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REVIEW ARTICLE

BIORELEVANT DISSOLUTION MEDIA: ITS CURRENT STATUS AND FUTUTRE PERSPECTIVES

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Abstract:

The landscape of dissolution media has been dynamically evolving with newer drugs entering the market among which most of them are either BCS class II or BCS class IV drugs exhibiting poor solubility. Conventional dissolution raises lot of doubts in the pharmaceutical industry owing to its composition which fails to adequately mimic in-vivo conditions. The effect of bile salts, enzymes are prevalent factors which are often neglected by conventional dissolution media. To overcome these hurdles biorelevant media was suggested by several research groups and several developments have been witnessed to maintain a proximity to the required in-vivo conditions during dissolution. Media such as simulated gastric fluid, simulated intestinal fluid, milk-based dissolution media (Ensure®) have been suggested for fast and fed state taking into the considerations several factors like pH, surfactant concentration, etc. The recent improvement has been on fast state simulated intestinal fluid (FaSSIF- V2) where replacement of acetic acid by maleic acid is evident. Though biorelevant dissolution media has solved the primary hurdle of mimicking in-vivo conditions, it has still not been accepted as a quality control tool to test batch to batch consistency in pharmaceutical industry. The reasons being cost, time consumption and basic requirement of dissolution as test as discriminatory tool where biorelevant media fails at certain fronts where conventional dissolution passes fairly well. The use of surfactants like bile salts in biorelevant media shows increased dissolution profile of different batches and fails to discriminate between them. It is a common understanding that those compounds

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which show increased dissolution in conventional media are obvious to show better dissolution in biorelevant media. This is the reason why conventional media are preferred over biorelevant media during quality control evaluation and batch to batch variability tests to save cost and time. Biorelevant media are being used today majorly during developmental stages and still need to go a long way to be used as quality control tool.

Introduction:

The concept of dissolution was sown more than century ago in 1897 when Noyes & Whitney studied the dissolution of two sparingly soluble compounds, benzoic acid and lead chloride. The experimental set up was conceptually similar to the modern-day dissolution set up having a cylinder immersed in water held at constant temperature and contents inside rotated at constant speed [1]. The work led to the establishment of basic dissolution equation which is Noyes-Whitney equation. By 1950s the value of dissolution and it's testing in rate of absorption of dosage form was well understood. Today dissolution is not only used for drug development and quality control to check lot-to-lot consistency but also to check the bioequivalence, as an assistive tool in IVIVC [2], SUPAC, and biowaiver of BCS class I and III drugs [3]. The effectiveness of a formulation inordinately depends on the bioavailability which is the rate and extent of drug in systemic circulation that can be available at the site of action [4]. This can be easily estimated by in-vivo tests which takes in-to account valuable parameters like Cmax and AUC to estimate bioavailability[5]. In-vivo bioavailability estimation is an indispensable part of study in getting approval for New Drug Applications and novel formulations. But performing in-vivo tests to obtain bioavailability for Abbreviated New Drug Applications (ANDA) is unnecessary and it becomes a herculean task to estimate in-vivo data for large number of batches as it involves huge cost, loss of time and hindrances associated with animal ethics. Therefore, in-vivo bioequivalence is often predicted with help of in-vitro tests which follows a set of guidelines, protocols and methodology set by regulatory agencies all over the world. The USFDA has given several guidance documents on dissolution testing and acceptance criteria for Immediate Release (IR) dosage forms and also for SUPAC- IR/MR dissolution requirements. Apart from the instrumental and process parameters associated with dissolution methodology, the other most important aspects are dissolution media, the type used, volume used etc. A carefully set dissolution specifications will avoid the inconsistencies among different lots, better quality and life-cycle of product [6]. This review emphasizes on the advances in dissolution media with special focus on biorelevant dissolution media and its use in pharmaceutical industry today.

