

Simulated Biological Fluids with Possible Application in Dissolution Testing

Margareth R. C. Marques^{1,*}, Raimar Loebenberg², and May Almukainzi²

e-mail: mrm@usp.org

¹U.S. Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852, USA

²Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6J 2L7

ABSTRACT

This literature review is a compilation of the composition and, in most cases, the preparation instructions for simulated biological fluids that may be used as dissolution media in the evaluation of dissolution profiles and amount of drug released from pharmaceutical dosage forms. The use of simulated biological fluids can give a better understanding of the release mechanisms and possible in vivo behavior of a product and enhance the predictive capability of the dissolution testing. A summary of the major characteristics of the most used routes of administration that may affect dissolution and absorption of drug substances is presented. The routes and simulated biological fluids covered by this review are:

- **Parenteral:** simulated body fluid and simulated synovial fluid.
- **Oral:** fasted-state simulated gastric fluid, fed-state simulated gastric fluid, fasted-state simulated intestinal fluid, fed-state simulated intestinal fluid, simulated colonic fluid, fasted-state simulated colonic fluid, and fed-state simulated colonic fluid.
- **Buccal and sublingual:** simulated saliva.
- **Pulmonary:** simulated lung fluid.
- **Vaginal:** simulated vaginal fluid and simulated semen.
- **Ophthalmic:** simulated tears.

Simulated sweat is also included. Some examples of how these simulated biological fluids are used to evaluate dosage forms are included in each route of administration.

INTRODUCTION

Until some decades ago, most of the conventional pharmaceutical dosage forms were essentially injections, oral formulations (solutions, suspensions, tablets, and capsules), topical creams, and ointments. With the progress of drug delivery technology, novel dosage forms have been developed to overcome the problems associated with conventional drug delivery. Research has been directed toward the use of alternatives to the parenteral route for drugs that cannot be delivered orally. Potential alternative portals of drug entry to the systemic circulation include buccal, sublingual, nasal, pulmonary, and vaginal routes, among others. These routes of administration are also used for the local delivery of drugs directly to the site of action, with consequent reduction in the dose needed to produce a pharmacological effect, eliminating problems related to first-pass metabolism and possibly minimizing systemic side effects (1).

An important application of dissolution testing is the prediction of in vivo performance of pharmaceutical dosage forms. The media typically used for quality control dissolution testing do not represent all aspects of the physiological conditions of the most used routes of administration and do not allow correlation with in vivo data. Prediction of dosage form performance in the site where most of the absorption occurs requires adequate simulation of the in vivo conditions.

This paper describes some characteristics of the parenteral, oral, buccal and sublingual, pulmonary, ophthalmic, and vaginal routes that are important to consider when developing simulated media. A compilation of the composition and preparation of several simulated biological fluids, including simulated sweat, with potential use as dissolution media is presented.

PARENTERAL ROUTE

The most used routes of injection are intramuscular, intravenous, and subcutaneous and are normally associated with short-term effects. Novel implant devices that can adequately control drug release and provide a prolonged duration of effect have been developed (1). To evaluate the in vitro drug release from these dosage forms, the dissolution medium should have ion concentrations almost equal to those of the human plasma. Table 1 describes the ionic composition of simulated body fluid (SBF) and human blood plasma. Simulated body fluid was developed initially to evaluate the surface structural changes of glass-ceramics used to manufacture artificial vertebrae, ileum, tooth roots (2), and bioactive material used to repair hard tissues such as artificial middle-ear bone and maxillofacial implants (3). This simulated body fluid was prepared using the reagents listed in Table 2. These reagents were added to 700 mL of water in the order given in Table 2, one by one, after each reagent was completely dissolved. The pH was adjusted to 7.4 with 1 M

*Corresponding author.

Table 1. Ionic Concentration of Simulated Body Fluid and Human Blood Plasma

Ion	Simulated Body Fluid (mM)	Human Blood Plasma (mM)
HCO ₃ ⁻	4.2	27
K ⁺	5	5
Cl ⁻	148.8	103
Na ⁺	142	142
Ca ⁺²	2.5	2.5
Mg ⁺²	1.5	1.5
HPO ₄ ⁻²	1	1
SO ₄ ⁻²	0.5	0.5
tris(hydroxymethyl) aminomethane	50	50
hydrochloric acid	45	45
Properties		
pH	7.25	7.25

hydrochloric acid, and the final volume adjusted to 1 L with water (4). In most of the studies, a volume ranging from 15 to 200 mL of simulated body fluid was used to evaluate the samples (2–4).

In 2003 an updated version of simulated body fluid (Table 3) with detailed instructions for its preparation was submitted to the Technical Committee ISO/TC150 of International Organization for Standardization as a solution for in vitro measurement of the apatite-forming ability of implant materials. The volume of the updated SBF to be used for sample evaluation is calculated by the formula $V_s = S_a/10$, where V_s is the volume of updated SBF and S_a is the apparent surface area of the sample (mm²).

Table 2. Reagents for Preparing Simulated Body Fluid

Reagent	Amount (g/L)
sodium chloride	7.996
sodium bicarbonate	0.350
potassium chloride	0.224
potassium phosphate dibasic trihydrate	0.228
magnesium chloride hexahydrate	0.305
1 M hydrochloric Acid	40 mL
calcium chloride	0.278 g
sodium sulfate	0.071
tris(hydroxymethyl) aminomethane	6.057

Table 3. Reagents for Preparing the Updated Simulated Body Fluid

Reagent	Amount for 1 L of SBF
sodium chloride	8.035 g
sodium bicarbonate	0.355 g
potassium chloride	0.225 g
potassium phosphate dibasic trihydrate	0.231 g
magnesium chloride hexahydrate	0.311 g
1 M hydrochloric acid	39 mL
calcium chloride	0.292 g
sodium sulfate	0.072 g
tris(hydroxymethyl) aminomethane	6.118 g

For porous materials, the volume of SBF should be greater than the calculated V_s (5).

