Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 3, 2013

Research Article

DISSOLUTION ENHANCEMENT OF ATOVAQUONE THROUGH CYCLODEXTRIN COMPLEXATION AND PHOSPHOLIPID SOLID DISPERSION

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Received: 15 Apr 2013, Revised and Accepted: 03 Jun 2013

ABSTRACT

Objective: The main aim of this study was to enhance the dissolution rate of a poorly water-soluble antiprotozoal drug, Atovaquone, by fabricating its cyclodextrin (CD) complex and Phospholipid solid dispersion.

Methods: CD complex was prepared employing Hydroxypropyl beta cyclodextrin (HPβCD) and beta cyclodextrin (βCD) using Rotary Evaporation (Rot) and Kneading (KN) methods. Phospholipon 90H, Lipoid S100 and Lipoid EPCS were used to prepare Phospholipid Solid dispersion using solvent evaporation method. These formulations were characterized by *in vitro* dissolution, Differential Scanning Calorimetry (DSC), X-ray diffraction (XRD), Fourier Transform Infrared (FTIR) spectroscopy and Scanning Electron Microscopy (SEM).

Results: On the basis of *in vitro* dissolution, βCD was superior to HPβCD and Lipoid S100 was more effective than Phospholipon 90 and Lipoid EPCS. Solid state characterization revealed amorphization of Atovaquone with βCD while no change in crystallinity was seen with Lipoid S100.

Conclusion: It was interesting to note that at lower carrier concentration, the solid dispersion of Atovaquone with Lipoid S100 presented a dissolution profile similar to its complex with βCD at higher carrier concentration.

Keywords: Atovaquone; Hydroxypropyl beta cyclodextrin (HPβCD); Beta cyclodextrin (βCD); Phospholipon 90H; Lipoid S100; Lipoid EPCS.

INTRODUCTION

Atovaquone is a unique naphthoquinone with broad-spectrum antiprotozoal activity. It is effective for the treatment and prevention of *Pneumocystis carinii* pneumonia (PCP), Malaria and Babesiosis [1]. In spite of this wide spectrum of pharmacological activity, its use in pharmaceutical field is limited because it suffers from low aqueous solubility (less than 0.2µg/ml) and belongs to class II of the biopharmaceutical classification system (BCS). As a result it exhibits poor dissolution and insufficient oral bioavailability [2, 3]. Thus, an efficient oral formulation of Atovaquone with an enhanced dissolution rate and hence, an improved bioavailability is highly desired.

Reducing the particle size was an alternative to improve the oral bioavailability of Atovaquone, but was associated with some major limitations. The use of conventional jet milling method was incapable of reducing the particle size below 6 µm without causing fracture of the crystal structure. Complexity of the method resulting in longer processing time coupled with high cost of the equipment and its maintenance were the drawbacks associated with the microfluidization technique [4]. As a result, there is a need for alternative methods to increase aqueous solubility of Atovaquone which in turn would lead to increased bioavailability.

Amongst the various methods employed to improve the solubility and bioavailability of poorly water soluble drugs, solid dispersions and cyclodextrin (CD) inclusion complexes are the most frequently used [5, 6].

Amongst solid dispersions, lipid based carriers have demonstrated a higher success rate in enhancing the bioavailability of Class II drugs. One such promising group of carrier is Phospholipid. They possess the advantage of improving the dissolution rate of poorly soluble drugs at a low carrier concentration [7-11]. Cyclodextrins are frequently used in drug formulations as solubility enhancers because of their ability to form water-soluble inclusion complexes with poorly water-soluble drugs [12, 13]. β-Cyclodextrin (βCD) and its hydroalkyl derivative, hydroxypropyl-β-cyclodextrin (HPβCD) were selected as they are the most promising and widely employed CD derivatives [14, 15].

The aim of this work was to investigate the influence of cyclodextrin complexation and Phospholipid solid dispersion on the dissolution rate and physicochemical characteristics of Atovaquone.

MATERIALS AND METHODS

Materials

Atovaquone was a gift from USV Pharmaceuticals (Mumbai). Phospholipon 90H, Lipoid S100 and Lipoid EPCS were kindly supplied by Lipoid (Ludwigshafen, Germany). Betacyclodextrin and Hydroxypropyl betacyclodextrin was a gift from Gangwal Chemicals (Mumbai, India). All other chemicals and solvents used in the study were of analytical reagent grade.

Preparation of Cyclodextrin Complex

Phase Solubility Study

The phase–solubility of Atovaquone was conducted according to Higuchi and Connors [16]. An excess amount of Atovaquone (10 mg) was added to 10ml of deionized water containing increasing amounts of βCD / HPβCD (ranging from 0 to 0.016 M) in amber coloured vials. The resulting mixture was equilibrated by placing the vials on a rotary shaker (RS-12R DX, Remi, Mumbai, India) at 25^{0}C for 48hrs. At equilibrium aliquots were withdrawn, filtered through 0.45 µm cellulose acetate membrane filters and suitably diluted. Concentration of Atovaquone was determined spectrophometrically at 276 nm.

A plot of total molar concentration of Atovaquone against the total molar concentration of CDs gave phase solubility diagrams from where the apparent stability constant K_s was calculated according to the following equation:

$$K_s = \frac{\text{Slope}}{S_0\,(1 - \text{slope})}$$

Where, S_0 is the solubility of Atovaquone in absence of CD.

Preparation of Inclusion Complexes

The CDs used for the preparation of inclusion complexes were βCD and HPβCD. Atovaquone-CD inclusion complex was prepared in 1:1 molar ratio by using two different methods namely by (1) Rotary evaporation method and (2) Kneading method

Rotary Evaporation Method (Rot)

CD, βCD or HPβCD was accurately weighed and dissolved in distilled water in a Round Bottom Flask (RBF). The solution was held at 60-

65°C and stirred on a magnetic stirrer (Remi, Mumbai, India). Atovaquone was added to this solution followed by addition of 25% ammonia solution and 2-Propanol to aid in dissolution of drug. The solution was then flash dried under vacuum at 85-90°C using Rotary Flash Evaporator (PBU-6, Superfit, Mumbai, India). The powdered complex obtained was then passed through sieve no.80 and stored away from light [4].