Physiological Conditions:

The absorption of drug from Gastro-Intestinal (GI) tract depends on the dissolution which depends on solubility of

drug in the gastric or intestinal fluids. The vital factors responsible for dissolution of dosage form in the lumen include the composition, volume and the hydrodynamics of the content present there. This is followed by permeability across the GI tract. Therefore, the major concern becomes the solubility of drug in GI media. This is affected by the pH and composition of media. The median pH in the upper gastric region in fasted state is 1.7 and in fed state is 5.0 in stomach [7]. Composition of contents depends on whether it is fasted state or fed state. In fasted state the gastric volume is around 15-50ml [8]it is hypo-osmotic in nature (d"200 mOsm/kg) [9], [10], with Na^+ and Cl^- being responsible. The gastric fluid has a surface tension of around 30 to 50 Nm/m, less than water(approximately70Nm/m) as reported by different researchers. Efentakis and Dressman found values of surface tension around 35-45 mN/m [11]. Finholt and Peterson in 1968 suggested through their research that the surface tension was in the range of 36-51 mN/m [12]. Pederson and his group conducted study of surface tension of gastric fluids on five healthy subjects which found surface tension in the range of 28-42 mN/m [13]. On the other hand, in fed state, the pH of gastric fluid rises and ranges from 4.3 to 5.4 [7]. Generally, the pH osmolarity and surface tension greatly depends on the type of food consumed. During fed state as the food moves towards small intestine, in duodenum the slightly alkaline secretion of liver and pancreas which is bile and pancreatic juices mix up with the chyme and raise the pH to 5.5-6.5 which is lower than that during fasted state in small intestine. There is irregular falling and rising of pH in proximal duodenum, but as the chyme moves along the small intestine fluctuations decrease. All these factors along the GI tract are responsible for modulating the solubility, dissolution and finally the rate and extent of drug absorption. In order to best mimic the in-vivo conditions to predict the behavior of solubility and dissolution inside the body, it is essential to replicate the in-vitro behavior in the best possible manner. Therefore, dissolution medias have been specified in the pharmacopoeias and by regulatory agencies so that there is similarity in dissolution testing. Though the most commonly used dissolution media has been the one specified by FDA in its guidance documents which are 0.1N HCl, pH_4.5 acetate buffer and pH_6.8 phosphate buffer which covers the pH of the GI range from pH 1.2 to 6.8, there have been other medias like simulated gastric and intestinal fluid and biorelevant dissolution media. The type of dissolution media selected as per the purpose, if it is for development purpose simulated or biorelevant media is mostly used and for quality control purposes compendial medias are used as specified by the regulatory agencies.

Dissolution media for Generic Drugs:

Dissolution methods specifications are often described in Pharmacopoeias of different countries or regions, If the product is intended to be marketed in US,

and that product is official in USP, USP specifications are followed. If the product is not official in USP, then, dissolution guidance recommended by FDA is followed. If FDA-recommended dissolution method is not available then a new dissolution method is developed which should include the pH solubility profile of the drug, dissolution in different media from pH 1.2, pH 4.5 acetate buffer and pH 6.8 phosphate buffer. For poorly soluble drugs surfactants can be used. The FDA in its guidance document for dissolution testing of IR dosage forms has sad that, efforts should be made to perform dissolution testing in physiological conditions as it will help to interpret dissolution data with regard to in-vivo product performance. At the same time flexibility in mimicking GI environment should be allowed for and absolute adherence to maintaining GI environment in routine dissolution testing is not necessary. Dissolution test conditions should be based on physicochemical characteristics and environmental conditions the dosage form might be present when administered orally. The suggested volume of dissolution medium is 500ml, 900ml or 1000ml where sink conditions are prudent but not binding. When aqueous media are employed it should be used in range of pH 1.2 to 6.8 (ionic strength of buffers the same as in USP). While mimicking simulate intestinal fluid (SIF), a dissolution medium of pH 6.8 should be utilized. Moving on to a higher pH should be reasoned appropriately depending on the case, in general, should be limited to pH 8.0. Gastric fluid should be simulated with a dissolution medium of pH 1.2 in absence of enzymes. The use of enzymes in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) should be reasoned appropriately depending on the case involved. Past knowledge through practical knowledge has indicates the need for use of (enzymes pepsin with SGF and pancreatin with SIF) to dissolve pellicles, in case formed, to allow drug dissolution. Source of water if varied and due to the active and inactive ingredients can be of a major concern leading to changes in pH and surface tension and hence water as a dissolution medium is not encouraged. For water insoluble or sparingly water-soluble drug products, use of a surfactant such as sodium lauryl sulphate is recommended [14]. The necessity for and the quantity of the surfactant should be reasoned appropriately. Use of a hydro-alcoholic medium is not encouraged. For ER and DR dosage forms similar protocol is followed for selecting dissolution media. In addition to developing any new dissolution method, dissolution profile should be generated in pH 1.2, pH 4.5 and pH 6.8. The ultimate aim should be selecting such a dissolution media which is discriminatory and replicating in-vivo physiological conditions.

