Pharmaceutical Sciences, 2024, 30(2), 274-278
doi:10.34172/PS.2024.2
https://ps.tbzmed.ac.ir/

Video Article

A Description on the Shake-Flask and Laser Monitoring-Based Techniques for Determination of the Drug's Solubility

Aynaz Zarghampour[1], Kimiya Jouyban[2], Vahid Jouyban-Gharamaleki[3,4], Abolghasem Jouyban[1,5], Elaheh Rahimpour[1,6*]

[1]*Pharmaceutical Analysis Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.*
[2]*Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran.*
[3]*Kimia Idea Pardaz Azarbayjan (KIPA) Science Based Company, Tabriz University of Medical Sciences, Tabriz, Iran.*
[4]*Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*
[5]*Faculty of Pharmacy, Near East University, PO BOX: 99138 Nicosia, North Cyprus, Mersin 10, Turkey.*
[6]*Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*

Article Info

Article History:
Received: 1 Nov 2023
Accepted: 6 Jan 2024
ePublished: 15 Feb 2024

Keywords:
-Laser
-Shake-flask method
-Solubility

Abstract

In the current work, the performance of the shake-flask and laser monitoring-based techniques for the determination of the solubility is described as a video file. The video is prepared in three parts. The first part is a general explanation of solubility and its manual and automatic measurement methods. The second part is about how to perform the shake-flask method to measure drug solubility, and the third part is about how the laser monitoring -based automatic device works to measure drug solubility.

Introduction

In the pharmaceutical sciences, solubility is one of the challenging topics in drug discovery and development studies.[1] More than half of the newly extracted/synthesized materials have been abandoned from the developing process owing to the low aqueous solubility. Taking drugs orally is the most common and easiest used method in drug delivery due to the administration ease, high patient acceptance, affordability, less need for sterile conditions, and more flexibility in designing different drug forms and adjusting their dosage.[2] For this reason, most pharmaceutical companies tend to produce drugs in oral forms. The crucial challenge in designing oral pharmaceutical forms is their low bioavailability. The low bioavailability of drugs depends on various factors, including dissolution rate, solubility, drug permeability, and drug metabolism before entering the systemic bloodstream. Among these factors, low aqueous solubility and low permeability are the main reasons for the low bioavailability of the drugs.[3] Solubility is one of the important physicochemical parameters in creating the biological effects of drugs such as pharmacological effects, side effects, toxicity, etc., and in addition to oral dosage forms, it is also of particular importance in injectable formulations.[4] There are two concepts for solubility (*i.e.* thermodynamic or equilibrium and kinetic solubility) which both are used to understand the solubility behavior of compounds in a solvent. The thermodynamic solubility refers to the concentration of a compound in a solution when it is in a state of equilibrium with an excess of solid material that remains in the solution after the dissolution process is complete. This is considered the true solubility of the compound from a thermodynamic perspective. The kinetic solubility refers to the concentration of a compound in a solution just before the point at which precipitation begins to occur. This value represents the maximum solubility that can be achieved for the fastest-precipitating species of the compound in the chosen solvent medium. High-throughput kinetic aqueous solubility assays are designed to measure the solubility of compounds in aqueous or aqueous buffered solutions by detecting the precipitate formation. These assays involve adding small, predetermined amounts of the compound to aqueous (or aqueous buffered) solutions at specific time intervals until the solubility limit is reached.[5] In kinetic solubility assays, there is a possibility of observing a decrease in transmission after reaching a maximum transmission of liquid. This can occur when the compound being tested forms a solubility limit, which is the maximum concentration that can be dissolved in the solvent at a given temperature and pressure. Once this limit is reached, any further addition of the compound will result in the formation of precipitate,

*Corresponding Author: Elaheh Rahimpour, E-mail: rahimpour_e@yahoo.com

which can scatter light and decrease the transmission of the solution. Therefore, in some cases, the transmission of the solution may initially increase as the compound dissolves, but then decrease as precipitation begins to occur. This is a key feature of kinetic solubility assays, as they allow for the detection of the solubility limit and the formation of precipitate in real-time.

