reference programs to study the effect of temperature and pH changes on the drug release behavior of the four IR formulations. These programs were identical to test program A in terms of duration, emptying kinetics, and motility events (Figure 2) and differed only in terms of temperature (E) or pH (F). In program E, the temperature was kept constant at 37 °C (program E) and in program F, the pH was constantly at pH 1.2.

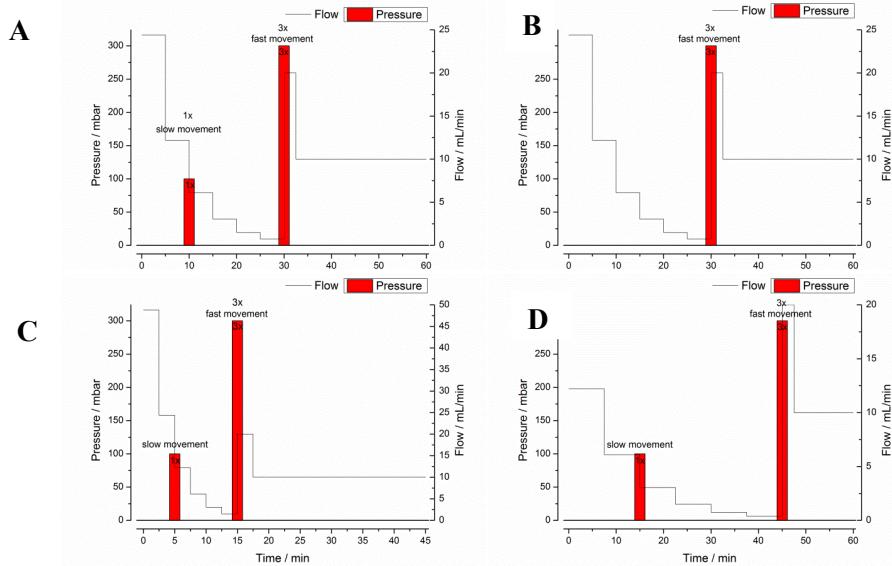


Figure 2. Graphical illustration of the GastroDuo test programs (A–D) with pressure and flow rates over time. Text boxes additionally indicate slow or fast movements.

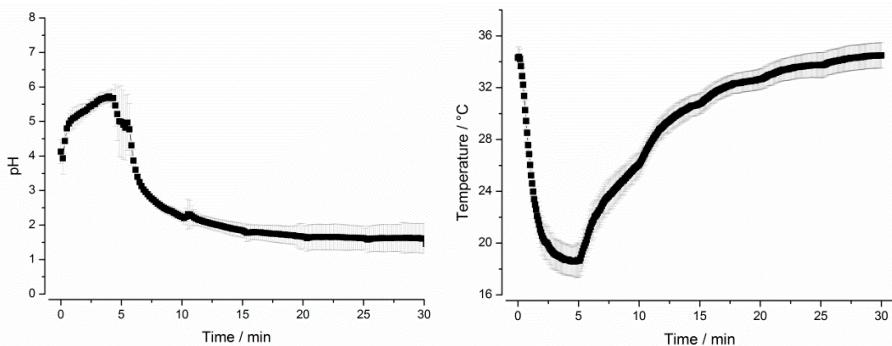


Figure 3. *Left:* pH profile simulated in test program A. *Right:* Temperature profile simulated in test program A (mean \pm SD, $n = 6$).

2.4. Determination of In Vitro Disintegration Parameters

Drug concentrations in the outflow of the gastric cells and the acceptor vessels were determined with a spectrophotometer (Cary 60, Agilent) that was equipped with a 16-channel fiber optic system. The detection wavelength was 272 nm.

On the basis of the drug concentrations measured in the outflow of the gastric cell, the initial capsule or tablet disintegration (iDT) was calculated to describe the in vitro disintegration behavior. The iDT was defined by the measurement of a caffeine concentration that exceeds the lower limit of quantification (LLOQ = 15 μ g/mL).

2.5. In Vivo Study with the Salivary Tracer Method

An in vivo study based on the recently reported salivary tracer method was performed to investigate the disintegration behavior of the four IR formulations in the human GI tract. The procedure applied in this study followed the description of a recent publication “Low dose caffeine as a salivary tracer for the determination of gastric water emptying in fed and fasted state: A MRI validation study” [14].

2.5.1. Study Design

In this study, 14 healthy volunteers of both sexes were included. The information about the characteristics of the study participants is provided in Table 2.

Table 2. Demographic data of the study volunteers.

Age	22–31 years
Body Mass Index	20–25
Female/Male	6/8
Ethnics	Caucasian
Pre-existing conditions	none
Number of volunteers	14
Regular coffee consumers	12

Written informed consent was obtained from every participant. The study was conducted in compliance with the “Declaration of Helsinki” (revised version from 2013, Fortaleza) and the “(Model) Professional Code for Physicians in Germany” (revised version from 2011, Kiel). A positive vote was given by the ethics committee of the University Medicine Greifswald for the study named “Nutzung von Speichelmarkern zur in vivo Charakterisierung von Arzneiformen” (registration date: 17 November 2017, registration number: BB 172/17). For every participant, insurance was concluded to all risks associated with participating.

The study was conducted in a randomized four-way, cross-over design. Thus, all volunteers received the four formulations in a randomized manner on different days with a wash-out period of at least 48 h. After an overnight fast of at least 10 h, the volunteers had to take the dosage form in an upright position together with 240 mL of tap water (time point 0 min). Afterwards, the oral cavity was rinsed three times with around 100 mL of tap water without swallowing for cleaning purposes. Saliva samples were collected by drooling at the time points: −5, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 26, 31, 36, 41, 51, 61 min plus every 15 min until 241 min.

2.5.2. Preparation and Analysis of Saliva Samples

All saliva samples were stored at −80 °C immediately at the end of each study day until analysis. Saliva sampling and probe preparation was based mainly on the procedure described in the recent publication [14].

Determination of caffeine in the saliva samples was performed by use of an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a triple quadrupole mass spectrometer API4000 QTRAP (AB Sciex, Darmstadt, Germany) via the electrospray ionization source Turbo V™. The LC-MS/MS system was controlled by the validated Analyst 1.6 software (AB Sciex, Darmstadt, Germany).

Caffeine was separated from hydrophilic saliva components such as mucins and other glycoproteins by isocratic elution. The mobile phase consisted of a 50/50 (*v/v*)-mixture of ammonium acetate buffer (5 mM; pH 3.8) and methanol. The flow rate was set to 250 µL/min. The reversed-phase column Xterra®MS (C18, 3.5 µm, 2.1 × 100 mm; Waters, Dublin, Ireland) was tempered at 40 °C. The injection volume was 20 µL.

The chromatographic flow was directed to a $0.5\text{ }\mu\text{m}$ filter device (PEEK, Supelco, Taufkirchen, Germany) to avoid particulate contamination. The HPLC was connected to the mass spectrometer interface (Turbo VTM ionization source) operated in the positive ion mode. The following gas parameters were used: temperature, $550\text{ }^{\circ}\text{C}$; gas 1, 60 psi; gas 2, 60 psi (all nitrogen); voltage, 4000 V; collision-activated dissociation (CAD), 12 (arbitrary unit). The Analyst[®] 1.6 software was applied to evaluate the chromatograms using the internal standard method and peak-area ratios for calculation (quadratic regression, $1/x$ weighting).

The analytical method was validated with respect to linearity, precision (within-day and between-day), accuracy (within-day and between-day), selectivity, and rack stability regarding the FDA Guidance for Industry “Bioanalytical Method Validation” (Issue: May 2001). All validated criteria met the requirements of the FDA guideline. Lower limit of quantification (LLOQ) was 5 ng/mL determined in the matrix.

2.5.3. Determination of the In Vivo Disintegration Parameters

Similar to the in vitro parameters, two in vivo parameters were chosen to describe the in vivo disintegration. Initial capsule or tablet disintegration (iDT) was defined by reaching a salivary caffeine concentration which exceeded the triple limit of quantification (15 ng/mL). This time point had to be supported by the concentration of the next sampling point, which should be either more or not less than 5% of the concentration value before. This procedure was described in more detail in a recent publication [18]. In order to describe the kinetics of in vivo disintegration, the difference (ΔT_{\max}) between salivary caffeine T_{\max} and iDT was calculated. Generally, a low ΔT_{\max} indicated a rapid and complete disintegration, whereas a higher value for ΔT_{\max} was an indicator for a slow disintegration of the dosage form.

2.5.4. Statistical Comparison

The statistical comparison was performed for the two in vivo parameters iDT and ΔT_{\max} as one single value for every in vivo experiment between the four groups. As statistical analysis of the experiments reviled non-normality of the observations, nonparametric tests were used. In order to determine if the observations originated from the same distribution, the Kruskal–Wallis test was used. For the direct comparison between the four groups, the Wilcoxon signed-rank test was used. All p -values in the manuscript are given based on this test.

3. Results

3.1. In Vitro Experiments

3.1.1. Compendial Dissolution Testing

The results obtained with USP paddle apparatus are presented in Figure 4. It can be seen that the hard gelatin capsules (HGC) showed the fastest dissolution with a drug release of more than 80% within 5 min. In contrast, the longest delay in drug release was observed for the Vcaps[®] plus capsules, for which a drug release of more than 80% was obtained only after 13.5 min. Compared to the HGC, both HPMC capsules showed higher variability during drug release as can be seen from the higher standard deviations.

