Advances in drug release investigations: Trends and developments for dissolution test media

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Received: Received: 2 July 2020 / Accepted: 17 July 2020

Abstract: Dissolution research started more than a century ago in the field of physical chemistry and went through several significant developments since. Yet, the explicit attention in drug-related dissolution has only started growing in the 1950s, after the researchers realized that drug release from orally administered solid pharmaceutical formulations significantly influences the bioavailability. Researches show that solubility and permeability of the drugs are key factors in the correlation between the *in vitro* examination and the *in vivo* determinations (IVIVC). This review aims to summarize the most relevant developments in chronological order, ranging from simple disintegration studies to biorelevant methods. Biorelevant methods can be used to recognize the effects of food on dissolution, as well as to identify solubility limitations and stability issues. The development of a biologically relevant dissolution method for oral dosage forms needs to take the physiological conditions of the gastrointestinal (GI) tract into account that may influence the drug dissolution. This review presents the simplest dissolution media, the composition of biorelevant media simulating gastrointestinal fluids, and the latest updates of the field.

Keywords: drug dissolution, bioavailability, biorelevant methods, biorelevant media, gastrointestinal (GI) tract

Introduction

Dissolution tests in the pharmaceutical industry are not only used for the prediction of the expected bioavailability but also for quality control purposes. In product development, dissolution tests are used to select the labeled preparation, while in research to determine the influence of the critical manufacturing parameters (for example binder effect, mixing effect, granulation process, excipients) [1]. Predicting the *in vivo* bioavailability of the drug is extremely important to provide the best modeling of the digestive system. Dissolution methods should simulate the biological system in which the tablet passes through media of varying pH, ionic strength and bile acid content until it reaches the site of dissolution and absorption. Laboratory imaging of these conditions is particularly important in case of formulations where the dissolution of the active pharmaceutical ingredient (API) in the stomach is not advantageous and therefore provided with a gastroprotective coating. The digestive tract consists of three important parts: stomach, small intestine and large intestine [2]. Its diverse conditions have a major influence on the *in vivo* dissolution profile of the API [3]. The digestive system excretes various substances (e.g. water, enzymes, surfactants, hydrochloric acid) that amongst others deter-

mine the pH, buffer capacity and molarity [4]. All of these factors influence the dissolution and absorption of the API [5]. The stomach and the small intestine play vital role in the dissolution process, while primarily the latter is crucial for the absorption in most cases. Absorption can also happen in the large intestine, but its role therein is generally less important than the small intestines.

The Biopharmaceutical Classification System (BCS)

In terms of bioavailability, active pharmaceutical ingredients can be described with two main physicochemical properties: solubility and permeability. Experiments demonstrate that these are key factors in the correlation of *in vitro* drug dissolution and *in vivo* bioavailability [6,7]. According to BCS, the drugs can be categorized into four basic groups based on their solubility and permeability (Figure 1) [6].

An active ingredient is considered to be highly soluble when its highest dose is soluble in max. 250 mL aqueous medium at pH 1.0-7.5, otherwise the active ingredient is poorly soluble [8]. The 250 mL volume estimate is derived from the typical bioequivalence testing protocols that require the patients to take the drug with a glass of water [9].

Figure 1 The Biopharmaceutical Classification System.

The European Medicines Agency has defined the pH to be in the range of 1.2 and 6.8 for solubility testing. The pH of the medium must be measured before and after the addition of the active ingredient [10]. In the case of poorly water-soluble active ingredients, the solubility may be enhanced by the use of solubilizers. Furthermore, according to recent studies, the addition of formulation auxiliaries may also alter the permeability of the active ingredient [11].

During the early stages of developing drug candidates, it is possible to change the physicochemical properties of a molecule by modifying its chemical structure. The addition of polar substituents [12] or the formation of salts [13] can improve poor water solubility, while lipophilicity can be changed by e.g. the addition of fluorine substituents. In many cases, it is not possible to alter the chemical structure of a drug candidate because it may also alter its therapeutic effect, metabolism, toxicity or synthesis. Therefore, specific formulation methods might provide a better solution to overcome poor water solubility.

