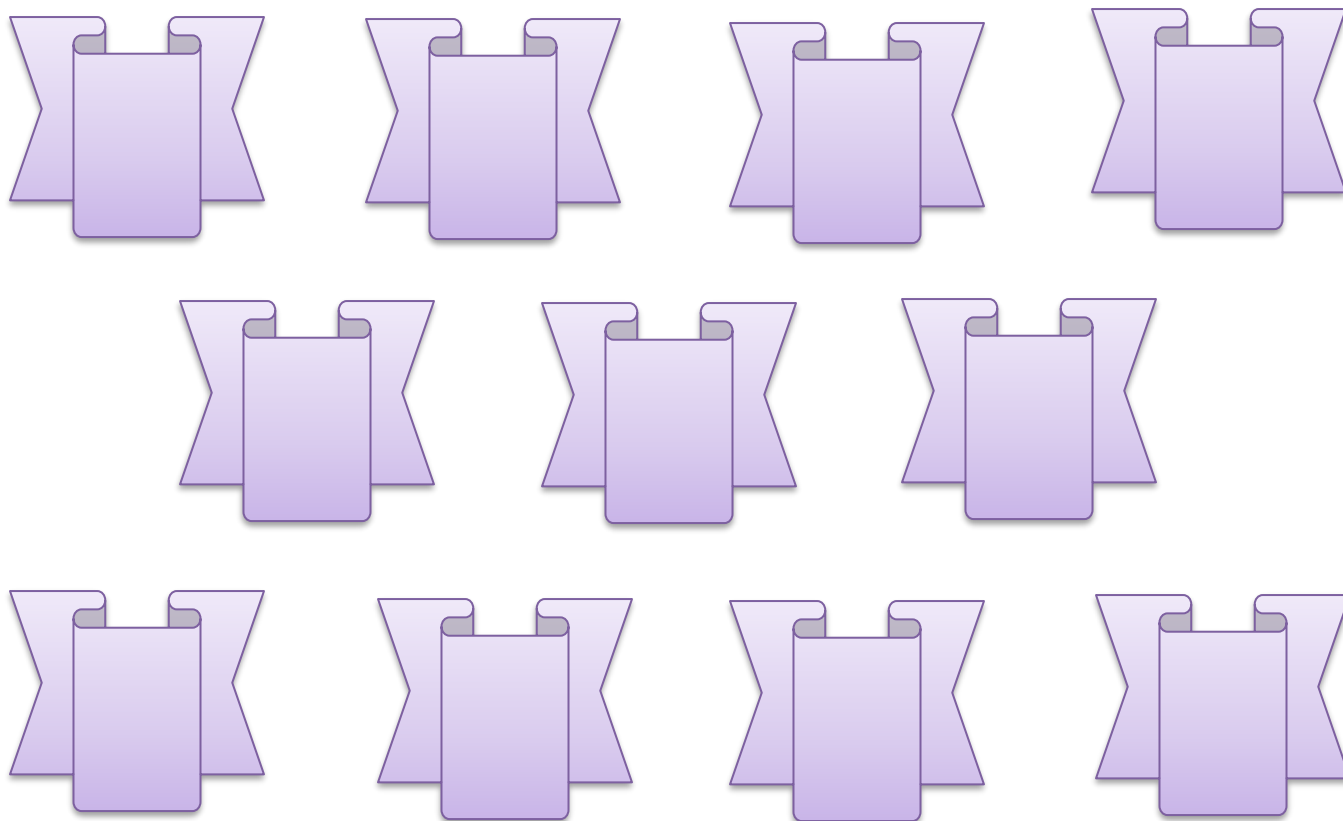


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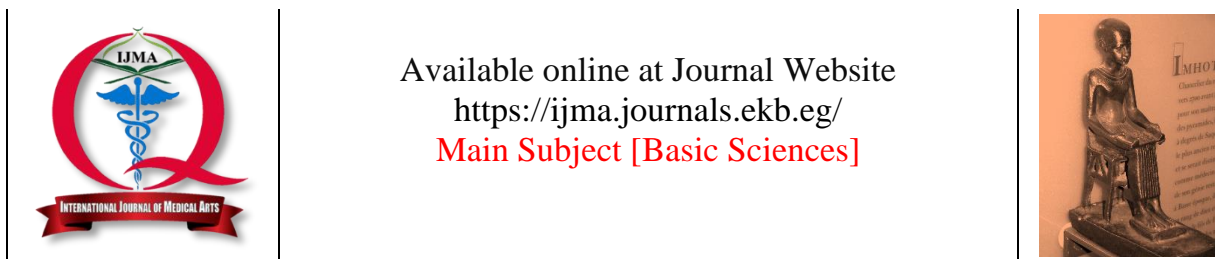
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## Original Article

# Liquid-Liquid Phase Separation at the Surface of Dissolving Drug Salt Particles: Prediction

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## ABSTRACT

This study aimed to show how the amount of liquid in a liquid mixture separates, and how it relates to the science of heat and energy.

