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Preparation and Physicochemical Evaluation of Lumefantrine-2-Hydroxypropyl-β-cyclodextrin Binary Systems

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AJA and COO conceived the study idea, study design and participated in the study protocol development. Authors EO, OOO, JOS and AJA participated in data gathering and data analysis. Author AJA wrote the first draft of the manuscript while Authors EO, OOO, JOS, COO and OOB reviewed the manuscript. All authors read and approved the final draft of the manuscript.

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ABSTRACT

Lumefantrine contributes significant roles in artemisinin-based combination therapy for malaria treatment but associated with a limitation of poor aqueous solubility and low permeability. This study investigated lumefantrine-2-hydroxypropyl-β-cyclodextrin complex to improve its solubility profile. A phase-solubility analysis and molecular modelling were carried out before the preparation of the complex by physical mixture, kneading, co-evaporation and freeze-drying methods. Fourier transform infrared (FT-IR) spectroscopic and powder X-ray diffractometric (PXRD) techniques were used to characterised the complex. The phase-solubility studies showed a type AL diagram with an apparent stability constant value of 243.4 M^{-1} suggesting the formation of a soluble and stable

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complex. Significant improvements in aqueous solubility was achieved, notably the freeze-dried system gave a 3-fold and 11-fold increase in solubility in simulated gastric and intestinal fluids respectively. The FT-IR spectra and PXRD patterns of co-evaporated and freeze-dried systems indicated stronger interactions and complexation of lumefantrine in the 2-HP-β-CD cavities. Our findings suggest that the host-guest binary system of lumefantrine-2-HP-β-CD is achievable, structural stable via intermolecular interactions consisting of hydrogen bonding and van der Waals interaction. The inclusion complex is considered a formulation option to ameliorate the poor aqueous solubility of lumefantrine which might improve the absorption and therapeutic efficacy of the drug.

Keywords: Antimalarial; solubility; bioavailability; inclusion complex.

1. INTRODUCTION

Lumefantrine, an aryl-amino alcohol antimalarial drug, is systematically named as (Z)-2-(dibutyl amino)-1-(2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl) ethanol (Fig. 1). It is a weakly basic and highly lipophilic drug with a log P value of 8.34 [1]. Although its mechanism of antimalarial action is yet to be fully elucidated [2], data from in vitro studies suggest that lumefantrine, like other antimalarial drugs such as chloroquine and mefloquine, binds to haem produced during the breakdown of haemoglobin. This binding process prevents detoxification of the haem which occurs through the formation of a crystalline malaria pigment called hemozoin [3]. The accumulation of free haem results in the killing of the parasites.

Lumefantrine serves a prominent role as a component of artemisinin-based combination therapy (ACT), a regimen now adopted as a drug of choice for uncomplicated malaria [4,5]. It is co-partnered with artemether as an orally administered antimalarial medicine both in adult and pediatric populations [5]. It is known to offer protective benefits to artemether as it helps to eliminate remnant plasmodium, which have the potential to develop resistance in the presence of sub-therapeutic plasma levels of artemether, post-treatment [1,4,6]. As a result of its long elimination half-life, lumefantrine also provides post-treatment prophylaxis [7,8]. However, it is associated with low aqueous solubility; therefore, despite its pharmacological benefits, concerns remain about its poor bioavailability following oral administration [9-11].

Lumefantrine belongs to the Biopharmaceutic Classification System (BCS) class IV group [1] with low permeability and very low aqueous solubility of 0.02 mg/mL [12]. The absorption of most drugs with aqueous solubilities < 0.1 mg/ml is limited by poor dissolution [13]. It is also known that following oral administration, lumefantrine undergoes an extensive pre-systemic metabolism contributing to a further reduction in its bioavailability [14]. Therefore, the low aqueous solubility and permeability of lumefantrine [1,12,15] coupled with its extensive pre-systemic metabolism predispose the drug to erratic absorption and low bioavailability. The consequence of this unpredictable absorption and reduced bioavailability is the use of larger doses of the drug to ensure that therapeutic plasma drug concentrations are achieved

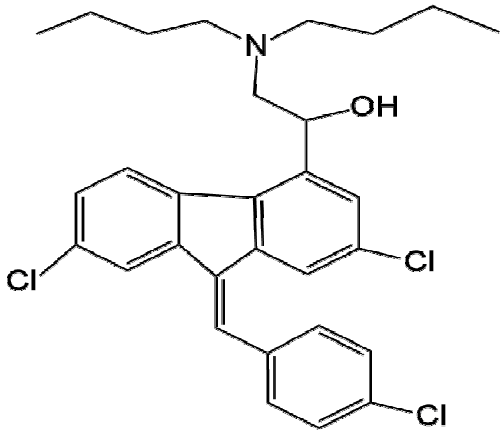

Fig. 1. Structure of lumefantrine

Studies have been undertaken to improve the oral bioavailability of lumefantrine through various strategies. For example, an improvement in the bioavailability of lumefantrine has been reported when an artemether-lumefantrine treatment was administered with fatty meals [10]. Although this has been shown to produce up to 16-fold enhancement of its bioavailability [16], the possibility of accepting this strategy to optimise the bioavailability of lumefantrine is limited because of variations in diets across the globe, which may warrant standardisation of the fat contents of food from various sources [17]. It

is also known that patients with uncomplicated malaria often present with vomiting and anorexia with little or no adherence to dietary advice [18]. Of recent, few formulation interventions or attempts have been directed towards enhancing the bioavailability of lumefantrine. Jain et al. reported a novel solid dispersion formulation (SDF) as an option to significantly improve the bioavailability of lumefantrine [10] while another group investigated the use of novel lipid-based formulation (LBF) and self nano-emulsification (SNE) with fatty acid to augment the pharmacokinetic profile of the drug [19,20]. However, the processes involved in both SDF, LBF, and SNE are known to require high-tech formulation expertise.

