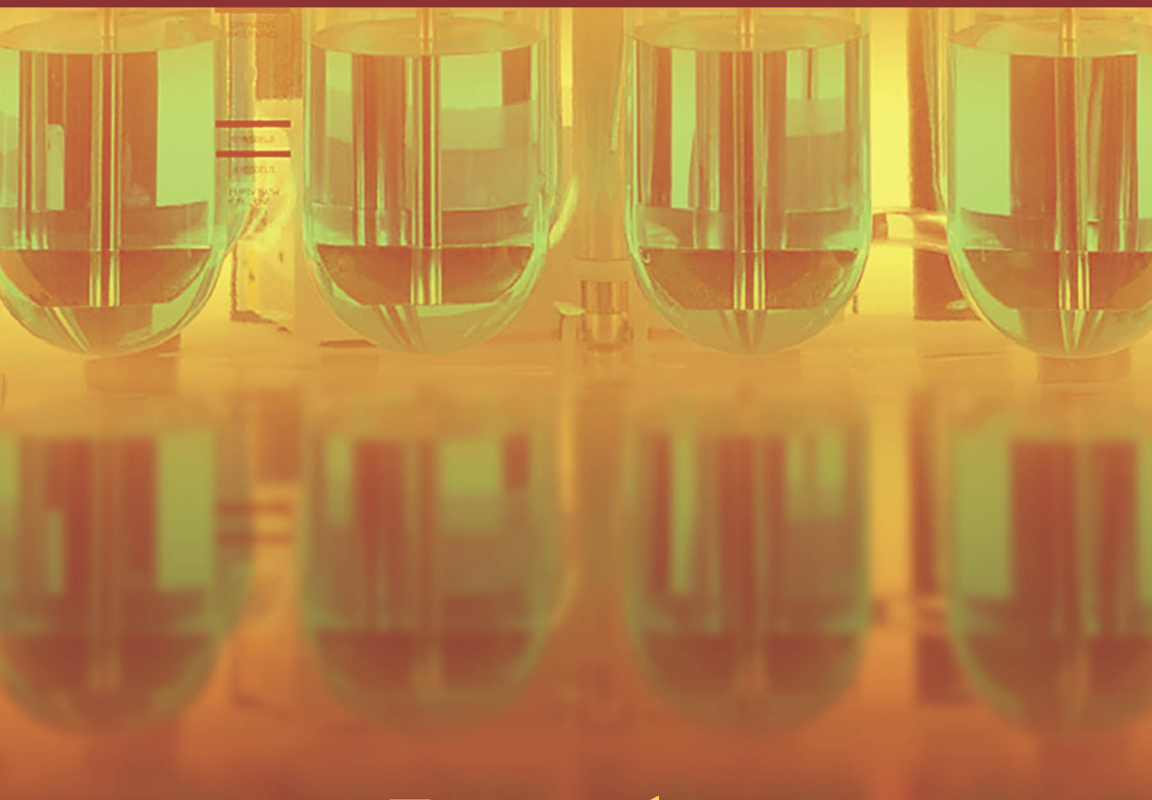
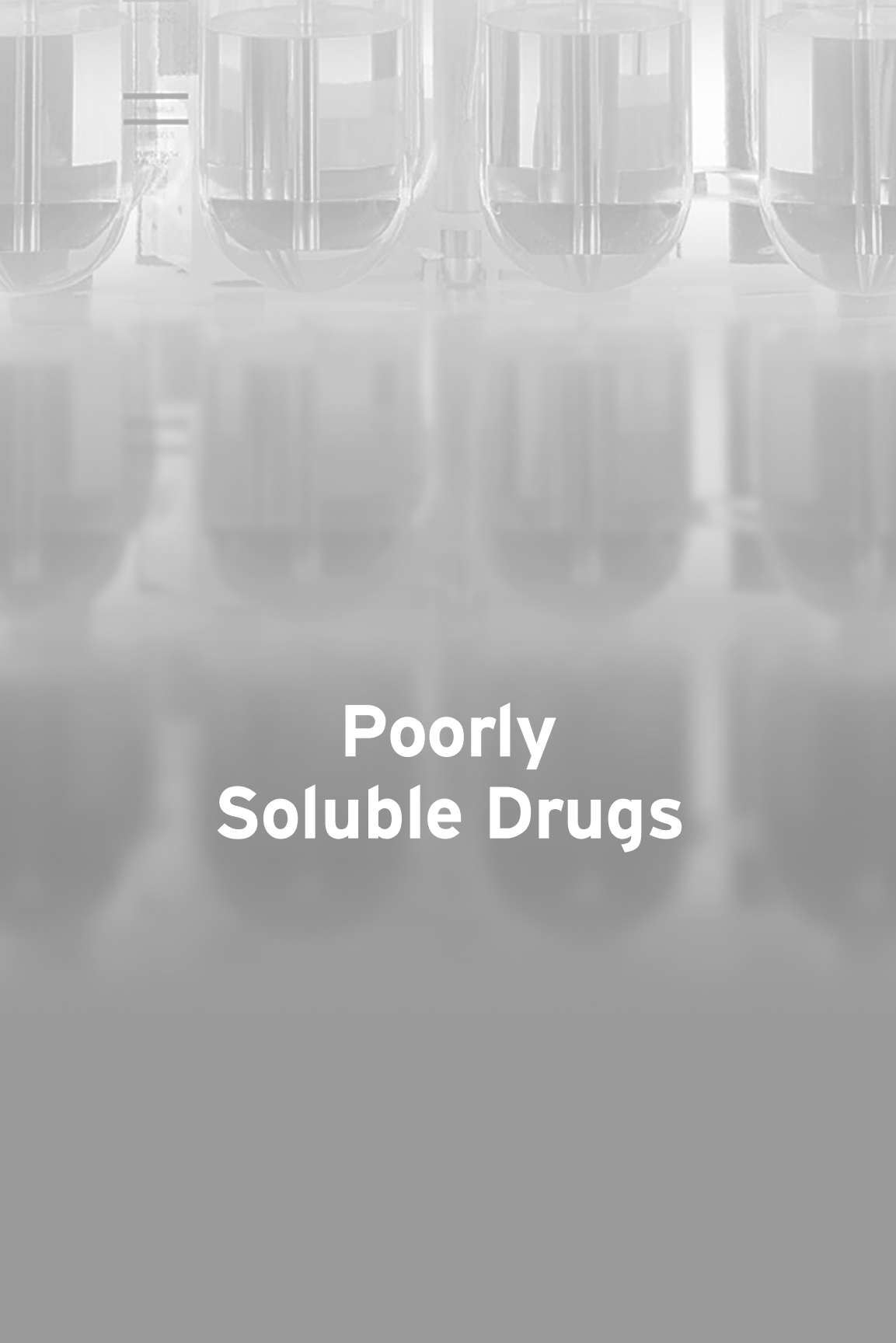
Pan Stanford Series on Pharmaceutical Analysis
Volume 1



# Poorly Soluble Drugs

## Dissolution and Drug Release

edited by
Gregory K. Webster | J. Derek Jackson | Robert G. Bell



# Poorly Soluble Drugs

## Pan Stanford Series on Pharmaceutical Analysis

Gregory K. Webster
*Chief Editor*

J. Derek Jackson and Robert G. Bell
*Editors*

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Gregory K. Webster, J. Derek Jackson, and Robert G. Bell, eds.

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# Foreword

Roughly twenty years ago, it became clear that drug product development had entered a new era of difficulty with the increased throughput of therapeutically effective but poorly soluble drug candidates. Gone are the days when all drug candidates were rapidly dissolving and absorbing drugs that were relatively easy to formulate and even easier to test. Now the development of an in vitro method for poorly soluble drugs is not boring. Rather, it's a stimulating endeavor.

High-throughput screening has contributed to the invention and discovery of many new poorly soluble molecules. This book, *Poorly Soluble Drugs: Dissolution and Drug Release*, is most timely as the authors are up to the challenge of sharing the knowledge and tools to tackle the in vitro testing and manufacturing of these products. This work is unique in that it has provided the linkage between testing and formulating the products with equal importance given to each aspect.

In vitro testing of poorly soluble drug products is especially challenging and important since dissolution is the rate-limiting step to drug absorption and exposure. The path forward is clear: methods must be able to take advantage of this characteristic by providing meaningful elucidation of the release rate or, in some cases, the actual release mechanism, and hence giving critical clinically relevant information.

Poorly soluble drugs require special attention during formulation and manufacturing to enhance the effectiveness of the drug through methods as simple as reducing particle size to the much more complex areas of formulation manipulation and engineering technology to increase in vivo concentrations and adsorption.

The practical matter is that the demands from regulators, the globalization of pharmaceuticals, and the competitive arena of

market share drive the need to quickly educate and strengthen the knowledge of scientists working on these products. This book is quite essential to this effort.

The development of clinically relevant dissolution methods for drug products with limited water solubility has been a challenge for scientists in the drug industry as well as the regulatory agencies. The trend has started with the powerful tools available through quality by design (QbD) to create a clinically relevant dissolution test. Designing robust dosage forms of poorly soluble actives employs a thorough understanding of the components, matrix, and variability, thus following QbD concepts. This book gives a thorough investigation of the role of QbD with poorly soluble dosage forms, including design of experiments (DOE).

Development scientists are tasked with making these compounds soluble in a medium that is foreign to the poorly soluble drug but is necessary for oral drug formulation absorption. Aqueous solubility is the primary gauge of the success or failure of a drug and drug product. Solubility and dissolution performance in the gastrointestinal tract are critical for the bioavailability, and hence efficacy, of the product.

There are some emerging topics that are starting to acquire additional in-depth understanding—in particular, topics such as sink versus non-sink conditions in the dissolution method, contribution of solid-state properties, the chemistry of surfactants, in silico modeling, dose dumping, and capsule properties. The chapters in this book give these and other new topics well-referenced and refreshingly up-to-date attention. The work in this book bridges with established art and then builds links to, in some cases, entirely new directions.

The authors are from industry and academia, giving a well-rounded approach to this unique topic that has not been treated in book form to date. The subject is treated well beyond current guidances and USP chapters, a step much further than the status quo. I know the authors personally or by reputation, and they are experts in their areas. Many have a long history of direct involvement with the in vitro release test from the simpler testing equipment and methods to more complex and in some cases closer to the in vivo condition.

In vitro testing shows that the product is dissolved and therefore available for absorption and therapeutic effect thus linking what occurs in the patient's body to the efficacy of the product. The FDA and USP have emphasized the dissolution test for this reason as a proof that a commercial product on the market for many years will still be efficacious if it passes that test developed with the biobatch formulation. Hence the push to improve and make more robust the dissolution methods to link to in vivo performance. A way to forecast the in vivo performance is by making the dissolution test conditions as close to in vivo conditions as is possible. Approaches to assist the analyst in developing a sensitive method to characterize the release rate are explored thoroughly in this book along with the topics of in vitro and in vivo correlations and relationships.

Historically, a defining moment for poorly soluble drugs is the Biopharmaceutics Classification System, where the poorly soluble drug was described and characterized with some clarity. At that time, it became apparent that biowaivers for poorly soluble dosage forms were in most part unobtainable. With the exception of in vitro and in vivo correlations, clinical studies seemed to always be necessary, and little has changed over the years in this regard. The book offers insight into the development of predictive dissolution methods. Furthermore, knowing that poorly soluble drugs are uniquely sensitive to the testing environment (e.g. equipment design, vibration and de-aeration) is helpful when interpreting dissolution results.

Formal education of the industry analyst may not be provided for this topic. Because developing methodology for poorly soluble drugs demands more resources and research, this work will be helpful to the analyst to work more efficiently and solve problems more rapidly with this new knowledge in hand.

I commend the authors for their very considerable effort in bringing out this valuable publication.

**Vivian Gray**
Managing Director
Dissolution Technologies, Inc.
Hockessin, DE, USA
October 2015

Chapter 1

# The Modern Pharmaceutical Development Challenge: BCS Class II and IV Drugs

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## 1.1 Introduction

Since the 1960s, pharmaceutical companies have been charged with monitoring the characteristics of their oral dosage forms dissolving in controlled media. Early dissolution testing focused on the quality control of the dosage form manufacturing. Dissolution testing provided unique capabilities in monitoring integrated production parameters that affect the dissolution rate: tablet hardness, excipient control, particle size, etc. The technique became required for the routine testing of oral dosage forms worldwide.