The classification of permeability is based directly on the Human Intestinal Absorption of the API and is considered to be good above 85% [7]. A drug substance is considered highly permeable if the extent of intestinal absorption is 90% or higher. Otherwise, the drug substance is considered poorly permeable [7,14,15]. Based on the BCS, a drug is classified as rapidly dissolving when at least 85% of the labeled amount of the active ingredient is dissolved in 30 min in USP (United States Pharmacopeia) Apparatus I at 100 rpm or in USP Apparatus II at 50 rpm in maximum 900 mL of each of the following media: a.) acidic media, such as 0.1 N HCl or USP simulated gastric fluid (SGF) without enzymes, b.) a pH 4.5 buffer, and

c.) a pH 6.8 buffer or USP simulated intestinal fluid (SIF) without enzymes. Otherwise, the drug product is considered to be a slowly dissolvable product [14] [16].

According to literature, an increasing proportion of the new drug molecules, estimated to be about 70%, are poorly water-soluble BCS Class II drugs, which sets a major challenge for both pharmaceutical researchers and developers. At least 30% of the drugs currently on the market also fall into this category [17].

The evolution of dissolution tests

Dissolution tests are one of the most essential techniques in the pharmaceutical industry. Not only used for development and quality control purposes, but the in vitro drug release measurements have always aimed to forecast the in vivo performance of pharmaceutical products [1]. From the simple disintegration assays the technique has greatly evolved, as it also enables the conduction of dissolution studies of immediate release (IR) products for quality control purposes [18].

The first dissolution studies were reported by Noyes and Whitney in 1897, who studied the dissolution of benzoic acid and lead chloride, two compounds well known for their poor solubility. The materials were placed into glass cylinders that were submerged into water tanks. The cylinders were rotated with constant speed at constant temperature. The authors noticed proportional correlation between the rate of dissolution and the difference between the instantaneous concentration C at time t . The saturation solubility C_s and k are constant (Eq. 1) [18,19]:

$$\frac{dC}{dt} = k \cdot (C_s - C) \quad (1)$$

The Nernst-Brunner equation, published in 1904, is based on the diffusion layer concept and Fick's second law (Eq. 2).

$$\frac{dC}{dt} = \frac{DS}{Vh} \cdot (C_s - C) \quad (2)$$

where $k = D/(Vh)$, D is the diffusion coefficient, h is the thickness of the diffusion layer and V is the volume of the dissolution medium.

Later more alternative explanations were avail-

able. Danckwerts' classic surface renewal model was presented in 1951. It gives a quantitative description of gas absorption at the surface of a turbulent liquid. According to this model, the gas-liquid interface, where the absorption occurs, is continuously rejuvenated by fresh liquid elements arriving from the liquid bulk [20]. In 1961, Higuchi reviewed the interfacial barrier model and due to the high activation energy level of the interfacial transport, he considered it as the limiting step, rather than diffusion through the film [21].

In 1934, there was a disintegration test for tablets that used water as medium at 37 °C and periodical shaking. It was the first test that was published in *Pharmacopeia Helvetica* [22]. Several other disintegration methods were developed (using tubes [23], meshes [24], etc.) that tried to use rather biorelevant conditions, like simulated gastric fluid as disintegration media [25]. In 1948, Filborn introduced an artificial stomach model that simulated the *in vivo* conditions, including the pH, peristalsis, fed state, volume of the gastric juice, and the hydrostatic pressure [26]. Notwithstanding the advances in *in vitro* dissolution, the tests were not used widely until the early 1950s.

Although a lot of experiments on physicochemical dissolution were carried out, the first official dissolution tests were accepted by the British Pharmacopoeia in 1945, and later, in 1950 by the USP [27]. It was only in the early 1950s that scientists began to recognize that the importance of dissolution – absorption rates for oral drugs [22]. Assuming that the drug is rapidly absorbed from the gastrointestinal tract, dissolution may be the rate-limiting step that controls its appearance in the bloodstream. This finding was first published by Edwards in 1951 [28]. He assumed that if aspirin tablets were dissolved in the stomach, the intestine would be the factor influencing the absorption into the bloodstream [22].

However, in 1957 Nelson was the first to explicitly link the *in vitro* dissolution rate with the blood levels of orally administered theophylline salts [29]. Only in the mid 1960s was the impact of dissolution on the therapeutic effect of orally administered drugs recognized. Two reports were published in 1963 [30] and 1964 [31] that drew attention to the lack of clinical efficacy of the two brands of tolbutamide marketed in Canada. The tablets showed much slower disintegration and release compared to other brands [32]. Such observations have also been confirmed with other tablets, like chloramphenicol and diphenylhydantoin

[5]. In 1968 Martin et al. reported significant differences in the bioavailability of diphenylhydantoin, chloramphenicol, and sulfisoxazole-beading products of different brands [33].

