Review of Literatures

Dissolution testing was first established more than half a century ago and for many years was mainly performed to address questions of quality control. However, in recent years, there has been a strong push to identify bioavailability (BA) problems of a drug formulation based on the results of appropriately designed dissolution experiments. Thus, the scope of dissolution testing has expanded considerably to include screening formulations and predicting the in vivo performance of drug formulations. To answer questions in terms of the BA of oral drug formulations, it is crucial to run dissolution tests under conditions that closely resemble the key parameters of human gastrointestinal physiology. In addition to the choice of adequate equipment and appropriate instrument parameters, the use of physiologically relevant dissolution media is of great importance (13). Drug dissolution (or release) testing in an analytical technique used to assess release profiles of drugs in pharmaceutical products, generally solid oral products such as tablets and capsules. For a dosage form to produce its effect, drug must be released and generally should be dissolved in the fluids of the gastrointestinal tract (14). Drug dissolution in the physiological environment of the GI tract is the primary step in the oral absorption process from a pharmaceutical dosage form. Since only dissolved drug can permeate the mucosa at the absorptive sites in the GI tract, both the solubility of the drug and its dissolution rate are crucial for its in vivo behavior. First theories on the dissolution process were described more than a century ago by Noyes and Whitney in 1897 and extended by Nernst and Brunner in 1904 by applying Fick’s law of diffusion. From the following equation, based on Nernst–Brunner and Levich modifications of the Noyes–Whitney model (15, 16), the factors important to the rate of drug dissolution (DR) can be identified:

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\frac{dX_d}{dt} = \frac{A X D}{\delta V} \times (C_s - X_d)
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where A is the effective surface area of the drug, D is the diffusion coefficient of the drug, \( \delta \) is the effective diffusion boundary thickness adjacent to the dissolving surface, \( C_s \) is the saturation solubility of the drug under luminal conditions, \( X_d \) is the amount of drug already in solution, and \( V \) is the volume of the dissolution medium. Some of these parameters are mainly determined by the physicochemical properties of the drug itself, but several are strongly affected by the conditions in the gastrointestinal
tract and also by the composition of the dosage form. The composition of the gastrointestinal content may significantly influence drug solubility. For lipophilic drugs, fat level and bile salt concentration have been shown to be the most pertinent factors. In the case of ionizable drugs, buffer capacity and pH are also relevant to the dissolution rate (13). Dissolution from the dosage form involves mainly two steps: liberation of the drug from the formulation matrix (disintegration) followed by the dissolution of the drug (solubilization of the drug particles) in the liquid medium. The overall rate of dissolution depends on the slower of these two steps. In the first step of dissolution, the cohesive properties of the formulated drug play a key role. For solid dosage forms, these properties include disintegration and erosion. If the first step of dissolution is rate-limiting, then the rate of dissolution is considered disintegration controlled. In the second step of dissolution (i.e., solubilization of drug particles), the physicochemical properties of the drug such as its chemical form (e.g., salt, free acid, free base) and physical form (e.g., amorphous or polymorph and primary particle size) play an important role. If this latter step is rate-limiting, then the rate of dissolution is dissolution controlled. Dissolution test plays important role during life cycle of drug development. During preclinical development, candidates are selected and formulated to assess their safety and tolerability. Adequate bioavailability is a prerequisite, and poorly water soluble compounds present major challenges. Biorelevant dissolution plays an important role in the selection of appropriate drug candidates and formulations for development (17).