A host-guest cyclodextrin (CD) complexation is now considered more promising to enhance the solubility of lipophilic drugs [21]. CDs are cyclic oligosaccharides which are products from the enzymatic degradation of carbohydrates [22]. They have been used to enhance the solubility, stability, and bioavailability of poorly soluble drugs through the formation of complexes with such drugs [23,24]. Based on the numbers of glucopyranose units, CDs are categorised as α, β, or γ-CDs, and each has been derivatised further for various pharmaceutical purposes. The basic structure of CD contains a relatively hydrophilic outer surface because of the presence of hydroxyl groups and a hydrophobic central cavity [13]. Among various derivatives, hydroxypropyl-β-cyclodextrin (HP-β-CD) possesses a unique capability to enhance aqueous solubility and has been widely utilised in pharmaceutical applications [25].

Reports in the literature [21,26,27] indicate that artemether, an orally co-administered drug with lumefantrine, formed a binary inclusion complex with 2-HP-β-CD to improve its aqueous solubility and possibly enhance its bioavailability profile. However, no attempt has been made to prepare and assess a binary system between lumefantrine and 2-HP-β-CD. Thus, this study aimed to investigate the formation of complexes between lumefantrine and 2-HP-β-CD prepared by various techniques. Besides, the study aimed to evaluate the extent of enhancement of the aqueous solubility of the drug through the cyclodextrin complexation and to charaterise the complexes using Fourier transform infrared (FT-IR) spectroscopic and Powder X-Ray Diffractometric (PX-RD) techniques. Besides, the structural stability of the lumefantrine-CD binary inclusion complex was investigated using molecular dynamics simulation.

2. MATERIALS AND METHODS

2.1 Materials

Lumefantrine (M_W. ~ 528.94 g/mol) was obtained from AK Scientific Inc. (Union City, CA, USA) while 2-hydroxypropyl-β-cyclodextrin, (average M_W. ~ 1460 g/mol) with molar substitution of approximately 0.8 was gotten from Sigma-Aldrich Chemical Company (Darmstadt, Germany). Methanol and other reagents are of analytical grade.

2.2 Preparation of Calibration Curve for Lumefantrine

Before solubility measurements, calibration curves were made from concentrations of methanolic solution of lumefantrine ranging from 2.5 – 80 µg/mL, and UV spectrophotometric absorbance measured at the maximum wavelength of 333 nm. The calibration curve had a linearity range of 2.5 to 80 µg/mL with a straight-line equation: $y = 0.0723x + 0.066$ and R^2 = 0.9995

2.3 Phase-Solubility Studies

These were conducted in line with the method previously described by Higuchi and Connors [28]. Aliquots of 20 mg lumefantrine, an amount found to be higher than its aqueous solubility value of 0.02 mg/mL [12], were weighed and transferred into screw-capped tubes containing 10 ml solutions of 2-HP-β-CD with molarities varying from 0 to 30 mM. The tubes were shaken for 60 h at room temperature (300 K) on a mechanical shaker. In the course of shaking, 1.0 ml aliquots were taken after 12 h and underwent filteration using a 0.45 µm nylon disc filter. The samples were appropriately diluted and analysed for lumefantrine by UV-Visible spectrophotometry at the maximum 333 nm wavelength using blanks prepared in the same molarity of cyclodextrin in water. Further samplings were carried out at 12-h intervals until three successive determinations which had no significant changes in lumefantrine concentrations were obtained. The phase-solubility studies were conducted in triplicate, and the average solubility of lumefantrine from the three experiments was used to construct the phase-solubility curve. Subsequently, the curve

was used to calculate the apparent stability constant (Kc) in line with the hypothesis of a 1:1 stoichiometric ratio of complexes using the following equation:

$$K_C = \frac{S}{[I\,(1-S)]}$$

Where,

S and *I* are the slope and intercept of the phase-solubility diagram respectively. In cases K_C is taken as $K_{1:1}$, the complexation efficiency calculated was estimated according to the Loftsson method [29]:

$$K_{1:1} \times I = \frac{[Drug/CD]}{[CD]}$$

2.4 Preparation of Solid Binary Systems

The preparations of lumefantrine-β-cyclodextrin inclusion complexes were performed at 1:1 molar ratio by various techniques as described below:

Physical mixture (PM): Amounts of lumefantrine and 2-HP-β-CD to produce a 1:1 molar ratio were weighed into a glass mortar. Subsequently, both were homogeneously mixed using a spatula.

Kneading method (KM): The physical mixture of lumefantrine and 2-HP-β-CD in a 1:1 molar ratio was formed as presented above. The mixture was further mixed using a small volume of water-methanol (1:1, v/v) solution in a mortar to produce a homogenous paste. The paste was kneaded for 1 h, during which a sufficient volume of water-methanol was added to maintain an appropriate consistency. The resulting paste was dried for 24 h in oven at 45°C, after which it was pulverised into a fine powder and sieved (106 µm).

Co-evaporated method (CM): Lumefantrine and 2-HP-β-CD were weighed in a 1:1 molar ratio. Lumefantrine was dissolved in 40 ml of methanol, while 2-HP-β-CD was dissolved in 10 ml of distilled water. Subsequently, the aqueous solution of 2-HP-β-CD was added to the methanolic solution of lumefantrine, and the content was gently stirred for 1 h with a glass rod. The mixture was dried under vacuum at 45°C using a rotary evaporator. The solid residue obtained was further dried at 40°C and pulverised into a fine powder and sieved (106 µm).

