In the event that the package cannot be delivered as scheduled, the package should be returned to the mailing pharmacy.

Risk Management System

Risk Management System strategies should ensure that each organization's best interests are served by adhering to proper practices, controls, and procedures, including but not limited to the following: the nature of the drug products; distribution requirements on the readable container labeling; exposure to adverse environmental conditions; number of stages/receipts in the supply chain; manufacturer's written instructions; contractors; and drugs at risk from freezing (vaccines, insulin, and biological products) or elevated temperatures (fatty-based suppositories, vaccines, insulin, and biological products).

Examples of risks include the following: (1) vibration that can cause aggregation of some drug products such as proteins and peptide-based drugs; (2) temperature excursions that may lead to phase changes (melting or freezing); (3) loss of container–closure integrity in transit that could cause glass fractures or loss of sterility in sterile drug product containers; and (4) ingress of water or oxygen that could lead to an increase in degradation products. Appropriate firms such as applicant holders are recommended to convey relevant environmental requirements when needed to support deviations or excursions. There may be alternate ways of determining acceptable environmental conditions and these should be documented and justified.

Pharmaceutical manufacturers should ensure that suppliers of drug product transportation are monitored. Auditing transportation firms should be carried out routinely to ensure adequate product handling. The manufacturer's change control system should capture and evaluate changes in logistic factors such as warehouse or receiving areas and vehicle changes.

CONCLUSION

The practices and processes set forth in this general information chapter apply to storage and distribution as part of the life-cycle management of drug products. All involved should ensure the product to its point of use, creating a contiguous supply network that is collaborative and emphasizes preventive measures to protect drug product quality. The increase in global processes coupled with products requiring special environmental controls highlights the need for a strong QM program. QM should provide the foundation for maintaining the storage and distribution practices in a continual improvement program and part of an overall management system review by each entity, as appropriate, in the supply chain.

It is equally important to stay current and be ready to change as new solutions evolve. These new technologies should be considered in developing strategies for good distribution practices, controls, and procedures.■2S (*USP35*)

⟨1088⟩ IN VITRO AND IN VIVO EVALUATION OF DOSAGE FORMS

Change to read:

▪PURPOSE

This chapter provides an overview of the methodology for characterizing the physicochemical properties of a drug substance as well as its associated drug product and discusses the relationship of these methods and properties to the pharmacokinetic and pharmacodynamic properties of the drug product. Results of in vitro methods are linked with information from in vivo evaluations through an in vitro–in vivo correlation (IVIVC).

SCOPE

The ultimate goal of these characterization studies is an understanding of the relationship between the physicochemical and pharmacological properties of the drug substance to the pharmacokinetic properties and in vitro performance of the drug product. This chapter outlines the in vitro and in vivo testing that goes into the development of the body of data that informs decision making relating to the formulation, manufacturing, and related regulatory activities necessary for the development, regulatory approval, and marketing of any drug product. The chapter complements the information in general chapters, *Assessment of Drug Product Performance—Bioavailability, Bioequivalence, and Dissolution* ⟨1090⟩ and *The Dissolution Procedure: Development and Validation* ⟨1092⟩ by detailing the essential in vitro and in vivo data elements underlying an understanding of bioequivalence and bioavailability. The chapter text recognizes that regulatory guidances and a wealth of text books are available to elaborate on the content provided, and it is not the purpose to provide an exhaustive disquisition on the subjects presented but rather to provide a guide and listing of the issues of interest.

BACKGROUND INFORMATION

Establishing a meaningful relationship between dissolution behavior and in vivo drug performance (i.e., IVIVC) has long been sought from the perspectives of both bioavailability (BA) and bioequivalence (BE) and quality control considerations. In setting dissolution acceptance criteria for a product monograph, USP's policy has been to give predominant consideration to valid BA or BE studies, when available.

The earliest achievable in vitro characteristic thought to predict an acceptable in vivo performance was tablet and capsule disintegration. A test for disintegration was adopted in *USP XIV* (1950). At that time, no quantitative work was done to attempt to demonstrate such a relationship, especially with regard to in vivo product performance. Advances in instrumental methods and analytical precision ultimately opened up prospects for this work. The USP–NF Joint Panel on Physiologic Availability recognized that the disintegration test was insufficiently sensitive and in 1968 directed the identification of candidate articles for the first 12 official dissolution tests that used *Apparatus 1*.

USP requires drug release testing via the USP performance test in the majority of monographs for non-solution oral, sublingual, and transdermal dosage forms. In the current state of science, in vivo testing is necessary during the development and evaluation of both immediate-release and

modified-release dosage forms. In some cases, depending on the Biopharmaceutics Classification System (BCS) classification of the drug, and depending on regulatory policy, in vivo testing may not be necessary. The special sensitivity of the dissolution test to changes in composition or method of manufacturing that do not result in significant changes in performance in vivo is well recognized. An understanding of the full complement of information given by in vitro and in vivo evaluation of the drug substance and product is the starting point in the development of a meaningful in vitro performance test.

IN VITRO EVALUATION

Physicochemical Properties—Drug Substance

Physicochemical information typically includes polymorphism, stability, particle size distribution, solubility, dissolution rate, lipophilicity, permeability, and other release-controlling variables of the drug substance under conditions that may mimic the extremes of the physiologic environment experienced by the dosage form.

Physicochemical Properties—Drug Product

The variables tested to characterize the physicochemical properties of the drug product should be the same as those that are tested to characterize the drug substance. Dissolution profiles over a relevant pH range, usually from pH 1–6.8, should be obtained with particular attention to formulation effects. Characterization of formulations that are insoluble in aqueous systems may require the addition of sodium lauryl sulfate or another surfactant. The BCS classification of the drug substance should be determined, especially for immediate-release dosage forms.

Dissolution Testing

Dissolution testing is required for all non-solution oral, including sublingual, Pharmacopeial dosage forms in which absorption of the drug is necessary for the product to exert the desired therapeutic effect. Exceptions include tablets that meet a requirement for completeness of solution, products that contain radiolabeled drugs, or products that contain a soluble drug and demonstrate rapid (10–15 min) disintegration. Dissolution testing should be conducted on equipment that conforms to the requirements in *Dissolution* ⟨711⟩ and on which a performance verification test has been conducted when one is available. On its website, USP provides a guidance for optimizing dissolution instrument performance by mechanical calibration and performance verification testing (http://www.usp.org/pdf/EN/dissolutionProcedureToolkit2010-03.pdf).