Techniques employed for determining the solubility data are categorized into two classes including manual methods and smart/synthetic methods.[6] The manual methods for solubility determination are kinetic, thermodynamic and potentiometric methods.[7] The kinetic method is based on the identification of soluble sediment in an aqueous or buffer solution. In this method, specific and different volumes of the stock solution prepared with dimethyl sulfoxide are added to the aqueous solution (buffer) to determine the range and amount of solubility. According to this method, there are two approaches for measuring solubility: The first approach is a separation of the sediment from the liquid phase and determining the concentration of the solute in the solution phase with a spectrophotometer. The second one is using the formed sediment as an indicator in determining the range of solubility of the solute.[8] In a thermodynamic method, the solute concentration is measured after a solid-liquid equilibrium at a constant temperature. Most of the solubility measurement methods are based on this technique.[8] Potentiometric method as another method for solubility determination was introduced for the first time by Avdeef[9] and it can be used for ionizable compounds, especially compounds that their equilibration time is too long and cannot be completely dissolved. In this method, using acid-base titration and pH change, the solubility of the solute can be measured at different pHs.[10]

Among these methods, thermodynamic methods are popular ones. The shake-flask or shaking-flask method is the most common and the oldest equilibrium thermodynamic method for solubility measuring.[11-13] In this method, the excess amount of the drug is dispensed into the solvent with pre-determined weight percentages in lined flasks, and the samples are placed on a shaker inside an incubator at the desired temperature to obtain an equilibrium saturated solution. Then, a suitable amount of the supernatant solution is removed and filtered or centrifuged, and after proper dilution, it is recorded with a separation method such as high-performance liquid chromatography (HPLC)-UV or spectrophotometery which both is commonly used to analyze solute molecules in a sample solution. Experimental techniques such as the shake-flask method can produce highly accurate solubility data. However, in many cases, determining the ideal solvent composition to achieve the desired drug solubility in a given solution volume can be a tedious and time-consuming task. This trial-and-error approach often leads to limitations in the discovery and development of new drugs, despite the certainty of the experimental measurements. Synthetic and/or smart methods are the next methods for obtaining solubility data which present few limitations in practice applications. The main principle for these methods was offered by Alex Ejew in 1886 and later developed by other research groups.[14] Some reported synthetic and/or smart devices are Particle Video Microscopes, Focused Beam Reflectance Measurement, three Dimensional Optical with Selective Multi depth Focus, in combination with Infrared spectroscopy, Raman spectroscopy, HPLC, nephelometry and UV-spectroscopy etc. which are a combination of qualitative and quantitative methods for sensing and evaluating the solubility variations.[6]

The main aim of this work is to explain the implementation steps of the shake-flask method as a classical method and a laser monitoring technique as a smart method for solubility determination. This video will outline the principles of these methods, and demonstrate the basics of sample preparation and operation in the laboratory in detail.

Methods

Method 1: Principal and experimental steps for a shake-flask method

When conducting solubility studies using the shake-flask method, a mono or mixed solvent, which may be binary or ternary, is usually saturated with the solute being investigated. Following this, an oversaturated solution is prepared and used to determine the saturation point.

The experimental steps for conducting a shake-flask method are summarized here and schematically is given in Figure 1. The solvent mixtures are prepared by mixing appropriate mass fractions of each solvent into the desired vials. The excess amount of solute is dispersed into the vials, then the vials are sealed tightly and located on a shaker in an incubator equipped with a temperature-controlling system. The mixtures are allowed to equilibrate for a pre-determined time. At the end of the equilibration time, the saturated solutions are centrifuged or filtered if necessary, diluted with water: ethanol mixture and analyzed. Various techniques can be used for measuring the solute concentration in the diluted mixture including UV-vis spectrophotometry, spectrofluorimetry, HPLC coupled with different detectors, etc. It should be noticed that before the mixture's analysis, a calibration curve had been plotted using the chosen instrument.

Method 2: Principal and experimental steps for a laser monitoring technique

For the laboratory-developed, automated laser-based method (depicted in Figure 2), the process of saturating the solution involves gradually introducing solid drug particles to the solvent mixture that is below saturation level, until it reaches the saturation point. The instrument employs a laser monitoring method to examine the alteration in the drug particles into the solution. Solubility data is obtained through measurements of the amount of drug added to the dissolution vessel, taken by means of gravimetric techniques.