Freeze-drying method (FM): The solution of 2-HP-β-CD in water and solution of lumefantrine in methanol were mixed as described in the co-evaporation process, but instead of stirring manually for 1 h, the mixture was agitated with a magnetic stirrer for 24 h to attain thermodynamic equilibrium. The content was frozen at -20°C and then lyophilised in a freeze dryer (CHRIST Beta 1-8 LD plus, Belgium) at -55°C and 6.1 mBar. The resultant product was pulverised into a fine powder and sieved (106 µm).

2.5 Physicochemical Assessment of Solid Complexes

2.5.1 Solubility measurements

Aqueous solubility measurements of lumefantrine and its binary systems with 2-HP-β-CD were investigated by introducing a 40 mg of lumefantrine or the binary system, containing to 40 mg of lumefantrine, into 5 mL aqueous solutions of pH 1.2, composed of 2.0 g/L NaCl and 0.065 M HCl in water to simulate the gastric fluid without enzyme (SGF) according to United State Pharmacopoeia (USP) 2003 all in glass tubes. The above step was replicated with aqueous solution of pH 6.8 composed of 6.805 g/L potassium dihydrogen orthophosphate (KH_2PO_4) and 0.896 g/L sodium hydroxide dissolved in water to simulate the intestinal fluid without enzyme (SIF) (USP 2003). The glass tubes were screw-capped, immersed in a water bath regulated at 37 ± 0.5°C, and intermittently shaken every 6 h until equilibrium was attained. After 60 h, solutions were cooled to room temperature, and 1 mL aliquots of the supernatant liquid were taken, filtered through 0.45 µm membrane filters, appropriately diluted, and assayed for lumefantrine as previously described. Three determinations were performed on each sample and the statistical differences in the mean solubilities in SGF and SIF were tested for significance using a Two-way ANOVA.

2.5.2 Fourier transform- infrared (FT-IR) spectroscopy

FT-IR spectra were generated for standard lumefantrine powder, cyclodextrin, and each of the four preparations of lumefantrine-CD complexes using an FT-IR Cary 630 Agilent technologies (Santa Clara, CA, USA). Each sample was milled into a fine powdery form, loaded into the sample holder and the infrared (IR) scanning was run from 650 to 4000 cm^{-1} to obtain the spectrum for each of the binary

systems and the reference standards of lumefantrine and cyclodextrin.

2.5.3 Powder X-ray diffraction (PXRD) analysis

The crystal structure of the lumefantrine, cyclodextrin and the binary systems were obtained by using Powder X-ray diffraction (PXRD). The structure analysis was carried out by using a Bruker BV 2D PHASER Best Benchtop X-ray diffraction (XRD) analyser with reflection geometry ranging from two thetas (2θ) values of 5° - 70°. Each sample was milled into a fine powdery form, transferred into the sample holder, and operated with a monochromatised CuKα_1 radiation source (λ = 0.15406 nm) at 30 mA and 50 kV. The step size during analysis was 0.005° with a counting time of 5.240 s per step.

2.5.4 Molecular docking and dynamics simulation

A binary inclusion complex was generated with Chem3D of the ChemOffice suite by constructing 3D models for lumefantrine and 2-hydroxypropyl-β-cyclodextrin. The centres-of-mass of both 3D coordinates were subsequently superimposed following which geometric optimisation was performed using the steepest descent algorithm. The optimised structure, representing a single microstate in the coordinate space only, was subsequently subjected to 1 ns molecular dynamics (MD) using the NPT ensemble coupling the system to a 1 bar barostat and 298 K thermostat, and a 2 fs time step. The resulting 1 ns was subsequently analysed with respect to the time-dependent system potential energy, root mean squared deviation (RMSD) and a lumefantrine-CD separation distance. These observables were then plotted to gain insight into the overall energetics and stability of the inclusion complex.

3. RESULTS AND DISCUSSION

3.1 Phase Solubility Study

The phase solubility study carried out permits the assessment of the affinity between lumefantrine and cyclodextrin in water. The profile is needful to determine the stability and nature of the host-guest complexation. The aqueous solubility of lumefantrine was determined with increasing molarities of 2-HP-β-CD. The phase solubility diagram is presented in Fig. 2, and the linearity of the curve implies that the aqueous solubility of lumefantrine is positively correlated with an increase in the concentration of cyclodextrin (R^2 = 0.9835). Higuchi and Connors described this linear relationship as type A_L indicating that a soluble complex is formed [28]. The slope of this plot was found to be 0.0066. It is known that the slope of a phase-solubility diagram that is less than 1, is suggestive of a molar ratio of 1:1 for the complex formation. According to the Higuchi and Connors's phase-solubility model, the apparent stability constant, K_C, represented as $K_{1:1}$, is estimated from the intrinsic aqueous solubility of the drug in the absence of 2-HP-β-CD (the intercept at y-axis) and the slope of the phase solubility diagram.