Radiation synovectomy is one of the ways to treat rheumatoid arthritis. This technique involves intra-articular injection of radioactive particles. An ideal particulate system for radiation synovectomy would contain a pure beta-emitting radioisotope and would be biodegradable. Currently radiocolloids and tagged macroaggregates are the preferred systems. Radiocolloids are composed of nanosized radioisotopes in resin, silicate, or citrate form. Tagged macroaggregates are much larger than radiocolloids and are formed when a substance is precipitated in a solution containing a radioactive isotope; one example is ferric hydroxide macroaggregates tagged with ¹⁶⁵Dy (dysprosium). Conzone et al. (6, 7) evaluated the in vitro dissolution of dysprosium lithium borate microspheres using simulated synovial fluid (Table 4).

If the solution is prepared with only the first four reagents, it is known as phosphate buffered saline solution (PBS). Hyaluronic acid is a mucopolysaccharide and is the primary diffuse macromolecule that exists in human

Table 4. Simulated Synovial Fluid

Reagent	Amount (g/L)
sodium chloride	8
potassium chloride	0.2
sodium phosphate dibasic	1.44
potassium phosphate monobasic	0.24
hyaluronic acid	3
Properties	
pH	7.4

synovial fluid. A volume ranging from 10 to 20 mL of this simulated fluid was used in the in vitro tests (6, 7).

The synovial fluid was used to evaluate the in vitro bioactive behavior (in vitro bone-bonding ability) of tissue-engineered osteochondral (bone-cartilage) composite used in the treatment of lesions of the articular cartilage. The simulated synovial fluid was continuously circulated in physiological conditions (pH 7.4 and 37 °C) using a peristaltic pump for up to 14 days with an available volume of 50 mL of fluid (8).

ORAL ROUTE

The oral route is the most common and convenient administration method for the systemic delivery of drugs. It affords high patient-acceptability, compliance, and ease of administration. Moreover, the cost of oral therapy is generally much lower than that of parenteral therapy (1).

Nevertheless, the oral route is not without disadvantages, particularly with respect to labile drugs such as peptide- and oligonucleotide-based pharmaceuticals. During the last decades, numerous novel oral drug delivery systems such as mucoadhesives, matrix systems, reservoir systems, microparticulates, and colon-specific drug delivery systems have been developed to overcome some of these limitations (1).

The pH of the fluids in the fasted-state stomach is typically below 2, but can range between 1 and 7.5. Food intake results in an almost instantaneous increase of the gastric pH. Depending on the contents of the meal, the fed-state gastric pH increases to values between 4 and 7. Soon after food intake, the gastric pH gradually returns to the fasted-state pH. Determinations of the fasted-state pH in the proximal small intestine often result in mean values of approximately 6.5, but can fluctuate momentarily between 2 and 7. Prior to emptying of the stomach content into the small intestine, the duodenal pH is in the fasted-state range (i.e., approximately 6.5). Postprandially, the pH in this region gradually decreases as increasingly acidic gastric content enters the intestine, before returning to fasted-state values at the completion of the digestive period. In the distal small intestine (ileum), the pH rises to approximately 7.5 because of bicarbonate secretion. Furthermore, the effect of food intake on pH diminishes toward the distal end of the small intestine. Because of the formation of short-chain fatty acids by fermentation, the pH falls considerably from the terminal ileum to the ascending colon. Some studies show that the pH in the caecum/ascending colon is on average just over 6, but values between 5.7 and 8.4 have also been reported. The short-chain fatty acids are quickly absorbed and metabolized during transit through the proximal colon, and together with bicarbonate secretion, result in an increase in the luminal pH toward the distal colon. The rare observations of pH in the descending colon/rectum point toward a value closer to 7 (9).

Drug absorption may be affected by several physiological factors including volume and composition of

Table 5. Simulated Gastric Fluids

Composition	FaSSGF	FeSSGF		
		Early	Middle	Late
sodium taurocholate (μM)	80	-	-	-
lecithin (μM)	20	-	-	-
pepsin (mg/mL)	0.1	-	-	-
sodium chloride (mM)	34.2	148	237.02	122.6
acetic acid (mM)	-	-	17.12	-
sodium acetate (mM)	-	-	29.75	-
orthophosphoric acid (mM)	-	-	-	5.5
sodium dihydrogen phosphate	-	-	-	32
milk/buffer	-	1:0	1:1	1:3
hydrochloric acid/sodium hydroxide q.s.	pH 1.6	pH 6.4	pH 5	pH 3
Properties				
pH	1.6	6.4	5	3
osmolality (mOsm/kg)	120.7 ± 2.5	559	400	300
buffer capacity (mmol/L/pH)	-	21.33	25	25
surface tension (mN/m)	42.6	-	-	-

gastrointestinal fluids, the pH and buffer capacity of these fluids, digestive enzymes, contraction patterns, and bacterial flora in the gut. In addition, the extent of drug absorption and bioavailability may be further affected by gastrointestinal transit, the presence of cellular transporters, and metabolic enzymes. Several of those factors are affected by intake of food. The effects of food on the physiology and consequently on the in vivo drug release and absorption are most pronounced in the stomach. In fact, even coadministration of water with a dosage form may influence the conditions in the stomach because of dilution effects (9). The oral bioavailability of many, but not all, drugs is affected by food intake. The absorption of a drug can be increased, decreased, delayed, or accelerated depending not only on the physicochemical characteristics of the drug substance but also on the type of dosage form, composition of the ingested food, and general state of the gastrointestinal tract (10).