We did experiments to study the liquid phase separation, as well as the ability of drug-like molecules to dissolve in crystal form. The CLME equation was derived based on how heat and energy affect things, like how much substance can dissolve in a liquid, how strong the concentration is, and the temperature at which it melts [denoted as  $T_m$ ]. The equation is  $[\log_{10} s_o^c = \log_{10} s_o^{lps} - 0.0095 [T_m - 310]]$  for 310 K. The scientists tested 31 drugs by changing the pH or solvent of the substance and using lasers to see if there were any changes in how they looked. To ensure the material didn't form crystals within 10 seconds, I used a special microscope that utilizes polarized light to conduct some tests.

The measured and calculated values showed a strong similarity [with a small error of 0.40 log units]. The average error was 0.32 log units.

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**Keywords:** Liquid-liquid phase separation; Intrinsic solubility; Melting point; Drug-like.



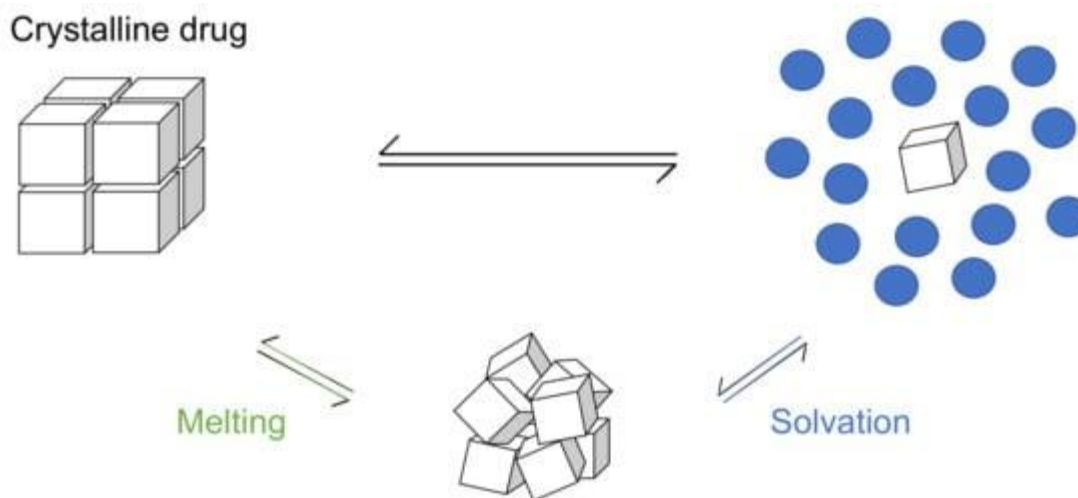
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## 1. INTRODUCTION

The natural ability of a drug to dissolve in water is referred to as intrinsic aqueous solubility [ $s_o^c$ ]. It is an important quality for a good medicine. Many ways of making predictions have been talked about in research papers [1-3]. I can use statistical methods to predict  $s_o^c$  based on the chemical structure by studying observations

and conducting experiments [4-6]. However, even after studying this method for a long time, it is still hard to accurately predict  $s_o^c$  [7].

Another option is to divide the process of dissolving a substance into two steps: melting it and combining it with a liquid. This is about studying how heat and energy influence how chemicals behave [Figure 1].



**Figure [1]:** The diagram shows how a solid drug dissolves in a liquid [8]

The general solubility equation [GSE] is a way to figure out how well a substance can dissolve by using a series of calculations. A rule of thermodynamics is used to make GSE [9],

$$\log_{10} s_o^c = 0.5 - \log_{10} P_{oct} - 0.01 [T_m - T] \quad [1]$$

The melting point is called  $T_m$ , and temperature is represented by  $T$ . The octanol/water partition coefficient is denoted as  $P_{oct}$ . GSE was made using heat principles without changing anything. The GSE constant [0.5] is determined by how well the drug can dissolve in octanol. A drug's ability to dissolve in octanol is called 0.5 when it can fully mix with the liquid octanol [10].