The most serious bioavailability problems were reported in the United Kingdom and the United States (1971), and in Australia and New Zealand with phenytoin (1968). In the first case, a sevenfold difference was found in digoxin serum levels between the different formulations. This observation prompted the Food and Drug Administration (FDA or USFDA) to investigate the dissolution of 44 digoxin products of 32 manufacturers. Large differences were detected in the dissolution profiles in the *in vitro* assays [19,34]. In the latter case, the phenytoin containing IR product, increased toxicity was observed when lactose was used as excipient instead of calcium sulfate [35]. The lower phenytoin absorption in the original case was attributed to precipitation of insoluble calcium phenytoin salt [36]. However, in 1979 Chapron et al. found no calcium-induced effect on the bioavailability of phenytoin when tested with calcium gluconate. These results indicate that the presence of lactose lead to the undesired increase in plasma concentrations that reached beyond the narrow therapeutic range (10-20 µg/mL). He considered that the highly hydrophile lactose increased the dissolution rate of phenytoin and thereby the bioavailability and consequently higher plasma concentrations [37].

This period pointed out the critical relationship between solubility and bioavailability. As a result, dissolution tests became one of the elemental tools for quality control purposes. As a consequence of these developments, the basket-stirred-flask test (USP Apparatus I, designed by M. Pernarowski) was accepted in 1970 as an official dissolution tester apparatus by the United States Pharmacopoeia (USP) and the National Formulary (NF) [1,18,27,22,38]. Since then the dissolution test was improved, standardized and calibration tests were also launched.

Pharmacopoeial Methods

Tablet disintegration devices have evolved into dissolution test devices, that usually contain 6-8 vessels. In addition to the samples blank or standard samples may also be used.

The rotating basket method first appeared in 1971. At the beginning of each test, the sample is placed in the basket made of stainless steel or

equivalent. This method is generally used to measure capsules since this dosage form would float after being thrown into the dissolution medium. Nowadays, the official pharmacopoeial (Ph.Eur; USP; JP) baskets with standard size and design are made of stainless steel. Unfortunately, the long-term use of these baskets in acidic or other corrosive media can damage them, thus causing the dissolution tests to fail. Pure gold coating up to 2.5 μm thickness is allowed by the USP for use in acid media, or in case the active drug reacts with the plain steel basket. PTFE (polytetrafluoroethylene), FEP (fluorinated ethylene propylene), PFA (perfluoroalkoxy alkane) coatings are ideal for the protection of components when highly corrosive or solvent conditions exist.

The paddle method (USP Apparatus II) was introduced in 1978 [27, 22]. These devices are either made of stainless steel or another inert material, but inert surface coating is also approved. This test method is mainly applied on tablets that sink to the bottom of the vessel before mixing. When the Apparatus II is used for testing certain dosage forms, such as hard-gelatin capsules [39], sticky tablets or slowly disintegrating tablets, a sinker is needed to prevent the sample from floating [27]. Placing the samples in sinkers (for example 8 mesh basket sinker Japanese pharmacopeia size, spiral capsule sinker, O-ring style sinker, U-type sinker, etc.) may resolve these concerns allowing the use of the paddle apparatus [40]. In dissolution testing, according to the U.S. Pharmacopeia, typically a nonreactive stainless steel wire helix is used when normally floating dosage forms are tested. In 1987, Soltero et al. fabricated, tested and classified various new sinker designs. Four classes of sinkers were defined: (a) Longitudinal sinkers contact the dosage forms on the long axis; (b) Lateral sinkers wrap around the capsule or contact with it in the middle; (c) Screen enclosures are either cage-shaped and hold the entire capsule or circular and placed on top of the capsule; (d) Internal weights consist of two steel ball bearings [39].

While the use of sinkers may solve floating problems, their final position in the dissolution vessel may vary, thus contributing to deviance in test results. These can be eliminated by using the suspended stationary basket (for example felodipine stationary basket). Reproducibility and accuracy problems have been reported in connection with the USP Apparatus II. The primary source of variability is in the conventional cylindrical vessel,

especially under the rotating paddle. The new peak vessels effectively displace the unstirred cone, forcing the tested API into the region of appropriate hydrodynamics, where the whole product is constantly and uniformly exposed to the medium [41].