Simulated Gastric Fluids

Vertzoni et al. (11) developed a simulated gastric medium that more adequately reflects the physiological conditions of the fasted state (see FaSSGF, fasted-state simulated gastric fluid in Table 5). This medium contains pepsin and low amounts of bile salt and lecithin. The

Table 6. Fasted-State Simulated Intestinal Fluid (FaSSIF)

Composition	FaSSIF
sodium taurocholate (mM)	3
lecithin (mM)	0.2
maleic Acid (mM)	19.12
sodium hydroxide (mM)	34.8
sodium chloride (mM)	68.62
Properties	
pH	6.5
osmolality (mOsm/kg)	180 ± 10
buffer capacity (mmol/L/pH)	10

reduced surface tension of this medium is created by a mixture of pepsin, bile salts, and lecithin (11).

A major issue when trying to simulate the intragastric environment in the fed state is the composition change with time as digestion proceeds and emptying occurs. Media that simulate the initial composition after food intake include full-fat milk (3.5%), which simulates a light meal, and Ensure Plus, which has physicochemical properties that are similar to those of the standard meal recommended by FDA for the effects of food in bioavailability and bioequivalence studies (12). One way of modeling composition changes is to develop “snapshot” media, each corresponding to a certain time after ingestion of the meal. It has been suggested that the most suitable medium representing the fed-state stomach is the homogenized form of the meal used in clinical studies, but difficulties in the analysis of the drug released into the medium put some limitations on its use (13). The composition of each snapshot medium was calculated to reflect the pH value, buffer capacity, and osmolality of the gastric aspirates during the first 75 min (early), from 75 to 165 min (middle), and 165 min (late) after meal ingestion (see FeSSGF, fed-state simulated gastric fluid, Table 5) (13, 14).

Simulated Intestinal Fluids

Biorelevant media simulating preprandial and postprandial conditions in the upper small intestine, including fasted-state simulated intestinal fluid (FaSSIF) and fed-state simulated intestinal fluid (FeSSIF), were introduced some years ago. These media were intended to simulate additional important aspects of the GI fluids, including bile salts and lecithin. Some adjustments to these media have been made to improve predictions of in vivo performance. From studies in healthy human volunteers, it was verified that the concentrations of bile salts observed in vivo are somewhat lower than in FeSSIF. The original FeSSIF contained no lipolysis products, which together with the bile can enhance the solubility of poorly soluble drugs.

Table 7. Fed-State Simulated Intestinal Fluid (FeSSIF)

Composition	FeSSIF			
	Early	Middle	Late	FeSSIF-V2
sodium taurocholate (mM)	10	7.5	4.5	10
lecithin (mM)	3	2	0.5	2
maleic acid (mM)	28.6	44	58.09	55.02
sodium hydroxide (mM)	52.5	65.3	72	81.65
sodium chloride (mM)	145.2	122.8	51	125.5
glyceryl monocho- late (mM)	6.5	5	1	5
sodium oleate (mM)	40	30	0.8	0.8
Properties				
pH	6.5	5.8	5.4	5.8
osmolality (mOsm/kg)	400 ± 10	390 ± 10	240 ± 10	390 ± 10
buffer capacity (mmol/L/pH)	25	25	15	25

Additionally, recent data in humans indicate that the pH in the upper small intestine decreases rather slowly after meal intake (Table 6) (13, 14).

As for the gastric fluids, the composition of the intestinal fluids changes over time in the fed state. Three snapshot media were developed to reflect conditions in the upper small intestine during the digestion process (see FeSSIF in Table 7) (13, 14).

As with the gastric media, it is practical to select compositions that globally reflect conditions in the upper small intestine before and after meals for the purposes of comparing formulations or assessing food effects. For the fasted state, FaSSIF (Table 5) is suitable. For the fed state, an improved composition was developed (see FeSSIF-V2 in Table 6) (13, 14).

The volume of fluids in the fasted stomach is usually only about 30–50 mL. Considering the coadministered fluid (250 mL, as standardized in most in vivo studies), the final volume is around 300 mL. Therefore, a realistic volume to simulate the total fluid available in the stomach in the fasted state would fall in the range of 250–300 mL. To evaluate drug release in this volume, the use of USP Apparatus 3 or mini-vessels may be appropriate. Because the minimum volume of medium that can be used with USP Apparatus 2 (paddles) is slightly higher than 300 mL (otherwise the paddle is not completely immersed in the medium), a volume of 500 mL is recommended. To simulate fasted conditions in the small intestine, a volume of up to 200 mL would be appropriate. Volumes of up to

Table 8. Simulated Colonic Fluid 1 (SCoF1)

Composition	Amount (g/L)
potassium chloride	0.20
sodium chloride	8
potassium phosphate monobasic	0.24
sodium phosphate dibasic	1.44
Properties	
pH	7

one liter would be recommended for the fed-state small intestine (11–14).

The preparation of these simulated fluids is time consuming, requires organic solvents, makes daily preparation necessary, and sometimes does not result in a usable medium. The possible residual organic solvents may affect physicochemical properties and dissolution behavior. One way of reducing or eliminating these problems is to use a simplified method for preparation of the media mentioned in Tables 3, 4, and 5, which can be found in the literature (15), or use freeze-drying methods for bulk preparation of storable, instant media (16).

Use of FaSSIF and FeSSIF as the apical media in Caco-2 permeation studies may improve the biorelevance of the model to evaluate the oral absorption in humans (17). The composition of these media may be modified for more compatibility with Caco-2 cells (18).

