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Experimental determination of the physicochemical properties of lumefantrine

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Abstract

Background: The physicochemical properties of lumefantrine, a first line combination medicine for the treatment of uncomplicated falciparum malaria have been determined experimentally rather than theoretically as a guide to understanding its disposition in human.

Method: The solubility of lumefantrine in various organic solvents was evaluated by estimating the volume of solvent that completely dissolved 15 mg of the drug. Melting point determination was carried out using a melting point apparatus. Dissociation constant of the drug was determined potentiometrically in 0.1M perchloric acid and partition coefficient was by the method of Leo Hansch, using ratio of the concentration of organic to aqueous phase.

Result: Lumefantrine has a melting point of 128 – 131°C. Its solubility in selected solvents range from 0.013% in acetonitrile (very slightly soluble) to 7.5% in chloroform and dichloromethane (soluble), and it is practically insoluble (0.002%) in water. The ionization constant (pKa), determined in 0.1 M perchloric acid was found to be 9.35. The Log P lies in the range 2.29 - 3.52, confirming the lipophilicity of lumefantrine.

Conclusion: The physicochemical properties of lumefantrine reveal that it is highly lipophilic, weakly basic and readily dissolves in non-polar and/or aprotic organic solvents. While these properties will favour its distribution across cellular membranes, the rate-limiting step will be at the dissolution-absorption stage which will require biopharmaceutical modifications.

Keywords: *Lumefantrine, physicochemical properties, solubility, dissociation constant, partition coefficient*

Résumé

*Contexte:*Les propriétés physico-chimiques d'amodiaquine, une première ligne combinaison médicament pour le traitement des complications le paludisme à falciparum ont été déterminées expérimentalement plutôt que théoriquement comme un guide permettant de comprendre sa disposition en l'homme.

*Méthode :*La solubilité d'amodiaquine chlorhydrate dans divers solvants organiques a été évalué par l'estimation du volume de solvant que complètement dissous 15 mg du médicament. Détermination du point de fusion a été effectuée à l'aide d'un appareil à p oint de fusion. Constante de dissociation de la drogue a été déterminé potentiométrie en 0.1M acide perchlorique et coefficient de partition était par la méthode de Leo Hansch, utilisant ratio de la concentration de matières organiques en phase aqueuse.

Résultat : Lumefantrinehasamelting point de 128 - 1310 C. Sa solubilité dans les solvants sélectionnés vont de 0,013 % dans l'acétonitrile (très légèrement soluble) à 7,5 % dans le chloroforme et de dichlorométhane (soluble), et il est pratiquement insoluble (0,002 %) dans de l'eau. La constante d'ionisation (pKa), déterminée à 0,1 Mperchloric acide a été trouvé à 9,35 . Le journal Pliesintherange 2,29 - 3,52 , confirmant la lipophilie oflumefantrine.

*Conclusion :*Les propriétés physico-chimiques d' amodiaquine indiquent qu'il est fortement lipophiles, faiblement basique et se dissout facilement dans non polaires et/ou aprotique solvatant solvants organiques. Tandis que ces propriétés en faveur de sa répartition à travers les membranes cellulaires, la limitation de débit étape sera à la dissolution-phase d'absorption qui exigera modifications biopharmaceutique.

Introduction

Lumefantrine (Fig.1) was synthesized originally by the Academy of Military Medical Sciences in Beijing, China [1]. It conforms structurally, physicochemically and in mode of action to the aryl amino alcohol group of anti-malarial agents including quinine, mefloquine, and halofantrine [2]. It is a racemic 2,4,7,9-substituted flourene derivative. The dibutyl amino-1-ethanol substitution at the 4 position permits formation of dextro- and laevo-rotatory enantiomers which have equal antimalarial activities [1].

The antimalarial activity of lumefantrine involves lysosomal trapping of the drug in the intra-erythrocytic parasite, followed by binding to toxic haem

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that is produced in the course of haemoglobin digestion. This binding prevents the polymerization of haem to non toxic malaria pigment [1].

