

Article

Thermodynamic Correlation between Liquid–Liquid Phase Separation and Crystalline Solubility of Drug-Like Molecules

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Abstract: The purpose of the present study was to experimentally confirm the thermodynamic correlation between the intrinsic liquid–liquid phase separation (LLPS) concentration (S_0^{LLPS}) and crystalline solubility (S_0^C) of drug-like molecules. Based on the thermodynamic principles, the crystalline solubility LLPS concentration melting point (T_m) equation (CLME) was derived ($\log_{10} S_0^C = \log_{10} S_0^{LLPS} - 0.0095(T_m - 310)$ for 310 K). The S_0^{LLPS} values of 31 drugs were newly measured by simple bulk phase pH-shift or solvent-shift precipitation tests coupled with laser-assisted visual turbidity detection. To ensure the precipitant was not made crystalline at <10 s, the precipitation tests were also performed under the polarized light microscope. The calculated and observed $\log_{10} S_0^C$ values showed a good correlation (root mean squared error: 0.40 log unit, absolute average error: 0.32 log unit).

Keywords: liquid–liquid phase separation; intrinsic solubility; melting point; drug-like



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1. Introduction

Intrinsic aqueous solubility (S_0^C) is one of the most important physicochemical properties of a crystalline drug. Many S_0^C prediction methods have been reported in the literature [1–3]. In most cases, S_0^C is directly predicted from the chemical structure by empirical statistic approaches [4–6]. However, even though this approach has been investigated for more than several decades, S_0^C prediction is still challenging [7].

An alternative approach would be to separate the solubilization process into the melting and solvation terms based on the theory of thermodynamics (Figure 1).

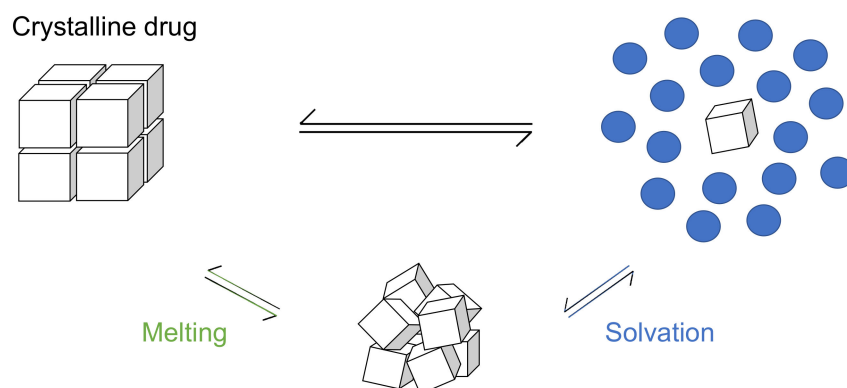


Figure 1. Thermodynamic scheme of crystalline drug solubilization.

The general solubility equation (GSE) is one of this kind of stepwise prediction approach. Based on the thermodynamic principles, GSE has been formulated as [8],

$$\log_{10} S_0^C = 0.5 - \log_{10} P_{oct} - 0.01(T_m - T) \quad (1)$$

where T_m is the melting point, T is the temperature, and P_{oct} is the octanol/water partition coefficient. GSE was derived from the thermodynamic principles without parameter fitting. The constant of the GSE (0.5) is attributed to the solubility of a drug in octanol. When a liquid-state drug is completely miscible in octanol, the solubility of the liquid-state drug in octanol is expressed as 0.5 [9].

One merit of this kind of stepwise prediction approach is that it can be used to evaluate the relative contribution of the solvation and crystal lattice energy terms such as $0.5 - \log_{10}P_{oct}$ and $0.01(T_m - T)$ in GSE, respectively. Therefore, it is of great help to navigate drug design. In addition, it also helps formulation design because a solubilizing formulation suitable for a drug differs depending on the cause of poor solubility being solvation or crystal lattice energy. Another merit of the stepwise approach is that the intermediate parameters such as P_{oct} and T_m can be directly experimentally measured to confirm the prediction accuracy for each process.

Recently, the liquid–liquid phase separation (LLPS) of a drug attracted a lot of attention in drug research [10–14]. Several methods have been reported to measure the intrinsic LLPS concentration (S_0^{LLPS}) [15]. As explained in the Theory section, LLPS is synonymous with the solvation phenomena shown in Figure 1. Therefore, in theory, S_0^c can be approximately described by S_0^{LLPS} and melting point (T_m) (see Theory section). However, the accuracy of this approximation has not been experimentally confirmed for drug-like molecules. This information is important for clarifying which energy term is responsible for the inaccuracy of S_0^c prediction, which may lead to breakthroughs in *in silico* predictions.