Kneading method (KN)

Required quantity of CD was wetted in a ceramic mortar with hydro-alcoholic solution (1:1 v/v) until a paste was obtained. The required amount of Atovaquone was then slowly added and the slurry was kneaded for about 60min. During this process an appropriate quantity of hydro-alcoholic solution was added in order to maintain a suitable consistency. The paste was dried in a vacuum oven (Metalab, India) and then sieved through 80 mesh sieve and stored away from light.

Physical Mixture (PM)

A physical mixture consisting of Atovaquone and βCD or HPβCD in 1:1 molar ratio was prepared by homogeneously blending in a mortar for 15 min.

Preparation of Phospholipid solid dispersion

Solid dispersion of Atovaquone and phospholipids was prepared by solvent method, using chloroform as the solvent [17]. Weighed amounts of phospholipid and Atovaquone were added to the solvent in a RBF and dissolved with gentle stirring. The solvent was then removed at 40^{0}C using a Rotary Flash Evaporator. Further drying was accomplished in a vacuum oven to ensure complete removal of solvent. Solid dispersions were passed through 80-mesh sieve and stored away from light.

***In vitro* Dissolution Studies**

Dissolution studies were conducted using USP Type II (paddle) Dissolution Apparatus (TDT-08L, Electrolab, Mumbai, India). The dissolution medium consisted of 900 ml Phosphate buffer containing 40% Iso propyl alcohol (IPA) at pH 7.4. The temperature was maintained at 37 ± 0.5^{0}C with paddle speed set at 50 rpm throughout the experiment. Sieved samples (250 mg of Atovaquone or Atovaquone equivalent formulation) were dispersed on the surface of the dissolution medium at the beginning of the study. An aliquot of the released medium (5 ml) was withdrawn at pre-determined time intervals and an equivalent amount of fresh dissolution media pre-warmed to 37°C was replaced. The aliquots were filtered after withdrawal and analyzed by UV spectrophotometer (V-550, Jasco, USA) at 277 nm.

Solid-state Characterization

Differential Scanning Calorimetry (DSC)

DSC analysis was performed using Differential Scanning Calorimeter (Metller Toledo, DSC 821e, USA). Accurately weighed samples were placed in open, flat bottom, aluminum sample pans. Thermograms were obtained by heating the sample at a constant rate of 10^{0}C/minute. A dry purge of nitrogen gas (20ml/min) was used for all runs. Samples were heated from 25^{0}C – 250^{0}C. The instrument was calibrated using Indium (melting point, 156.61^{0}C; enthalpy of fusion 28.71 J/g).

Powder X-ray Diffraction (PXRD)

The XRD patterns of the powder formulation were obtained using X-ray diffractometer (Xpert PRO MPD, PANlytical, Netherlands). The measuring conditions were as follows: Cu K-alpha radiation, 45 kV voltage; and 40 mA current with X'celerator detector (λ = 1.5405 angstroms). All samples were run at 1^0 (2h) min^{-1}from 10^0 to 70^0 (2h). Samples (~50 mg) were run as a smear mount on a glass slide on which a thin layer of grease was applied. The excess sample was removed such that only a monolayer was examined.

Fourier Transform Infrared (FTIR) spectroscopic analysis

The Infrared spectra (IR) spectra were obtained using Fourier Transform Infrared spectrophotometer (IRAffinity 1, Shimadzu, Japan) by the conventional KBr pellet method. Data was collected over a spectral region from 4000-500 cm^{-1} and the resolution was 4cm^{-1}.

Scanning Electron Microscope (SEM)

The surface morphology of the powders was investigated by SEM (Zeiss, LS10 EVOSEM). Samples were fixed on a brass stub using double-sided adhesive tape and were made electrically conductive by coating with a thin layer of gold and SEM images were recorded at variable accelerating voltage from 0.1kv to 30 kv.

RESULTS AND DISCUSSION

Phase Solubility Study

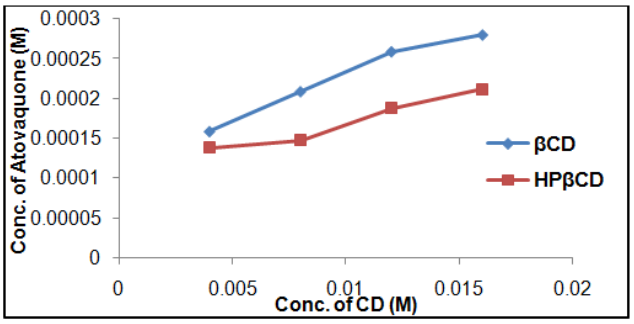


Fig. 1: It shows Phase solubility diagram of Atovaquone-CD system in water

The Atovaquone-CD solubility curve is presented in Fig.1. The apparent solubility of Atovaquone increased linearly as a function of CD concentration, over the entire concentration range studied. Linearity was characteristic of the AL-type system [14]. Furthermore, the slope values were lower than 1.0, indicating that inclusion complexes in a molar ratio of 1:1 between the guest (Atovaquone) and host (CDs) molecules were formed. The K_s value was found to be 104.07M^{-1} for βCD and 66.44 M^{-1} for HPβCD suggesting that βCD formed a more stable inclusion complex with Atovaquone than HPβCD. This phenomenon could be due to the steric hindrance of the hydroxypropyl groups of HPβCD, which can hamper the inclusion of guest molecules within the CD cavity [18].

