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A Critical Assessment of Current Practices of Drug Dissolution Testing – Irrelevancies, their Causes and Suggestions to Address These

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Abstract

In principle, drug dissolution testing should be one of the simplest analytical techniques, however, in practice it is perhaps the most confusing, complex and frustrating techniques often lacking scientific and/or logical considerations. Perhaps the strange aspect of current practices is that despite the availability of hundreds, if not thousands, of methods and numerous regulatory guidance, if given a blinded sample of a simple tablet or capsule product one cannot determine its dissolution characteristics. On the other hand, fortunately, if one applies common and well-established scientific principles and logical judgements, drug dissolution testing can become a powerful analytical tool based on a simple set of experimental conditions. The purpose of this article is to highlight some of the critical irrelevancies of current practices. Describing basic principles of underlying science, a number of suggestions are made for simplifications and improvements of testing and thus product evaluations such as; A single product and drug independent approach/method for conducting dissolution tests alleviating the need for current dissolution method development practices. A simple and practical approach based on convolution technique, termed as *in vitro*-to-*in vivo* profiling or IVIVP, for estimating plasma drug concentrations-time profiles from dissolution results using Excel spreadsheet software. Using the improved dissolution testing approach to determine related quality parameters, such as identity, assay/potency and content uniformity, thus providing significant simplicity and saving of resources. In addition, a discussion is provided to circumvent issues of low solubility of drugs, requirements of a sink condition, and *in vitro-in vivo* correlation (IVIVC) practices.

Keywords: Dissolution method development; *In vitro-in vivo* correlation (IVIVC); Sink condition; *in vitro*-to-*in vivo* profiling (IVIVP); Convolution; Biopharmaceutic classification system

Basic and Fundamental Considerations for Dissolution Testing

A drug dissolution test is conducted to evaluate dissolution or release characteristics of the drug from a product, in particular tablet or capsule. Drug dissolution and release are to be considered one and the same thing, thus the terminologies are used interchangeably throughout this article. Drug dissolution testing is one of the most important and useful techniques and is extensively used for the development and manufacturing , along with a worldwide regulatory requisite to establish safety, efficacy and quality, of solid oral dosage forms, in particular tablet and capsule [1-4]. Dissolution tests are conducted to evaluate drug release characteristics of a product *in vivo* or in the gastrointestinal (GI) tract. The reason for such an assessment is that for a product to provide its therapeutic effects it should be present in the systemic circulation (blood). To be present in the blood, the drug needs to be absorbed from the GI tract which requires the drug release from the product and then be dissolved in an aqueous content of the GI tract. Therefore, the products administered through the GI tract (i.e., oral route) require a dissolution step and *in vitro* dissolution testing is conducted to evaluate this step. It is important to note that *in vitro* dissolution test is conducted to evaluate the *in vivo* dissolution. This forms the basis of conducting a dissolution test.

A dissolution test is considered a quality control tool as well during the manufacturing of the products. The underlying assumption for such a practice is that if a product is capable of providing expected drug dissolution *in vitro*, then it will be of expected quality in humans thus the quality control or assurance test. Just like a thermometer which only monitors body temperature but does not have any capability to monitor body functions, a dissolution test monitors dissolution characteristics of the end product (tablet/capsule) not the functioning or operation of the manufacturing. Just like a normal healthy person showing a body temperature of 37°C, all things being equal and normal, does not raise any concerns about the health of the person, similarly observing an expected drug dissolution characteristic, while all things being equal, the product is considered of expected quality for human use. It is important to note that the role of drug dissolution testing, as a quality control test, is dependent on the drug release evaluation of the product which is linked to the *in vivo* drug release, not to the operation of the manufacturing.

As the drug dissolution tests are conducted to assess the *in vivo* dissolution therefore these must also be conducted using experimental conditions simulating the *in vivo* conditions or environment as closely as possible.

Representing Dissolution Testing Environment for Products Evaluation

When an oral product, usually a tablet or capsule, is taken, it almost instantaneously goes into the stomach (gastric compartment). The gastric environment can be described as acidic, mostly a hydrochloric acid (HCl) based aqueous solution (pH 1 to 3, mostly 1) with a churning (moving and mixing) process. Assuming a disintegrating type product, the product will disintegrate into solid particles/aggregates. Once in

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this disintegrated form, the drug will behave exactly like granules in dilute acidic solvent, with mild stirring, in a beaker or flask. In case of non-disintegrating type tablets, the drug will be released or leaked-out from the unit into the acidic solvent.

If the drug is soluble then it will move into the intestine as a solution, otherwise as a slurry or suspension. The important aspect to note here is that it is with some delay, the drug will move into the intestinal compartment. Here the acidic solution, or suspension, will be mixed with a strong buffer turning the acidic liquid to basic, or more accurately less acidic, in the pH range of 5 to 7. Considering the variability in contents, the rates of entrance of the two liquids i.e. slurry from the stomach and the buffer from the pancreas, it is almost impossible to accurately determine or establish the pH of the mixture. However, for absorption purposes, pH in the intestine can be assumed in the range of 5 and 7 [5-8]. Therefore, for all practical and standardization purposes one can use a pH of 6, the average of 5 and 7.

After reaching the intestinal compartment, the drug will be in a solution or suspension/slurry form as well, depending on its solubility characteristics at pH of 6, exactly like it would be in a beaker containing an aqueous solvent having a pH of 6.

The drug for all practical purposes, can be considered in solution (dissolved), or in a mixture (slurry or precipitated), form in the two compartments, i.e., stomach and intestine at the body temperature. Therefore, to represent this situation *in vitro* one would require that the drug should also be present in the aqueous phase, having pH 1 or 6. Further, as the drug is constantly moving forward in the GI tract resulting in constant mixing and churning, *in vitro* testing should also provide this stirring and mixing environment.

Selecting Dissolution Medium Representing Physiological Environment

From the previous explanation, one should conclude that for dissolution, and absorption, purposes the characteristics of a drug may be considered exactly like a drug in a beaker, either in a weak acidic (HCl) solution or an almost neutral (pH) aqueous solution. If one considers such behaviour of a drug, then it becomes easier to understand and/or evaluate the behaviour of drug dissolution and/or absorption in the body.

The process of dissolution and absorption may be explained with the following analogy. Consider if one is given an assignment to extract propranolol (PL), a drug commonly used, from a mixture of microcrystalline cellulose (MC) and a propranolol·HCl (PL·HCl). In a sense, it will be a fractional extraction procedure where one would exploit differences in the chemical or physical properties of these two compounds. The difference one would observe will be in their aqueous solubilities; MC is not soluble in water, but PL·HCl is. So, one can separate PL·HCl from MC simply by adding some water and filtering it. The PL·HCl will remain in solution form but MC will be separated out as precipitate. However, PL will still be in its hydrochloride form. To extract the PL, one may require a liquid-liquid extraction step. In this regard, one first needs to adjust the pH of the aqueous solution so that the HCl part can be neutralized and PL·HCl should be available as PL which could then be extracted with an organic solvent (e.g., hexane or dichloromethane). Adding some alkaline solution to PL solution will increase the pH of the solution to a much higher level, e.g. pH 12. Most of the PL will now be in undissociated form and can be extracted into the organic phase. One or two extraction repeats will transfer PL into the organic phase which may be removed by evaporation, leaving behind pure PL in its native or basic form.

On the other hand, if one is unable to increase the pH of the solution to 12 to avoid potential complications then a lower pH may be used. The same extraction step can be used; however, one would require an increased number of extraction repeats for complete extraction of PL from the aqueous solution. The end result will be the same, i.e., complete extraction of PL form in its native or basic form in the organic solvent.

It is important to note that one can also perform the above described extraction in one step (i.e., without separating MC by filtration first). In this case, adding some milder buffer having a pH around 7 to the mixture, and extracting with organic solvents.

Now let us assume that this extraction process of PL is to occur in the intestinal tube rather than in a glass test tube as explained above. The content of the intestinal tube are at a pH, between 5 and 7, and contains multiple endogenous and exogenous compounds including PL and MC. The organic phase for test tube experiment is replaced with the lipid layers of the intestinal tube walls. When the undissociated PL comes in contact with the lipid part of the intestinal tube it will get extracted or absorbed. A schematic representation of this extraction/absorption process is shown in Figure 1. This process will occur almost an infinite number of times (considering the vast surface area of the intestines). Once PL, or any other drug, gets absorbed it will be transferred to the blood stream.

This process of liquid-liquid drug extraction is commonly referred to as absorption of drugs in humans by passive diffusion. However, the process in both cases (*in vitro* vs. *in vivo*) is almost the same, if not exactly the same. It is worth remembering that the majority of drugs are absorbed from the GI tract by a passive diffusion process [9].

What this means is that when one takes a solid oral product (tablet and capsule), the drug must first comes out of the product, and then dissolve as a non-polar (undissociated) drug in the intestinal fluid. The drug does not have to be completely dissolved but to the extent that the

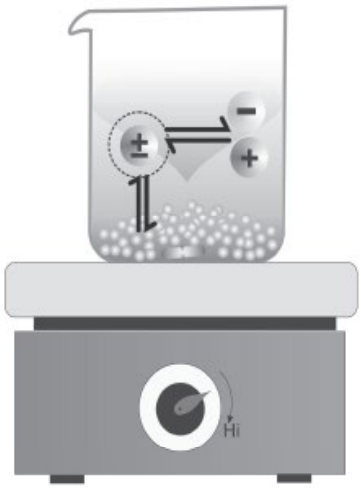

Figure 1: Schematic representation of the equilibriums of different drug entities in an aqueous solution reflecting availability of an undissociated drug usually responsible for drug absorption through the GI tract. Such equilibriums facilitate efficient drug absorption even for relativity low solubility drugs without requiring high solubility of a drug and/or a large volume of dissolution medium.

continuous process of extraction/absorption can occur efficiently and sufficiently within the intestine.