Simulated Colonic Fluid (SCoF)

A major indication for colon-specific drug delivery is in the treatment of local disorders of the colon, such as inflammatory bowel disease (IBD, which includes ulcerative colitis and Crohn's disease) and carcinoma of the colon. The colon can also be used as an absorption site for the delivery of drugs to the systemic circulation. Although absorption from the colon is generally much lower than from the small intestine, systemic drug delivery via the colon has some advantages such as prolonged residence time, relatively low enzyme secretion, and delivery directly into the systemic circulation, avoiding hepatic first-pass effect (1).

Recently, there has been increasing interest in targeting peptide and protein drugs to the colon because of the relatively low activity of proteolytic enzymes in the colon. One of the possible platforms for targeted drug delivery to the colon is to modify the structure of starch to improve its resistance to digestion. This prevents enzymatic digestion and acidolysis in the upper digestive tracts but subjects it to degradation by microorganisms in the colon. These modified starches are known as enzyme-resistant starches. Chen et al. (19) evaluated the in vitro release of bovine serum albumin from matrixes made of these modified starches using the SCoF1 described in Table 8.

Table 9. Simulated Colonic Fluid 2 (SCoF2)

Composition	Concentration (mM)
acetic acid	170
sodium hydroxide	~157
Properties	
pH	5.8
osmolality (mOsmol/kg)	295
buffer capacity (mEq/L/pH)	29.1
ionic strength	0.16

The in vitro release of metronidazole from modified Pulsincap dosage forms targeted to the colon in a time-dependent manner was evaluated using pH 6.8 phosphate buffer (20). The same buffer was used in the in vitro evaluation of colon-specific formulations for orally administered diclofenac sodium (21). Pectinolytic enzyme was added to pH 6.8 phosphate buffer to assess the in vitro drug-release behavior of pectin cross-linked with glutaraldehyde nanogel particles, a matrix for colonic drug delivery (22).

The pH in the initial part of the large intestine has been reported to range with time and composition of food intake from 4.8 to 7. In this location, enzymes produced by bacteria metabolize several residual nutrients. Carbohydrates and proteins are broken down during bacterial fermentation to short-chain fatty acids, ammonia, hydrogen, carbon dioxide, and sometimes methane. Acetate, propionate, and butyrate are the principal organic anions found in colonic contents. The average concentration of total short-chain fatty acids in the ascending colon has been reported as 120–130 mM. Further, about 1–1.5 L of a nearly isotonic fluid enters the colon daily. Most of the fluid is absorbed, so the volume excreted in feces is only about 50–200 mL. With regard to electrolytes, short-chain fatty acids stimulate absorption of sodium and water, whereas chloride is absorbed in exchange for bicarbonate (secreted to neutralize the organic acids produced). Based on these observations, Fotaki et al. (23) developed a fluid to simulate the contents of the proximal colon (Table 9). This medium was used to predict the input profiles of extended-release products containing isosorbide-5-mononitrate.

Recently, Vertzoni et al. (24) developed media simulating the physicochemical characteristics of the ascending colon in the fasted and fed states.

Fasted-State Simulated Colonic Fluid (FaSSCoF)

Tris/maleate buffer solution is prepared by dissolving 5.5 g of tris(hydroxymethyl) aminomethane (Tris) and 8.8 g of maleic acid in water. The pH is adjusted to 7.8 with about 240 mL of 0.5 M sodium hydroxide, and the final volume is adjusted to 1 L with water. About 450 mL of this

solution is transferred to a 1-L round-bottom flask, and 0.113 g of bile salt extract is added. Two separate solutions are prepared by dissolving 0.222 g of phosphatidylcholine and 0.026 g of palmitic acid separately in 3 mL of dichloromethane each. These two solutions (3 mL each) are transferred into the round-bottom flask. The dichloromethane is evaporated under vacuum at 40 °C until a clear solution having no perceptible odor of dichloromethane is obtained. The volume of the solution is adjusted to 1 L with tris/maleate buffer, and 3 g of bovine serum albumin are added and dissolved by gentle agitation with a magnetic stirrer. The final solution is lightly turbid. The pH of this medium is about 7.8.

Fed-State Simulated Colonic Fluid (FeSSCoF)

Tris/maleate buffer solution is prepared by dissolving 3.7 g of tris(hydroxymethyl) aminomethane (Tris) and 3.5 g of maleic acid in water. The pH is adjusted to 6.0 with about 33 mL of 0.5 M sodium hydroxide, and the final volume is adjusted to 1 L with water. About 450 mL of this solution is transferred to a 1-L round-bottom flask, and 0.451 g of bile salt extract is added. Two separate solutions are prepared by dissolving 0.370 g of phosphatidylcholine and 0.051 g of palmitic acid separately in 3 mL of dichloromethane each. These two solutions (3 mL each) are transferred into the round-bottom flask. The dichloromethane is evaporated under vacuum at 40 °C until a clear solution having no perceptible odor of dichloromethane is obtained. The volume of the solution is adjusted to 1 L with tris/maleate buffer, and 2 g of sodium chloride, 14 g of glucose, and 3 g of bovine serum albumin are added and dissolved by gentle agitation with a magnetic stirrer. The final solution is lightly turbid. The pH of this medium is about 6.0.

BUCCAL AND SUBLINGUAL ROUTE

Localized drug delivery to the mouth is used for the treatment of conditions of the oral cavity, principally aphthous ulcers, fungal conditions, and periodontal disease. However, in addition to topical delivery, there has been considerable interest in the possibility of oral transmucosal delivery to achieve the systemic delivery of drug moieties via the mucous membranes of the oral cavity. Oral transmucosal drug delivery can be subdivided into (1) sublingual, via the mucosa of the ventral surface of the tongue and the floor of the mouth under the tongue; and (2) buccal, via the buccal mucosa, the epithelial lining of the cheeks, the gums, and also the upper and lower lips (1). Drugs absorbed here are not exposed to gastrointestinal secretions that may cause degradation. Moreover, they bypass the liver in the first circulatory pass and are not exposed to microsomal enzymes before entering the general circulation, as is usually the case in gastrointestinal absorption (25, 26).