The purpose of the present study was to experimentally confirm the thermodynamic correlation between the intrinsic liquid–liquid phase separation (LLPS) concentration (S_0^{LLPS}) and crystalline solubility (S_0^c) of drug-like molecules. In this study, the S_0^{LLPS} values of 31 drugs were newly measured by a simple precipitation test using laser-assisted visual turbidity detection (LAVTD).

2. Theory

The intrinsic solubility ratio of a crystalline drug (S_0^c) and a liquid drug (S_0^L) equals the ideal solubility ratio of the crystal drug (X_0^c) and the liquid drug (X_0^L),

$$\frac{S_0^c}{S_0^L} = \frac{X_0^c}{X_0^L} \quad (2)$$

The ideal solubility is the mole fraction solubility of a drug in an ideal solution (in the liquid drug). By rearranging this,

$$\log_{10}S_0^c = \log_{10}S_0^L + \log_{10}\frac{X_0^c}{X_0^L} \quad (3)$$

Assuming that the change in heat capacity upon melting is equal to zero [16], the ideal solubility ratio (X_0^c/X_0^L) can be estimated as,

$$\log_{10}\frac{X_0^c}{X_0^L} = -\Delta S_m \frac{T_m - T}{2.303RT} \quad (4)$$

where ΔS_m is the entropy of melting, and T_m is the melting point.

Approximating S_0^L by the intrinsic liquid–liquid phase separation concentration (S_0^{LLPS}), S_0^c is expressed as

$$\log_{10}S_0^c = \log_{10}S_0^{LLPS} - \Delta S_m \frac{T_m - T}{2.303RT} \quad (5)$$

This formula is hereinafter referred to as the crystalline solubility LLPS concentration melting point equation (CLME). It should be noted that S_0^{LLPS} and S_0^L do not exactly match because water and a liquid phase drug are mutually miscible to some extent. In addition, LLPS and the glass–liquid phase separation were not distinguished in this study [17].

According to Walden's rule, $\Delta S_m = 56.5 \text{ J/K}\cdot\text{mol}$. Therefore, at 298 K (25 °C) and 310 K (37 °C), Equation (5) becomes

$$\log_{10}S_0^C = \log_{10}S_0^{LLPS} - 0.0099(T_m - 298) \quad (6)$$

$$\log_{10}S_0^C = \log_{10}S_0^{LLPS} - 0.0095(T_m - 310) \quad (7)$$

It should be noted that CLME was derived from the thermodynamic principles without any parameter fitting.

3. Materials and Methods

3.1. Materials

Sodium hydroxide aqueous solution (8 mol/L) (NaOH), 6 mol/L hydrochloric acid (HCl), sodium chloride (NaCl), sodium dihydrogen phosphate dihydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$), *N,N*-dimethylacetamide (DMA), boric acid, methanol, 0.1% trifluoroacetic acid-acetonitrile, (S)-(+)-naproxen, diphenhydramine hydrochloride, haloperidol, ibuprofen, indomethacin, ketoprofen, niflumic-acid, papaverine hydrochloride, ($\pm$)-propranolol hydrochloride, quinine, and warfarin sodium were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). 2-Naphthoic acid, acetaminophen, bifenazone, bupivacaine hydrochloride, carprofen, chlorpromazine hydrochloride, diclofenac sodium salt, dipyridamole, fenofibrate, flufenamic-acid, flumequine, flurbiprofen, furosemide, glipizide, ketoconazole, ketotifen fumarate, losartan potassium, loxoprofen, mefenamic-acid, meloxicam, phenylbutazone, probenecid, procaine hydrochloride, procaine, propafenone hydrochloride, rebamipide, sulfasalazine, sulindac, thioridazine hydrochloride, and verapamil hydrochloride were purchased from Tokyo Chemical Industry (Tokyo, Japan). Meclofenamic-acid sodium salt and phenytoin sodium were purchased from Sigma-Aldrich (Arklow, Ireland). Benzocaine, lidocaine hydrochloride, and terbinafine hydrochloride were purchased from Combi-Blocks (San Diego, CA, USA). Orphenadrine hydrochloride was purchased from Chem Cruz (Huissen, Netherlands). 0.1% trifluoroacetic acid-distilled water was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Pramoxine hydrochloride was purchased from Cayman Chemical (Ann Arbor, MI, USA). Warfarin free acid was prepared by adding 1 N HCl to warfarin sodium dissolved in distilled water. Propafenone free base was prepared by adding 1 N NaOH to propafenone hydrochloride dissolved in distilled water.