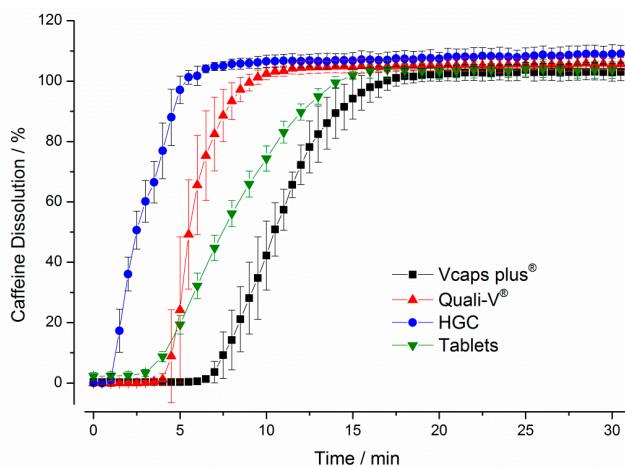


Figure 4. Dissolution of caffeine over time for four immediate release dosage forms in the paddle apparatus. The stirring speed was 75 rpm, the medium temperature was $37 \pm 0.5^{\circ}\text{C}$ and a total volume of 900 mL simulated gastric fluid pH 1.2 without pepsin was used. The drug concentrations were determined by fiber optic UV measurement at 272 nm (mean \pm SD, $n = 6$).

3.1.2. GastroDuo Experiments

As can be seen from Figure 5, the drug release profiles measured by using the GastroDuo were different to the profiles obtained by using the USP paddle apparatus. In the following sections, we will briefly describe the results of the in vitro experiments for every formulation.

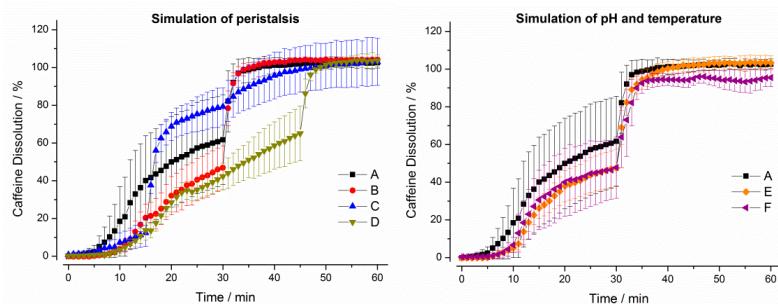


Figure 5. Dissolution profiles of immediate release tablets in the GastroDuo. *Left:* Programs simulating the influence of peristalsis (A–D). *Right:* Programs simulating the influence of temperature and pH value (A,E,F). The concentration was measured in the outflow of the gastric cell by fiber optic UV measurement (mean \pm SD, $n = 6$).

For the IR tablet, drug release was not complete in any of the six programs until the simulation of gastric emptying. In test program A, only around 60% of the drug was released until this time point. Notably, the initial stress event had only minor effects on drug release. In programs B and D, drug dissolution was again slower due to lower mechanical (pressure) and hydrodynamic (flow rates) stresses in the first 30 min. The simulation of constant pH and temperature did not lead to relevant differences in drug dissolution.

For Quali-V® capsules, only small effects of simulated peristalsis as well as of pH and temperature on drug release were observable in the in vitro experiments (Figure 6). The profile was mainly dictated by the process of gastric emptying.

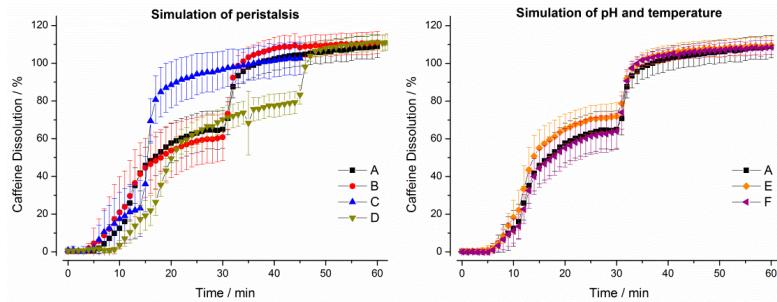


Figure 6. Dissolution profiles of Quali-V® capsules in the GastroDuo. *Left:* Programs simulating the influence of peristalsis (A–D). *Right:* Programs simulating the influence of temperature and pH value (A,E,F). The concentration was measured in the outflow of the gastric cell by fiber optic UV measurement (mean \pm SD, $n = 6$).

It can be seen from Figure 7 that the simulation of mechanical and hydrodynamic stresses was important for drug release from Vcaps® plus. Whereas the short gastric transit time in program C led to a fast dissolution of the caffeine, drug release was slow in test programs B and D, where lower mechanical stresses were present. The simulation of pH and temperature profiles did not lead to any relevant changes.

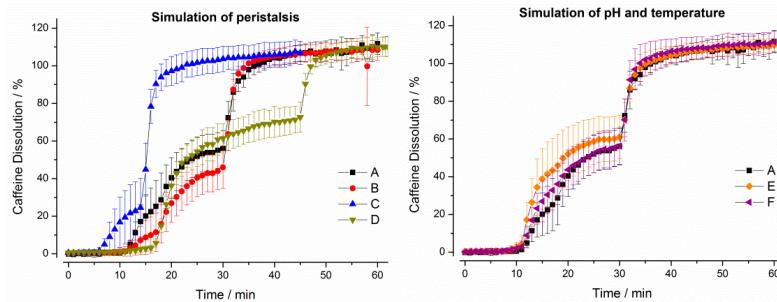


Figure 7. Dissolution profiles of Vcaps® plus in the GastroDuo. *Left:* Programs simulating the influence of peristalsis (A–D). *Right:* Programs simulating the influence of temperature and pH value (A,E,F). The concentration was measured in the outflow of the gastric cell by fiber optic UV measurement (mean \pm SD, $n = 6$).

It can be seen from Figure 8, that the constant higher temperature of 37 °C (program E) as well as the constantly low pH of pH 1.2 (program F) in the gastric cell accelerated drug release from the hard gelatin capsules. Moreover, dissolution was also accelerated in programs characterized by higher mechanical and hydrodynamic stresses in the first 30 min (A and C).

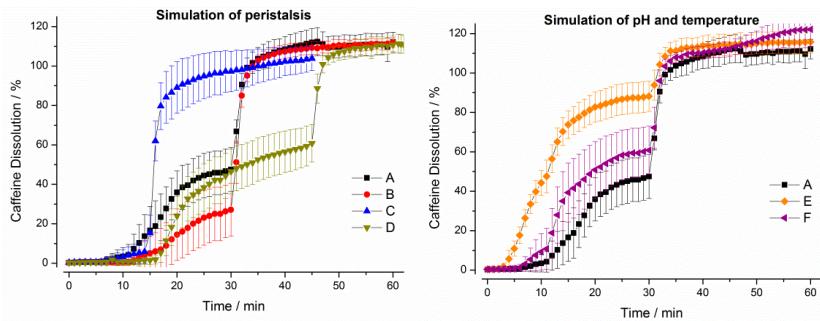


Figure 8. Dissolution profiles of hard gelatin capsules in the GastroDuo. *Left:* Programs simulating the influence of peristalsis (A–D), *Right:* Programs simulating the influence of temperature and pH value (A,E,F). The concentration was measured in the outflow of the gastric cell by fiber optic UV measurement (mean \pm SD, $n = 6$).

The initial disintegration times (iDT) are given in Figure 9. For the USP II data iDT was based on the individual dissolution profiles, whereas GastroDuo data was based on the caffeine concentrations measured in the outflow of the gastric cell. The highest variability in between the test programs was observed for the HGC ranging from 2.5 to 17 min, whereas Quali-V® and the film-coated tablet exhibited a lower variability ranging from 3 to 12 min. The lowest variability between test program A was observed for Vcaps® plus.

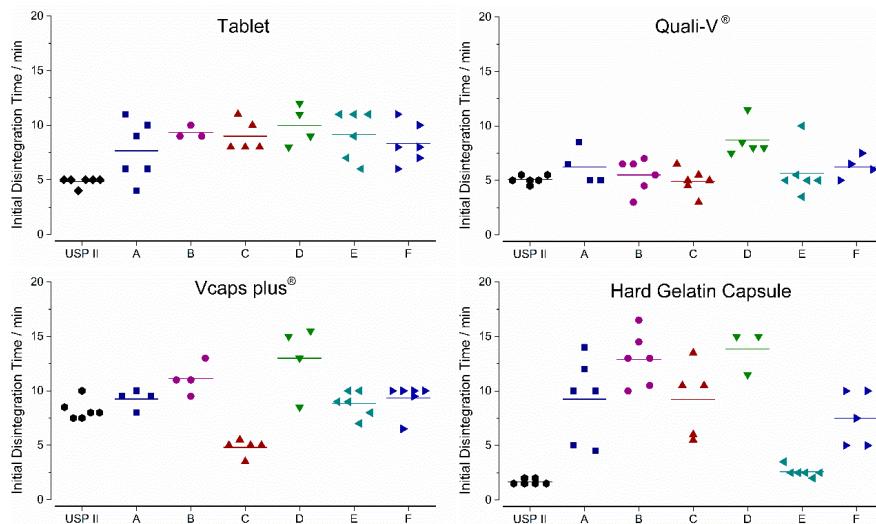


Figure 9. In vitro initial disintegration times (iDT) of four immediate release dosage forms. Given for all six test programs of the GastroDuo and the compendial dissolution. Diamonds indicate individual values and solid lines indicate means.

3.2. In Vivo Experiments

In all 14 subjects, caffeine concentration-time profiles were successfully measured in the saliva. These profiles were used to calculate the initial disintegration time (iDT) and the ΔT_{\max} . An exemplary caffeine saliva concentration-time profile is given in Figure 10.