***In vitro* Dissolution Studies**

Atovaquone-CD complex

Although phase solubility studies are of great importance from the theory point of view, in practice the most informative solubility method is the dissolution test [18]. As seen in Fig. 2 and 3, Atovaquone showed a low dissolution profile indicative of its poor solubility and wettability. The dissolution of Atovaquone from PM with CDs was slightly higher than the pure drug. This may be attributed to improved drug wettability due to the presence of hydrophilic CD which is capable of reducing the interfacial tension between poorly soluble drug and dissolution medium. Furthermore, in early stages of the dissolution process, CD molecules may operate locally on the hydrodynamic layer surrounding the particles of Atovaquone, resulting in an in situ inclusion process and thus producing a rapid increase in the amount of dissolved drug [19-22].

Both βCD and HPβCD enhanced the dissolution rate of Atovaquone in comparison to their respective PMs. Complexation of Atovaquone with βCD gave a 1.9 fold rise in the dissolution rate whereas with HPβCD a 1.4 fold rise in dissolution rate of Atovaquone was seen. This increase in dissolution rate of Atovaquone might be due to several reasons such as the formation of soluble inclusion complex, amorphization of the drug and consequently solubility increase, better wettability and reduction of particle size [23].

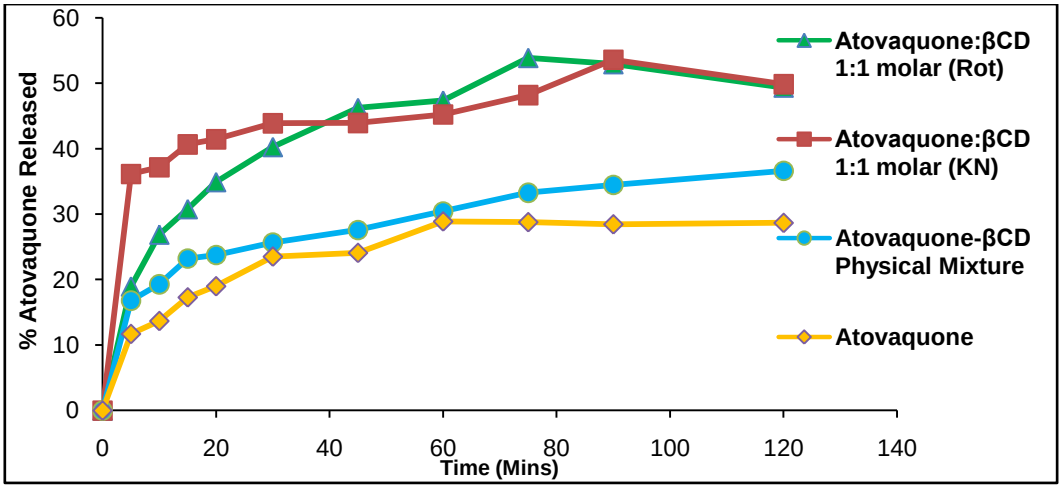


Fig. 2:It shows Dissolution profile of Atovaquone and βCD complex

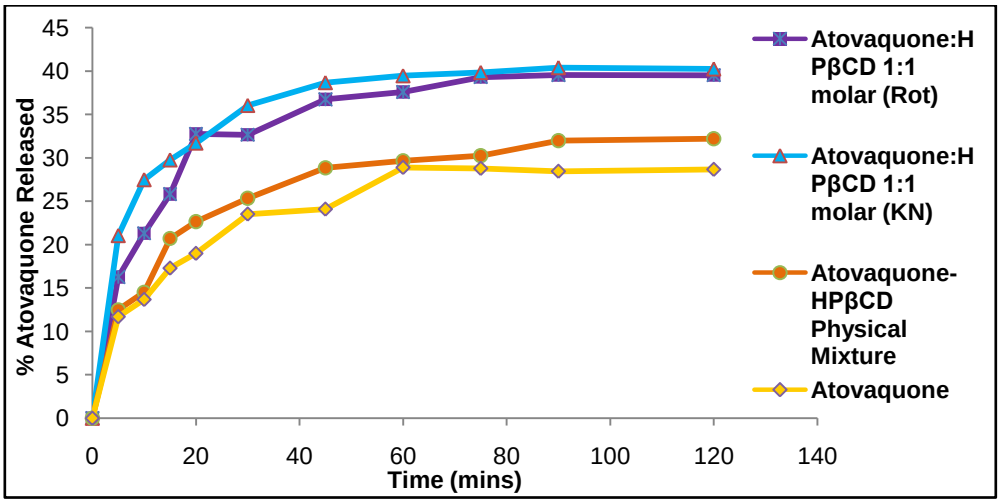


Fig. 3: It shows Dissolution profile of Atovaquone and HPβCD complex

In accordance with the phase solubility study, dissolution studies also indicated the superiority of βCD over HPβCD in enhancing the dissolution rate of Atovaquone.

When the dissolution profiles of Atovaquone-βCD inclusion complex prepared by Rot and KN were compared (Fig.2) it was seen that both gave a similar rise in dissolution rate at the end of 120 mins. However, with KN the enhancement was achieved in a shorter time span in comparison to Rot as indicated by their Initial Dissolution rate (IDR). IDR is defined as the amount of drug released in 5 mins. As compared to pure drug, KN gave a 3.1 fold rise in IDR while Rot gave a 1.6 fold rise in the same suggesting the superiority of KN method over Rot in preparation of Atovaquone-βCD inclusion complex.The reason for this rise in IDR is discussed in section given ahead.

Atovaquone-Phospholipid Solid Dispersion

In order to identify the most potent dissolution rate enhancer for the model drug, the 10:1 (drug-to-carrier ratio, w/w) binary solid dispersions of Atovaquone with varying carrier phospholipids such as Phospholipon 90H, Lipoid S100 and Lipoid EPCS were initially prepared and subsequently evaluated in terms of their dissolution performance [11].