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The early history of dissolution testing is well documented in the literature.[1–3]

The additional advantage of early dissolution testing was the relative simplicity of the active pharmaceutical ingredients (API) and their associated dosage forms. These soluble drugs were readily bioavailable as they tended to be both highly soluble and highly permeable in the gastrointestinal tract. Thus, for highly soluble and permeable drugs, dissolution assessment was adequate to ensure clinical performance through simple standardized solutions such as water and acidic media. Discrimination focused primarily on the disintegration and dispersion from solid oral dosage forms with the active ingredients behaving predictably. Under these conditions, the dissolution test was effective in predicting performance in human clinical trials and was denoted as a clinically relevant method. With further evolution in drug formulation technologies, dissolution testing harmonized on the four basic apparatus still in use today and commonly referred to by their United States Pharmacopeia (USP) designations of apparatus 1, apparatus 2, apparatus 3, and apparatus 4.[4] Other USP apparatus designations, such as apparatus 7, tend to be for non-oral dosage forms.

Today, dissolution testing has broadened its applications and scope in moving forward from a simple quality control test to use in predicting in vivo/in vitro correlations (IVIVC) for soluble and permeable dosage forms and clinical relevance for many others. The simple formulations found in the developing years of dissolution testing have given way to more complex technologies to advance the bioavailability of less soluble molecules. The goal of this book is to move past the existing dissolution texts referenced earlier, which primarily denotes dissolution testing for soluble drugs, and to focus on the issues of dissolution testing current with the molecules in development today. This text builds upon the solid foundation of the earlier works[1–3] to the current application of dissolution and drug release technologies with an emphasis on poorly soluble drugs.

## 1.2 Changing Drug Emphasis

The dissolution platforms of USP apparatus 1–4 were key technologies in facilitating soluble drugs to market. The techniques,

instruments, and the simple buffer systems used ideally characterized the dosage forms being developed. However, as the active pharmaceutical ingredients (API) became increasingly more complex (in terms of solubility and permeation in the gastrointestinal tract), these simple drug release mechanisms did not correlate. The Biopharmaceutics Classification System was developed by Amidon et al.[6] and published as a guidance by the U.S. Food and Drug Administration (FDA) for predicting the intestinal drug absorption of oral dosage forms.[7] The BCS system has become the gold standard to categorize and estimate oral drug absorption based on the drug's solubility and intestinal permeability characteristics.

### 1.2.1 BCS Classification System

The BCS categorization of drugs is based on the premise that as a drug dissolves, this concentration is available to move across the membrane and correlate to intestinal absorption. Gastric solubility is established through in vitro chemical testing at various conditions and pH's representative of the human gastrointestinal tract. The permeability of the drug is based on initial lipophilicity testing and further studied in animal models, tissue studies, cultured epithelial cells such as Caco-2 testing, and ultimately in humans (mass balance, absolute bioavailability intestinal perfusion testing, etc.).

With the drug solubility and permeability established, the BCS system segregates drugs into four classes, as illustrated in Table 1.1.

Per the FDA Guidance, the target drug is deemed "highly soluble" when the highest dose strength is soluble in $<250$ mL of aqueous media over a pH range of 1 to 7.5. The drug is deemed "highly permeable" if the extent of absorption in humans is determined to

**Table 1.1** BCS drug classification

| | **High solubility** | **Low solubility** |
|---|---|---|
| **High permeability** | **Class 1**<br>High solubility<br>High permeability | **Class 2**<br>Low solubility<br>High permeability |
| **Low permeability** | **Class 3**<br>High solubility<br>Low permeability | **Class 4**<br>Low solubility<br>Low permeability |

be >90% of an administered dose. In addition, a drug formulation is deemed "rapidly dissolving" when > 85% of the labeled amount of drug substance dissolves within 30 minutes using USP apparatus 1 or 2 and a volume of ⩽500 mL of buffered media.

Knowing the drug's BSC category allows the pharmaceutical scientist to evaluate the rate-limiting step in the absorption of the drug. For class 1 drugs the high solubility and high permeability of the API indicate that the absorption in the gastrointestinal tract should be dissolution rate limited. For class 2 drugs, because the drug is less soluble but still very permeable, this class of drugs should also be dissolution rate limited. With the solubility being high in class 3 but permeability of the drug low, the API in this class becomes absorption rate limited. For this class of drugs, dissolution is seldom clinically relevant. The FDA has recently issued a guideline denoting that, under special cases, a class 3 drug can be addressed with class 1 specifications for biowaiver studies.[8] However, this guidance does not propose that class 3 drugs can readily achieve IVIVC via traditional dissolution testing. IVIVC for class 1 and 3 drug products is not likely unless drug dissolution is significantly slowed due to formulation (e.g., MR formulation) or the compound is borderline BCS 1 with respect to solubility. This is acknowledged in the FDA Guidance for Industry for Dissolution Testing of IR solid oral dosage forms.[9] Class 4 drugs rely upon transporters and other biological means to transport across the membrane. Dissolution may be able to characterize this transport, but as with BCS class 3 drugs, dissolution is challenged to characterize the absorption of these drugs.

### 1.2.2 Poorly Soluble Drugs

A main focus of this book is to focus on the dissolution and drug release of BCS class 2 drugs. These drugs are permeable but with limited solubility. As of 2006, BCS class 2 drugs made up approximately a third of the global pharmaceutical market.[10] As will be illustrated in Chapter 5, formulation technologies have gone a long way to increasing the bioavailability of these molecules. Typically, these formulations are often working with amorphous or nanoparticle material technologies.

## 1.3 The Dissolution Market

Dissolution is a significant technology found in the laboratories of every major pharmaceutical business concern with oral dosage forms and predominately uses chromatography and spectroscopy for final quantitative analysis. As such, perhaps it is time that dissolution testing warrants a chapter in undergraduate instrumental analysis textbooks. In 2009, approximately 3400 instruments were sold with a market expecting to grow at 8% annually.[11] Today this market is valued at over $150 million and tied directly to the pharmaceuticals market. The pharmaceutical industry accounts for approximately 75% of these sales. The remaining 25% is split between contract research organizations, biotech, academia and agriculture.

As seen in Fig. 1.1, quality control testing dominates the demand for dissolution testing.

As Fig. 1.2 shows, in 2009, the largest dissolution vendor was Varian/Agilent. The market was diversified with several vendors; however, even with this diversity, the technique still today revolves around the standard USP designations of apparatus 1 and 2, with less significant portion of the market operating apparatus 3 and 4.

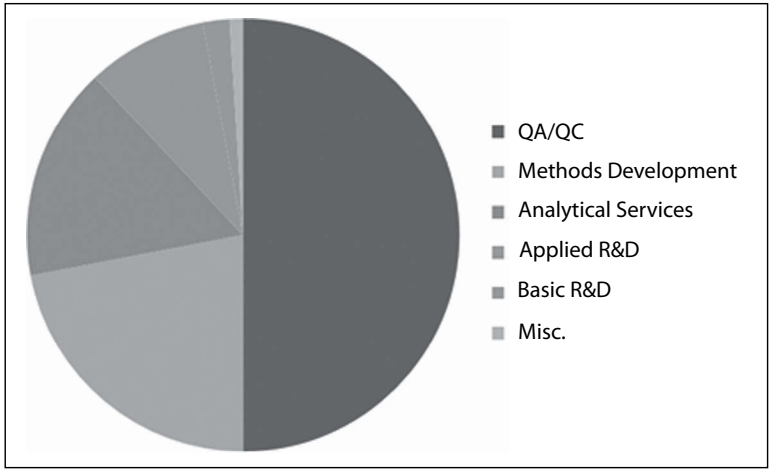


**Figure 1.1** Dissolution testing by function in 2009 (data from Ref. 11).

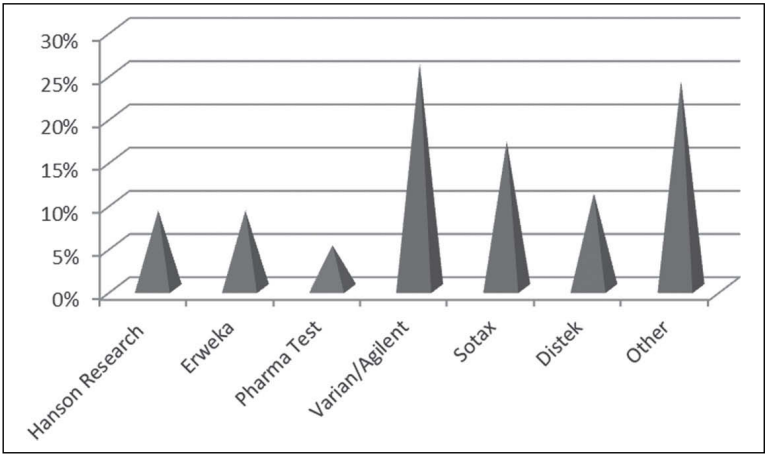


**Figure 1.2** Vendor diversity in 2009 (data from Ref. 11).

However, as will be seen in Chapters 8 and 9, these later instrument technologies may play a more significant role in the dissolution and drug release testing in the years to come.

## 1.4 Dissolution and Drug Release in the Pharmaceutical Industry

The goal of this text is for leading scientists in the field to discuss the current applications of dissolution and drug release to poorly soluble molecules. Each chapter provides a current view from leading practitioners of the material presented. While much of the instrumentation of dissolution and drug release has not changed dramatically from the traditional USP apparatus, the approaches and applications have moved forward with the changing molecules being brought to market. This book builds upon the technique from its initial and early developments. Details on the development and theory of dissolution and drug release can be found in the literature.[1–3]

### 1.4.1 Solubility Determinations for Pharmaceutical API

Aqueous solubility for the active pharmaceutical ingredient (API) in the final formulation is a significant factor that influences the pharmacokinetic profile of a drug. There are various analytical methodologies and computational models for the prediction of solubility of the API. The solubility in the body can often be different from that determined in common in vitro buffer systems that are typically used for pharmaceutical quality control processes. The quality of computational models is also affected by the accuracy of the experimental solubility data. High-throughput discovery processes have driven the development of high throughput screening processes for measurement of physicochemical determinations including lipohilicity, p$K_a$ and solubility, but the accuracy of these is often compromised by the requirement for speed and the use of non-biorelevant media in the determination. Kofi Asare-Addo and Barbara R. Conway from the University of Huddersfield will discuss the nuances regarding the solubility determinations for APIs.

### 1.4.2 Use of Surfactants in Dissolution Testing

Before any relevant dissolution or drug release testing can occur in formulated product, the analytical target must be dissolved in the test matrix. In dissolution testing, this is often achieved by adding surfactants to the dissolution media. The primary goal for the surfactant is to keep the target analyte in solution after it dissolves. Surfactants also aid in material wetting by reducing the surface tension in the media. However, ideally the surfactant should not alter the mechanism of release by accelerating the dissolving or erosion of the drug formulation itself. Amit Gupta of Zydus reviews the use of surfactants in dissolution media to expand this technique to poorly soluble drugs as well.

### 1.4.3 Intrinsic Dissolution Evaluation of Poorly Soluble Drugs

Michele Georges Issa and Humberto Gomes Ferraz of the University of San Paulo discuss the aspects involved in the intrinsic dissolution

assay and its use in the evaluation of poorly soluble drugs. The most commonly used apparatuses and the calculation of the intrinsic dissolution rate (IDR) are discussed, as well as some relevant examples of applications and a brief explanation of the variables of the method and the use of experimental designs. Applying IDR throughout all stages of a medicinal compound's development—from the synthesis of a new chemical entity up to the quality assurance of the API—development scientists can better understand key parameters in selecting lead candidate parameters for optimum bioavailability using relatively small quantities of material.