In recent times, the regulatory perspective of dissolution has shifted due to improved knowledge and understanding of dissolution science and mechanisms. FDA’s Guidance for Industry on the Biopharmaceutics Classification System (BCS Guidance) emphasizes how the dissolution test can be used to grant biowaivers for highly soluble and highly permeable drugs formulated in rapidly dissolving IR solid oral dosage forms. The BCS is a framework for classifying drug substances based on their solubility and permeability, and for providing a scientific rationale to justify the granting of biowaivers for certain products. The BCS system (18, 19) when integrated with the dissolution of the drug product takes into account three major factors; namely, dissolution, solubility, and intestinal permeability. It is well-established that these three factors govern the rate and extent of drug absorption from IR solid oral dosage forms. According to the BCS Guidance, biowaivers may be granted for BCS class 1 (high-solubility and high-permeability drug substance) products if the drug product is rapidly dissolving. An IR drug product is considered rapidly dissolving when no less than 85% of the labeled amount of the drug substance dissolves within 30 min (20).
Dissolution method development generally requires optimization of parameters such as (a) medium, (b) apparatus/agitation rate, (c) study design, (d) assay, and (e) acceptance criteria of dissolution. Physical and chemical data for the drug substance and dosage unit need to be determined before selecting the dissolution medium. Two key properties of the drug are the solubility and solution state stability of the drug as a function of the pH value. When selecting the composition of the medium, the influence of buffers, pH value, and surfactants on the solubility and stability of the drug need to be evaluated. Key properties of the dosage unit that may affect dissolution include release mechanism (immediate, delayed, or modified) and disintegration rate as affected by hardness, friability, presence of solubility enhancers, and presence of other excipients. Generally, while developing dissolution procedure, one goal is to have sink conditions, defined as the volume of medium at least three times that required in order to form a saturated solution of drug substance. When sink conditions are present, it is more likely that dissolution results will reflect the properties of the dosage form. A medium that fails to provide sink conditions may be acceptable if it is shown to be more discriminating or otherwise appropriately justified. Using an aqueous–organic solvent mixture as a dissolution medium is discouraged; however, with proper justification this type of medium may be acceptable. The dissolution characteristics of an oral formulation should be evaluated in the physiologic pH range of 1.2 to 6.8 (1.2 to 7.5 for modified-release formulations). Typical media for dissolution may include the following (not listed in order of preference): dilute hydrochloric acid, buffers in the physiologic pH range of 1.2 to 7.5, simulated gastric or intestinal fluid (with or without enzymes), water, and surfactants (with or without acids or buffers) such as polysorbate 80, sodium lauryl sulfate, and bile salts. Normally, for basket and paddle apparatus, the volume of the dissolution medium is 500 mL to 1000 mL, with 900 mL as the most common volume. The choice of apparatus is based on knowledge of the formulation design and the practical aspects of dosage form performance in the in vitro test system. USP has provided seven different apparatus such as Paddle, Basket, Reciprocating cylinder, Flow through cell (21), Paddle over disk, Rotating cylinder, and Reciprocating Holder (22) to conduct dissolution test. For solid oral dosage forms, Apparatus 1 and Apparatus 2 are used most frequently. When Apparatus 1 or 2 is not appropriate, another official apparatus may be used. For immediate-release capsule or tablet formulations, Apparatus 1 (baskets) at 100 rpm or Apparatus 2 (paddles) at 50 or 75 rpm are most commonly used. Other agitation speeds and apparatus are acceptable with appropriate justification. Rates outside 25 to 150 rpm are usually inappropriate because of the inconsistency of hydrodynamics below 25 rpm and because of turbulence above 150 rpm. For immediate-release dosage forms, the duration of the procedure is typically 30 to 60 minutes; in most cases, a single time point specification is adequate for Pharmacopeial purposes.
Industrial and regulatory concepts of product comparability and performance may require additional time points, which may also be required for product registration or approval. A sufficient number of time points should be selected to adequately characterize the ascending and plateau phases of the dissolution curve. For an extended-release dosage form, at least three test time points are chosen to characterize the in vitro drug release profile for Pharmacopeial purposes. Additional sampling times may be required for drug approval purposes (23).

Validation studies should address the variations associated with different profile time points. For products containing more than a single active ingredient, the dissolution method needs to be validated for each active ingredient. Parameters such as specificity, linearity, recovery, precision, solution stability and robustness should be checked during method validation study of dissolution test method. (24, 25, 26, 27).

The absorption of electromagnetic radiation of wavelengths between 200 and 800 nm by molecules which have π electrons or atoms possessing unshared electron pairs can be employed for both qualitative and quantitative analysis; as such, it is known as spectrophotometry. As a wide variety of pharmaceutical substances absorb radiation in the near-ultraviolet (200–380 nm) and visible (380–800 nm) regions of the electromagnetic spectrum, the technique is widely employed in pharmaceutical analysis. The relationship between the concentration of analyte and the intensity of light absorbed is the basis of quantitative applications of spectrophotometry. In addition, features of absorption spectra such as the molar absorptivity, spectral position, and shape and breadth of the absorption band are related to molecular structure and environment and therefore can be used for qualitative analysis. The absorption of near-ultraviolet or visible light by molecules occurs as a result of the interaction of the electric field associated with a light wave or photon with molecular electrons. The basic spectrophotometer generally consists of a light source from which a given wavelength or range of wavelengths is selected by a wavelength selection device. The radiation selected is directed through the analytical sample and the transmitted light monitored by a detector. The light intensity measured by the detector is subsequently compared to that transmitted by a reference substance, the ratio being displayed usually as an absorbance but less commonly as a percent transmittance on a readout device (28). Development of simple UV – spectrophotometric method will prove helpful towards rapid quantification of analyte from the sample. Acceptability of these methods should be checked by validating them for accuracy, linearity, precision, specificity etc. (29). HPLC can be used to analyze dissolution samples that otherwise cannot be analyzed by UV methods either because of significant
interferences or poor UV absorbance. As formulation development tends toward increasing complexity, HPLC methodology will be embraced even more in dissolution testing, as it can help solve unique analytical challenges posed either by the media or by dosage form characteristics.

