Development and examination of solubility measurement methods for drug solubility determination

Thesis of doctoral (PhD) dissertation

Edit Baka

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1 Introduction

The solubility processes in human body are very important in pharmaceutical chemistry. The 60 percent of human body is water; the biological and chemical reactions are taken place in this medium in soluble form. The active agent of drug has to dissolve and be absorb in the body to reach adequate concentration in the near of receptor.

In drug development there is an increasing importance of solubility measurements. The importance of precise prediction of drug solubility from chemical structure or physico-chemical parameters also increase.

Solubility in water is also required for pharmacokinetic and stability examinations and for drug formulations. Knowledge of solubility is essential for making drug both in industry and in pharmacy because it helps to choose appropriate auxiliary materials in drug formulations.

The known of solubility characteristics is important for the choosing of appropriate ingredient, carrier substances and drug formulation. The inappropriate ingredients can cause incapability during drug manufacturing, which can be responsible for the degradation of quality or effectiveness.

It is unlikely that a potentially drug candidate with slight absorption characteristic can be developed to drug. Slight solubility in water can cause slight absorption. That's why it is important to know the solubility of substances. This knowledge can predict the pharmacokinetic features and can help in the selection of drug candidates. Biopharmaceutics Classification System is based on this finding. Four groups (I.-IV.) are determined according to the (good or bad) solubility and (good or bad) permeability of the drug substances. Based on these groups the absorption features can be predicted. The methods for determining solubility are different in the different phases in drug innovation. In the early research phase indicating data are appropriate but later, during development exact results are needed.

The development of science and technique gives moderner, faster and automated methods for the measurement of solubility. At the same time the old, classic methods (saturation shake-flask) are still important, on which the new methods are based.

2 Objectives

2.1 Examination of influential parameters on saturation shake-flask method and standardization of shake-flask method

The classic saturation shake-flask method is widely spread for the determination of the equilibrium solubility of drug molecules. It has been used for decades to measure the thermodynamic solubility but its literature is very

heterogenic. For this reason and because of the problems occurred during our measurements the first aim of my doctoral research was to standardize this method.

In this part of my Ph. D. thesis the parameters that affect the saturation shake-flask method were investigated.

2.2 Development and standardization of new shake-flask protocol

According to the results got from the investigation of the influential parameters and the standardization of the method our goal was to create a new protocol for shake-flask method. On the basis of the results we suggest the use of shortened protocol for the determination of the solubility of drug substances. Some circumstances (buffer solution, temperature, phase separation techniques) must be taken into consideration and must be chosen carefully.

To prove the suitability of the protocol we investigated the equilibrium solubility of 5 drug substances with both protocols (standard and new). During the investigation we validate the new one.

2.3. Validation of CheqSol method

The third goal of my study was to validate and to initiate the CheqSol method, which was developed by Sirius Analytical Instruments Ltd.

This research and develop laboratory published a new method in 2005 for potentiometric solubility measurement, which is detailed in my dissertation. To validate the method we choose numerous compounds and we planned to measure their thermodynamic solubility with saturation shake-flask method and to compare the results with the data of CheqSol. To analyze reproductivity we planned to compare our CheqSol results with the results of the developing laboratory.

2.4. Examination of validity of Henderson-Hasselbalch relationship

The Henderson-Hasselbalch relationship has been used in drug development for a long time to calculate the apparent solubility data of the compound on biological pH (stomach, intestine, plasma). In this calculation the intrinsic solubility ($\log S_0$) and pKa is used. Our aim was to determine the usability of HH relationship.

With this object we planned to determine the $\log S_0$ value of the compounds with CheqSol and the pH dependence of the solubility with shake-flask method. The measured and calculated results will be compared to investigate the validity of HH relationship.

3 Methods

3.1 Determination of specific absorptivity

In the classic saturation shake-flask method the concentrations of saturated solutions was determined with UV spectrophotometry. Therefore the specific absorptivity of the compounds on the exact pH in the buffer solution must be determined earlier. This was measured using two or more dilution series then the calibration curve was calculated using Lambert-Beer law. The slope of the curve gave specific absorptivity.

3.2 Determination of solubility by shake-flask method

The examined compound was solved in solid excess in 1-10 mL buffer (Britton-Robinson, Sörensen-citrate, Sörensen-Phosphate) in a glass vial.

