Physiological Considerations for Drug Dissolution Testing

Saeed A. Qureshi, Ph.D. (www.drug-dissolution-testing.com)

It is often suggested that drug dissolution testing should be conducted using a physiological environment to better characterise a product (dissolution/absorption) behaviour in humans. It is, therefore, important for an analyst to have a clear understanding of *the environment and processes* of the human gastrointestinal tract relevant to the absorption of food/nutrients which are extended to the drugs taken. The words *environment and processes* are intentionally used to avoid confusing or distressing analysts who may not have formal training in human physiology. Therefore, if it helps the analysts, they should consider these exactly like chemical environments and processes. If further details are desired they may consult a basic physiology book. However, in this article, terminologies of physiology and *the environment and processes* are used interchangeably. *The digestion step in humans is literally a chemical process just like is a chemical reaction in a rector followed by an extraction step.* Therefore, for the convenience of understanding, the following paragraphs first describe the physiological aspects and then are translated into simple chemical equivalents.

Common books of physiology and anatomy often show colourful pictures of internal organs, for example see (link), however, in simple terminology the GI tract consists of a long hollow tube with six interconnected compartments having different shapes and sizes (see Figure 1a): (1) mouth (2) esophagus (3) stomach (4) small intestine, as it has a smaller diameter which is divided into three segments; duodenum, jejunum and ileum (5) large intestine or colon and (6) rectum. If these compartments are to be described with chemical/mechanical terminology, then these will be: (1) grinder (2) conveyer (3) mixer/blender (4) extractor (5) concentrator and (6) waste dump/bucket, respectively. In addition to these compartments, there are two other important tissues/organs which play a critical role in the absorption process, and are also considered part of the GI tract, they are the liver and pancreas. In non-physiological terminology, these may be considered as suppliers of needed chemical ingredients which are emulsifiers and catalysts commonly known as bile and enzymes, respectively (see Figure 1b). From the chemistry aspect, bile is mixture of salts of cholic acids. These are emulsifiers and are necessary to breakdown the fat globules for easy hydrolysis to fatty acids which are absorbed into the body. On the other hand, the pancreas provides enzymes for breaking down the fats, proteins and, carbohydrates for absorption. The mechanical and chemical processes within the GI tract are nature's way of processing the food we eat so that the useful and required ingredients should be absorbed into the body for our health and nourishment.

It is important to note that we use these chemical and mechanical processes of absorption to our advantage to deliver the drugs to the body. More importantly, a formulator's objective is to use the absorption processes to his or her advantage while avoiding any of the potential negative impacts of these processes which may breakdown the drug or reduces its absorption.

Let us consider, in a step by step manner, what happens in the GI tract, starting with the mouth. In the mouth one chews (masticate) so that food gets broken down into smaller pieces and mixes with saliva (mixture of water and enzymes in particular lipase) forming a soft bolus which is pushed into the stomach through the esophagus. The transit time in the esophagus is very short, in seconds. However, once the bolus reaches the stomach the crushed food is stored in the stomach. When the meal is complete, the stomach receives gastric juice which is the name given to a solution containing mostly hydrochloric acid (HCl), mucous and enzyme (pepsin). At this stage, the stomach churns to provide mixing (see video), and further breakdown the food resulting in a thick slurry called chyme. There exists a stop-valve (called the pylorus) which controls the exit of the chyme from the stomach. Normally particles larger than 5 mm in size are pushed back (regurgitation) for further grinding, however, smaller particles and the slurry passes through the pylorus to the intestine, in periodic expels. This process of regurgitation and transfer of slurry continues until all food is transferred from the stomach which may take from 3 to 6 hours, and is commonly referred to as stomach emptying time.

Different type of foods take different amounts of time to get transferred, for example fats take the longest time while carbohydrates the shortest, the proteins in between. Another aspect which also controls the stomach emptying and churning is the amount of content present in the first part of the small intestine (duodenum). If the amount is not absorbed or processed by this part i.e. duodenum "feels full" then the digestion step stops or slows down.

The first part of the intestine, i.e. duodenum, receives the chyme which is acidic (pH 1-3) in nature and is neutralized by the pancreatic juice which is high in content of bicarbonate buffer. This neutralization step (higher pH), not only protects the duodenum from high acidity but is also required for the pancreatic enzymes to work which are also delivered as a part of pancreatic juice. At the same time, the duodenum also receives the bile juice from the bile duct directly from the liver or mostly from the gallbladder where bile is stored while on the way to the intestine from the liver when not in need. With the addition of more juice, the process of mixing and dilution continues at this stage, however, significant absorption of ingredients occurs here.