In vitro dissolution testing generally should attempt to mimic in vivo dissolution, but such in vitro conditions cannot be selected reliably a priori. A range of in vitro dissolution test conditions (e.g., media of varying pH, surfactant, and apparatus rotational speed) should be evaluated. Knowledge of drug substance properties, product formulation, gastrointestinal physiology, in vitro dissolution, and in vivo pharmacokinetics will aid in the selection of in vitro dissolution test conditions and specifications.

For products that contain more than a single active ingredient, dissolution typically should be determined for each active ingredient. When a dissolution test is added to an existing monograph, the disintegration test is deleted, but in the case of sublingual preparations and orally disintegrating tablets, disintegration may be a critical quality attribute in addition to dissolution. In such cases one or both tests can be included in the monograph.

When a single set of specifications cannot be established for multisource products described in monographs, multiple dissolution tests are allowed, and labeling is required to indicate the appropriate dissolution test for the specific product.

Detailed information about method development and validation can be found in *The Dissolution Procedure: Development and Validation* ⟨1092⟩.

IMMEDIATE-RELEASE DOSAGE FORMS

For immediate-release dosage forms the in vitro dissolution process typically requires no more than 60 min, and in most cases a single time-point specification is adequate for Pharmacopeial purposes. To allow for typical disintegration times, test times of less than 30 min should be based on demonstrated need.

EXTENDED-RELEASE DOSAGE FORMS

For extended-release products in vivo dissolution generally is rate limiting, which results in protracted drug absorption and thus facilitates the identification of in vitro test conditions that may be predictive of in vivo dissolution. Multiple sampling time points, therefore, are necessary to define a dissolution profile for a modified-release dosage form.

The choice of apparatus should be based on knowledge of the formulation and actual dosage form performance in the in vitro test system. *Apparatus 1* (basket) or *Apparatus 2* (paddle) may be more useful at higher rotation rates (e.g., the paddle at 100 rpm). *Apparatus 3* (reciprocating cylinder) has been especially useful for bead-type modified-release dosage forms. *Apparatus 4* (flow cell) may offer advantages for modified-release dosage forms that contain active ingredients that have limited solubility. *Apparatus 7* (reciprocating disk) is applicable to nondisintegrating oral modified-release dosage forms, as well as to transdermal dosage forms. *Apparatus 5* (paddle over disk) and *Apparatus 6* (cylinder) also are useful for evaluating and testing transdermal dosage forms.

At least three timepoints are chosen to characterize the in vitro drug release profile of an extended-release dosage form for Pharmacopeial purposes. Additional sampling times may be required for drug approval purposes. An early time point, usually 1–2 h, is chosen to show that dose dumping is not probable. An intermediate time point is chosen to define the in vitro release profile of the dosage form, and a final time point is chosen to show essentially complete release of the drug.

IN VIVO EVALUATION OF DOSAGE FORMS

In evaluating a drug product's performance, analysts fundamentally must ask what type of study should be performed to give reasonable assurance of BE of a marketed product to the clinical trial product that demonstrated safety and efficacy. Although they provide important information concerning the release characteristics of the drug from the dosage form, in vitro dissolution studies at present are used primarily for setting or supporting specifications for drug products (e.g., shelf life) and manufacturing process control (e.g., scale-up or postapproval changes). Normally BE is best demonstrated by in vivo evaluation but can sometimes be replaced by in vitro studies.[1] BE assessment of modified-release dosage forms is best achieved by observing in vivo drug pharmacokinetic and/or pharmacodynamic behavior by means of well-designed clinical studies. Multiple guidances for the conduct of such studies are provided by regulatory agencies. Moreover, when a well-defined, predictive relationship exists between plasma concentrations of a drug

[1] 21 CFR 320.22 Criteria for waiver of evidence of in vivo bioavailability or bioequivalence.

or its active metabolites and the clinical response (therapeutic and adverse), it is possible to use plasma drug concentration data alone as a basis for the approval of a modified-release dosage form that is designed to replace an immediate-release dosage form.

Although human pharmacokinetic studies often are used to assess BE of immediate-release solid oral dosage forms, in some cases in vitro studies can be used to assess BE. The principal advantage of in vitro studies is that they reduce development costs. For example, an in vitro test is preferable when one is testing BCS Class I drugs with rapid dissolution. Some regulatory agencies permit this type of testing in lieu of in vivo testing.

The following discussions are intended to provide guidance for drug substance evaluation and the design, conduct, and evaluation of studies involving dosage forms. Although these guidelines focus on oral drug delivery systems, the principles may be applicable to other routes of drug administration (e.g., transdermal, subcutaneous, intramuscular, etc.).

CHARACTERIZATION OF DRUG SUBSTANCE

The Biopharmaceutics Classification System (BCS)

FDA has issued a guidance titled "Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System" (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070246.pdf). A key assumption in the approach is that drug release and dissolution is sufficiently rapid so that an in vitro–in vivo correlation is not possible and/or useful. When applicable, the BCS allows dissolution rate data in lieu of BA or BE studies for product approval.

Pharmacokinetic Properties

Analysts should thoroughly characterize the input absorption profile of the active drug entity from a formulation that shows rapid BA (an intravenous solution, oral solution, or a well-characterized immediate-release drug product). In turn, this formulation serves as a reference to evaluate the input profile of the modified-release dosage form. This information, together with the pharmacokinetics of the active drug entity, can characterize drug absorption and can predict changes in drug BA when input is modified as in modified-release dosage forms. For example, if the active drug entity exhibits saturable first-pass hepatic metabolism, a reduction in systemic availability could result after oral administration if the input rate is decreased.

In designing an oral modified-release dosage form, analysts may find it useful to determine the absorption of the active drug entity in various segments of the gastrointestinal tract, particularly in the lower gastrointestinal tract (colon) for delayed-release dosage forms that release drug in this region. Food effects also may be important and should be investigated.

Drug Disposition

The information required to characterize drug disposition may include the following.

1. Disposition parameters—clearance, area under the time—plasma concentration curve (AUC), maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), volume of distribution, half-life, mean residence time, or model-dependent parameters.
2. Linearity or characterization of nonlinearity over the dose or concentration range that could be encountered.
3. Drug/metabolite accumulation.
4. Metabolic profile and excretory pathway, with special attention to the active metabolites and active enantiomers of racemic mixtures.
5. Enterohepatic circulation.
6. Protein-binding parameters and effect of dialysis.
7. The effects of age, gender, race, and relevant disease states.
8. Plasma: blood ratios.
9. A narrow therapeutic index or a clinical response that varies significantly as a function of the time of day (chronopharmacokinetics).