Herein, all explained steps for a shake-flask method are automatic and the results could be obtained after 8-72

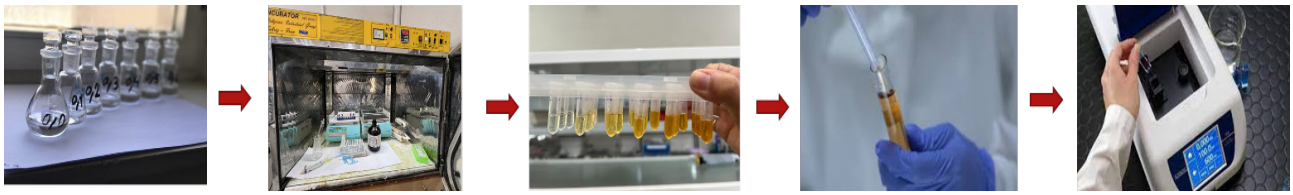

Figure 1. Experimental steps for conducting a shake-flask method.

hours depending on the dissolution rate of the drug in the solvent system. In a robotic system, the processing time amounts to 10-15 minutes and involves preparing the solvent and weighing the powder at the start and conclusion of the test. An alteration in the particles is detected by employing a laser beam source with a wavelength ranging from 650 to 750 nm. The least and greatest quantity of solvent used is 100 mL and 320 mL, respectively, in this technique. The mixing of the solution is done in a closed space which minimizes the risk of evaporation as much as possible and facilitates the probing of the solubility in binary, multiple, aquatic, or non-aquatic solvents. To carry out an experiment using a smart system, one needs to commence by setting the temperature before dispersing the solute powder into the dissolution vessel. This is accomplished with the aid of a robotic arm, and the vessel features mono- or mixed solvents. During laser monitoring, the solution is stirred using a magnet mounted in the dissolution vessel. The robot consistently injects the solute powder until an under-saturated solution converts into a saturated one, discernable from the activation of a green light on the device. The saturation point is repeatedly checked according to the programmed instructions, and to establish the solubility value, the powder is weighed upon dispensing it into the dissolution vessel.

Results

In both techniques, the solubility value, which is defined as the concentration of a solute in a solvent, is obtained for each investigated mono- or mixed solvent. In the shake-flask method, after proper dilution of the saturated mixtures, the absorbance or emission (dependence on the used technique) of the analyte is recorded and their concentrations are obtained from interpolation in a previously plotted calibration curve.

In the introduced laser-based technique, the solute concentration is obtained by weighting the dispensed powder into the dissolution vessel. The weight of the drug vessel is recorded in the initial and final steps of the procedure and their difference provides the mass of the dispensed drug in the known volume of mono or mixed solvent which the solute concentration could be computed using these findings.

The above steps are repeated for each mass fraction of solvents at each investigated temperature and their diagrams are plotted as solubility values (solute concentration) against co-solvent mass fraction and temperature. The solubility profile could be different depending on the studied solute or selected solvents. Figure 3 shows two types of general solubility profile. As can be seen, the solubility profiles could have an upward trend and increase with co-solvent increasing or show a maximum in the intermediated mixtures which can be explained using the different interactions between solute and solvents. However, temperature mostly has a positive effect on the solubility values.

Discussion

Most of the drugs that are taken orally must first be dissolved in the body fluids to be effective, and the higher the dissolution rate of these drugs in the body fluids caused a better effect in the action site. Most 40%

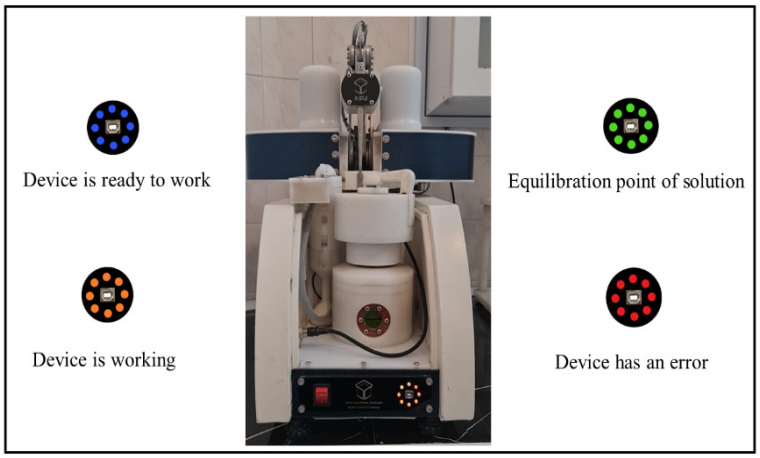

Figure 2. A lab-made automated laser-based instrument for the determination of solubility.