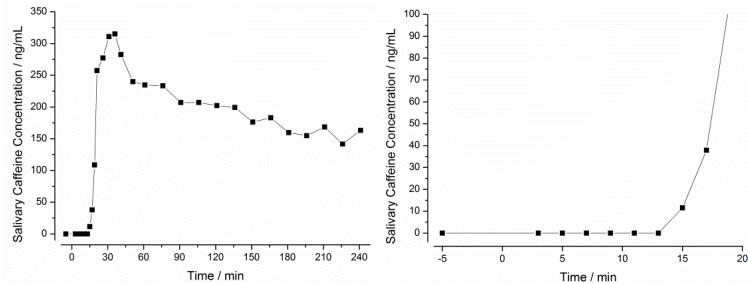


Figure 10. Salivary caffeine concentration over time after administration of a Vcaps® plus capsule with 25 mg caffeine to one healthy volunteer after a 10 h fasting period. *Left:* Whole observation time frame. *Right:* Detail of the first 20 min.

In this example, the first caffeine concentration above LLOQ (5 ng/mL) was observed after 15 min (11 ng/mL). As described earlier, the definition of the iDT was based on a concentration value at least three times above LLOQ (15 ng/mL). Since the first concentration above this value was measured after 17 min, this time was defined as iDT. Since Salivary caffeine t_{max} in this example was observed after 36 min, a ΔT_{max} of 19 min was calculated.

In Figure 11, the iDTs of all four formulations are depicted. Interestingly, the highest variability was observed for Quali-V® and Vcaps® plus capsules. These two IR formulations also showed the highest mean initial disintegration times (>20 min). In between these two formulations, a statistically significant difference could not be observed. In contrast, the tablets (13.8 ± 5.2 min) and the hard gelatin capsules (15.5 ± 4.3 min) started to disintegrate earlier with no statistical difference between these two dosage forms. Notably, between these two groups (i.e., capsules based on HPMC and tablets/hard gelatin capsules) statistically significant differences were present.

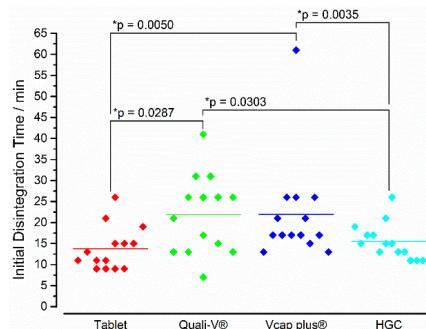


Figure 11. In vivo iDT of the four immediate release dosage forms. Diamonds indicate individual values and solid lines indicate means. The * p -value was based on the two-tailed Wilcoxon signed-rank test ($n = 14$), $p < 0.05$ indicating a significant difference.

With respect to the kinetics of the in vivo disintegration process, a different picture was seen for ΔT_{max} than for iDT (Figure 12). Whereas the tablet showed the fastest iDT, it had the longest ΔT_{max} . The lowest ΔT_{max} was seen for HGC and this value was significantly lower than for the tablet and the Quali-V® capsule. It has to be noted, that with the except of the HGC, all formulations showed individual values diverging around 40–50 min to the mean value.

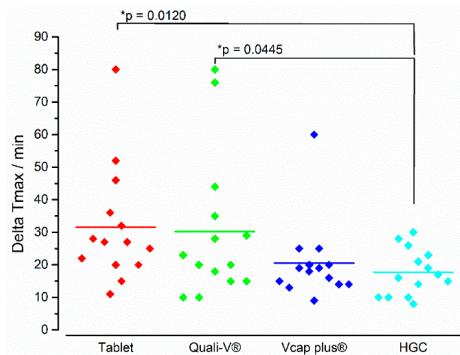


Figure 12. In vivo ΔT_{max} of the four immediate release dosage forms. Diamonds indicate individual values and solid lines indicate means. The * p -value was based on the two-tailed Wilcoxon signed-rank test ($n = 14$), $p < 0.05$ indicating a significant difference.

4. Discussion

In this work, the disintegration and dissolution behavior of four different IR formulations were investigated in vitro by using a novel dissolution tool, the GastroDuo, and in vivo by using the salivary tracer technique. The four formulations tested in this work included one tablet formulation and three different capsule formulations. They were all based on the same powder mixture composed of caffeine, lactose monohydrate, and croscarmellose in order to directly compare the behavior of the formulations and to minimize the effect of further parameters (e.g., certain excipients, particle size, etc.). The powder mixture was filled manually into three different capsule shells that were based either on gelatin (hard gelatin capsules) or HPMC (Quali-V®, Vcaps® plus). In case of the tablets, the powder mixture was compressed to tablets on a rotary tablet press and coated with HPMC (5% weight gain) in a subsequent step.

Compendial dissolution testing with the paddle apparatus revealed that the hard gelatin capsule disintegrated first, whereas Quali-V® capsules had the longest lag time until beginning of disintegration. In case of the tablets and the Vcaps® plus capsules, the starting point of disintegration was comparable for both formulations, but subsequently the capsules released their content faster. This may be due to the fact that after the capsule shell ruptured, the particles were released and, thus, presented a larger surface area available for drug dissolution. In line with this hypothesis, we also observed similar dissolution rates for the other two capsule shells.

In contrast to compendial dissolution test methods, the GastroDuo was able to simulate variable gastric emptying kinetics, dynamic pH, and temperature profiles as well as gastric peristalsis. The basis for these simulations were recent in vivo studies that were performed with healthy subjects [3,4,6,19]. For instance, the application of MRI allowed us to describe the kinetics of gastric emptying in high temporal resolution [6], whereas telemetric capsules such as SmartPill provided deeper insights into luminal conditions in terms of pH value, temperature, and pressures [3]. In order to reflect the large variability of these physiological parameters in the fasted state, six test programs were defined. Thereby, program A represented the expected ‘average’ profile: gastric emptying of water following first-order kinetics within 30 min, an early pressure event of 200 mbar after 10 min, and the presence of MMC phase III activity after 30 min. The other five programs represented modifications of single parameters in order to cover the extremes that may occur in the human stomach. By this, the variability of drug release that may occur in vivo should be investigated.

The GastroDuo experiments demonstrated that the simulated physiological variability of the different parameters was highly important for the disintegration behavior of the four formulations. Compared to what has been observed with compendial dissolution testing, the starting point of disintegration was clearly later for all formulations in the GastroDuo. This effect could be explained

by the mild conditions inside the GastroDuo in terms of shear rates and stresses that were simulated in the GastroDuo. In contrast, the high shear rates applied in the paddle apparatus overestimated the disintegration process of the different formulations as was shown later *in vivo*. In test program A, which was regarded as the standard program, the disintegration times were more or less comparable with values between 5 and 10 min. However, especially in case of the hard gelatin capsule the disintegration times were highly variable with values ranging from 5 to 15 min. At the end of simulated gastric transit, around 40%–60% of the drug had been transferred to the acceptor vessel.

As can be seen from the comparison of the results of test programs A and B, the presence of an initial pressure event was highly relevant especially for the hard gelatin capsules and for the tablets but was of minor importance for the HPMC-based capsules. Apparently, only small pressures were needed to initiate the disintegration of the formulations. The presence of single pressure events has been observed also in recent *in vivo* studies and their timing may contribute significantly to the *in vivo* variability. In test programs C and D, the pressure event was simulated after 5 and 15 min, respectively. Interestingly, it was only in case of Vcaps® plus that an earlier pressure event also caused an earlier starting point of disintegration. For the other formulations, this event was of minor importance. This effect may have to do with penetration of water into the tablet and with the swelling of the polymer in case of the capsule shells. Apparently, 5 min was not sufficient to cause a relevant softening of the dosage form. Thus, they could withstand the simulated pressure. The simulation of a later pressure event in program D initiated the disintegration of Vcaps® plus capsules and of hard gelatin capsules. In case of the tablets and the Quali-V® capsules, disintegration started before this event and, thus, the simulation of this pressure event only accelerated drug release by improving mixing of released contents. Thus, peristaltic contractions are not only important to initiate disintegration of the dosage form, but also to allow mixing of undissolved particles which will facilitate dissolution. Analogous effects have been observed for hydrogel matrix tablets in combined pharmacokinetic and magnetic marker monitoring studies. The drug release rate of felodipine matrix tablets was shown to be highly dependent on the intragastric localization. An onset of drug plasma concentrations could not be observed as long as tablets were located in the fundus [20]. This effect can also be explained by the absence of peristaltic contractions. In all programs and for all formulations, the simulation of intense gastric peristalsis and of high flow rates, by which we simulated the emptying of any residues from the stomach during MMC phase III, resulted in the rapid increase of caffeine levels in the acceptor vessel. This highlights the importance of the MMC for the onset of drug plasma concentrations after oral drug administration in fasted state. The same effect has been recently demonstrated *in vivo* by van den Abeele and colleagues [21].

As the timing of MMC phase III activity with respect to the time point of drug administration appears to be highly relevant, we simulated different gastric transit times [21]. Especially for short gastric transit times, the contribution of this final event to the overall drug transfer to the acceptor vessel was great for all formulations. For the hard gelatin capsule, more than 90% of the caffeine was released as a result of an early phase III activity. In contrast, prolonged gastric transit times (program D) resulted in different effects. In the case of the tablets, decreased transfer rates also resulted in a delayed onset of caffeine levels in the acceptor vessel. On the contrary, the onset of caffeine levels in the acceptor vessel was similar to the standard program for the capsule formulations. Thus, at the end of the longer simulated gastric transit (45 min), a higher amount of caffeine was present in the acceptor vessel compared to the standard program.

Recent studies have already shown that the simulation of temperature profiles is of high relevance for hard gelatin capsules since the gelatin starts to dissolve only above temperatures of 32 °C [15]. Therefore, the simulation of physiologically pH and temperature profiles was regarded as crucial for the development of the biorelevant dissolution tool presented herein. In line with the aforementioned study, we could show that the temperature dip which is caused by the intake of an oral formulation together with a glass of water at room temperature delays the disintegration of hard gelatin capsules. As can be seen from the results of program E but also from the compendial dissolution experiment, disintegration

occurs earlier if the temperature is constantly at 37 °C. For the other formulations, the temperature of the medium was only of minor importance. Similar effects were observed for the simulated pH profile as neither the caffeine nor the used excipients experienced a pH-dependent dissolution behavior. Drug release from the tablets was slightly slower but this effect was not considered relevant.