### 1.4.4 Strategies for Oral Delivery of Poorly Soluble Drugs

The advances in combinatorial chemistry and high-throughput screening in the past couple of decades have enabled discovery of new chemical entities (NCEs) for a variety of complex and diverse biological targets. Many biological targets are highly lipophilic or hydrophobic in nature and there has been a significant increase in the number of NCEs with poor aqueous solubility. While oral delivery continues to be the most commonly used route of administration for NCEs, poor aqueous solubility can result in significant development challenges such as incomplete absorption, highly variable bioavailability, and highly variable pharmacokinetic profiles in preclinical species and humans. A number of conventional and enabling formulation technologies are now available to address poor solubility and the resulting poor biopharmaceutical performance of NCEs. However, a systematic evaluation and proactive selection of the optimal formulation technology is typically not available during discovery and a number of promising NCEs are terminated because of poor biopharmaceutics and lack of adequate exposure for preclinical safety assessment. The goal of this chapter is to describe a systematic approach for generating a cross-functional package of data comprising physicochemical, biopharmaceutical, ADME, PK/PD, and delivery technology evaluation to enable oral delivery of poorly water-soluble NCEs. Akash Jain, Dev Prasad, and Sudhakar Garad discuss the general concepts of formulating poorly soluble molecules into viable dosage forms from pre-clinical through commercial development.

### 1.4.5 A Staged Approach to Pharmaceutical Dissolution Testing

The application of dissolution methodologies develops as the target molecule goes from discovery through the various stages in pharmaceutical development. As more information becomes known about the drug and its chosen dosage form(s), the expectation of the dissolution method performance increases as well. By late Phase 2b/3 of clinical development, the dissolution method should be in its finalized form. The results of the clinical tests and manufacturing history are reviewed to propose relevant specifications for commercially marketed material. Gregory Webster, Paul Curry, and Xi Shao of AbbVie review the development of the dissolution method as the drug traverses the stages of pharmaceutical development.

### 1.4.6 Development and Application of in vitro Two-Phase Dissolution Method for Poorly Water-Soluble Drugs

A two-phase dissolution test, containing both aqueous and organic phases, is designed to be more physiologically relevant than conventional single-phase dissolution tests with the involvement of an "absorptive environment." This in vitro test simulates both kinetic processes of drug dissolution and partitioning into the intestinal membrane. Case studies reported in this chapter illustrate that this two-phase dissolution test enables a better opportunity for establishing in vitro and in vivo relationship (IVIVR) for several drugs, including immediate and extended dosage forms, compared with conventional single-phase dissolution methods. In particular, the two-phase dissolution test may be extremely useful in assessing the metastable supersaturated state of BCS II drugs, including the duration and degree of supersaturation, effect of polymeric precipitation inhibitors, and an overall effect upon drug partition. In addition, theoretical models of the two-phase dissolution systems with emphasis on drug partition kinetics are discussed. Ping Gao, Yi Shi, and Jon Miller of AbbVie review the application of biphasic dissolution to poorly soluble drugs.

### 1.4.7 Use of Apparatus 3 in Dissolution Testing of Poorly Soluble Drug Formulations

Brian Crist of Agilent Technologies discusses the use of apparatus 3 in the dissolution of poorly soluble drug formulations. The USP apparatus 3 reciprocating cylinder was originally designed for testing extended release products and exposing drug formulation to pharmacokinetic and mechanical properties similar to those found in the GI tract. The instrument has proven useful for poorly soluble compounds, chewable formulation, immediate release, delayed release, and numerous modified-release products. At the time of this writing, adaptions to the reciprocating cylinder to contain microspheres within a dialysis membrane are under evaluation so this may provide additional benefits to biorelevant drug release profiling micro- and nanoparticles. Because of the apparatus's ability to characterize the release profile of early drug formulation candidates, the apparatus has the potential to provide knowledge-based assessments of formulations developed through Quality by Design (QbD) and further characterization of the products design space for post-approval manufacturing.

### 1.4.8 Use of Apparatus 4 in Dissolution Testing of Poorly Soluble Drug Formulations

Geoffrey N. Grove of Sotax along with Rajan Jog and Diane Burgess of the University of Connecticut provide a brief history of the development of the USP 4 technique, pharmacopeial considerations, and regulatory considerations to help in laying the groundwork for when to choose USP 4. It also covers an overview of a variety of different system configurations and flow cell selection and design covering both compendial and non-compendial design choices. It further presents method parameters for considerations such as pump selection and flow rates and highlights choices which may be pertinent to working with poorly soluble drugs.

The chapter also offers a comprehensive review of tested drugs, including article titles, instrumentation, and system design notes. Experimental conditions including media types, filter types, flow rates, and various other parameters are summarized with emphasis

placed on the relevance of the results for choosing USP 4. It is the authors' hope that this review of the current literature will serve as a useful reference and decision-making tool.

### 1.4.9 Dissolution of Nanoparticle Drug Formulations

John Bullock of Shire LLC discusses some of the many challenges associated with the dissolution of nanoparticle drug formulations. Numerous types of nanoparticle-based drug formulation technologies have been developed over the last couple of decades in order to provide a range of different enhanced drug delivery properties for therapeutic and diagnostic agents. In the field of oral drug delivery, the most common nanoparticle structures can be categorized as either pure drug nanoparticles consisting of essentially 100% drug or an assortment of different nano-sized structures in which the active drug is encapsulated or dispersed in a solid, semi-solid, or liquid state within a formulation matrix. Regardless of which of these two categories of drug nanoparticles are used in an oral dosage form, the fundamental challenges encountered and approaches pursued for developing suitable techniques to measure drug release are generally similar for both pure drug nanoparticles and matrix-type nanoparticle formulations. Two of the more distinguishing characteristics of dissolution methods for these nanoparticle formulations include the challenges encountered in appropriately sampling and processing dissolution samples containing small nano-size drug particles and the potential for much faster dissolution rates compared to formulations manufactured with conventional-size drug particles. This review starts with a brief discussion of the theoretical underpinnings controlling solubility and dissolution of drug particles in general as well as certain considerations more important for nano-size drug particles, with an emphasis on pure drug nanoparticles. Following this discussion, practical guidelines to lead the practitioner in developing a suitable method are provided that include considerations for the dissolution media used, instrumentation considerations, and sampling and processing approaches that have proven successful for a number of different types of nanoparticle formulations. In conjunction with the discussion of instrumentation considerations, a variety of in situ

analysis techniques that have recently emerged and demonstrated advantages for dissolution testing of rapidly dissolving nanoparticle formulations are reviewed, and some alternative in vitro release techniques that have been described for evaluating the performance of nanoparticle drug formulations are examined.

### 1.4.10 Dissolution of Lipid-Based Drug Formulations

Stephen Caffiero from Boehringer Ingelheim describes the general nature of lipid-based drug formulations and their dissolution behavior. The availability of the drug for in vivo absorption can be enhanced by presentation of the drug as dissolved within a colloidal dispersion. The industry trend of an increasing number of poorly soluble drugs formulated in lipophilic matrices has resulted in a collaboration of expert groups at United States Pharmacopeia (USP) authoring a general chapter with specific focus on dissolution testing of these formulations (general chapter USP <1094>Liquid-Filled Capsules—Dissolution Testing and Related Quality Attributes), which will be discussed, as well as relevant topics such as a capsule rupture test, emulsion droplet size determinations, and a case study.

### 1.4.11 Dissolution of Stabilized Amorphous Drug Formulations

Justin Hughey of Banner Life Sciences discusses the critical role of dissolution testing in characterizing stabilized amorphous drug formulations (SADFs). Amorphous systems continue to be an important and growing technology in the oral delivery of BCS class 2 drugs. The use of high-energy forms of a drug substance, such as amorphous forms, coupled with precipitation inhibiting excipients has proven a valuable strategy in solubility and bioavailability enhancement. This chapter explains how the interplay of chemistry, physical form, functional excipients, supersaturation, and thermodynamics come together in dissolution testing to provide insight into the solution-mediated mass transfer and phase transition phenomena associated with SADFs.

The underlying considerations in drug delivery system design, dissolution method parameters, media selection, and the nuances of sink and non-sink conditions are discussed with illustrative case studies. These studies clearly demonstrate the power of dissolution and precipitation testing in understanding and advancing this important drug delivery approach.

### 1.4.12 Dissolution of Pharmaceutical Suspensions

A suspension consists of insoluble solid particles dispersed in a liquid medium. The most common reason to develop a suspension dosage form is limited aqueous solubility of the active pharmaceutical ingredient (API) at the dosage required. Another common reason to use suspensions is that they typically offer improved chemical stability compared to solutions. Suspensions also offer advantages in taste masking and a more convenient dosage form for certain patients (e.g., pediatrics). In this chapter, Beverly Nickerson, Michele Xuemei Guo, Kenneth Norris, and Ling Zhang of Pfizer focus on dissolution and drug release of suspensions in dosage forms that are dosed by the oral route of administration and include oral suspensions, suspensions for reconstitution, and suspensions in capsules.

### 1.4.13 Biorelevant Dissolution

Mark McAllister and Irena Tomaszewska of Pfizer discuss testing poorly soluble drugs in biorelevant dissolution conditions. In general, the approaches adopted to improve the physiological relevance of a dissolution test can be broadly categorized into one of two groups. The first group encompasses mechanistic approaches in which a single aspect of the dissolution process is controlled to study the impact of a physiological variable such as media or hydrodynamics. The second group includes methods and equipment designed to simulate multiple aspects of the gastrointestinal tract and deliver a holistic simulation of luminal conditions encompassing fluids, digestion, transit, and absorption. It is recognized that for particular aspects of a dosage form or API dissolution that a mechanistic (reductionist) approach

may allow a detailed assessment of individual phenomena such as supersaturation, precipitation, and re-dissolution. In contrast, a holistic simulation approach considers the summation or net effects of multiple processes such as digestion, transit, and absorption on dosage form performance, for example when assessing complex food effects. This chapter describes the development of biorelevant dissolution testing for both mechanistic and holistic approaches and assesses the biological relevance of modifications made to media (composition and volume), hydrodynamics, and integration of an absorptive component within the dissolution test.

### 1.4.14 Clinically Relevant Dissolution for Low-Solubility Immediate-Release Products

Paul Dickinson, Talia Flanagan, David Holt, and Paul Stott of AstraZeneca discuss clinically relevant dissolution for low-solubility immediate-release products. A structured approach to development that endeavors to identify and evaluate risk to clinical performance and, where appropriate, test the impact of these risks in vivo is presented. Each compound should be considered on its own merits but the application of a proposed 5 step approach will ensure that all important factors are considered and will ultimately lead to the establishment of a robust control strategy. The benefits of such an approach include enhanced security of product supply, the ability to optimize the manufacturing process and demonstrate the (lack of) impact of any proposed change, and an improved assurance of the clinical quality of product supplied to patients. Dissolution testing and its associated specification is expected to serve a number of purposes as the demands of discriminatory power and demonstration of complete release can be at conflict. Further work in our scientific understanding and regulatory harmonization are required if we are to realize the full benefit of a move towards more clinically relevant dissolution specifications. The authors are firmly of the belief that the development of increased knowledge and understanding proposed under the auspices of QbD are predicated on an insight into and control of in vivo performance.

### 1.4.15 Method Validation and QbD for Dissolution Testing of BCS Class II/IV Products

Alger Salt of GlaxoSmithKline discusses the QbD approach to method development and validation for dissolution testing. QbD principles provide a structured approach to the development and delivery of products, technologies, and processes. Historically, QbD has been directed at manufacturing processes and product attributes. QbD principles are now being applied to analytical methods. Method transfers are part of a successful product lifecycle because work initiated in R&D is ultimately moved into the QC laboratories at manufacturing sites.

Prior to embracing the QbD principles analysts learned (and many times re-learned) that problems with analytical methods were often discovered during method transfers. This is not a good time to discover such problems, because it is usually difficult to make changes to the method and fixing the problems can be frustrating and expensive.

The four stages of the QbD approach are listed below. Definitions follow for each, but the control definition stage is the focal point in this chapter and is widely considered to be the primary component of the QbD approach.

1. Design intent
2. Design selection
3. Control definition
4. Control verification

### 1.4.16 Regulatory Considerations in Drug Release Testing of BCS Class II/IV Products

Robert Bell of Drug & Biotechnology Development and Laila Kott of Takeda Pharmaceuticals summarize the current guidances and how they are related to compounds of low solubility. We review a history and a progression of the instrumentation, as well as the evolution of the guidance documents. A summary of three classification systems that are used to describe all types of compounds is presented. The classifications systems include the 1995 biopharmaceutics

classification system (BCS) for drug products, the biopharmaceutics drug disposition classification system (BDDCS) published in 2005, and the developability classification system (DCS) presented for the first time in 2010.

