Miniature Device for Aqueous and Non-aqueous Solubility Measurements During Drug Discovery

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Received March 30, 2004; accepted June 1, 2004

Purpose. A miniature device was developed for the measurement of aqueous and non-aqueous equilibrium solubility during drug discovery. The solubility values obtained using the miniature device were compared to those obtained using the conventional shake-flask method.

Methods. The aqueous solubility of six structurally diverse compounds, the solubility of carbamazepine in various cosolvent systems, and the pH-solubility profile of saquinavir were determined using the miniature device. The device contains a multichannel cartridge pump and a Tygon tubing that is mounted on the pump with two ends linked by a syringe filter. The drug slurry was filled into the tubing and circulated inside, continually passing through the syringe filter. At the end of the experiment, the filtrate was collected and analyzed directly by High-Pressure Liquid Chromatography (HPLC). The solubility was also determined by the shake-flask method.

Results. The solubility values determined by the miniature device were in good agreement with those measured by the conventional shake-flask method.

Conclusions. The miniature device provides a unique way of testing aqueous and non-aqueous equilibrium solubility in a microscale setting. With ≈1 mg of compound, it is possible to determine the entire pH-solubility profile. The device is useful for solubility screening during lead optimization and candidate selection in early drug discovery, when compound supply is limited. It can also be used for screening solubility in non-aqueous systems to select vehicles for preclinical *in vivo* studies.

KEY WORDS: drug discovery; equilibrium; microscale; miniature; physicochemical properties; solubility.

INTRODUCTION

Aqueous solubility is an important physicochemical property that plays a significant role in various physical and biological processes. Poor aqueous solubility is likely to result in low bioavailability or increased formulation difficulties during clinical development (1–3). Evaluation of solubility at early stages of lead optimization and candidate selection is therefore essential during the drug discovery process. However, during early discovery, large numbers of compounds are generated in 1- to 5-mg quantities, making the experimental testing of equilibrium solubility challenging. It would therefore be valuable to develop a miniaturized device for solubility measurement in a microscale setting.

Traditionally, equilibrium solubility is determined by the shake-flask method in which the compound is shaken in the solvent of choice for at least 24 h or until no more solids can be dissolved. After filtration of the slurry, the concentration of the dissolved compound in the filtrate is determined by High-Pressure Liquid Chromatography (HPLC) or other suitable analytical methods. The shake-flask method typically requires a few milligrams of compound per determination. This may become an issue for solubility screening at early stages of drug discovery when the compound supply is limited and many other properties related to potency, metabolism, toxicology, and permeability need to be evaluated.

In recent years, a variety of novel methods have been developed for solubility measurement during drug discovery and early development. For example, a potentiometric acid-base titration method has been reported for determining the intrinsic solubility and pH-solubility profile (4). In another study, solubility in aqueous and non-aqueous systems has been determined by differential scanning calorimetry (5). However, these methods require several milligrams of compounds for accurate determination. During the past several years, high-throughput screening methods have been developed for fast determination of aqueous solubility of drug candidates supplied as dimethylsulphoroxide (DMSO) stock solution in 96-well plates (6–7). Although such methods may require small amount of compound, the solubility values measured by these screening assays may not reflect true equilibrium solubility due to short equilibration time, the presence of cosolvent, and use of DMSO stock solutions to precipitate the drug.

The purpose of the current study was to develop a validated miniaturized method for equilibrium solubility measurement in aqueous and non-aqueous systems where the pH of the slurry can be monitored, if necessary. Though several studies have been published on the microscale measurement of physicochemical properties such as ionization constant (8–9), partition coefficient (8), and viscoelastic properties (10), no microscale method for solubility has been reported.

MATERIALS AND METHODS

Chemicals

All compounds tested in the current study were purchased from Sigma (St. Louis, MO, USA). All reagents were analytical grade and used without further purification.

