Determination of Aqueous Solubility by Heating and Equilibration: A Technical Note

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INTRODUCTION

The Biopharmaceutical Classification System (BCS) teaches that the 2 main indicators of drug bioavailability are the aqueous solubility and the ability of the drug molecules to permeate biologic membranes.[1] Still, drug development technologies, such as combinatorial chemistry and high throughput screening, are based on the basic principles of medicinal chemistry, teaching that the most reliable method to increase in vitro potency is to add lipophilic moiety at appropriate position of the lead structure. This has lead to an increase in the number of lipophilic and poorly soluble molecules being investigated for their therapeutic activity.[2] Determination of solubility, where a solid compound is allowed to equilibrate with an aqueous medium, is usually too time consuming and requires too large a sample to be feasible for high throughput screening. Instead the kinetic solubility is measured in which dimethyl sulfoxide solution of the compound is gradually added to an aqueous media and the solubility determined as the concentration at which precipitation is formed as detected by light scattering. The advantages of the kinetic method are that it is relatively rapid, requires only small sample and that it is easily automated.[3] The disadvantages of this method are the presence of dimethyl sulfoxide in the final medium (frequently 0.5%-5% vol/vol) and potential formation of supersaturated solutions. Automated and miniaturized methods for determination of solubility of solid compounds have been developed,[4,5] but these methods require equilibration time that can be several days or weeks for slowly dissolving drugs. Inadequate equilibration time can result in significant underestimation of the solubility. Alternatively, aqueous drug solubility can be estimated from easily obtainable properties, such as the melting point, the octanol-water partition coefficient, the hydrogen-bonding capacity of the molecule, and its nonpolar surface area.[4,6-8] Presently such computational methods for solubility estimation are far from accurate. The drug training sets used to create the methods tend to be overrepresented by low molecular weight drugs and uncharged drugs that are somewhat soluble in water, and the sets are subject to an unknown degree of experimental error.[7] Drug-like molecules, especially those that possess ionizable moieties, are ill-represented in these training sets. Training sets containing drug-like compounds of wide molecular diversity might allow better methods to be developed.[7,8] For the past decade we have used a modified shake-flask solubility method, where we shorten the equilibration time through heating prior to equilibration at desired temperature. In this method the equilibrium solubility is approached from supersaturation and accelerated precipitation through addition of the original solid compound after cooling to room temperature. Here we report solubility of 48 different drugs and pharmaceutical excipients in pure water at room temperature.

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MATERIALS AND METHODS

The drug solubility data were generated in our lab over the past decade during various drug preformulation studies.[9] The solubility of the drugs was determined in pure glass-distilled water. First the stability of the drug to be tested was evaluated by dissolving small amount of the drug in an aqueous cyclodextrin solution, typically 5% (wt/vol) 2-hydroxypropyl-β-cyclodextrin solution. The cyclodextrin was included as solubilizer. The solution was then divided into 4 sealed glass vials that were heated in an autoclave for 1, 2, 3, and 4 heating cycles; each cycle consists of heating to 121°C for 20 minutes. The drug concentrations in the vials were then determined by a high-performance liquid chromatographic (HPLC) method. If the drug degradation was less than 1% during one cycle, then the heating method in an autoclave was applied. If the degradation was greater, then heating in the autoclave was replaced by heating in an ultrasonic bath for 1 hour at 60°C to 70°C. The maximum allowable drug degradation during the solubility studies was under all circumstances 1%. The drug solubility was then determined as follows:

1. An excess amount of the drug to be tested was added to pure water (1 mL) in, for example, a 2-mL disposable crimp-top glass vial.
2. The suspension formed was then heated in a sealed glass vial in an autoclave (121°C for 20 minutes) or

Table 1. Comparison of the Experimental Solubilities (S_0) in Pure Water at Ambient Temperature (22°C-24°C) With the Calculated Solubilities According to the Yalkowsky Equation and the Literature Values (room temperature).