A discussion on the applicability of using biorelevant media for formulation development and the possibility of its use for quality control (QC) testing is presented. The guidance, as it stands, on this topic is discussed and dissected. Included is an outline on the setting of dissolution specifications as they relate to current guidance and industry trends. Finally, there is a summary of the current key guidance documents and emerging regulatory topics.

### 1.4.17 Dissolution of Capsule-Based Formulations

The availability of a compound formulated in a liquid-filled capsule for absorption depends on the initial dissolution and rupture of the capsule shell and subsequent release and dissolution of its fill contents in the GIT fluids. These two processes need to be monitored at the time of release and during the shelf life of a capsule product. Liquid-filled capsules pose unique challenges during the development and application of dissolution methods because of the complex nature of the shell and fill materials. The shell material is prone to changes in its mechanical properties or cross-linking of gelatin, which results in changes in its solubility. The fill material, on the other hand, may exhibit changes in particle size distribution or the polymorphic nature of the suspended material in the suspension fill or crystallization of a solubilized compound from a solution fill. In the latter case, the crystallization of the solubilized compound can occur either in the capsule dosage form or when the fill material encounters in vitro and in vivo aqueous fluids.

Dissolution testing is a highly valuable tool to characterize liquid-filled capsule products in vitro and is used routinely (a) to assess batch-to-batch quality, (b) to monitor changes in the quality of a product during its shelf life, (c) to assess product sameness after scale-up and post-approval changes (SUPAC), (d) to comply with biowaiver requirement for a lower strength of a product, and (e) to comply with biowaiver requirement for a product intended for local action in the GIT. In addition, a dissolution method designed

to produce in vitro/in vivo correlations (IVIVC) or in vitro/in vivo relationship (IVIVR) can be used to predict potential bioequivalency or bioinequivalency between products. The intent of this chapter by Rampurna Gullapalli from Dart Neurosience LLC is to provide an in-depth discussion on the factors affecting in vitro and in vivo dissolution of liquid-filled capsule products, development of dissolution methods for their routine QC testing, and modifications to these methods to produce potential IVIVC and IVIVR.

### 1.4.18 Emerging and Non-compendial Drug Release Techniques

Dissolution testing of conventional and non-conventional dosage forms was introduced in the 1960s, and now serves as an essential compendial test, which is used by all pharmacopoeias for evaluating drug release. Apart from the standardized tests, there are several non-compendial methods that are qualified and validated for use in drug release testing.

This chapter is divided into three sections. The first section briefly discusses existing compendial methods along with any non-compendial modifications. The second section describes non-compendial apparatus for different types of dosage forms. The final section explains the various detection techniques such as UV imaging with Raman spectroscopy, FTIR-ATR, and fiber optics. Namita Tipnis and Diane Burgess of the University of Connecticut provide their review of emerging technologies on the horizon for dissolution and drug release testing in pharmaceutical laboratories.

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## Chapter 2

# Solubility Determinations for Pharmaceutical API

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## 2.1 Introduction

The complex and time-consuming process of drug development, from discovery of a new chemical entity (NCE) to the authorization of marketing for a new drug, can span a period of 12–20 years.[1] Undesirable physicochemical attributes are a major cause of attrition in the drug development process. In a typical physicochemical screening process (i.e., p$K_a$, solubility, permeability, stability, and lipophilicity), poor solubility is a key factor limiting successful development.[1,2] Compounds with insufficient solubility are more likely to fail during discovery and development as inadequate solubility not only impacts other property assays, masking additional undesirable properties, but also influences both pharmacokinetic and pharmacodynamic characteristics of the

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**Table 2.1** The Biopharmaceutics Classification System (BCS)

| Biopharmaceutics class | Permeability | Solubility |
|---|---|---|
| I | High | High |
| II | High | Low |
| III | Low | High |
| IV | Low | Low |

compound.[3] The Biopharmaceutics Classification System (BCS) is the scientific framework that allows the classification of drug substances based on their dissolution, aqueous solubility, and intestinal permeability.[1–4] A drug's bioavailability depends primarily on its solubility in the gastrointestinal (GI) tract and its permeability across the cell membranes upon oral administration.[5] This BCS system was proposed by the Food and Drug Administration (FDA) as a bioavailability/bioequivalence (BA/BE) regulatory guideline and assigns drugs into four groups illustrated in Table 2.1. According to the BCS framework, solubility is determined by obtaining the pH-solubility profile of the drug substance in question in an aqueous medium of pH range 1–8 at an established temperature of $37 \pm 1^{\circ}$C.

A drug substance according to the solubility classification in the BCS is thus considered to be highly soluble when its highest dose strength proves to be soluble in 250 mL or less of an aqueous medium over the pH range of 1–8.[6–8] If not, the drug substance is considered as poorly soluble. The 250 mL volume estimate is from bioequivalence study protocols which prescribe drug product administration with a glass of water to fasting volunteers.[7] A drug substance is considered highly permeable when the extent of intestinal absorption is determined to be 90% or higher; if not, the drug substance is considered to be poorly permeable.[7] Permeability classification is thus based directly on the extent of intestinal absorption of a drug substance in humans or indirectly on the measurements of the rate of mass transfer across the human intestinal membrane.[7]

BCS class I compounds have high permeability and high solubility with examples including captopril, propranolol, diltiazem, metoprolol and antipyrine. The rate of drug dissolution limits the

in vivo absorption of these drugs.[9] Class II compounds include naproxen, itraconazole, carbamazepine and piroxicam.[9–11] These drugs exhibit high permeability but fail to meet the criterion for high solubility across the physiological pH range of pH 1–8. Solubility, as such, limits the absorption flux. The restriction can be equilibrium-based, whereby either the composition or volume of the contents in the GI tract preclude complete dissolution of the drug. Alternatively, the volume and composition of the GI contents can, in theory, facilitate complete dissolution of the drug, but the dissolution rate is too slow in the appropriate absorption site in the intestine (kinetic-based). Class III compounds are those with high solubility, but low permeability. Drugs in this group include cimetidine, atenolol, and acyclovir, with permeability being the rate-determining step affecting absorption.[5,6,12–18] BCS class IV drugs are compounds exhibiting both low solubility and low permeability. In this group, the rate of the in vivo absorption of the drug depends on the relative interplay between the two and also whether the drug's low permeability is borderline or whether it is as a result of GI metabolism. Drugs in this group include cyclosporine and terfenadine.[12–18] Recent adaptations to the BCS include the Biopharmaceutical Drug Disposition Classification System (BDDCS) based on solubility and intestinal permeability rate, which is related to the extent of metabolism, to predict drug disposition and potential drug-drug interactions in the intestine and/or liver.[19] Both systems, although they differ in the criterion for permeability and have different purposes, are based on classifying drugs and new molecular entities into four categories using the same solubility criteria. The solubility of a drug is therefore a key consideration when systemic delivery is desired as low aqueous solubility can either delay or limit drug absorption.[5]

## 2.2 Drug Solubility Assay Development

Aqueous solubility and rate of dissolution are pivotal attributes in drug discovery and development as they affect both in vitro and in vivo assay results. Determination of these parameters will facilitate understanding the interplay among absorption,

**Table 2.2** USP descriptive classification of drug solubility[24]

| Definition | Parts of solvent required for one part of 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 | >10,000 |

distribution, metabolism, excretion, and toxicity (ADMET) parameters as insufficient solubility can interfere with pharmacokinetic and pharmacodynamic properties.[1] Along with other pharmacodynamic and pharmacokinetic parameters, one can utilize solubility to help prioritize early drug candidates, to not only accelerate the discovery and development of NCEs but also improve the attrition rate for drug candidates in late development.[4,20–22] The two major causes of poor aqueous solubility are high lipophilicity and strong intermolecular interactions, which render solubilization of the solid energetically unfavourable.[23] The United States Pharmacopeia (USP) classifies drug solubility using seven classes with definitions in Table 2.2.

In discovery and development phases, the determination of solubility, along with other physicochemical parameters, can allow screening out of unsuitable candidates and can also inform formulation optimization and salt selection. Solubility is considered a thermodynamic parameter being defined as the saturation concentration of a solute in solution under specific conditions, where an equilibrium between the solute and solvent is achieved thermodynamically. This equilibrium balances the energy of solvent and solute interacting with themselves against the energy of solvent and solute interacting with each other (Fig. 2.1).[3,20,25–30] The overall intent of measuring solubility in the drug development context is to determine an intrinsic property that influences the absorption of a potential compound. Solubility and permeability tend to be inversely related; hence close attention should be given to several physicochemical properties when increased oral absorption is desired.[31,32]

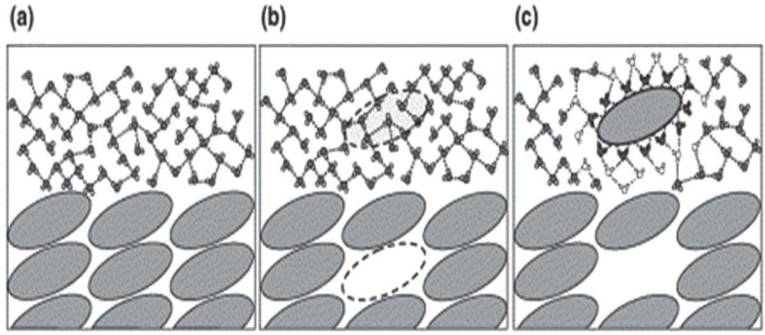


**Figure 2.1** The intermolecular forces that determine thermodynamic solubility. (a) Solvent and solute are segregated; each interacts primarily with other molecules of the same type. (b) To move a solute molecule into solution, the interactions among solute molecules in the crystal (lattice energy) and among solvent molecules in the space required to accommodate the solute (cavitation energy) must be broken. The system entropy increases slightly because the ordered network of hydrogen bonds among solvent molecules has been disrupted. (c) Once the solute molecule is surrounded by solvent, new stabilizing interactions between the solute and solvent are formed (solvation energy), as indicated by the dark gray molecules. The system entropy increases owing to the mingling of solute and solvent (entropy of mixing), but also decreases locally owing to the new short-range order introduced by the presence of the solute, as indicated by the light gray molecules.[27]

The methods of determining aqueous solubility include

- thermodynamic or equilibrium (the concentration of a compound in saturated solution when excess of solid is present, and solution and solid are at equilibrium),
- semi-equilibrium (close to equilibrium), and
- kinetic methods (the concentration of a compound at the time when an induced precipitate first appears in the solution).

These terms are used commonly in pharmaceutical literature.[25,33–36] In the general sense, assays that are equilibrium-based and start with the solid form of the compound are thermodynamic whereas assays that start with the compound predissolved in organic solvent generally have shorter incubation durations and are

kinetic. The true thermodynamic solubility of a compound should be determined using the purified crystalline form of the compound because there can be very large differences in the solubility values between amorphous and crystalline forms.[32,37,38] Despite this, the solubilising and storage of compounds in solvents such as dimethyl sulfoxide (DMSO) and the acceptance of DMSO stocks in discovery for solubility testing is because the physical form of the compound at that stage of discovery is usually not the pure crystalline form.[4,32,39] Physical forms such as different polymorphs or solvates give rise to measurable differences in their physical properties due to the different lattice energies and entropies associated with them.[34] For example, salt formation will affect properties such as physical and chemical stability, melting point, electrostatic behaviour, crystal habit, refractive index, solubility, mechanical, dissolution rate, density, and biopharmaceutical properties, with the particular salt formed determining these properties.[37,40] However, thermodynamic or equilibrium solubility determinations tend not to be very feasible in the early stages of drug discovery owing to the fact that a relatively large sample is required, the sample preparation can be considered laborious, the compound's purity and physical form may have yet to be determined and there is a low throughput of the compound.[24,27,41] Despite this, it is becoming increasingly common to measure solubility as early as feasible in the discovery process due to the fact that the solid-state properties of compounds such as purity, degree of crystallinity, particle size, and polymorphism can be studied and characterized in detail.[1]

Typically more than one solubility determination methodology is applied to support the different stages of drug discovery and development. In the early phases of discovery, kinetic solubility or semi-equilibrium methods are often used facilitated by high-throughput (HT) assays for profiling large numbers of compounds, and medium-throughput (MT) and/or low-throughput (LT) equilibrium methods are often used for later stages of drug discovery and development. Although kinetic solubility can be used as an alternative for the thermodynamic method, it cannot, however, be used as a complete substitute since the method uses solvents, normally DMSO, meaning that any impact of the crystal lattice and the presence of polymorphic forms of the drug are lost. As such,

kinetic methods tend to be most suitable for the drug discovery stage and the thermodynamic methods of determining solubility tend to be more appropriate at the drug development stage (Table 2.3).[1,24]