The reciprocating cylinder method (USP Apparatus III), based on the disintegration of tablets and capsules, appeared in 1991 [42,43]. This method is used for modified (sustained or delayed) release formulations. However, it simulates the vital physicochemical parameters and is undoubtedly more effective than USP Apparatus I-II for making in vivo predictions [44,45], the method was still extremely labor-intensive and there were limitations with regards to automatization [18,42].

Later, in 1995, the USP Apparatus IV was introduced as a flow-through cell method designed to mimic the bowel movement by pulsating flow. The design allows the medium to vary continuously thereby inducing the pH gradient, thus the effect of this gradient and the continuous removal of the medium can be investigated [46]. This device also enables the dissolution testing of sustained-release formulations as the product always contacts with the fresh medium as if absorbed from the intestinal tract. The in vitro-in vivo correlations (IVIVC) of the poorly soluble compounds have been made easy with this flow-through apparatus [47].

Sampling is also an important issue for dissolution studies. Today's modern equipments are capable of sampling, which means it is removed, filtered, and collected from a specific location at a given time. It is possible to measure the dissolved drug in the sample on-line with a spectrophotometer connected to the system. To ensure the accuracy of the measurements rapid testing is inevitable along with the integrity of the equipment. Since the insoluble or yet undissolved particles (API or excipient) in the medium mustn't leave the vessels, the applied filters are of great importance in dissolution testing. In general, the filters are made of UMWH PE (ultra-high-molecular-weight polyethylene), but on occasions, when chemical compatibility is an issue, PVDF (polyvinylidene fluoride or polyvinylidene difluoride) filters can also be used. PVDF exhibits increased chemical resistance and compatibility among thermoplastic materials and known for its inert resistance (Figure 2).

Pharmacopoeias also regulate the procedure of the dissolution tests. For example, two pharmaco-

poelial approaches are listed in the European Pharmacopoeia for the examination of delayed-release solid dosage forms. The tablets are stirred for two hours in (a) 750 – 750 mL or (b) 1000 – 1000 mL 0.1 M degassed hydrochloric acid (HCl) at 37 ± 0.5 °C. Afterward, the pH is raised by adding buffer solutions: (a) 250 mL 0.02 M trisodium phosphate dodecahydrate solution is added to the dissolution vessel. If necessary, the pH is adjusted to 6.8 ± 0.05 with 2.0 M hydrochloric acid or 2.0 M sodium hydroxide solution. (b) After two hours of dissolution in 0.1 M HCl, the hydrochloric media is drained from the vessels and replaced with 1000 mL pH 6.8 phosphate buffer. The buffer solution is prepared by mixing 0.1 M HCl solution (3 parts) and 0.2 M aqueous solution of trisodium phosphate dodecahydrate (1 part). If necessary, the pH is adjusted to 6.8 ± 0.05 with 2.0 M hydrochloric acid or 2.0 M sodium hydroxide solution. The solution exchange can also be accomplished by replacing the acidic vessel with new vessels previously filled with the 1000 mL of degassed pH 6.8 buffer solution and thermostated to 37 ± 0.5 °C and transferring the baskets. In both cases, the devices are used for additional 45 minutes or a specified period. Then a sample of the solution is removed from the bulk and analyzed by suitable qualitative and quantitative tests [48].

In vitro forecasting methods

Bicarbonate buffers are often used for in vitro pre-

dictions as they simulate the physiological pH ranges (between pH 5.0 and 8.4) of luminal fluids present in the digestive tract well. The pH of the bicarbonate buffer is the result of a complex and dynamic interaction among the bicarbonate ions, the carbonic acid and the dissolved and solvated carbon dioxide, as well as the partial pressure above the solution [49]. Liu et al. developed the bicarbonate system by modifying Hanks' buffered saline (pH 7.4) [50], that has too high pH but has too low buffer capacity compared to human intestinal fluids [51]. Therefore, this buffer was modified to create a pH 6.8 bicarbonate buffer with higher buffer capacity. The Hanks' solution is primarily a bicarbonate buffer in which hydrogen carbonate (HCO_3^-) and carbonic acid (H_2CO_3) are present simultaneously and carbon dioxide ($\text{CO}_{2(\text{aq})}$) is formed by the dissociation of the latter [52,53] (Eq. 3).