One advantage of this step-by-step prediction approach is that it can assess how much the solvation and crystal lattice energy terms contribute like  $0.5 - \log_{10} P_{oct}$  and  $0.01 [T_m - T]$  in GSE, respectively. So, creating drugs is made simpler with this. Additionally, it helps in creating a helpful liquid for a medicine, which can change depending on whether the reason for the medicine not dissolving well is because of being mixed with a solvent or the energy of its crystal structure. "A step-by-step approach has another advantage because it lets us use in-between measurements or values",

like  $P_{oct}$  and  $T_m$  I can directly measure something in an experiment to make sure the prediction is correct for each process

Lately, scientists have been studying how drugs can be divided into different liquid parts. [11-15]. Scientists have discovered methods to measure the regular levels of LLPS concentration in nature. [ $s_o^{llps}$ ] [16]. The Theory part says that LLPS is like the solvation shown in Figure 1. In theory, this means that  $s_o^c$  can be approximately described by  $s_o^{llps}$  and melting point [ $T_m$ ], however, I have not tried to see how close this estimation is for molecules that are like medications. This information is very important for figuring out which energy term is causing the prediction of  $s_o^c$  to be incorrect. This could help improve computer-based predictions.

The aim of this research was to use experiments to demonstrate the relationship between the amount of liquid-liquid phase separation and thermodynamics. [ $s_o^{llps}$ ] and crystalline solubility [ $s_o^c$ ] of drug-like molecules. In this research, I measured  $s_o^{llps}$  value in the drug effects of 31 medications using a simple test that detects cloudiness with the help of lasers.

## 2. THEORY

The natural ability of a solid drug to dissolve in a liquid  $[s_o^c]$  and a liquid drug  $[s_o^l]$  equals the ideal solubility ratio of the crystal drug  $[x_o^c]$  and the liquid drug  $[x_o^l]$ ,

$$\frac{s_o^c}{s_o^l} = \frac{x_o^c}{x_o^l} \quad [2]$$

The ideal solubility is when a drug can completely dissolve in a liquid drug at the right amount. we can make the sentence easier to understand,

$$\log_{10}s_o^c = \log_{10}s_o^l + \log_{10}\frac{x_o^c}{x_o^l} \quad [3]$$

Assuming that there is no change in heat capacity when melting occurs [17], the ideal solubility ratio  $[x_o^c/x_o^l]$  can be calculated as,

$$\log_{10}\frac{x_o^c}{x_o^l} = -\Delta S_m \frac{T_m - T}{2.303RT} \quad [4]$$

Where  $\Delta S_m$  is the entropy of melting, and  $T_m$  is the melting point

Detecting  $s_o^l$  by the intrinsic liquid-liquid phase separation concentration  $[s_o^{llps}]$ ,  $s_o^c$  is expressed as

$$\log_{10}s_o^c = \log_{10}s_o^{llps} - \Delta \frac{T_m - T}{2.303RT} \quad [5]$$

This formula is called the CLME equation. It should be noted that  $s_o^{llps}$  and  $s_o^l$  Water and a liquid medicine can combine, but they are not completely identical. Furthermore, this research did not distinguish between the merging of liquid droplets and the division of liquid and solid phases [18].

Regarding to Walden's rule,  $\Delta S_m = 56.5$  J/K.mol. So, at 298 K [25 °C] and 310 K [37 °C], Equation [5] becomes

$$\log_{10}s_o^c = \log_{10}s_o^{llps} - 0.0099 [T_m - 298] \quad [6]$$

$$\log_{10}s_o^c = \log_{10}s_o^{llps} - 0.0095 [T_m - 310] \quad [7]$$

We made CLME by following simple rules about how heat works, without needing to change any specific things.

## 3. MATERIALS AND METHODS

### 3.1. Materials

I purchased some chemicals from a company called FUJIFILM Wako Pure Chemical Corporation in Osaka, Japan.

The chemicals I bought include sodium hydroxide solution, hydrochloric acid, sodium chloride, sodium dihydrogen phosphate dihydrate, N,N-dimethylacetamide, boric acid, methanol, trifluoroacetic acid-acetonitrile solution, [S]-[+]-naproxen, diphenhydramine hydrochloride, haloperidol, ibuprofen, indomethacin, ketoprofen, niflumic acid, papaverine hydrochloride,  $[\pm]$ -propranolol hydrochloride, quinine, and warfarin sodium. 2-Naphthoic acid, acemetacin, bifonazole, 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 bought from Tokyo Chemical Industry [Tokyo, Japan]. I bought two medicines from a company called Sigma-Aldrich in Arklow, Ireland.