The dissolution experiments performed with the GastroDuo nicely revealed that drug release even from very relatively simple formulations of a highly soluble drug can be influenced to different extents by various physiological parameters. The four IR formulations investigated in this study clearly showed that the simulation of peristalsis strongly affected in vitro drug release. These effects showed varying tendencies in between the formulations, although they were generally considered as comparable. Furthermore, the GastroDuo was able to discriminate between formulations, which was not possible in compendial experiments. The next step was the investigation whether these differences could be confirmed *in vivo*.

The salivary tracer technique represented an interesting tool to check the *in vivo* relevance of the data that were obtained by using the GastroDuo. In a recent study, it has been confirmed by MRI that this technique can be used to determine *in vivo* disintegration times with an acceptable accuracy [18]. For this reason, we determined the disintegration behavior of the four formulations in 14 healthy subjects by using salivary caffeine concentrations. In comparison to the results from scintigraphic studies [22–26], in which the starting point of disintegration of hard gelatin capsules was typically in the range of 4–12 min, the mean iDT for HGC in this study was 16 ± 4 min and, thus, clearly longer. Similar effects were also observed for the other capsules. This difference can be at least partly explained by the fact that scintigraphy is a direct imaging method, whereas the salivary tracer technique requires additional processes such as dissolution, gastric mixing, and emptying of the tracer. These processes probably caused an offset between the disintegration of the formulation and the appearance of caffeine in saliva. In a recent study, the offset between the salivary tracer method and MRI as a direct imaging method was on average approximately 5 min [21]. Due to this limitation, the initial disintegration times determined *in vitro* and *in vivo* cannot be compared directly. However, the *in vivo* data could be used to rank-order the four formulations as the offset was expected to be independent from the formulation.

In terms of the starting point of *in vivo* disintegration, two groups could be identified between which a significant difference could be detected. The starting point of disintegration of the tablets and the hard gelatin capsules was after around 15 min, whereas the HPMC-based capsules began to disintegrate on average only after more than 20 min. Within these groups, i.e., between tablets and HGC as well as between Quali-V® and Vcaps® plus, we could not find statistically significant differences. Especially in case of Quali-V® capsules, these data were in contrast to the *in vitro* data obtained with the GastroDuo. Based on the GastroDuo data, we would have expected that the disintegration of Quali-V® capsules occurs earlier or at similar time points compared to the tablet or the HGC. One reason for this effect might have been the gastric peristalsis again. For the sake of reproducibility, the blades within the gastric cell performed slow movements every 3 min. However, the mechanical forces that were caused by these movements may have been high enough to initiate the rupture of the capsule shell of the HPMC based formulations. This sensitivity towards small mechanical pressures may also explain the large variability that was seen *in vivo*. Another explanation may be based on the special characteristics of the gelling agent, which was carrageenan in case of the Quali-V® capsules. The sulphate group of carrageenan is known to interact with polar substances, even in acidic media. This interaction can cause a delayed disintegration of the capsule shell, which may have occurred *in vivo* [27,28]. In contrast to the observations made in this study, Tuleu and colleagues determined disintegration times of 9 ± 2 min for Quali-V® capsules with the aid of scintigraphy [24]. Thus, further studies will be needed to study this effect in more detail.

In general, the *in vitro* data obtained with the GastroDuo provided a good impression of the *in vivo* situation, but with some limitations. One of the most surprising *in vivo* observations was the relatively low variability of the *in vivo* iDT of the hard gelatin capsules. Based on the *in vitro* dissolution experiments, a higher variability compared to the other IR formulations was expected. An

explanation for this overestimation of variability in the GastroDuo was the weighting of the six test programs. It should be noted that four of the six test programs were mimicking physiological extrema (low and high peristalsis, constant pH, constant temperature, etc.) which are not always likely to occur in a such relatively small number of subjects. Based on the in vivo observations, we would emphasize that the test programs A and D are more likely to occur than the other test programs. On the other hand, under the controlled conditions of BE/BA studies, a situation like in program E (constant gastric temperature of 37 °C) is rather unlikely. However, in terms of real-life variability, such a case may be likely, for instance, when a patient administers a drug product with a warm or hot drink such as coffee or tea. Similar effects could be observed with respect to motility and gastric emptying kinetics. Grimm and colleagues showed recently that gastric emptying of 240 mL of water can take between 10 and 50 min [6]. Thus, it is likely that several individuals in this study showed gastric emptying times that were outside the range simulated in vitro (i.e., 15–45 min). With these considerations in mind, the dissolution results should be interpreted not only based on the data but also with respect to the likelihood of each test program to occur in vivo.

Interestingly, we also observed in vivo disintegration times of 41 or 61 min in this study. Such long in vivo disintegration can be caused by various factors. Apart from a lack of mechanical stresses acting on the dosage forms, such long times can also be caused by exceptionally long esophageal transit times. The retention of capsules in the esophagus was reported in the past for hard gelatin and also for HPMC capsules [29–31]. Furthermore, the localization of the dosage form in the stomach can be variable. As the gastric emptying of fluids can be relatively fast, it is possible that dosage form localization in areas with limited fluid volumes may hamper its disintegration. Additionally, the floating of the formulation on top of the gastric contents, which is typically observed in vivo, as well as sticking to the stomach wall can also contribute to the observed in vivo variability of disintegration.

For the delivery of drugs by immediate release formulations the time point of the beginning of disintegration is not the only relevant parameter; it is also the subsequent phase of drug release, especially for drugs which are subject to a concentration dependent absorption [32]. A rapid and complete drug release typically results in higher concentrations, which can drive absorption and, thus, lead to changed pharmacokinetics compared to a slow and stepwise disintegration. A straightforward parameter to describe the kinetics of drug release from an IR formulation in this study was the time difference between t_{max} and the beginning of disintegration (ΔT_{max}). A small value indicated a rapid drug release whereas larger values indicated slower drug release. With mean values of 31 and 30 min for ΔT_{max} , the tablet and Quali-V® exhibit the slowest drug release. In contrast HGC showed a very fast and reproducible dissolution within 18 min. Vcaps® plus released the drug slightly slower within 20 min but with a higher variability. The in vivo data revealed that although Vcaps® plus and Quali-V® started to disintegrate at similar times, Vcaps® plus showed a mean ΔT_{max} that was 6 min shorter compared to Quali-V®. One likely explanation for this difference is that the disintegration of the capsule shell must have been different. The highest ΔT_{max} was reported for the tablet, which could be explained by the fact that tablet disintegration is typically based on a more or less continuous erosion, whereas in case of the capsule, the powder was released within a relatively short time and subsequently, presented a large surface available for drug dissolution. However, it must be considered that ΔT_{max} was based on salivary caffeine t_{max} , and, thus, certainly influenced by gastric emptying. It cannot be excluded that the formulation disintegrated fully in the stomach, but the released content was not emptied completely into the small intestine. It is generally possible that larger parts of the drug are retained in the stomach, especially if the coadministered water was already emptied. In this case, t_{max} would depend largely on the occurrence of the phase III activity of the MMC. Hence, we would underestimate the rate of drug release. Nonetheless, this effect was assumed to be similar for all four formulations that were investigated in this controlled cross-over study.

In contrast to human GI physiology, the GastroDuo is a more rigid system that allows a certain level of standardization which is needed for dissolution experiments. This standardization of the test programs has the advantage that the conditions can be fully controlled and changed independently from

each other. This allows the identification of crucial parameters for both disintegration and dissolution of an oral formulation early on. Thus, the GastroDuo should always be regarded as a compromise between a physiologically relevant simulation of the *in vivo* situation and a fully controlled dissolution test system which allows the monitoring of all relevant parameters.

5. Conclusions

In this work, the GastroDuo was applied to study the disintegration and drug release behavior of four different IR formulations in the fasted stomach under physiologically relevant conditions. This novel dissolution tool enabled a realistic simulation of gastric emptying, gastric motility, as well as luminal pH and temperature profiles. With the aid of six test programs, the physiological range of these parameters should be covered. The results of this study revealed that the GastroDuo was able to detect certain differences between the tested IR formulations, which could also be confirmed in an *in vivo* study conducted in healthy volunteers by using the salivary tracer technique. However, compared to *in vivo* data, the *in vitro* variability was larger for certain formulations. This suggests that not all test programs were likely to occur under the controlled and standardized situation of clinical trials. It should be considered that some of them have represented physiological extremes which may occur only in certain patient populations or under certain conditions. However, they allow to study the sensitivity of certain formulations towards individual physiological factors. Therefore, the GastroDuo represents a valuable tool to understand disintegration and dissolution of oral dosage forms.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1999-4923/11/12/659/s1>. The video file “GastroDuo_Video.MOV” can be found in the supplementary materials.