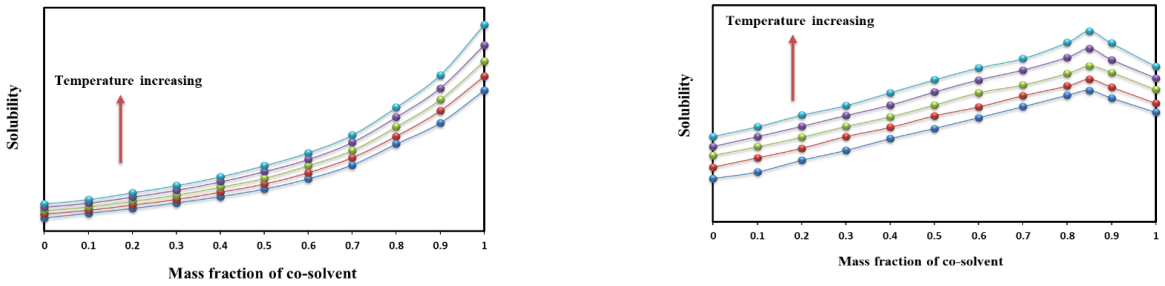

Figure 3. Different types of solubility profiles.

of drugs have the aqueous solubility problem. Therefore, any attempt to enhance the solubility of a targeted drug will be important in pharmaceutical research. Knowing the solubility is valuable even for the salt form of a drug which has a high-water solubility. Because in addition to formulations, solubility can be effective in other fields such as recrystallization in the purification process, extraction, the development of analysis methods, etc. Many methods have been reported to increase the solubility of poorly soluble drugs which cosolvency is one of the simplest and oldest of these methods. Herein, we reported the performance of the shake-flask method as a classical cosolveny method. Due to the simplicity and convenience of the shake flask method, from the first step which is solvent preparation to the concentration determining by the optical method, it is particularly popular among researchers compared to other proposed methods for cosolvency procedure. However, the main limitations and considerations in the method are 1) fluctuation in the set temperature; although the whole procedure, including equilibration and dilution, is performed in a temperature set incubator, the incubator surrounding is not isolated and its temperature affect by the room temperatures; and 2) errors in the sample preparation or dilution step; personal errors in the computational part of the study whether in the sample preparation step or the dilution step sometimes cause inaccurate results for solubility values which can have unpleasant consequences. In addition to personal errors, instrumental errors can also produce a bias in the obtained results. Some of the errors are resulted from using non- calibrated sampler, pipette and employed spectroscopic instruments. In the following of this study, we reported the performance of the laser-based technique as a smart and new emerging cosolveny method which compensated most of the above-mentioned limitations. For instance, the technique is based on laser monitoring in a completely isolated environment which less affects by the temperature of the surrounding environment. Moreover, a technique only needs a recording weight in the initial and final step and no dilution procedure are perfumed. The elimination of the dilution steps removes all personal or instrumental errors related to this part. In addition, due to the elimination of boring dilution procedures in the laser-based technique, it is considered as a simpler method compared to the shake-flask method. However, the main drawback of this smart method can be the depreciation of mechanical parts of the device, *e.g.* injection robotic arm, over time.

Additionally, there is a crucial issue when measuring the solubility of a solute that can exist in multiple polymorphic forms. It is essential to consider the specific form of the solute for solubility measuring. The solubility measurement may correspond to a specific polymorph or a mixture of polymorphs, depending on the experimental conditions. Determining the exact polymorph being measured can be challenging and requires additional characterization techniques. Methods such as X-ray diffraction (XRD), differential scanning calorimetry (DSC), and solid-state nuclear magnetic resonance (ssNMR) spectroscopy can be employed to identify and differentiate polymorphs.[15] These techniques provide structural information about the solid-state form of the solute and help confirm the identity of the polymorph present in the solubility measurement. If the solute exists in multiple polymorphic forms at the measurement temperature, it is important to consider the potential impact of this polymorphism on the solubility results. Different polymorphs and even crystal habits can have distinct solubility behaviors, leading to variations in solubility values. Therefore, it is necessary to carefully control experimental conditions, including temperature, solvent choice, and stirring rate, to ensure consistent measurements and accurate determination of the solubility for the specific polymorph of interest.

Solvate formation can also affect a solute solubility. Solvate formation refers to the process by which a compound forms a solvated crystal structure in the solid state, where one or more molecules of a solvent are incorporated into the crystal lattice. This can have a significant impact on the physical and chemical properties of the compound, including its solubility, melting point, and stability. Unfortunately, solvate formation cannot be directly detected using common analytical techniques such as liquid chromatography or mass spectrometry, as these methods are typically used to analyze solutions or dissolved compounds. Instead, specialized techniques such as DSC, powder PXRD, and other crystallography methods are used to investigate solvate formation.