One medicine is called Meclofenamic-acid sodium salt and the other medicine is called phenytoin sodium. I bought three different substances from a company called Combi-Blocks in San Diego. The substances are called benzocaine, lidocaine hydrochloride, and terbinafine hydrochloride. Orphenadrine hydrochloride was purchased from Chem Cruz in Huissen, Netherlands. I purchased a product called 0. The liquid made of 1% trifluoroacetic acid mixed with distilled water is from a company called Kanto Chemical Co., Tokyo is the capital city of Japan. I purchased Pramoxine hydrochloride from Cayman Chemical, a company based in Ann Arbor, MI, USA. Warfarin free acid was created by mixing a powerful acid with warfarin sodium dissolved in water.

To make propafenone free base, we mix propafenone hydrochloride with water and then add sodium hydroxide.

## 3.2. Methods

### 3.2.1. Crystallization Time Measurement

Before the  $s_o^{llps}$ , they used a special microscope to test how long it took for 47 drugs to form crystals.

In the pH-shift precipitation method, a medicine that can change its charge was mixed in pure water as a salt or by adding a little bit of NaOH [for weak acids] or HCl [for weak bases]. A tiny bit of powerful acid or base was placed on a glass slide. Then, a liquid medicine was poured in and a piece of glass was placed on top to cover it.

In a method called solvent-shift precipitation, a hard-to-separate drug was dissolved in a chemical called N, N-dimethylacetamide. I added a very small quantity [19. Add 8 microliters of pure water to the medicine, which has a volume of 0. The solution had different amounts of drugs. For propafenone, it was 20 millimoles per litre. For lidocaine, it was 200 millimoles per litre. For terbinafine, it was 18 millimoles per litre. For fenofibrate, it was 198 millimoles per litre. And for haloperidol, it was 2 millimoles per litre. To maintain the temperature at 310 K, I used a glass plate heater from a company called BLAST Inc., which is located in Kanagawa, Japan. The scientists carefully observed the substances using a special microscope called a PLM. The microscope had special tools to make it easier to see the different colours of the substances. The microscope's brand was Olympus CX-43 and it was created in Tokyo, Japan by a company named Olympus Corporation. The substance was identified as having a solid form that looks like crystals when polarization was found.

**3.2.2.  $s_o^{llps}$  Measurement by the precipitation tests coupled with laser-assisted visual turbidity detection [lavtd] can be simplified as using a method that combines precipitation tests with laser technology to measure turbidity visually**

The drugs with a crystallization time  $> 10s$  were selected for the  $s_o^{llps}$  measurements. The  $s_o^{llps}$  level was figured out by doing tests where the acidity of the liquid was changed or using a different liquid to see how cloudy it gets with a laser. This way has been detailed before [19]. Each medicine blend was prepared as previously described. To do the experiment at a temperature

of 310 degrees Kelvin, we heated up the medicine solution, a solution containing 1 normal sodium hydroxide [NaOH], a solution containing 1 normal hydrochloric acid [HCl], and glass test tubes, in a container filled with water. I put 100  $\mu$ L of a strong base called sodium hydroxide or a strong acid called hydrochloric acid into a glass tube for drugs that can turn into ions. Next, I inserted it into the LAVTD device. Afterward, I poured the drug solution [900 microliters] into the glass test tube and vigorously shook it immediately. For drugs that cannot be divided, I put a little bit of the drug [10  $\mu$ L] into a tube made of glass. Then I added a bigger amount of purified water [990 microliters]. I could see how cloudy something was really fast by using a red laser [635 nm] in just 10 seconds. The drug in the solution was slowly made stronger by adding little bits at a time, by 0 each time. The initial amount was adjusted to be between 001 to 01 millimoles [mM] and the final results showed three significant digits. The  $s_o^{llps}$  level was determined as the point at which the drug concentration in the solution started to make it look cloudy. The  $s_o^{llps}$  measurement was performed in triplicate.

To check if the results match with previous studies, I also measured  $s_o^{llps}$  using the same settings as the reference study [20]. Diclofenac sodium was combined with methanol and completely dissolved. I placed the medicine in a tiny glass tube [around 10 microliters]. I put a liquid called a phosphate buffer [990 microliters] with a very sour pH level of 2. 0 into a small glass tube. The liquid had 50 millimolar of a substance called phosphates and 128 millimolar of another substance called sodium chloride. After that, I shook the tube very hard at a temperature of 298 degrees Kelvin. The  $s_o^{llps}$  value was determined as described above.