**Table 2.3** Differences between the methods used for solubility measurement in drug discovery and drug development processes[1]

| | **Discovery** | **Development** |
|---|---|---|
| Compounds tested | | |
| Number | 100–1000 | 10 |
| Quantity available | A few mg | >g |
| Purity | Limited | Improved |
| Solid state | Amorphous or partially crystalline (not characterized) | Stable, crystalline material (characterized) |
| Distribution | Generally in DMSO stock solutions | Generally in solid form |
| Methods | | |
| Type of solubility measured | Kinetic solubility (fully dependent on experimental conditions) | Thermodynamic solubility |
| Throughput | High | Low 25–50 compounds a week |
| Automation | Fully | Only partially automated |
| Format | 96-well or 384-well microplates | Small scale (single tube) |
| Incubation time | Minutes | Hours or days |
| Detection | UV, turbidity | HPLC-UV, HPLC-MS |
| Media | Aqueous | >20 (aqueous, organic, biorelevant media, formulations, excipients …) |
| Data generated and intended purpose | Solubility in screening bioassay media to avoid misinterpretation | Solubility and dissolution in biorelevant media |
| | Rank-order hits | Evaluation of formulations<br>Characterization and optimization of solid state<br>Selection of promising compounds<br>Development of adequate strategies to overcome solubility problems |

## 2.2.1 Media for Solubility Studies

### 2.2.1.1 Biorelevant media

Normally, to achieve desirable systemic exposure after oral dosing, the active drug substance or compound must be in solution in the aqueous environment of the intestine in order to cross the luminal wall; hence the importance of drug solubility and drug dissolution for orally administered drugs.[42] During the early screening process, drug candidates with low water solubility may be excluded from further development because failure in later stages is likely.[42] However, although drug candidates with solubility below an acceptable predetermined solubility may prompt a degree of concern, it is important to note that low aqueous solubility also may not always constitute a sufficient reason for the compound's elimination as it could still have relatively high intraluminal solubility.[3,42,43] The composition of GI fluids has a large impact on the solubility and dissolution of poorly soluble API in the GI tract, and hence a large influence on the drug absorption. The ability to predict solubility in the upper GI tract would thus be advantageous to drug discovery and development. Gastric and intestinal fluids under fasted and fed conditions vary with regards to pH, buffer capacity, osmolarity, surface tension, and lipid concentration of GI fluids.[44]

The two most widely implemented approaches for estimating drug solubility in the GI tract are determination of solubility in fluids aspirated from the human gastrointestinal tract, and determination of solubility in biorelevant media.[44,45] The solubilizing capacity of the GI environment may be much higher than aqueous buffer systems such as those used in pharmacopeial dissolution testing methods[46] and as such solubility measurements are also performed in biorelevant media to identify potential in vitro–in vivo *correlations* (IVIVCs). Bile salt composition is variable throughout the duodenum and jejenum (Fig. 2.2).[44] It is important that simulating media for solubility screening in early drug development reflect the solubilizing capacity of human intestinal fluid rather than having an identical composition to ensure functional relevance prevails over compositional biorelevance.[47]

This allows the evaluation of the impact of solubility on absorption. For example, Sunesen et al.[48] showed that the solubility

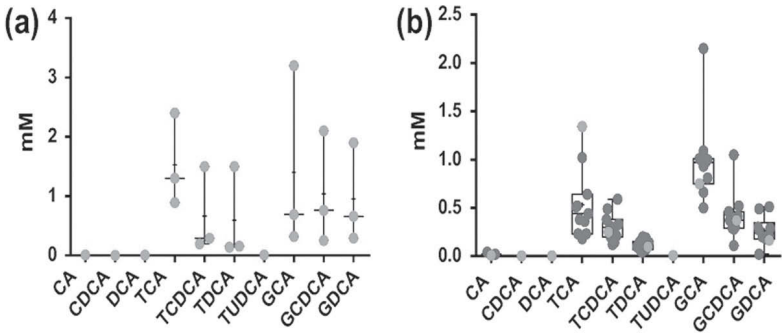


**Figure 2.2** Bile salt composition in human (a) duodenum and (b) jejunum. CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; TCA: taurocholic acid; TCDCA: taurochenodeoxycholic acid; TDCA: taurodeoxycholic acid; TUDCA: tauroursodeoxycholic acid; GCA: glycocholic acid; GCDCA: glycochenodeoxycholic acid; GDCA: glycodeoxycholic acid. Box–whisker plots show minimum and maximum values, as well as 25, 50, and 75 percentile. The cross indicates the mean value. Each data point represents a group of participants ($n$ = 1–10 gray, $n$ = 11–20 dark gray) reported in one publication.[44] Reprinted from Bergström et al.,[44] with permission from Elsevier.

of danazol increased from 0.42 μg/mL in water to 1.61 and 2.04 μg/mL in gastric and intestinal fluids respectively, contributing in part to a higher bioavailability than predicted. Several authors have shown drug solubility to be enhanced in biorelevant media compared to aqueous buffers.[49–52] These authors together with Dressman et al.,[53] Naylor et al.,[54] Pederson et al.,[55] and Galia et al.[56] showed that bile salts (sodium taurocholate as a model) and lecithin can self-assemble and form micelles, as well as act as wetting agents, thereby increasing the solubility and dissolution rate of lipophilic compounds and are included in biorelevant media.

Jantratid et al.[57] updated the components or constituents of the biorelevant media taking into consideration fed and fasted states in man. Takács-Novák et al.[58] evaluated four poorly soluble drugs encompassing a range of lipophilicities and solubilities in biorelevant media and confirmed the primary role of ionization in governing the solubility in the biorelevant media. They also found the higher concentration of solubilizing agents in Fed State Simulated Intestinal Fluid (FeSSIF) to improve solubility. Using 17 model

drugs, Clarysse et al.[42] evaluated the solubilizing capacity of Fasted State Simulated Intestinal Fluid ($FaSSIF_c$) and $FeSSIF_c$ (subscript indicates the use of crude taurocholate) and different concentrations of D-$\alpha$-tocopheryl polyethylene glycol 1000 succinate (TPGS) in phosphate buffer and correlated it with the solubilizing capacity of human intestinal fluids (HIF) in the fasted and the early postprandial state. They found a good correlation between solubility in fasted HIF and FaSSIF and also between solubility in fed HIF and $FeSSIF_c$. Comparable values were also obtained for the 0.1% TPGS for the fasted state and 2% TPGS for the fed. They then concluded that FaSSIF and FeSSIF could be considered as biorelevant media for intestinal solubility estimations and the simpler TPGS-based system may be a valuable alternative with improved stability and lower cost. However, Ottaviani et al.[59] evaluated the solubility of 25 chemically diverse compounds in modified simulated intestinal fluid (FaSSIF-V2) and in aqueous phosphate and maleate buffers and found that the solubility of the ionized acids did not increase in FaSSIF-V2. They attributed this behaviour to electrostatic repulsions with the media components but found lipophilicity to play an important role mainly for charged bases with a $\log P > 4$ (or $\log D_{6.5} > 1.9$). They also found that when the aqueous solubility is mainly driven by lipophilicity, the FaSSIF-V2 components improved the solubility of basic compounds. This increase was less for compounds whose solubility is limited by crystal packing, leading them to conclude that ionization, lipophilicity, and crystal packing all play important but peculiar roles in controlling solubility in FaSSIF-V2 compared to that in aqueous buffer.

As the constituents of FaSSIF and FeSSIF are comparatively expensive and the preparation process is labour intensive, requiring fresh preparation daily,[60] their application in solubility studies has tended to be limited to later and advanced stages of the drug development process. However, an instant powder is available to facilitate the preparation of FaSSIF and FeSSIF, which may address some of these issues[42] and a high-throughput screening UV method for measuring biorelevant solubility showed excellent correlation with solubility data obtained using the shake-flask method without DMSO.[61] This may provide a reliable method for

measuring intestinomimetic solubility during the early stages of drug discovery.

#### 2.2.1.2 Organic solvents (DMSO)

Various methods have been developed for the measurement of solubility in the last two decades, including turbidity and UV plate scanner-based detection systems.[62] These are ranking assay methods for high-throughput screening where the buffered sample solutions are prepared by adding aliquots of 10 mM dimethyl sulfoxide (DMSO) stock solutions.[63] With regard to the turbidimetric methods, small aliquots of the stock solutions are added to pH 7 buffer solutions at fixed intervals until turbidity is detected. A plot of volume versus turbidity is extrapolated to zero turbidity after the addition of a few more aliquots to ascertain the onset of precipitation and anywhere from 50–300 compounds can be analyzed with this methodology.[64] The fast UV plate spectrophotometer method allows solubilities at a single or multiple pH values to be rapidly determined. In this method,

- a dry powder or a DMSO solution of a known quantity of sample is added to a known volume of a universal buffer solution of known pH (it is important that the amount of sample is sufficient to cause precipitation in the solution or an excess of solid),
- the saturated solution is allowed to reach equilibrium,
- the solid material or precipitate is removed by filtration,
- a suitable water-miscible cosolvent is added to the sample solution and the UV spectrum of the solution determined, and
- the compound's solubility is then calculated from the ratio of the reference spectrum and the sample spectrum taking the dilution factors into consideration.

If determining the compound's solubility at one pH, up to about 400 compounds can be analyzed in a day. Figure 2.3 shows the pH solubility profile of piroxicam determined using this method.[64]

As DMSO increases solubility, assays that use stock compounds in DMSO have a limited maximum solubility, owing to the specified %

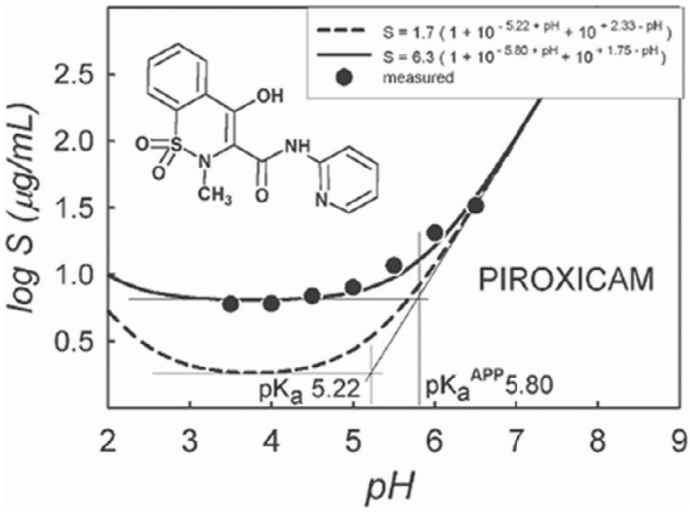


**Figure 2.3** Solubility-pH profile of piroxicam, determined by the fast-UV method. The apparent curve (solid line) is shifted from the true aqueous curve (dashed line) due to the presumed formation of aggregates, as evidenced by the shift in the p$K_a$ value of the drug. Reprinted from Avdeef and Testa,[64] with permission from Wiley.