Pharmacodynamic Properties

Before developing a dosage form, analysts should obtain concentration–response relationships over a dose range sufficiently wide to encompass important therapeutic and adverse responses. In addition, the equilibration-time[2] characteristics between plasma concentration and effect should be evaluated. For modified-release products that typically have larger drug doses in the dosage form, these concentration–response relationships should be sufficiently characterized so that a reasonable prediction of the safety margin can be made if dose dumping should occur. If there is a well-defined relationship between the plasma concentration of the active drug substance or active metabolites and the clinical response (therapeutic and adverse), the clinical performance of a new modified-release dosage form could be characterized by plasma concentration–time data. If such data are not available, clinical trials of the modified-release dosage form should be carried out with concurrent pharmacokinetic and pharmacodynamic measurements.

CHARACTERIZATION OF THE DOSAGE FORM

Pharmacokinetic Properties: Immediate-Release Products

The types of pharmacokinetic studies that should be conducted are based on how much is known about the active drug substance, its clinical pharmacokinetics, and its BCS Class. For example, a new chemical entity requires greater pharmacokinetic characterization than does an FDA-approved formulation that is undergoing scale-up and postapproval changes (SUPAC) evaluation.

The latter is seen when an FDA-approved drug product undergoes changes in the manufacturing of the product after the product has been approved. Such changes are common and can be caused by expansion in the size of the lots manufactured, new manufacturing locations, or the introduction of new technology. Necessary in vitro dissolution tests and/or in vivo BE tests are described in the FDA "Guidance for Industry: Immediate-release Solid Oral Dosage Forms: Scale-up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation" (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070636.pdf).

[2] Equilibration time is a measure of the time-dependent discontinuity between measured plasma concentrations and measured effects. The discontinuity is more often characterized by the degree of hysteresis observed when the effect-concentration plot for increasing concentrations is compared with that for decreasing concentrations. Where the equilibration time is very short (i.e., rapid equilibration with no active metabolites generated), there will be little or no hysteresis. That is, the same effect will be observed for a given concentration independent of the interval between the time of dosing and the time that measurements are made.

Similar requirements apply to a generic equivalent of an approved immediate-release dosage form that must be BE to the innovator drug, known as the reference listed drug. The two most frequently used methods for meeting bioequivalence requirements are in vivo pharmacokinetic studies and BCS-based in vitro studies.

Pharmacokinetic Properties: Modified-Release Products

Like the approaches for immediate-release products, the types of pharmacokinetic studies that should be conducted for modified-release products are based on how much is known about the drug substance, its pharmacokinetics, biopharmaceutics, and whether pharmacokinetic studies are intended to be the sole basis for product approval. At a minimum, two studies are required to characterize the product when no reference modified-release product exists: (1) a single-dose crossover study for each strength of a modified-release dosage form and (2) a multiple-dose, steady-state study using the highest strength of a modified-release dosage form. A food effects study to evaluate the potential for dose dumping from extended-release dosage forms also is required as a separate study or is included as an arm of a crossover study. In the demonstration of interchangeability, a single-dose, fasting crossover study vs. the reference product usually will suffice. In some cases, a food-effects study is required if the reference product has demonstrated a food effect on BA. Some appropriate single-dose crossover and multiple-dose steady-state studies are described below.

For modified-release products, intravenous solutions, oral solutions, or well-characterized immediate-release drug products are possible reference products to evaluate a modified-release formulation. For example, if the active drug entity exhibits saturable first-pass hepatic metabolism from the small intestine, a reduction in systemic availability could result after oral administration if the input rate is decreased. An increase in systemic availability could be observed if a drug is absorbed from the colon from a delayed-release dosage form that targets the colon, thus avoiding a first-pass effect.

In some modified-release capsule dosage forms, the strengths differ from each other only in the amount of identical beaded material contained in each capsule. In this case, single-dose and multiple-dose steady-state studies at the highest dosage strength are sufficient. Other strengths can be characterized on the basis of comparative in vitro dissolution data.

The pharmacokinetic studies described below are needed for most modified-release dosage forms. These studies may be the basis for characterization of the dosage form. If regulatory approval is sought without conducting clinical trials, manufacturers should consult with the regulatory authorities to ensure that an adequate database exists for the approval. The types of pharmacokinetic studies generally conducted can be categorized as follows.

CASE A

Case A applies to an original modified-release oral dosage form for a drug already marketed in an immediate-release dosage form and for which extensive pharmacokinetic/pharmacodynamic data exist.

Single-dose crossover study: A single-dose crossover study should include the following treatments: the modified-release dosage form administered under fasting conditions; a dosage form that is rapidly available administered under fasting conditions; and the modified-release dosage form administered immediately after a high-fat standardized meal. The food effects study should control the ambient-temperature fluid intake (e.g., 6–8 oz.) at the time of drug administration. The dosage form should be administered within 5 min after completion of the meal. Ideally all subjects should consume the meal in approximately 15 min. If there are no significant differences in the rate or extent of bioavailability (AUC, C_{max}, and T_{max}) as a function of the meal, then additional food effect studies are not necessary. If significant differences in bioavailability are found, researchers must define how food affects the modified-release dosage form,[3] as well as how the food–drug effect relates to time.

Use the following guidelines in evaluating food effects.

1. If no well-controlled studies have previously defined the effects of a concurrent high-fat meal on an immediate-release dosage form, studies should be performed to determine whether a food effect is a result of problems with the dosage form. Does the dosage form show food-related changes in release, or are there food effects that are unrelated to the dosage form, e.g., changes in the drug's absorption from the gastrointestinal tract or changes in the drug's disposition that are independent of absorption? The cause of the food effect should be determined by a single-dose crossover study comparing the solution (or immediate-release dosage form) under fed and fasting conditions. If there is no food effect, then one concludes that there are problems with the dosage form. If there is a food effect, then one concludes that the effect is unrelated to the dosage form.
2. The influence of timing on the food effect should be tested by a four-way crossover study, in which the modified-release dosage form is administered under the following treatment conditions: fasting, taken with a high-fat meal, 1 h before a high-fat meal, and 2 h after a high-fat meal.
3. If the food effect on an immediate-release dosage form is determined to result from changes in the dissolved drug's absorption from the gastrointestinal tract or from changes in drug disposition, studies should define the appropriate relationship between drug dosing and meals.
4. Alternative appropriate studies can be conducted if the applicant labels the drug for administration with a meal that is not fat loaded. In this case, an alternative meal composition should be considered.
5. Analysts should monitor the entire single-dose, modified-release absorption profile. Where appropriate (e.g., in a multiple-dose study) for specific drugs and drug delivery systems, blood samples should be taken following breakfast on the second day, before the second dose is administered. This sampling schedule is particularly important for once-a-day products.
6. For delayed-release (enteric-coated) dosage forms, analysts should perform BA studies to characterize food effect and to support the dosing claims stated in the labeling.