In conclusion, drug absorption occurs as a liquid-liquid extraction process for which dissolution of the drug within the GI tract is one of the most critical steps. However, it is generally neither necessary nor required that the drug has to be completely dissolved at a given time for successful and efficient drug absorption. Efficient and successful drug absorption can occur by continuously replenishing the extracted/absorbed (undissociated or native) portion of the drug, as low solubility drugs (mostly non-polar) often show quite high and efficient drug absorptions, as explained later.

On the other hand, this continuous extraction/absorption process does not occur *in vitro*. *In vitro* all one is interested in knowing if the product is capable of releasing the entire drug present in a product in an expected and reproducible manner. How would one know this? It can be evaluated by placing a product in a container having some volume of solvent, i.e., dissolution medium, with stirring and then measuring the drug in solution at a specific time. The amount of drug in solution measured, as amount/mL or percentage of total expected amount, will indicate whether the drug has been released from the product. The only difficulty in this step is that if the drug is not freely soluble in the limited volume used for dissolution testing, then the entire drug released will not be in solution form and cannot be measured, because for sampling and measuring the drug has to be in solution form. The drug will stay as precipitate in the dissolution medium even though it has been released from the product. As explained earlier, this situation does not arise *in vivo*, where the non-polar (fatty) wall continuously extract the drug from solution even though drug is present as precipitate there as well. This situation *in vitro* is alleviated by adding some non-polar substance, which acts as a solubilizer. Commonly, sodium lauryl sulphate (SLS) is used for this purpose, which indeed contains a long chain hydrocarbon (fat component) to help in dissolving non-polar drugs which otherwise have low solubilities in water. The solubilizer does not have to be SLS, it can be any other compound of similar characteristics i.e. it should maintain the pH of the medium around 6 and should not cause a negative impact on the drug and/or its excipients. Therefore, for dissolution testing purposes, one needs an aqueous solvent or buffer with some solubilizer if the drug is of low aqueous solubility, and a stirring and mixing mechanism.

The next question is which pH for the medium is to be chosen for dissolution testing, as there are two sets of pH values observed in the GI tract, pH 1-3 (stomach or gastric) and 5-7 (intestinal). As dissolution is a necessary step for drug absorption and it is a well-established fact that most, if not all, drug absorption occurs from the intestinal site because of its large and porous surface area [10-13]. Therefore, one should select a dissolution medium representing the intestinal area, i.e., pH 5-7 or pH 6 for standardization.

A quick review of literature would clearly show that dissolution media are used having a pH in range of 1 to 7 or 8 [14,15], which, as explained above, would not accurately reflect the physiological environment for absorption purposes and thus would not provide physiologically relevant testing or results.

In short, for *in vitro* drug dissolution testing, the physiological aspect dictates that the dissolution medium should be aqueous based having a pH of 6. If a drug is not expected to dissolve in the volume used, one should add some solubilizer to enhance its solubility in the dissolution medium.

Selecting Agitation (Stirring and Mixing) Representing Physiological Environment

As explained above, the drug moves through the GI tract which for all practical purposes is a long, flexible and convoluted tube of relatively narrow diameter. The drug/product moves through the intestine along with turning or tossing which provides an efficient mechanism of mixing its content and perhaps more importantly facilitating contact of drug in solution with the surface of the intestine for absorption. As such, the physiological environment does not have a rotating stirrer, however, its process (turning/tossing) results in mixing and stirring. So, the question is, for simulating a physiological environment how should one simulate this process and its effect? In reality, it is not only practically impossible to duplicate/simulate exactly the same process *in vitro*; it is in fact not necessarily required. However, it is the intestinal process of turning or tossing which dictates that dissolution step requires thorough mixing and interaction of the drug/product with the dissolution medium. It is just like the use of a thermometer for monitoring body temperature. One does not have to relate the processes in the body of providing higher and lower temperatures for developing a thermometer. If one is interested in monitoring body temperature, all one needs is an instrument which would be capable of monitoring the temperature. A thermometer is good for this purpose. If a person has a fever, a thermometer is expected to only indicate higher temperature. The mechanics of rising body temperature is studied separately.

Similarly, for dissolution testing one requires a soft but thorough mixing environment, which could be achieved in any number of different ways, e.g., using a bench-top mechanical stirrer, magnetic-bar stirrer, shakers, or roller type mixers. It will depend on our convenience and preference. Eventually the requirement of a (soft) stirring and mixing has to be fulfilled.

In currently recommended practices this requirement is fulfilled using stirrers commonly known as paddle and basket apparatuses (Figure 2). It is important to note that for all practical purposes these testers (paddle/basket) are simple stirrers. No matter how efficient the marketing is, these are simple stirrers and must be treated as such, without any sophisticated and elaborate requirements of specifications and/or calibrations. The requirements of specifications or calibrations for such testers should not be any more complex than the requirements for the magnetic stirrers and the associated stirring bars. These (paddles/baskets along with vessels) should be as "plug n play" type as any other

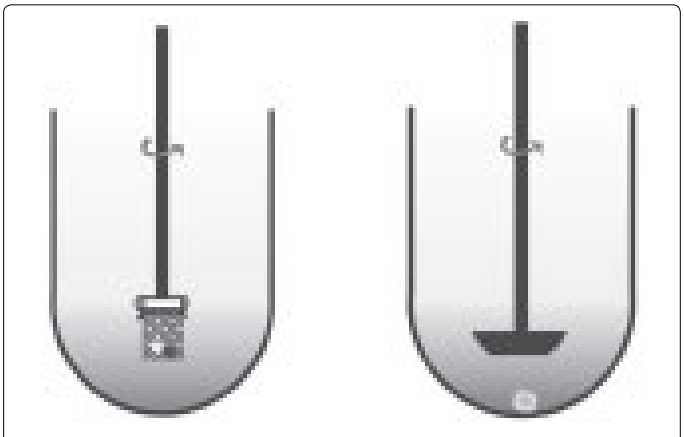

Figure 2: Schematic representation of the most commonly recommended and used dissolution testers known as the basket (left) and paddle (right) apparatuses.

simple laboratory equipment should be. The commonly suggested approaches for standardization known as enhanced mechanical calibration [16] or performance verification testing [17] appear to add limited value and may be considered as an unnecessary burden on the analysts and/or manufacturers [17,18].

For the sake of discussion, let us select the paddle as a stirrer/mixer and ask a question; would this fulfill the requirement or relevancy of physiological environment i.e. providing a soft and thorough stirring and mixing? The answer is clearly no. The reason being that anyone who would watch the operation of these apparatuses for a few minutes after dropping a product (e.g., tablet) which would provide thicker and heavier content settling at the bottom of the vessel (for easy observation), will conclude that the apparatuses do not provide stirring and mixing at all. A representation of poor stirring and mixing within a dissolution vessel using paddle apparatus is shown in Figure 3. The tester not only lacks the stirring and mixing processes, it almost forces the product and/or it's content to remain stagnant, thus producing a non-physiological environment. Similar behaviour of poor stirring and mixing can also be observed with a basket apparatus. It is surprizing that authorities have been recommending such apparatuses for dissolution testing which, in fact, do not provide stirring and mixing. It is simply (mechanically) impossible that these apparatuses can provide the necessary mixing and stirring, especially as required to simulate the physiological environment. Moreover, considering the flow-movement (or hydrodynamics) within the vessels, it can further be explained that using these testers, dissolution of the product will be dependent on the settling position of the product [19-22]. This settling of the product at the base of the vessel is a random phenomenon with a high degree of variability and uncertainty. Therefore, not only the apparatuses would not provide the required thorough mixing, they also would not provide results with acceptable reproducibility [23-26].

Considering the preceding discussion and the available scientific work in literature on the topic, there is really no logical reason that one should be using these apparatuses for dissolution testing purposes. Pharmaceutical manufacturers and regulatory authorities should take note of the serious deficiencies of these apparatuses.

The lack of bio-relevancy and poor reproducibility of these testers can further be emphasized by the fact that these apparatuses have never been qualified and validated for their intended purpose, i.e., for dissolution testing. There is no evidence available in literature showing that these apparatuses are indeed capable of providing reproducible and/or bio-relevant stirring and mixing and thus dissolution results and product characteristics. On the contrary, significant literature is available showing otherwise [e.g. see 19 and 27]. It is surprising that authorities require and/or accept the use of such flawed apparatuses for the evaluations of pharmaceuticals.