Dissolution media for New Drug Applications as per US-FDA should be selected on basis of experience gained during drug development process and in-vitro performance of suitable test batches.

They should be based on acceptable clinical, pivotal bioavailability, and/or bioequivalence batches. For, New Chemical Entity (NCE), the dissolution characteristics of the drug product should be developed based on consideration of the pH solubility profile and pKa of the drug substance.

Simulated Gastric fluid (Fasted and Fed):

In fasted state as mentioned previously the median pH lies in the range of 1.5 to 1.9. The presence of pepsin and lipase becomes comparatively less significant to influence the dissolution during the fasted state. The pepsin production is 0.8mg/ml which becomes 0.08gm/ml when the drug is ingested with 200-250ml of water [15], [16]. The lipase secretion is 0.1mg/ml which on dilution again becomes less significant with the fact that it remains active only in pH range 3-6 [17]. The bile salts content in stomach in fasted state has been researched quite extensively. An average of bile salt content in stomach was found to be in range of 100-275 μM in a study conducted by Efentakis & Dressman [18] and Lindahl et al [10]. In fasted state a study conducted by Rhodes *et al.* found the bile salt concentrations upto 80μM with a standard error upto 30μM [19]. Surface tension as discussed earlier has been found in the range of 28-51mN/m. Osmolality has been found to be and reported in the range of 190-200 mosm/kg by Lindahl et al [10] and Pederson et al. [13] in their respective studies. The major cations in fasted state include sodium ion (70 mM), potassium (15 mM), chloride (100 mM) and some multivalent ions like calcium present in sub-millimolar concentrations. Depending on the physiological conditions in fasted state the composition for simulated gastric fluid in fasted state in presence of surfactant was given by Vertzoni et al. described in Table 1 [16].

Table 1. Simulated Gastric Fluid (Fasted State)

Composition	**Quantity**
HCl	0.01-0.05 N
Sodium Lauryl Sulfate	2.5 G
Sodium chloride	2 G
Distilled water q.s.	1000mL

In fed state the contents of stomach vary significantly depending on the meal consumed. Some have suggested that homogenized meal be used with a specific quantity of oil reflecting the fats present in food and co-administering with water. Then the pH, osmolarity and buffer capacity can be adjusted to best simulate the fed state conditions before the start of dissolution test in clinical studies. Apart from this, use of whole milk [20], [21] or product Ensure® [22], [23] has been suggested to mimic the fed state of stomach as it best describes the ratio of carbohydrate: protein: fat that may be present.

Simulated Intestinal fluids:

Fasted State: The buffer capacity slightly varies of fluids in fast state and fed state. In simulated fast state use of bile salts, lecithin potassium phosphate and some other components without the use of pancreatin has been given [24] in Table 2.

Table 2: SIF fasted state

Composition	Quantity
KH_2PO_4	0.029 M
Sodium Hydroxide (qs)	pH 6.8
Sodium Taurocholate	5 mM
Lecithin	1.5 mM
KCl	0.22 M
Distilled water (qs)	1000 mL

During fed state in small intestine, the meal induces secretion of more bile in small intestine which can improve the solubility of many poorly soluble BCS class II and IV drugs leading to better absorption. As buffer capacity slightly increases during fed state with decrease in pH, acetic acid has been used instead of potassium phosphate along with other components described in Table 3.

Table 3 : SIF Fed state

Composition	Quantity
Acetic acid	0.144 M
Sodium Hydroxide	pH 5
Sodium Taurocholate	15 mM
Lecithin	4.0 mM
KCl	0.19 M
Distilled water (qs)	1000 mL

Biorelevant Dissolution media development:

A constant debate over the dissolution medium to best mimic the in-vivo drug release and absorption led to the development of biorelevant dissolution media. Tremendous advances have been done in developing biorelevant media and several researchers have explored and provided compositions for biorelevant media. Biorelevant dissolution media has been primarily being used in predictions of in-vivo conditions, in developmental stages and in IVIVC correlations.

Galia et al., evaluated the effects of various dissolution media on dissolution of various BCS class I and II drugs. It was found the effects of dissolution media on dissolution of Class I drugs was not significant but greatly increased the dissolution of Class II drugs (Danazol, Mefenamic acid and Ketoconazole) in FaSSIF and FeSSIF media (Table 5) when compared with SGF, milk and Simulated Intestinal fluid without pancreatin (SIF_{sp}) [25]. Work of Vertzoni and his group highlighted the importance of simulated gastric fluid media in in-vivo dissolution of lipophilic compound GR253035X and atovaquone in fasted state simulated gastric fluid (FaSSGF), and simulated gastric fluid (SGF). GR253035X showed decreased dissolution in FaSSGF as compared to SGF_{sls} and for atovaquone dissolution was more or less limited in both media. Media composition is given in Table 4 for FaSSGF and SGF [16].

