

Research Article

Improvement of Water Solubility of Josamycin by Inclusion Complex with γ -Cyclodextrin

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Received 2 November 2011; Accepted 20 November 2011

Academic Editors: D. Heimler and V. Stefov

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Josamycin propionate (JMP) is an antibiotic belonging to the family of macrolide. According to the Biopharmaceutical Classification System (BCS), this compound can be classed in class II, low solubility and high permeability. In order to increase its apparent water solubility, inclusion complexation between Josamycin propionate and γ -cyclodextrin (γ -CD) was studied. UV spectrophotometric method was employed to investigate the phase-solubility profile and the stability constant of the complexation in aqueous medium. Solid state of the binary system prepared by coevaporation (in 50%-50% ethanol/water) has been characterized using powder X-ray diffraction (XRD) and Fourier transformation-infrared spectrometry (FTIR). These techniques indicate that JMP forms an association complex with γ -CD. The shift in the nuclear magnetic resonance spectroscopy (¹H NMR) confirms the existence of the inclusion complex. Also the results obtained showed an enhancement of the solubility in water of Josamycin propionate.

1. Introduction

Cyclodextrins (CDs) (Figure 1) are macrocyclic oligosaccharides constituted by 6, 7, or 8 D-glucose units forming α -, β - and γ -cyclodextrin, respectively. They have the property of forming inclusion complex with various guest molecules with suitable polarity and dimension because of their special molecular structure/hydrophobic internal cavity and hydrophilic external surface. The most probable binding involves the insertion of the lipophilic portion of the guest molecule into the cavity of the host and displacement of the water molecules located inside the cavity [1–3].

The Complexation with cyclodextrins has been widely used to improve the solubility, dissolution rate, stability, bioavailability of poorly water-soluble drugs, and elimination of undesired properties of drug, such as unpleasant odor and taste [4–9]. In our knowledge no study has been reported in the literature which interested to improve solubility of Josamycin propionate.

Josamycin propionate (JMP) (Figure 2) is a macrolide antibiotic, produced by *Streptomyces narbonensis vary* [10–12], characterized by a large lactone ring, a ketone group and a glycosidically linked amino sugar attached to nucleus which explains the basicity of this antibiotic [13]. According to the Biopharmaceutical Classification System (BCS), this compound can be classed in class II, poorly soluble in aqueous medium but permeable [14, 15].

The aim of this study was to prepare and to characterize inclusion complexes of Josamycin propionate in γ -CD, to provide a way to increase their aqueous solubility. Phase-solubility diagram was used to evaluate the solubility of this macrolide and to determine its stability constant of the complex. The characterization of the binary mixtures as performed using differential scanning calorimetry (DSC), powder X-ray diffractometry, and Fourier transformation-infrared spectrometry (FTIR). Finally, the shift in the nuclear magnetic resonance spectroscopy (¹H NMR) was performed to confirm the existence of the inclusion complex.

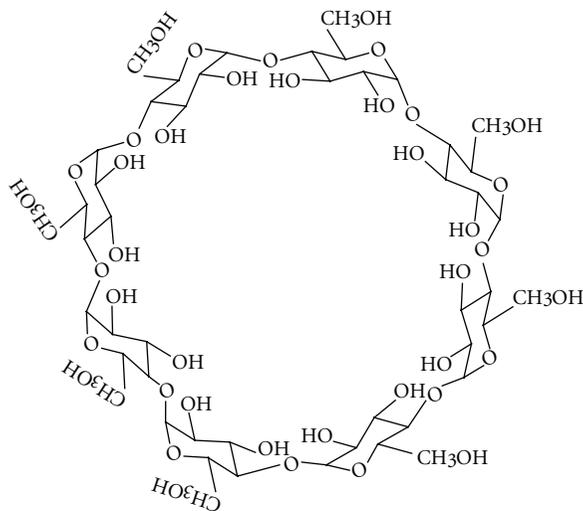
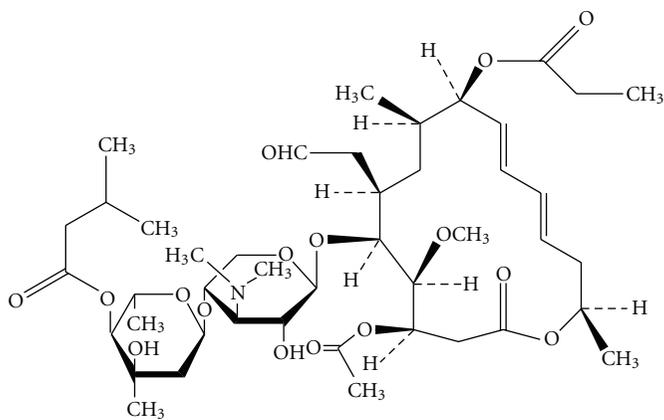
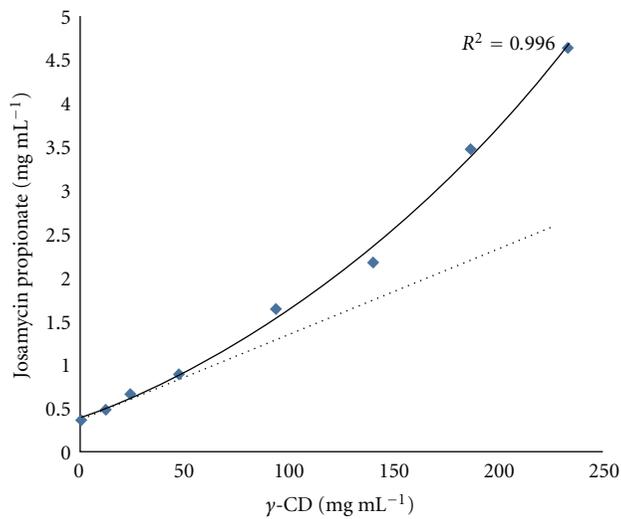
FIGURE 1: chemical structure of γ -cyclodextrin.