3.2. Methods

3.2.1. Crystallization Time Measurement

Before the S_0^{LLPS} measurements, the crystallization time of 47 drugs was measured by performing the precipitation test under the polarized light microscope (PLM). In the pH-shift precipitation method, an ionizable drug was dissolved in distilled water as a salt form or by adding 1–3 Eq of NaOH (for weak acids) or HCl (for weak bases). A total of 1.0 μL of 1 N HCl (for weak acids) or 1 N NaOH (for weak bases) was dropped onto a glass slide, then the drug solution (9.0 μL) was added and covered with a cover glass. In the solvent-shift precipitation method, an un-dissociable drug was dissolved in *N,N*-dimethylacetamide. Distilled water (19.8 μL) was added to the drug solution (0.2 μL). The drug concentration was set to 30 mM, except for propafenone (20 mM), lidocaine (200 mM), terbinafine (18 mM), fenofibrate (198 mM), and haloperidol (2 mM). For the measurements at 310 K, the temperature was maintained by a glass plate heater (BLAST Inc., Kanagawa, Japan). The precipitants were monitored under a PLM (crossed-Nicols with a sensitive-tint plate) (Olympus CX-43, Olympus Corporation, Tokyo, Japan). The solid state of the precipitant was diagnosed as crystalline when polarization was observed.

3.2.2. S_0^{LLPS} Measurement by the Precipitation Tests Coupled with Laser-Assisted Visual Turbidity Detection (LAVTD)

The drugs with a crystallization time > 10 s were selected for the S_0^{LLPS} measurements. The S_0^{LLPS} value was measured by the bulk phase pH-shift precipitation tests or the solvent-

shift precipitation tests coupled with laser-assisted visual turbidity detection (LAVTD) as previously reported [18]. Each drug solution was prepared as described above. For the measurements at 310 K, the drug solution, 1 N NaOH, 1 N HCl, and glass test tubes were pre-heated in a water bath. In the case of ionizable drugs, 1 N NaOH or 1 N HCl (100 μ L) was added to the glass test tube, then set to the LAVTD device (Figure S1). The drug solution (900 μ L) was then added to the glass test tube and immediately vigorously shaken. In the case of un-dissociable drugs, the drug solution (10 μ L) was added to the glass tube, then distilled water (990 μ L) was added. Turbidity was visually detected within 10 s with the assistance of a red laser (635 nm). The concentration of the drug solution was changed with 0.001 to 0.1 mM increments to give 3 significant digits. The S_0^{LLPS} value was defined as the drug concentration at which the solution started to show turbidity. The S_0^{LLPS} measurement was performed in triplicate.

To compare with the literature data, S_0^{LLPS} was also measured using the same medium conditions as the reference [19]. Diclofenac sodium was dissolved in methanol. The drug solution was added to the glass test tube (10 μ L). Phosphate buffer (990 μ L, pH 2.0, PO_4 : 50 mM, NaCl: 128 mM) was added to the glass test tube and immediately vigorously shaken at 298 K. The S_0^{LLPS} value was determined as described above.

3.2.3. S_0^{LLPS} Measurement by Turbidity Detection Using a UV/VIS Spectrophotometer

Each drug solution was prepared as described above. A 1 N NaOH or 1 N HCl solution (70 μ L) was added to the quartz cell and set to a UV/VIS spectrophotometer (UV-1850, Shimadzu Corporation, Kyoto, Japan). A drug solution (630 μ L) was rapidly added to the quartz cell and the absorbance was measured at 500 nm within 10 s at 298 K. This wavelength was set to be higher than the absorption wavelength of each drug. The S_0^{LLPS} measurement was performed in triplicate.

3.2.4. Intrinsic Solubility Measurement

Crystalline free-form drugs were used for the intrinsic solubility measurement. The intrinsic solubility was measured based on the harmonized protocol as previously reported [20]. Each drug was added to a test solution (10 mL) in a 15 mL tube. The samples were rotated at 40 rpm at 310 K except for procaine (1800 rpm). Before filtration, the sample was allowed to stand still for 1 min. The sample was then filtered (hydrophilic PVDF, 0.22 μ m pore size). The first few drops were discarded to avoid filter adsorption [21]. The drug concentration in the filtrate was determined by UV spectroscopy (UV-1850, Shimadzu Corporation, Kyoto, Japan). The residual solid was collected by vacuum filtration and analyzed by differential scanning calorimetry (DSC). The composition of the medium, the amount of the added drug, the incubation time, and the detection wavelength are summarized in Table 1. The achievement of equilibrium was confirmed by time-course measurements up to 48 h.

Table 1. Experimental conditions of intrinsic solubility measurement.

Drug	Medium	Amount of Drug (mg)	Incubation Time (h)	Wavelength (nm)
Bifonazole	pH 9.0 borate buffer ¹	30	72	255
Carprofen	0.1 N HCl	30	48	300
Flurbiprofen	0.1 N HCl	30	48	248
Loxoprofen	0.1 N HCl	50	48	220
Phenylbutazone	0.1 N HCl	30	48	264
Procaine	0.01 N NaOH	500	1	280
Propafenone	0.01 N NaOH	30	48	305
Quinine	0.01 N NaOH	100	48	350
Sulfasalazine	0.1 N HCl	30	48	369
Sulindac	0.1 N HCl	50	48	331
Warfarin	0.1 N HCl	30	48	275

¹ The concentration of boric acid was adjusted to 50 mM.