The combination of Artemether and Lumefantrine (Co-artem®) is a well tolerated oral antimalarial drug effective even against multidrug resistant falciparum malaria and recommended by World Health Organization (WHO) as a first line antimalarial therapy. It is one of the most commonly used antimalarial in Africa including Nigeria. Pharmacokinetic data which are dependent on physicochemical properties are available for lumefantrine from several trials. The half-life of lumefantrine was estimated as 87 hours in 2 Chinese trials, 74 and 107 hours in 2 Thai trials and only 30 hours in an European trial [1]. Lumefantrine is highly protein bound (>99%), and binds mainly to high density lipoproteins [3]. Absorption of lumefantrine is highly dependent on the intake of food, especially lipids. Its oral bioavailability varied sixteen-fold in fasting healthy Thai volunteers compared to volunteers who have taken a fatty meal [4].

A study of the physicochemical properties of a drug substance is a pre-requisite for product formulation and an aid in understanding the inter-relationship between a drug molecule and its action [5]. Physicochemical properties help to control the processes of drug absorption, distribution, metabolism, excretion (pharmacokinetics) and interaction of the drug at the active sites. Despite the acclaimed usefulness and potential of lumefantrine, properties of the drug such as solubility in different solvents other than dichloromethane and water, its ionization constant and partition coefficient have neither been elucidated nor published in literature. Some information from literature was obtained from theoretical simulations [15]. Therefore, there is a need for practical determination of these physicochemical properties. This study is therefore aimed at elucidating some of the properties of lumefantrine such as solubility in several solvents, partition coefficient, and ionization constant experimentally.

Materials and equipment

Lumefantrine reference sample (kindly supplied by National Agency for Food Drug Admi nistration and Control (NAFDAC), Coartem® tablets (Novartis Pharmaceutical Corporation), acetonitrile High Performance Liquid Chromatography (HPLC grade), ethyl acetate, dichloromethane, and chloroform. Ultraviolet-Visible spectrophotometer (Perkin Elmer, Spectrum BX 11), Fourier Transform Infrared Spectrophotometer, Stuart melting point apparatus (Gallenkamp London, Model MFB-595).

Method

Isolation and purification of lumefantrine from artemether-lumefantrine tablet

A pack of Coartem® tablets (24 tablets) were weighed and uniformity of weight determined. These were crushed and dissolved in 150 ml of ethyl acetate to separate the excipients from the two active principles – artemether and lumefantrine. The solution was filtered and the filtrate was evaporated to dryness on a water bath. The dried residue was dissolved in 150 ml acetonitrile at room temperature. Lumefantrine is slightly soluble in acetonitrile but artemether is very soluble, therefore the solution was filtered and the lumefantrine crystals on the filter paper were collected. The lumefantrine crystals were dissolved in 50 ml dichloromethane and 100 ml acetonitrile was again added to re-crystallize the drug out of solution. Dissolution and re-crystallization in dichloromethane and acetonitrile respectively was repeated thrice so as to obtain pure crystals of lumefantrine that served as secondary standard.

Preparation of lumefantrine secondary standard

Powdered sample of lumefantrine isolated from tablets were standardised using a reference standard by IR spectrum and melting point determinations. The standardised lumefantrine sample then served as secondary standard for subsequent analysis.

Thin layer chromatography (TLC)

5 ì l solutions of both the primary and secondary lumefantrine standards were spotted on a silica gel GF_{254} pre-coated TLC plate. The plate was placed in a TLC tank containing a mobile phase that consisted of ethyl acetate: ether: glacial acetic acid (8:2:1 v/v/v). The plate was allowed to run and then was examined under daylight, iodine vapour and also viewed under UV lamp at 254 nm.

Spectrophotometric analysis

Equivalent amounts of crystals for both the primary and secondary lumefantrine standards were placed on an IR sodium chloride plate. Liquid paraffin for IR spectrophotometry was added to form a null and this was covered with a second plate. The plate was placed in the spectrophotometer and the IR spectra generated from both standards were superimposed on one another.