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6.6 In vivo characterization of enTRinsic™ Drug Delivery Technology capsule after intake in fed state: A cross-validation approach using salivary tracer technique in comparison to MRI (*Journal of Controlled Release* 2019, 313, 24 – 32)

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In vivo characterization of enTRinsic™ drug delivery technology capsule after intake in fed state: A cross-validation approach using salivary tracer technique in comparison to MRI



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ABSTRACT

The instability of various small molecules, vaccines and peptides in the human stomach is a complex challenge for oral drug delivery. Recently, a novel gastro-resistant capsule – the enTRinsic™ Drug Delivery Technology capsule – has been developed. In this work, the salivary tracer technique based on caffeine has been applied to study the *in vivo* disintegration of enTRinsic™ capsules in 16 healthy volunteers. In addition, magnetic resonance imaging (MRI) was used to visualize GI transit and to verify the disintegration times determined by using the salivary tracer technique. The enTRinsic™ capsules filled with 50 mg of caffeine and 5 mg of black iron oxide were administered in the fed state, *i.e.* 30 min after a light meal (500 kcal). In the first hour after capsule intake, the subjects were placed in supine position in the MRI scanner and scans were performed in short time intervals. After 1 h, the subjects could leave the MRI scanner in between the MRI measurements, which were performed every 15 min until disintegration of the capsule was confirmed (maximum observation time: 8 h). Saliva samples were obtained simultaneously with MR imaging. Caffeine concentrations in saliva were determined by LC/MS-MS. The starting point of capsule disintegration was determined visually by inspection of the MR images as well as by the onset of salivary caffeine concentrations. In 14 out of 16 subjects, the capsule disintegrated in the small intestine. In one subject, the enTRinsic™ capsule was not emptied from the stomach within the observation time. In another subject, disintegration occurred during gastric emptying in the antronyloric region. In this study, we demonstrated that the enTRinsic™ capsules are also gastro resistant when taken under fed state conditions. Furthermore, we demonstrated the feasibility of using low dose caffeine as a salivary tracer for the determination of the disintegration of an enteric formulation.

1. Introduction

Various small molecule drugs, peptides, nucleic acid-based therapeutics, vaccines or biotherapeutic products are degraded in the acidic environment of the stomach and therefore, should be protected during gastric transit. The gastro-resistance or enteric properties of tablets and capsules is typically obtained by depositing a seal coating of pH-

sensitive polymers on the dosage form by pan coating or fluid bed coating. The selected polymers (*e.g.* methacrylate copolymers, hydroxypropyl methylcellulose acetate succinate and cellulose acetate phthalate) are insoluble in the acidic environment of the stomach and readily dissolve in the small intestine, where neutral pH values are present. Critical quality attributes of such dosage forms are the homogeneity and thickness of the polymer film as they are important for the

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efficacy of the gastro-resistance. Moreover, the development and scale-up of the underlying coating processes is complex and can also induce variations of critical attributes such as the disintegration time [1,2].

The gastric transit time, the availability of luminal fluids as well the pH values in gastric and small intestinal fluids are highly important physiological factors that affect the *in vivo* behavior of gastro-resistant dosage forms. The lag phase before the onset of drug absorption and thus, drug action is affected by the gastric transit time. Thus, the physiological variability in gastric transit times translates directly into a variability of the PK profile [3]. Therefore, it is important to understand how factors such as the properties of the dosage form, the intake conditions or the co-medication affect the transit behavior of oral drug products. Generally, the administration of non-disintegrating oral dosage forms in the fed state leads to longer residence times in the stomach. Due to the small opening diameter of the pylorus in the fed state, larger objects like tablets and capsules are normally not able to leave the stomach as long as food stuff is present in the gastric lumen [4]. Thus, monolithic gastro-resistant formulations can leave the stomach and reach the small intestine typically only after return of the fasted state motility [3].

For the characterization of the GI transit of gastro-resistant dosage forms, different techniques such as scintigraphy, magnetic marker monitoring or magnetic resonance imaging (MRI) can be applied [5,6]. Scintigraphic investigations by Wilding and co-workers have demonstrated that gastric residence time and disintegration time of enteric coated tablets can be highly variable (Fig. 1) [7–9]. In both conditions, fasted and fed state, the time to disintegration after the tablets were emptied from the stomach was in the range of several minutes up to more than 2 h.

This high variability of disintegration times of enteric-coated tablets cannot only be explained by the variability in gastric transit time. The complex physiological environment, to which a dosage form is exposed during GI transit, must also be considered. Recently, we have developed a simple and robust approach to study gastric emptying of fluids – the salivary tracer technique. This technique is based on the determination of caffeine concentrations in the saliva after caffeine is administered in form of an ice capsule or hard gelatin capsule [10,11].

After an enteric coated dosage forms enters the small intestine, the coating has to be dissolved in order to release the drug. Thus, the availability and the composition of luminal fluids in the small intestine are crucial for the *in vivo* behavior of gastro-resistant dosage forms. The distribution of fluids in the small intestine can be studied by MRI. Several MRI studies revealed that the luminal fluid volume in the small intestine can range from 5 to 319 mL. This volume is distributed in a non-homogeneous manner along the small intestine in several fluid pockets [12–14]. Thus, the fluid available in the small intestine is underlying dynamic changes, which further contributes to the variability

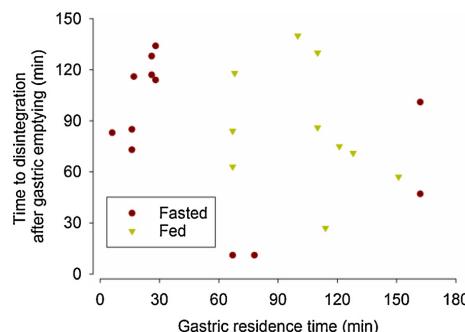


Fig. 1. Time to disintegration after gastric emptying in dependence of gastric residence time for enteric coated tablet. Reproduced from Wilding et al. [7].

in disintegration of gastro-resistant formulations.

Recently, a new capsule with intrinsic gastro-resistant properties called enTRinsic™ Drug Delivery Technology (DDT) capsule was developed using six sigma process. The capsule shell comprises a pH-sensitive polymer with a well-controlled shell thickness that protects the capsule content in acidic pH and rapidly delivers the formulation in the upper intestine when the pH rises. The enteric properties of this capsule were already proven *in vitro* and *in vivo* in fasted conditions [15]. The ability of the capsule to maintain its gastro-resistant properties in fed conditions has not yet been demonstrated and is challenging as fed conditions induce various physiological modifications in the gastrointestinal (GI) tract, most notably a raise of gastric pH that can provoke the opening of capsules in the stomach.

The primary aim of the present work was to determine the *in vivo* disintegration times of enTRinsic™ DDT capsules administered in the fed state by using the salivary tracer technique and MRI. By combining these two methods, we aimed to get further insights into the site of disintegration as well as into the kinetics of drug release after disintegration. The application of these two techniques also allowed to describe the role of gastric emptying for the disintegration of enTRinsic™ DDT capsules.

2. Materials and methods

2.1. Materials

Caffeine was obtained from Sigma Aldrich (Steinheim, Germany). Black iron oxide (E172) and Aerosil® were purchased from Caesar & Loretz GmbH (Hilden, Germany). Mannitol was obtained from Fagron GmbH & Co. KG (Barsbüttel, Germany). All excipients were supplied in pharma grade. The enTRinsic™ Drug Delivery Technology capsules (size 0) were provided by Lonza (Illkirch-Graffenstaden, France). The components of the meal white toast (Sammy's Super Sandwich, Harry Brot GmbH, Schenefeld, Germany), strawberry jam, strawberry yoghurt, orange juice (all manufactured for EDEKA, Germany, Brand: "Gut & Günstig") were purchased in a local supermarket.

All solvents used for LC-MS, *i.e.* water, methanol, formic acid and acetonitrile were purchased in LC-MS grade from VWR international (Fontenay-sous-Bois, France). Ammonium acetate was obtained from Merck KGaA GmbH (Darmstadt, Germany).

2.2. Capsule preparation

The enTRinsic™ DDT capsules are two-piece enteric capsules equivalent in appearance, shape and dimensions to regular two-piece hard gelatin capsule size 0. The capsule shells are made of cellulose acetate phthalate – a pharmaceutically accepted enteric cellulosic derivative polymer, which has intrinsic enteric properties. They are produced by a pin dipping process using conventional and industrial scale capsules manufacturing machines. Thus, the release profile of the capsules is fully compliant with USP/EP specifications for both disintegration test (*i.e.* no evidence of early disintegration, rupture or content release after 2 h in acid stage) and dissolution test (*i.e.* less than 10% of drug released after 2 h in acid stage and more than 90% of drug released after 30 min in buffer stage) for gastro-resistant dosage forms [16].

In this study, these size 0 capsules were filled with a powder mixture of 20% (w/w) caffeine, 2% (w/w) black iron oxide, 77% (w/w) mannitol and 1% (w/w) Aerosil® by using a capsule filling board. The powder mixture was prepared by mortar and pestle. The target content of each capsule was 50 mg of caffeine and 5 mg of black iron oxide.

2.3. In vivo study

2.3.1. Ethics

The study was conducted in compliance with the "Declaration of

Table 1
Inclusion criteria of the study population.

age	18 – 65 years
sex	male and female
ethnic origin	Caucasian
body mass index	$\geq 18.0 \text{ kg/m}^2$ and $< 30 \text{ kg/m}^2$
good health as evidenced by the results of the clinical examination, which are judged by the clinical investigator not to differ in a clinically relevant way from the normal state	
written informed consent	

Helsinki” (2013, Fortaleza, Brazil) and the “(Model) Professional Code for Physicians in Germany” (amended 2015 in Frankfurt, Germany). The study protocol was approved by the local ethics committee of the University Medicine Greifswald (registration number: BB 099/17). An insurance was taken out to cover commuting accidents and risks arising from the participation in the study for every participant. Written informed consent was obtained from every participant.

2.3.2. Subjects

The inclusion criteria of the presented study are given in Table 1. The most relevant exclusion criteria were presented below. For a full overview of all exclusion criteria, please refer to the supplementary materials.

- claustrophobia
- cardiac pacemakers, metallic implants (excluding dental retainers) or large tattoos
- known allergic reactions or food intolerances to components of the study meals
- known allergic reactions to caffeine and/or theobromine
- gastrointestinal diseases and/or pathological findings, which might interfere with gastrointestinal motility and emptying processes
- lactation and pregnancy test positive (urine test)
- participation in a clinical trial according to AMG during the last 1 month prior to the start of the study

In this study, 16 healthy volunteers (V01-V16) of both genders were included. A summary of the subject characteristics is given in Table 2. The sample size was based on a calculation assuming to use the Wilcoxon signed rank test to compare the observed disintegration times. The following parameters were used for the calculation, alpha error of 0.05, a power of 0.8 and a large effect size ($\Delta = 0.8$) [17].