DMSO in the assay and the DMSO stock concentration.[33] The DMSO stock solution can be removed by evaporation with subsequent buffer addition to the remaining material in an effort to minimize the contribution of DMSO to solubility measurement. The amount of DMSO used in solubility assays typically varies from 0 to 5% v/v and buffer pH is between 6.5 (intestine) and pH 7.4 (bioassay and blood). In addition to the use of DMSO, other issues cited with such assays include poor reproducibility for very sparingly water soluble compounds, differences between physical forms of candidates and an apparent lack of standardization, which limits comparisons between different sites.[64]

In drug discovery processes, kinetic solubility tends to be the most relevant parameter for rapidly evaluating whether the compound of interest, which is generally pre-dissolved in DMSO, stays in solution after dilution in specific screening media or not. It is important to note that incomplete solubility within the functional assay could lead to problems such as unreliable

results, underestimated activity, reduced high-throughput screening (HTS) hit rates and an inaccurate structure–activity relationship (SAR) when not identified.[1,65] In the development phase of drug research, equilibrium solubility of drug-like molecules is measured by different methods, among them potentiometric methods such as Dissolution Template Titration (DTT) or CheqSol®.[35,36,66–69] The DTT procedure simulates the entire titration curve before the assaying begins using the p$K_a$ and the estimated intrinsic solubility.[66,69] The basic method, however (against which new solubility methods are generally validated), remains the classical saturation shake-flask method.[25]

#### 2.2.1.3 Detection methods

It is important to note that the different analytical tools used for compound detection, such light scattering/turbidity, UV plate readers, LC-UV, and LC–MS/LC-CLND (chemiluminescent nitrogen detection), can potentially impact assay results (Table 2.4). Other factors that can affect the ability to determine "true" thermodynamic solubility of compounds in solvents include the compound's purity; the reliability of compound's particle size measurement, shape factor, and particle growth, all of which can cause a change in the surface area. For example, Fini et al.[70] reported a linear relationship between the efficiency of dissolution and the shape factor of dissolving particles of a diclofenac salt crystallized from various solvents and mixtures of solvents suggesting importance of shape irregularity in affecting dissolution rate differences ($r^2 = 0.9077$); amount of residual solid, changes in solid properties of the compound (polymorph, amorphous, solvate formation); the crystallization solvent; solvent purity; mixing conditions which may affect the homogeneity of mixing and particle size reduction; the efficiency of temperature control within the host vessel; uncertainty regarding the time taken to establish equilibrium; degree of ionization; compound's stability in solution; pH of the solution at start and end of procedure; compound aggregation due to the formation of promiscuous aggregates; self-association of molecules or surface reduction by aggregates and filter pore or the effect on centrifugation on solid/liquid separation.[3,62,70–82]

**Table 2.4** Advantages and limitations of common detection methods for solubility measurements[33]

| Detection methods | Advantages | Limitations |
|---|---|---|
| Light scattering/ turbidity | Universal, fast, economical | Interference from certain colored compounds and impurities, sensitive to sedimentation and particle size, low sensitivity, measures precipitates rather than solution concentration |
| UV plate reader | High sample coverage, fast, economical, sufficient sensitivity for solubility measurement, good linearity over wide dynamic range | Requires UV chromophore, interference from impurities and matrix material |
| LC-UV | High sample coverage, less interference from impurities and matrix material, sufficient sensitivity for solubility measurement, good linearity over wide dynamic range | Requires UV chromophore, might need different HPLC methods for special compounds, not as fast and economical as UV plate readers |
| LC–MS | High sensitivity, high selectivity, low interference | Less universal, moderate sample coverage, low dynamic range for linearity, too sensitive for solubility measurement (need large dilution), high maintenance, costly |
| LC-CLND | No standard curve needed | Only for nitrogen containing compounds, interference from nitrogen containing solvents, significant signal loss for adjacent nitrogen |

Kramer et al.[83] investigated kinetic solubility by nephelometry for over 700 drug-like compounds and built three classification criteria; insoluble (<20 μM), moderately soluble (20–200 μM) and soluble (>200 μM) using a forest algorithm for which the datasets' performance was evaluated to provide appropriate data for early phases of drug discovery. As such, the environment in which a solubility assay is run and the primary focus of the assay dictate how assays are currently set-up and performed in the drug discovery and development process (Fig. 2.4).[3] Although high-throughput kinetic solubility determination provides a cost-effective and fast solution, its application in projecting the impact of solubility in vivo for formulation is limited due to a poor correlation with conventional solubility data from equilibrated thermodynamic conditions.[20,84] Kinetic solubility, however, seems to becoming more prevalent due to reasons such as compounds being solubilized in DMSO for storage and distribution and allowing poorly soluble compounds to be available in the aqueous phase.[32] As both kinetic and thermodynamic solubility play crucial roles in drug discovery and development, it is important to remember that the effective solubility (the goal for high-throughput solubility assays) of compounds in the screening environment is kinetically driven, and the intrinsic solubility of the compound is driven by a thermodynamic process and with regards to the detection methods, they offer different advantages depending on the question being addressed.[32]

## 2.3 Drug Solubility Determination

### 2.3.1 Shake-Flask Method

The shake-flask (SF) method is a relatively simple procedure and is considered the method of reference for solubility measurement but it is time-consuming and requires a lot of manual processing. Due to potential compound degradation or polymorph formation, both of which can affect solubility, it is important that the compound's dissolution profile is investigated alongside solubility measurements. This allows the determination of the shortest time

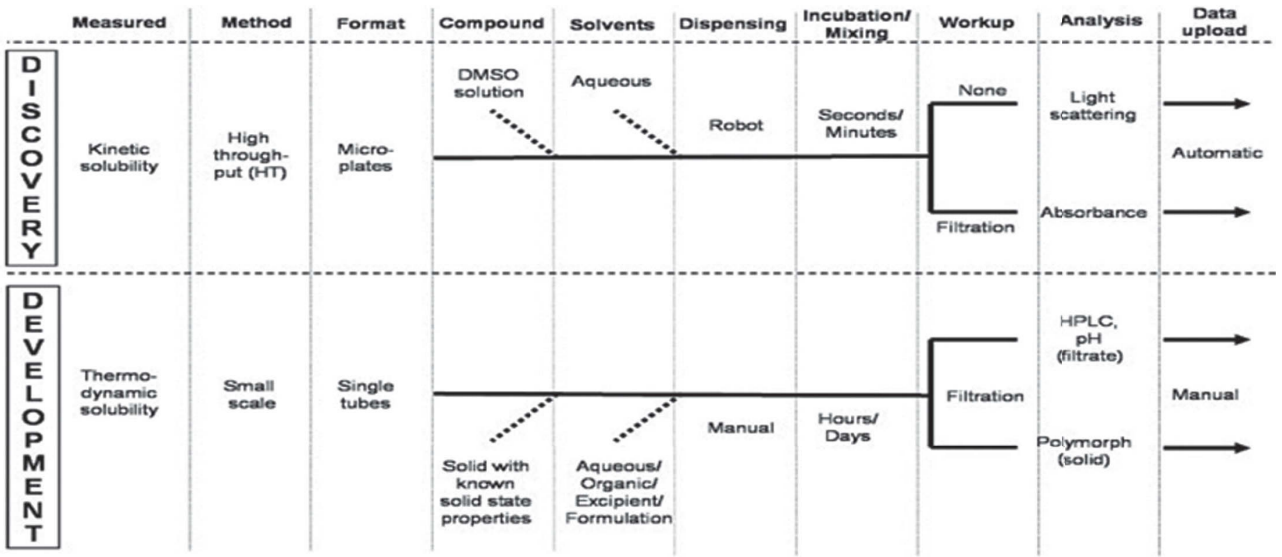


**Figure 2.4** Solubility determination in drug discovery and development: key elements of traditional solubility assay workflows. Reprinted from Alsenz and Kansy,[3] with permission from Elsevier.

required for deducing the establishment of an equilibrium. It is also important to note that manipulations such as vortexing, sonicating, or the use of small glass microspheres prior to equilibration can be incorporated to offset problems of low wettability of poorly soluble compounds and also thus reduce equilibrium time. Although filtration is often used to separate phases for analysis, with the other main technique being centrifugation, the sorption of especially low soluble compounds on the filter can lead to errors in solubility values. Precautions such as pre-rinsing the filter with a saturated solution can reduce this error.

UV spectrophotometric analysis tends to be the most commonly used method in solubility determination; however, HPLC may often be a more suitable method, as it can detect impurities and degradation products if any are present.[24,85] As a result of lack of standardization of this method, there is a huge range of published literature showing variations not only in experimental conditions but also in separation techniques which can lead to differences between solubility data reported in literature for some compounds.[66,70,78,86–93]

This widely used thermodynamic or equilibrium solubility measurement method was proposed by Higuchi and Connors.[94] It involves the following steps:

- An excess of the compound of interest is added to a solubility-determining medium such that a saturated solution exists in equilibrium with some solid phase.
- The sample is then shaken at a controlled temperature for a specific time. Acidic or basic drugs should be dissolved using an unbuffered medium (usually water) as the solubility of compound of interest could change as a result of a change in pH arising from further addition of the compound. Thus the final pH of a saturated solution of the compound may be far from pH 7 due to self-buffering.[3]
- The sample is then filtered and the concentration of the compound in the filtrate determined after equilibrium (usually after 24 hours using HPLC). Data obtained can then be compared with literature to determine similarity or give an indication into the accuracy of the method used.

This method takes into consideration the effect of the crystal lattice and polymorphic forms of the compound being tested as these properties can greatly affect solubility. During solubility measurement, a compound may transform to a more stable polymorph. It is, therefore, especially important to isolate the solid at the end of the assay, to ensure no polymorphic transitions have occurred and the form of the compound is unaltered.[73,95,96] Pudipeddi and Serajuddin,[73] determined the solubilities of 55 compounds, with previously documented solubilities, each of which had multiple polymorphs. For the non-solvated crystal forms, there was only a two-fold difference between the most soluble and the least soluble polymorphs. The highest ratio of difference in solubilities between different forms of the active was 23, for premafloxacin. Solubility ratios of amorphous to crystalline forms were generally higher (about 10-fold). They proposed that "unstable solid forms with extraordinarily high free energies might have eluded detection due to rapid conversion to lower energy forms, thus limiting the observable range of measurable solubility differences."[73,97] As such thermodynamic solubility is often referred to as the "true" solubility of a compound.[1,63] Despite the benefits of this method of solubility measurement, it has several drawbacks:

- It is time-consuming and difficult to automate requiring an individual weighing step.
- It requires relatively high amounts of compounds (usually several mg).
- A relatively lengthy incubation time is needed for equilibrium attainment.
- The method cannot distinguish between soluble monomers and soluble aggregates of the drug molecules (which may range from dimers to micelles) unless more sophisticated experiments are performed.[23,98]
- Solubility measured at a fixed pH value may be highly dependent on the nature and concentration of the counterions present in the medium. This may be critical for poorly soluble compounds being highly ionized at the pH of the measurement.[23,98]

This method is therefore often used in the later development stages and advanced stages of lead compounds.

Baka et al.[25] evaluated the experimental conditions that affect equilibrium solubility by use of the classical saturation shake-flask method using hydrochlorothiazide as a model compound. They found modifications in temperature, sedimentation time, composition of aqueous buffer and the technique of separation of solid and liquid phases all strongly influence the equilibrium solubility results. Variations in the amount of excess solid and stirring time, however, were found to have less of an influence. A new shorter protocol was then developed by the same authors with the recommendations below to ensure a reduction in experimental error of solubility measurements to about 4%.

- The measurements must be carried out at controlled, standard temperature.
- Sörensen's phosphate buffer can be used between pH 3–7; Britton–Robinson buffer solution can be used between pH 2.5–11.5; HCl of appropriate concentration can be used below pH 2. (There is potential for salt formation with ionizable compounds which could affect the solubility hence the importance of isolating the solid for characterization as discussed in Section 2.2 and Section 2.3.2).
- To avoid difficulties in sampling, only a small excess (~5–10 mg/5 mL) of solid should be present.
- A minimum of 24 hours is necessary to reach the thermodynamic equilibrium; this time should consist of 6 hours of stirring plus 18 hours of sedimentation; but in case of very sparingly soluble compounds longer stirring time may be necessary for equilibrium, so in the most rigorous application of the shake-flask method, solubility would be measured after checking the required equilibration time from compound to compound.
- The recommended technique for phase separation is sedimentation, which assures the existence of a heterogeneous system until equilibrium has been achieved.

The equilibrium solubilities of five other drugs (Table 2.5) were measured using these recommendations and the results were in

excellent agreement with the standard protocol used with the log *S* of a compound determined in less than one and a half days.[25]

Loftsson and Hreinsdottir[99] modified the classical shake-flask solubility method by shortening the equilibration time by application of a heating process to determine the solubility of 48 different drugs and pharmaceutical excipients in pure water at room temperature. Despite finding this modified shake-flask method generated reliable and reproducible data, the mean calculated solubilities were lower than experimental solubilities according to the Yalkowsky equation (see Section 2.3.2). This was due to the equation only being valid for non-electrolytes whereas many of the compounds tested were partly ionized in the aqueous solutions.