It is almost impossible to duplicate the properties of human saliva because of its particular characteristics.

Saliva is a mixture of fluids secreted by several salivary glands, it is a system with numerous constituents, and it is variable according to the time of day, diet, and so forth (27). The dilution and rapid elimination of the drugs from the oral cavity are consequences of speech, mastication, and continuous saliva production. Normal, healthy saliva in the oral cavity has a pH between 6.7 and 7.4, but it can temporarily drop below 5 when sweets, carbonated and fruit drinks, and other dietary acids are consumed. Some drugs, such as beta blocking agents, nitrates, and diuretics, as well as tobacco smoking can also reduce salivary pH (28).

Duffo and Castillo (27) developed an artificial saliva (SS1 in Table 10) for studying the corrosion behavior of dental alloys. In this solution, all components dissolve completely. It is recommended to add potassium bicarbonate just before use to avoid loss of CO₂ and changes in the pH. Bicarbonate is the major buffering agent of saliva, and is readily lost on standing when exposed to the air. This loss is the principal, if not the sole, cause of rise in pH that is observed on short-term standing. SS1 can be stored at 5 °C for at least 8 days without modification of its properties.

SS2 was used to develop computational models to predict local effects on the mouth from carcinogenic compounds present in tobacco smoke (28).

The interaction of benzethonium-copolymer complex, used in mouth rinse or dentifrices, was evaluated using SS3 (29).

Davis et al. (25) used SS4 in a dynamic dialysis system to investigate the interactions that might occur between drug molecules and the constituents of salivary secretions. Such interactions, if they occur, might influence drug absorption through the oral mucosa.

SS 5 (300 mL) was used to monitor the release of salbutamol sulphate from oral fast-dissolving films using USP Apparatus 2 (paddles) (30, 31).

PULMONARY ROUTE

Pulmonary drug delivery is used primarily to treat conditions of the airways, delivering locally acting drugs directly to their site of action. The lung may also be employed as a route for delivery of drugs into the systemic circulation and onward to an effect site located elsewhere in the body.

Mucus does not exist in the alveolar region, but is present as a viscoelastic layer in the tracheobronchial region. Respiratory mucus is a complex mixture that includes glycoproteins, proteins, and lipids. Its composition is also highly variable in disease states, with infections being accompanied by high levels of DNA.

Epithelial type-II cells constantly secrete and reutilize lung surfactant. It consists of lipid-rich lipoproteins with the lipid composition dominated by phosphatidylcholine with a high dipalmitoyl content. About 85–90% of isolated surfactant is lipid of which 95% is phosphoglycerols with cholesterol as the main neutral component. Many proteins

Table 10. Simulated Saliva (SS)

Composition	SS 1 (g/L)	SS 2 (g/L)	SS 3 (g/L)	SS 4 (g/L)	SS 5 (g/L)
potassium chloride	0.720	0.720	-	0.149	
calcium chloride dihydrate	0.220	0.220	0.228	-	
sodium chloride	0.600	0.600	1.017	0.117	8.00
potassium phosphate monobasic	0.680	0.680	-	-	0.19
sodium phosphate dibasic	0.866 (dodecahydrate)	0.866 (dodecahydrate)	0.204 (heptahydrate)	-	2.38
potassium bicarbonate	1.500	1.500	-	-	
potassium thiocyanate	0.060	0.060	-	-	
citric Acid	0.030	0.030	-	-	
magnesium chloride hexahydrate	-	-	0.061	-	
potassium carbonate hemihydrate	-	-	0.603	-	
sodium phosphate monobasic monohydrate	-	-	0.273	-	
sodium bicarbonate	-	-	-	2.100	
submaxillary mucin	-	-	1.000	-	
alpha-amylase	-	-	2.000	2.000	
mucin gastric	-	-	-	1.000	
Properties					
pH	6.5	7.4	-	-	6.8

are also present, and in addition to albumin, which might be a contaminant, include four non-serum apoproteins (SP-A, SP-B, SP-C and SP-D) (1).

Table 11 lists some of the most used simulated lung fluids (SLF). SLF1 has been used to evaluate human exposure to particulates from environmental emissions. Artificial lysosomal fluid (ALF) and Gamble's solution were used to simulate different interstitial conditions in the lung. ALF is analogous to the fluid with which inhaled particles would come into contact after phagocytosis by alveolar and interstitial macrophages in the lung. Gamble's solution represents the interstitial fluid deep within the lung. When preparing Gamble's solution, the components should be added in the order presented in Table A to avoid salt precipitation. Citrate was used instead of proteins to avoid foaming and acetate instead of organic acids. Gamble's solution has a pH of 7.4, whereas ALF has a pH of 4.5 and has a much higher organic content than Gamble's solution (32).

SLF2 is a modified version of Gamble's solution; it was used to estimate potential human exposure to Hg due to inhalation of airborne calcine particulates from mine waste. This simulated fluid was designed to model interactions of particles with extracellular lung fluids (33).

SLF3 models the interstitial fluid (ionic equivalent amount of citrate used to replace protein because of foaming). Care must be taken in mixing the components to prevent precipitation. The starting volume of water should be at least 95% of the desired final volume; otherwise, a precipitate may form when some compounds are added (34). This simulated fluid was used to evaluate the in vitro release of insulin from poly(lactide-co-glycolide) (PLGA)/cyclodextrin porous particles intended for pulmonary delivery of insulin (35).

