MMSL
Military Medical
Science Letters

Mil. Med. Sci. Lett. (Voj. Zdrav. Listy) 2017, vol. 86(1), p. 11-16
ISSN 0372-7025
DOI: 10.31482/mmsl.2017.003

ORIGINAL ARTICLE

METHOD OPTIMIZATION FOR DETERMINATION OF DRUG SOLUBILITY LIMIT

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Received 10th February_ 2017.
Revised 18th February_ 2017.
Published 10th March 2017.

Summary

In the early stage of drug development the solubility of drug candidate is the most crucial physicochemical parameter to be defined for the selection of lead compound. Conventional shake flask method of solubility determination has now been replaced with more precise measurements like ultraviolet absorption, nephelometry, nuclear magnetic resonance and potentiometry. The development of a simple, rapid, sensitive and precise spectrophotometric method for the routine quantitative determination of samples will definitely reduce unnecessary tedious sample preparations and the cost of materials and labour. This article accounts for the measurement of solubility limit of few selected drugs by spectrophotometry using dilution technique. This has been done to optimize the method for rapid and convenient determination of drug solubility limit. Concentration of saturated solution of drug was determined from the absorbance versus concentration plots of various diluted solutions of drug as per Beer-Lambert law and was reported as drug solubility limit.

Key words: drugs; solubility limit; absorbance; spectrophotometry

INTRODUCTION

Drugs can be classified into BCS Class I (highly soluble and permeable), Class II (highly permeable but poorly soluble), Class III (highly soluble but poorly permeable), and Class IV (poorly soluble and poorly permeable) according to Biopharmaceutics classification system [1]. Classes II and IV are poorly water-soluble and normally characterized as high molecular weights, large log P values, and poor water solubility, generally have problems with drug bioavailability. Factors like high lipophilicity and strong intermolecular interactions are responsible for the poor aqueous solubility of a drug. Augmentation of aqueous solubility of poorly-soluble drugsis quiet challenging in pharmaceutical analysis and formulation because drug efficiency is directly linked to its solubility [2-5]. For proper transportation of drug across the biological membranes and absorption,it must be properly soluble. Various techniques are being employed to increase drug solubility like physical modifications which may include: particle size reduction, micronization, nanosuspension, homogenization, wet milling etc.[6]. Several organic solvents like methanol, chloroform, alcohol, dimethyl formamide, and benzene have been employed for the solubili-

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zation of poorly water soluble drugs for spectrophotometric estimations. Drawbacks of organic solvents include higher cost, toxicity, pollution, and error in analysis due to volatility.Table 1 shows categorization of compounds in descriptive terms of solubility.

Table 1. Expression for approximate solubility [7]

Descriptive terms for solubility	Relative amounts of solvents to dissolve 1 part of solute
Very soluble	Less than 1
Freely soluble	From 1-10
Soluble	From 10-30
Sparingly soluble	From 30-100
Slightly soluble	From 100-1000
Very slightly soluble	From 1000-10,000
Insoluble or practically insoluble	More than 10,000

More than 40% of drug suffers from poor aqueous solubility [7]. We decided to conduct UV-Vis spectrophotometric analysis for measurement of drug solubility limit as it is the simplest, eco-friendly, economic, and accurate way of measurement however it has a limitation that it cannot be applied to compounds lacking chromophores. For such compounds other sensitive techniques like HPLC, nephelometry and potentiometry can be used.

MATERIALS

Chemicals

All the investigated oximes (Chart 1) were prepared and characterized at the Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defence, Czech Republic. Synthesis and characterization details are published elsewhere. All the reagents used were of analytical grade. Triply distilled water was used throughout.

METHODS

Determination of drug solubility limit in phosphate buffer of pH 7.4

A series of standard solutions of the investigated compound were prepared. The absorbance of the standard solutions was measured and used to plot a calibration curve. Following the Beer's Law, the slope and intercept of that line provided a relationship between absorbance *A* and concentration C from the eq(1).

$$A = slope.C + intercept \qquad \text{…….eq(1)}$$

The unknown (**saturated**) solution was then analyzed. Using the slope and intercept values eq(1)the absorbance of the unknown solution, *Au*, was calculated according to eq(2).

$$C = (A\text{-intercept})/slope \qquad \text{…….eq(2)}$$

For Calibration curve: a known concentration of drug in PBS (range 10^{-4} M) (Stock A) was prepared. The absorbance of Stock A at the maximal wavelength was measured by using Synergy HT spectrophotometer (Biotek, USA). Three to four diluted solutions of Stock A were prepared in order to reach absorbance not exceeding value of 2 (ideally between 0.5-1). Dilutions were carried by phosphate buffer solutions. A graph of concentration vs ab-