Apparatus

A schematic diagram of the experimental apparatus is shown in Fig. 1. The Master-Flex L/S 12-channel, 6-roller cartridge pump head system (Cole-Palmer Instrument Co., Vernon Hills, IL, USA) was used for the current study. Specifically, the drug slurry was filled into a L/S precision pump tubing (0.8 mm or 1.6 mm internal diameter, 25 cm in length, Cole-Palmer Instrument Co.) that was mounted on to the pump. The two ends of the tubing were then connected by a syringe filter (Acrodisc 13 or 4, 0.45 μm, Gelman Sciences, Ann Arbor, MI, USA). By applying pressure to the tubing via the Master-Flex pump, the slurry was circulated in the tubing, passing through the syringe filter constantly. For pH measurement, a T-connector was linked to the tubing, and a micro-

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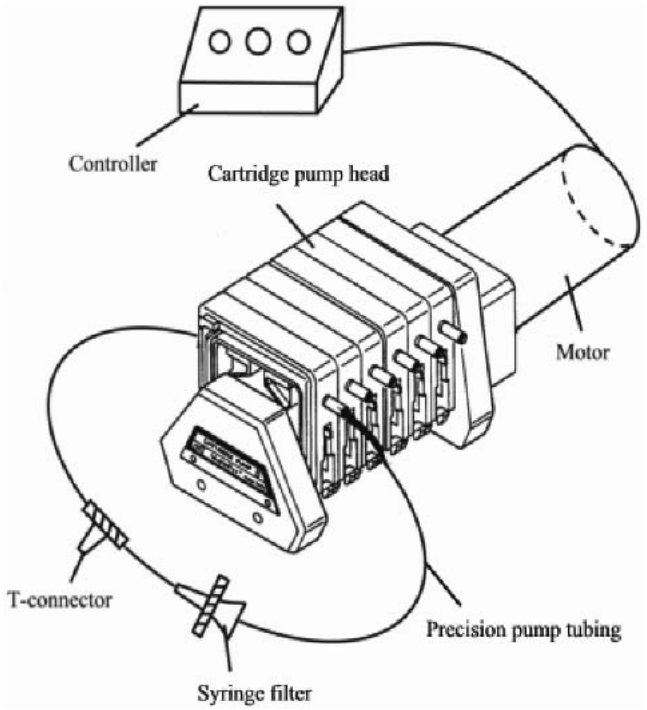


Fig. 1. Schematic representation of the miniature device.

electrode (1.3 mm tip diameter, Model 98-10, Orion Research Inc., Beverly, MA, USA) was inserted to the opening of the T-connector. In addition, the T-connectors can be used for injecting or drawing liquid. For all measurements, the amount of drug used for testing was ≤0.5 mg and the total volume in the tubing was 125 μl for 0.8-mm-i.d. tubing and 500 μl for 1.6-mm-i.d. tubing. The experimental details are listed in Table I.

Equilibrium Solubility Determination by the Miniature Device

Six compounds with diverse chemical structures were selected for this study. Drug slurry was filled into the tubing and circulated in the system at a speed of 4.0 ml/min (for 0.8-mm-i.d. tubing) or 14 ml/min (for 1.6-mm-i.d. tubing). The filtrate was collected from one end of the tubing at 6 and 24 h, and the concentration of dissolved compound was determined by HPLC upon appropriate dilution. All experiments were performed at room temperature. The volume of filtrate collected at 6 and 24 h was ~20 μl and the volume was not replaced at 6-h time point. The small loss of volume would not significantly affect the solubility measurement, as the undissolved solid remains in the system and the saturated condition is maintained. If equilibrium is not reached at the end of 6 h, the undissolved solid can further dissolve into the remaining solution.

Table I. Experimental Details of the Miniature Device

Tubing	Internal diameter	Volume[a]	Flow rate	Filter
L/S 13	0.8 mm	125 μl	4.0 ml/min	Acrodisc 4, 0.45 μm
L/S 14	1.6 mm	500 μl	14 ml/min	Acrodisc 13, 0.45 μm

[a] The length of the tubing used in the current study was 25 cm.