As presented in Fig.4, it is clearly seen that regardless of the carrier employed, the dissolution rate of drug from solid dispersion was higher when compared to that of control Atovaquone. This may be attributed to the ability of Phospholipids to increase the wettability of hydrophobic drug particles. In addition, Phospholipids form myelinic structures (liposomes) on hydration which sequester substantial quantities of drug in the lipidic compartment of liposomes formed which accounts for rapid IDR and increase in the amount of drug dissolved at the end of 120 mins [7,8].

The analysis of IDR and limiting concentrations after 120 min yielded by the solid dispersion revealed that Phospholipon 90H had minimal effect on the dissolution rate of Atovaquone. This may be credited to its high phase transition temperature (Tc) of about 55°C. As a result under experimental conditions (37^{0}C) Phospholipon 90H would have remained predominantly in the solid crystalline state showing little effect on the dissolution kinetics of the drug. On the other hand Lipoid S100 and Lipoid EPCS both have Tc lower than 37 ^{0}C and thus experienced the phase transition at the experimental temperature and consequently demonstrated utmost enhancing effect on the dissolution rate of the model drug [11, 24].

Among all the phospholipids used, Lipoid S100 exerted the greatest effect on both the rate and extent of dissolution of Atovaquone. In order to evaluate the effect of Lipoid S100 weight fraction on the dissolution rate of Atovaquone, solid dispersions with varying drug-to-carrier ratios of 5:1, 10:1 and 15:1 w/w were prepared and evaluated in terms of their dissolution performance.

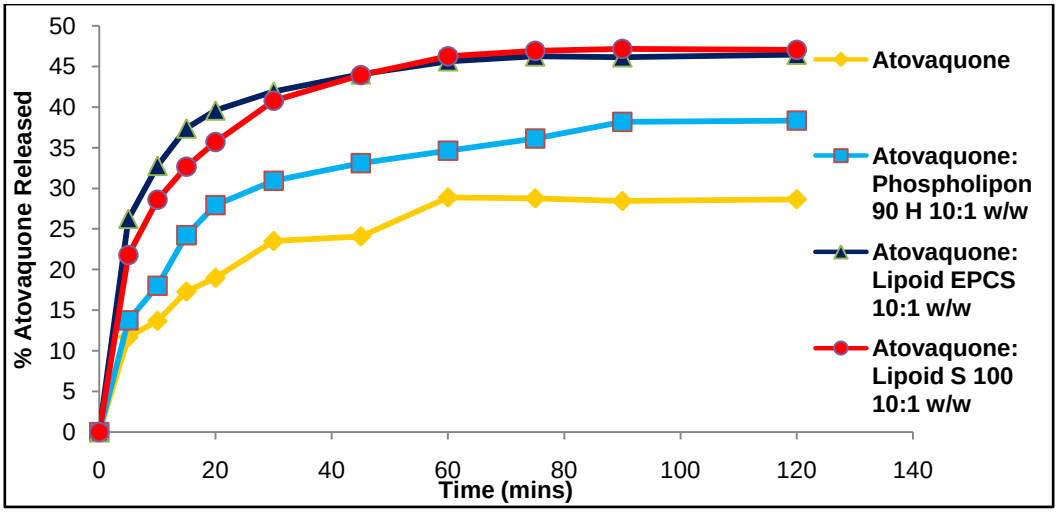


Fig. 4: It shows Dissolution profile of Atovaquone and various phospholipid solid dispersions of Atovaquone

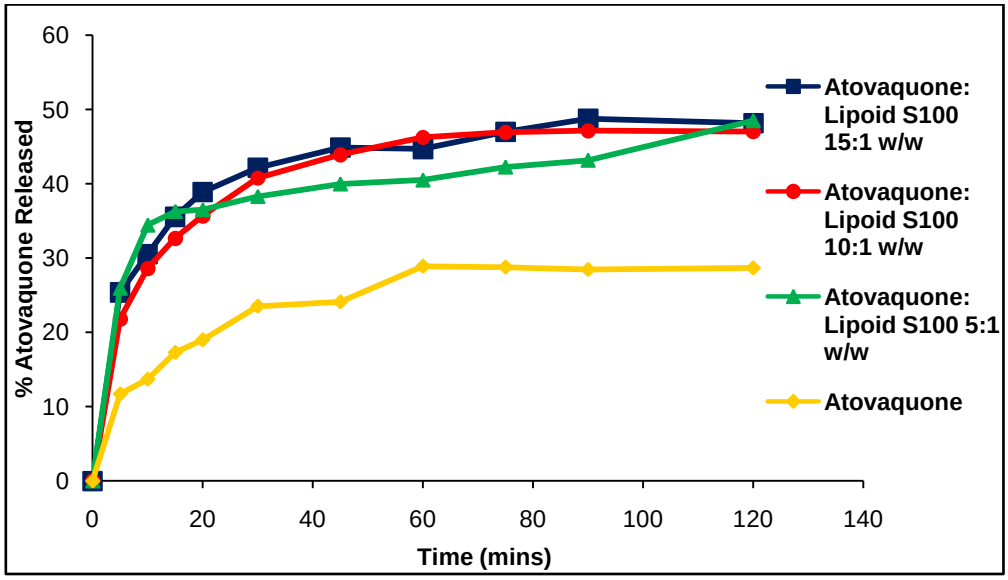


Fig. 5: It shows Dissolution profile of Atovaquone and Atovaquone-Lipoid S100 solid dispersion at different ratios

Fig.5 shows that incorporation of low amount of Lipoid S100 at 15:1 drug-to-carrier ratio w/w gave 2.2 fold enhancement in IDR and 1.7 fold enhancement in dissolution rate of Atovaquone. On further increasing weight fraction of Lipoid S100 in the formulation, no additional effect on the dissolution rate enhancement was seen. This is interpreted to mean that there is a limit to the quantity of Lipoid S100 that can be included and that the excess beyond this limit is deposited on the crystal surfaces and plays no role in the dissolution process [8].