Table 4: Simulated Gastric Fluid Vs Fasted State Simulated Gastric fluid

Physicochemical Properties	**SGF**	**FaSSGF**
Sodium lauryl sulfate (% w/v)	0.25	-
Pepsin	-	0.1 mg/ml
Sodium Taurocholate	-	80 µm
Lecithin	-	20 µm
NaCl	34.2 mM	34.2 mM
Surface tension	33.7 mN/m	42.6 mN/m
Osmolarity (mosm/kg)	180±3.6	120.7±2.5
pH	1.2	1.6

Table 5: Fasted State and Fed State simulated Intestinal Fluid [25]:

	FaSSIF	FeSSIF
pH	6.5	5.0
Osmolality	270±10 mOsmol	635±10 mOsmol
Sodium Taurocholate	3.0 mM	15 mM
Lecithin	0.75 mM	3.75 mM
KH_2PO_4	3.9 g	-
Acetic acid	-	8.65 g
KCl	7.7 g	15.2 g
Sodium Hydroxide qs	pH 6.5	pH 5.0
Deionized water qs	1 L	1 L

Composition of Fed State Simulated Gastric Fluid (FeSSGF) for early, middle and late gastric conditions was suggested by Jantratid et al., to simulate postprandial conditions is given in Table 6, and an advanced prototype of Fasted State Simulated Intestinal fluid (FaSSIF-V2) was proposed with decreased lecithin and osmolality as compared to FaSSIF, and pH of 6.5 was maintained with help of maleate buffer, described in Table 7 [26].

Table 6: Biorelevant simulated FeSSGF

	Early FeSSGF	Middle FeSSGF	Late FeSSGF
Sodium chloride (mM)	147	237.02	122.6
Acetic acid (mM)	-	17.12	-
Sodium acetate (mM)	-	29.75	-
Ortho-phosphoric acid (mM)	-	-	5.5
Sodium dihydrogen phosphate (mM)	-	-	32
Milk/buffer	1:0	1:1	1:3
Hydrochloric acid/ sodium hydroxide	q.s. pH 6.4	q.s. pH 5	q.s. pH 3
pH	6.4	5	3
Osmolality (mOsm kg^{-1})	559	400	300
Buffer capacity (mmol l^{-1} ΔpH^{-1})	21.33	25	25

Table 7: FaSSIF- V2

Composition	Quantity
Sodium taurocholate (nM)	3
Lecithin (mM)	0.2
Maleic acid (mM)	19.12
Sodium hydroxide (mM)	34.8
Sodium chloride (Mm)	68.62
pH	6.5
Osmolality (mOsm/kg)	180±10
Buffer capacity (mmol/L ΔpH^{-1})	10

Ghazal et al. studied the effects of fats, protein and carbohydrates on the solubility and dissolution behavior of itraconazole where an increased solubility and dissolution was observed. Dissolution of itraconazole increased in FaSSIF media and further increased in FeSSIF media suggesting increased drug dissolution in post-prandial conditions [27]. In another study by Ghazal and his group, studied the intrinsic dissolution rate (IDR) of ketoconazole in biorelevant media. The IDR of ketoconazole varied with pH, with high IDR in pH 1.2 with decrease in IDR in SIF pH 6.8 [28]. Omuku et al., compared the dissolution of etoricoxib in different dissolution media including FaSSIF and FeSSIF and using computer simulations justified that etoricoxib could be classified as intermediate BCS class I/II rather than class II based on its solubility behavior. Though etoricoxib is a weak base no precipitation was seen during transition from low pH to high pH of biorelevant FaSSIF and SIF, indicating the role of bile salts and lecithin in aiding the drug to remain in solubilized form which suggests that during in-vivo conditions high solubility without precipitation can be expected in intestine [29]. Fagerberg et al., studied the dissolution and apparent solubility of ten BCS class II drugs in biorelevant dissolution media which led to the suggestion of upgrading five of the drugs to BCS class I category. Biorelevant media were also used to study the molecular factors like rigidity, hydrophilicity, permeability diffusivity etc. which were responsible for increased solubility in biorelevant media with help of multivariate analysis [30]. Clarysse et al., predicted that the solubilizing capacity of GI fluids might be higher than aqueous buffer systems. Clarysse et al. in another study compared the solubility of 17 model drugs in Human Intestinal fluid (HIF) with $FaSSIF_c$ and $FeSSIF_c$ and validated the same, suggesting such FaSSIF and FeSSIF can be predictive and alternative media for