However, in this study, the slope of the phase-solubility profile is relatively very low which suggestive of a lower complexation efficiency compared to previous reports on halofantrine [30], pyrimethamine [31], and artemether [21]. Complexation efficiency was estimated as 0.18 which can be improved by increasing either the intercept or K_C or both. It has been asserted that the intercept or K_C of CD complexes of weakly basic drugs can be enhanced through the formation of a water-soluble salt with an organic acid such as lactic acid or by adding small amounts of the water-soluble polymer [29,32]. In this study, the $K_{1:1}$ of lumefantrine-2-HP-β-CD was calculated as 243.4 M^{-1}. Previous studies reported that, K_C within the range of 200 - 5000 M^{-1} is acceptable for the formation of a drug-CD inclusion complex to enhance the bioavailability of highly hydrophobic drugs like lumefantrine [25,30-33]. Weak interaction or poor stability is associated with the inclusion complexes with very low K_C values below 200 M^{-1} whereas in cases when the K_C values are greater than 5000 M^{-1}, the drug/CD complexes are very strong and stable, which result in a partial release of the drug from the cavity [34-36]. Thus, the lumefantrine-2-HP-β-CD is expected to have a superior bioavailability profile compared to pure lumefantrine.

3.2 Aqueous Solubility

The aqueous solubility of the lumefantrine-2-HP-β-CD in each binary system is presented in Table 1. The result indicates that the binary systems had a superior aqueous solubility compared to the pure lumefantrine both in pH 1.2 and pH 6.8. The solubility was tested in pH 1.2 and 6.8 to simulate the milieu within the gastrointestinal tract. The baseline pH of gastric acid in the human stomach is within the range of

1.2 to 3.5 and it is kept within the range under normal physiological conditions by the proton pump H^+/K^+ ATPase [37]. On the contrary, the pH of the small intestine is maintained at 5.9 - 6.8 [37,38]. Basic drugs are ionisable under acidic conditions and will furnish more ionised species at a low pH (1.2). Therefore, the aqueous solubility of lumefantrine is comparatively higher at pH 1.2 than in pH of 6.8. The results (Table 1) indicate that extent of enhancement of solubility of the drug was dependent on the method of preparation of the complex and the order was Freeze drying method (FM) > Co-evaporation method (CM) > Kneading Method KM > Physical mixture (PM). The differences in the aqueous solubilities between these binary systems in both the SGF and SIF are significant ($p < 0.05$). Enhancement of aqueous solubility of lumefantrine observed in the present study is in agreement with other previous studies on cyclodextrin complexation of halofantrine, pyrimethamine and simvastatin [30,31,39]. The co-evaporated and freeze-dried systems had approximately a 3-fold and 11-fold increase in the aqueous solubility at pH 1.2 and pH 6.8, respectively, compared to the pure lumefantrine. Lumefantrine is expected to be absorbed mainly in the small intestine in the unionised form. Therefore, an 11-fold increase in solubility of the binary systems in the SIF indicates that the absorption of lumefantrine might be significantly improved when administered as a CD inclusion complex. This finding is useful considering the effect of poor aqueous solubility of lumefantrine on its oral absorption and bioavailability.

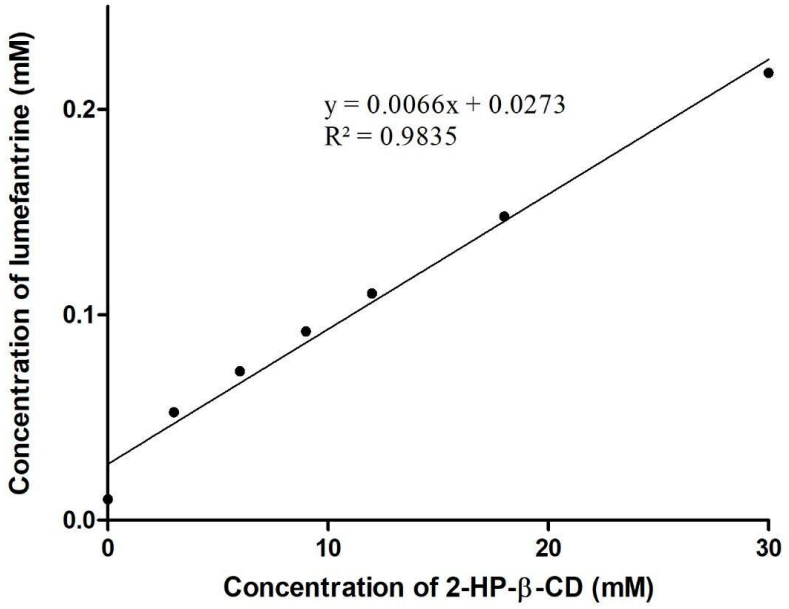

Fig. 2. Phase solubility profile of lumefantrine in aqueous 2-hydroxypropyl-β-cyclodextrin solution

Table 1. Solubility of lumefantrine and its binary systems with 2- hydroxypropyl-β-cyclodextrin

Compound	Aqueous solubility (mg/ml)	
	*SGF	**SIF
Pure Lumefantrine	0.95 ± 0.02[a]	0.09 ± 0.01[a]
Physical mixture	1.84± 0.24[b]	0.26 ± 0.01
Kneaded system	2.01± 0.23[c]	0.35± 0.01
Co-evaporated system	2.98 ± 0.28[d]	0.81± 0.03[b]
Freeze-dried system	3.18 ± 0.37[c]	0.98± 0.03[c]

**SGF is a simulated gastric fluid (pH 1.2); **SIF is a simulated intestinal fluid (pH 6.8). The values are represented as mean ± SD values, means with different alphabets are significantly different from each other (p<0.05)*