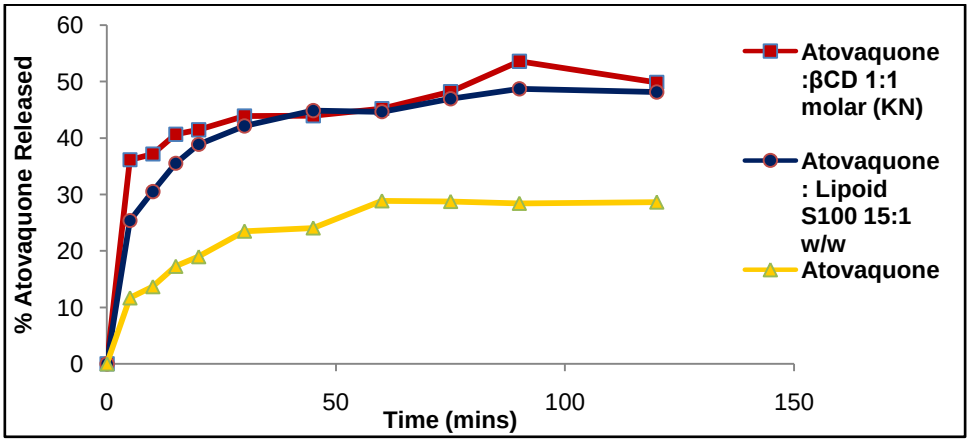


Fig. 6: It shows Comparison of dissolution profile of Atovaquone from solid dispersion and βCD complex

Comparison of Cyclodextrin complexation with Phospholipid Solid dispersion

Fig.6. presents the dissolution profile of Atovaquone from Lipoid S100 solid dispersion (15:1 drug-to-carrier ratio w/w) and βCD complex (1:1 molar ratio) prepared by KN. It can be seen that both the carriers had a similar effect on dissolution profile of Atovaquone. Further it is interesting to note that in their respective formulations, Lipoid S100 is effective at carrier concentration of 6.25% w/w, while βCD is required at 75.61% w/w to show a similar release profile suggesting the superiority of Lipoid S100 solid dispersions over complexation with βCD in enhancing the dissolution rate of Atovaquone.

Solid-state characterization

DSC

DSC thermograms of Atovaquone, Lipoid S100 and βCD are presented in Fig.7. Atovaquone showed a melting endotherm peak at 220.46° C indicative of its crystalline anhydrous state. Broad peak was observed for Lipoid S100 around 50 to 220°C. The thermogram of βCD showed an intense, broad endothermal band, in the 80–120° C range with a peak at 103.63°C .The broad endothermic peak is related to dehydration of water molecules that bind to CD [21, 22, 25].

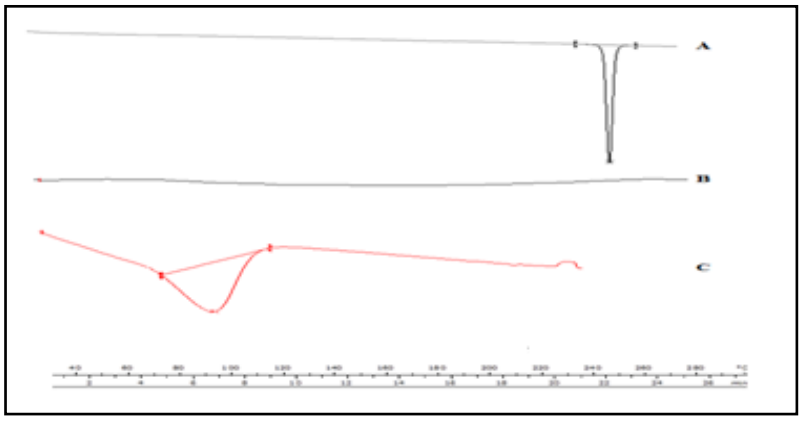


Fig. 7: It shows DSC thermograms of [A] Atovaquone [B] Lipoid S100 [C] βCD

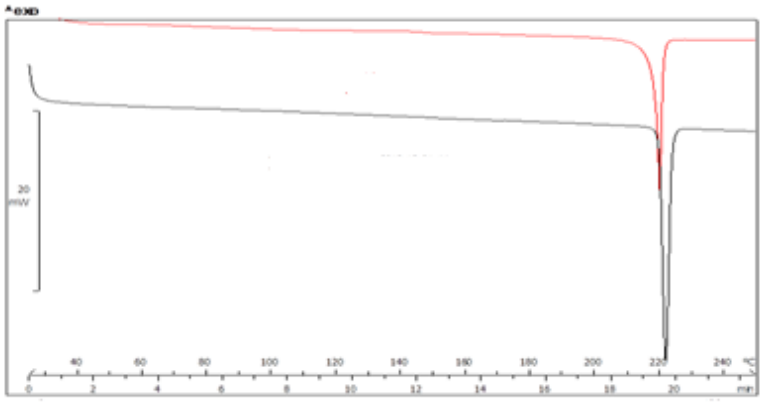


Fig. 8: It shows DSC thermogram of Atovaquone (bottom) and Atovaquone-Lipoid S100 Solid Dispersion 15:1 w/w (top)

Fig.8 shows that the DSC endotherm of Atovaquone from solid dispersion did not differ significantly from that of pure drug. This would indicate that for Atovaquone, the enhanced dissolution is due to the increased surface area of the drug crystallites after formation of the solid dispersions and not due to a change from a crystalline to an amorphous state as observed for Ibuprofen by Hussain *et al* [24].