Designing a standardized dissolution method applicable to the lung is not an easy task, because the lung has unique features that are difficult to replicate in vitro, such as the extremely small amount of aqueous fluid and lung surfactant. Son and McConville (36) described a potential standardized test method to characterize the dissolution properties of a multitude of formulation types available for pulmonary delivery. SLF3 and SLF4 (SLF3 modified with the addition of 0.02% [w/v] dipalmitoylphosphatidylcholine [DPPC]) were used. To prepare SLF4, 200 mg of DPPC was weighed into a 500-mL round-bottom flask and dissolved in 40 mL of a mixture of chloroform and methanol (1:1). The solvent was evaporated using a rotary evaporator. The dry film was dissolved with 200 mL of

Table 11. Simulated Lung Fluid (SLF)

Composition	SLF1		SLF2	SLF3	SLF4	SLF5
	ALF (g/L)	Gamble's Solution (g/L)	(mg/L)	(g/L)	(g/L)	mMol/L
magnesium chloride	0.050	0.095	-	0.2033 (hexahydrate)	MgCl ₂ hexahydrate 0.2033	-
sodium chloride	3.21	6.019	6800	6.0193	6.0193	116
potassium chloride	-	0.298	-	0.2982	0.2982	-
disodium hydrogen phosphate (Na ₂ HPO ₄)	0.071	0.126	1700 (monohydrate)	-	-	-
sodium sulfate	0.039	0.063	-	0.0710 (anhydrous)	0.0710 (anhydrous)	-
calcium chloride dihydrate	0.128	0.368	290	0.3676	0.3676	0.2
sodium acetate	-	0.574	580	0.9526 (trihydrate)	0.9526 (trihydrate)	-
sodium hydrogen carbonate (NaHCO ₃)	-	2.604	2300	2.6043	2.6043	27
sodium citrate dihydrate	0.077	0.097	-	0.0970	0.0970	0.2
sodium hydroxide	6.00	-	-	-	-	-
citric acid	20.8	-	420 (monohydrate)	-	-	-
glycine	0.059	-	450	-	-	5
sodium tartrate dihydrate	0.090	-	-	-	-	-
sodium lactate	0.085	-	-	-	-	-
sodium pyruvate	0.086	-	-	-	-	-
ammonium chloride	-	-	5300	-	-	10
phosphoric Acid	-	-	1200	-	-	-
sodium carbonate	-	-	630	-	-	-
potassium acid phthalate	-	-	200	-	-	-
sulfuric acid	-	-	510	-	-	0.5
sodium citrate dihydrate	-	-	590	-	-	-
sodium phosphate monobasic monohydrate	-	-	-	0.1420	0.1420	1.2
L-cystine hydrochloride	-	-	-	-	-	1.0
DPPC ^a	-	-	-	-	0.02% (w/v)	-
DTPA ^b	-	-	-	-	-	0.2
ABDCB ^c	-	-	-	-	-	50
Properties						
pH	4.5	7.4	7.4	7.4	7.4	-

^aDPPC: dipalmitoylphosphatidylcholine.

^bDTPA: diethylenetriaminepentaacetic acid.

^cABDCB: alkylbenzyl dimethyl ammonium chloride 50% by volume.

water at 55 °C and agitated for 2 h. The warm suspension was sonicated at 55 °C for 1 h. The concentrated DPPC solution was stored at 4 °C and diluted with SLF3 before use. SLF4 was used to evaluate the in vitro release of itraconazole from nebulized nanoparticle dispersions (37).

Cheng et al. (38) used SLF5 to evaluate the dissolution of titanium tritide particles used as components of neutron generators. These particles may be released into the air as aerosols during fabrication, assembling, and testing of components or in accidental or fugitive releases.

VAGINAL ROUTE

Until recently, vaginal preparations on the market were restricted to those that were topically effective for localized treatment of a variety of conditions. Current technologies in vaginal drug delivery are concerned with the systemic delivery of drugs such as estrogens, progesterones, and prostaglandins. New technologies explore the systemic delivery of therapeutic peptides and proteins via the vaginal route, for example.

Although the vaginal epithelium is relatively aglandular (in comparison with other possible delivery sites such as the gastrointestinal tract), it is usually covered with a surface film of moisture, “the vaginal fluid.” The vaginal fluid is composed of cervical fluid (the vagina receives approximately 2 g of mucus per day from the cervix) and small amounts of the secretion from Bartholin’s glands in the vaginal wall. The vaginal secretions, which serve as a protective barrier for infections, contain a variety of antimicrobial substances including lysozyme, lactoferrin, fibronectin, polyamines such as spermine, and secretory IgA. The fluids also contain carbohydrate from the epithelial glycogen, amino acids, aliphatic acids, and proteins.

The bioavailability of drugs administered via the vaginal route depends on both the effective dissolution of solid drug particles, if present, in the vaginal fluid prior to absorption and the degree of deactivation by enzymes present in the fluids.

The pH in the vaginal lumen is controlled primarily by lactic acid produced from cellular glycogen, or by carbohydrates produced by the action of the normal vaginal microflora. Vaginal pH in an adult female varies between 4.0 and 5.0, depending on the particular stage of the menstrual cycle. During pregnancy, the pH is lower and varies between 3.8 and 4.4 (due to an increase in cellular glycogen content); in the postmenopausal state or after oophorectomy, the pH increases to 7.0–7.4 because of the decrease in cellular glycogen content. Vaginal pH affects the degree of ionization of drugs, which can affect their absorption properties. Physiological changes in the pH of the vaginal fluids can also result in fluctuations in vaginal bioavailability.