A similar process of mixing stirring continues in rest of the intestine (jejunum and ileum). However, a very important point to remember is that most of the absorption occurs in the small intestine. The main reason for such absorption is the availability of an extremely large surface area of the intestine. By the time the content pass through the small intestine, it passes through about two tennis court equivalent of absorption surface.

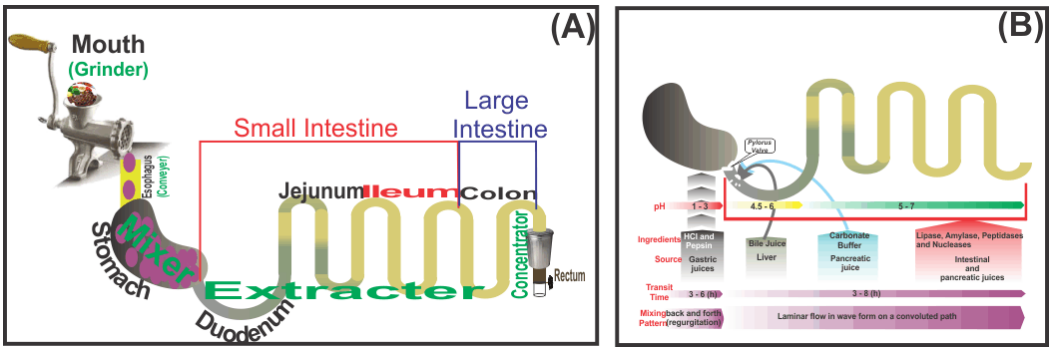

Figure 1: Various compartments of a GI tract

Although, the absorption can also occurs in the stomach, due to the limited available surface area and lack of well processed food, absorption here is relatively non-existent. On the other hand, considering the large surface area almost all the absorption occurs in the small intestine.

The process of absorption of water along with some other small nutrients starts once the content of the small intestine reaches the large intestine. The content starts concentrating which prepares un-absorbable content of the food for defecation which is stored in the last part of the colon and in the rectum.

From the drug efficacy aspect, which depends on the absorption, two compartments, the stomach and the small intestine, are the most relevant (see Figure 1b). As products (tablets and capsules) are often swallowed whole thus the mouth and esophagus play a little role other than a passage channel. On the other hand, most of the drug is expected to be absorbed through the small intestine, thus compartments after the small intestine would also be of little relevance as well, at least in most cases.

The environment and processes, therefore, in the stomach and small intestine are the most important to consider. Considering the discussion provided above, these environments and processes can be divided into two categories: (1) mechanical and (2) chemical. The mechanical aspect relates to the stirring and mixing aspects of the stomach and the intestine (see video). In this regard, the stomach provides a fairly intense churning and mixing motion while the intestine provides a relatively softer motion but highly efficient mixing. As most of

the drug would be absorbed in the small intestine, thus the mixing and stirring environment of this area would be most important.

On the other hand, a chemical environment means aqueous phase (slurry) containing salts, emulsifiers (bile acids or salts) and enzymes. These are summarized as follows:

Major ingredients of gastrointestinal (GI) tract contents	
Stomach (pH=1-3)	Enzyme (Pepsin): source=gastric juice Hydrochloric Acid: Source=gastric Juice
Small Intestine (pH=4.5-7)	Enzymes (peptidase, sucrase, maltase, lactase, lipase, amylase, nuclease): Source=intestinal and pancreatic juices Bicarbonate solution: Source=pancreatic juice Bile acid/salts: Source=bile from liver/gall bladder

All these contents are maintained and/or processed at 37 °C.

From the aspect of drug dissolution one has to keep these two mechanical and chemical environments/processes in mind which are to be used to our advantage to provide efficient and predictable drugs into to human body through blood circulation. From the mechanical aspect, products in particular tablets should be compressed appropriately so that they should be able to disintegrate under physiological stirring and mixing environment.

From the chemical aspect, drugs are expected to be stable at pH 1-3 (in the stomach), otherwise it would not be possible for the drugs to reach to the intestine for absorption. In cases where drugs are sensitive to the higher acidity of the stomach they are formulated with a pH 1-3 resistant coating and these products are commonly known as enteric-coated products. For all practical purposes, one may consider the presence of a drug in a thin slurry, containing components of food (fat, proteins, starch) which would now be mostly in the form of fatty acids, amino acids, and carbohydrates (mono/disaccharides) along with bile components and the enzymes.

The drug or drugs have to live and survive in this environment and be absorbed into blood circulation. This is the idea behind the drug development in particular for those which are to be administered through the oral route, i.e., drugs should be stable and absorbable in this environment.

