Comparison of a Miniaturized Shake-Flask Solubility Method with Automated Potentiometric Acid/Base Titrations and Calculated Solubilities

A. GLOMME,[1,2] J. MÄRZ,[1] J.B. DRESSMAN[2]

[1]Merck KGaA, Medicinal Chemistry, Frankfurter Straße 250, 64271 Darmstadt, Germany

[2]Institute of Pharmaceutical Technology, Biocenter, Johann Wolfgang Goethe University, Marie Curie Street 9, 60439 Frankfurt (Main), Germany

Received 27 February 2004; revised 3 June 2004; accepted 17 June 2004

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20212

ABSTRACT: Solubility is one of the most important parameters for lead selection and optimization during drug discovery. Its determination should therefore take place as early as possible in the process. Because of the large numbers of compounds involved and the very low amounts of each compound available in the early development stage, it is highly desirable to measure the solubility with as little compound as possible and to be able to improve the throughput of the methods used. In this work, a miniaturized shake-flask method was developed and the solubility results were compared with those measured by semiautomated potentiometric acid/base titrations and computational methods for 21 poorly soluble compounds with solubilities mostly in the range 0.03–30 μg/mL. The potentiometric method is very economical (approximately 100 μg 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. The miniaturized shake-flask method can be used for all compounds and a wide variety of media. Its precision and throughput proved superior to the potentiometric method for very poorly soluble compounds. Up to 20 compounds a week can be studied with one set-up. Calculated solubility data seem to be sufficient for a first estimate of the solubility, but they cannot currently be used as a substitute for experimental measurements at key decision points in the development process. J Pharm Sci 94:1–16, 2005

Keywords: solubility; high throughput; logP; physicochemical properties; druglike properties; pH-profile; calculation potentiometric titration; solubility measurement

INTRODUCTION

Technologies such as combinatorial chemistry have changed the drug discovery process substantially in the last decade. With these methods, hundreds of thousands of new, diverse compounds can be synthesized per year. At the same time, high-throughput screening technologies have become available to examine the potency *in vitro* of the ever-increasing number of compounds. Together, these technologies were expected to accelerate the identification of lead substances and to shorten the time to market. The success of these new technologies is somewhat debatable and the early expectations have had to be scaled back.

One reason that the rate of discovery of new drugs did not improve lies in the diversity of the compounds that can be found in the high-throughput screening pool. It was originally intended that the compounds should have a broad structural

A. Glomme's present address is F. Hoffmann-La Roche Ltd., Pharmaceutical and Analytical R&D, CH-4070 Basel, Switzerland.

Correspondence to: J.B. Dressman (Telephone: +49-69-7982-9680; Fax: +49-69-8982-9724;
E-mail: dressman@em.uni-frankfurt.de)

Journal of Pharmaceutical Sciences, Vol. 94, 1–16 (2005)

diversity, covering the complete range of chemical and physical properties. For example, the solubility of a molecule can span more than 15 orders of magnitude (fg/mL to g/mL). The usual solubility range for drugs, however, is considerably more restricted. Compounds with an aqueous solubility of >100 μg/mL are unlikely to show solubility-related problems in development, but compounds with solubilities between 1 and 100 μg/mL may require development of a special formulation to overcome poor absorption properties associated with their low solubility, whereas those with even lower solubility usually represent a real formulation challenge.

Another reason for the failure of the new technologies to produce a large number of new drugs is attributed to the almost exclusive focus on specific binding to the receptor/enzyme to optimize the structure, with insufficient consideration of the pharmacokinetic properties.[1] Kennedy[2] examined the reason for the failure of 198 clinical candidates. For at least 40% of the candidates, development was terminated because of poor pharmacokinetics. This number may in fact be even larger, because lack of efficacy (30% of development terminations) could also be attributed to inappropriate pharmacokinetics, which could result in insufficient concentrations to elicit a response at the site of action. In general, optimization of structures by focusing exclusively on the receptor binding has led to compounds that are too big, very poorly soluble, and far too lipophilic. To address these issues, much attention has recently been devoted to identifying which physicochemical characteristics lead to a "druglike" compound. Lipinski et al.[3] examined the compounds on the market and established the so-called Rules of Five, according to which a compound is "druglike" 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.

Despite these attempts to develop "early warning" tools, solubility problems often are recognized for the first time when an oral, solid dosage form or a parenteral is to be developed. This late identification of poor solubility is attributable to the widespread use of organic solvents at the early stages of drug discovery, and even in the first animal experiments. At the time when the pharmaceutical (formulation) development starts, it is usually no longer practical to chemically change the active compound. Unfortunately, attempts to save the drug candidate through suitable formulation are expensive, time-consuming, and not always successful. Therefore, the solubility of new substances should be established as early as possible.

Because of the large number of drug candidates being screened, it would be highly desirable to develop an accurate, reliable, and fast method to measure solubility. Furthermore, the method should require a minimum amount of compound, and be flexible in terms of its ability to evaluate the pH dependency and influence of other biorelevant components on the solubility. For solubility evaluation at the level of the binding tests and first hit evaluations, calculation of the solubility may be sufficient. There are several solubility calculation programs available that produce adequate results for these purposes. With ongoing development of a class of compounds or for the formulation development of a promising candidate, however, an accurate assessment of solubility behavior is needed and values should be experimentally determined.

In this work, a miniaturized shake-flask method was developed and the results were compared for a wide range of poorly soluble compounds with those measured by the pSol method (Sirius Analytical Instruments Ltd.), which determines solubility using a semiautomated potentiometric acid/base titration. Results were additionally compared with calculated solubilities.

THEORETICAL

Solubility Calculations

After the introduction of high-throughput screening and combinatorial chemistry, the number of very lipophilic and poorly soluble compounds in development increased markedly. The early calculation of the aqueous solubility became very important to assist in weeding out compounds with undesirable characteristics and to assist in lead optimization. Several computer programs, with which the solubility of a compound can be computed directly from the chemical structure, are currently available. The accuracy of the calculated values is sufficient to serve the synthesis chemist as an orientation for further synthesis plans. The Hansch equation (eq. 1) was one of the most important early approaches to solubility calculation.[4] The following correlation can be applied to many organic lipids:

$$\log S = A \log K_{OW} + B \quad (1)$$

where $\log K_{OW}$ (= logP) is the octanol/water