The enzymatic activity in the vaginal epithelium is thought to be lower than in the gastrointestinal tract. The influence of the ovarian cycle on protease activity in the vagina has also been demonstrated. The activity of

Table 12. Simulated Vaginal Fluid (SVF)

Composition (in water)	SVF1 (g/L)	SVF2 (g/L)
sodium chloride	3.51	3.51
potassium hydroxide	1.40	1.40
calcium hydroxide	0.222	0.222
bovine serum albumin (BSA)	0.018	0.018
lactic acid	2.00	2.00
acetic acid	1.00	1.00
glycerol	0.16	0.16
urea	0.40	0.40
glucose	5.00	5.00
mucin	-	1.5% (w/v)
Properties		
pH	4.2	4.2

β-glucuronidase, acid phosphatase, alkaline phosphatase, and esterase all vary in the vaginal tissue of premenopausal and post-menopausal women (1).

Owen and Katz (39) developed a simulated vaginal fluid (SVF1) (Table 12) that models the fluid properties originating in the vagina, specifically the vaginal secretion found in healthy, nonpregnant, premenopausal women.

The volume of vaginal fluid can be measured in two ways, the amount of fluid present at any one time or the amount of fluid produced over an extended time interval. Some studies suggest that the daily production of vaginal fluid is around 6 g/day, with approximately 0.5–0.75 g present in the vagina at any one time. A volume of 0.75 mL of simulated vaginal fluid may be appropriate to evaluate some types of formulations (39).

SVF1 was used to evaluate the in vitro dissolution of polymer–lipid-based mucoadhesive microspheres containing econazole nitrate with the USP Apparatus 1 (basket) in a volume of 900 mL. SVF2 was used to hydrate these microspheres before evaluating their mucoadhesive properties (40).

The in vitro release of clindamycin from bioadhesive gel was performed using 15 mL of SVF1 in a vertical diffusion cell (41). This dosage form was composed of chitosan, which acts as both bioadhesive and permeation enhancer, and gellan gum, an ion-activated polymer that gels when in contact with ions (i.e., vaginal fluid). A similar evaluation was carried out using SVF1 (10 mL/2 g of product) with poloxamer 407-based vaginal in situ gel containing acyclovir (40).

The swelling behavior, the mucoadhesion, and the in vitro release of nystatin from a mucoadhesive vaginal delivery were evaluated using SVF1 (43).

Table 13. Simulated Semen Solution 1

Composition (final volume 100 mL)	Amount
0.123 M sodium phosphate monobasic monohydrate	5.46 mL
0.123 M sodium phosphate dibasic anhydrous	49.14 mL
sodium citrate dihydrate	813 mg
potassium chloride	90.8 mg
potassium hydroxide	88.1 mg
fructose	272 mg
glucose anhydrous	102 mg
lactic acid	62 mg
urea	45 mg
bovine serum albumin	5.04 g

Simulated Semen (SSe)

One of the applications of simulated semen is to evaluate its influence in the performance of vaginally applied topical therapeutic, contraceptive, and prophylactic formulations.

Human semen is a mixture of components produced by several different glands. These components are incompletely mixed during ejaculation; hence, the initial ejaculate is not an entirely homogeneous mixture. The prostate is the main source of acid phosphatase, citric acid, inositol, calcium, zinc, and magnesium. The seminal vesicles' contribution is rich in fructose, ascorbic acid, and prostaglandins, while the concentrations of L-carnitine and neutral alpha-glucosidase are indications of epididymal function. The final composition of SSe presented below models the properties of semen (after complete mixture) produced by healthy male donors.

Solution 2: 101 mg of calcium chloride dihydrate in 15.13 mL of water.

Solution 3: 92 mg of magnesium chloride hexahydrate in 15.13 mL of water.

Solution 4: 34.4 mg of zinc chloride in 15.13 mL of water.

First, add Solution 2, then Solution 3, and finally Solution 4 to Solution 1 (Table 13). The pH is adjusted to 7.7 with sodium hydroxide. The final solution is sterilized by filtration and can be kept frozen until the moment of use. This simulated semen has a viscosity of about 1.3 cP. Its viscosity can be increased to 4 cP by the addition of 0.17% of methyl cellulose (44).

Data from the literature indicate that the average volume of semen ejaculated is about 3.4 mL. This volume was used to evaluate drug release from vaginal gel formulations (44).

Table 14. Simulated Tears 1 and 2

Composition (in water)	Simulated Tears 1 (mg/mL)	Simulated Tears 2 (w/w)
sodium bicarbonate	192.4	0.2%
potassium chloride	111	-
calcium chloride	2.29	0.008% (as CaCl ₂ ·2H ₂ O)
sodium chloride	672.8	0.67 %
albumin	669	-
glucose	2.5	-
Properties		
pH	7.4	7.4

OPHTHALMIC ROUTE

This route is used only for the treatment of local conditions of the eye and cannot be used as a portal of drug entry to the systemic circulation. The pH of normal tears has a mean value of 7.4, but there are wide variations. It is lowest on awakening because of acid by-products associated with relatively anaerobic conditions in prolonged lid closure and increases because of loss of carbon dioxide as the eyes open. The tears are more acid in contact-lens wearers because of the impediment of the efflux of carbon dioxide, and more alkaline in diseases such as dry eye, severe ocular rosacea, and lachrymal stenosis. The surface tension of the tear fluid at the eye temperature is 43.6–46.6 mNm⁻¹ for normal eyes and 49.6 mNm⁻¹ for patients with dry eyes. The osmolality of tears is important, since optical integrity of the cornea is significantly influenced by the tonicity of the tears. The normal osmolality of tears varies from 290 to 310 mOsm kg⁻¹, which is almost equivalent to that of normal saline solution (1). Absorption of drugs into the eye requires good pre-corneal penetration and prolonged contact time with corneal tissues. Ideally, the formulation should sustain drug release and maintain contact with the eye for extended periods of time. The extent of absorption into the eye is severely limited by the physiological constraints such as reflex tearing and blinking. Further, drug loss occurs from tear turnover, solution drainage by gravity, and binding of drugs to proteins and other components of tears. As a result, typically only 1–2% of the instilled drug is bioavailable (45). The volume of tears in the eye is about 5–10 μL. The normal secretion is about 1–2 μL/min (46).