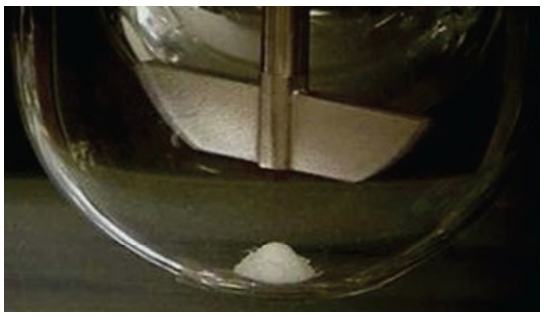

Figure 3: Operation of a paddle apparatus set at 50 rpm with a USP prednisone performance tablet highlighting the flaw of poor stirring and mixing environment within a dissolution vessel causing incorrect characterization of dissolution/quality attribute of the product.

An interesting and fortunate aspect, however, is that these deficiencies can be addressed quite easily by replacing the paddle/basket with another type of stirring and mixing spindle. One such example is the use of crescent shape spindle. This spindle is a modified version of the paddle/basket spindle to address the deficiencies of the paddle and basket apparatuses. That is, it does not allow the stagnation of the product at the bottom of the vessel, while providing the soft and smooth stirring and mixing within the vessel. The literature provides examples of its successful and useful applications [28-30].

Drug/Product Specific vs. "Universal" Dissolution Testing

Let's now consider the setting of some specific experimental conditions for *in vitro* testing. As explained above, *in vitro* drug dissolution testing is required to assess the *in vivo* dissolution which is essential for drug absorption. This absorption occurs from the intestinal part as explained above as well. Furthermore, as explained earlier, the intestinal environment can be simulated by an aqueous solvent such as water itself or an aqueous buffer having a pH of 6. Before moving further, one should ask the following question; do these physiological environments/requirements change from drug to drug, or product to product, i.e., is the physiological environment drug or product dependent?

The fact is that the physiological environment remains consistent, i.e., drug/product independent, which means dissolution tests must also be conducted using product independent experimental conditions. A quick review of literature, however, would show that not only are the experimental conditions used are drug and product dependent but also emphasized that such testing must be product dependent as well [15,31-34]. It is not clear what the reasons for such a requirement and practice are when these are clearly against the scientific principles and logical considerations. To be scientifically valid and logically correct, a dissolution environment must remain constant and product independent, i.e., dissolution tests should always be conducted using water or buffer having pH 6. Any deviation from such practice should be considered a scientifically invalid practice [35].

The quantity or volume of the dissolution medium is not critical, and can be used as appropriate but should be capable of dissolving the expected amount of drug with or without a solubilizer. For standardization purposes, however, one should preferably use a commonly suggested volume of 900 mL. In addition, temperature of dissolution medium is to be maintained at 37°C which is physiologically relevant and commonly used as well.

Next item is the use of a stirrer and its rpm. As noted above any stirrer can be used, set at any appropriate rpm which should provide soft and thorough stirring and mixing. However, once a stirring approach and rpm is established that should also be kept constant, i.e., independent of drug and product as the choice of the medium. In this regard, an rpm of 25 for crescent-shape has been suggested, which provides soft and thorough mixing (and stirring) and also the bio-relevant dissolution results. Here bio-relevant means that the test is capable of representing product behaviour *in vivo,* i.e., test provides faster dissolution results for an immediate release product and corresponding slower dissolution for an extended release product under exactly the same experimental conditions [29].

To summarize the above mentioned experimental conditions it can

be stated that bio- or physiologically relevant dissolution tests can be conducted using 900 mL of water or buffer (pH 6) with or without a solubilizer as dissolution medium, with stirring using a crescent-shape spindle set at 25 rpm. What this means is that if given a product (tablet/capsule) it should be tested using this set of experimental conditions, and the results would represent the dissolution characteristic of the test product. On the other hand, if the results do not represent *in vivo* dissolution characteristics of the test product then the formulation or manufacturing attributes of the product are to be adjusted to achieve desired dissolution characteristic or *in vivo* dissolution characteristics.

Often, in literature, suggestions are made for changing or adjusting experimental conditions to achieve certain desired dissolution behaviour of the product. This practice obviously does not make sense and should be avoided at all costs, otherwise there is no need for developing a product, all one has to do is to adjust experimental conditions to achieve the desired dissolution characteristics of any product.

Irrelevancy of Developing and Using Drug and Product Specific Dissolution Methods

There are about 500+ dissolution methods listed in the US FDA database [15] and about 600+ methods (monographs) in the USP [36]. In addition to these, there are many more, perhaps in the hundreds, dissolution methods described in literature. Moreover, as part of new product development exercises, it is a common and an expected practice to develop additional new or revised methods.

It may be interesting to note that the objective of drug dissolution testing, in particular from a product development perspective, has never been to develop dissolution methods, but to use the method for determining or estimating drug dissolution/release characteristics of products. By developing drug and/or product specific dissolution tests, one in fact would never know or determine the actual (or "true") dissolution characteristics of any product. The current practices of method development simply defeat the purpose of products evaluation.

For product evaluation one requires a test/method which is independently developed and established. Therefore, current practices of method developments are not only scientifically invalid and useless resulting in waste of time and resources.

Since practically each and every drug, and product, comes with its own set of experimental conditions at present, it would not be possible to establish whether the dissolution characteristics are reflective of the product or suggested experimental conditions. Furthermore, if given a blinded sample of a product, a common and often required analytical chemistry practice, it is not possible to determine dissolution characteristics of such a product, or any product. Obviously this defeats the purpose of conducting a test which is unable to provide an answer for the parameter for which it has been developed, a serious deficiency in the current practices of dissolution method development and their uses.

The use of the crescent shape spindle with a common set of experimental conditions has been suggested to address the current difficulties. The suggested approach not only practically eliminates the need for method development exercises, in particular product dependent, but also provides an unbiased assessment of dissolution characteristics of a product in a scientifically sound and valid manner [28,30].

For convenience, the following summarizes some of the additional unique features and advantages of using crescent-shape spindle not available with the use of current USP dissolution testers, in particular paddle and basket:

1. As the spindle sits at the bottom of the vessel, it eliminates unstirred and stagnant areas in the vessels, thus the tester is free from the artefacts of the "cone" formation and/or positioning effects of the product yielding higher consistency (repeatability and reproducibility) of dissolution results.

2. The use of the spindle results in a universal dissolution tester having drug and/or independent test conditions, i.e., 900 mL of distilled water as medium (37°C) with spindle rotation speed set at 25 rpm. Small amounts of solubiliser may be required for drugs with low aqueous solubility to facilitate appropriate sample withdraw and quantitation. Same method would be useable for both IR and ER products.

3. The same set of experimental conditions is applicable for both product development and QC purposes.

4. As the spindle snugly fits at the bottom of the vessel yielding a rugged design thus testing is free from sensitivities of usual and expected vibrations and variations in vessel/spindle alignments. In addition, if formed, air-bubbles would easily be broken or eliminated thus de-aeration of the medium is not required.

5. Provides complete extraction and dissolution of the drug from the product leading to dissolution results matching those obtained from assay and content uniformity tests. Therefore, dissolution tests using crescent shape spindle would make the separate assay and content uniformity testing unnecessary (as explained later).

Evaluating Dissolution Results for their Physiological Relevance

After conducting a dissolution test, the next step is to evaluate product characteristics based on the results obtained. Dissolution results are often reported as percent of drug dissolved at times. Suppose, one has done a dissolution experiment and obtained dissolution results of 80% drug dissolved in 30 minutes. The question is what should one do with this number, or numbers, if one measures dissolution at multiple intervals? This number is exactly like a number an analyst obtains for the absorbance of a solution from UV spectrophotometer or peak height/area from a chromatogram. One cannot use this number (results) in any useful way until these numbers are linked to the objective of the test. What is the objective of the dissolution test? As stated above, a dissolution test is conducted to simulate or estimate dissolution of a drug in the GI tract. So, if one has dissolution results from the GI tract, i.e., *in vivo* dissolution results, then one can compare them directly. However, unfortunately, one cannot measure the *in vivo* dissolution results directly, at least in most cases. These *in vivo* dissolution results are measured indirectly using plasma drug levels, therefore, one has to know how these *in vivo*, and by extension *in vitro*, dissolution results are linked to the plasma drug levels. One may argue that the job of a dissolution scientist would not be completed until the scientist/analyst provides the simulated plasma drug levels calculated/derived from the dissolution results.

How should one convert dissolution results into plasma levels? For such a conversion, the analyst is required to combine the drug dissolution results with the pharmacokinetic parameters of the drug to determine plasma drug levels. Such combining is achieved by a method known as convolution technique and for simplicity sake may be described as *in vitro*-to-*in vivo* profiling or IVIVP (Figure 4).

Although details of the methodology are explained in literature [37-41], the essential steps are briefly described below.

The suggested procedure is based on six steps: (1) Conducting a dissolution test using product independent experimental conditions; (2) Converting percent drug release values from a dissolution test into discrete doses (amounts, in mg, etc.) within every sampling interval; (3) Converting the discrete percent doses into amounts available in plasma by multiplying it with the drug's bioavailability factor, obtained from literature; (4) Calculating decreasing amount of drug in plasma with time, separately for every dose/amount segments, using the drug's elimination rate (or rate equation), obtained from literature; (5) Adding all the calculated drug levels (amounts) for every time; (6) Dividing total drug amount in plasma at every time by volume of distribution of the drug, obtained from literature, to calculate the concentration of the drug in plasma. This will provide the expected or predicted plasma drug concentration-time (C-t) profiles. An example of such a conversion is shown in Figure 5, for two diltiazem products, one 60 mg immediate-release (IR) and the other 120 mg extended-release (ER) [36]. The estimated, or predicted, plasma drug concentration-time profiles can then be evaluated exactly as their counterpart obtained from the *in vivo* studies.