2.3.3. Study protocol

In this study, every subject underwent the same treatment within only one study day. After an overnight fast of at least 10 h, the subjects were admitted to the Radiology department of the University Medicine Greifswald. Upon arrival, an initial MRI scan was performed to guarantee fasted state conditions in each subject. Subsequently, the subjects had to consume a light breakfast (approx. 500 kcal) consisting of 200 mL orange juice, two slices of white toast with 5 g butter and strawberry marmalade as well as 200 mL of strawberry yoghurt. Thirty minutes after beginning of meal intake, one enTRinsic™DDT capsule was administered in an upright position together with 240 mL of tap water (room temperature). The time point of capsule intake was defined

as $t = 0 \text{ min}$.

Immediately after intake of enTRinsic™DDT capsule, the subjects were placed in the MRI scanner in supine position. They had to stay inside the MRI scanner for the first 60 min. During this time, saliva samples were collected while lying in the MRI. Afterwards, the measurements had an interval of 15 min. In between two measurements, the volunteers were taken out of the MRI scanner. MRI measurements were continued in a 15 min-interval until capsule disintegration was confirmed visually with aid of the MR images.

The specific imaging and sampling scheme can be summarized as follows:

- *before capsule administration*: one MRI measurement was performed before intake of the meal ($t = -35 \text{ min}$) and two measurements were done after meal intake, but before capsule administration ($t = -10 \text{ min}$ and $t = -5 \text{ min}$)
- *after capsule administration*:
 - 0–20 min: measurement interval of 2 min
 - 20–40 min: measurement interval of 5 min
 - 40–60 min: measurement interval of 10 min
 - after 60 min: measurement interval of 15 min until capsule disintegration was completed as confirmed visually by the MRI or end of imaging time (420 min) was reached

The saliva samples were collected always 1 min after each MRI measurement. Saliva was also collected directly before and after the meal to confirm the abstinence from caffeine and to exclude accidental caffeine contamination arising from meal intake. Volunteers had to abstain from caffeine for 72 h before begin of the study.

2.3.4. Magnetic resonance imaging

All investigations were performed with a 1.5 T MAGNETOM Aera from Siemens Healthcare (Erlangen, Germany), located at the Institute of Diagnostic Radiology and Neuroradiology of the University Medicine Greifswald. All measurements were performed in supine position (subject lying on the back, head forward) by using a six-element phase array abdominal receiver coil and four spine coils inside the MRI desk, which were used for signal detection. Only coronary image sets were obtained, but reconstruction of image sets in other orientation was possible as well. One subject was investigated per study day.

For the investigation of the transit and disintegration behavior of the capsules as well as for the anatomical assignment and the evaluation of gastric volumes, a T1/T2-weighted TRUFI (*True Fast Imaging with Steady Precession*) sequence was used. The sequences were particularly sensitive to susceptibility artefacts of iron oxide and had a repetition time (TR) of 3.55 ms, an echo time (TE) of 1.48 ms, a slice thickness of 5 mm, an interslice gap of 0.75 mm, a flip angle of 65°, a matrix of 256 × 243 and a resulting voxel size of 0.04 mL. In order to reduce motion artefacts, subjects were asked to hold their breath for 23 s for each image set.

2.4. Image analysis

Imaging data were analyzed using the Software OsiriX Lite v.7.0 32-bit (Pixmeo SARL, Switzerland), which was updated stepwise during this study up to version v9.5. The regions of interest (ROI) for gastric content volumes (GCV) were tracked manually in each slice by marking the contours of gastric content. Air was not included into these calculations. The volumes were subsequently calculated using an integrated software tool under consideration of slice thickness and interslice gap. Due to the different anatomy of the subjects, tracking and assignment to the gastrointestinal compartments were performed manually. The classification was performed in T1/T2-weighted TRUFI sequences with respect to geometrical orientation, bowel diameter, roughness of intestinal wall, filling volumes and transit times (Fig. 2). All assignments were done by two different and independent investigators, which were

Table 2
Demographic data of the study volunteers. Age and BMI given as mean \pm standard deviation.

Age / years	24.9 \pm 2.2
Body Mass Index / kg/m^2	24.0 \pm 1.9
Female / Male	9 / 7
Ethnics	Caucasian
Pre-existing conditions	None
Number of Volunteers	16

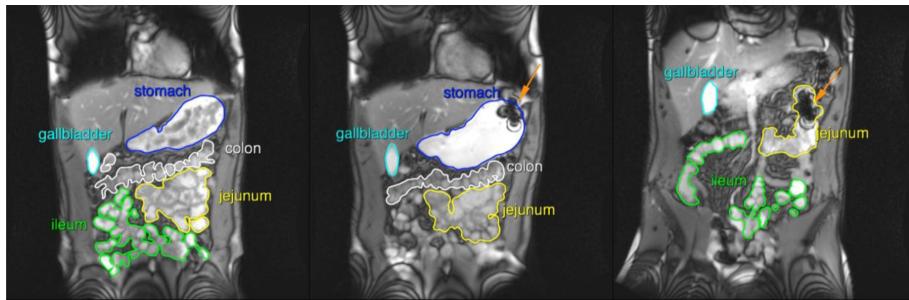


Fig. 2. Exemplary abdominal MR images of one subject obtained by using T1/T2-weighted TRUFI sequence. The presented images were taken before (left), 2 min after (middle) and 195 min after capsule intake (right). The capsule could easily be detected by the artefacts caused by iron oxide (orange arrows). The different parts of the gastrointestinal tract are also highlighted in this figure.

both well-trained in MR image analysis. In case of differences, these cases were analyzed by a third independent person.

In most cases, the capsules could be tracked on-site during the investigations. In case of uncertain detection during the image acquisition at the study site, measurements were repeated immediately within 1 min to secure reliable tracking.

2.5. Preparation and analysis of saliva samples

Saliva sampling and probe preparation was based mainly on the procedure described in a recent publication [10]. Determination of caffeine in the saliva samples was performed by use of an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a triple quadrupole mass spectrometer API4000 QTRAP (AB Sciex, Darmstadt, Germany) via the electrospray ionization source Turbo V™. The LC-MS/MS system was controlled by the validated Analyst 1.6 software (AB Sciex, Darmstadt, Germany).

Caffeine was separated from hydrophilic saliva components such as mucines and other glycoproteins by isocratic elution. The mobile phase consisted of a 50/50-mixture of ammonium acetate buffer (5 mM; pH 3.8) and methanol. The flow rate was set to 250 µL/min. The reversed-phase column XTerra®MS (C18, 3.5 µm, 2.1 × 100 mm; Waters, Dublin, Ireland) was tempered at 40 °C. The injection volume was 20 µL.

The chromatographic flow was directed to a 0.5 µm filter device (PEEK, Supelco, Taufkirchen, Germany) to avoid particulate contamination. The HPLC flow was connected to the mass spectrometer interface (Turbo V™ ionization source) operated in the positive ion mode. The following gas parameters have been used: temperature, 550 °C; gas 1, 60 psi; gas 2, 60 psi (all nitrogen); voltage, 4000 V; collision-activated dissociation (CAD), 12 (arbitrary unit). The Analyst 1.6 software was applied to evaluate the chromatograms using the internal standard method and peak-area ratios for calculation (quadratic regression, 1/x weighting).

The analytical method was validated with respect to linearity, precision (within-day and between-day), accuracy (within-day and between-day), selectivity and rack stability regarding the FDA Guidance for Industry “Bioanalytical Method Validation” (Issue: May 2001). All validated criteria met the requirements of the FDA guideline. Lower limit of quantification (LLOQ) was 5 ng/mL determined in the matrix.

2.6. Interpretation of capsule disintegration

Based on the MRI images, the capsule disintegration was defined as any alteration of the artefact caused by iron oxide. This could have been growth, separation or disappearance of the artefact, as well as the occurrence of additional artefacts, which could have only been caused by

a leakage in the capsule shell. Thus, if disintegration took place in-between to measurements, the later one will be identified as the timepoint of disintegration.

The determination of capsule disintegration based on the salivary tracer technique was based on reaching a salivary caffeine concentration which exceeded the value of triple LLOQ (15 ng/mL). Additionally, this value had to be supported by the concentration of the next measurement, which should have been higher or not smaller than 5% of the prior concentration.

2.7. Statistics

The disintegration times determined by MRI and STT were compared based on linear regression. For both regression curves, slope, intercept and coefficient of determination (R^2) were calculated. Furthermore, the disintegration times of the two methods were analyzed with respect to significant/not significant differences by the Wilcoxon signed-rank test. In this test, the null hypothesis assumed the absence of significant differences. This non-parametric statistical hypothesis test was used because the disintegration times of the two methods were not distributed normally based on the Shapiro-Wilk test for normality (p -values < 0.05).

3. Results

3.1. Gastric emptying

Fig. 3 illustrates the gastric content volumes (GCV) over time as determined by MRI. The residual GCV was between 0 and 75 mL. It can be seen that the consumption of the light meal led to a GCV of 500–600 mL ($t = -10$ min). The subsequent intake of water caused an increase to maximum GCV values of 700–800 mL. Interestingly, some variability between the subjects could be observed for the initial period of gastric emptying. Whereas in subject V03, the GCV decreased by more than 210 mL within the first 10 min, in subject V07 the decrease was only 70 mL in the same interval.