The purpose of these studies is twofold: first, to determine whether a need exists for labeling instructions describing special conditions for administration with respect to meals; and second, to provide information concerning the pattern of absorption of the modified-release dosage form compared to that of the immediate-release dosage form. Drug input function should be defined for modified-release dosage forms. This will aid in the development of an appropriate in vitro dissolution test. For dosage forms that exhibit high variability, a replicate study design is recommended.

Multiple-dose, steady-state studies

Study I—When data demonstrating linear pharmacokinetics exist for an immediate-release dosage form, a steady-state study should be conducted with the modified-release dosage form at one dose rate (preferably at the high end of the usual dosage regimen) using a comparable total daily dose of an immediate-release dosage form as a control. At least three trough plasma drug concentration (C_{min}) determinations at the same time of day should be made to demon-

[3] Wagner–Nelson, Loo–Riegelman, and other deconvolution methods are found in textbooks on biopharmaceutics.

strate that steady-state conditions have been achieved. Plasma drug concentration determinations, over at least one dosing interval of the modified-release dosage form, should be made in each phase of the crossover study. It may be preferable (as in the case of rhythmic variation in absorption or disposition of the drug) to measure concentrations over an entire day in each phase. The presence or absence of circadian variation should be verified. The modified-release dosage form should produce an AUC that is equivalent to that of the immediate-release dosage form if the extent of absorption from the modified-release dosage form is comparable to the immediate-release dose. The degree of fluctuation for the modified-release product should be the same as, or less than, that for the immediate-release dosage form given by the approved regimen. Appropriate concentration measurements should include unchanged drug and major active metabolites. For racemic drug entities, analysts should consider measurement of the active enantiomers.

Study II—When comparisons of the pharmacokinetic properties of an immediate-release dosage form at different doses are not available, or when the data demonstrate nonlinearity, steady-state crossover studies comparing effects of the modified-release dosage form and those of the immediate-release dosage form should be conducted at two different dose rates: one at the low end of the recommended dosing range and the second at the high end of the dosing range. In each case, the modified-release dosage form must meet the criteria described in *Study I* with respect to AUC and fluctuations in plasma drug concentrations. If there are significant differences between the modified-release dosage form and the immediate-release dosage form at either the low or the high dosing rate, these data alone are not adequate to characterize the product. Data can be misleading when obtained from subjects with atypical drug disposition or physiologic characteristics relative to the target population. Therefore, subject selection should be from an appropriate target population with randomized assignment to dosage form population. If the modified-release dosage form is for use in a specific subpopulation (e.g., for children), it should be tested in that population. Whether a drug exhibits linear or nonlinear pharmacokinetics, the basis for characterization is equivalence of AUC and of the relative degree of fluctuation of concentrations of the modified-release and immediate-release dosage forms.

Steady-state studies in selected patient populations or drug interaction studies may also be necessary, depending on the therapeutic use of the drug and the types of individuals for whom the modified-release dosage form will be recommended. For drugs that have narrow therapeutic indices, it may be necessary to perform more extensive plasma concentration measurements to determine the potential for unusual drug-release patterns in certain subpopulations. In such studies, researchers should perform more than one AUC measurement per patient to assess variability with both the modified-release and the immediate-release dosage forms.

CASE B

Case B applies to a non-oral, modified-release dosage form of an already marketed active drug entity for which extensive pharmacokinetic and pharmacodynamic data exist.

Case A studies (omitting the food effects studies) are appropriate for the evaluation of a modified-release dosage form designed for a non-oral route of administration if the pattern of biotransformation to active metabolites is identical for the two routes. If the biotransformation patterns are different, then clinical efficacy studies should be performed with the modified-release dosage form. In addition, special studies may be necessary to assess specific risk factors related to the dosage form (e.g., irritation and/or sensitization at the site of application of a transdermal drug delivery system).

CASE C

Case C applies to a generic equivalent of an approved modified-release dosage form, which should be BE to the reference drug in its rate and extent of drug exposure (i.e., AUC, C_{max}, C_{min}, and degree of fluctuation) in crossover single-dose studies. For an oral modified-release dosage form, the food studies described under *Case A* also should be performed.

CASE D

Case D applies to an FDA-approved product that has undergone SUPAC. Necessary in vitro dissolution tests and/or in vivo bioequivalence tests are described in the FDA guidance, *SUPAC-MR: Modified Release Solid Oral Dosage Forms; Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation* (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070640.pdf).

Statistical Analysis of In Vivo Bioequivalence

An appropriate statistical method should be selected. (See *Assessment of Drug Product Performance—Bioavailability, Bioequivalence, and Dissolution* ⟨1090⟩).

IN VITRO–IN VIVO CORRELATIONS

The term IVIVC first appeared in the pharmaceutical literature as a result of the awareness of the importance of bioavailability concepts and in vitro dissolution rate determinations. IVIVC refers to the establishment of a rational relationship between a biological property, or a parameter derived from drug plasma concentrations produced by a dosage form, and a physicochemical property or characteristic of the same dosage form. The biological properties most commonly used are one or more pharmacokinetic parameters such as C_{max} or AUC, obtained following the administration of the dosage form. The physicochemical property most commonly used is a dosage form's in vitro dissolution behavior (e.g., percent of drug released under a given set of conditions). The quantitative relationship between the two properties, biological and physicochemical, is an IVIVC. The most important use of an IVIVC is for predictability. In many cases the actual drug plasma concentration profile can be predicted from in vitro dissolution data.

Historically, IVIVC analysis has been more successful for extended-release products than for immediate-release products. This difference probably reflects the application of specific data analysis techniques and interpretations that require dissolution rate–limited drug absorption. However some correlations with immediate-release products have been demonstrated using methods that rely on the current, broad availability of computers and nonlinear regression software, along with new correlation methods.

General Considerations

With the proliferation of modified-release products, it becomes necessary to examine IVIVC in greater detail. Unlike immediate-release dosage forms, modified-release products, particularly extended-release dosage forms, cannot be characterized using a single time point dissolution test. These products are designed to deliver drug so that a patient has a specific plasma level profile over a prolonged period, usually 12–24 h. Analysts require an in vitro means of ensuring that each batch of the product will perform identically in vivo. An IVIVC satisfies this requirement. Initially, it was thought that developing a meaningful correlation for imme-

diate-release dosage forms would be an easier task than for extended-release products. However, because of the nature of the principles on which each type is based, analysts now believe that an IVIVC is more readily achieved for modified-release dosage forms.