## 2.3.2 Miniaturized Shake-Flask Method

Despite application of Lipinski's rule of five as an early warning tool (see Section 2.3.4), the extensive use of organic solvents in the early stages of drug discovery can lead to a delay in determination of true solubility (see Section 2.2.1.2). With this in mind and also because of the large numbers of compounds screened and the very small amounts of compounds produced in the early development stage, Glomme et al.[85] developed a miniaturized shake-flask method for solubility determination that could be applied earlier in the discovery process. They determined the solubility of 21 compounds (solubility range 0.03–30 mg/mL) and compared them with those measured by a semi-automated potentiometric acid/base titrations and computational methods (Table 2.6). They showed their method's precision and throughput to be superior to the potentiometric method and that the miniaturized shake-flask method could be used for all compounds and a wide variety of media.[85]

### 2.3.2.1 Sample preparation and analysis

The theory and method is detailed in Glomme et al.,[85] but in brief, it is as follows:

**Table 2.5** The solubility of compounds measured by the standard and the new shake-flask protocols[25]

| Compound | MW | μg/mL | Solubility, S (M) | log *S* (M) | Solubility, S (μM) | log *S* (μM) | n |
|---|---|---|---|---|---|---|---|
| Standard protocol | | | | | | | |
| Hydrochlorothiazide | 297.7 | 556 ± 13.2 | 0.001868 | −2.73 | 1867 | 3.27 | 18 |
| Furosemide | 330.8 | 20.4 ± 2 | 0.000062 | −4.21 | 61.7 | 1.79 | 8 |
| Nitrofurantoin | 238.2 | 109.5 ± 3 | 0.00046 | −3.34 | 460 | 2.66 | 8 |
| Piroxicam | 331.4 | 5.95 ± 0.4 | 0.000018 | −4.75 | 17.9 | 1.25 | 2 |
| Quinine-HCl | 360.4 | 201 ± 10 | 0.000558 | −3.25 | 558 | 2.75 | 6 |
| Trazodone | 371.4 | 138 ± 10 | 0.000372 | −3.43 | 370 | 2.57 | 6 |
| New protocol | | | | | | | |
| Hydrochlorothiazide | 297.7 | 571 ± 8.6 | 0.001918 | −2.72 | 1918 | 3.28 | 12 |
| Furosemide | 330.8 | 18.7 ± 1.2 | 0.000057 | −4.25 | 56.4 | 1.75 | 8 |
| Nitrofurantoin | 238.2 | 99 ± 4.1 | 0.000416 | −3.38 | 416 | 2.62 | 8 |
| Piroxicam | 331.4 | 6.36 ± 0.04 | 0.000019 | −4.72 | 19.2 | 1.28 | 3 |
| Quinine-HCl | 360.4 | 285 ± 30 | 0.000791 | −3.10 | 717 | 2.86 | 5 |
| Trazodone | 371.4 | 176 ± 1.8 | 0.000474 | −3.32 | 473 | 2.67 | 12 |

*Note*: Britton-Robinson (BR) buffer used for all samples except furosemide, which was measured in 0.01 M HCl solution.

**Table 2.6** Solubility determined by the miniaturized shake-flask and pSol methods at several pH values for ionizable compounds[85]

| | | Method | |
|---|---|---|---|
| **Compound** | **pH** | **Shake-flask solubility (μg/mL)** | **pSol solubility (μg/mL)** |
| Dipyridamole | 3.5 | 2199.4 ± 99.4 | |
| | 4.2 | 342.7 | |
| | 5 | 54.1 ± 2.2 | 39.9 ± 4.98 |
| | 6 | 10.5 ± 0.94 | 5.5 ± 0.66 |
| | 7 | 4.9 ± 0.13 | 2.1 ± 0.25 |
| | 7.8 | 6.0 ± 0.22 | 1.8 ± 0.22 |
| Glyburide | 2 | 0.07 ± 0.002 | 0.06[a] |
| | 3 | 0.06 ± 0.01 | 0.06[a] |
| | 5 | 0.10 ± 0.06 | 0.01[a] |
| | 6 | 0.62 ± 0.15 | 0.56[a] |
| | 7 | 5.62 ± 0.72 | 5.13[a] |
| | 9 | 51.2 ± 0.29 | 51.2[a] |
| | 9 | 98.6 ± 0.83 | |
| | 11.8 | 531.6 ± 9.60 | |
| Mefenamic acid | 2 | 0.06 ± 0.002 | 0.06 ± 0.02 |
| | 3 | 0.07 ± 0.01 | 0.06 ± 0.02 |
| | 5 | 0.65 ± 0.03 | 0.19 ± 0.08 |
| | 6 | 7.18 ± 0.15 | 1.41 ± 0.58 |
| | 7 | 67.2 ± 3.27 | 13.7 ± 5.62 |
| | 8 | 357.0 ± 2.79 | |
| | 9 | 486.2 ± 3.13 | |
| Phenytoin | 5 | | 20.62 ± 2.67 |
| | 6 | | 20.70 ± 2.69 |
| | 7 | 31.00 ± 2.03 | 21.49 ± 2.81 |
| | 8 | | 29.52 ± 3.98 |
| Levothyroxine | 5 | 0.26 ± 0.1 | 0.22[a] |
| | 6 | 0.27 ± 0.1 | 0.24[a] |
| | 7 | 0.49 ± 0.1 | 0.45[a] |

[a]Measured with cosolvent; pSol method is discussed in Section 2.3.3.

- It is recommended that the drug's approximate solubility is estimated using an in silico method outlined in Section 2.4 to ensure a minimal amount of drug is used.
- An amount of drug, corresponding to twice the calculated solubility, is weighed and filled into the Whatman UniPrep chamber (Fig. 2.5). 2 mL of appropriate medium is added

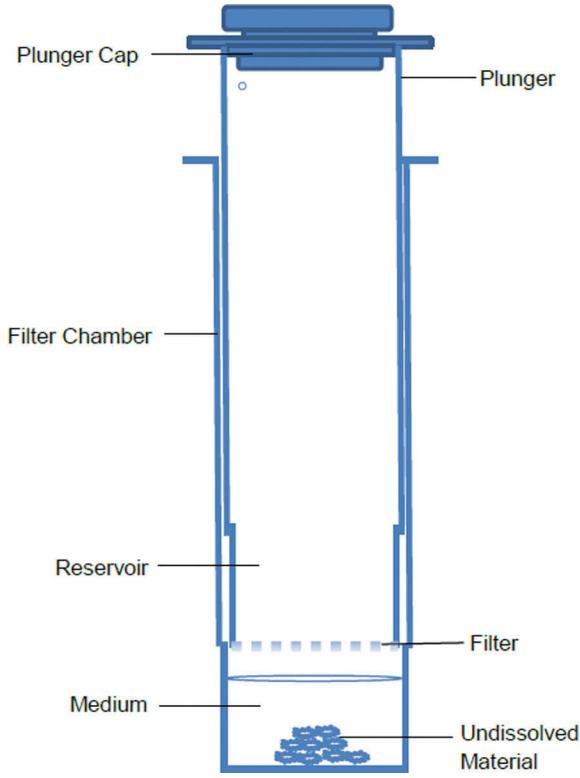


**Figure 2.5** Schematic of Whatman Processor™. Adapted from Glomme et al.[85]

and chamber closed with a plunger. The sample is then shaken at 450 rpm and 37°C for 24 hours.

- With the exception of neutral compounds, after several hours, the pH and presence of precipitation should be checked. Additional sample can be added if dissolution is complete.

It is important to note that with regards to salts of strong acids and bases, an adjustment with 0.1 M NaOH/0.1 M HCl may be needed to maintain a constant pH value due to the small volume of medium used and a low buffering capacity.

- The plunger is inserted into the sample-containing chamber after shaking is completed and depressed, thereby forcing the filtrate into the reservoir of the plunger.
- The concentration of compound in the filtrate is determined after filtration using HPLC with UV detection, against a standard solution.

Bergstrom et al.[90] investigated the extent to which the Henderson–Hasselbalch equation could be used to predict the pH-dependent aqueous solubility of 25 weakly basic cationic drugs using a small-scale shake-flask method and found that the equations do not accurately predict the pH dependence at 25°C. This was attributed to the formation of aggregates/precipitates with the 0.15 M phosphate buffer used.[97] The authors deduced from solid state characterization of the aggregates/precipitates using differential scanning calorimetry (DSC) that the ionized samples had a higher melting point compared to that of the free base. It was concluded that the aggregates were as a result of the formation of phosphate salts. Procedures similar to this have been adapted to fast LC/MS detection and throughputs of about 50–200 compounds per day have been reported.[4,100] Zhou et al.[20] developed a high-throughput equilibrium solubility assay method using a miniaturized shake-flask method for the early drug discovery process and found it to offer a fast, reliable, and a cost-effective screening tool for solubility assessment.

### 2.3.3 Potentiometric Titration Methods

Although equilibrium solubility measured by the classical shake-flask method is accurate if performed to a high standard, it is also slow to carry out. With advances in high-throughput screening for discovery of NCEs, kinetic solubility determinations may provide a faster alternative. Kinetic solubility measurements are based on the precipitation of a compound pre-dissolved in a co-solvent or aqueous medium by pH adjustment for ionizable compounds after dilution in a given medium (Fig. 2.6).[1,3,101] Determinations are based on the shift in pH values caused by loss of compound due to precipitation. It is important to note that kinetic solubility

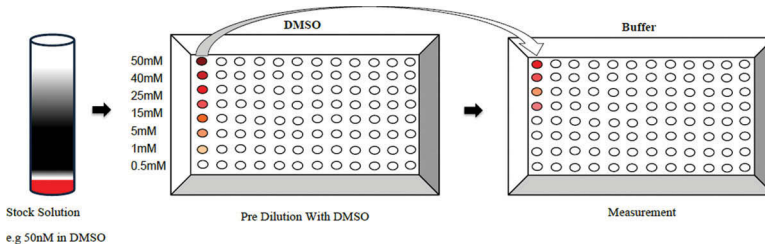


**Figure 2.6** Principle of kinetic solubility determination exemplified by the Roche in-house Parallel Incremental Solubility Assay (PISA) adapted from Alsenz and Kansy.[3]

measurement is not a direct substitute for evaluation of the thermodynamic solubility, for reasons such as solubility is not measured at equilibrium, the appearance of a precipitate is strongly time-dependent and the disruption of the crystal lattice is not taken into consideration in this process.

The potentiometric titration method generating a plot of pH against the titrant volume added (Fig. 2.7a) was introduced by Avdeef[35,36,66] and is the reference method for kinetic measurements. The method of potentiometric measurements is detailed in[9] but in brief;

- 2–20 mL of water or mixed solvent comprising water and an organic water-miscible co-solvent such as DMSO, acetonitrile, 1,4-dioxane or methanol is used to dissolve about 50–500 μM of the sample to be assayed.
- Known volumes of strong standardized acids such as HCl or base such as KOH or NaOH are added to the ionizable compound solution of interest under vigorous stirring.
- Using a precision combination glass electrode, pH is continuously measured between the intervals of pH values 1.5–12.5.

To improve the precision of the measurement and to better mimic physiological states, an inert water-soluble salt (such as 0.15 M of KCl or NaCl) can be added to the reaction vessel which is thermostated at 25°C whilst the solution surface is bathed with a heavy inert gas (normally argon).

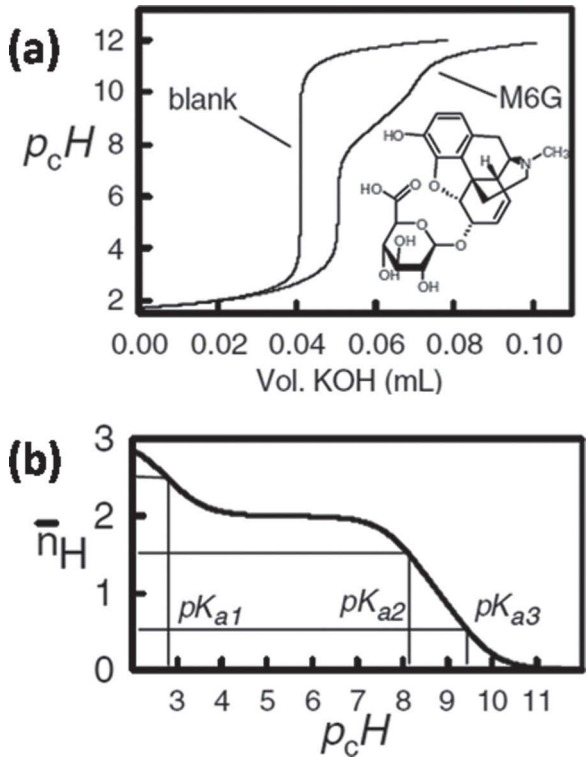


**Figure 2.7** (a) Potentiometric titration curve (b) Bjerrum plot for a three-p$K_a$ molecule.[9] M6G is morphine-6-glucuronide.