Simulated Tears 1 (Table 14) in the volume of 8 mL was used to evaluate the in vitro release kinetic of poly-anionic oligonucleotide macromolecules from cationic nanoemulsions with potential use for age-related macular degeneration treatment (47). Simulated Tears 2 (Table 14) in the volume of 4 mL was used to evaluate drug-loading efficiency and in vitro release of pilocarpine from

Table 15. Simulated Tears 3

Composition (in water)	Amount (mg/mL)
lysozyme	2.68
D-glucose	6.50
gamma globulin	1.34
sodium chloride	6.50
bovine serum albumin	2.68
CaCl ₂ ·2H ₂ O	0.08
Properties	
pH	7.4

hydrogels. These hydrogels behave as a drug-depot for controlled-release applications (48). The same simulated medium was also used to evaluate the in vitro release of pilocarpine from nanoparticles composed of chitosan and polyacrylic acid (49). Pandit et al. (45) developed in situ forming sodium alginate gels for the extended release of indomethacin to the eye. They evaluated the in vitro gelation using Simulated Tears 2 and Simulated Tears 3 (Table 15) and assessed the in vitro release using Simulated Tears 2 (200 mL/2 mL of sample).

SIMULATED SWEAT (SSW)

The main composition of human sweat is shown in Table 16. Shimamura et al. (50) prepared several simulated fluids (Table 17) using different proportions of the ions found in human sweat. The numbers in parentheses represents milliequivalents of calcium ions. Each ion concentration and pH in SSW(3) was adjusted to the median values of the human sweat in Table 16. For SSW(60), SSW(120), and SSW(240), each ion concentration except for calcium was adjusted to the upper limit and the pH to the lower limit of human sweat.

These simulated media were used to evaluate the in vitro release of nonsteroidal anti-inflammatory drugs from plasters. One of the major problems encountered in

Table 16. Main Composition of Human Sweat

Ion	Concentration (mEq/L)	Ion	Concentration (mEq/L)
sodium	9.7–94.1	chloride	0–65.1
calcium	0.2–6	phosphorus	0.003–0.014
magnesium	0.03–4	sulfur	0.022–0.231
iron	0.022–0.068	bromide	0.002–0.006
copper	0.002	fluoride	0.011–0.095
manganese	0.001–0.003	iodine	0.043–0.096
zinc	0.016–0.052	potassium	4.3–10.7

Table 17. Simulated Sweat

Salt	SSW(3) ^a	SSW(60) ^a	SSW(120) ^a	SSW(240) ^a
sodium chloride	2.92	5.49	5.49	5.49
calcium chloride	0.166	3.32	6.64	13.28
magnesium sulfate	0.12	0.24	0.24	0.24
potassium phosphate monobasic	1.02	1.36	1.36	1.36
Properties				
pH	5.4	4.5	4.5	4.5

^aThe numbers in parentheses indicate milliequivalents of calcium ions.

the in vitro drug-release test using aqueous solution was the swelling of the plaster due to the penetration of water, because the systems studied consisted of water-soluble polymers. Because the swelling of water-soluble polymers is suppressed by some metal ions, especially calcium, and by low pH, the use of simulated sweat with different concentrations of calcium and different pH was evaluated. As the formulation of plasters varies from manufacturer to manufacturer, SSW of different ionic concentrations is necessary to minimize the swelling. The in vitro release of fentanyl from transdermal systems was evaluated using 500 mL of SSW(60) using USP Apparatus 5 (paddle over disk) (51).

The British Standard Specification for the Reference Test Method for Release of Nickel from Products Intended to Come into Direct and Prolonged Contact with the Skin (52) has a recommended composition for simulated sweat (SSW2, Table 18), and the recommended volume of simulated fluid is about 1 mL per cm² sample area.

Nickel can induce contact sensitization when metallic objects made with nickel alloys are corroded by sweat. The estimation of the allergenic power of these metallic objects is based on an understanding of the electrodisso-lution mechanism of nickel in sweat. Colin et al. (53) characterized the corrosion layer of copper–nickel alloys in SSW2, with its pH adjusted to 6.5 with ammonia. SSW2 with an adjusted pH of 5 (54), an adjusted pH of 5.5 (55), and with pH values between 4.8 and 5.8 (56) was used to evaluate the corrosion behavior of magnesium alloys used in consumer electronic products. The amount of medium used in most of these studies was about 20 mL/cm² of sample area.

Table 18. Simulated Sweat 2 (SSW2)

Compound	Amount (% in mass)
sodium chloride	0.5
lactic acid	0.1
urea	0.1

The release of potential allergens from latex gloves was studied using 500 mL of SSW2 at several pH values, namely, 5, 6, 6.5, 7, 7.2, 7.4, and 8 (57).

CONCLUSION

Dissolution testing is most often used for the in vitro assessment of the in vivo behavior of a pharmaceutical dosage form, both for development and quality control. Early prediction of the absorption of drug candidates is a key factor in the development of pharmaceutical products. In routine quality control dissolution testing, simple dissolution media like aqueous buffer solutions are preferred because of cost, ease of preparation and recovery, and reproducibility. For development purposes, the use of more complex media, simulating the composition of the biological fluids of the site where the drug product will be administered and/or absorbed, is relevant. These media will subject the pharmaceutical dosage form to conditions that are closer to those encountered in vivo, which can increase the possibility of predicting differences between formulations, provide better understanding of drug-release mechanisms, and allow establishment of in vivo–in vitro correlation (IVIVC).

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