There are significant advantages of using this technique over the current practices.

1. It is a simple procedure and plasma levels can be obtained using spreadsheet software, i.e., there is no need of using sophisticated, proprietary and expensive software.

2. No concurrent bioavailability studies of the test products are required. Pharmacokinetic data from literature can be used, thus this technique can be used at the product development stage to estimate potential drug levels of a test formulation. In fact, it (IVIVP) is the only technique which can be used at the stage of product development for determining plasma drug levels.

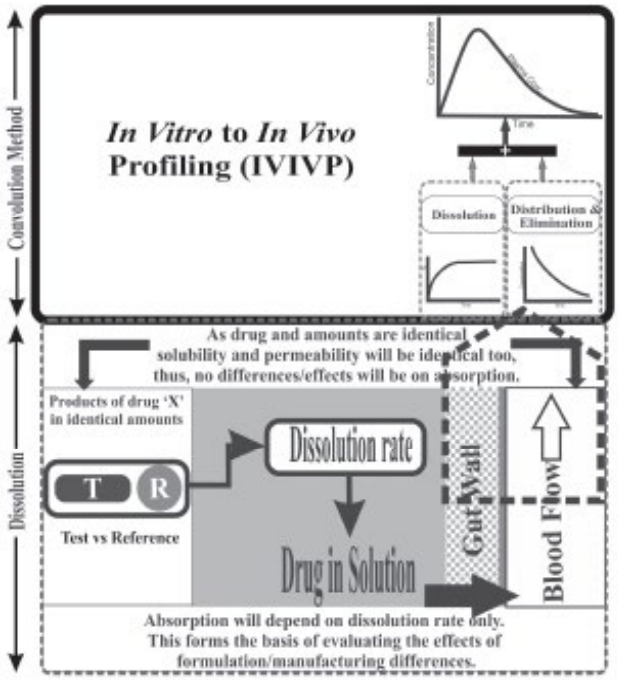

Figure 4: Schematic representation of the IVIVP (*in vitro*-to-*in vivo* profiling) concept in which drug dissolution tests are to be conducted using product independent and physiologically relevant test conditions. This is followed by merging (convoluting) of dissolution results with pharmacokinetic parameters of the drug to obtain (estimate/predict) plasma drug concentration-time profiles.

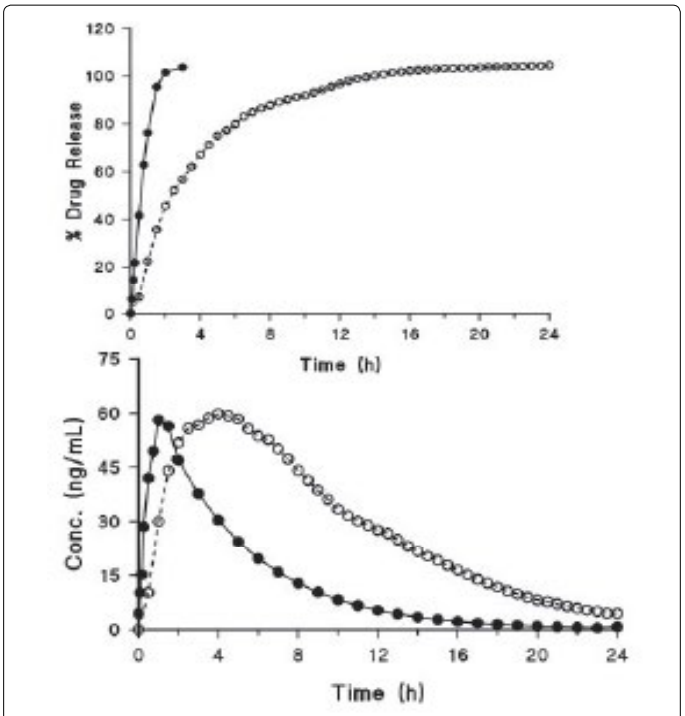

Figure 5: Above: Drug dissolution profiles of two diltiazem products (60 mg IR and 120 mg ER) using the same experimental conditions for dissolution testing which were 900 mL of water as dissolution medium maintained at 37°C using crescent shape spindle set at 25 rpm. Below: The corresponding predicted plasma drug concentration-time profiles obtained following the IVIVP concept.

It may be possible to compare *in vitro* dissolution (cumulative percentages with time) profiles with C-t profiles of different products/formulations. However, it is strongly suggested that direct relating or comparing of dissolution results/profiles to plasma profiles should be avoided. This is because there is always a possibility of incorrect interpretation, since dissolution profiles are reported based on a linear scale while plasma profiles are based on the exponential or logarithmic progressions or declines.

At present there are two approaches commonly suggested for the evaluation and comparison of dissolution results: (1) USP tolerances; (2) based on a similarity factor (f2). The USP tolerances are based on a 3-stage, progressively relaxed, acceptance ranges dependent on results of individual units (tablet/capsule). It can be argued that these are not based on any statistical evaluation, i.e., tolerance appears more on a chance-based rather than scientific based. It is surprising that there is no requirement of % RSD even though dissolution tests are conducted mostly in a set of 6 units and often in a set of 12 or 24 units. If one follows the example of the USP tolerances, and converts these into % RSD equivalents, it would be evident that, these (USP tolerances) represent extremely high % RSD values as show in Table 1. Perhaps this is the reason of not requiring usual RSD-based statistical approach in reporting/assessment of dissolution results for pharmacopeial purposes. An interpretation of this observation is that one should expect a very high degree of variability and unpredictably in dissolution results using

	1	2	3	4	5	6	Mean	% RSD (CV)
Stage 1	96	88	65	110	66	65	82	23
Stage 2	95	100	91	90	98	102	96	5
Stage 3	55	61	92	98	105	102	85	26
	103	87	77	97	93	89	91	10

Table 1: Simulated data representing four sets (n=6) of drug dissolution results for an immediate release product as per USP <711> Tolerances highlighting expected variability in results.

current USP, or other pharmacopeial, methods.

Another serious flaw of the USP approach of setting tolerances is that it requires certain percentage of drug dissolve/release (e.g., 80%) at a certain time, which is usually less than 100% of drug released. It is important to note that usually 100% of the drug is present in the products, at least on average, confirmed by assays, content uniformity and bioavailability/bioequivalence assessments. It is not clear, in fact scientifically incorrect, as to why dissolution tests are required to show dissolution/release of only 80% of the drug or less. Dissolutions tolerance should be based on 100% drug release at the set time points. The point being, current tolerances do not reflect physiological relevancy or true product characteristics.

The other criterion most often used for comparing dissolution profiles is known as the similarity factor (or f2) [39,40]. Basically, it represents a form of pooled, or average, differences in dissolution results of two dissolution profiles resulting in a value between 0 and 100. Zero being no similarity between the two dissolution profiles while 100 means no differences, i.e., complete overlaps of the profiles. A value between 50 and 100 is considered representing similarity of two products reflecting possible observance of no difference in drug release in humans [40]. Although the range 50 to 100 is used to reflect similarity of dissolution characteristics in human, there is no data, however, available to establish validity of such a claim. In general, wider range of f2 can be shown to be of no negative physiological consequences.

It may be argued that this parameter does not appear to provide any added advantage. On the other hand, the statistical approach based on % RSD can be simpler and effective for the evaluations of dissolution results. Otherwise, preferably the other approaches such as the one described above based on converting dissolution results to plasma levels (IVIVP) appears more physiologically relevant thus offers a better alternative.

Irrelevancy of Developing and Requiring *In vitro-In vivo* Correlations (IVIVC)

The development of IVIVC is described as: an important concept and a tool for the development and evaluation of pharmaceutical dosage forms, especially modified release dosage forms. The objective of developing an IVIVC is to establish a predictive mathematical model describing the relationship between an *in vitro* property and a relevant *in vivo* response [4].

The objective of IVIVC, therefore, is to establish a relationship between *in vitro* (dissolution) and *in vivo* response (*in vivo* dissolution). The *in vivo* dissolution is derived or calculated from a C-t profile. Conversely, IVIVC is also described as a relationship between C-t profile and *in vitro* response (dissolution). In this case, *in vitro* dissolution results are to be converted to C-t profiles. These *in vitro* and *in vivo* responses are then plotted against each other to obtain a straight line to reflect a relationship (which is considered as the mathematical model) as shown in Figure 6.

Aside from practical and procedural difficulties and complexities, which are enormous, for deriving and/or converting *in vitro* dissolution results to *in vivo* response (C-t profile) and vice versa, let us assume that one obtains working relationships, as straight lines with a correlation coefficient (r)=0.999 as shown in Figure 6. The question is what would one do with these relationships or developed models? That is, how should these relationships, or lines, are used for predicting corresponding *in vivo* and/or *in vitro* product characteristics? For example, suppose an analyst has dissolution values or plasma drug levels at different sampling times for a product which is under development. How should the analyst use the values on these (IVIVC) graphs, since these graphs only have percentages or concentrations on both axes, while the analyst have the values/results with sampling times? Obviously these relationships/lines cannot be used, it is a mathematical impossibility.