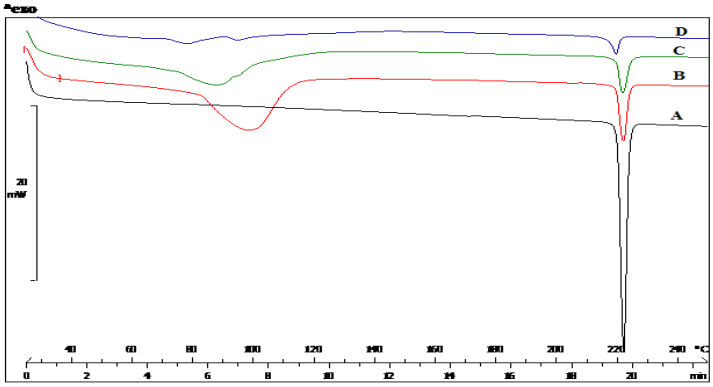


Fig. 9: It shows DSC thermogram of [A] Atovaquone [B] Atovaquone- βCD physical mixture [C] Atovaquone- βCD complex 1:1 molar (Rot) [D] Atovaquone- βCD complex1:1 molar (KN)

As seen in Fig.9, the thermogram of Atovaquone-βCD PM showed a broad endothermic effect at around 100°C due to the cyclodextrins' dehydration process [26]. Reduction of drug endothermic peak in PM may be due to the formation of partial complex with βCD molecules. The decrease in the endothermic peak of Rot and KN products indicates amorphization of Atovaquone or a complex formation between Atovaquone and βCD molecules [27]. Compared to the Rot system, the KN system gave a substantial reduction in drug's endothermic peak and also shifted the drug's melting point to a lower temperature (216.67°C). This may be associated with higher amorphization of drug by KN.

Preparation of complex by KN also resulted in loss of water molecules associated with the sample as indicated by absence of any peak around 100^{0}C, resulting in the formation of a dehydrated sample. This would have resulted in higher wettability and greater affinity of drug towards water improving its surface area and favouring the dissolution of drug in the medium. This factor would have accounted for the higher enhancement in IDR of Atovaquone from complex prepared by KN [28].

XRD

Fig.10 shows the X-ray diffractograms of Atovaquone, βCD, Atovaquone-βCD PM, Atovaquone-Lipoid S100 solid dispersion, Atovaquone- βCD complex (Rot) and Atovaquone- βCD complex (KN).

It can be suggested that both Atovaquone and βCD are present in crystalline form since they exhibited several well defined peaks at a diffractogram angle of 2θ (Fig.10.a and 10.b). The diffractogram of Atovaquone-βCD PM (Fig.10.c) showed sharp peaks similar to the superimposition of each component indicating that the drug maintained its initial crystallinity.

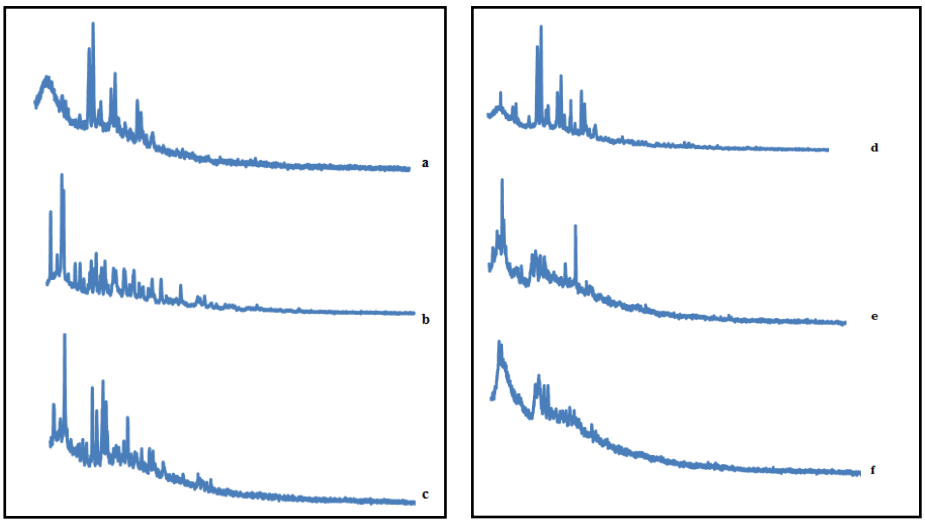


Fig. 10: It shows X-ray diffraction pattern of [a] Atovaquone [b] βCD [c] Atovaquone-βCD PM [d] Atovaquone LipoidS100 solid dispersion 15:1 w/w [e] Atovaquone- βCD complex 1:1 molar (Rot) [f] Atovaquone- βCD complex 1:1 molar (KN)

The marked differences between the diffraction patterns of the physical mix and the Rotary and Kneaded Products (Fig.10.e and 10.f) indicate the formation of a new phase upon complexation. In addition, the decrease in peak intensity indicates the formation of an amorphous complex [29].

In accordance with the DSC results, KN gave a higher amorphization of drug evidenced by a more diffused XRD pattern.

The XRD pattern of Atovaquone from Lipoid S100 solid dispersion does not differ from that of pure drug (Fig.10.d). This would indicate that there is no difference in crystallinity between the two as indicated by DSC analysis.

FTIR

For Atovaquone (Fig.11.A), the band at 3379.29 cm^{-1} is assigned to Phenol group vibration, 3334.92 cm^{-1} is assigned to the aromatic C-H stretching vibration, 1656 cm^{-1} and 1645 cm^{-1} are assigned to the stretching vibration of the band of the C=O group and 727.16 cm^{-1} is assigned to aromatic chloride. For Lipoid S100 (Fig. 11B), the band at 1735.93 cm^{-1} is attributed to C=O stretching vibration due to the ester group and 1238.30 cm^{-1} due to the Phosphate group. The bands appearing at 3300-3400 cm^{-1} are due to O-H stretching vibration and 3000-2800cm^{-1} are assigned to the stretching vibration of the bonds in –CH and –CH_2 groups [30, 31].