Procaine showed hydrolysis in alkali conditions [22]. Therefore, the concentration of procaine was determined by HPLC (Shimadzu Prominence LC-20 series; Colum: Zorbax Eclipse Plus C18, 2.1×50 mm, particle size: $3.5 \mu\text{m}$; mobile phase: acetonitrile/0.1% trifluoroacetic acid (5: 95); flow rate: 0.6 mL/min; temperature: 40°C ; injection volume: $10 \mu\text{L}$). The achievement of equilibrium was confirmed by time-course measurements.

3.2.5. Differential Scanning Calorimetry Measurement

The solid form of the residual particles in the intrinsic solubility measurement was determined by differential scanning calorimetry (DSC). The sample was placed in a non-sealed aluminum pan and analyzed by DSC (Shimadzu DSC60 plus, Shimadzu Corporation Kyoto, Japan) under nitrogen gas (50 mL/min). Heat flow was set to $10^\circ\text{C}/\text{min}$.

3.2.6. Thermodynamic Correlation between the Intrinsic Liquid–Liquid Phase Separation Concentration and Crystalline Solubility

The S_0^c value was calculated by CLME (Equation (7)). The experimental T_m values were obtained from the literature when available (Table 2). The S_0^c values at 310 K were also obtained from the literature when available. The correlation between the calculated and observed S_0^c values was evaluated by the average absolute error (AAE), the root mean square error (RMSE), and the coefficient of determination (r^2). The AAE and RMSE were calculated by

$$AAE = \frac{\sum |\log_{10} S_{0,calc}^c - \log_{10} S_{0,obs}^c|}{N} \quad (8)$$

$$RMSE = \sqrt{\frac{\sum (\log_{10} S_{0,calc}^c - \log_{10} S_{0,obs}^c)^2}{N}} \quad (9)$$

where the subscript calc and obs indicate the calculated and observed values.

The S_0^c value was also calculated by GSE using the experimental $\log_{10} P_{oct}$ values in the literature.

4. Results

4.1. Crystallization Time

Before the precipitation tests, the crystallization time for each compound was determined under PLM. The results are summarized in Table S1. Because LAVTD requires 10 s, the drugs that crystallized within 10 s were excluded from the following studies (31 remained out of 47 drugs).

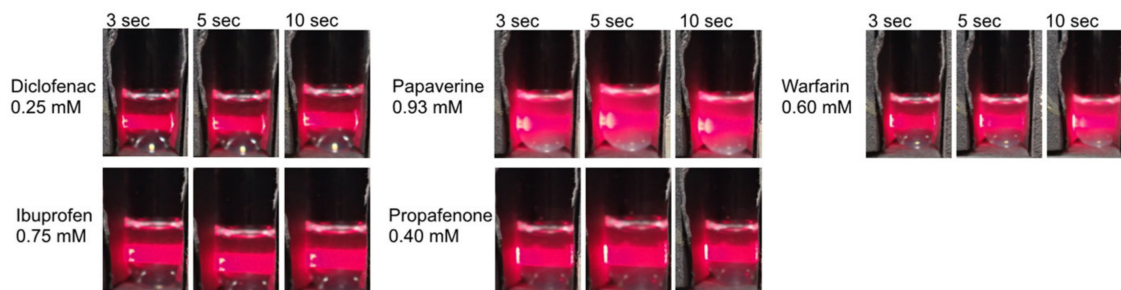
4.2. Validation of the Precipitation Tests Coupled with Laser-Assisted Visual Turbidity Detection

In LAVTD, the turbidity of a solution was detected by visual inspection. However, visual detection could cause a measurement error. To validate LAVTD, turbidity measurements were also performed by UV/VIS spectrometry for several drugs (diclofenac, ibuprofen, papaverine, propafenone, and warfarin). The photograph of the LAVTD method and the absorbance vs. concentration profiles measured by the UV/VIS spectrometry are summarized in Figure 2. The S_0^{LLPS} value was determined as the concentration intercept value. The S_0^{LLPS} of diclofenac, ibuprofen, papaverine, propafenone, and warfarin were determined as 0.25 ± 0.00 mM, 0.64 ± 0.01 mM, 0.83 ± 0.00 mM, 0.38 ± 0.00 mM, and 0.56 ± 0.00 mM, respectively by the UV/VIS method. The S_0^{LLPS} values measured by LAVTD were almost identical to those measured by the UV/VIS method (Figure 3). The coefficient of determination was 0.997.