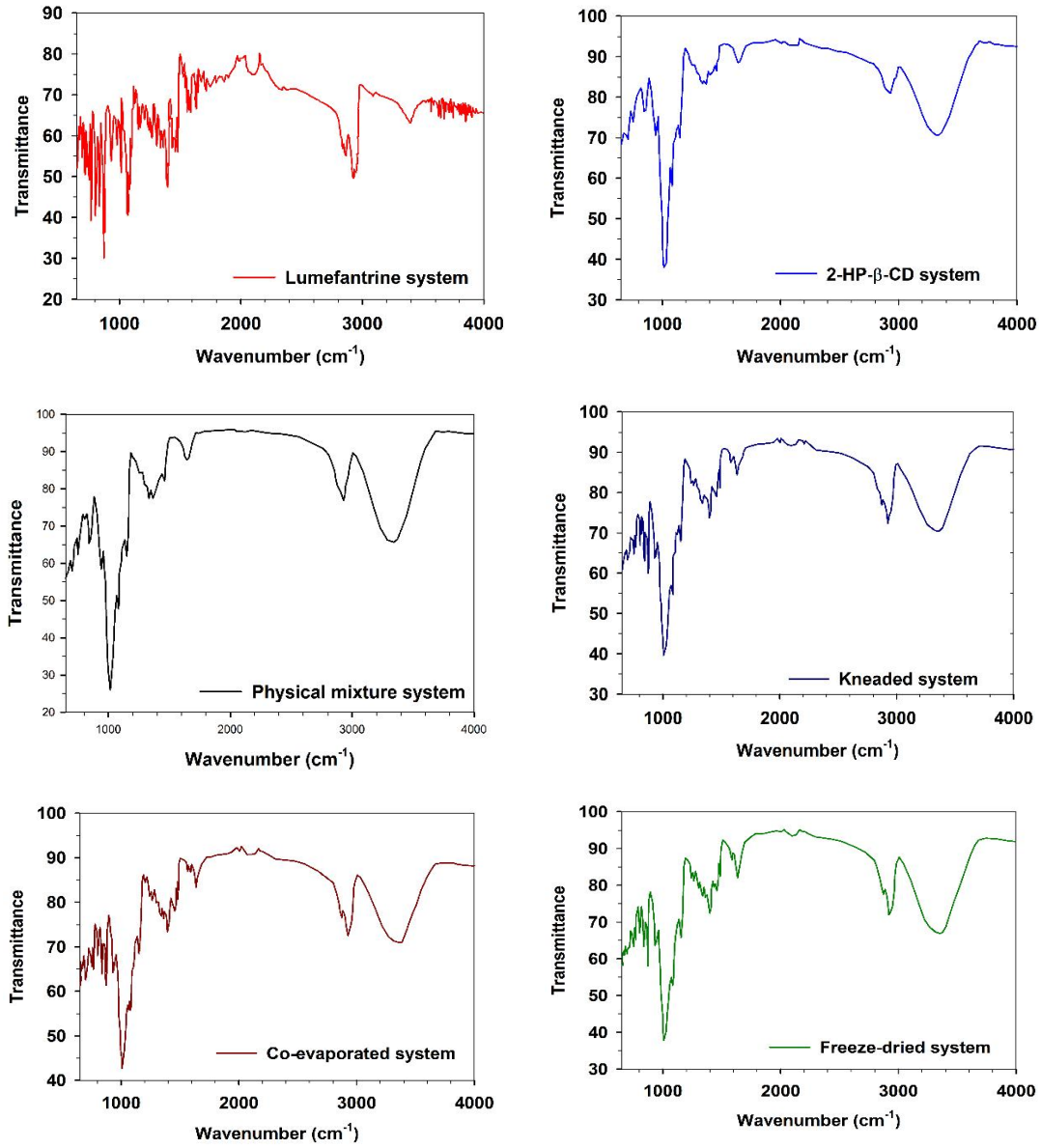

Fig. 3. FT-IR Spectra of lumefantrine (a), 2-HP-β-CD (b), physical mixture (c), kneaded (d), co-evaporated (e) and freeze-dried (f) systems

3.3 FT-IR Spectroscopy

The spectrum of lumefantrine in Fig. 3A shows a characteristic peak at 3399.0 cm^{-1}(OH stretching vibration) with possible interactions with the lone pair of electrons on the tertiary nitrogen and the OH group. The O-H stretching vibration on lumefantrine is diagnostic because of the high tendency of formation of intramolecular hydrogen bond due to the co-occurrence of the tertiary amine group and a secondary hydroxyl group. On the other hand, Fig. 3B indicates that a prominent broad peak characterises the 2-HP-β-CD molecule at 3332.4 cm^{-1} (OH stretching vibration). The 3399.0 cm^{-1} peak attributed to the hydroxyl group in lumefantrine was suppressed in the binary complexes. However, the OH peaks appeared in the binary systems at 3349.0 cm^{-1}, 3362.1 cm^{-1}, 3363.9 cm^{-1} and 3345.3 cm^{-1} respectively in the physical mixture, kneaded, co-evaporated and freeze-dried systems. These peaks appeared broader and resembled the OH peak found in the pure cyclodextrin sample suggesting an interaction of

the drug with 2-HP-β-CD molecule possibly, due to the formation of weak hydrogen bonds. The broadening of the peak in the binary systems in comparison to that of the pure drug was probably as a result of the restriction of bending and stretching vibration of the lumefantrine molecule due to the 2-HP-β-CD cavity.