**3.2.3.  $s_o^{llps}$  Measurement Using a UV/VIS spectrophotometer**

Each batch of drugs was made as previously described. A little bit [70  $\mu$ L] of a liquid called 1N sodium hydroxide [NaOH] or 1N hydrochloric acid [HCl] was placed into a special container made of quartz. Next, the container was put in a machine called a UV/VIS spectrophotometer [specifically, the UV-1850 model made by a company called Shimadzu Corporation located in Kyoto, Japan]. I added a



little bit of medicine into a container and checked how much light it soaked up at a certain colour in a short time of 10 seconds at normal room temperature. This wave was designed to be longer than how much each drug can soak up. The  $S_o^{lps}$  measurement was performed in triplicate.

### 3.2.4. Intrinsic Solubility Measurement

I took drugs that were not in liquid form to see how quickly they could dissolve. We used the same method as before to measure how well the substance dissolves [21]. Each medicine was placed into a small amount of liquid, about 10 millilitres, in a special container called a 15-millilitre tube. The objects being tested were rotated at 40 times every minute and heated to 310 degrees Kelvin, except for procaine, which was rotated at 1800 times per minute. Before I

separated the sample, I allowed it to rest without being disturbed for 1 minute. Next, the sample was divided using a unique filter made of a substance that pulls water towards it. The filter had extremely small openings, which were 0.22  $\mu\text{m}$  in size. The first few drops were discarded so that they wouldn't be soaked up by the filter. [22]. They used a special machine called a UV spectroscope to measure the medicine in the liquid. This machine was created by Shimadzu Corporation in Kyoto, Japan. The remaining solid was collected using a special filter that uses suction and then analysed using a technique called differential scanning calorimetry [DSC] [Table 1] gives a summary of information about what the medium is made of, how much of the drug was put in it, how long it was left to incubate, and what wavelength was used to detect it. I was able to see that things were even by checking measurements made during 48 hours.

**Table [1]:** The way to measure how well a substance can dissolve in a liquid in a controlled experiment

Drug	Medium	Amount of Drug [mg]	Incubation Time [h]	Wavelength [nm]
Bifonazole	pH 9.0 borate buffer <sup>1</sup>	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

<sup>1</sup> The concentration of boric acid was adjusted to 50 mM.

Procaine breaks down when exposed to alkali conditions. So, they used a machine called HPLC to see how much procaine was in something [23]. The machine they used was called Shimadzu Prominence LC-20 series. They also used a small tube called Zorbax Eclipse Plus C18, which was 2.1 mm wide and 50 mm long. The tiny pieces in the tube were 3.5 micrometres in size. They combined two substances, acetonitrile and a small amount of trifluoroacetic acid [0.1%], in a proportion of 5 parts acetonitrile to 95 parts trifluoroacetic acid. They made these liquids move through the tube at a rate of 0.6 millilitres every minute. They also made sure that the temperature was 40 degrees Celsius. They put a very small amount of the sample, 10 microliters, into the machine. I checked if things were balanced by using time.

### 3.2.5. Differential Scanning Calorimetry Measurement

The scientists used a technique called differential scanning calorimetry [DSC] to discover the solid type of the small particles that remained after measuring how readily a substance dissolve.

The object being examined was placed in an open aluminium pan and observed using a machine called DSC. The machine was created by a company called Shimadzu Corporation in Kyoto, Japan. The researchers used nitrogen gas for their study. The speed at which heat moved was 10 degrees Celsius per minute.

### 3.2.6 The relationship between the concentration at which liquid separates into two phases and the ability of a substance to dissolve in a crystalline form

The  $s_o^c$  value was calculated using a math equation called CLME [Equation 7]. The scientists collected the  $Tm$  measurements from earlier studies if they were available. If the measurements of  $s_o^c$  at 310 K were in the literature, we also looked at them. I checked how close the calculated and  $s_o^c$  values were using three methods: average absolute error, root mean square error, and coefficient of determination [ $r^2$ ]. The AAE and RMSE were figured out by

$$AAE = \frac{\sum |\log_{10} s_o^c, calc - \log_{10} s_o^c, obs|}{N} \quad [8]$$

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

Where the subscript calc and obs indicate the calculated and observed values

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

## 4. RESULTS

Crystallization Time is the time it takes for something to change from a liquid to a solid crystal. Before doing the tests to see if the substance can form crystals, I checked how long it takes for each substance to form crystals using a method called PLM. The LAVTD test is quick, only taking 10 seconds. Any drugs that created crystals in that time were not used in the rest of the research. So, out of 47 medicines, only 31 were selected for more study.