To further validate LAVTD, the S_0^{LLPS} value of diclofenac measured by LAVTD was compared with the literature values measured by the UV/VIS method and the fluorescence spectroscopy method [19]. The S_0^{LLPS} value measured by the LAVTD methods, the UV/VIS method, and the fluorescence spectroscopy method were 0.20 mM, 0.18 mM, and 0.17 mM,

respectively (at 298 K). Therefore, LAVTD yielded the S_0^{LLPS} value close to the previously reported values.

(A) LAVTD method



(B) UV/VIS method

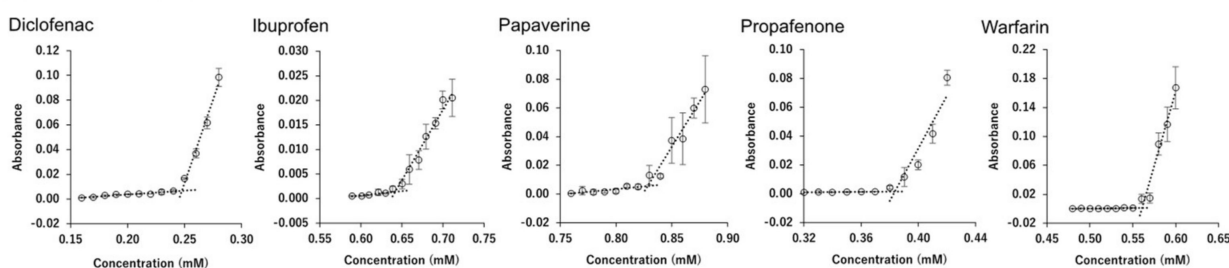


Figure 2. (A) The photograph of LAVTD method at 298 K. (B) Concentration vs. absorbance profiles in S_0^{LLPS} measurement by turbidity detection by UV/VIS spectroscopy at 298 K. The S_0^{LLPS} value was determined as the concentration intercept value.

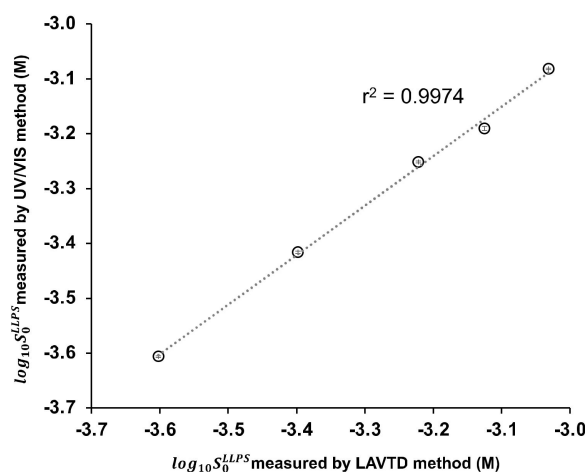


Figure 3. Comparison of the S_0^{LLPS} values measured with the UV/VIS method and the LAVTD method (mean $\pm$ S.D., $n = 3$) (298 K).

4.3. Thermodynamic Correlation between the Intrinsic Liquid–Liquid Phase Separation Concentration and Crystalline Solubility

The molecular weight (MW), acid–base dissociation constant (pK_a), T_m , $\log_{10} P_{oct}$, $\log_{10} S_0^{LLPS}$, and $\log_{10} S_0^C$ of each drug are summarized in Table 2. Many of the S_0^{LLPS} differed from the S_0^C values more than 10-fold and up to 158-fold. The correlation between the calculated and observed S_0^C values is shown in Figure 4. The drugs with a melting point below 310 K were excluded from the analysis. In addition, terbinafine was excluded because it was difficult to prepare the crystalline-free base due to its low melting point (314 K). The AAE, RMSE, and r^2 values are summarized in Table 3.

The correlation between P_{oct} and S_0^{LLPS} is shown in Figure 5. When the P_{oct} value increased, the S_0^{LLPS} value decreased.

Table 2. MW, pK_a , T_m , P_{oct} , S_0^{LLPS} , and S_0^C of model compounds.