Solvate formation can also have an indirect effect on the

solute UV absorption, as the solvate may have a different solubility and refractive index than the initial powder. This can lead to a change in the absorption of UV light, which can impact the compound's photostability and other light-dependent properties. Therefore, it is important to consider the potential for solvate formation when designing and interpreting the experiments.

Acknowledgments

This work was supported by Research Affairs of Tabriz University of Medical Sciences, Tabriz, Iran under grant number of 72173.

Author Contributions

Aynaz Zarghampour: Investigation. Kimiya Jouyban: Investigation. Vahid Jouyban-Gharamaleki: Methodology, Data Curation. Abolghasem Jouyban: Conceptualization, Writing - Review & Editing. Elaheh Rahimpour: Conceptualization, Writing - Original Draft.

Conflict of Interest

The authors declare no conflict of interest. V. Jouyban-Gharamaleki and A. Jouyban patented the automated solubility setup in Iranian patent office.

Supplementary Data

Supplementary data and video article, are available at https://doi.org/10.34172/PS.2024.2. The videos in other languages are available in https://parc-fa.tbzmed.ac.ir/uploads/user/7547/1.mp4.

References

1. Martínez F, Jouyban A, Acree Jr WE. Pharmaceuticals solubility is still nowadays widely studied everywhere. Pharm Sci. 2017;23(1):1-2. doi:10.15171/PS.2017.01
2. Pouton CW. Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. Eur J Pharm Sci. 2006;29(3-4):278-87. doi:10.1016/j.ejps.2006.04.016
3. Beg S, Swain S, Rizwan M, Irfanuddin M, Shobha Malini D. Bioavailability enhancement strategies: Basics, formulation approaches and regulatory considerations. Curr Drug Deliv. 2011;8(6):691-702. doi:10.2174/156720111797635504
4. Savjani KT, Gajjar AK, Savjani JK. Drug solubility: Importance and enhancement techniques. ISRN Pharm. 2012;2012:195727. doi:10.5402/2012/195727
5. Jouyban A, Fakhree MAA. Experimental and computational methods pertaining to drug solubility. In: Acree Jr. WB, editor. Toxicity and Drug Testing. IntechOpen: London; 2012.
6. Jouyban-Gharamaleki V, Jouyban A, Acree WE, Rahimpour E. Smart systems for determination of drug's solubility. Drug Dev Ind Pharm. 2019;45(2):177-87. doi:10.1080/03639045.2018.1529786
7. Bergström CA, Avdeef A. Perspectives in solubility measurement and interpretation. ADMET and DMPK 2019;7(2):88-105. doi:10.5599/admet.686
8. Veseli A, Žakelj S, Kristl A. A review of methods for solubility determination in biopharmaceutical drug characterization. Drug Dev Ind Pharm. 2019;45(11):1717-24. doi:10.1080/03639045.2019.1665062
9. Avdeef A. Solubility of sparingly-soluble ionizable drugs. Adv Drug Deliv Rev. 2007;59(7):568-90. doi:10.1016/j.addr.2007.05.008
10. Avdeef A, Berger CM. Ph-metric solubility. 3. Dissolution titration template method for solubility determination. Eur J Pharm Sci. 2001;14(4):281-91. doi:10.1016/s0928-0987(01)00190-7
11. Zhou L, Yang L, Tilton S, Wang J. Development of a high throughput equilibrium solubility assay using miniaturized shake-flask method in early drug discovery. J Pharm Sci. 2007;96(11):3052-71. doi:10.1002/jps.20913
12. Tinjacá DA, Martínez F, Almanza OA, Jouyban A, Acree WE. Solubility, correlation, dissolution thermodynamics and preferential solvation of meloxicam in aqueous mixtures of 2-propanol. Pharm Sci. 2022;28(1):130-44. doi:10.34172/PS.2021.39
13. Jafari P, Rahimpour E, Jouyban A. Solubility of Bosentan in polyethylene glycol 400+ water mixtures: experimental and mathematical computations. Pharm Sci. 2023;29(2):228-35. doi:10.34172/PS.2022.13
14. Ward HL. The solubility relations of naphthalene. J Phys Chem C. 2002;30(10):1316-33. doi:10.1021/j150268a003
15. Pudipeddi M, Serajuddin AT. Trends in solubility of polymorphs. J Pharm Sci. 2005;94(5):929-39. doi:10.1002/jps.20302