One expects all extended-release products to be dissolution rate limited. For these products, the formulation significantly contributes to the prolongation of drug release from the dosage form. Because of the impact of formulation on BA from an extended-release product, numerous attempts have been made to correlate one or more pharmacokinetic parameters determined from in vivo studies with the amount released in a given time during an in vitro dissolution test. Single-point correlations can indicate that increasing or decreasing the in vitro dissolution rate of the modified-release dosage form would result in a corresponding directional change in the product's performance. However, such single-point correlations reveal little about the overall plasma level curve, which is a major factor for drug performance in the patient. Rather, correlation methods that utilize all plasma drug concentration data and all in vitro dissolution data are preferred. Three correlation procedures are available that use all dissolution and plasma data, along with statistical moment analysis. Each procedure displays important differences in the quality of the correlation. These methods are discussed in terms of the advantages of each along with its potential utility as a predictive tool for pharmaceutical scientists.

Correlation Levels

Three correlation levels have been defined and categorized in descending order of quality. The concept of correlation level is based on the ability of the correlation to reflect the entire plasma drug concentration–time curve that results from administration of the given dosage form. The relationship of the entire in vitro dissolution curve to the entire plasma concentration–time profile defines the strength of the correlation and, therefore, the predictability.

LEVEL A

This level is the highest category of correlation. It represents a point-to-point relationship between in vitro dissolution and the in vivo input rate (absorption rate of the drug from the dosage form). For a *Level A* correlation, a product's in vitro dissolution curve is compared to its in vivo input curve, i.e., the curve produced by deconvolution of the plasma profile. Deconvolution can be accomplished using mass balance model-dependent methods, such as the Wagner–Nelson or Loo–Riegelman methods, or by model-independent, mathematical deconvolution. In an ideal correlation, the in vitro dissolution and in vivo absorption rate curves are superimposable or can be made superimposed by the use of a constant offset value of the time scale. The equations describing each curve are the same. This procedure often is found with modified-release dosage systems that demonstrate an in vitro release rate that is essentially independent of the dissolution media and stirring speeds used in a dissolution apparatus. Superimposition is not an absolute requirement for a *Level A* correlation. If the dissolution and absorption curves are different and a mathematical relationship can be developed to relate the two, the plasma level profile still is predictable from the in vitro dissolution data. This relationship must be true not only at that single input rate but also over the entire quality control dissolution range for the product. Furthermore, when the dissolution rate depends on mixing speed, the two curves can be made to superimpose by either increasing or decreasing the in vitro mixing speed or some other alteration of the dissolution method.

The advantages of a *Level A* correlation are as follows.

1. It develops a point-to-point correlation. This is not found with any other correlation level. It is developed using every plasma level and dissolution point collected at different time intervals, so it reflects the complete plasma level curve. As a result, in the case of a *Level A* correlation an in vitro dissolution curve can serve as a surrogate for in vivo performance. A change in manufacturing site, method of manufacture, raw material supplies, minor formulation modifications, and even product strength using the same formulation can be justified without the need for additional BA-BE studies.[4,5]
2. A truly meaningful quality control procedure that indicates in vivo performance and is predictive of a dosage form's performance is defined for the dosage form.
3. The extremes of the in vitro quality control standards can be justified either by convolution (simulating the plasma level profile from the dissolution curve) or by deconvolution (using the upper and lower confidence interval limits).

LEVEL B

This correlation uses the principles of statistical moment analysis. The mean in vitro dissolution time is compared to either the mean residence time or the mean in vivo dissolution time. As with a *Level A* correlation, *Level B* uses all of the in vitro and in vivo data but is not considered a point-to-point correlation. It does not correlate the actual in vivo plasma profiles but rather a parameter that results from statistical moment analysis of a plasma profile component such as mean residence time. Because a number of different plasma profiles can produce similar mean residence time values, one cannot rely on a *Level B* correlation alone to predict a plasma profile from in vitro dissolution data. In addition, in vitro data from such a correlation cannot be used to justify values at the extremes of quality control standards.

LEVEL C

This category relates one dissolution time point ($t_{50\%}$, $t_{90\%}$, etc.) to one pharmacokinetic parameter such as AUC, C_{max}, or T_{max}. It represents a single-point correlation and does not reflect the complete shape of the plasma profile, which best defines the performance of modified-release products. Because this type of correlation is not predictive of actual in vivo product performance, generally it is useful only as a guide in formulation development or as a production quality control procedure. Because of its obvious limitations, a *Level C* correlation has limited usefulness in predicting in vivo drug performance and is subject to the same caveats as a *Level B* correlation in its ability to support product and site changes as well as justification of the extreme values in quality control standards. The FDA Guidance "Extended-Release Solid Oral Dosage Forms—Development, Evaluation, and Application of In Vitro/In Vivo Correlations" (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070239.pdf) states that manufacturers can obtain biowaivers based on multiple *Level C* correlations. The guidance shows how manufacturers can achieve this correlation. The FDA also indicates that if such a

[4] FDA Guidance SUPAC-MR: Modified Release Solid Oral Dosage Forms—Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation (1997).

[5] FDA Guidance Extended-Release Solid Oral Dosage Form—Development, Evaluation, and Application of In Vitro/In Vivo Correlations, "If an IVIVC is developed with the highest strength, waivers for changes made on the highest strength and any lower strengths may be granted if these strengths are compositionally proportional or qualitatively the same, the in vitro dissolution profiles of all the strengths are similar, and all strengths have the same release mechanism."

correlation is achievable, it is likely that the development of a *Level A* correlation is also feasible.

DEVELOPING A CORRELATION

This chapter does not define the only procedures for developing an IVIVC, and any well-designed and scientifically valid approach is acceptable. To assist the pharmaceutical scientist, one possible procedure for developing a *Level A* correlation is described below:

1. In order to perform deconvolution properly, analysts should be familiar with the pharmacokinetics of the drug itself as well as when it is incorporated into a modified-release dosage form. For example, if a drug is known to be fully absorbed but demonstrates saturable first-pass kinetics, it is best to assume 100% bioavailability for purposes of absorption rate calculation. This is based upon the fact that the drug is fully absorbed, but because of liver metabolism, one sees less than if the drug were administered as an immediate-release bolus. If one utilizes the extent of absorption relative to an immediate-release or solution dosage form, the input profiles will not superimpose with that calculated assuming 100% absorption. However, point-to-point correlations most likely will be possible.
2. Different dissolution profiles of a formulation should be obtained as illustrated in *Figure 1*. The formulation should be modified only sufficiently to produce different dissolution profiles so that the formulation has the same excipients in all the lots that will be tested. The formulation modifications used in these batches should be based on factors that would be expected to influence the product's modified-release rate and could occur during normal product manufacture. In vitro drug release is performed on the batches that will be used in the bioavailability study, and the effect of varying the dissolution conditions is investigated. Some of the variables that should be studied are the apparatus (it is preferable to use official dissolution equipment), mixing intensity, and dissolution medium (i.e., pH value, enzymes, surfactants, osmotic pressure, ionic strength, etc.). The dissolution behavior of the dosage form need not be studied under all of the conditions indicated. The number of conditions investigated depends largely on whether a correlation can be developed with the in vitro results obtained under the more commonly investigated conditions such as apparatus, agitation intensity, or dissolution medium and pH value. Each formulation and every drug represents an individual challenge. The resulting dissolution profiles from the use of different dissolution media are illustrated in *Figures 1* and *2* in which the same formulations were tested in water and an acid buffer.