The respective peaks in the IR spectrum for pure lumefantrine at 2926.0 cm^{-1} and 2946.5 cm^{-1}, 2870.1 cm^{-1} and 2840.2 cm^{-1} are attributed to C-H stretching vibration; 1586.6 cm^{-1}, 1507.7 cm^{-1}, 1559.8 cm^{-1} and 1541.3 cm^{-1} are attributed to aromatic nuclei, while the peaks at 1097.7 cm^{-1} and 1013.3 cm^{-1} are respectively accredited to C-Cl stretching vibration and C-N stretching vibration. The C-H stretching vibration peak (2926.0 cm^{-1}, 2946.5 cm^{-1}, 2870.1 cm^{-1}, and 2840.2 cm^{-1}) were suppressed or removed in the binary systems which may be due to formation of a hydrophobic bond. The central cavity of 2-HP-β-CD offers a hydrophobic milieu to host lipophilic lumefantrine molecules suggesting hydrophobic interactions as the attractive nonpolar interaction for the formation of inclusion complex [25]. In the physical mixture, only one of the C-H peaks observed in the pure drug was suppressed, indicating that the physical mixture formed a partial interaction of the pure drug and 2-HP-β-CD.

Significant changes were recorded in the IR spectrum of the inclusion complexes prepared by the kneading, co-evaporation and freeze-drying method. Almost all peaks of lumefantrine were smoothened, indicating a robust physical interaction between pure drug and 2-HP-β-CD. Other peaks that might associate with complex formation are 1647.5 cm^{-1} (H-O-H bending), 1023.2 cm^{-1} (C-O-C). The presence of H-O-H suggests the possibility of dipole interaction within the central cavity of the host molecules. Besides, the broad peak of 2-HP-β-CD at 1647.5 cm^{-1} due to water of crystallisation narrowed down in the binary systems, and this is because of the replacement of water molecules by lumefantrine inside the 2-HP-β-CD cavity indicating the formation of the inclusion complex in the solid-state.

The fingerprint regions of the IR spectra produced by the pure lumefantrine, 2-HP-β-CD and binary systems are characteristically different. The differences might be attributed to the possibility of interactions between the two molecules. Such an interaction either results in complete or partial suppression of absorption bands within the fingerprint region. Also observed is the slight shifting of the peak positions shown for binary systems in the fingerprint region relative to that of parent compounds. This further provides evidence of the interaction between lumefantrine and 2-HP-β-CD. It is worth noting that no new peak was observed in all the binary systems of lumefantrine-2-HP-β-CD, indicating non-covalent interaction in the inclusion complex.

3.4 PXRD Studies

The PXRD patterns of lumefantrine, cyclodextrin and the complexes from the physical mixture, kneaded, co-evaporated and freeze-dried binary systems are presented in Fig. 4. In the PXRD pattern of lumefantrine as shown in Fig. 4a, the peaks are sharp and narrow, suggesting good crystallinity of the sample. In contrast, the peaks for cyclodextrin are weak and broad, indicating its nature as an amorphous compound [40]. PXRD is a useful technique for detecting cyclodextrin inclusion complexes in solid states as the height and sharpness of each peak are proportional to the crystallinity of a given sample. A distinct PXRD pattern of the binary system from the patterns presented by either lumefantrine or cyclodextrin is an indication of the formation of a true inclusion complex. The relative degree of crystallinity (RDC) of a complex to the pure sample is estimated as the ratio of certain representative peak heights of the binary systems to that of the pure sample.

Table 2. Relative degree of crystallinity of the binary systems of lumefantrine-2-hydroxypropyl-β-cyclodextrin

Compounds	PI	RDC (PI_{BS}/PI_{PS})
Lumefantrine (RS)	50000	1
Physical mixture	45000	0.9
Kneaded BS	30000	0.6
Co-evaporated BS	28000	0.56
Freeze-dried BS	17500	0.35

PI represents the peak intensity at 23°, RDC is the relative degree of crystallinity, RS is the pure sample of lumefantrine and BS is the binary system