The buffer pH can be adjusted by altering the acid (H_2CO_3) and conjugate base (HCO_3^-) concentration corresponding to the Henderson-Hasselbalch equation (Eq. 4):

$$\text{pH} = \text{pKa} + \frac{\text{HCO}_3^-}{\text{H}_2\text{CO}_3} \quad (4)$$

Bicarbonate buffers are less commonly used in the solubility studies of solid formulations. The reason lies in the thermodynamic instability of hy-

Figure 3 The GIS dissolution model.

drogen carbonate solutions due to the complex equilibrium between the various ions [49]. Spontaneous CO_2 loss from the solution results in irregularly increase in pH. To keep the pH at the desired level, either the CO_2 loss must be completely avoided, or the leaked CO_2 must be replaced by quantitative substitution, so an appropriate amount of gas has to be introduced to obtain the required pH again [54].

To avoid difficulties, Al-Gousous et al. developed a phosphate-based dissolution method (pH 6.5 phosphate buffer) that resulted in similar dissolution profile compared to the carbonate system [55]. This method provides a better biopredictability in the fed state. The dissolution behavior of enteric polymers is more complex than the small molecules, therefore such approaches have been used where the molarity of phosphate gave similar disintegration times with the physiological bicarbonate buffer [55].

Biorelevant forecasting methods

Although the parameters and the biorelevant media can simulate the gastrointestinal tract better, the biorelevant methods provide a more complex, but less feasible way of studying the dissolution behavior of drugs [56]. In these cases, smaller volumes (250 mL) of biorelevant fluids (e.g. FaSSIF – Fasted State Simulated Intestinal Fluid, FeSSIF –

Fed State Simulated Intestinal Fluid, FaSSGF – Fasted State Simulated Gastric Fluid, FeSSGF – Fed State Simulated Gastric Fluid) are used to simulate the composition of fluids occurring at the different parts of the digestive tract. This way the experiments can provide a more accurate picture of in vivo dissolution [57,58,59,60]. These media also model buffer capacity and surfactants (bile acids, lecithin) at exact pH levels. However, the quantitative prediction of in vivo performance directly from the dissolution test results can be challenging, since various physiological parameters (e.g. gastric emptying, permeability through the intestinal membrane, transit time, pH, etc.) can also influence the bioavailability [61]. In general, the three most important absorption sites are modeled: stomach, duodenum and jejunum. The pH values of the media representing these sites are 2.0, 6.5 and 6.8. The stirring of the medium is ensured by mixing elements and the test is kept at constant temperature (37 ± 0.5 °C).

Some methods that can be used to predict the behavior of the human body, such as the ASD (Artificial Stomach-Duodenum), a digestive system simulator that models three parts of the gastrointestinal tract: the stomach, the duodenum and the jejunum [62,63] (Figure 3). The precision pumps provide the fluid supply for gastric and intestinal secretion, as well as the transfer of chamber content between the gastric-duodenum and duode-

Figure 4 Gut-on-a chip device.

num-jejunum. The speed of the pumps and the liquid temperature are adjustable. Furthermore, the pH can be controlled while the rotation speed of paddles is kept constant [64].

Gut-on-a chip

The small intestine serves as a barrier against orally administered drugs, so it is highly important to predict its function at drug development. The biggest problem of animal models is that, they can not always predict the processes that take place in the human body [65,66]. This poses particular problems with the management of nutrient metabolism, transport, and oral absorption [67]. For these reasons, there has been a growing interest in developing an in vitro model of human intestinal function, including cell culture systems using transwell filter inserts that allow transepithelial inhibition. None of the existing in vitro intestinal models summarize the mechanically active intestinal microenvironment (e.g. peristaltic movements and intraluminal fluid flow) [68]. To evaluate this feature, Kim et al. developed a gut-on-a chip device (Figure 4) with an optical sensor system that allows continuous stimulation of

Caco-2 cells with physiological stimuli, such as shear stress and cyclic mechanical stress, that stimulate peristaltic movements in vivo. The gut-on-a chip device was made by chip microfabrication using a flexible material, polydimethylsiloxan (PDMS) polymer with an adaptation of a soft lithographic technique. Therefore, the device has two independent compartments separated by a micro-porous membrane on which small intestinal model (Caco-2) cells were cultured.

Cyclic aspiration caused by peristaltic movements of the human gut was performed with a computer-controlled vacuum tube. The authors demonstrated that the in vitro model reproduced the results of the previously described animals and humans, thus concluding that the gut-on-a chip device could be used to analyze intestinal pathophysiology and dissect disease in vitro [69].