Solubility profile of lumefantrine in various solvents

Solubility of lumefantrine in ethyl acetate, dichloromethane and chloroform was determined by placing 15 mg of the drug in a test tube and adding 0.1 ml aliquots of solvent with continuous agitation

until all particles were completely dissolved and the solution had attained saturation point. Solubility in methanol, *n*-hexane, *n*-octanol, diethyl ether and acetonitrile was performed by placing 5 mg of the drug in a test tube, and adding 0.5 ml aliquots of solvent with continuous agitation until all particles were completely dissolved. For its dissolution in water, it was carried out by placing 5 mg of the drug in a 500 ml flask with addition of aliquot amounts of water until dissolution was achieved. Solubility in water at elevated temperature (40°C) was carried out by sonicating for 2 hours.

Determination of ionization constant (pKa)
Ionization constant (pKa) was determined potentiometrically using 0.1M perchloric acid after standardization. The pKa of lumefantrine was determined graphically as pH at half neutralization (pH = pKa) and confirmed with first order derivative plot [6].

Determination of partition coefficient (Log P).
Lumefantrine dissolved in n-octanol was scanned in UV spectrophotometer and a maximum absorption (λmax) of 270 nm was obtained. Calibration curves of lumefantrine (2 - 20 μg/ml) were obtained at this λmax for *n*-octanol. Thereafter the partition coefficient, log P was determined according to Leo Hansch method for lipophilic substances using a 1:100 ratio of organic phase to aqueous phase [7]. An aliquot (2 ml of 0.2 mg/ml) of lumefantrine in *n*-octanol was placed in a separatory funnel and partitioned with water (200 ml) once, twice (100 ml + 100 ml) and four times (50 ml + 50 ml + 50 ml + 50 ml). 100 inversions were made and the solution was allowed to settle for 45 minutes for separation of the two layers. The *n*-octanol phase was analysed spectrophotometrically while the drug in water layer was obtained by difference as the levels were below detection limit in UV. Partition coefficient, log P, was calculated as concentration of lumefantrine in n-octanol divided by concentration of lumefantrine in water $\{P = [\text{lumefantrine}]_{oct}./[\text{lumefantrine}]_{H2O}\}$. All determinations carried out in this study were done in quadruplicates and at room temperature (27°C) except ionization constant determination.

Result
The melting point for lumefantrine was 128 – 131°C. On TLC analysis, significant solute migration was observed with the mobile phase ethyl acetate: ether: glacial acetic acid (8:2:1) with R_f of 0.86. IR spectral characteristics and overlay of both primary and secondary lumefantrine standards were obtained as shown in Fig. 3. The solubility in different solvents:

Table1:Solubility of lumefantrine in various solvents

Solvent	1 part of solute in parts of solvent	Mean Solubility (%w/v)	Inference
Ethylacetate	1 part in 48.67 parts	2.05	Sparingly soluble
Chloroform	1 part in 13.33 parts	7.5	Soluble
Dichloromethane	1 partin 13.33 parts	7.5	Soluble
N-hexane	1 part in 566 parts	0.177	Slightly soluble
Methanol	1 part in 3934 parts	0.025	Very slightly soluble
N-octanol	1 part in 2900 parts	0.034	Very slightly soluble
Acetonitrile	1 part in 7266 parts	0.013	Very slightly soluble
Ether	1 part in 86 parts	1.163	Sparingly soluble
Water	1 part in >40,000 parts	<0.0002	Practically insoluble
Warm Water	1 part in >40,000 parts	<0.0002	Practically insoluble

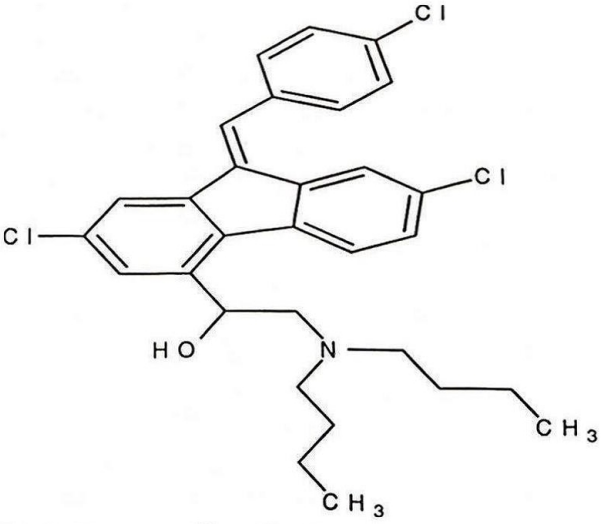

Fig.1: Structure of lumefantrine
$C_{30}H_{22}Cl_3NO$
Relative molecular mass:528.9
Chemical name:2-dibutylamino-1-(2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluorene-4-yl)-ethanol