It is important to note that the shape of the curve produced can give an indication of the amount of substance present and its acid-base characteristic properties.[9] The solubility determinations are based on the difference between the aqueous drug or compound's p$K_a$ measured in the absence of a solid phase and the apparent p$K_a$ determined in the presence of an excess of solid compound. The shift produced or observed is thus proportional to the loss of compound, and thus to the solubility.[1] The advantages of this method include its ability to provide tangible solubility screening data in early stages, it is very economical (approximately 100 mg of a poorly soluble compound is needed) and is able to create a pH/solubility profile with one single determination, but is limited

to ionizable compounds. It is used for calibrating high-throughput solubility methods and computational methods due to its ability to provide reliable data without the use of co-solvent, providing a complete solubility pH profile with a limited number of experiments and providing a better insight into potential solubility behaviour through the GI tract.[21,66,85,87,102] However, the simplified protocol for the determination of kinetic solubility is prone to inaccuracies and variation, e.g., insufficient incubation time, improper handling of the phase separation process, presence of DMSO, and indirect readout of solubility data can result in false positives or negatives due to discrepancies in solubility values sometimes up to 1–3 times in order of magnitude.

Figure 2.7a suggests that the compound, M6G, has a p$K_a$ of ~8.8; however, the wrong conclusions can be deduced from a simplistic reading or interpretation of a titration curve and as such, Bjerrum plots which can be obtained from the transformation of titration curves are necessary.[103–108]

Knowledge of the amount of strong acid and base and how many dissociable protons the compound of interest brings means that, irrespective of the on-going equilibrium reaction, one would know the total hydrogen ion concentration in solution. The concentration of the bound hydrogen ions is the difference between the total and the free concentrations of the hydrogen ion concentration. Dividing this concentration by the concentration of the sample, the average number of bound hydrogen atoms per molecule of substance, $\bar{n}_H$ can be calculated. The Bjerrum curve is thus a plot of $\bar{n}_H$ against the pH scale based on hydrogen ion concentration ($p_cH$). Bjerrum plots are an important graphical tool in the initial stages of titration data analysis[9] and can be obtained by:

- Subtracting the "blank" (titration curve with no sample) from a titration curve with sample present at fixed pH values (Fig. 2.7a).
- The difference is plotted and then rotated to show emphasis on $\bar{n}_H$ and pH being the dependent and independent variables, respectively (Fig. 2.7b).

Figure 2.7b shows the three p$K_a$ values of M6G as $p_cH$ values at half integral $\bar{n}_H$ positions, thus demonstrating the capability

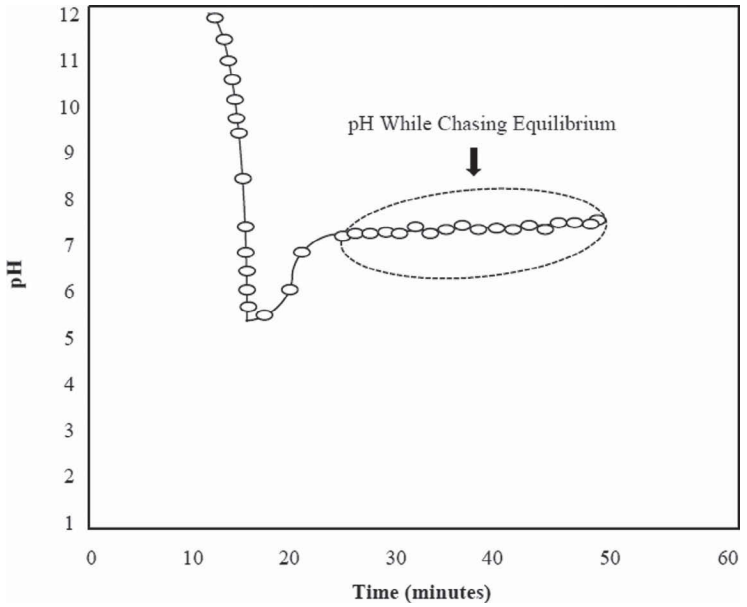


**Figure 2.8** pH versus time curve for warfarin. The pH while chasing equilibrium corresponds with the pH at the CheqPoint. Adapted from Box et al.[26]

of the technique for studying compounds with overlapping p$K_a$ values. Two commercial systems for potentiometric solubility measurement, pSol® and CheqSol®, are currently available.

Box et al.[26] developed a novel potentiometric procedure for rapid measurement of equilibrium aqueous solubility values of organic acids, bases, and ampholytes that form supersaturated solutions. In this procedure, the equilibrium solubility is actively sought by changing the concentration of the neutral form by adding HCl or KOH titrants and monitoring the rate of change of pH due to precipitation or dissolution in a process called Chasing Equilibrium (Fig. 2.8).[68] The results were reported in terms of intrinsic solubility, which for the acids and bases represented the concentration of their unionized species in a saturated solution at the pH where the compound was fully unionized. The Chasing Equilibrium method developed was used in the determination of the kinetic and equilibrium solubilities of the same 16 compounds.[26] Out of the 16 small organic

compounds ranging from high to moderate to poorly soluble, 10 of the compounds showed an excellent correlation between the aqueous equilibrium solubility measured by the shake-flask protocol and the Chasing Equilibrium solubility method (Table 2.7). The kinetic solubilities, however, were higher than the equilibrium solubilities for the compounds that "chased equilibrium" but correlated well with the "non-chasers." The authors also found this new method to provide equilibrium solubility values in about an hour per sample.[26]

This chasing equilibrium technique, which is based on the same principle as the traditional potentiometric titration method, proved to be faster since the intrinsic solubility of the compound is determined rather than the entire pH/solubility profile. The Henderson–Hasselbach relationships can then be used in determining the approximate pH/solubility profile.[1]

### 2.3.4 High-throughput Solubility Determinations

Experimental and computation approaches for the estimation of permeability and solubility followed the establishment of Lipinski's so-called Rules of Five, according to which a compound is "drug-like" if its molecular weight is $<500$ Da, its octanol/water partition coefficient $<5$ (log scale), and it possesses $<10$ hydrogen bond acceptors and $<5$ hydrogen bond donors after examining the compounds on the market.[63] This provided an impetus for the development of the high-throughput approach for solubility determinations and allowed the estimation of aqueous solubility in the early stages of drug discovery to become a critical parameter in lead selection and optimization. The collection of such data via high-performance liquid chromatography (HPLC) or liquid chromatography mass spectrometry (LC/MS) was commonly carried out in manual format thus being time-consuming, labour-intensive, and costly. Its use is often restricted to late discovery or early development phases to test a few, selected compounds. As there are large number of compounds and small samples of compounds available at the early stages of drug discovery, small-scale, compound-sparing and fully automated methods of determining drug solubility are often desirable. A first estimation of solubility, to help define the absorption, distribution, metabolism, excretion, and toxicity (ADMET) space of a compound

**Table 2.7** Summary of results from SF and Chasing equilibrium study[26]

| Compound | Shake-flask measurement pH [unionized percent] | Spectroscopic data: $A_{1cm}^{1\%}$ @ $\lambda_{max}$ (nm) | Intrinsic solubility (μg/mL) | Intrinsic solubility (μM) | Intrinsic solubility (log *S*) (mol/L) | Potentiometric range of sample weights (mg) (6 assays) | Time taken (min) | Kinetic solubility (μg/mL) | Intrinsic solubility (μg/mL | Intrinsic solubility (μM) | Intrinsic solubility (log *S*) (mol/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chasers | | | | | | | | | | | |
| Amodiaquin (ampholyte) | 10 | † | † | † | † | 9.0–12.5 | 40 ± 3 | 9 ± 4 | 0.4 ± 0.1 | 1.2 ± 0.1 | −5.94 ± 0.04 |
| Flumequine (acid) | 2.50 [99.99] | 403 @ 311 | 20.7 ± 0.8 | 79.2 ± 3 | −4.10 ± 0.02 | 9.3–12.4 | 48 ± 2 | 121 ± 10 | 34.2 ± 2.1 | 130.8 ± 8.2 | −3.88 ± 0.03 |
| Furosemide (diacid) | 2.00 [97.55] | 1225 @ 234 | 20.4 ± 2 | 61.7 ± 6 | −4.21 ± 0.04 | 9.7–11.2 | 46 ± 1 | 96 ± 17 | 19.7 ± 0.8 | 59.5 ± 2.4 | −4.23 ± 0.02 |
| Maprotiline (base) | 11.50 [93.66] | 45.3 @ 271 | 8.05 ± 3[c] | 29 ± 9 | −4.54 ± 0.12 | 9.8–10.8 | 74 ± 25 | 26.8 ± 4.7 | 5.8 ± 0.3 | 20.9 ± 1.1 | −4.68 ± 0.02 |
| Miconazole (base)e | 11.5 | † | † | † | −5.85[i] | 6.2–26.4 | 55 ± 8 | ∼ 2.3[f] | 1.0[d] ± 0.2 | 2.3[d] ± 0.5 | −5.63 ± 0.09 |
| Niflumic acid (ampholyte) | 3.35 [86.02] | 408 @ 254 | 29.5 ± 3 | 104.5 ± 9 | −3.98 ± 0.04 | 10.3–11.5 | 45 ± 2 | 59 ± 12 | 9.5 ± 0.5 | 33.8 ± 1.8 | −4.47 ± 0.03 |
| Nitrofurantoin (acid) | 2.50 [99.99] | 510 @ 265 | 109.5 ± 3 | 460 ± 10 | −3.34 ± 0.01 | 9.7–16.8 | 70 ± 7 | 441 ± 81 | 112 ± 7 | 472 ± 28 | −3.33 ± 0.03 |
| Phthalic acid (diacid)h | 0.50 [99.37] | 65 @ 275 | 5950 ± 200 | 35800 ± 1000 | −1.45 ± 0.02 | 506–549 | 78 ± 14 | 10104 ± 5223 | 5330 ± 47 | 32080 ± 287 | −1.49 ± 0.01 |
| Piroxicam (ampholyte) | 3.73 [96.03] | 640 @ 361 | 5.95 ± 0.4 | 17.9 ± 1 | −4.75 ± 0.03 | 9.8–13.4 | 54 ± 2 | 234 ± 88 | 5.9 ± 0.9 | 17.9 ± 2.8 | −4.75 ± 0.06 |
| Warfarin (acid) | 2.50 [99.63] | 430 @ 282[b] | 5.25 ± 0.2 | 17.0 ± 0.7 | −4.77 ± 0.02 | 10.1–11.3 | 61 ± 9 | 119 ± 2 | 5.3 ± 0.2 | 17.2 ± 0.7 | −4.77 ± 0.02 |
| Non-chasers | | | | | | | | | | | |
| Chlorpromazine (base) | 11.50 [99.45] | 1045 @ 254 | 2.41 ± 0.3 | 7.56 ± 0.9 | −5.12 ± 0.05 | 9.7–10.2 | 84 ± 15 | 2.7 ± 0.1 | 2.7 ± 0.1 | 8.5 ± 0.2 | −5.07 ± 0.01 |
| Imipramine (base) | 11.50 [98.92] | 261 @ 251[a] | 21.7 ± 3[c] | 77.6 ± 10 | −4.11 ± 0.06 | 9.5–12.9 | 77 ± 3 | 17.3 ± 0.3 | 17.2 ± 0.4 | 61.3 ± 1.5 | −4.21 ± 0.01 |
| Nortriptyline (base) | 11.50 [95.12] | 384 @ 238 | 49.3 ± 2 | 187 ± 6 | −3.73 ± 0.01 | 9.9–10.9 | 95 ± 11 | 27.3 ± 0.9 | 27.0 ± 1.4 | 102.6 ± 5.3 | −3.99 ± 0.02 |