intraluminal drug solubility estimation during drug discovery and early drug development phase [31]. Sachin et al., developed a novel probiotic biorelevant media which could serve as substitute to animal sacrificed based simulated colon media to test the dissolution of polysaccharide-based colon specific drug delivery. Sulfasalazine coated with different polysaccharide polymers were used to prepare spheroids that were tested in simulated colonic medium which were probiotic culture-based Fluid Thioglycolate medium (FTM), rat caecal contents, and human faecal slurries. The dissolution studies suggested no significant difference in dissolution profiles of different polysaccharide coated sulfasalazine in different media and also highlighted the benefits of simulated probiotic based FTM medium over other animal and human based colon specific dissolution medium [32].

Alhayali, Tavellin and Velaga studied the dissolution and precipitation behavior of solid dispersions of drug ezetimibe (EZ) in fasted state gastric and intestinal biorelevant media at different temperatures (25ºC and 37ºC). The study aimed at creating supersaturation of drug in intraluminal regions for enhanced absorption and bioavailability. The amorphous solid dispersions prepared by melt quenching (MQ) technique and spray dispersion (SD) techniques were evaluated for their precipitation behavior in biorelevant FaSSIF and FaSSGF and predict the in-vivo supersaturation behavior in best possible manner. The supersaturation and precipitation behavior of both formulations were temperature and media dependent. At 25ºC, the MQ was more soluble than SD of ezetimibe in FaSSIF, but at 37ºC precipitation behavior was disparate for both formulations in FaSSIF. However, in FaSSGF, irrespective of temperature the MQ gave enhanced solubility behavior than SD [33]. Madsen et al tried to establish the in-vivo supersaturation behavior of zafirlukast (ZA) in in-vitro conditions using biorelevant media in miniaturized dissolution apparatus µDissoProfiler™. Supersaturation behavior observed was disparate in different types of FedM and FastedM intestinal media. Supersaturation was of shorted time period in FedM than in FastedM, but concentration of drug dissolved during supersaturation period was higher in FastedM than FedM. Biorelevant dissolution media was able to distinguish between the effects of two polymers HPMC and PVP on supersaturation behavior of ZA, wherein precipitation of ZA was seen in FedM but not in FastedM indicating negative food effect on supersaturation of ZA in solution [34].

Some of the drug examples for which FDA guidance has been suggested or researched upon for performing dissolution in biorelevant media are;

Table 8: FDA suggested guidance, dissolution methods and research with biorelevant media

DRUGS	BIORELEVANT MEDIA	PURPOSE
Nitazoxanide [35]	Biorelevant FaSSGF Biorelevant FeSSGF Biorelevant FaSSGF-V2 Biorelevant FeSSGF-V2	Bioequivalence study
Canagliflozin (Can)/ Metformin HCl (Met) [36]	Met: Simulated Gastric Fluid [SGF] without enzyme, pH 1.2	Dissolution method
Linagliptin/Metformin HCl [36]	Simulated Gastric Fluid (SGF) without enzyme (pH 1.2) (degassed)	Dissolution method
Acetriptan [37]	Biorelevant FaSSGF Biorelevant FaSSIF-V2	Dissolution method development and Bioequivalence studies for generic version.
Furosemide (model drug) [38]	Simulated Gastric fluid Simulated Intestinal fluid	Exploring the new FloVitro™ technology
Danazol (100mg capsules) [38]	Fasted and Fed state media	In-vitro dissolution in FloVitro™ technology

Alcohol in Biorelevant Media:

It would not be fair to not consider alcohol in biorelevant media as alcohol acts as a co-solvent and helps in increasing the dissolution of most of the drugs. It is further justified by taking into account the world alcohol consumption data, as per National Institute of Alcohol Abuse and Alcoholism (NIAAA), in US approximately 56-70% of population above 18 consumed alcohol [39] and as per WHO, in Europe approximately 70% of adults consumed alcohol [40]. Alcohol and the resulting dose dumping is a serious concern incase of modified release dosage forms and abuse deterrent formulation. If the polymer or coating of MR dosage is such that it is soluble in alcohol then there are high chances that alcohol consumption

may induce dose dumping. The resulting adverse event will also depend on the therapeutic index, pharmacokinetics, etc. Taking into consideration the large population consuming alcohol and bitter history with alcohol dose dumping in a case like that of Palladone (a hydrocodone multiparticulate capsule), in 2005, led the regulatory agencies, of the European Union and United States to draw some guidance on effects of alcohol on dissolution [41]. Both agencies have given conditions of different alcohol concentrations in which dose dumping studies should be performed, Table 9. The use of alcohol (ethanol) as biorelevant media is should be on a case by case basis. It should be mandatory to test modified release dosage forms having drugs with narrow therapeutic window and with formulations of potential abuse. It also essential to consider the purpose of dissolution test whether it is to be used for quality control purpose or alcohol dose dumping studies.

Table 9: In-vitro Testing of Formulations at risk for ADD as per FDA and EMA [41].

	FDA	EMA
Requirements	Dissolution medium- 0.1N HCl Alcohol Concentrations: 0%, 5%, 20% & 40% Time- every 15mins until 2hrs	Dissolution medium: Same as that used in routine testing Alcohol Concentrations: 5%, 10%, 20%. Time: Not defined
Products to be tested	All modified release products especially opioid drugs and those having dose dumping risk	All oral modified release products

Biorelevant Dissolution Media (BDM): Future Perspectives

Biorelevant dissolution media has been majorly utilized in drug developmental studies. Its primary purpose has been to evaluate how well the drug molecule behaves in-vivo. Use of biorelevant media as a quality control tool has still not been very significantly proposed and the primary reason for it may be the increased cost and time involved in development of BDM and the consequent testing involved. All BDM have bile salts kind of surfactants added in them which can increase the solubility of poorly soluble drugs and hence sometimes fail to discriminate between experimental batches. Biorelevant media have been used in several cases to show that drug molecule

has good solubility and dissolution when characteristics when the same has failed in conventional dissolution media. The use of biorelevant media has still not found strong reasons to completely replace the conventional dissolution media, as the drug candidates which show good dissolution characteristics in conventional dissolution are obvious to show better dissolution characteristics in biorelevant dissolution media due to presence of bile like surfactants. Thus, biorelevant media surely is an essential tool to mimic physiological conditions and helpful in drug developmental stages, but its use as quality control tool to discriminate between experimental batches raises lot of questions.

References:

1. Whitney, Arthur A. Noyes & Willis R., "The rate of solution of solid substances in their own solutions," Journal of American Chemical Society, 1897;(19): pp. 930-934.

2. Uppoor, Venkata Ramana S., "Regulatory perspectives on in vitro (dissolution)/in vivo (bioavailability) correlations," journal of controlled release. 2001; (72): pp. 127-132.

3. Lawrence X. Yu, Gordan L. Amidon James E. Polli, Hong Zhao, Mehul U. Mehta, Dale P. Conner, Vinod P. Shah, Lawrence J. Lesko, Mei-Ling Chen, Vincent H. L. Lee, and Ajaz S. Hussain "Biopharmaceutics Classification System: The Scientific Basis for Biowaiver Extension," Pharmaceutical Research. 2002; (19): pp. 921-925.

4. Raimar Lobenberg, Gordon L. Amidon, "Modern bioavailability, bioequivalence and biopharmaceutics classification system. New Scientific approaches to international regulatory standards," European Journal of Pharmaceutics and Biopharmaceutics. 2000; (50): pp. 3-12.

5. Gordon L. Amidon, Hans Lennernas, Vinod P. Shah, John R. Crison, "A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of in vitro Drug Product Dissolution and in Vivo Bioavailability," Pharmaceutical Research.1995; (12): pp. 413-420.

6. V. P. Shah, "Dissolution: a quality control test vs. a bioequivalence test," Dissolution Technologies. 2001; (8): pp. 6-7.

7. Jennifer B. Dressman, Rosemary R. Berardi, Lambros C. Dermentzoglou, Tanya L. Russel, Stephen P. Schmaltz, Jeffrey L. Barnett, and Kathleen M. Jarvenpaa, "Upper Gastrointestinal (GI) pH in Young, Healthy Men and Women," Pharmaceutical Research. 1990; (7): pp. 756-761.