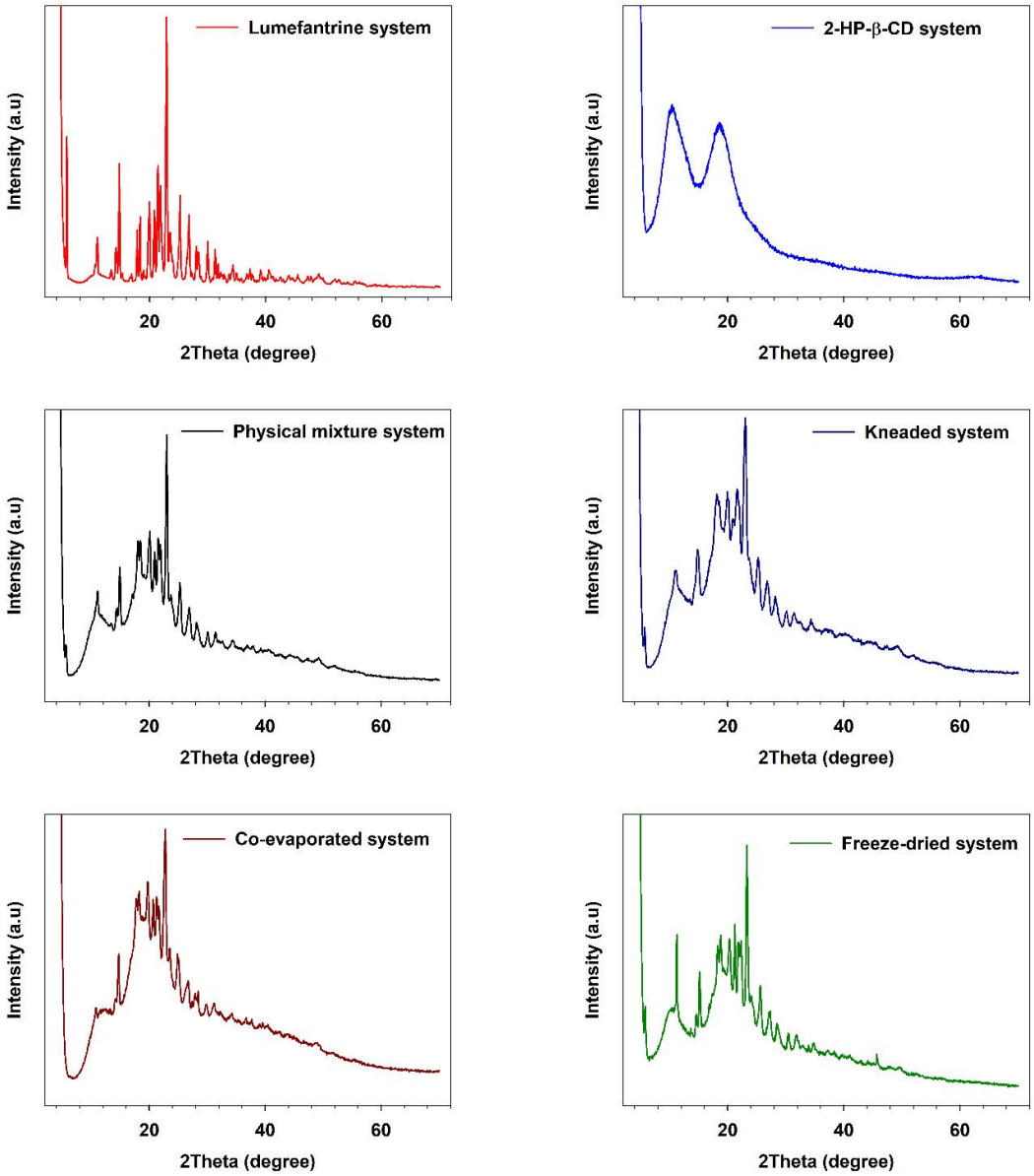

Fig. 4. PXR diffractograms of lumefantrine (a), 2-HP-β-CD (b), physical mixture (c), kneaded (d), co-evaporated (e) and freeze-dried (f) system

The pure drug at 23° was used for the estimation of the RDC values. The peak height of lumefantrine was taken as the reference peak height and used to calculate the RDC for each of the binary systems presented in Table 2. Results show a reduction in the RDC values for the physical mixture, kneaded, co-evaporated and the freeze-dried binary systems, indicative of a proportionate decrease in crystallinity. Among the binary systems, the complex prepared by the freeze-drying technique has the least value of RDC, indicating that it has the highest degree of amorphisation. In addition, by visual observation of the diffractograms region situated between 5° and 30°, the intensity and sharpness of the peaks were compared. The diffractogram of lumefantrine showed more intense and sharp peaks across the selected region, indicating and confirming its crystalline nature. The cyclodextrin, however, had two broad peaks situated between 5° and 22° and no considerable peak was

observed from 22° to 70°. This PXRD pattern is typical for 2-HP-β-CD reported in the literature with broad peak(s) and many undefined, diffused peaks with low intensities [41]. This pattern represents the amorphous nature of 2-HP-β-CD.

Compared to the diffractogram of lumefantrine, some of the peaks in PXRD of the binary systems were diminished, and some were suppressed. This is an indication of the inclusion of lumefantrine in the lipophilic central cavity of 2-HP-β-CD, resulting in a reduction in the crystallinity of the drug. In addition to the diminution of the peaks, the PXRD patterns of the four binary systems also showed a varying number of peaks. For example, for the physical mixture (Fig. 4c), prominent peaks are still present. However, with reduced intensity and this lends more credence to the formation of a partial inclusion complex in this binary system. The number and intensities of these peaks were further reduced in the kneaded binary systems indicating a more efficient complex formation with this technique compared to the physical mixture binary system. The diffractograms of the co-evaporated (Fig. 4e) and freeze-dried (Fig. 4f) binary systems showed still fewer peaks with much-reduced intensities compared to the patterns for the kneaded, physical mixture or pure lumefantrine. Thus, the patterns of PXRD suggest that the freeze-drying technique resulted in the most efficient inclusion complex formation, and this is similar to the finding with halofantrine complexation with 2-HP-β-CD [30].

3.5 Molecular Dynamics Simulation

Following steepest descent geometric optimisation, the lumefantrine-CD inclusion complex appeared to be stable *prima facie* (Fig. 5). But since the resulting structural assembly likely represents local energy minimal on the potential energy landscape (PES), a more involving conformational sampling is required to more extensively probing the PES from which profile the stability of the system may be inferred. Short MD simulation ranging from picosecond to nanosecond timescale is judged sufficient to examine the conformational dynamics of the relatively small-sized inclusion complex compared with macromolecular systems composed of thousands of atoms. A plot of both the time-dependent potential energy reveals a binary complex that is stable over the entire trajectory (Fig. 6). The separation distance over time between lumefantrine and cyclodextrin also revealed a stably bound complex stabilised by a combination of hydrogen bond formation and van der Waals attraction. So based both on thermodynamic quantity analysed (the potential energy) and the distance between the two partners, the binary system between lumefantrine and 2-HP-β-CD is stable.