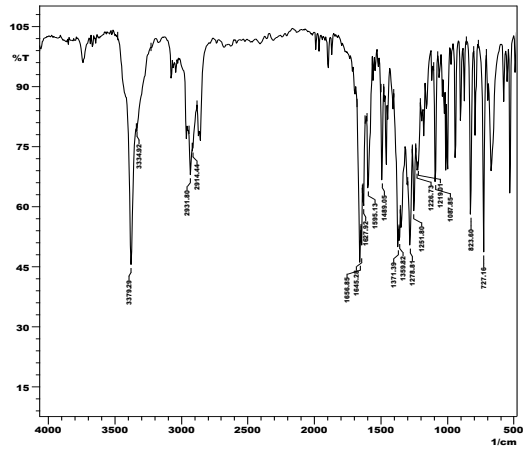


Fig. 11 A: It shows IR spectrum of Atovaquone

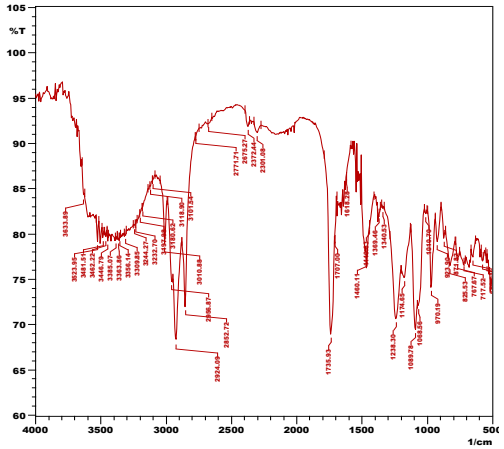


Fig. 11 B: It shows IR spectrum of Lipoid S100

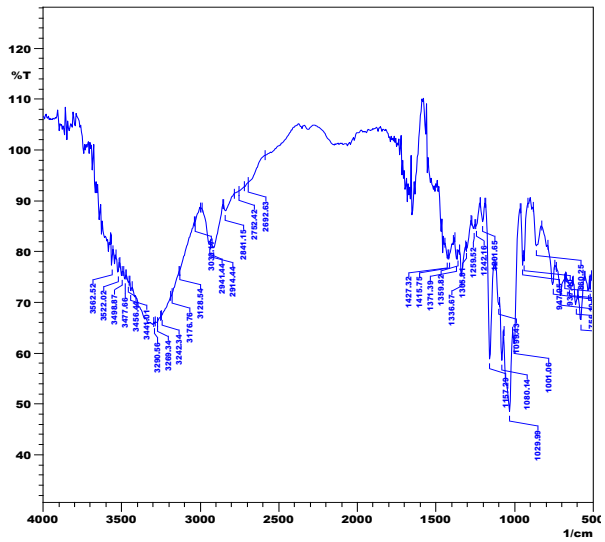


Fig. 11 C: It shows IR spectrum of βCD

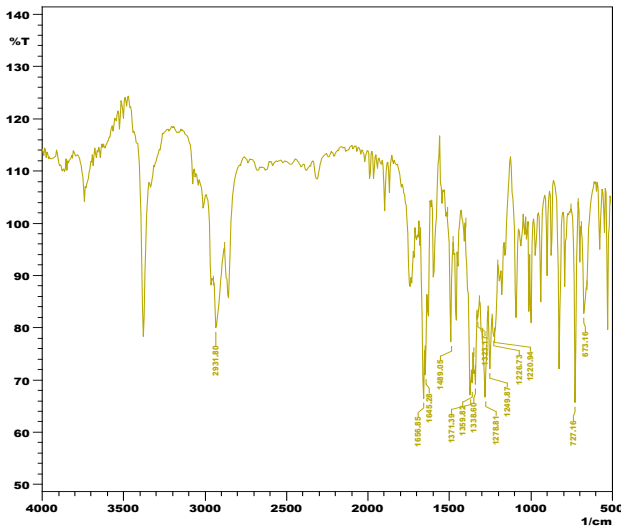


Fig. 11 D: It shows IR spectra of Atovaquone Lipoid S100 Solid Dispersion (15:1 w/w)

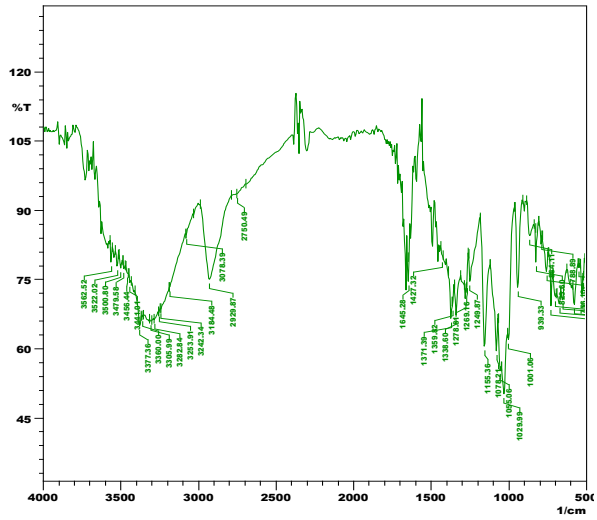
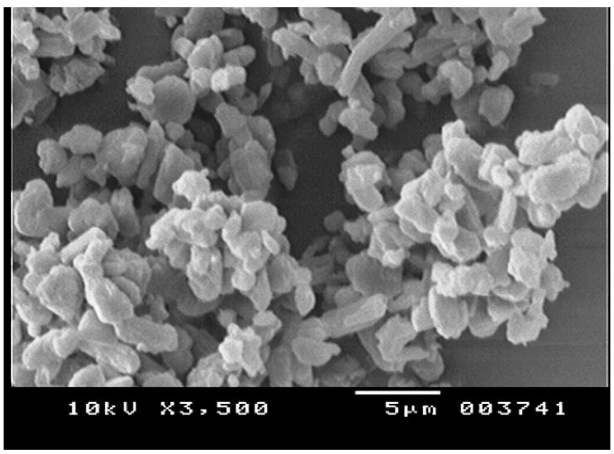
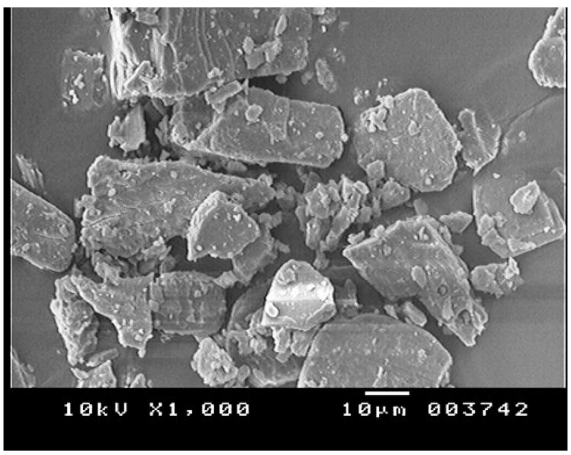