Before moving further regarding drug absorption and/or dissolution/release of drug from products (tablet and capsules), it is very important to recognize that the formation of slurry/soup in the intestine is an extremely controlled and reproducible process. Production of enzymes and bile and their delivery to the exact locations and in the exact amounts, movement of food/drug, maintenance of temperature are all extremely complex chemical processes but precisely controlled by perhaps even more complex feed-back mechanisms of hormones, neurons, and metabolism. However, the fact remains that even with such complexity, from a drug absorption perspective, fortunately, we often only have to deal with the relatively simple “soup”.

An analogy here may explain the situation better by describing the availability of orange juice in a 1 L bottle or carton. If some one likes to enjoy a glass of good orange juice to get a small “dose” of vitamin C, which is the net result of extremely complex production processes. These may include growing oranges (agriculture) which depends of the climate and availability of water or rain and then extraction, processing, packing/storage followed by delivery to a customer with an analytical report of ingredients to reflect consistency from batch to batch. The same sort of thing happens within the small intestine, where natural variation may occur but consistency and reproducibility of the slurry, like orange juice, will remarkably be similar or the same. Therefore for all practical purposes this environment of the small intestine may be considered highly reproducible and consistent.

Fasted and fed states may also provide differences from the drug absorption perspective which a formulator needs to keep in mind. During the fasted state the GI tract (stomach and intestine) is in a resting state or in limited agitation motions. Once in a while (about every 2 hours) a strong wave occurs in the stomach as a part of house-keeping waves (more formally known as a

migrating motor complex or MMC) and pushes the content of the stomach to empty or clean the intestine. These waves are usually very strong and cause the transfer of very large particles and items in the stomach (large tablets even coins) to push it through the pylorus. Similarly, in the small intestine processing remains more or less similar. Obviously bile acids and enzyme will be present but in much diminished quantities. The pH remains in the same range i.e. 1-3 for stomach and 4.5-7 for intestine.

The major differences which occur between fasted and fed state is that of the motility of the stomach. This may be explained as follows: let us assume a healthy volunteer takes in a light meal, the stomach will stir and mix and sort of slowly and continuously release its content into the intestine along with the drug present after disintegration of the tablet. If a person takes a heavy meal (fat/meat etc) the stomach will require a lot more time to digest it (preparing for intestine). This digestion step could be erratic and inconsistent. Therefore, if a drug product is present in the stomach while food is digesting, its delivery will also be delayed and erratic as well. What this means is that with the fed state the major impact will be on the delivery of the drug to the intestine, thus absorption will be delayed and erratic compared to the fasted state. To control this erratic delivery or absorption often drugs are given with standardized meals which help in reducing the variability of absorption but one cannot control inter-, even intra-, individual variability in the emptying of the stomach. This is why often one would see significant delays and erratic in drug absorption thus blood drug levels with meals. Often products such as suspensions, beads and osmotic based, which usually do not require a disintegration step, tends to be less variable as the delivery through pylorus may be considered somewhat constant and stable.

To conduct an in vitro dissolution test, therefore, one is required to simulate the in vivo (GI tract) environment as closely as possible and as relevant as possible. In this regard, the following discussion may help.

It appears that there is some confusion in this regard as the analysts/formulators tries to duplicate rather than simulate the "necessary" GI tract environment. For example, it is often suggested that, to represent the GI tract, "ideally" one needs to have tubular path with peristaltic movements (mixing) as are in the GI tract along with continuously changing (increasing) pH environment and containing enzyme and bile. However, it is not necessary. The analyst/formulator needs to focus on the relevant and necessary mechanics and chemistry of the GI tract. From the dissolution testing perspective stirring and mixing, pH and presence of bile appears to be the most critical.

Although, a product passes through the stomach, it plays a limited role in the absorption of drug, except potential disintegration of the product. In fact, the products/drugs are often protected from the high acidic environment of the stomach. Therefore, from the dissolution testing perspective the stomach and its environment plays a limited role. It is the small intestine and its environment which is the most relevant one. Thus one needs to simulate this environment. In this regard, one requires an aqueous solution having a pH in the range of 5-7 and some small amount of bile acids or their equivalent along with a soft but thorough stirring/mixing mechanism. The role or requirement of enzymes from dissolution purposes does not appear to be relevant or necessary. It is important to note that as analysts or formulators we are only concerned with transfer of drug from product (tablets/capsule) into solution. We are not even concerned about the absorption because absorption is dependent on the drug in solution. *Our objective for drug dissolution testing is only to evaluate the breaking down of the product and dissolution of the drug into a solution*.