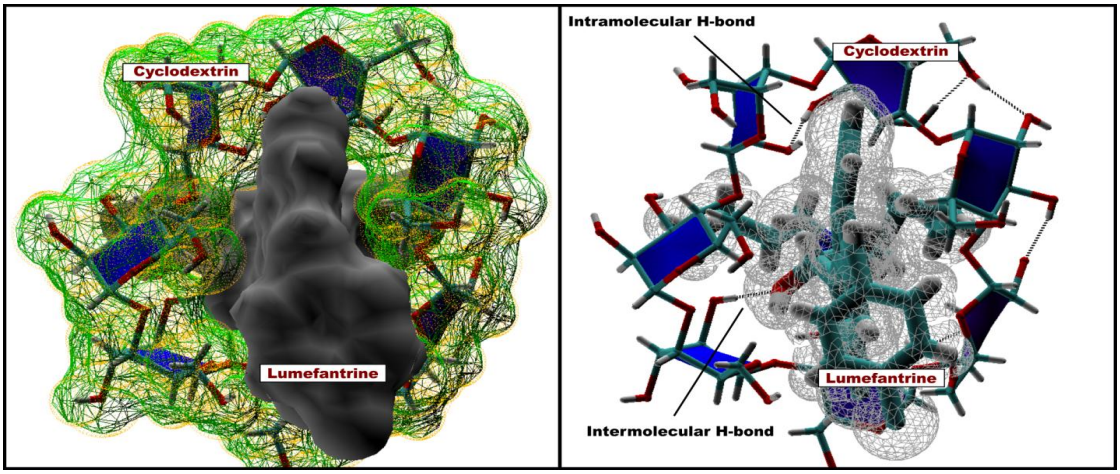

Fig. 5. Energy minimised 3D structure of the lumefantrine-2-HP-β-CD binary system. Lumefantrine is shown (left: with gray solid surface representation; right: transparent surface superimposed on its stick representation) inserted stably within the core of cyclodextrin (left: shown with green transparent surface representation superimposed on its stick representation; right: stick thinner representation). Both intermolecular hydrogen bonds between the hydroxyl groups of lumefantrine, and intramolecular hydrogen bond involving interacting units within cyclodextrin are highlighted

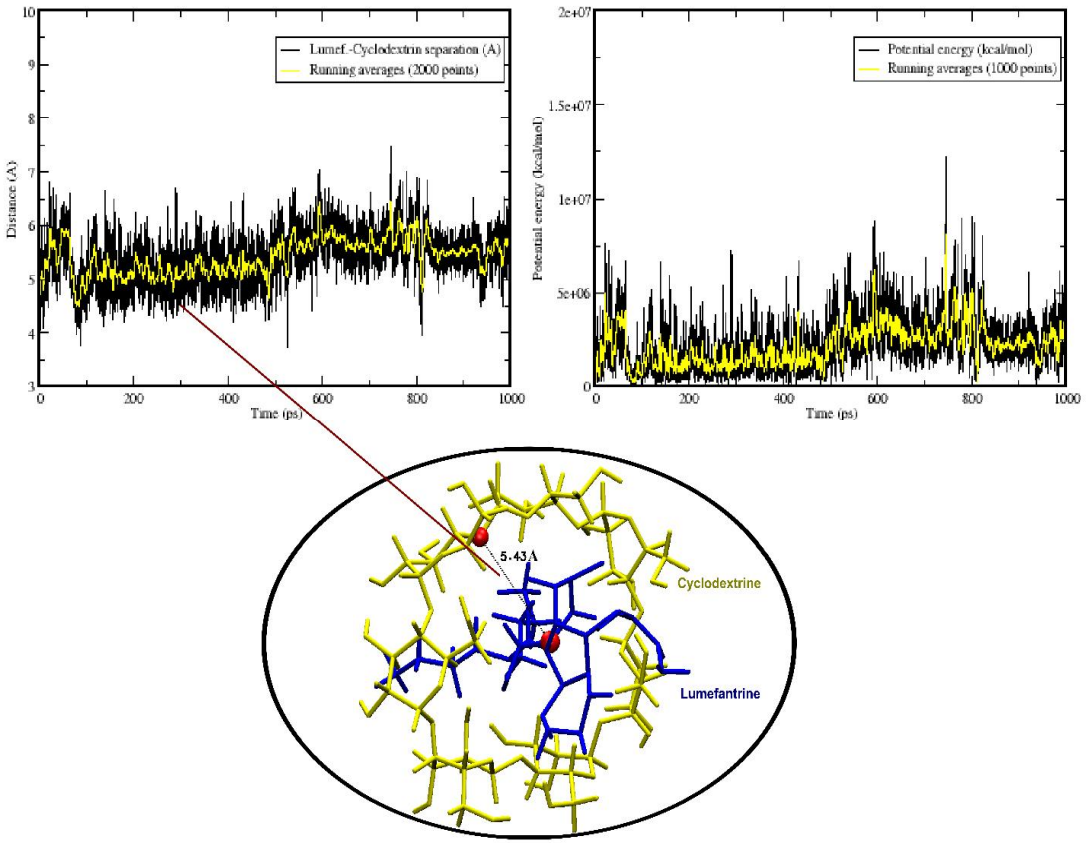

Fig. 6. Separation distance between lumefantrine and cyclodextrin (*top-left*) and system potential energy (*top-right*) plotted over MD simulation time revealed a structurally stable binary inclusion complex. The black plot indicates instantaneous values while the yellow lines show running averages over 2000 and 1000 data points, respectively. A graphical representation is presented in the lower panel to illustrate the reference distance employed

4. CONCLUSION

Inclusion complexes between lumefantrine and 2-HP-β-CD were successfully prepared and confirmed to be stable at 1:1 molar ratio. All the binary systems significantly increased the aqueous solubility of the drug. Still, the systems prepared by the freeze-drying process were the most efficient as it resulted in about 3- and 11-fold increases in drug solubility in SGF and SIF, respectively, compared to the pure drug. Our findings suggest that aqueous solubility of lumefantrine could be improved through cyclodextrin complexation, and there is a potential to apply cyclodextrin complexation of lumefantrine towards improving the therapeutic efficacy of this highly lipophilic drug.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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