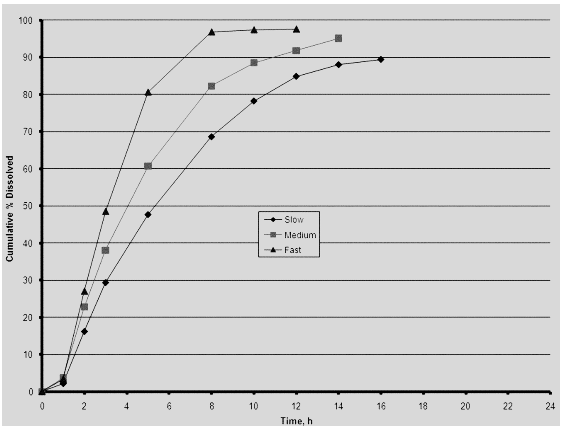

Figure 1. Mean dissolution profiles of three modifications of a new modified-release formulation (USP Apparatus 2, 50 rpm, 0.9 L water, 37°).

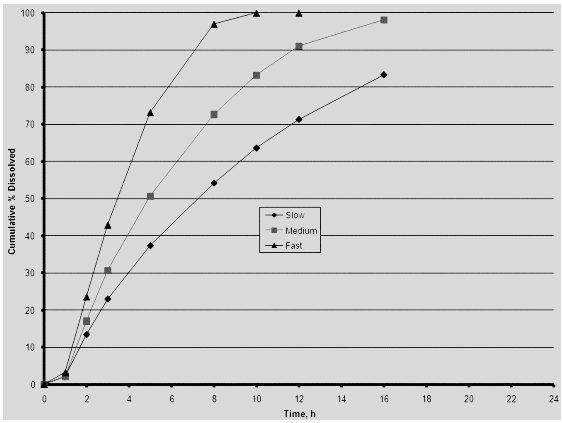

Figure 2. Mean dissolution profiles of a new modified-release formulation (USP Apparatus 2, 50 rpm, 0.9 L, pH 4.5 buffer, 37°).

3. The plasma level or urinary excretion data obtained in the definitive bioavailability study of the modified-release dosage form are treated by a deconvolution procedure. The resulting data may represent the drug input rate of the dosage form. They also represent in vivo dissolution when the rate-controlling step of the dosage form is its dissolution rate (i.e., drug absorption after dissolution is considered to be instantaneous). Any deconvolution procedure (e.g., mass balance or mathematical deconvolution) will produce acceptable results. *Figure 3* illustrates the results of numerical deconvolution of the plasma profiles obtained for the batches in *Figures 1* and *2*.

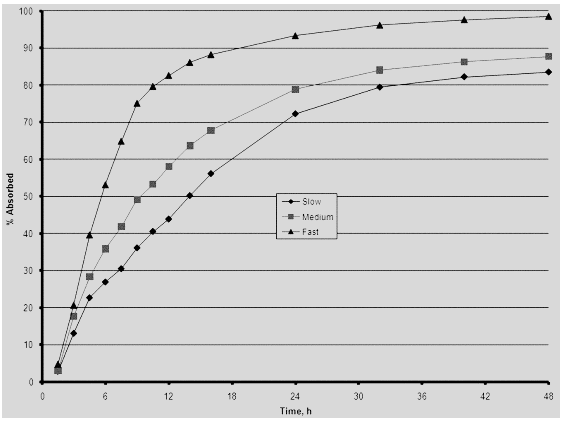

Figure 3. Mean absorption profiles from numerical deconvolution of plasma concentration–time plots.

4. The in vitro dissolution curve is then compared to the drug absorption rate curve. This can be performed by various methods. Simply positioning one curve on the other often can indicate the existence of a correlation. This may then be quantified by defining the equation for each curve and comparing the corresponding constants. The simplest way to demonstrate a correlation is to plot the fraction absorbed in vivo vs. the fraction released in vitro, as illustrated in *Figures 4* and *5*. With a *Level A* correlation, this relationship is often linear with a slope approaching 1. As illustrated in *Figures 4* and *5*, a correlation may be curvilinear. The intercept may or may not be zero depending upon whether there is a lag time before the system begins to release drug in vivo, or the absorption rate is not instantaneous, resulting in the presence of some finite quantity of dissolved but unabsorbed drug. In either case, it is a point-to-point or a *Level A* correlation when the least-squares fit of the line approaches a coefficient of determination, R^2, of 1. For the correlations illustrated in *Figures 4* and *5*, the IVIVC using the acid buffer dissolution profiles was superior to that obtained from water.

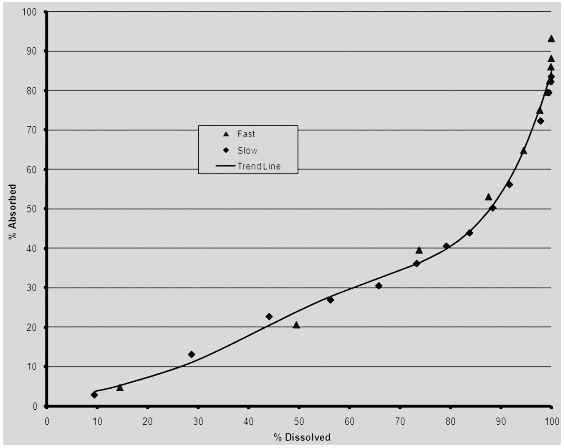

Figure 4. IVIVC attempt: water (using slow and fast formulations).