Components of the dissolution test media

The FDA Dissolution Database describes a large number of the different media from the simplest medium (water) or buffer solutions to solutions with added surfactants, organic solvents and enzymes [70]. The pH values of the most common

dissolution media according to the database are in the range of 1.0- 7.5 (ionic strength similar to USP). Their pH values align with the corresponding sections of the GI tract, thus they are considered physiologically relevant [70]. In fasted state the gastric emptying time is approximately 30 minutes [71], in fed state it takes longer, about 2 hours [72]. This period varies greatly in healthy adults (20-53 years) and healthy children aged 0-5 years [73].

Conforming to the database, various acidic aqueous solutions can be used as solvents. The most commonly used sour media is hydrochloric acid (0.1 M, 0.01 M, 0.001 M), but phosphoric acid (0.01 M) is also widely used [70]. To simulate the intestinal fluid (SIF), a medium at pH 6.8 should be used. As the source of water highly influences basic test conditions, like the pH or surface tension, water is not a recommended choice for dissolution medium. Likewise, owing to both the active and inactive ingredients, the circumstances may also change during the dissolution test itself [74]. Besides, its low buffering capacity is also disadvantageous. For water-insoluble or sparingly water-soluble drugs the use of surfactants, such as sodium lauryl sulfate (SLS) [4], Tweens (polysorbate 20 and 80), lauryl dimethylamine oxide (LDAO), Triton X [75], Brij 35 (polyoxyethylene lauryl ether) or cetyl trimethyl ammonium bromide (CTAB) is common [70]. Artificial surfactants can interfere with salt formation of weak bases, thus dissolution can be affected in an artefactual way [76]. The incidental use of higher pH should be justified and should not exceed pH 8.0 in principle [74]. Small intestine transit time is nearly similar in children [77] and healthy adults in fed state, it is about 7.5 hours [78], in fasted state it is much shorter, approximately 2.5 hours [79].

Although, the conduction of in vivo assays may often be opposed for ethical reasons, technical limitations or monetary considerations, a better understanding of the infantile digestive process would be greatly informative and helpful. Learning the process of infant formula disintegration in the infantile digestive system is a key in the development of new neonatal formulas with additional health benefits [80]. In recent years there was a little progress in the development of biorelevant media for suitable pediatric dissolution tests [81]. Predictive biopharmaceutical methods representing the in vivo drug dissolution in children would be of huge benefit for early formulation screening and influence assessment.

Simulated gastric fluids

In the fasted state the simplest dissolution medium is Simulated Gastric Fluid (SGF). This pepsin-containing hydrochloric acid has a pH value of 1.2, and its surface tension approximately equals to water [82]. To reduce its surface tension the use of surfactants, like SLS have been proposed [4]. Human stomach pH varies considerably (1.2-6.4) among individuals due to the prandial state, the quality and quantity of the consumed food. Typically the pH for fasted fluid ranges between 1.2 and 2.7 [83,84,85,86,87,88], for fed gastric fluid it can range from 3.0 to 6.4, depending on the stage of digestion [85,86].

In 2005, Vertzoni et al. developed a medium to simulate the fasted state of gastric fluid, which reflects the actual gastric composition according to published physiological data [89]. FaSSGF (pH = 1.6) contains physiologically relevant amounts of pepsin, bile salts and lecithin to obtain in vivo-like surface tension.

The content of the fed stomach is complex and its physicochemical properties change constantly over time as secretion, digestion and gastric emptying proceeds, therefore milk and/or nutritional liquid products can only be used to simulate initial gastric conditions in the fed state. Homogenized long-life milk (3.5% fat) is recommended as a solvent for simulating nutritional conditions [58,90,91,92,93]. The FeSSGF contains equal amounts of UHT-milk and acetate buffer. The mixture is stirred with a magnetic stirrer while the pH is adjusted to 5.0 with 0.1 N HCl. This medium can be used to predict food effects [94]. The pH in the stomach usually rises due to the buffering ability of the meal, and initially may be up to pH 7.0, depending on the composition of the meal. After several hours of continued secretion of gastric acid, the pH is reduced to the initial value [85,95,96].

In 2017 Passannanti et al. developed a dynamic in vitro digestive system based on infant physiology, including the oral, gastric and small intestinal phases of digestion to estimate the digestibility of rice starch- and rice cream-based baby foods [97]. In another study, cow- and soya milk formulas were used to simulate the gastric fluid in neonates [98] (Table 1).