Moreover, it is important to note that the drug dissolution tests are conducted utilizing the existence of underlying IVIVCs and not verifying or establishing such relationship for each and every drug and product. Therefore, it should be noted that developing an IVIVC, as presently described and required, is of no real practical use, and cannot be used, for the assessment and development of pharmaceutical products.

On the other hand, however, what is needed at the stage of product development is the prediction of C-t profiles from dissolution results of the test products. Such profiles can only be obtained by merging *in vitro* dissolution results with the pharmacokinetic parameters of the drug. This merging step is commonly, or formally, known as the convolution technique or method as described above. This merging or convolution step does not require the development of an IVIVC either, it is an independent step. In this regard, a simple and practical approach, based on convolution principles, to obtain plasma drug concentration-time profiles has been described above. The need during the products development and evaluation stage is the predictability of C-t profiles; therefore, one should use the proposed terminology of IVIVP, and not the IVIVC.

In conclusion, developing an IVIVC as suggested presently is an unnecessary practice and requirement and should be considered a waste of time. Furthermore, IVIVC cannot be used for the evaluation and development of pharmaceutical products. A simpler approach of IVIVP based on convolution method is more appropriate and useful.

Deficiency of Biopharmaceutics Classification System (BCS)

BCS is a classification approach in which drugs (Active Pharmaceutical Ingredients or APIs) are divided into four classes based on the extent (high or low) of their aqueous solubilities and

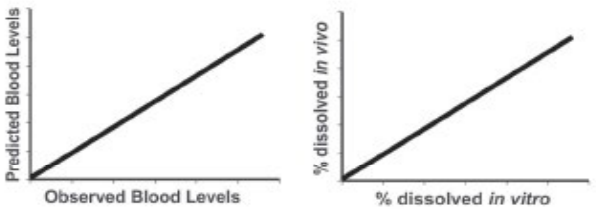

Figure 6: Representation of the target (correlation or mathematical model) for achieving IVIVC for drug products representing the fact that axes do not contain a time scale (axis) which is required for estimating or predicting plasma drug concentration time profiles from dissolution results.

permeabilities through the GI tract wall, in particular intestinal [42,43]. In this regard, these four classes are: (I) high solubility and high permeability drugs, (II) low solubility and high permeability drugs, (III) high solubility and low permeability drugs and, (IV) low solubility and low permeability drugs. It is critical to note that BCS relates only to drugs (APIs) and their characteristics and not to the products.

These two factors (solubility and permeability) may indicate, keeping all other factors equal, dissolution and absorption characteristics of drugs in humans. For example, if four drugs, one from each class in equal doses, are administered in solution forms, all of them would show differences in dissolution and/or absorption in the GI tract or appearances in the blood stream depending on their solubility/permeability characteristics. Potentially, the drugs in group I would show fast and high absorption (least hindrance to absorption), drugs in group IV would show slow and erratic absorption (highest hindrance) while the drugs in group II and III would show absorption in between. Therefore, BCS may provide a basis for assessing potential absorption behavior of a drug in humans.

The use of BCS may not, however, be extended for product evaluation and development. There are at least four reasons for this: (1) as described above BCS relates to drugs only and not the products; (2) for products evaluation, one assesses the effect(s) of formulation and manufacturing attributes while keeping the drug and its strength constant, thus solubility and permeability do not usually differ during product development; (3) BCS refers to aqueous solubility, however, the product may be evaluated in buffer solutions with or without solubilizers; (4) often reference is made about BCS in predicting the bioequivalence of two products, based on their *in vitro* release (dissolution) characteristics, e.g., in the case of developing IVIVC. It is important to note that, one does not use BCS criteria for evaluating bioequivalence (which is in fact assessment of *in vivo* drug dissolution/release); similarly, an assessment of *in vitro* equivalence of two products having the same drug and strength should not require the use of BCS classification as well.

In vitro drug release or dissolution tests are conducted to assess the dissolution of the drug in a medium in which the drug must be freely soluble. The choice of medium is made prior to conducting a dissolution test, so that the medium provides sufficient solubility. All drugs must be freely (highly) soluble in the dissolution medium. Therefore, for the evaluation of *in vitro* drug release, there are not two classes but one, i.e., high solubility of a drug in a medium.

Furthermore, apparently there is a serious oversight in classifying drugs based on their solubilities for this classification explained as follows. It is generally accepted that for a drug to be absorbed from the human GI tract, it should be in solution form which is established based on solubility/dissolution characteristics of the drug. It is also generally accepted that the higher the solubility of the drug, the higher the dissolution and absorption, and their corresponding rates, will be. In addition, it is also a well-established fact that absorption preferentially occurs from the non-polar or undissociated form of a drug [10]. On the other hand, the undissociated or non-polar moiety, of a drug often shows lesser aqueous solubility compared to its polar version.

For example, propranolol is a drug which is basic in nature with a pKa value of 9.42 and its aqueous solubility is of 61.7 mg/L or 1 part in ~ 16,000 [44]. Therefore, propranolol should be considered a low solubility drug. However, its products are usually manufactured using the drug in its hydrochloride salt form, i.e., propranolol·HCl which is freely or highly soluble in water. In water it would exist in its ionic/protonated form, which would be less absorbable than the native propranolol. On the other hand, propranolol is known to be highly absorbable/permeable (bioavailability higher than 90%) (10), which suggests that in reality the body sees propranolol as a non-polar/undissociated moiety. Therefore, for *in vivo* dissolution/absorption purposes the solubility of native propranolol should be considered, not of its salt form. This means that in reality propranolol (and other similar drugs) is a BCS class II drug and not a class I drug, as commonly considered [44,45].

In conclusion, for drug dissolution and absorption evaluation purposes, one should consider solubility characteristics of a drug in its native form and not that of its salt form. It appears that the use and application of BCS criteria for the evaluation of products requires a careful reconsideration and its use can be reduced without any negative impact on products development and evaluation, however, with potential gains in economical efficiencies.

Are Low Solubility Drugs Really Problematic and Require Special Attention?

Drug absorption from the GI tract is generally dependent on the dissolution characteristics of a product which in turn is dependent on the aqueous solubility of the drug. In general, it is assumed that the higher the solubility, the higher the expected drug absorption will be, and vice versa.

Before considering the link between absorption and solubility, it would be prudent to define and establish the solubility characteristics of a drug for absorption purposes. In this regard, it is a well-known fact that drugs are mostly absorbed from the intestinal part of the GI tract [10]. The liquid phase in the intestine is aqueous-based having a pH in the range of 5 to 7. For all practical purposes one may consider a pH of 6 (average of 5 to 7) for the intestinal fluid, as explained earlier as well. Thus, to represent intestinal fluid, for dissolution testing, one may use water itself, which usually has pH around 6 or a (phosphate) buffer having a pH of 6. Therefore, in the following discussion, solubility of drugs in water will only be considered.

The solubility values for compounds/drugs in water are commonly described in literature and the reported values are often for room temperature. However, for dissolution testing purposes one would require solubility values at 37°C. To be precise, one should determine the solubilities at 37°C. As a rule of thumb, one may use solubility values at room temperature, as solubilities at a higher temperature (i.e., 37°C) would most likely be higher, an advantageous situation for dissolution testing.

It is important to note that for absorption purposes, as explained above, it is the solubility of the undissociated (or native) form of the drug that is relevant and not that of the salt form. Drugs are often available and administered as salts such as chloride, sulphate, phosphate, citrate, etc., however, these salt components get dissociated. These drugs then exist in an undissociated form in equilibrium with a dissociated form with a counter ion depending on the nature of the environment (Figure 1). The absorption of the drug usually occurs from the undissociated form of the drug.

For dividing drugs into groups of high or low solubility, one is first required to define or establish criterion for this classification. An appropriate approach in this regard would be the one recommended by the pharmacopeias [e.g., see 46]. Pharmacopeias usually define solubilities in units of parts (drug) in parts (solvent). Commonly, these are reported as how many parts of solvent (water) would be required

to dissolve one part of a drug, usually in grams (~mL) of water. Table 2 provides the description of different solubility levels. As per the table, any drug which would require 30 parts (or 30 g) of water or less to dissolve 1 part (or 1 g) of drug is considered as soluble. However, if the drug requires more than 30 parts (30 g) of water then it will be considered a low solubility drug.

The next item to consider is absorption i.e. what does it mean and how is it reported? First of all, it is important to note that, just like solubility, absorption is a drug property as well and not the property of the products. Different products can have different absorption profiles of the same drug, e.g., IR vs. ER products where for IR products absorption would appear faster while for ER products it would appear slower. This difference in (rate of) absorption is not because of the drug, but due to the release of the drug from the product at the absorption site. The drug dissolution tests are conducted to assess this release, or differences in release, of a drug from the products.

Furthermore, it is also often assumed [47-50] that dissolution testing is used for the prediction of absorption which is not accurate. A dissolution test does not predict absorption, but linearly relates/links to absorption. It is just like the concentration of a drug in solution does not predict UV (coefficient of) absorption, as UV absorption is a drug characteristic. However, UV absorption of the solution is directly proportional to the concentration of the drug in the solution. Similarly, dissolution does not describe the absorption characteristics of the drug in humans, however, higher and faster dissolution will proportionally provide a higher and faster absorption outcome.