8. W Scott Jellish, Vyas Kartha, Elaine Fluder, Stephen Slogoff, "Effect of Metoclopramide on Gastric Fluid Volumes in Diabetic Patients Who Have Fasted before Elective Surgery," Anesthesiology: The Journal of the

American Society of Anesthesiologists. 2005; (102): pp. 904-909.

9. S Mahe, J F Huneau, P Marteau, F Thuillier, D Tome"Gastroileal nitrogen and electrolyte movements after bovine milk ingestion in humans," The American journal of clinical nutrition. 1992; (56): pp. 410-416.

10. Anders Lindahl, Anna-Lena Ungell, Lars Knutson, Hans Lennernas, "Characterization of Fluids from the Stomach and Proximal Jejunum in Men and Women," Pharmaceutical Research. 1997; (14): pp. 497-502.

11. Jennifer B. Dressman, Gordon L. Amidon, Christos Reppas, and Vinod P Shah, "Dissolution Testing as a Prognostic Tool for Oral Drug Absorption: Immediate Release Dosage Forms," Pharmaceutical Research. 1998; (15): pp. 11-22, 1998.

12. Per Finholt, Sissel Solvang, "Dissolution kinetics of drugs in human gastric juice- the of surface tension," Journal of Pharmaceutical sciences. 1968; (57): pp. 1322-1326.

13. Betty Lomstein Pederson, Anette Mullertz, Helle Bronsted, Henning Gjelstrup Kristensen, "A Comparison of the Solubility of Danazol in Human and Simulated Gastrointestinal Fluids," Pharmaceutical Research. 2000; (17): pp. 891-894.

14. Vinod P. Shah, John J. Konecny, Richard L. Everett, Brace McCullough, A. Carol Noorizadeh, "In Vitro Dissolution Profile of Water-Insoluble Drug Dosage Forms in the Presence of Surfactants," Pharmaceutical Research. 1989; (6): pp. 612-618.

15. R. Lambert, F. Martin and M. Vagne, "Relationship Between Hydrogen Ion and Pepsin Concentration in Human Gastric Secretion," Digestion. 1968; (1): pp. 65-77.

16. Maria Vertzoni, Jennifer Dressman, James Butler, John Hempenstall, Christos Reppas, "Simulation of fasting gastric conditions and its importance for the in vivo dissolution of lipophilic compounds," European Journal of Pharmaceutics and Biopharmaceutics. 2005; (60): pp. 413-417.

17. Frederic Carriere, Christophe Renou, Veronique Lopez, Josiane de Caro, Francine Ferrato, Hans Lengsfeld, Alain de Caro, Rene Laugier, Robert Verger, "The specific activities of human digestive lipases measured from the in vivo and in vitro lipolysis of test meals," Gastroenterology. 2000; (119): pp. 949-960.

18. Dressman, M. Efentakis and J.B., "Gastric juice as a dissolution medium: Surface tension and pH," European Journal of Drug Metabolism and Pharmacokinetics. 1998; (23): pp. 97-102.

19. J. Rhodes, D. Barnardo, S. Phillips, R. Rovelstad, A. Hofmann, "Increased reflux of bile into the stomach in patients with gastric ulcer," Gastroenterology. 1969; (57): pp. 497-502.

20. Sandra Klein, Jennifer B. Dressman, James Butler, John H. Hempenstall, Christos Reppas, "Media to simulate the postprandial stomach I. Matching the physicochemical characteristics of standard breakfasts," Journal of Pharmacy and Pharmacology. 2004; (56): pp. 605-610.

21. P. Macheras, M. Koupparis and C. Tsaprounis, "Drug dissolution studies in milk using the automated flow injection serial dynamic dialysis technique," International Journal of Pharmaceutics. 1986; (33): pp. 125-136.

22. L.J. Ashby, A.E. Beezer, G. Buckton, "In vitro dissolution testing of oral controlled release preparations in the presence of artificial foodstuffs. I. Exploration of alternative methododlogy: microcalorimetry," International Journal of Pharmaceutics. 1989; (51):pp. 245-251.

23. S. Klein, "Biorelevant dissolution test methods for modified release dosage forms," Shaker, 2005.

24. Jennifer B. Dressman, Gordon L. Amidon, Christos Reppas and Vinod P. Shah, "Dissolution Testing as a Prognostic Tool for Oral Drug Absorption: Immediate Release Dosage Forms," Pharmaceutical Research.1998; pp. 11-22, 1998.