Simulated small intestinal fluids

The most straightforward way to represent the

Table 1 Adult and paediatric biorelevant media (V=1L) [58,70,71,72,76,77,78,88,93,97]

	Fasted- state Simulated Gastric Fluid				Fed-state Simulated Gastric Fluid				
	Neonate	Infant	Adult	Adult in-vivo data [95,105,106]	Neonate-cow formula	Neonate-soy formula	Adult		
Pepsin (mg/mL)	0.015	0.025	0.1	~0.8	-	-	-		
Sodium taurocholate (μM)	20	60	80	~0.8	-	-	-		
Lecithin (μM)	5	15	20	-	-	-	-		
NaCl (mM)	34.2	34.2	34.2	68±29	100.35	94.79	237.02		
Acetic acid (mM)	-	-	-	-	7.25	7.25	17.12		
Sodium acetate (mM)	-	-	-	-	64.65	64.65	29.75		
Milk:buffer ratio	-	-	-	-	1:1	1:1	1:1		
pH	1.6	1.6	1.6	1.4-2.1	5.7	5.7	5.0		
Buffer capacity (mmol/l/ΔpH)	-	-	-	41.0±6.0	15	15	25		
Osmolality (mOsmol/kg)	120.7±2.5	120.7±2.5	120.7±2.5	191±36	340	240	400±10		
Gastric emptying time (h)	~0.5				~1.0		~2.0		
	Fasted-state simulated intestinal fluid		Fed- state simulated intestinal fluid						
	FaSSIF	FaSSIF-V2	Neonate-breastfed	Neonate-cow formula	Infant-cow formula	Adult	“Early”	“Middle”	“Late”
Sodium taurocholate (mM)	3	3	2.5	2.5	7.5	15	10	7.5	4.5
Lecithin (mM)	0.75	0.2	0.5	0.5	1.5	3.75	3	2	0.5
Dibasic sodium phosphate (mM)	28.65	-	-	-	-	-	-	-	-
Maleic acid (mM)	-	19.12	55.02	55.02	55.02	-	28.6	44	58.09
Sodium hydroxide (mM)	8.7	34.8	81.65	81.65	81.65	101	52.5	65.3	72
Sodium chloride (mM)	105.85	68.62	95	111.73	107.35	173	145.2	122.8	51
Glyceryl monooleate (mM)	-	-	5	6.65	5	-	6.5	5	1
Sodium oleate (mM)	-	-	0.8	1.06	0.8	-	40	30	-
Acetic acid (mM)	-	-	-	-	-	144	-	-	0.8
pH	6.5	6.8	5.8	5.8	5.8	5.0	6.5	5.8	5.4
Buffer capacity (mmol/l/ΔpH)	12	10	25	25	25	76	25	25	15
Osmolality (mOsmol/kg)	270±10	180±10	330±10	330±10	390±10	635±10	400±10	390±10	240±10
Small intestinal transit time (h)	~2.5		~8.0			~7.5			

small intestinal conditions is by using Simulated Intestinal Fluid (SIF), a pancreatin-bearing medium with pH = 6.8. Generally it is used for quality control purposes and its suitability for IVIVC is restrained. For example, the effect of food on the absorption of a drug cannot be predicted [99]. In

the small intestine, the pH gradually increases between the duodenum and the ileum [2,100,101]. At the beginning of the transit, the pH changes rather slowly [102]. In the fasting state, the small intestine has a pH between 6.0-7.2, that decreases after a meal to a pH of about 5.0-6.0, since the food is

mixed with the gastric juices that reduce the initial alkaline pH of the small intestine [55,103]. After reaching the colon, the pH decreases to approximately 5.0 [100,102].

In 1998, Dressman et al. introduced a medium to simulate the fasting state in the small intestine based on physiological data [4,58]. In addition to pH, osmolality and buffer capacity, FaSSIF takes the solubility of intestinal fluids into account. FaSSIF (pH = 6.5) contains sodium taurocholate and phospholipids (4:1). In 2008, Jantratid et al. came up with the updated composition of FaSSIF. FaSSIF-V2 contains reduced amount of lecithin (0.75 mM in FaSSIF to 0.2 mM), it has lower osmolality and the phosphate buffer is substituted to maleate buffer (Table 1) [94].

The use of bicarbonic media in the small intestine requires attention, as carbon dioxide introduction is necessary to maintain the pH level, buffer capacity, ionic strength and osmolality [104]. Phosphate buffer is used instead to avoid the incidental pH instability.