Fig. 11 E: It shows IR spectra of Atovaquone βCD Complex 1:1 molar (KN)

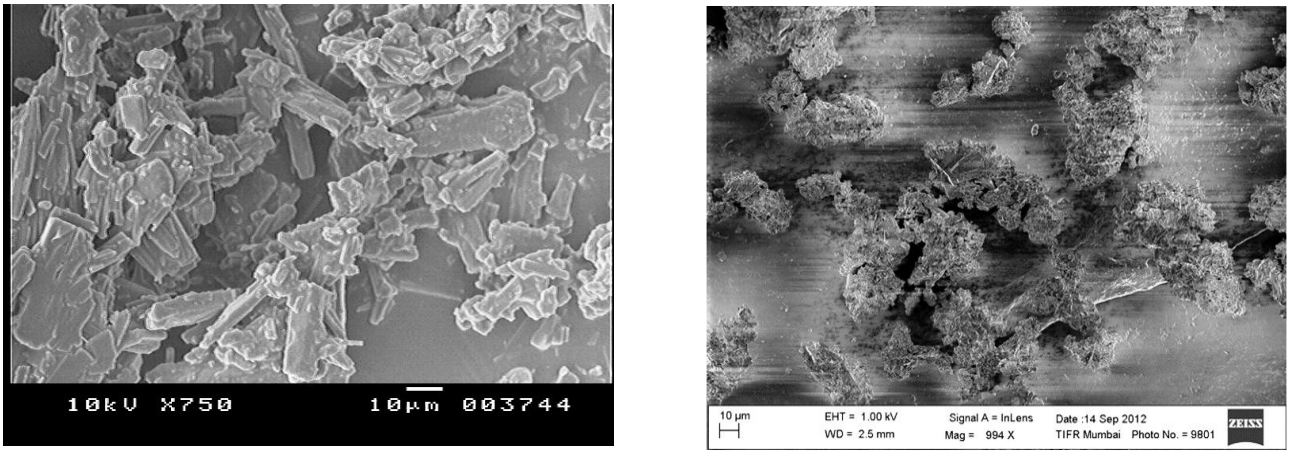
The spectrum of βCD (Fig.11.C) showed intense bands at 3300-3500 cm^{-1} due to O-H stretching vibration and 3000-2800cm^{-1} due to stretching vibration of the bonds in –CH and $–CH_2$ groups [32, 33]. No additional peaks in FT-IR spectra of Atovaquone solid dispersion and βCD complex (Fig. 11.D and 11.E) were found. In addition the characteristic peaks of Atovaquone were intact in the formulation. This indicates the absence of chemical interaction between the drug and formulation ingredients.



A



B



C **D**

Fig. 12: It shows Scanning electron microphotograph of (A) Atovaquone (B) βCD (C) Atovaquone-Lipoid S100 Solid Dispersion (15:1 w/w) (D) Atovaquone-βCD Complex 1:1 molar (KN)

SEM

Scanning electron microphotograph of Atovaquone and βCD (Fig.12.A and 12.B) depict their crystalline character. Scanning electron microphotograph of Atovaquone-Lipoid S100 Solid Dispersion (Fig.12.C) shows the presence of drug particles bearing a crystalline nature similar to pure drug. In accordance with DSC and XRD results the drug seems to have maintained a crystalline nature.

The original morphology of both Atovaquone and βCD appeared clearly changed in Fig.12.D, where it was not possible to differentiate the two components. These morphological changes indicate interaction between Atovaquone and βCD molecules where the changes in appearance and size of particles indicate the formation of an inclusion complex [29, 35].

CONCLUSION

The study demonstrated that βCD was more effective than HPβCD for preparing inclusion complex with Atovaquone while Lipoid S100 was superior to Phospholipon 90H and Lipoid EPCS for forming Solid dispersion. DSC, XRD and SEM analysis revealed that for Lipoid S100 solid dispersion the improvement in dissolution rate was due to wetting property of phospholipid and formation of liposomes on hydration and no change in crystal structure was observed and on the other hand for βCD complex the enhancement was due to the amorphization of drug. Further, it was interesting to note that both the carriers were almost equally effective in enhancing the dissolution rate of Atovaquone. While Lipoid S100 was effective at a carrier concentration of 15:1 drug-to-carrier ratio w/w, βCD was required at a concentration of 1:3 drug-to-carrier ratio w/w. Thus, Lipoid S100 solid dispersion proves to be an efficient formulation in enhancing the dissolution rate of Atovaquone.

ACKNOWLEDGEMENTS

Authors are thankful to USV Pharmaceuticals (Mumbai, India) for the gift sample of Atovaquone. Further, we extend our thanks to Lipoid (Germany) and Gangwal Chemicals (Mumbai, India) for kind gifts of excipients used in this study. The authors wish to thank Mrs. Bhagyashree Chalke, Dept. of Solid-State Physics and Mr. Nilesh Kulkarni, Tata Institute of Fundamental Research (TIFR), Mumbai, India for providing facility and assistance during SEM studies and X-ray diffraction studies.

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