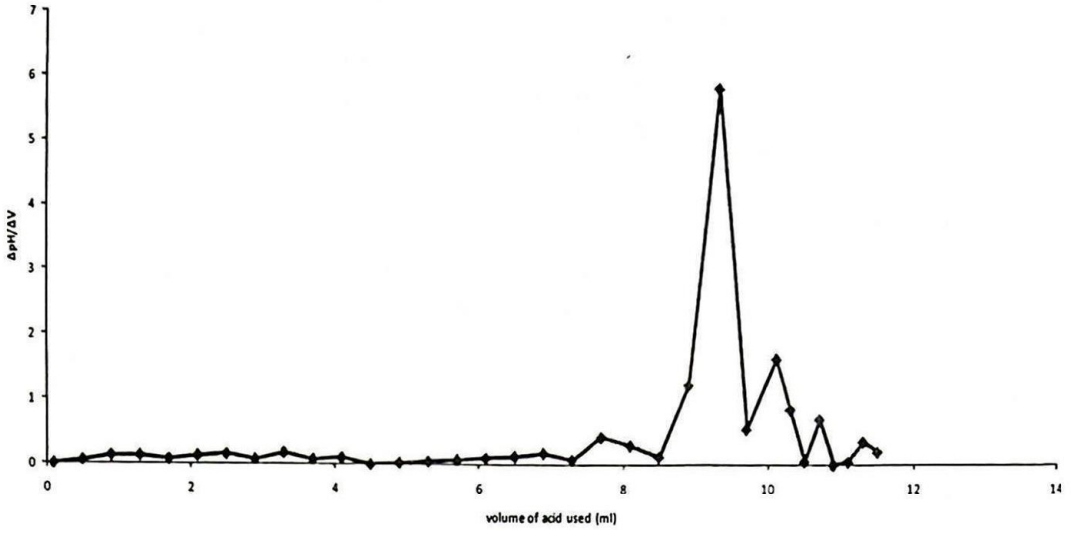

Fig.2: First derivative plot for pka of lumefantrine in 0.1% acetic acid

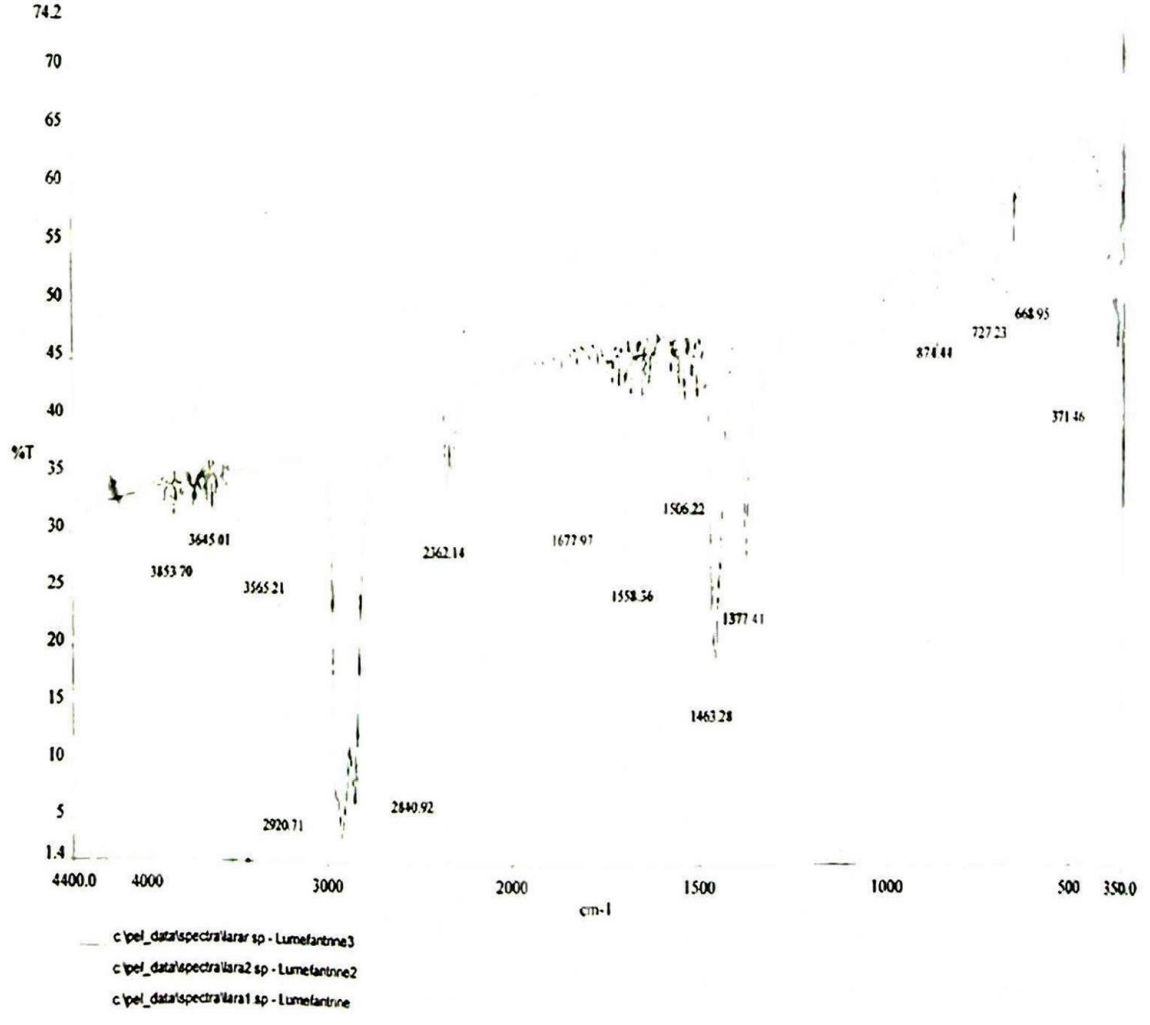

Fig.3: Spectra overlay of isolated lumefantrine on chemical reference standard of lumefantrine

ethyl acetate, chloroform, dichloromethane, *n*-hexane, methanol, *n*-octanol, acetonitrile, diethyl ether, water and water at elevated temperature (40°C) are as shown in Table 1, with their respective interpretations according to Alfred *et al* [8]. The first order derivative plot shows the ionization constant pKa, for lumefantrine as 9.35 [Fig. 2]. The Log P value for lumefantrine ranged between 2.29 ± 0.08 - 3.52 ± 0.18 with r^2 of 0.999.

Discussion

This study gave the melting point of lumefantrine as 128 -131°C, which is the same with the value stated in the current edition of The International Pharmacopoeia, (Fourth Edition). The fourth edition comprises of Volumes 1 and 2 published together in 2006 and the First (2008) and second supplement [13]. The R_f value for lumefantrine secondary standard also corresponded to that of the reference standard. UV-Vis spectroscopic analysis of the reference and secondary standards in methanol gave peaks at 201 nm, 234 nm, 265 nm, 302 nm and 335 nm. Also in *n*-octanol a prominent peak was observed at 270 nm with a minor peak at 350 nm when spectrum was observed between 200 nm and 800 nm. IR spectral analysis carried out for both reference and secondary standards were super imposable confirming the identity and purity of the secondary standard used [Fig. 3].