At least three, at the most six samples were prepared from every compound. The solutions were stirred for 48 hours on magnetic stirrer under thermostated circumstances until the solubility equilibrium. The time of stirring depended on the quality of the substance. When the examined compound was disintegradable or photosensitive, the time of stirring was decreased or it was stirred in dark.

To separate phases the solutions were left to sediment for 24 hours under thermostated circumstances. When the solutions were not clear, filtration or centrifugation was needed. Aliquots were taken from the clear part of the solutions with micropipettes. The aliquots (5-500µl) were diluted. At least three, at the most six aliquots were taken to precise the measurement.

The absorption of the diluted aliquots was measured with UV spectrophotometer (Jasco V-550 UV/Vis). The concentrations of the aliquots were calculated using the previously determined specific absorptivity.

3.3 Potentiometric pK_a determination

GLpKa automated pK_a analyser (Sirius Analytical Instruments Ltd., Forest Row, UK) fitted with combination Ag/AgCl pH electrode was used for determination of dissociation constants. For bases and ampholytes, in each experiment, 10.00 ml of a 1 mM aqueous solution of sample was preacidified to pH 1.8-2.0 with 0.5 M HCl, and then titrated with 0.5 M KOH to an appropriately high pH, usually 12. In the case of acids, the titration was performed in the opposite direction. The titrations were carried out at constant ionic strength (I = 0.15 M KCl) and temperature (t = 25.0 ± 0.5 ºC), and under nitrogen atmosphere. The pK_a values of samples were calculated by RefinementPro^{TM}2.2 software (Sirius Analytical Instruments Ltd., UK).

The four-parameter technique (Four Plus^{TM} method) was used for electrode calibration both in aqueous medium, methanol-water mixtures and MDM-mixtures.

The pK_a values of sparingly water soluble compounds were determined in various methanol-water and MDM-water (methanol-dioxane-acetonitrile) mixtures by cosolvent method. Each sample was measured in at least three different organic solvent mixtures. To obtain the aqueous pK_a value from the cosolvent dissociation constants (p$_s K_a$ value) we used the Yasuda-Shedlovsky extrapolation.

3.4 Determination of intrinsic solubility by CheqSol method

GLpKa instrument (Sirius Analytical Instruments Ltd., UK) was used to determine potentiometrically the intrinsic solubility of the compounds. Titration was carried out under these circumstances: 1.8-12.2 pH range, stable ion strength, concentration of precipitation. UV detector with optical cable, which dived into the cell, detected the precipitation and stopped the titration. Then we change the pH slightly with alternating acid and base titrant, therefore the sample dissolves and reprecipitates. The equilibrium solubility can be calculated from the titration value that belongs to the zero pH gradient.

4 Results and conclusions

4.1. Examination of the factors that influence the measurements of solubility by saturation shake-flask method

The solubility can be affected by temperature, pressure, purity of the compound, pH, composition of the buffer solution and other characteristics (polymorphy, aggregation, and supersaturation). Our aim was to investigate the experimental circumstances of shake-flask method and that’s why the following was analyzed:

- solvent,
- composition of the buffer solution,
- amount of solid excess,
- temperature,
- saturation time,
- time for equilibrium,
- technique of phase separation.

The experimental factors were systematically varied to check their effect on thermodynamic solubility. The results were compared with those that were measured by the standard protocol. During the experiment the circumstances were constant and the examined parameter was changed only. Hydrochlorothiazide was chosen as a model compound. It is a stable, UV active acid. The measurements were carried out at pH 6.0, where the molecule is un-ionized. We found out that the quality of buffer solution and the temperature affect equilibrium solubility significantly. At the same time the intrinsic solubility did not depend on the amount of solid excess. The sedimentation was the most appropriate among phase separation techniques.

Incubation time was investigated carefully. In the shake-flask method, the achievement of equilibrium consists of two important but different parts: vigorous agitation (by stirring or shaking) and sedimentation. To discover which of these parts plays higher role in the formation of equilibrium, the time of stirring and the time of sedimentation were independently varied.

The time of stirring was varied between 30 minutes and 48 hours. As shown in figure 1. the measured solubility of hydrochlorothiazide increases with increasing stirring time and then reach a maximum value. There are no significant differences in the solubility results obtained after stirring for 6 hours or more. This suggests that 48 hours of stirring time is not required for the measurement of solubility of hydrochlorothiazide.