In this regards, it is appropriate to assume that enzymes, at least in most cases, play a limited role in breaking down the drug product and its dissolution. However, stirring/mixing, presence of bile acids and the pH of the medium play a significant role in the dissolution of drug in the in vivo environment. Therefore, these three variables need to be controlled appropriately. There are no absolute or exact characteristics or values for these parameters, but should be agreed upon a "norm". For example:

1. Stirring and mixing: What should be the most appropriate mechanism of stirring and mixing; peristaltic, regurgitation, simple or complex mixing etc.? It appears that a simple stirring and mixing

mechanism serves the purpose adequately. The vessel based apparatuses appears appropriate with a modified stirring mechanism. *As the paddle and basket stirrers do not provide appropriate stirring and mixing thus their use should be avoided.*

2. Medium: The purpose of the dissolution medium is to represent liquid content of the GI tract. As most of the absorption of drugs occurs in the small intestine, the liquid should represent this environment. The liquid should be aqueous based having a pH in the range of 5-7. There appears to be a misconception where it is often assumed that pH varies in the GI tract as a gradient in the range of 1 to 7. However, most often pH of the liquid phase exists in two sets of ranges 1-3 (stomach) and 4.5-7 in the small intestine. As the absorption occurs in the small intestine, therefore, pH of the dissolution medium between 5 and 7 should be appropriate. In this regard water (distilled) appears to serve the purpose well. The volume of dissolution medium should be such that it should able to dissolve the expected amount of drug. Commonly 900 mL of water is used which should be considered as a first choice. However, if 900 mL volume does not allow the drug to dissolve then some small amounts of solubiliser should be considered (further details below).

3. Use of solubilisers: Often limitations of the volume of dissolution medium causes a problem with dissolution testing, as it is difficult to dissolve the expected amount of drug from a product in the medium (900 mL). This is strictly an in vitro environment or testing issue. Therefore, the analyst is required to use/add a solubiliser to enhance the solubility capacity of the dissolution medium. The nature of the solubiliser should be such that the medium maintains its GI tract relevancy. In this regard, often bile acids equivalents are often used. The most commonly used solubiliser in this regard is sodium lauryl sulphate (SLS) which is similar in properties as of bile salts thus provides GI relevance.

Therefore, a vessel based apparatus ***with a modified stirrer***, 900 mL water with or without SLS maintained at 37 °C provides an appropriate dissolution testing environment for most of the drug products. Although, one often finds suggestions of numerous dissolution media and test conditions in literature, mostly drug and/or product dependent, this practice is neither valid nor reflects the GI environment or physiology. As the human physiology remains the same and is independent of products, dissolution tests must be conducted using a product independent environment as well.

For a more detailed discussion on the subject of conducting in vitro drug dissolution tests, some relevant links and references are provided.

To conclude:

The GI tract consists of different interconnected compartments. From a drug absorption perspective the stomach and small intestine are the most important ones. The mechanical and chemical environments within these two compartments dictate drug dissolution testing conditions. The mechanical environment refers to the stirring and mixing aspect, where this stirring and mixing is usually quite intense within the stomach, however softer but thorough in the small intestine. The chemical environment refers to the presence of enzymes, bile, acids, HCl and buffers (bicarbonate) while maintaining the pH in the ranges of 1-3 (stomach) and 4.5-7 (intestine). *From a drug dissolution perspective, these environments appears quite harmless to products and their excipients (inactive ingredients). Sensitivities of drugs to and from these chemical environments can be a cause of concern which should be evaluated using drugs in solutions, prior and separate to the evaluations of the products*. Considering the relevant GI tract environment, drug dissolution testing may be conducted using a vessel based apparatus, ***but with a modified stirrer***, using water (with or without a solubiliser) as a dissolution medium maintained at 37 °C. It is important to note that dissolution test conditions must be product independent and represent a human physiological environment.

Some relevant references with links:

1. In Vitro-In Vivo Correlation (IVIVC) and Determining Drug Concentrations in Blood from Dissolution Testing – A Simple and Practical Approach (Link)

2. Limitations of Some Commonly Described Practices in Drug Dissolution Testing and Suggestions to Address These. (Link)

3. Drug Dissolution Testing: Selecting a Dissolution Medium. (Link)

4. A new crescent-shaped spindle for drug dissolution testing - but why a new spindle? (Link)

5. "Typical" variability in drug dissolution testing: study with USP and FDA calibrator tablets and a marketed drug (glibenclamide) product (Abstract)

6. Defining a dissolution apparatus (Link)

7. In Vivo vs In Vitro Bioequivalence (Link)

8. A bio-relevant dissolution test? (Link)

9. Dissolution-Absorption Link. (Link)

10. IVIVC – Conflict between practices and objective/intent. (Link)

11. Product dependent dissolution testing – a scientifically invalid practice (Link)

Feedback to indicate errors, omissions and/or suggestions in general will be greatly appreciated (moderator@drug-dissolution-testing.com).