The integrity of the filter membrane was checked at the end of the experiment by filtering a suspension through the membrane and examining the physical appearance of the filtrate. The filtrate was clear all the time, indicating that the filter membrane was intact throughout the experiment.

Equilibrium Solubility Determination by Shake-Flask Method

An excess amount of drug was added to 1 ml of water, and the slurry was shaken at room temperature for 24 h. The slurry was then filtered through a syringe filter, and the filtrate solution was diluted appropriately for HPLC analysis. The solubility values reached equilibrium by 24 h because no more solid dissolved after this time.

pH-Solubility Profile of Saquinavir

The pH-solubility profile of saquinavir was determined using the miniature device. Specifically, the 1.6-mm-i.d. tubing containing 500 μl water and 0.5 mg of saquinavir was used. A T-connector was attached to the tubing for pH adjustment and pH measurement. The pH adjustment was done by adding 0.1 N HCl to the slurry via a microsyringe, and pH was measured by a microelectrode linked to the T-connector. Aliquots of filtrate were collected for HPLC assay. The pH solubility profile of saquinavir was also determined by the conventional shake-flask method.

Solubility of Carbamazepine in Different Solvent Vehicles

Slurries of carbamazepine were prepared in 25% cremophor EL, 20% polysorbate 80, or 50% PEG 400 and loaded into the miniature device. After 24 h of equilibration at room temperature, the filtrates were collected for HPLC analysis. The samples reached equilibrium after 24 h, as no more solid dissolved after this time. The solubility of carbamazepine in various cosolvent vehicles was also determined using the shake-flask method.

HPLC Assays

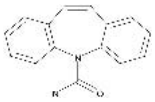
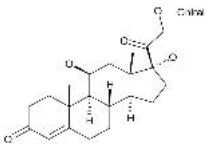
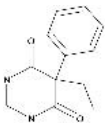
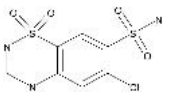
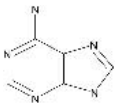
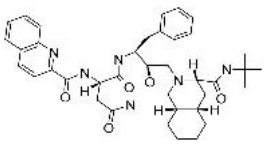
A reversed-phase HPLC system was used for analysis in the current study. The system includes a Waters 2690 Separations Module, a Waters 2487 Dual Wavelength Absorbance Detector, and a C-18 column (Waters, Milford, MA, USA). A gradient method was developed using mobile phase containing acetonitrile and deionized water with 0.05% trifluoroacetic acid.

RESULTS AND DISCUSSION

Aqueous Solubility of Various Compounds

As shown in Table II, the solubility values of six model compounds obtained from the miniature device were consistent with values determined by the shake-flask method. Also as shown in this table, tubing size did not affect the solubility values obtained from the miniature device. Thus, when compound supply is limited, which is usually the case in early discovery stages, a small amount of compound (as low as 0.2 mg) can be added to the smaller tubing (L/S13, 0.8 mm i.d.)

Table II. Aqueous Solubility of Compounds

Compound	Structure	MW	logP (ACD)	Tubing[a]	Solubility (mg/ml)[b] Miniature device 6 h	Miniature device 24 h	Shake-flask 24 h
Carbamazepine		236.3	2.67	L/S 14	0.15 (0.01)	0.15 (0.02)	0.12 (0.02)
				L/S 13	0.17 (0.03)	0.15 (0.03)	
Hydrocortisone		362.5	1.43	L/S 14	0.38 (0.02)	0.34 (0.03)	0.31 (0.01)
				L/S 13	0.50 (0.07)	0.38 (0.02)	
Primidone		218.3	−0.95	L/S 14	0.44 (0.02)	0.47 (0.05)	0.49 (0.01)
				L/S 13	0.41 (0.01)	0.47 (0.02)	
Hydochlorothiazide		297.7	−0.07	L/S 14	0.61 (0.04)	0.61 (0.06)	0.52 (0.04)
				L/S 13	0.65 (0.05)	0.60 (0.04)	
Adenine		135.1	−0.09	L/S 14	0.75 (0.06)	0.77 (0.07)	0.67 (0.05)
				L/S 13	0.69 (0.06)	0.71 (0.05)	
Saquinavir		670.9	4.5	L/S 14	0.06 (0.02)	0.06 (0.01)	0.05 (0.01)
				L/S 13	0.06 (0.02)	0.05 (0.01)	

[a] L/S 13: 0.8-mm i.d.; L/S 14: 1.6-mm i.d.
[b] All measurements were performed at 25 ± 3°C; results expressed as mean (standard error) (n = 3).

for solubility measurement. The length of the tubing (and thus the volume) can be further reduced, if necessary, to further conserve compound.