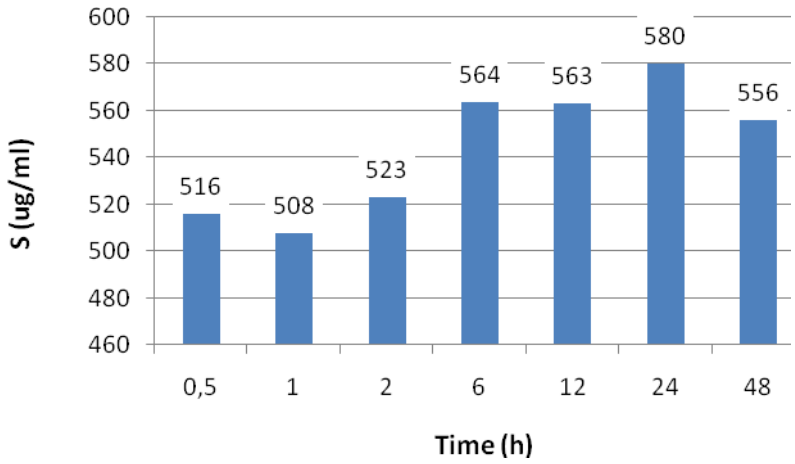

Figure 1. The effect of stirring time on the equilibrium solubility of hydrochlorothiazide

This phenomenon exists in the case of almost every compound. The results of this study suggest that it is reasonable to start with 6h of stirring time.

The time of sedimentation was varied between 1 and 24 hours. The results

show the opposite tendency as in the previous test (Figure2). The solubility values of hydrochlorothiazide were higher in the 1-8 hours interval. At the beginning a supersaturized solution is arisen then the compound precipitate and the thermodynamic equilibrium is reached.

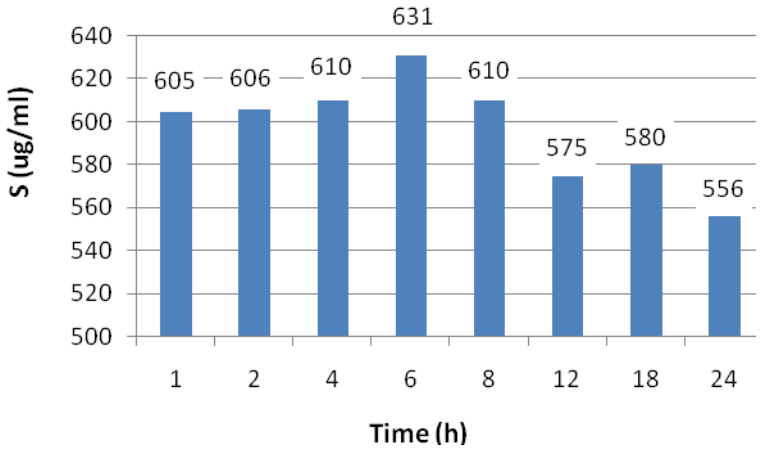

Figure 2. The effect of sedimentation time on equilibrium solubility of hydrochlorothiazide

According to our results the time of sedimentation affect firmly the development of equilibrium solubility. Longer sedimentation time is recommended for the precised measurement.

Results also show that the time of sedimentation plays greater role in development of thermodynamic equilibrium solubility than the time of stirring.

4.2. Development and validation of new protocol

A new, shortened protocol was prepared and validated based on the results of the investigation of the saturation shake-flask method and on our experiences. The parameters of the new protocol are in Table 1.

Parameters of new protocol	
Buffer	Britton-Robinson buffer
Solid excess	small excess
Temperature	25 ± 0,1 ^{o}C
Stirring time	6 hours
Sedimentation time	18 hours
Phase separation	sedimentation
Concentration measurement	UV-spectrophotometry

Table 1.: Parameters of new protocol of saturation shake-flask method.

To prove the suitability of the new protocol it was validated. During the process the intrinsic equilibrium solubility of six samples was determined with both processes. Table 2 shows that the results correlated.