The absorption characteristic of a drug is a standalone property and is to be determined experimentally. In most cases, for product evaluation purposes, this absorption value can be obtained from literature. In this regard, one should be clear regarding the differences between absorption and bioavailability terminologies. The differences in these two may be explained as follows: Disappearance of a drug from the intestine (assuming no degradation or metabolism in the GI tract) represents absorption while appearance of the drug in the blood stream represents bioavailability. If a drug disappears from the intestine and appears in the blood, without any loss, then absorption and bioavailability becomes the same or equivalent. However, if a drug disappears from the intestine, and appears in lesser amounts in blood then it means that the drug has a lower bioavailability. The loss between the disappearance from the intestine and appearance in the blood is commonly known as the "first pass effect" and represents "filtering" or metabolic conversion of the drug by liver. For example propranolol is a drug which is rapidly and almost completely (>90%) absorbed, however its bioavailability is only 36%. This means that the liver removed about 64% of the absorbed drug and the blood sees only 36%. For dissolution testing purposes, it is only the absorption part which is relevant and applicable, because bioavailability or the metabolic effects of the liver is a drug property and this effect occurs after drug leaves (or disappears) the intestine. From a drug dissolution testing aspect, one should focus on the drug absorption (not the bioavailability per se) characteristics. Often, absorption is reported as "rapidly and completely absorbed" or in percentages [51].

Now let us evaluate the relationship between these two properties, solubility and absorption. For the purpose of the discussion, one may divide drugs into two groups, i.e., drugs which require 30, or less, parts of solvent for 1 part of the drug as "high" solubility drugs and the ones which require greater than 30 parts as "low" solubility drugs. The solubility and absorption characteristics of some commonly used drugs were obtained from literature [52,53], and are listed in Table 3, where further detailed discussions on permeability and/or bioavailability aspects may also be found.

In Table 3, the drugs are listed with decreasing solubilities, i.e., requiring increasing amounts of solvent to dissolve the same amount (1 g) of drug. However, note that all drugs, with few exceptions, show rapid and complete absorption, without any particular order. In addition, it is to be noted that the majority of the drugs in this list are of low solubility to practically insoluble. If one considers the commonly held belief that solubility and absorption are linked or linearly related then absorption characteristics of the drugs listed in Table 3 should also linearly decrease [54-57]. This, however, is not the case.

It should be interesting to note that out of these 30 drugs listed in Table 3 only 4 would meet the criteria of being soluble, while the rest would be sparingly to practically insoluble. On the other hand, the interesting aspect is that most of the drugs show very high absorbability characteristics. What this means is that absorbability of a drug is not directly linked or related to solubility of the drug in a "traditional sense". This observation is not in line with the currently held view where it is believed that low solubility drugs may cause absorption difficulties.

How should one explain this discrepancy? One of the possible explanations is that while considering the solubility aspect, unfortunately, it is assumed that the physiological system (GI tract, especially intestinal) is a closed system, i.e., the entire drug is expected to be in solution form in the available volume at a given time. However, the GI tract provides an open or continuous extraction site which can continuously extract extremely small amounts of dissolved drugs repeatedly for absorption (Figure 1 and 7), thus neither requires large volumes of solvent nor high solubility of a drug [57].

Secondly, scientists have become accustomed to considering paddle and basket vessels/apparatuses as the dissolution tester, representing the GI tract environment. This is not an accurate view or assumption either. The stirring environment within a dissolution vessel is such that not only does it not facilitate dissolution, it in fact retards and hinders dissolution. Consider for example, dissolution testing of USP prednisone (performance verification or PV) tablets. The drug is capable of dissolving completely in 900 mL of water; however, it often shows dissolution of around 40% in 30 minutes. The reason being, the drug (prednisone) sits at the bottom of the vessel without any stirring and mixing (Figure 3). The dissolution testers do not provide an efficient mixing environment as one observes *in vivo*, thus dissolution would appear problematic in particular for lower aqueous solubility drugs [30]. This is not the dissolution or absorption problem rather a stirring and mixing problem within a dissolution tester.

Thirdly, scientists have accustomed to the view that the drug or dose has to be dissolved in 900 mL or a smaller volume to be considered as a soluble drug. However, this practice of using 900 mL volume is for our convenience or traditional, in reality it can be 500 mL or 2000 mL. This limitation of volume can easily be addressed by adding a small amount

Descriptive terms	Parts of solvent needed for 1 part solute
Very soluble	<1
Freely soluble	1-10
Soluble	10-30
Sparingly soluble	30-100
Slightly soluble	100-1000
Very slightly soluble	1000-10,000
Practically insoluble or insoluble	>10,000

Table 2: Classifying solubilities of drugs as per US Pharmacopeia (USP, 2011).

Drug	Solubility	Absorption Characteristics (BA=Bioavailability)
Isoniazid	8	Readily absorbed (BA=91%)
Stavudine	12	Apparently fast absorption (BA 86- 100%)
Lamivudine	14	BA >82%
Doxycycline Hyclate	20	>95%
Ciprofloxacin	33	Rapid absorption (BA ~ 70)
Levofloxacin	40	Rapid (BA ~ 100%)
Ranitidine	40	Rapidly absorbed (BA 50-60%)
Zidovudine	50	Rapidly and almost completely absorbed
Pyrazinamide	67	Fully absorbed
Acetaminophen	68	Readily absorbed (BA=62%–89%)
Cimetidine	88	Rapidly, yet incompletely (BA 56-68%)
Metronidazole	100	Rapidly absorbed with BA >90%
Ibuprofen	571	Rapid and complete (BA 100%)
Acyclovir	714	Erratic (BA 10 to 30%)
Rifampicin	714	Well absorbed (BA ~ 93%)
Atenolol	1010	Incomplete absorption, (BA 40-60%)
Acetazolamide	1389	Rapidly and almost completely
Quinine	2000	Rapidly and almost completely absorbed
Prednisolone	4348	Rapidly absorbed (BA >75–98%)
Metoclopramide	5000	Rapid absorption (BA 30-100%).
Prednisone	8333	Rapidly absorbed (BA 80–100%)
Propranolol	16207	Almost completely absorbed (>90%)
Furosemide	54795	Fairly rapidly absorbed (BA 60–70%)
Choroquine	94340	Rapidly and almost completely (BA ~ 89%)
Efavirenz	100000	Absorption? (BA 40-45%?)
Ketoprofen	100000	Readily absorbed (BA=92%)
Verapamil	223714	Rapidly and almost completely (BA 10-20%)
Diclofenac	421941	Rapid and complete (BA ~ 60%)
Amitriptyline	500000	Well absorbed (BA ~ 48%)
Amodiaquine	>1000000 (?)	Readily absorbed

Table 3: Solubility and absorption characteristics of different drugs (solubility is defined as per Table 1).

of solubilizer to enhance the solubility of the drug in the dissolution medium. As long as the medium remains aqueous and at a pH around 6, with or without solubilizer, not changing the chemical nature of the drug or excipient, this should be an acceptable dissolution medium for dissolution testing.

The condition of limited volume and/or poor stirring environment within dissolution vessels often creates problems for proper dissolution assessment which scientists tend to extend to *in vivo* as well. However, this is in reality an *in vitro* and analytical problem and does not reflect an *in vivo* situation. It is important to note that low solubility drugs do not seem to be problematic for absorption as described above. However, *in vitro* they do create significant problems because of a lack of stirring and mixing environment and availability of small volume of medium in particular for paddle and basket apparatuses. This issue should be addressed by adding some amount of solubilizer and improving the stirring and mixing environment. The use of a crescent spindle addresses this problem very well [28,30] and most dissolution tests can be conducted using a simple and single set of experimental conditions. In short, as long as system/testing is based on using a medium in a vessel capable of dissolving an expected amount of drug present in a product and availability of a gentle but thorough stirring/mixing set-up, it should be able to reflect *in vivo* dissolution/absorption characteristics, appropriately.

On the other hand, if a drug is indeed insoluble (like sand particles) in water or aqueous buffer (with a pH of 6), then the question should be asked how is it getting absorbed through the GI tract, because absorption requires dissolution. In such cases, the drug may be getting absorbed through other mechanisms (such as pinocytosis) which are not diffusion based. Here dissolution or its testing should not be required and one should not be conducting a drug dissolution test at all for such drugs for their product developments and/or evaluations. However, fortunately, such situations are rare and should be considered as exceptions. Most drugs are indeed absorbed by diffusion mechanism which would require a dissolution step as a pre-requisite.

In conclusion, for absorption purposes, solubility characteristics of the undissociated (native) drugs should be considered. Drugs having low aqueous solubility often provide better absorption characteristics *in vivo*, however, may provide some challenges in monitoring dissolution characteristics *in vitro*. In such situations, if appropriate testing conditions (stirring and mixing with solubilizers) are used for dissolution testing then the products with low solubility drugs can be developed and evaluated as easily as high solubility drugs.