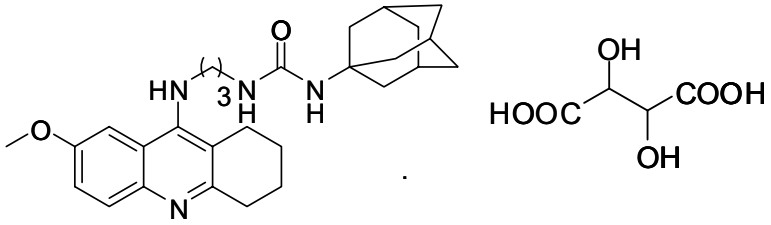
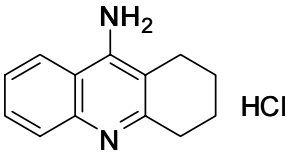
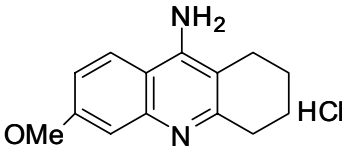
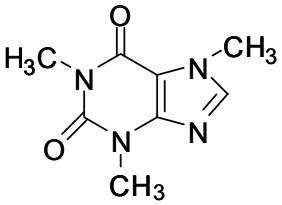
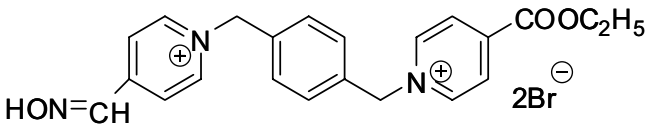
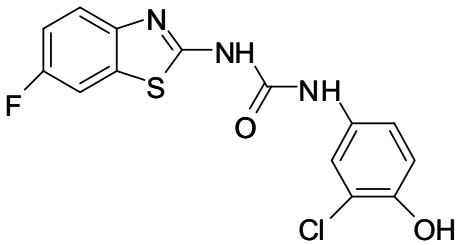
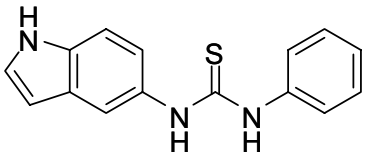
Compound	Structure	M. Wt. (g/mol)
KS-40		612.71
Tacrine		234.72
7-MEOTA		265.72
Caffeine		194.19
K747		537.24
K690		337.76
K748		267.35

Chart 1. Compounds used for the calculation of solubility limit

sorbance was plotted to yield a straight line. The values of slope and intercept were calculated from this plot. R2 value was also determinedand the equation of the line(y=Ax+B) was obtained, for further assessment of concentration of the saturated solution.

For Concentration of Saturated Solution: a saturated solution of drug was prepared by dissolving the solid drug in definite amount of PBS. In order to gain maximum solubility of drug, the solution wassubjected to shaking (VELP Scientifica Vortex mixer) for 2-3 min., sonication for 5 min and incubation at 37° C for 20 min. Once the drug attained solubility limit, the saturated solution was centrifuged at 1000 rpm for 10 minutes to obtain the clear supernatant (**Stock B**). The absorbance of saturated supernatant B was measured. If the absorbance was not in proper range of possible measurement, successive dilutions of B were performed (the extent of dilutionswas recorded). The concentration of stock B was calculatedby using the eq (3)

$$c = (y-B)/A \qquad \text{.....eq(3)}$$

Values of A and B were taken from calibration curve (line equation: (y=Ax+B) performed earlier. 'y' is the measured absorbance of the saturated solutions. C is the molar concentration of saturated solution of drug (moles/liter). Solubility limit of drug can be reported in gram/liter or mg/ml.

RESULTS and DISCUSSIONS

Table 2. Solubility limit of investigated compounds at 37° C in phosphate buffer at pH 7.4

Compound	**Solubility limit** (g/lit)
KS-40	0.274
Tacrine	200.7
MEOTA	0.069
Caffeine	24.04
K747	179.9
K690	0.018
K748	0.213

The prerequisite of UV-spectrophotometric method of solubility determination is that a UV-absorbing molecule should bear specific chromophores in the structure that absorb at a particular wavelength so that they can be employed for their quantitative determinations by spectroscopic method [8]. In the present investigation, the calibration curve was obtained for few selected drugs in the concentration range of 0.2-5.0 x 10^{-4} M. The calibration curve was found to be linear and hence we fixed this method as suitable technique for estimatingthe drug solubility limit using concentration versus absorbance profiles. The slope, intercept and correlation coefficient were calculated for each measurement. Regression analysis of Beer's law plot revealed a good correlation for all studied drugs. The precision was measured in terms of repeatability, which was determined by a sufficient number of aliquots of a homogeneous sample. Thus, the developed UV spectroscopic method has been adopted as a fast, convenient and economic way for analysis of drug solubility limit.