Drugs	MW	pK_a ¹	T_m (K)	$\log_{10} P_{oct}$	$\log_{10} S_0^{LLPS}$ (M)	$\log_{10} S_0^C$ (M)	Ref.
Atazanavir	705	4.5 (B)	481	5.8	−4.03	−5.83	[23,24]
Bifonazole	310	5.7 (B)	422 ²	4.8	−4.70 ± 0.00 ²	−5.42 ± 0.03 ²	[25,26]
Carprofen	274	4.2 (A)	484 ²	4.3	−3.66 ± 0.00 ²	−4.80 ± 0.02 ²	[27]
Celecoxib	381	11.1 (A)	437	3.4	−3.95	−5.50	[28–30]
Chlorpromazine ³	319	9.2 (B)	<298	5.4	−4.70 ± 0.00 ²	-	[27]
Cilnidipine	493	None	387	5.7	−5.33	−6.89	[31–33]
Clotrimazole	345	5.9 (B)	417	5.2	−4.65	−5.80	[23,25,34]
Clozapine	327	3.8 (B), 7.5 (B)	458	4.1	−3.38	−4.57	[15,25,34]
Danazol	338	None	498	4.5	−4.43	−6.21	[35–38]
Diclofenac	296	4.0 (A)	453	4.5	−3.52 ± 0.00 ²	−4.96	[18,27,39]
Diphenhydramine ³	255	9.1 (B)	<298	3.4	−2.94 ± 0.00 ²	-	[27]
Dipyridamole	505	6.4 (B)	436	2.2	−3.80 ± 0.00 ²	−4.70	[40–42]
Efavirenz	316	10.2 (A)	412	5.4	−4.23	−4.59	[15,43–45]
Enzalutamide	464	None between pH 3–11	470	4.0	−4.04	−5.20	[46,47]
Felodipine	384	<2	415	5.6	−4.59	−5.61	[10,15,25,48]
Fenofibrate	361	None	354	4.6	−4.70 ± 0.00 ²	−6.08	[49,50]
Flurbiprofen	244	4.0 (A)	388 ²	4.2	−3.37 ± 0.00 ²	−4.15 ± 0.01 ²	[27]
Ibuprofen	206	4.4 (A)	349	4.0	−3.12 ± 0.00 ²	−3.55	[27,51]
Ketoconazole	531	3.3 (B), 6.2 (B)	423	4.3	−3.80 ± 0.00 ²	−5.31	[25,52–54]
Ketoprofen	254	4.2 (A)	368	3.2	−2.76 ± 0.00 ²	−3.00	[25,55–57]
Ketotifen	309	6.7 (B)	430	2.1	−3.41 ± 0.00 ²	−4.28	[58,59]
Lidocaine	234	8.0 (B)	342	2.4	−1.74 ± 0.00 ²	−1.90	[27,60]
Loratadine	383	5.3 (B)	409	5.2	−4.70	−5.38	[15,41,61]
Losartan	423	3.2 (A)	457	3.5	−2.07 ± 0.00 ²	−3.47	[62–65]
Loxoprofen	246	4.2 (A)	358 ²	2.3	−2.21 ± 0.00 ²	−2.22 ± 0.00 ²	[66,67]
Meclofenamic-acid	296	4.1 (A)	530	5.9	−4.52 ± 0.00 ²	−6.68	[27,68]
Miconazole	416	6.1 (B)	358	4.9	−4.88	−5.62	[25,35,69]
Orphenadrine ³	269	9.0 (B)	<298	3.8	−3.26 ± 0.00 ²	-	[27]
Paclitaxel	854	None	493	3.9	−4.43	−6.38	[35,70,71]
Papaverine	339	6.4 (B)	421	3.0	−3.03 ± 0.00 ²	−4.35	[27,72]
Phenylbutazone	308	4.4 (A)	379 ²	3.3	−3.64 ± 0.00 ²	−4.49 ± 0.01 ²	[27]
Posaconazole	701	3.6 (B), 4.6 (B)	442	3.8	−4.89	−6.41	[73–75]
Pramoxine ³	330	7.1 (B)	<298	3.6	−3.09 ± 0.00 ²	-	[27]
Procaine	236	2.3 (B), 9.0 (B)	333 ²	2.1	−1.71 ± 0.00 ²	−2.02 ± 0.01 ²	[27]
Propafenone	341	9.3 (B)	364 ²	4.6	−3.42 ± 0.00 ²	−4.62 ± 0.03 ²	[76,77]
Propranolol	259	9.0 (B)	369	3.5	−2.78 ± 0.00 ²	−3.07	[27,78]
Quinine	324	4.2 (B), 8.6 (B)	449 ²	3.5	−2.82 ± 0.00 ²	−3.26 ± 0.00 ²	[27]
Rebamipide	371	3.3 (A)	579	2.6	−3.09 ± 0.00 ²	−5.29	[79–81]
Ritonavir	721	2.4 (B)	391	3.2	−4.58	−5.74	[15,82–84]
Sulfasalazine	398	2.4 (A), 8.0 (A), 10.9 (A)	532 ²	3.6	−4.15 ± 0.00 ²	−5.92 ± 0.04 ²	[27]
Sulindac	356	4.1 (A)	460 ²	3.4	−3.70 ± 0.00 ²	−4.60 ± 0.00 ²	[27]
Telaprevir	680	0.3 (B), 11.8 (A)	519	4.0	−3.87	−5.17	[17,85]
Terbinafine ³	291	7.1 (B)	314	6.2	−5.22 ± 0.00 ²	-	[25,86]
Thioridazine ³	371	8.9 (B)	<298	5.3	−4.30 ± 0.00 ²	-	[87–89]
Verapamil ³	455	8.7 (B)	<298	4.0	−4.10 ± 0.00 ²	-	[27]
Warfarin	308	4.9 (A)	436 ²	3.5	−3.20 ± 0.00 ²	−4.54 ± 0.01 ²	[27]