25. E. galia, E. Nicolaides, D. Horter, R. Lobenberg, C. Reppas and J.B. Dressman, "Evaluation of Various Dissolution media for Predicting In Vivo Performance of Class I and II Drugs," Pharmaceutical Research. 1998; (15): pp. 698-705.

26. Ekarat Jantratid, Niels Janssen, Christos Reppas and Jennifer B. Dressman, "Dissolution Media Simulating Conditions in the Proximal Human Gastrointestinal Tract: An Update," Pharmaceutical Research. 2008; (25): pp. 1663.

27. Heba S. Ghazal, A. Mark Dyas, James L. Ford, Gillian A, Hutcheon, "In vitro evaluation of the dissolution behavior of itraconazole in bio-relevant media," International Journal of Pharmaceutics. 2009; (366): pp. 117-123.

28. Hebas A . Ghazal, A. Mark Dyas, James L. Ford & Gillian A. Hutcheon, "The impact of food components on the intrinsic dissolution rate of ketoconazole," Drug development and industrial pharmacy. 2015; (41): pp. 1647-1654.

29. Arthur Okumu, Marie DiMaso, Raimar Lobenberg, "Computer simulations using GastroPlus to Justify a biowaiver for etoricoxib solid oral drug products," European Journal of

Pharmaceutics and Biopharmaceutics. 2009; (72): pp. 91-98.

30. Jonas H. Faferberg, Oksana Tsinman, Na Sun, Konstantin Tsinman, Alex Avdeef and Christel A.S. Bergstrom, "Dissolution Rate and Apparent Solubility of Poorly Soluble Drugs in Biorelevant Dissolution Media," Molecular Pharmaceutics. 2010; (7): pp. 1419-1430.

31. Sarah Clarysse, Joachim Brouwers, Jan Tack, Pieter Annaert, Patrick Augustijns, "Intestinal drug solubility estimation based on simulated intestinal fluids: Comparison with solubility in human intestinal fluids," European Journal of Pharmaceutical Sciences. 2011; (43): pp. 260-269.

32. Sachin Kumar Singh, Ankit Kumar Yadav, G. Prudhviraj, Monica Gulati, Punnet Kaur, Yogyata Vaidya, "A novel dissolution method for evaluation of polysaccharide based colon specific delivery systems: A suitable alternativee to animal sacrifice," European Journal of Pharmaceutical Sciences. 2015; (73): pp. 72-80.

33. Amani Alhayali, Staffan Tavellin & Sitaram Velaga, "Dissolution and precipitation behavior of ternary solid dispersions of ezetimibe in biorelevant media," Drug Development and Industrial Pharmacy. 2017; (43): pp. 79-88.

34. Ceilie Maria Madsen, Ben Boyd, Thomas Rades, Annette Mullertz, "Supersaturation of zafirlukast in fasted and fed state intestinal media with and without precipitation inhibitors," European Journal of Pharmaceutical Sciences. 2016; (91): pp. 31-39.

35. "U.S. Food & Drug Administration," November 2018. [Online]. Available: https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm179184.pdf. [Accessed 25th-Monday February 2019].

36. "U.S. Food & Drug Administration," [Online]. Available: https://www.accessdata.fda.gov/scripts/cder/dissolution/dsp_SearchResults.cfm. [Accessed 25th- Monday February 2019].

37. "U.S. Food & Drug Administration," Sunday April 2018. [Online]. Available: https://www.fda.gov/downloads/Drugs/.../UCM304305.pdf. [Accessed 25th-Monday February 2019].

38. "U.S. Food & Drug Administration," 3rd October 2015. [Online]. Available: http://pqri.org/wp-content/uploads/2015/10/03-PQRI-Dissolution-Buhse-2015.pdf. [Accessed 25th- Monday February 2019].

39. "National Institute on Alcohol Abuse and Alcoholism," August 2018.

[Online]. Available: https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/alcohol-facts-and-statistics. [Accessed Tuesday March 2019].

40. "World Health Organization," [Online]. Available: http://www.euro.who.int/en/health-topics/disease-prevention/alcohol-use/data-and-statistics/fact-sheet-alcohol. [Accessed Tuesday March 2019].

41. Thomas P. Friebe, Firouz Asgarzadeh, Ann Gray, Kevin Hughes, Johann-Philipp Hebestreit, Yvonne Rosiaux, Mahmud Yunis, Amina Faham, "Regulatory Considerations for Alcohol-Induced Dose Dumping of Oral Modified-Release Formulations," Pharamceutical Technology. 2015; (38): pp. 40-46.