3.2. Capsule tracking and disintegration

3.2.1. Capsule tracking and disintegration: Magnetic resonance imaging

The capsules were tracked individually in each volunteer until complete disintegration was confirmed by MRI. Since MRI allows the identification of the localization of the capsules marked with iron oxide, specific transit times such as the gastric emptying time (GET) could be obtained. In most cases the capsule was not emptied until the chyme was emptied from stomach. Accordingly, the mean GET amounted to 210 ± 84 min. In three subjects (V02, V08, V09), the capsule was

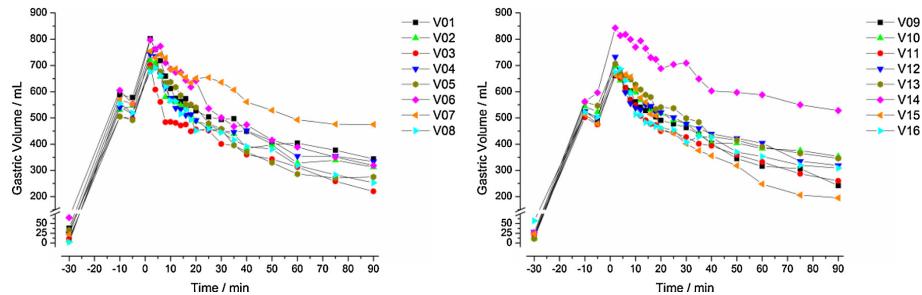


Fig. 3. Individual gastric content volume kinetics determined by MRI over 90 min for all 16 subjects. At $t = 0$ min the enTRinsic™ DDT capsule filled with 50 mg caffeine was administered together with 240 mL tap water. At $t = -30$ min the subjects consumed a light meal (500 kcal) within less than 20 min.

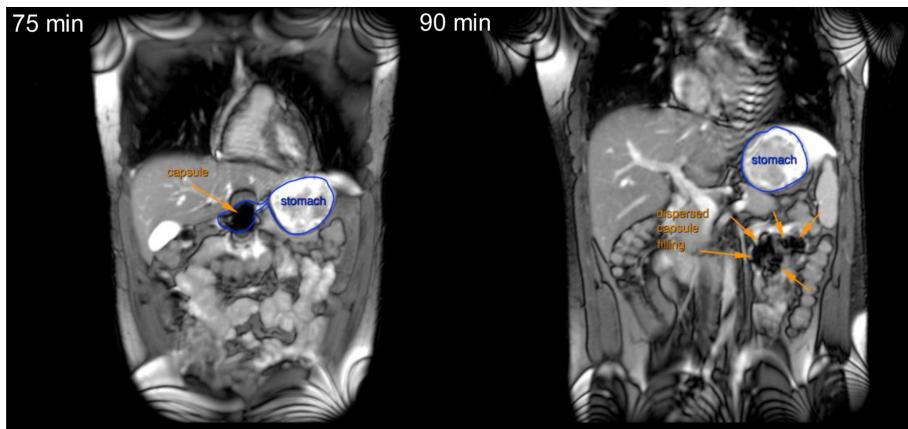


Fig. 4. MRI pictures of volunteer V09 75 min (left) and 90 min (right) after administration of the enTRinsic™ DDT containing 50 mg of caffeine and 5 mg of black iron oxide. The blue region of interest indicates the stomach, whereas the orange arrows show the susceptibility artefact caused by the iron oxide. Whereas after 75 min, only one large artefact is visible in the stomach, after 90 min several artefacts can be seen in the small intestine, which reveals that capsule has disintegrated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

emptied earlier, which was typically followed by a rapid disintegration. In subject 9 (V09), the capsule disintegrated during or immediately after gastric emptying. This can be concluded since in the last image sequence where the capsule was detectable the capsule was inside the stomach without any signs of rupture, in the next image sequence 15 min later the magnetic label was completely dispersed inside the small intestine (Fig. 4).

In these three cases of early capsule emptying from the stomach the capsules were deposited in the antropyloric area immediately after intake which facilitates mechanical stress and likeliness of emptying. One capsule (subject V14) was not emptied from the stomach within the maximal imaging time of 420 min. Therefore, disintegration was not observed in MRI for V14. The duodenal transit was very fast in most subjects and sometimes not even detectable within the imaging interval. The transit details are given in Fig. 5.

3.2.2. Capsule disintegration: salivary caffeine kinetics

Based on the salivary caffeine kinetics displayed in Fig. 6, capsule disintegration could be determined with the salivary tracer method. The time of disintegration could be easily identified by a steep increase in salivary caffeine concentration within 15 min. Only for three subjects (V01, V13 and V14), the increase in salivary caffeine concentration was slow. As mentioned before, it should be noted that for subject V14, no disintegration was observed by MRI within the 420 min imaging time.

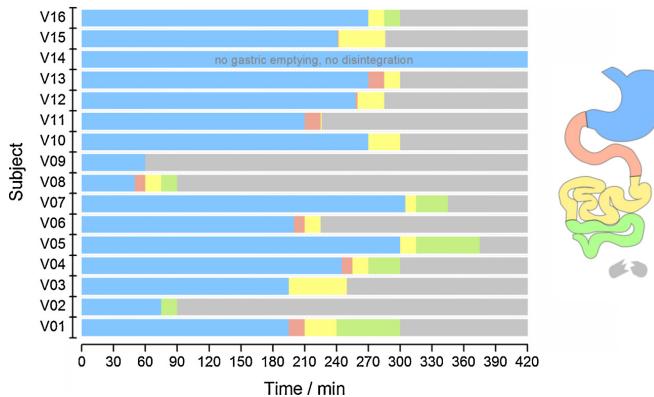
3.2.3. Capsule disintegration: cross-validation

A point-by-point comparison of the disintegration results for each individual obtained by the MRI and Saliva method is shown in Fig. 7. The mean disintegration time determined from saliva samples was 243 ± 96 min, while MRI yielded a mean disintegration time of 248 ± 96 min. The mean difference between the disintegration times observed by saliva and MRI was 26 ± 36 min with the highest difference of 134 min for subject V01. In 6 out of 16 volunteers the difference in the disintegration times was only 1 min. The comparison of the disintegration times by the Wilcoxon signed-rank test showed no significant differences ($p = 0.60$). Additionally, the correlation of disintegration times STT over MRI, resulted in a R^2 of 0.8132 (Fig. 8). The individual disintegration times can be found in the supplementary materials.

4. Discussion

In the present study, we demonstrated the feasibility of using caffeine as a salivary tracer technique for the determination of the disintegration of a gastro-resistant enteric formulation. The technique was used as an independent method to validate the disintegration time determined by using MRI.

Black iron oxide is a food additive with GRAS (generally recognized as safe) status. It is not absorbed from the human GI tract. It generates



even in low amounts characteristic susceptibility artefacts in MRI. Therefore, black iron oxide is a safe and reliable labeling substance for the MRI based determination of the gastrointestinal transit behavior of dosage forms [18–20]. It can also be used for the visualization of the disintegration or even drug release behavior of dosage forms. However, in this application its use is hampered by the fact that not every disintegration or drug release will immediately result in a clearly detectable change in the artifact caused by the magnetic label. Therefore, in case of the investigated capsules small ruptures or leakages in the capsule shell do not necessarily have to be visible.

Therefore, we used a cross-validation approach in this work as the results of one non-validated method were verified through the results of another non-validated method. From our point of view, the cross-validation of MRI method and the salivary tracer technique for the evaluation of disintegration of enTRinsic™ DDT capsules was successful, as both methods led to comparable results. With a R^2 of 0.81 and a mean difference of 26 min between both methods for the determination of the disintegration time of the capsules, it can be assumed that both methods are closely reflecting the *in vivo* capsule disintegration. Furthermore, these differences were not statistically significant. However, it must be noted that the sampling offset between the two methods was at least 1 min and that the sampling interval was 15 min in both methods at the time when capsule disintegration occurred. With the exception of two capsules, all capsules were located inside the small intestine at the time of disintegration. Owing to the fast dissolution and absorption of caffeine in the small intestine and the subsequent fast distribution into saliva, caffeine appears in saliva immediately after capsule

Fig. 5. Gastrointestinal transit through the different compartments (stomach: blue, duodenum: red, jejunum: yellow, ileum: green, disintegrated: grey) until disintegration for the enTRinsic™ DDT capsules administered in fed state. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

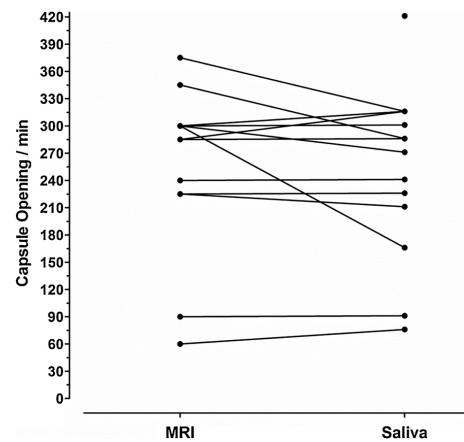


Fig. 7. Comparison of *in vivo* disintegration times (before-after plot) of the enTRinsic™ DDT capsules filled with 50 mg caffeine determined by two different methods. Administration of the capsule together with 240 mL tap water was performed 30 min after intake of a light meal (500 kcal). In one case (subject V14) no disintegration was observed with the MRI method until the last imaging performed at 420 min.

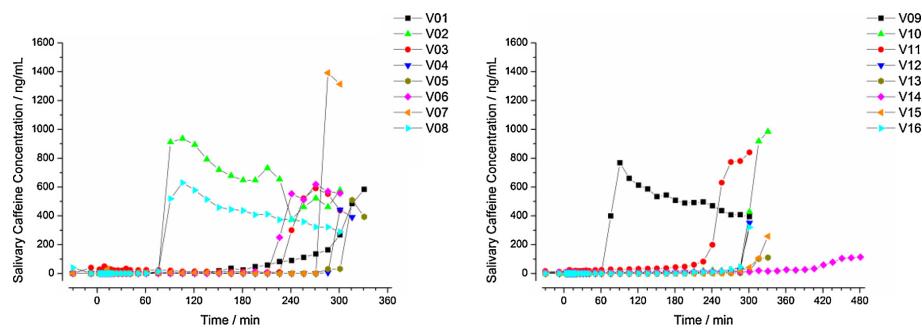


Fig. 6. Individual salivary caffeine kinetics over time for 16 subjects. Timepoint t = 0 min represents the timepoint at which the enTRinsic™ DDT capsule filled with 50 mg caffeine was administered together with 240 mL tap water. Thirty minutes prior to capsule administration, all subjects consumed a light meal (500 kcal) within maximum 20 min. Saliva samples were collected for at least 300 min or until disintegration was observed with the help of MRI.