¹ A: acid, B: base. ² Measured values in this study. The S_0^{LLPS} and S_0^C values were measured at 310 K. Detailed information on the S_0^C measurements (DSC curves of residual particles, the pH values of suspension after reaching equilibrium, and calibration curves) are summarized in Figure S4 and Figure 5, and Table S2. ³ Excluded from the S_0^C prediction.

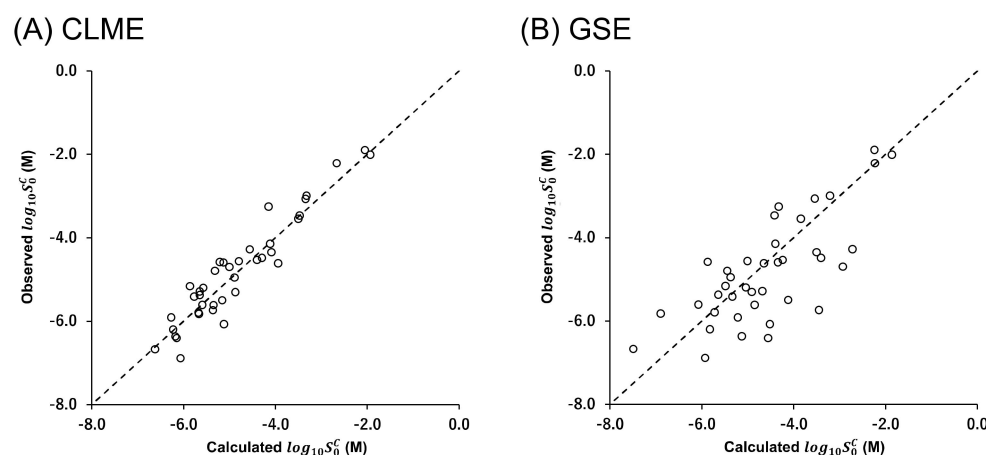


Figure 4. Correlation between the calculated and observed S_0^C values at 310 K by (A) Equation (7) (CLME with S_0^{LLPS} and T_m) and (B) Equation (1) (GSE using P_{oct}). The black dotted line indicates the calculated value equals the observed value.

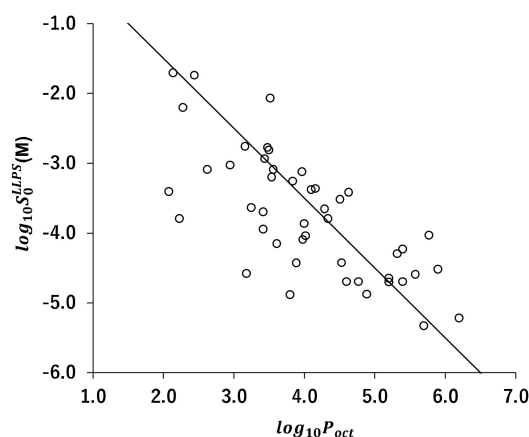


Figure 5. Correlation between P_{oct} and S_0^{LLPS} . The solid line is $\log_{10}S_0^{LLPS} = 0.5 - \log_{10}P_{oct}$.

Table 3. Statistical correlation of CLME and GSE (N = 39).

	CLME (with S_0^{LLPS})	GSE (P_{oct})
AAE (log unit)	0.32	0.71
RMSE (log unit)	0.40	0.91
r^2	0.90	0.56

5. Discussion

In this study, we first validated the bulk phase pH-shift and solvent-shift precipitation tests coupled with laser-assisted visual turbidity detection (LAVTD). This method is simple, rapid, robust, and requires minimum instrumental resources (only a red laser pointer). Because LAVTD requires less than 10 s, it enables the S_0^{LLPS} measurements of rapidly crystallizing drugs. However, visual detection could cause a measurement error. Therefore, to validate LAVTD, the S_0^{LLPS} values were compared with those measured by the UV/VIS spectrophotometric method and the fluorescence spectroscopy method. There was a good agreement between the S_0^{LLPS} values measured by these methods.