Checking the accuracy of the rainfall tests with the help of laser-assisted visual detection of cloudiness.

In LAVTD, I could tell if a solution was cloudy just by looking at it. However, when we rely on our eyes to see something, there is a possibility of making errors when trying to

measure it. To make sure LAVTD is correct, I also used UV/VIS spectrometry to measure how cloudy certain drugs are. These drugs include diclofenac, ibuprofen, papaverine, propafenone, and warfarin. The LAVTD method and absorbance measurement are being compared using a picture. The amount of focus measured by the UV/VIS spectrometry is displayed in Figure [2]. The  $s_o^{llps}$  value means the concentration intercept value. The level of diclofenac, ibuprofen, papaverine, propafenone, and warfarin in the sample was measured to be  $0.25 \pm 000$  millimolar,  $064 \pm 001$  millimolar,  $083 \pm 000$  millimolar,  $038 \pm 000$  millimolar, and  $0.56 \pm 000$  millimolar, respectively using the UV/VIS method. The values obtained using the LAVTD method were very similar to the values obtained using the UV/VIS method. The coefficient of determination is a number that shows how closely data points fit on a line. In this situation, the coefficient of determination was 0.997, which means that the data points fit very nicely on the line.

To check if LAVTD is correct, the value of diclofenac measured by LAVTD was compared to values measured by the UV/VIS method and the fluorescence spectroscopy method in previous research [figure 2] [20]. The measured values of  $s_o^{llps}$  using different methods were 0.20 mM, 018 mM, and 0.17 mM at 298 K. So, LAVTD gave a result that was similar to what had been reported before.

This text explains how much of a substance can separate into two liquids and how much of that substance can dissolve in a solid.

The heaviness of molecules, the power of acid or base, the temperature at which a molecule shifts its shape, and the difference between predicted and observed values.

**Focus:** The idea of focusing on something versus thinking about many different things at once. Measuring the absorbance profiles in  $s_o^{llps}$  using turbidity detection with UV/VIS spectroscopy at 298 K, the  $s_o^{llps}$  value was found to be the concentration intercept value.



(B) UV/VIS method

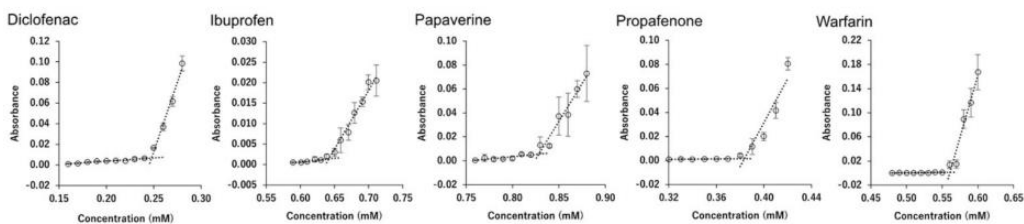


Figure [2]: The picture of the LAVTD method at a temperature of 298 Kelvin

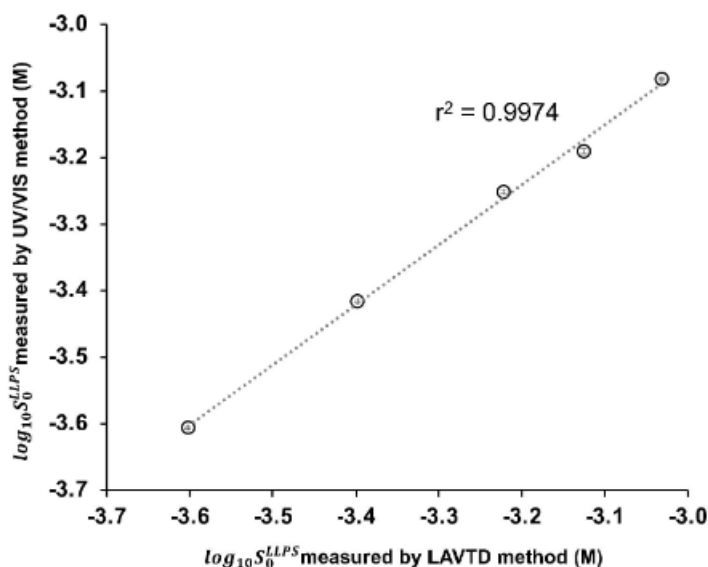


Figure [3]: The  $S_0^{llps}$  values were compared using two methods: UV/VIS and LAVTD. The measurements were done at 298 K and the results are expressed as mean plus or minus standard deviation, based on three replicates