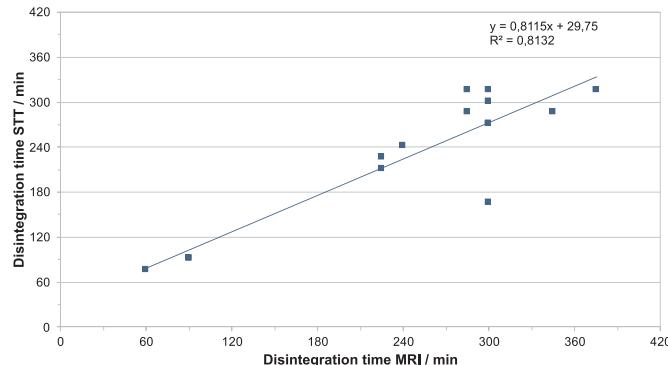


Fig. 8. Correlation analysis of disintegration times observed with the salivary tracer technique (STT) of disintegration times determined with magnet resonance imaging (MRI) based on a linear regression.

disintegration [21]. This assumption was supported by the fact that in most cases, the caffeine appearance in saliva was earlier than the suspected time point of capsule disintegration by using MRI. The mean disintegration time was 243 min for the salivary tracer technique and 248 min for MRI. Depending on the nature of disintegration, it may be difficult to determine capsule disintegration by using MRI only. In the case of a small rupture or leakage in the capsule shell, it may be possible that the iron oxide remains in the capsule or only small amounts of the iron oxide are able to leave the capsule. Thus, the artifacts necessary for confirmation of capsule disintegration are lacking.

The combination of the two presented *in vivo* methods has clear advantages for the *in vivo* evaluation of dosage form behavior in the human GI tract. The unique advantage of MRI is the visualization of the anatomy, and therefore, the possibility to locate objects in the human body (Fig. 2). The salivary tracer technique provides sensitive information on capsule disintegration. If the capsule disintegrates rapidly and with several ruptures in the shell, a fast onset and high salivary caffeine concentration will result. In contrast, if the capsule shell disintegrates slowly or incomplete, the onset of caffeine in saliva will be slow and the maximum concentrations will be lower. Such a slow disintegration occurred in subjects V01 and V14. Salivary caffeine kinetics can also be influenced by the individual fluid volumes in the small intestine as caffeine has to be dissolved before absorption. Previous studies have demonstrated that the volume in the small intestine after intake of a meal is around 54 ± 41 mL and relatively static [12]. Based on the aqueous solubility of 15 mg/mL, it can be assumed that caffeine was dissolved quickly and completely in the intestinal fluids. However, it is known that the fluids in the small intestine are distributed non-homogeneously in various smaller fluid pockets, which can potentially influence disintegration and dissolution [12,14,22]. However, the artifact caused by the magnetic label prevented the visualization of the direct environment of the capsules. Therefore, we could not clarify whether the capsules were embedded in an intestinal water pocket during disintegration or not.

In comparison to conventional enteric coated tablets, the enTRinsic™ DDT capsules provided fast *in vivo* disintegration times in fed conditions with comparable individual variability (Fig. 9) [7]. After gastric emptying, disintegration started on average after 35 min for the capsules. For gastro-resistant products, a fast and robust disintegration after gastric emptying is generally desired to favor a fast onset of drug plasma concentrations. Nonetheless, it must be noted, that further studies with larger and more heterogeneous populations are desired to investigate the *in vivo* disintegration of the enTRinsic™ DDT capsules in more depth. Nevertheless, the present study provided a robust investigation of the *in vivo* capsule behavior in healthy subjects with a

significant correlation between the two presented methods.

Due to the fact that in this study, enteric capsules were used, we were particularly interested in the kinetics of gastric emptying. It was shown in the past that large, non-disintegrating or slow disintegrating dosage forms are retained in the fed stomach, as gastric emptying of large solids like the capsules require strong peristaltic waves of the Migrating Motor Complex (MMC) [4,23–25]. In the first hour, the kinetics of the gastric content volume was dominated by the gastric emptying kinetics of the co-administered 240 mL of water. As it had already been demonstrated in previous studies, water ingested after meal intake emptied quickly from the fed stomach, which once more confirms the presence of a “Magenstraße” (stomach road) [14,26,27]. As described recently for various meals including the FDA standard breakfast and a light meal, the ingested water was able to bypass the stomach and to flow around the contents present in the stomach. Thus, the gastric emptying of the water is as fast as in the fasted state [28]. The GCV showed a decrease of around 200 mL within 10–50 min, which was a strong indicator for the presence “Magenstraße” in this study. After this initial period of fluid emptying, the gastric content volume decreased with a rate of 2.4 ± 0.6 mL/min. Based on the product specifications of the administered food, this corresponds to 3.0 ± 0.8 kcal/min. This value is well in the range of 2 to 4 kcal/min, which is frequently reported in literature [19,29]. Furthermore, the time to reach the level of the GCV that was measured prior to food and capsule intake, was calculated for every subject based on a linear regression model. On average, this time was 237 ± 128 min. This value was calculated to assess whether the enTRinsic™ DDT capsules was emptied by fasted or fed state motility. Although the gastric emptying time of the capsule was slightly shorter than the time needed for complete gastric emptying (210 ± 84 min vs. 237 ± 128 min), it can be assumed that gastric emptying of the capsules was mostly accomplished by fasted state motility. Cassilly and co-workers have demonstrated in a recent study that the MMC reappears if around 90% of a meal are emptied. These observations are in line with observations in this study.

5. Conclusion

In conclusion, the time point of disintegration of the enTRinsic™ DDT capsules was successfully evaluated *in vivo* with the aid of MRI and the salivary tracer technique. In 14 out of 16 subjects, the capsule disintegrated in the small intestine proving its ability to protect the formulation in the stomach even in fed conditions. Both methods used in this study came to similar results, although the salivary tracer technique tended to be more sensitive. However, a combination of both

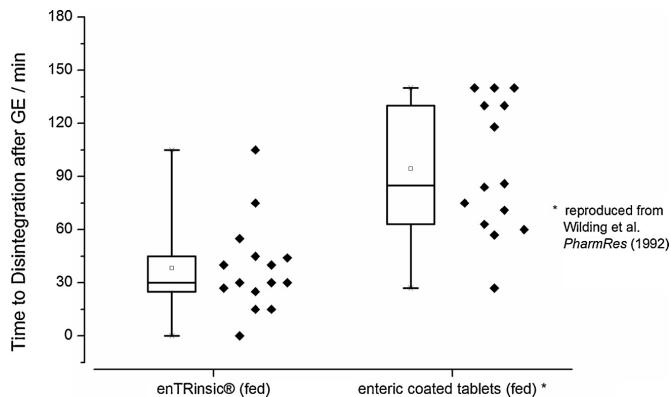


Fig. 9. Comparison of *in vivo* disintegration times for enteric coated tablets ($n = 14$) and enTRinsic™ DDT capsules ($n = 15$, observed by MRI) after gastric emptying. Single values and box-plot whisker graphs are given. Reproduced from Wilding et al. [7].

methods can be even more valuable in terms of providing further information. In principal, both methods can be used to determine the time point of disintegration, but MRI can also provide information about the localization and transit behavior of oral dosage forms as well as the availability of fluids. In contrast, the salivary tracer technique does not allow the localization of a dosage form, but it allows describing the dissolution kinetics of the incorporated drug. Future work will therefore focus on the combination of both methods to achieve deeper insights into the fate of oral dosage forms in the human GI tract.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jconrel.2019.10.023>.

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7 Veröffentlichungen

7.1 Publikationen

Koziolek, M., Grimm, M., Schneider, F., Jedamzik, P., Sager, M., Kühn, J.-P., Siegmund, W., Weitschies, W. *Navigating the human gastrointestinal tract for oral drug delivery: Uncharted waters and new frontiers.* Adv. Drug Deliv. Rev. **2016**, 101, 75–88.

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Sager, M.; Jedamzik, P.; Merdivan, S.; Grimm, M.; Schneider, F.; Kromrey, M.-L.; Hasan, M.; Oswald, S.; Kühn, J.; Koziolek, M.; Weitschies, W. *Low Dose Caffeine as a Salivary Tracer for the Determination of Gastric Water Emptying in Fed and Fasted State: A MRI Validation Study.* Eur. J. Pharm. Biopharm. **2018**, 127, 443–452.

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7.2 Vorträge

- ◊ “UNGAP Workshop: *In Vivo Tools*”, University of Greifswald, Greifswald, Germany **2019**
- ◊ “Transporttage”, Greifswald, Germany **2018**
- ◊ “11th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology”, PBP World Meeting, Granada, Spain **2018**
- ◊ “Northern Pharma Network Meeting”, University of Southern Denmark, Odense, Denmark **2018**

7.3 Posterbeiträge

- ◊ “11th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology”, PBP World Meeting, Granada, Spain **2018**
- ◊ “OrBiTo F2F Meeting“, Cambridge, United Kingdom, **2017**
- ◊ “DPhG Jahrestagung“, Saarbrücken, Germany, **2017**
- ◊ “OrBiTo ‘F2F Meeting‘”, Helsinki, Finland, **2017**
- ◊ „AAPS Annual Meeting“, Denver, USA **2016**
- ◊ “10th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology”, PBP World Meeting, Glasgow, United Kingdom **2016**

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