In the pH-shift LLPS measurements, in the case of ionizable drugs, the pH value was shifted by adding a small volume of 1 N HCl or 1 N NaOH to a drug solution (1:9). This pH-shift procedure can avoid inducing a local high drug concentration that could facilitate drug crystallization. In the case of un-dissociable drugs, a concentration drug solution in DMA was diluted by adding distilled water (the final DMA concentration was 1.0%). In

this case, a high drug concentration may be locally formed at the initial stage of dilution. In this study, the LLPS concentration was sought by changing the initial drug concentration rather than stepwise titration with a concentrated drug solution. Stepwise titration changes the concentration of a rich solvent. In addition, during the stepwise titration process, crystallization could be induced before reaching LLPS concentration.

In this study, to shift the pH value of a drug solution, 1 N HCl or 1 N NaOH was used for weak acids and bases, respectively. Therefore, the S_0^{LLPS} measurement was conducted at a pH where a drug is undissociated. In other words, LLPS observed in this study is a phenomenon that undissociated non-electrolytes phases separately to form a drug-rich phase (liquid drug phase). The drug-rich phase is a transient state before crystallization. However, when LLPS was formed, a pseudo-equilibrium state was created between the drug-rich phase and the drug molecules molecularly dissolved in water.

In this study, the S_0^{LLPS} values of 31 drugs were newly determined. These values were combined with those reported in the literature to be used for the evaluation of CLME (a total of 39 drugs). CLME appropriately described the S_0^C values. This result encourages that in silico S_0^C prediction can be improved by dividing the prediction scheme into two processes, S_0^{LLPS} prediction and T_m prediction. The development of an in silico S_0^{LLPS} model is currently under investigation. For drug-like molecules, a correlation was observed between P_{oct} and S_0^{LLPS} (Figure 5). Therefore, the same parameters for P_{oct} prediction from chemical structure (hydrogen bonds, molecular volumes, etc.) might also be used to predict S_0^{LLPS} [90,91]. A large amount of the S_0^{LLPS} data set is required to construct an in silico model. The LAVTD-based method is suitable for high throughput (HTS) measurements. The HTS S_0^{LLPS} measurement method is also currently under investigation.

Although the number of drugs used in this study was limited, CLME showed a significantly better correlation than GSE ($p = 0.0004$, comparison of two independent Pearson's correlation coefficients) (Figure 4). In GSE, P_{oct} is employed to represent the solvation term. In the octanol phase, drug molecules are surrounded by octanol molecules (octanol-drug mixture). On the other hand, in the case of LLPS, in the drug-rich phase, drug molecules are surrounded by themselves (drug-drug mixture). As shown in Figure 1, the solvation process is the same as the partitioning of a drug between the drug-rich phase and the water phase.

There may be three possible ways to further improve the correlation by CLME. First, in this study, the same ΔS_m value (56.5 J/K·mol) was used for all drugs. However, the ΔS_m values are different for each compound [92]. Second, the activity of the liquid phase (drug-rich phase) saturated with water should be considered. S_0^{LLPS} is not exactly the same as S_0^L because water and a liquid drug phase are mutually miscible to some extent [93,94]. Third, the heat capacity terms should be considered in the ideal solubility ratio calculation [95].

In this study, the S_0^{LLPS} values of drugs that crystallized within 10 s were not measured. To measure the S_0^{LLPS} value of quickly crystallizing drugs, a crystallization inhibitor such as polymers (e.g., polyvinylpyrrolidone) may be used [96]. However, the S_0^{LLPS} value varies depending on the type and concentration of a polymer [97,98]. Therefore, the selection of a polymer for S_0^{LLPS} measurements would be important. Alternatively, the results of this study suggest that the S_0^{LLPS} value can be calculated from the S_0^C value and the melting point when the S_0^{LLPS} value is difficult to measure.

In conclusion, S_0^C can be described by CLME with reasonable accuracy. The results of this study are important for a good understanding of drug solubility and shed light on the way to improve in silico S_0^C prediction. S_0^{LLPS} is a drug intrinsic parameter that determines the maximum achievable concentration of molecularly dissolved drugs in aqueous media. The S_0^{LLPS} measurement using LAVTD is simple and easy. It would be especially useful for highly lipophilic drugs for which S_0^C and P_{oct} measurements are often difficult. Therefore, similar to the other drug intrinsic parameters such as pK_a and P_{oct} , S_0^{LLPS} should be routinely measured in drug discovery.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pharmaceutics14122560/s1>, Figure S1: Illustration of the laser-assisted visual turbidity detection device.; Table S1: Crystallization time of drugs at 310 K; Figure S2: The examples of PLM observation of crystallization time measurement.; Figure S3: The chemical structure and CAS number of model drugs; Figure S4: DSC curves of residual particles in intrinsic solubility measurement.; Table S2: The pH value after reaching the equilibrium.; Figure S5: Calibration curves of intrinsic solubility measurement.

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