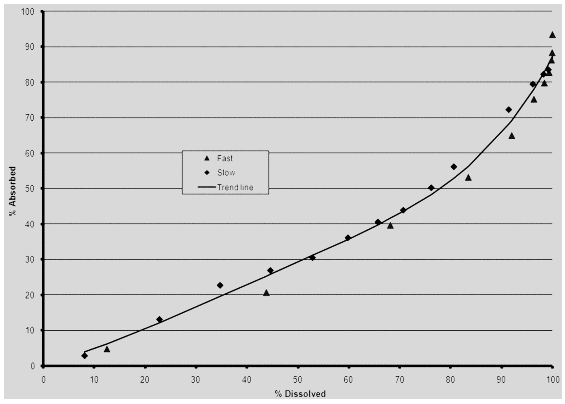

Figure 5. IVIVC attempt: pH 4.5 buffer.

5. If from the studies indicated in the in vitro dissolution evaluation, given above, the modified-release dosage form exhibits dissolution behavior that is independent of the variables studied and a *Level A* correlation is demonstrated when the in vitro dissolution curve is compared to the drug input rate curve, then it is likely that the correlation is general and can be extrapolated within a reasonable range for that formulation of the active drug substance. If the dosage form exhibits dissolution behavior that varies with the in vitro conditions, analysts must determine which set of dissolution conditions best correlates with in vivo performance. One can then establish whether the correlation is real or an artifact. This is achieved by preparing at least two formulations that have significantly different in vitro behavior. One should demonstrate a more rapid release and the other a slower release than the clinical bioavailability lot (biobatch). A pilot BA-BE study should be performed with these formulations, and the previously established correlation should be demonstrated for both. The formulation modifications of these batches should be based upon formulation factors that would be expected to influence the product's modified-release mechanism, and modification of these formulation factors are expected to influence the dosage form's release rate.
6. Alternatively, the in vivo performance of the biobatch formulation can be simulated based on the correlation developed with these formulations that were used in the BA-BE study. Analysts then can compare the predicted and experimentally determined values, the prediction error. The exercise illustrated in *Figures 6* and *7* serves as an internal validation of the *Level A* correlation. An external validation would involve simulating data for a formulation batch that was not included in the *Level A* correlation calculations. Such a validation was performed using the in vivo data from the medium lot of the formulation, and the results are illustrated in *Figure 8*.

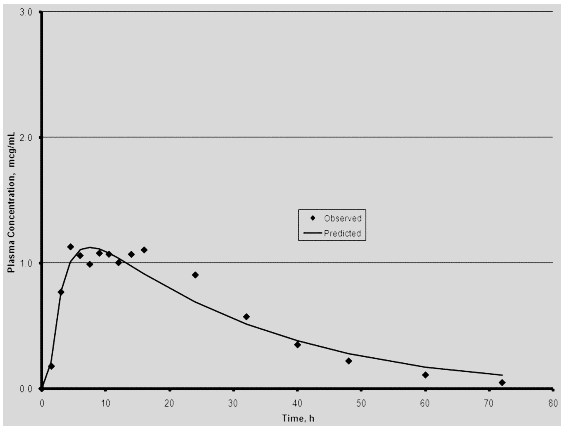

Figure 6. Observed and predicted mean plasma profiles: slow formulation.

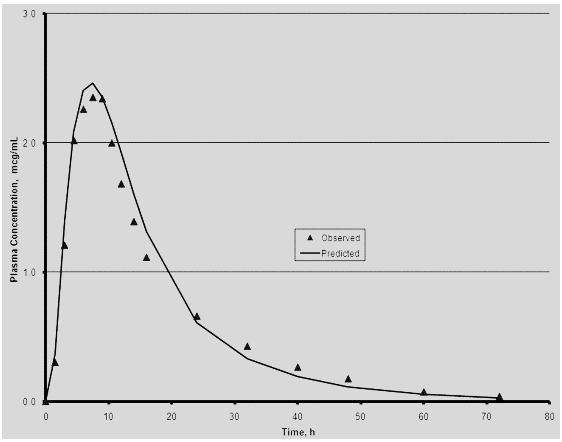

Figure 7. Observed and predicted mean plasma profiles: fast formulation.

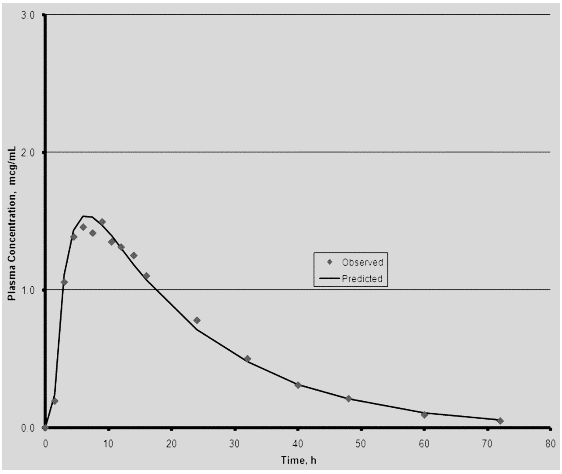

Figure 8. Observed and predicted mean plasma profiles: medium formulation.

7. Once a *Level A* correlation is established, in vitro testing can be used to establish dissolution specifications, biowaivers to facilitate SUPAC, and changes in dosage form strength for the same formulation. It is questionable whether such an extrapolation with *Level B* and *C* correlations is possible.

Establishment of Dissolution Specification Ranges

It is relatively easy to establish a multipoint dissolution specification for a modified-release dosage form. The dissolution behavior of the biobatch can be used to define the amount that will be released at each time point. The difficulty arises in the variation that will be allowed around each time point. In the case of a *Level A* correlation, this can be done in two ways, both of which use IVIVC: convolution and deconvolution.

CONVOLUTION

Reasonable upper and lower dissolution values are selected for each time point established from the biobatch. Historically, dissolution specifications have been selected by using the average dissolution of the development batches, with a range of ±2.5–3 standard deviations. It is now expected that the average dissolution values be approximately the same as those of the biobatch. The dissolution curves defined by the upper and lower extremes are convoluted to project the anticipated plasma level curves that would result from administration of these formulations to the same patients to whom the biobatch was administered. If the resulting plasma level data fall within the 95% confidence intervals obtained in the definitive BA-BE study, these ranges can be considered acceptable. An alternative acceptance approach that can be used after the therapeutic window for a drug has been defined, is to establish whether the upper and lower limits of the convolution results fall within the therapeutic window, even if they fall outside the confidence interval. If they fall outside the window, a more limited range must be established. This procedure should be continued until the predicted values meet the desired ranges.

DECONVOLUTION

An acceptable set of plasma-level data is established both for a batch of material demonstrating a more rapid release and for one demonstrating a slower release than that of the biobatch. These can be selected by using the extremes of the 95% confidence intervals or ±1 standard deviation of the mean plasma level. These curves are then deconvoluted, and the resulting input rate curve is used to establish the upper and lower dissolution specifications at each time point. In the case of *Level B* and *C* correlations, batches of product must be made at the proposed upper and lower limits of the dissolution range, and it must be demonstrated that these batches are acceptable by a BA-BE study.