The fed state simulating fluid (FeSSIF) was introduced for better simulation of the small intestinal surroundings [4,58]. FeSSIF has a pH of 5.0, the osmolality and buffer capacity were adjusted to approach the in vivo data like FaSSIF. In fed state (FeSSIF) the sodium taurocholate and phospholipids concentrations are higher than in fasted state (FaSSIF) [105].

Based on novel studies, Jantratid et al. have revised the composition of FeSSIF, as data indicated the rather slow pH decrease in the jejunum (upper part of the small intestine) after food intake [94]. The updated media (FeSSIF-V2) also contains various lipolysis products (glyceryl monooleate and sodium oleate).

So called snapshot media were developed to mirror the influence of digestive processes on the composition of fed state gastric media. “Early”, “middle” and “late” phases represent the different time-frames of the composition of the small intestine after the ingestion. FeSSIF-V2 is suggested as a representative medium for the conditions in the small intestine [94]. Table 1 also includes the media used to simulate the intestinal fluids of neonates and infants [98] as well as the transit time of the small intestine [77,78,79].

Simulated colon fluids

The colon can also serve as an absorption site, an alternative drug delivery route to the systemic cir-

culation. Although the absorption from the colon is less significant than from the small intestine, the systemic drug delivery via the colon has its advantages: namely the prolonged retention time, the relatively low enzyme secretion and the direct delivery to the systemic circulation avoiding the hepatic first-pass effect. Previous studies have shown that the pH in the initial part of the large intestine ranges with time and the composition of food intake from 4.8 to 7.0 [106]. In 2005, Fotaki et al. [107] developed a Simulated Colonic Fluid (SCoF) based on the pH values and short-chain fatty acid concentration according to available physiological data. SCoF has slightly acidic pH (pH 5.8). In 2010, Vertzoni et al. developed media that simulates the physicochemical characteristics of the ascending colon in the fed and fasted states [108]. Fasted state simulated colonic fluid (FaSSCoF) contains tris/maleate buffer solution and the pH is adjusted to 7.8. The same components are present in the fed state simulated colonic fluid (FeSSCoF) but in different concentrations, and the pH is adjusted to 6.0 [106]. Significant differences can be observed in the colon transit times of different age groups. While in children this period is about 17.5 hours long [77], in adults it takes remarkably more, around 39 hours [109] while in the elderly (75-80 years) this can extend even up to 66 hours [110] (Table 2).

Comparison of in vitro dissolution profiles

Among the numerous model-dependent and model-independent methods investigated for dissolution profile comparison, the use of fit factors are the simplest. The method was introduced by Moore and Flanner, and nowadays it is recommended by several global regulatory authorities. It is a model-independent mathematical approach to analyze the dissolution curves applying the similarity factor f_2 [111,112]. The method compares the dissolution values (in percentage) of the reference

Table 2 Components of SCoF (V=1 L) [76,108,110,111]

	SCoF
Acetic acid (mM)	170
NaOH (mM)	157
pH	5.8
Osmolality (mOsmol/kg)	295
Buffer capacity (mmol/l/pH)	29.1
Ionic strength	0.16
Transit time (h)	Children: ~17.5 Adult: ~39

and sample preparation at time and can be described by the following equation (Eq. 5).

$$f_2 = 50 \lg \left[\left(1 + \frac{1}{n} \sum_{i=1}^n (R_t - T_t) \right)^{-0.5} \cdot 100 \right] \quad (5)$$

where n is the number of sampling points, R_t is the cumulative percentage of the drug dissolved in the reference product at T_t the cumulative percentage dissolved at the selected times of the test product [113].

Profile tests and f_2 calculations are not necessary if more than 85% of the drug is dissolved in 15 minutes. If the similarity factor, f_2 is higher than 50, the dissolution profiles can be considered similar.

Conclusions

The dissolution methods used in quality control tests are simple, standardized tests that are well regulated by the pharmaceutical authorities. However, the conditions of these studies are often far from biologically relevant conditions, their in vivo predictability is limited. Biorelevant dissolution methods are used as in vitro representatives of in vivo performance. The updated biorelevant dissolution media simulate the physiological in vivo and in vitro hydrodynamic conditions accurately and also attempt to mimic the luminal hydrodynamics. These media can be used to predict the performance of different formulations and the effect of food in vivo. The proper selection of in vitro circumstances that closely simulate the in vivo conditions can lead to successful predictions of in vitro – in vivo correlation (IVIVC) for oral formulations.

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