The ICH guideline Q1A on Stability Testing of New Drug Substances and Products emphasizes that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and/or efficacy must be done by validated stability-indicating testing methods. The ICH guideline Q3B entitled ‘Impurities in New Drug Products’ emphasizes on providing documented evidence that analytical procedures are validated and suitable for the detection and quantitation of degradation products. It is also required that analytical methods should be validated to demonstrate that impurities unique to the new drug substance do not interfere with or are separated from specified and unspecified degradation products in the drug product. The ICH guideline Q6A, which provides note for guidance on specifications, also mentions the requirement of stability-indicating assays under Universal Tests/Criteria for both drug substances and drug products. Elaborate definitions of stability-indicating methodology are, however, provided in the United States-Food and Drug Administration (US-FDA) stability guideline of 1987 and the draft guideline of 1998. Stability-indicating methods according to 1987 guideline were defined as the ‘quantitative analytical methods that are based on the characteristic structural, chemical or biological properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured.’ This definition in the draft guideline of 1998 reads as: ‘validated quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference.’ (5).

Thus for routine analysis in a stability program, a stability-indicating method is required for analyzing both the API and impurities. Stability indicating analysis for an API is crucial since it measures the potency of the drug at an initial time point and the loss of potency during storage. The evaluation of impurities with a good stability-indicating method is also important in measuring the impurities and degradants, which could have toxicological effects when administered to humans. Forced degradation studies in which the product is artificially exposed to high stress conditions can be supportive in developing a stability-indicating assay method. (10)
Approach of forced degradation study (stress testing) can be applied for the development of stability-indicating HPLC technique for assay method and related substances method (impurity profiling). Stressing the parent compound under particular stress conditions can generate samples containing degradation products. These samples can then be used to develop suitable analytical procedures. It is important to note that the degradation products generated in the stressed samples can be classified as “potential” degradation products that may or may not be formed under relevant storage conditions. The knowledge gained from stress testing is useful for formulation and packaging development. Well-designed stress-testing studies can determine the susceptibility of a compound to hydrolysis, oxidation, photochemical degradation and thermal degradation. This information is then taken into consideration when developing the formulation and determining the appropriate packaging (30). Stress testing of the drug substance and drug product can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures developed (31, 32, 33, 34). The nature of the stress testing will depend on the individual drug substance and the type of drug product involved (35). Analytical method validation will ensure that the developed method is reproducible & reliable. Method validation is defined as the process of proving (through scientific studies) that an analytical method is suitable for its intended use. (36)

High-pressure liquid chromatography (HPLC) is used in analytical development to quantitate the active pharmaceutical ingredient (API) and to evaluate impurity and degradation product profiles of drug substances (DS) and drug products (DP). HPLC is a physical separation technique conducted in the liquid phase in which a sample is separated into its constituent components (or analytes) by distributing between the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column). An online detector monitors the concentration of each separated component in the column effluent and generates a chromatogram. HPLC is the most widely used analytical technique for the quantitative analysis of pharmaceuticals, biomolecules, polymers, and other organic compounds. Reversed-phase HPLC analysis is generally considered the most effective method of identifying most drug substance degradation or drug-excipient interactions. Hence, it is the typical choice for stability-indicating and stability-specific methods for small molecules. Reversed-phase HPLC accounts for more than 85% of stability-indicating methodologies for small molecular chemical entities as it is suitable for release testing, assay, and assessing impurities. With its well-established techniques, efficiency, robustness, and ease of use, HPLC plays a pivotal role in drug stability testing (10).