We believe that when the measured solubility is <1 uM, the compoundis unlikely to become a CNS drug [9]. Consequently, one can relate solubility with safety through its relationship with lipophilicity based on Yalkowsky's solubilityequation (Eq. 4) [10]. The equation states two factors, a lipophilicity term (C log P) and a melting point term (mp, often expressed in terms of crystallinity), are the major contributors to solubility (log Sw). Increasing lipophilicity and/or increasing crystallinity result in decreased solubility.

$$\log S_w = 0.5 - C \log P - 0.01(mp - 25°C) \qquad \text{.....eq (4)}$$

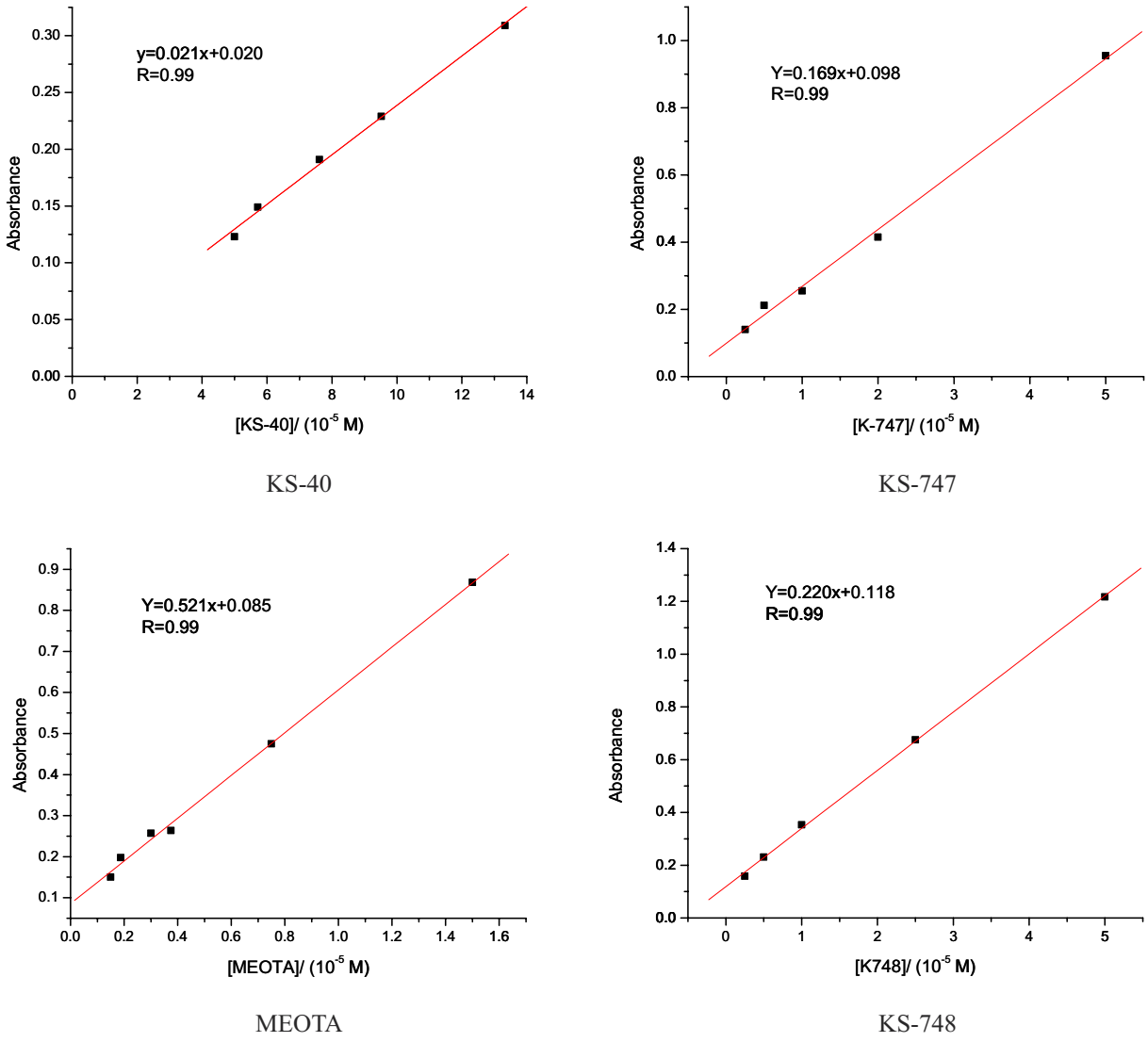


Figure 1. Calibration curve of selected drugs at 37° C.

CONCLUSIONS

There is increased importance of solubility measurements in drug development. It is, thus, concluded that the proposed method of analysis is simple, cost-effective, environment friendly, safe, accurate and reproducible. The simple method optimized in the present investigation for determination of drug solubility limit is convenient and can be adopted for routine analysis.

ACKNOWLEDGEMENTS

This work was supported by the Grant Agency of the Ministry of Education, Youth and Sports of the Czech Republic, An Evaluation of biochemical and pharmacological parameters of chosen acetylcholinesterase inhibitors, Long-term Development plan FMHS, Grant Agency of the Czech Republic No. GA15-16701S, International Post-Doc Research Fund of Charles University in Prague, Czech Republic and Long-term Development plan of Faculty of Military Health Sciences.

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