Drug	MW (Dalton)	MP† (°C)	pK_a†	$logK_{o/w}$‡	Solubility (experimental)		Yalkowsky§ (mM)	Literature† (mM)
					(mg/mL)	(mM)		
Acetazolamide (A)	222.3	260	7.2	-0.72	0.643	2.89	74.1	3.4
Alprazolam (B)	308.8	228	2.4	3.87	0.073	0.237	0.004	-
Benzoic acid (A)	122.1	122	4.2	1.87	2.40	19.7	4.57	24-27
Bupivacaine (B)	288.4	108	8.1	3.44	0.183	0.633	0.170	-
Butylated hydroxyanisole	180.2	52		3.50	0.396	2.20	0.537	-
Carbamazepine (B)	236.3	191	7.0	2.25	0.256	1.09	0.389	0.05-1.6
Chlorobutanol	177.5	78		2.09	8.11	45.7	7.59	45
Cholecalciferol	384.6	84		10.24	0.100	0.260	0.000	0.020-0.6
Clotrimazole	344.8	148		6.26	0.030	0.088	0.000	-
Cyclosporine A	1202.6	150		1.00	0.008	0.007	17.8	-
Dexamethasone	392.5	270		1.72	0.159	0.406	0.214	0.3
Dextromethorphan (B)	271.4	111	8.3	3.97	0.090	0.332	0.047	-
Diazepam (B)	284.7	133	3.3	2.70	0.057	0.199	0.525	0.1–0.3
Digoxin	780.9	240		0.5	0.986	1.26	7.08	0.1
Ergotamine (B)	581.7	213	6.4	2.53	0.002	0.004	0.123	-
Estradiol	272.4	176		3.94	0.090	0.331	0.011	0.01
Ethoxzolamide (A)	258.3	192	8.1	2.08	0.063	0.246	0.562	0.04
Finasteride	372.6	254		3.2	0.043	0.116	0.010	0.03
Flunitrazepam (B)	313.3	170	1.8	1.91	0.004	0.011	1.38	-
Fluoxetine hydrochloride (A)	345.8	138	8.7	4.65	15.2	43.8	0.005	40
Hydrocortisone	362.5	214		1.62	0.418	1.15	0.977	0.8–1.1
Ketoprofen (A)	254.3	95	4.5	3.00	0.010	0.039	0.631	0.2–0.4
Lidocaine (B)	234.3	69	7.9	1.66	3.58	15.3	25.1	-
Methazolamide (A)	236.3	213	7.3	0.33	0.704	2.98	19.5	4.1
Methylparaben (A)	152.1	131	8.4	2.00	3.16	20.8	2.75	16
Miconazole (B)	416.1	182	6.7	6.25	0.089	0.213	0.000	-
Midazolam (B)	325.8	159	6.2	4.33	0.024	0.073	0.007	-
Naproxen (A)	230.3	153	4.2	3.1	0.115	0.501	0.132	0.2–1.2
Omeprazole (A)	345.4	156	4.0	3.4	0.018	0.051	0.062	-
Oxazepam (B)	286.7	206	1.7	2.32	0.045	0.158	0.234	<0.01
Pentachlorophenol (A)	266.3	191	4.7	4.74	0.025	0.093	0.001	-
Phenol (A)	94.11	41	10.0	1.51	15.4	164	67.6	70
Prazepam (B)	324.8	146	2.7	3.99	0.004	0.013	0.020	-
Prednisolone	360.4	241		1.4	0.380	1.06	0.871	0.6–2.1
Pregnenolone	316.5	189		3.89	0.033	0.105	0.009	-
Progesterone	314.5	127		3.67	0.001	0.002	0.065	0.03–0.05
Propofol (A)‖	178.3	19	11.0	3.57	0.164	0.918	0.851	-
Propylparaben (A)	180.2	97	8.4	2.98	0.187	1.04	0.631	2.2
Retinol All-trans	300.4	62		7.62	0.044	0.146	0.000	0.003–0.03
Salicylic acid (A)	138.1	159	3.0	2.24	2.51	18.2	0.832	13
Sulfamethoxazole (A)	253.3	167	5.6	0.48	0.392	1.55	39.8	-
Temazepam (B)	300.7	158	1.6	2.15	0.604	2.01	1.05	-
Tenoxicam (A)	337.4	209	5.3	2.40	0.803	2.38	0.182	-
Triamcinolone acetonide	434.5	293		0.96	0.114	0.263	0.724	-
Triazolam (B)	343.2	234	2.4	3.96	0.045	0.130	0.003	-
Triclosan (A)	289.5	56	7.9	4.66	0.050	0.17	0.034	-
Trimethoprim (B)	290.3	204	6.6	0.73	1.37	4.70	9.55	1.4
Vanillin (A)	152.1	82	7.4	1.05	13.9	91.5	75.9	66