Interpreting the solubility according to standard terms specified by Alfred *et al.* [8], shows that lumefantrine is practically insoluble in cold and warm water (40 °C) (less than 0.0002%w/v), very slightly soluble in methanol, *n*-octanol and acetonitrile (0.013 – 0.025%w/v), slightly soluble in n-hexane(0.177%w/v), sparingly soluble in diethyl ether and ethyl acetate (1.163, 2.05%w/v respectively) and soluble in chloroform and dichloromethane (7.5%w/v). The structure, thus chemistry of lumefantrine can explain its varied solubility properties in the different solvents. Characteristically, like dissolves like, thus its insolubility in water even at elevated temperature can be accounted for by lack of polar moieties on lumefantrine capable of forming either hydrogen bonding or van der Waal forces. Presence of a single hydroxyl unit does not impact on its solubility in water due to steric hindrance by the surrounding bulky hydrocarbons. Contra wise, its dissolution in chloroform and dichloromethane can be accounted for by its rich halogenated-hydrocarbon character, making it a non-polar moiety. The International Pharmacopoeia (2011) stated that lumefantrine is practically insoluble in water and soluble in dichloromethane, comparing favorably with that obtained in this study. Until now its solubility in ethyl acetate had not been ascertained as it was said to be freely soluble in the Draft proposal for IP (October 2006) but is not included in the final text (2011). Also its solubility in diethyl ether, chloroform, *n*-hexane, *n*-octanol acetonitrile and warm water had not been elucidated.

Comparing lumefantrine solubility with the solubility of halofantrine [9], also an aryl amino alcohol, it reveals that halofantrine which is practically insoluble in water (both warm and cold) and very slightly soluble in acetonitrile compares well to lumefantrine's solubility profile in these solvents. The poor solubility of lumefantrine in polar solvents (methanol and water), is as a result of the contributory effects of the bulky aromatic rings and dibutyl side chains. The only polar hydroxyl group (OH) seems to be stearically overshadowed by the hydrophobic moieties but its slight solubility in polar solvents such as methanol could be as a result of the OH group and the presence of lone pair of electrons on the nitrogen atom as the dibutyl group has a positive inductive effect on the nitrogen centre. Lumefantrine is soluble in aprotic solvents such as ethyl acetate and dichloromethane. Its solubility in water poses a problem in analysis and also affects its absorption and distribution across body tissues [8].

The Log P in *n*-octanol/water, which is neither included in the IP monograph [13] nor in the material safety data sheet for lumefantrine, USP [14], was obtained in this study and ranged between 2.29 – 3.52 partitioning with water once, 1.77-2.43 when used twice and 1.91-2.84 when used four times. This shows that extracting twice is the most efficient procedure. Comparing the Log P of lumefantrine obtained from this study with that of halofantrine [9] with Log P 3.20-3.29 shows they both are highly lipophilic. The Log P value guarantees easy passage through the cell membrane in the body once the drug is in solution. According to Fick's law, the higher the Log P, the higher the rate of diffusion [10]. This value of Log P explains why highly lipid meals increase absorption of lumefantrine by up to 16 fold [9]. This is because solubility in lipid content makes the drug unionized aiding the easy passage across cell membrane.

An important physicochemical property of lumefantrine that is also not included in The International Pharmacopoeia is the ionization constant. The ionization constant obtained in the present study, 9.35, is a proof that lumefantrine is a weak base just as most antimalarials. Comparing the pKa of lumefantrine obtained in this study with that

of halofantrine (pKa = 8.18), shows that lumefantrine is slightly more basic than halofantrine. Absorption of most drugs takes place in the intestine due to the large surface area and only the unionized moieties are capable of traversing the cell membrane. Therefore the amount of unionized lumefantrine that will be present in the intestine (4.28% using the Henderson Hasselbalch equation), and its Log P will favour absorption in the intestine but its poor solubility in water may limit its absorption orally because absorption is subject to solubility of the drug in the body fluids. Interplay of all these factors may either contribute to effective or erratic absorption with consequences on the oral bioavailability. The values obtained however confirm the often poor, unpredictable and sometimes erratic absorption of lumefantrine seen in literature.

Conclusion

The physicochemical properties of lumefantrine have been determined, and in a wider scope than what obtain in literature presently. Its solubility in non-polar and aprotic solvents has been shown as compared to its insolubility in polar solvents. It is weakly basic in character with ionization constant of 9.35 for the pure base and it is highly lipophilic. Interplay of these physicochemical properties possibly account for the erratic absorption property associated with the oral intake of the drug but also is necessary in designing formulations that will yield optimum therapeutic concentrations when ingested. Therefore determining a drug's physicochemical properties can offer preliminary insight into the pharmacokinetic disposition of the drug in the body and also serves as an important factor in drug formulation.

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