Immediate-Release Dosage Forms

GENERAL CONSIDERATIONS

Because the mechanisms for drug release from modified-release dosage forms are more complex and variable than those associated with immediate-release dosage forms, one would anticipate that an IVIVC would be easier to develop with the latter formulations. Unfortunately, most of the correlation efforts to date with immediate-release dosage forms have been based on the correlation *Level C* approach, although there also have been efforts employing statistical moment theory (*Level B*). Although it is conceivable that the same *Level A* correlation approach can be used with immediate-release dosage forms, until data have been gathered to support this concept, *Level B* and *Level C* are the best approaches that can be recommended with these dosage forms.■2S (*USP35*)

Add the following:

■⟨1102⟩ IMMUNOLOGICAL TEST METHODS—GENERAL CONSIDERATIONS

INTRODUCTION

This general information chapter provides a high-level description of principles for immunological test methods (ITMs) that can be used in specified monograph tests, along with information and approaches to analytical development and validation for ITMs. The scope of this chapter is to provide general information that is applicable to all ITMs. The chapter provides a foundation for specific chapters about different types of ITMs, e.g., *Immunological Test Methods—Enzyme-Linked Immunosorbent Assay (ELISA)* ⟨1103⟩, *Immunological Test Methods—Immunoblot Analysis* ⟨1104⟩ (proposed), and *Immunological Test Methods—Surface Plasmon Resonance* ⟨1105⟩. This suite of general information chapters is related to the bioassay general information chapters. Use of ITMs for process monitoring, diagnosis, and evaluation of clinical response, assessment of pharmacokinetics/pharmacodynamics/absorption, distribution, metabolism, and excretion (PK/PD/ADME), and other product characterization (nonrelease testing) is outside the scope of this chapter.

The basis of all ITMs used to measure a quality attribute of a biologic drug substance or drug product is the highly specific noncovalent binding interaction between an antibody and antigen. The antigen typically is an analyte of interest (e.g., protein, carbohydrate, virus, or cell), and the binder is usually an antibody (e.g., monoclonal antibody or polyclonal antiserum). ITMs are applicable to molecules that are either directly antigenic (immunogens) or can be rendered indirectly antigenic (haptens). The measurand in ITM is directly related to a quality attribute of the product under test.

ITMs are valuable because they exhibit high sensitivity and specificity for an analyte in complex matrices. They typically are used for qualitative and quantitative assessment of both an antibody and antigen, but their application also extends to the measurement of hapten, complement, antigen–antibody complexes, and other protein–protein interactions. These properties of ITMs allow their use for assessing identity, potency (strength), purity, impurities, stability, and other quality attributes of biological drug substances and drug products.

ITMs are useful for many applications because they can measure molecules over a wide range of sizes and binding types. In general, antibodies are stable during various chemical modifications that do not have a significant adverse influence on interactions with an antigen. Antibody molecules tend to withstand moderate acidic and alkaline pH changes better than other proteins do. Because of this characteristic, a variety of ITMs with high degrees of sensitivity and specificity are possible. The ability to accelerate contact between an antigen and antibody enables ITM formats that provide rapid or real-time results.

Generally, ITMs have higher precision and shorter turnaround time than do traditional biologically-based (i.e., cell-based and animal) assays. Although in some cases these advantages can support the replacement of a biological assay with an immunoassay, such changes should be approached systematically and with caution. Often it is challenging to prove the equivalence, or comparability, of results from bioassays and immunoassays because the interaction between antigen and antibody may not reflect the functional attributes observed in bioassays.

One major limitation of ITMs compared to physicochemical methods (such as liquid or gas chromatography) is that the latter generally are more precise and can simultaneously identify a set of impurities or unexpected substance(s). Another major limitation is that generally ITMs operate at high molar dilutions at which they are sensitive to disturbances caused by environmental factors in the sample matrix (i.e., matrix effects). Matrix effects can depend on ITM format and are not fully understood. Their specificity, a hallmark of ITMs, is sometimes compromised by structural or sequence similarities between the analyte and a closely related molecular impurity (cross-reactivity).

Most ITMs reflect physical interaction (binding) between an antigen and antibody and not the analyte's functional properties. Therefore, analysts must pay attention in the selection and execution of ITM format. Cell-based ITMs that can provide functional information about the analyte are beyond the scope of this chapter.

GENERAL CHARACTERISTICS OF ITMs

ITMs are based on the principle of specific, noncovalent, and reversible interactions between an antigen and antibody. In general, the primary antigen–antibody reaction is brought about by complementarity, which creates macromolecular specificity. This noncovalent interaction determines the degree of intrinsic affinity. Intrinsic affinity contributes to functional and/or relative affinity that depends on factors like reaction phase and valency, which in turn determines the degree of reversibility of an interaction. A better understanding of factors that affect antigen–antibody interactions provides the rationale for the development of a suitable ITM format (e.g., solid or liquid phase, competitive or noncompetitive binding, etc.).

A defining characteristic of ITMs is that they employ an antigen (or hapten) and antibody. In addition, ITMs may contain companion molecules such as complement components. The components of ITMs are defined as follows:

- **Antigens**—Comprise a wide range of molecules that are capable of binding to the antibody in a specific interaction. Generally, part(s) of an antigen (the immunogenic epitope[s]) is/are capable of eliciting antibody response.
- **Haptens**—Small molecules that, by themselves, are not capable of eliciting an antibody response but are capable of eliciting an immune response when attached to a large carrier such as a protein. Antibodies produced to a hapten–carrier adduct also may bind to the small-molecule hapten in a specific interaction.
- **Complements**—Companion molecules that, under certain conditions, aid in the functionality of antigen–antibody complexes but are not required for antigen–antibody or hapten–antibody interaction.
- **Antibodies**—Proteins with regions that impart a high degree of specific binding to antigens (and haptens). The structural elements of an immunoglobulin G (IgG) antibody are shown in *Figure 1*.

In addition to these components, ITMs require some means to detect or monitor the binding reaction between the antigen and antibody.

TYPES OF ITMs

Measurement of antigen–antibody binding can be performed in a variety of assay types and formats: solid or liquid phase, manual or automated, labeled or nonlabeled, competitive or noncompetitive, qualitative or quantitative, homogeneous or heterogeneous, or combinations of some of these. The distinguishing characteristic of all these assays is the binding of an antibody or antigen to the analyte (which can be an antigen or antibody as well), followed by detection of the antigen–antibody complex. Although many different formats can be used for the binding reaction,