## 5. DISCUSSION

In this study, I tested to see if the bulk phase pH-shift and solvent-shift precipitation tests are accurate using a special laser-assisted method to detect cloudiness. This method is simple, quick, powerful, and only requires a red laser pointer. This tool can measure drugs that are formed quickly in less than 10 seconds. But sometimes, looking at something with your eyes can give you the wrong size. To prove that LAVTD is correct, I compared the  $S_0^{llps}$  values with measurements taken using the UV/VIS spectrophotometric method and the fluorescence spectroscopy method. The methods showed similar values for  $S_0^{llps}$ .

To measure pH-shift LLPS, I changed the acidity or alkalinity of a drug solution by adding a little bit of either HCl or NaOH. I added one-part acid or base to nine parts drug solution. This way of adjusting the pH level can stop

drugs from becoming too concentrated in one place, which could make them turn into crystals. To make some drugs weaker, a mix of them with distilled water was made. This mix had a concentration of 1. In this case, a large quantity of drugs can be found in one place when they are first mixed with another substance. I wanted to see when the concentration is high enough for LLPS to happen in this study. Instead of slowly increasing the amount of a powerful drug, I modified the initial levels of the drug. Stepwise titration slowly changes the amount of a powerful liquid. Also, when you gradually mix a substance, crystals can form even before reaching the needed concentration for the separation of liquids.

In this research, I used either a strong acid called 1 N HCl or a strong base called 1 N NaOH to change the level of acidity in a medicine solution. I used hydrochloric acid [HCl] for acids that aren't very strong, and sodium hydroxide [NaOH] for bases that aren't

very strong either. So, I did a test called  $s_o^{llps}$  at a certain pH level where a drug is not separated into smaller parts. In simple terms, LLPS in this study means that some things that don't break apart into ions gather to create a strong amount of medicine in liquid form. The drug-rich phase is a short-term period before crystals are formed. However, when LLPS was created, it balanced the drug's strong medicine part with the part where the drug molecules mixed with water on a small scale.

In this study, the researchers found the  $s_o^{llps}$  values for 31 different drugs. These values, along with the ones found in previous studies, were used to assess CLME. In total, 39 drugs were evaluated. CLME accurately explained the  $s_o^c$  values. This finding suggests that I can enhance the accuracy of predicting  $s_o^c$  by splitting the prediction process into two steps:  $s_o^{llps}$  prediction and Tm prediction. Currently, researchers are examining a model called *in silico*  $s_o^{llps}$ . In the study, scientists noticed a connection between Poct and  $s_o^{llps}$  for drug-like substances. So, the same factors that are used to predict Poct from chemical structure, like hydrogen bonds and molecular volumes, may also be used to predict  $s_o^{llps}$  [24, 25]. I need a lot of the  $s_o^{llps}$  data to make a computer model. The LAVTD-based method is good for doing a lot of measurements quickly methods].

CLME might be a more effective method of measuring something compared to GSE. We need to do more research to make sure that these discoveries are accurate.

In the field of GSE, we use Poct to represent the solvation term. In the mix of octanol and drugs, the drugs are covered by octanol. In LLPS, when there is a large amount of drug, the drug molecules stick together. In Figure 1, the solvation process can be thought of as separating a drug into two parts: one part that contains a lot of the drug and another part that contains water.

There are three ways to improve the correlation of CLME. In this study, all the drugs had a value of 56 for  $\Delta S_m$ . However, the change in entropy values [ $\Delta S_m$ ] is different for each compound. Next, I need to consider what happens to the liquid part of the medicine that contains a lot of drug when it mixes with water.  $s_o^{llps}$  is not exactly like SLO because water and a liquid drug can mix with each other to some

extent. Third, when calculating the ideal solubility ratio, one should take into account the heat capacity terms. However, in this study, the  $s_o^{llps}$  values of drugs that formed crystals within 10 seconds were not measured.

the comprehensive liquid mixture equations [CLME] are a good way to predict solubility, one errors that could occur that will lead to a decrease in the percent yield is the incomplete isolation. It is possible that instead of shaking the separatory funnel for a certain amount of time, it was done in a short amount of time. This will lead to a decrease in the movement of the analyte particles from the original solvent to the extracting solvent.