Explaining Dissolution and Absorption of Drugs Based on their Ionization Behaviours in the GI Tract

From a drug absorption aspect, one of the most critical aspects to consider is that one should watch for the undissociated drug in solution, which is often overlooked. The undissociated drug in solution is the one which is relevant for absorption. It is to be noted that an undissociated drug will almost always be present and available for absorption for both types of drugs (acidic and basic) both in the stomach and the intestine in small or large concentrations depending on the environments and the nature of the drugs. Confusion often occurs if one relates the appearance of a drug in solid form (or precipitate) as a reflection of lack of drug in solution form, which is not accurate. It is the invisible undissociated drugs in solution form, in equilibrium with the invisible ions, which are important and critical for drug absorption (See Figure 1). For low solubility drugs one often sees solid/precipitate, which is not relevant for absorption purposes. It is like sand to the body or GI tract. On the other hand, for soluble drugs one does not see the presence of the solid drug. However, in this case, the drug exits in solution and again in equilibrium with ions just like in the case of low solubility drugs. Note that just like the solid drug outside the solvent is irrelevant for absorption, ions in solution are almost equally irrelevant for absorption. The equilibriums between drug (solid) and drug in solution, and drug in solution and ions, have to be considered or watched for the drug absorption to occur efficiently (Figures 1 and 7).

Before moving further it is important to consider the relative concentrations of the dissociated and undissociated species in the stomach and the intestine. The ratio is defined by an equilibrium constant which is as follows: K_e= [A+] [B-] / [AB]. Let us assume that K_e=1 for two drugs here, where one drug has high water solubility (e.g., acidic) and the other one low water solubility (e.g., basic). If equal amounts of these drugs are delivered to the stomach where pH is low, the acidic drug is expected to dissolve to a limited extent (with significant quantity of undissolved solid) compared to the basic drug which will most likely be completely in solution form. Although equal amounts of the drugs are delivered, and both drugs are assumed to have the same K_e values, concentrations of drugs and ions in solution form will be different in these cases, to maintain the K_e, which will be lower in the case of the acidic drug and higher for the basic drug. This is opposite to common understanding where it is often assumed that the stomach would have a higher concentration of undissociated acidic drugs compared to the basic drugs. In fact, stomach will have a smaller amount of drug in solution, thus absorption, for acidic drugs as compared to the basic drugs on an equal amounts and K_e basis. This situation will be reversed in the intestine where pH is higher, therefore, acidic drugs should be more in solution, thus should absorb well compared to basic drugs.

Now, if the drug absorption is to depend on drug in solution only, then certainly basic drugs should preferentially be absorbed from the stomach and acidic drugs should preferentially be absorbed from the intestine. The assumption here is that the system is a closed and static one as in a beaker (Figure 1), and both the stomach and intestine physiologies are the same. However, the body or physiological (*in vivo*) system is neither closed nor static and have different stomach and intestine physiologies. This makes the described absorption model not bio-relevant and the observed absorption behavior of drugs is very different than described here.

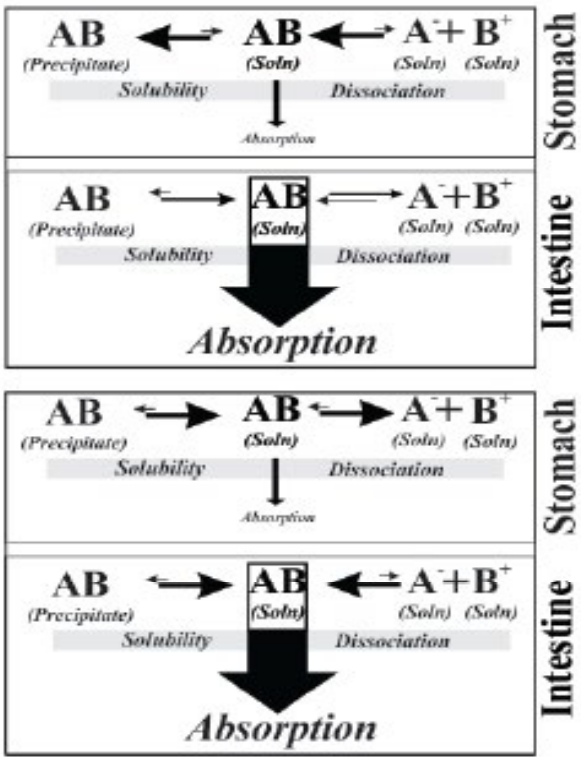

Figure 7: Schematic representation of the drug absorption process of weakly acidic (above) and basic (below) drugs in the GI tract based on their ionization/dissociation characteristics and the available surface areas (represented by downwards arrows).

In the physiological system (GI tract), there is another major contributing factor which is the availability of the surface areas of the GI tract or the absorption site/capacity with their associated blood flows. The available surface area for absorption is almost negligible in the stomach, compared to the enormous surface area in the intestine, which for all practical purposes can be ignored. However, the surface area and its associated blood flow play a major role for the drugs absorption in the intestine. Now, let us see how the combination of the two variables solubility/dissolution and dissociation/pH, and their equilibriums, interact together to provide appropriate drug absorption.

As the drug appears first in the stomach, it will behave as explained in Figure 7, i.e., the drug in solution form will be in equilibrium with ions and will also get absorbed (or leaks) into the stomach lining according to the availability of the drug in solution form. However, as the drug moves into the intestine, the equilibrium will be disturbed not only by the pH of the environment but also by the extraction capacity of the large surface area of the intestine. The large surface area of the intestine or its absorption capacity will have an enormous impact on the equilibrium and concentration of the drug in solution. Here (undissociated) drug from solution will be absorbed (extracted) in large quantities (drain vs leak). This will force the equilibrium to move greatly from undissolved drug to the dissolved drug (drug in solution)

or the dissociated ions towards the dissolved drug (drug in solution). Although pH will have its impact on the equilibrium (as shown in Figures 7) but the major impact will be because of the surface area. It is to be noted that a higher pH and higher surface area of the intestine concurrently increase the solution formation for a basic drug and thus absorption. This is a commonly known observation that basic drugs are absorbed more efficiently from the intestine compared to the acidic drug while both types of drugs get absorbed efficiently in the intestine.

Therefore, it is the intestine, because of its large absorption capacity, which plays a major role for drug absorption through the GI tract for both types of drugs, i.e., acidic and basic. The pH of the environment (stomach and intestine) plays a relatively smaller role in drug absorption. This explanation is for drugs which get absorbed through passive absorption mechanism, i.e., absorption based on undissociated molecules through cellular lipid layers, which in general represents absorption for the majority of drugs. It is important to note that drugs would seldom be in solution form completely at any given time or all the time. However, the extraction/absorption step keeps the solid drug moving into solvent/solution form that is the dissolution of drug which is necessary for the absorption of a drug.

So, if one likes to study absorption of a drug from a product, then one needs to evaluate the rate and extent of the drug going into solution form which is evaluated *in vitro* by dissolution testing. It is important to note that a dissolution test is used to evaluate only the formation of drug solution. Furthermore, as the solution formation is important for the absorption of drugs, which is required in the intestine, therefore, a dissolution test should always be conducted using the intestinal environment i.e. using a medium having a pH of 5-7 and with appropriate stirring and mixing.

It may be useful to note that in the area of solubility and dissolution another parameter pKa, and its use, is often described. In simple term pKa is defined as a pH value where dissociation constant (Ka) of a compound (drug) is 1, i.e., ratio of the ionized vs unionized moieties of the drug will be equal. In general, as the increased ionization of drug increases solubility of an ionic compound in water so its dissolution will as well. Therefore, reducing pH (acidic medium) for basic drugs would enhance the solubility thus dissolution, while increasing pH (basic medium) for acidic drugs would increase solubility thus dissolution. Considering the pKa and solubility/dissolution relationship behaviour often experimental conditions (pH of dissolution medium) are selected which are favorable to obtain increased solubility/dissolution. However, this practice of selecting drug dependent pH of dissolution medium should be avoided. The reason being that for relevant dissolution testing the selection of the medium including its pH is dependent or linked to the GI tract environment, which is drug/product independent. Therefore, dissolution tests should always be conducted at fixed pH of 6, as explained previously, independent of pKa of the drug.

It is often suggested that as the product goes through the stomach, one should also evaluate the impact of this (acidic environment) on the product first. Certainly, but this will not be part of dissolution testing but the evaluation of "stability" of a drug and/or its interaction with the excipients in the stomach (acidic environment). It is similar to stability studies conducted under harsher conditions to evaluate the impact of temperature and humidity on the drug and its product. Drug dissolution tests are conducted only to evaluate, as stated above, availability of a drug in solution form in the intestine.

Sink Condition, Physiological Relevancy or a Made-Up Requirement?

One of the requirements to conduct an appropriate drug dissolution test is to use a sufficient volume of dissolution medium, which should be able to dissolve the expected amount of drug released from a product. This ability of the medium to dissolve the expected amount of drug is commonly referred to as a "sink condition". It is important to note that a dissolution test should not be conducted in a volume of medium which would not dissolve the expected amount of drug present in a product completely and freely. This is because even if a product contains and releases 100% of the drug as expected, one cannot measure (quantify) it because for the quantitation/sampling the drug has to be in the solution. Therefore, it should be noted that this requirement of "freely soluble" or "sink condition" is the requirement for the quantitation (analytical chemistry) and has nothing to do with the physiological aspect. Considering that often limited volume is available for *in vitro* dissolution testing such as with the vessel based apparatuses, one is required to add some solubilising agent (e.g., SLS) to create the sink condition for quantitation of the drug.

It is to be noted that a physiological environment deals with the availability of limited volume in a completely different manner. Here, the drug is continuously absorbed into the blood, metabolised and eliminated, which provides a very efficient mechanism for providing a high solubility equivalent (Figure 7).