In addition, for most compounds tested in the current study, equilibrium was achieved within 6 h of circulation in the miniature system (Table II). This could be useful in reducing the turnaround time and increasing the throughput for solubility screening in drug discovery.

pH-Solubility Profile of Saquinavir

Figure 2 shows the pH-solubility profile of saquinavir. The profile obtained from the miniature device was similar to the one obtained from the shake-flask method. Fitting the data to the pH-solubility equation yielded an intrinsic solubility of 0.001 mg/ml and a pKa of 8.2 for saquinavir using both miniature device and conventional method.

Solubility of Carbamazepine in Cosolvent Systems

As shown in Table III, the solubility of carbamazepine in various cosolvent systems obtained by the miniature device was in reasonable agreement with the solubility values determined by the conventional method.

The miniature device may be used for preclinical vehicle selection studies when the desired target concentration is high and yet the amount of compound available for *in vivo* studies is low. In early drug discovery, pharmaceutics scientists are often asked to identify suitable cosolvent vehicles for phar-

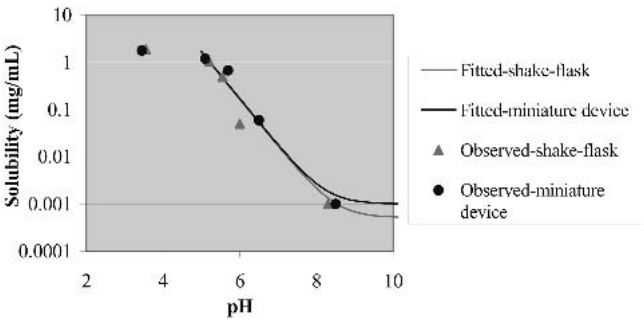


Fig. 2. pH-solubility profile of saquinavir.

Table III. Solubility of Carbamazepine in Cosolvent Systems

Vehicle	Solubility (mg/ml) at 25°C[a]	
	Miniature device	Shake-flask method
25% cremophor in water (w/v)	3.47	2.92
20% polysorbate 80 in water (w/v)	3.12	3.65
50% PEG 400 in water (w/v)	9.6	8.74

[a] Determined in L/S 13 tubing with 24-h circulation.

macokinetic or toxicological studies. These studies often require a target concentration of 20 mg/ml or higher while only several milligrams of compound are available. Traditional shake-flask method requires large amounts of compound and cannot be used for screening several vehicles simultaneously. The miniature device reported here solves this problem by reducing the amount of compound needed for solubility testing.

CONCLUSIONS

The miniature device was shown to be reliable for determining equilibrium solubility in aqueous and non-aqueous systems for a variety of compounds. It provides a unique way of measuring the pH of the slurry and provides a sample that can be directly injected into HPLC. It requires only a small amount of compound, which makes it a useful tool for solubility screening during lead optimization and candidate selection. Unlike the shake-flask method, in which filtration is performed at the end of equilibration, and drug adsorption to filter membrane could be a significant issue, the drug slurry in the miniature device is continually filtered through the membrane, and adsorption is minimized.

Unlike many high-throughput methods in which kinetic solubility is measured using DMSO stock solution, the current method determines equilibrium solubility using solid samples. The rapid equilibrium in the miniature device enables short turnaround time that is useful to medicinal chemists for building structure-solubility relationships. Finally, the current miniature device can be scaled for high-throughput measurements and automation, and future studies are planned to examine this possibility.

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