In order to find the  $s_o^{llps}$  value of drugs that crystallize quickly, a substance called a crystallization inhibitor, like polymers such as polyvinylpyrrolidone, can be used. However, the  $s_o^{llps}$  amount changes depending on the kind and strength of a material [26, 27]. So, it is important to choose the right polymer for  $s_o^{llps}$  measurements. In other words, if it's hard to measure the  $s_o^{llps}$  value, you can find it by using the  $s_o^c$  value and the melting point.

In the current study focuses on specific drugs, and the applicability of the findings to a broader range of pharmaceutical compounds as mentioned by **Fukiage et al.** [28] by using Inhibition of Liquid-Liquid Phase Separation for Breaking the Solubility Barrier of Amorphous Solid Dispersions to Improve Oral Absorption of Naftopidil and conclude that inhibition of LLPS by molecular complex formation could be a powerful strategy to improve the oral absorption of poorly soluble drugs.

As Liquid-liquid phase separation [LLPS] has been known to drive formation of biomolecular compartments, which can encapsulate RNA and proteins among other cosolutes. Such compartments, which lack a lipid membrane, have been implicated in origins of life scenarios as they can easily uptake and concentrate biomolecules, similar to intracellular condensates. Indeed, chemical interactions that drive LLPS *in vitro* have also been shown to lead to similar sub-cellular compartments *in vivo* [29].

In comparison with other studies using different methodologies **Gezahegn et al.** [30] aimed to develop a simple method for the extraction and determination of ciprofloxacin

residues in environmental water samples. A salting-out assisted liquid–liquid extraction [SALLE] method for the determination of ciprofloxacin in water samples by high-performance liquid chromatography with diode array detector [HPLC–DAD] was developed. The calibration curve was linear over the range of 0.1–100 µg/L with coefficient of determination [ $r^2$ ] of 0.9976. The limits of detection [LOD] and quantification [LOQ] of the method were 0.075 and 0.25 µg/L, respectively. The reproducibility in terms of relative standard deviation [% RSD] was less than 10%. The applicability of the developed method was investigated by analysing tap water, bottled mineral water and waste water and demonstrated satisfactory recoveries in the ranges of 86.4–120%. The method offered a number of features including wide linear range, good recovery, short analysis time, simple operation process and environmentally friendly. The developed method can be utilized as an attractive alternative for the determination of ciprofloxacin residues in environmental water matrices [30].

Using several additional compounds, it is further shown that by applying well-known thermodynamic principles, it is possible to predict the concentration at which phase separation occurs based on a consideration of the thermodynamic properties of the crystalline solid/supercooled liquid and the solution activity coefficients. Finally, through the use of an environmentally sensitive fluorescent probe, the liquid-like and hydrophobic character of the drug-rich phase is confirmed.

The approaches outlined in this study provide an alternative way to assess the properties of supersaturating systems.

LLE is often applied to a variety of matrices including blood, serum, urine, and gastric contents. Forensic laboratories have long relied on LLE for the extraction of additional matrices, such as bile and liver or kidney homogenates. Disadvantages of LLE include use of large solvent volumes, multiple extractions, and long extraction times. The extraction solvent used for LLE should be immiscible in the aqueous matrix so that the two liquids can easily be separated. Recent studies have revealed evidences that indicate that LLPS plays a vital role in human health and diseases. This highlights the need for an overview of the recent advances in

the field to translate our current knowledge regarding LLPS into therapeutic discoveries [31].

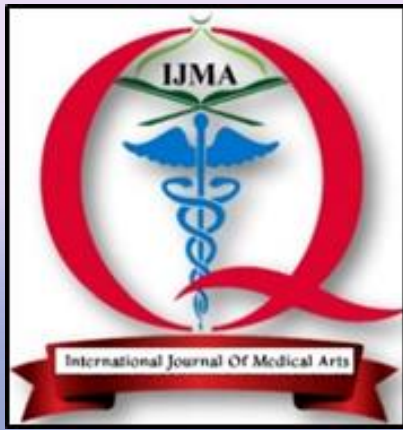
**In conclusion,**  $s_o^c$  can be well understood using CLME with a good level of accuracy. The findings of this research are significant for understanding how drugs dissolve in liquids and can help improve computer predictions of drug properties.  $s_o^{llps}$  is a factor that determines the highest number of drugs that can be dissolved in water. The measurement of  $s_o^{llps}$  using LAVTD is straightforward and not difficult. This would be very helpful for drugs that like to dissolve in fats, as it can be hard to measure their concentrations in the skin and outside the body. So, just like other important characteristics of a drug such as pKa and Poct, it's important to regularly measure  $s_o^{llps}$  in the process of finding new drugs.

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