On the other hand, the limitation of having a smaller volume *in vitro* is compensated by the use of a solubilizer. It is important to note, as has been highlighted in other situations as well, to conduct a dissolution test an analyst needs to simulate not duplicate, the *in vivo* environment. This can be explained with an analogy of using a mercury thermometer to monitor the body temperature. One does not require duplicating the body's mechanism to monitor the temperature. A human body does not have or require a mercury thermometer to monitor or maintain the body temperature. Furthermore, it also does not depend, what amount of mercury and the dimensions of thermometer used. It is for our own convenience that mercury thermometers are used with the objective that we like to know what the temperature of the body is at a certain time point.

Similarly, for dissolution testing one would like to see how a product will behave (release) in a physiological environment which is represented by an aqueous based medium having a pH of 6. If the product would be of a highly soluble drug, the analyst needs not to do anything further except taking the sample and measuring the drug to finish off the experiment/test. On the other hand, if it is required to test a product, having the same formulation as in the previous case, but with a low solubility drug, then the analyst may face a problem of quantitation. To address the problem of quantitation the analyst is required to modify the medium in such a way that it solubilizes the drug but should remain physiologically relevant as well. Therefore, for bile salts equivalents, which are present in the GI tract, such as SLS are used as the solubilizers. Thus, an analyst must first establish if a solubilizer will be required, or not, to meet the requirement of dissolving the expected amount of drug in the desired volume of the medium, commonly 900 mL.

Another requirement for the sink condition often described in literature [58] is that of the multiples (e.g., 3 times or more) of volumes of medium over and above the volume needed to saturate the medium with the expected amount of drug. It is very important to note that the only requirement for a sink condition is that the volume of the medium should be such that it would provide complete dissolution of the expected amount of the drug. Generally this condition is fulfilled if an analyst uses a little more (10 to 15%) volume than the volume of the medium (with or without solubilizer) required dissolving the expected amount of drug. The multiples as noted above and often

described in literature have no scientific basis and are not supported by any experimental evidence. Therefore, analysts can easily ignore such requirements, if they choose, without any deleterious impact on dissolution testing or product evaluation.

In short, a sink condition for dissolution testing is solely an *in vitro* requirement which is required to quantify the drug when it is released and dissolved in a dissolution medium. The sink condition may be defined as the volume of dissolution medium, with or without a solubilizer, needed to provide complete dissolution of the expected amount of drug present in the product. One may use any multiple of this volume if desired, however, scientific and experimental studies do not provide any support to such a requirement or practice.

Looking to the Future: A Simple and Unique Approach for Developing and Evaluating Products

Commonly pharmaceutical products are evaluated and developed based on four "quality" parameters/measurements: (1) Identity, to show that a product contains the expected drug; (2) Assay, to show that a product contains the expected amount of drug (dose); (3) Content uniformity (CU), to establish that the dose or drug content in each unit varies within an expected range; (4) Dissolution/release, to show that the drug will be released from the product in an expected manner.

All these tests are simple chemical tests based on solvent extractions, i.e. the drug is extracted from the product and measured using any of the quantitative techniques such as spectrophotometric or chromatographic. At present, there are no specific requirements for the use of an extraction procedure. Often, it is expected that the drug be ground and extracted by shaking, vortexing, blending or extracting, with a suitable solvent, filtered and then measured.

Let's consider the extraction step in a slightly different way. Rather than using the currently used practices of vortexing, shaking, blending or extracting, if one uses a dissolution apparatus, without calling it a dissolution apparatus but an ordinary extractor for the extraction of the drug from the product for determining assay and CU only. The extractions are to be performed using: (1) Distilled water as the solvent, which is maintained at 37°C and; (2) The stirring rod will be the crescent-shaped spindle set at 25 rpm, as shown on the top left corner in Figure 8. The solution samples are to be withdrawn at different time intervals to measure drug concentrations in solution until a plateau is reached.

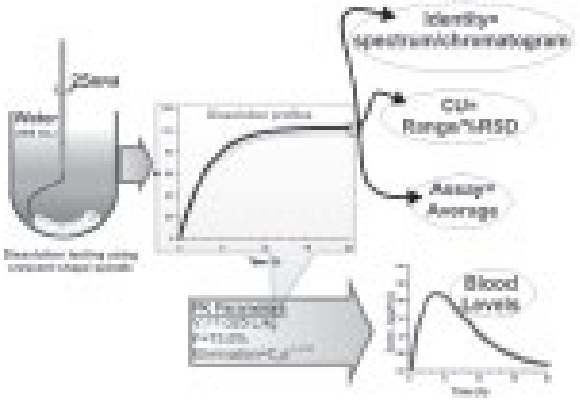

Figure 8: Representation of single (dissolution) test based quality assessment of solid oral pharmaceutical products such as tablets and capsules. As an example for determining plasma drug concentration-time profiles from dissolution results, pharmacokinetic parameters of acetaminophen, shown in the inset, are used.

Plot the extraction of drug (amount as cumulative percentages) against time using graph paper or an Excel spreadsheet. Note that the extractions results over time will in fact be a dissolution/release profile of the product. There is no need for conducting an extra test just for the dissolution testing purpose.

Before evaluating these results further, the following explanation is provided for the above mentioned approach. (1) If a drug is not soluble in water then how should one proceed? In such cases, one should first establish, before starting the extraction step, whether the drug will be soluble in water alone or with the use of some type of solubilizer (e.g., SLS). The requirement is that the extraction solvent should be water, with or without a solubilizer depending on the nature of the drug. (2) Why should one use the crescent-shaped spindle and not the more commonly used stirrers such as Paddle/Basket? The reason is that the paddle and basket apparatuses do not provide appropriate stirring and mixing therefore extraction would not be efficient and accurate. (3) Why should the test not be performed at higher speeds (rpm) to complete the experiment in a shorter time? The reason is that there is an interest in monitoring the difference between extraction profiles of different formulations of the same drug and/or different products, thus a softer/milder extraction process is beneficial.

The data set for the last sampling time represents the complete extraction of the drug, and its values should be on average around 100% of the dose. This result would represent the assay/potency of the product. As the test is conducted in multiple units (6, 12 or more) then the range of individual values or its % RSD values would represent CU (content uniformity). As the quantitation is conducted using UV or chromatographic techniques, then the matching spectra/peak retention time establishes the identity of the drug in the product [59,60].

The data set, as a whole, would represent extraction/release characteristics of the product and would reflect the dissolution characteristics of the product. There is no need for conducting a separate dissolution test. These results may further be manipulated mathematically using pharmacokinetic parameters of the drug to estimate the potential drug levels in humans as described above [37]. A schematic representation of this concept is described in Figure 8 as well.

In short, a single test will provide answers for all four of the parameters which, at present, commonly require 3 or 4 separate tests. In addition, there would not be any need for conducting additional tests for bio- or physiological relevancy, as the testing is conducted using bio-relevant conditions as described earlier, this data can directly be used for estimating blood-levels. Apart from simplicity, time and money savings, the approach provides the following additional advantages. (1) As assay, CU, identity and dissolution results using this approach are obtained from the same units (tablets/capsules), therefore, the approach provides improved overall evaluation of product characterization and quality. (2) The extraction solvent is distilled water, thus, offers significant experimental simplicity and physiological relevancy. The suggested approach eliminates the need for conducting separate bio-relevant dissolution studies. (3) Experimental conditions are product independent which can provide the required comparison of results (quality) within and between products. (4) The approach provides freedom from using paddle/basket apparatus which are known for their flaws and requirements of meeting unnecessary and irrelevantly large sets of specifications.

Conclusion

In general, dissolution tests are conducted ignoring their main and only purpose, i.e., assessment of potential drug release characteristics

of the products in the human GI tract. Considering the hydrodynamics within the vessels of the most commonly recommended apparatuses (paddle/basket), it is not possible to obtain relevant dissolution results as well as acceptable reproducibility. In addition, since these apparatuses have never been validated for their intended use therefore, credibility and relevancy of the data obtained from these will always be of doubtful nature and limited use even for QC purposes. Developing and using a drug and/or product dependent dissolution methods, as well as for applications such as bio-relevancy and quality control, should be considered a scientifically invalid practice which cannot provide true or unbiased dissolution characteristics of any product. Instead, a drug and product independent dissolution testing approach should be considered, as described here, which will not only be scientifically valid but also simplify testing significantly, circumventing unnecessary method development requirements. The practices of classifying drugs based on BCS and developing IVIVCs to estimate plasma drug levels for products from dissolution results require reconsideration as these approaches cannot be useful and/or helpful. Drug absorption behaviour can more appropriately be explained based on continuous extraction of undissociated drugs considering their ionization/dissociation characteristics. In addition, estimation of plasma drug levels from dissolution results, or *in vitro*-to-*in vivo* profiling (IVIVP), may be achieved using convolution technique which is the only method available for such estimations at the product development stage. As suggested in the article, if dissolution tests are conducted by addressing the flaws of the paddle/basket apparatuses, e.g., using a crescent-shape spindle, other quality parameters such as assay, CU, identity can also be obtained from a single and the same dissolution test. Such an approach would result in savings of significant resources as well as efficient and cost effective product developments and evaluations.

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This article is partially based on contributions by the author on a weblog (www.drug-dissolution-testing.com) reflecting current scientific issues, and potential resolutions, on the subject. Further detailed information on the topics covered in this article may be obtained by visiting the weblog.

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