*MW indicates molecular weight; MP, melting point; $logK_{o/w}$; the logarithm of the experimental octanol/water partition coefficient; (A) acid; and (B) base.
†Literature values.[4,5,10-19]
‡Estimated values, the 10-logarithm of the octanol/water partition coefficient (logP) (www.syrres.com).
§Calculated solubility using the Yalkowsky equation.[20]
‖Liquid.

sonicated in an ultrasonic bath (eg, at 70°C for 1 hour). After cooling to ambient temperature, the vial was opened, a small amount of the solid drug was added to the vial to promote drug precipitation, and the vial resealed.

3. After equilibration at ambient temperature (22°C–23°C) in a sealed vial under constant agitation for 3 to 7 days, the suspension was filtered through a 0.45-μm membrane filter (discarding approximately the first third of the filtrate), and the solution analyzed by HPLC. The time needed to reach equilibrium solubility was determined by analyzing samples of the equilibrating solution at different time points to establish constant drug solubility.

Results and Discussion

The results are shown in Table 1. In all, 48 compounds were tested; mean molecular weight (MW) was 314 Dalton (range, 94-1202 Dalton) and mean melting point was 158°C (range, 19°C-293°C). Experimentally determined solubilities range from 2 μM (1 μg/mL) to 164 mM (15.4 mg/mL) with a mean solubility of 9.4 mM (1.5 mg/mL). Heating of the aqueous drug suspension, in an autoclave or in an ultrasonic bath, accelerates the drug dissolution and frequently results in formation of a supersaturated drug solution upon cooling to room temperature, even in the presence of excess drug and especially if its melting point is close to or below the heating temperature. Addition of a small amount of the original solid drug promotes precipitation and generates reproducible solubility data. Thus, seeding with solid drug after heating and cooling to room temperature, but before equilibration, is of uttermost importance. In this study, the pH of the medium was not controlled, and the pK_a values indicate that ionization during dissolution in pure water could affect the solubility of almost half of the drugs listed in Table 1.

For comparison, the solubilities (S) of the compounds were calculated according to the Yalkowsky equation[20]:

$$log\ \ S = -0.01 \cdot (MP - 25) - log\ K_{o/w} + 0.5 \qquad (1)$$

In this equation the strength of the crystal lattice is represented by the melting point (MP in degrees Celsius) and the interaction between water and drug by the octanol/water partition coefficient ($K_{o/w}$). The mean calculated solubility (7.5 mM) is somewhat lower than the experimental solubility, which is understandable since the Yalkowsky equation is only valid for nonelectrolytes, but many of the compounds listed in Table 1 are partly ionized in pure aqueous solutions. However, even for the nonelectrolytes, the calculated values differ significantly from the experimental ones. This is especially true at solubilities close to or below ~0.3 mM (0.1 mg/mL), which is frequently referred to as the minimum solubility for avoiding dissolution controlled absorption of orally administered drugs. More sophisticated methods, which can be applied to estimate solubilities at different pHs, salt concentrations, and even in different solvents, do exist but they still produce fairly inaccurate solubility estimates.[7,21] It is hoped that the experimental data presented in Table 1 can be of some help during development of more precise computational methods for solubility estimation.

Summary and Conclusion

A modified shake-flask solubility method, where the equilibration time was shortened through heating, was used to determine the solubility of 48 different drugs and pharmaceutical excipients in pure water at room temperature. The heating process accelerates dissolution of the solid compound and frequently results in supersaturated solution. Seeding with the solid compound after heating and cooling to room temperature promotes precipitation of the solid compound in its original stable form. This modified shake-flask method generates reliable and reproducible solubility data.

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