Compounds	Solubility				
	µg/ml	± SD	mol/l	logS	n
Standard protocol					
Hydrochlorothiazide	556	13,2	0,001868	-2,73	18
Furosemide	20,4	2	0,000062	-4,21	8
Nitrofurantoin	109,5	3	0,000460	-3,34	8
Piroxicam	5,95	0,4	0,000018	-4,75	2
Quinine-HCI	201	10	0,000558	-3,25	6
Trazodone	138	10	0,000372	-3,43	6
New protocol					
Hydrochlorothiazide	571	8,6	0,001918	-2,72	12
Furosemide	18,7	1,2	0,000057	-4,25	8
Nitrofurantoin	99	4,1	0,000416	-3,38	8
Piroxicam	6,36	0,04	0,000019	-4,72	3
Quinine-HCI	285	30	0,000791	-3,10	5
Trazodone	176	1,8	0,000474	-3,32	12

logS: logarithm of mol/l concentration solubility

Table 2. The solubility of compounds measured by the standard and new

The intrinsic solubility can be measured with the new protocol in the major case of compounds. The advantage of it is the shorter measurement time since the early 4-day measurement time decreased to one and a half day.

4.3. Validation of CheqSol method

During the validation of CheqSol method the solubility results of 14 compounds were analyzed. Saturation shake-flask was used as a reference method. The correlation analysis of the results get from the two methods is in good agreement (Figure 3.)

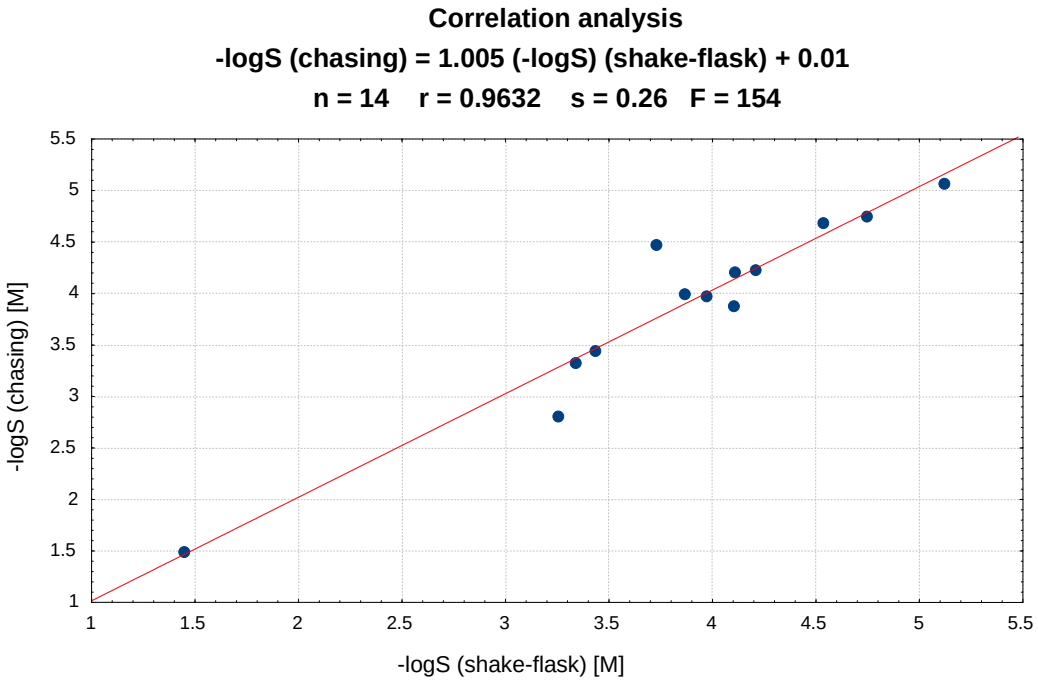

Figure 3. Correlation analysis of the results measured with the two methods

Taken the mistakes of the two methods into consideration, the statistical parameters of the regression curve are good. The slope and intercept of the regression equation are close to the ideal values 1 and 0, respectively. The correlation coefficient is $r^2 = 0{,}96$ and the standard deviation is $s = \pm 0{,}26$.

4.4. Study of the pH dependence of solubility. Revisit of Henderson-Hasselbach relationship

The pH dependence of solubility was investigated on 6 structurally diverse drug compounds: papaverin, prometazin, profenon, tiklopidin (monoprotic bases), quetiapine (diprotic base) and dezvenlafaxine (ampholyte compound). pKa values were determined potentiometrically. $LogS_0$ values were determined with two independent methods (new prototcol of saturation shake-flask and CheqSol method). The given results were used to represent Henderson-Hasselbach (HH) curves.

The solubility values were measured at wide pH range. The interpolation of the experimental results and the theoretical curve were investigated.

It was observed that precise pK_a and $logS_0$ values follow the HH equation until the limit of salt solubility (Figure 4.).

At low pH values the common ion effect had significant influent on solubility. This was evidently stronger in the case of diprotic bases than monoprotic bases.

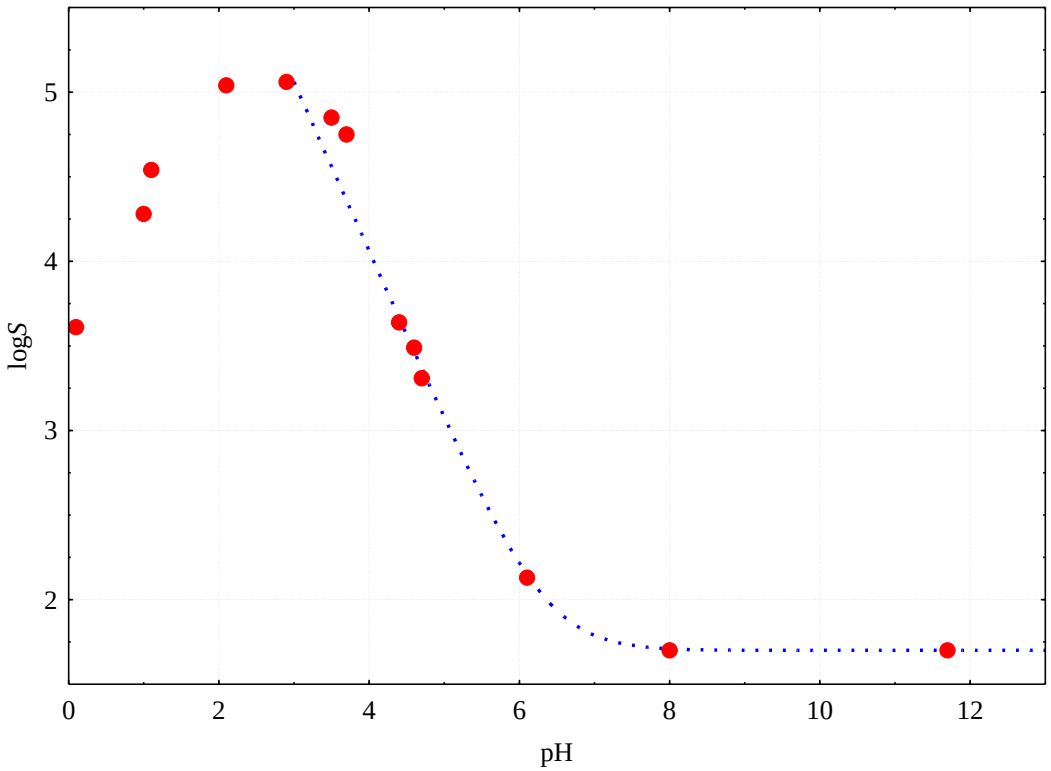

Figure 4. A pH-dependent solubility profile of papaverine hydrochloride

It was an essential observation that pH values in the shake-flask measurements must be precisely measured and also confirmed at the end of incubation period. It was found that the deviations from HH equation are often due to the inaccuracy of the applied methods.

5. The use of results

1. During the investigation of the parameters that have effect on saturation shake-flask method we realized that some parameters must be taken into consideration. Based on these observations a new protocol was prepared. According to this protocol the equilibrium time was decreased to 24 hours. The solubility measurement was shortened so pharmaceutical laboratories can save time and energy.

2. With the initiation of the new and validated potentiometric method (CheqSol) has chance to measure the intrinsic solubility of ionizable compounds in 1-2 hours. In pharmaceutical practice it is important to measure the equilibrium solubility fastly and precisely. Besides this value -with HH

relationship- can be used for the determination of the pH dependence of the compounds.

3. The applicability of the HH relationship was proved by systematic measurements. The relationship can be applied for the prediction of water solubility until the maximum of salt solubility. It was observed that some parameters could have disturbing effect, which could cause deviation from HH relationship. It was also observed that precise starting pKa and $\log S_0$ values were needed for the calculation of HH curves. At last we gave a practical guide for the solubility measurements in pharmaceutical companies.

6. References

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