FIGURE 2: Chemical structure of Josamycin propionate.

FIGURE 3: Phase-solubility diagrams for JPM with γ -CD at 27°C.

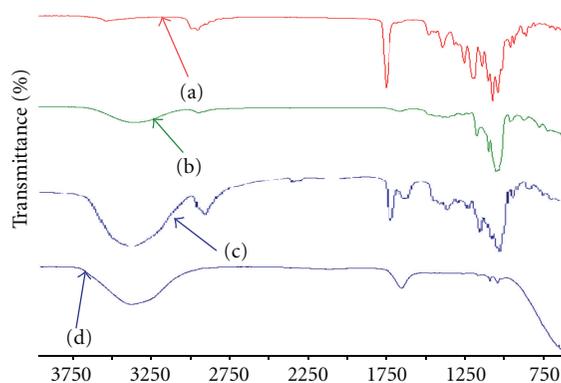


FIGURE 4: IR spectra of pure γ -CD (a), JMP (b), physical mixture (c), and co-evaporated product (d). % transmittance versus wave number (cm^{-1}).

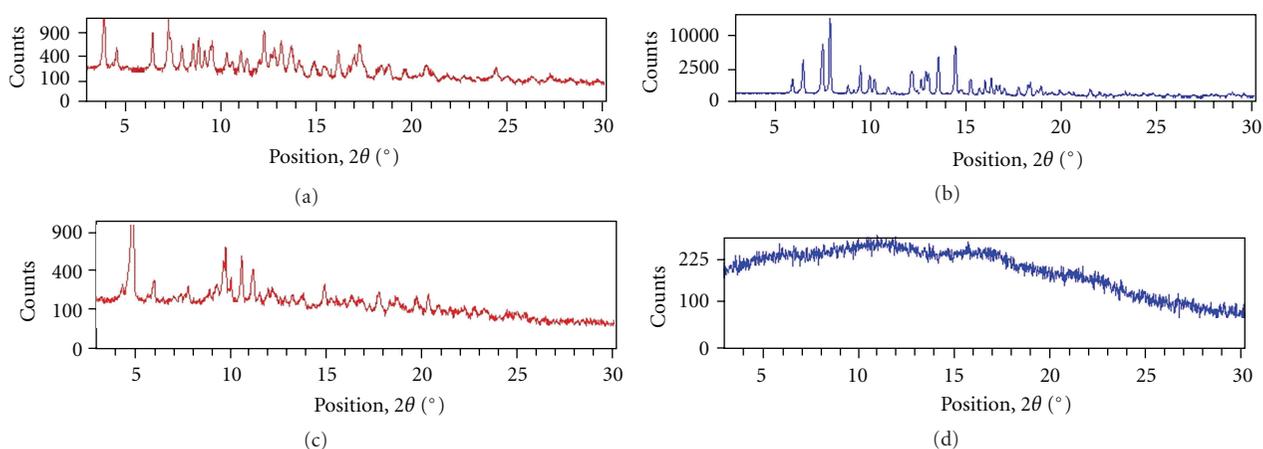


FIGURE 5: X-ray curves of pure γ -CD (a), JMP (b), physical mixture (c), and co-evaporated product (d).

2. Materials and Methods

2.1. Materials. Josamycin propionate (pharmaceutical grade) was obtained graciously from National Laboratory of Drug Control (Morocco). γ -cyclodextrin was purchased from Sigma; all other reagents were of analytical grade. Distilled water was used throughout the experiment.

2.2. Phase Solubility Studies. Phase solubility diagrams were obtained at room temperature (27°C) in water. An excess amount of the guest molecule JMP (100 mg) was added to a series of 5 mL flasks each containing increasing quantities of γ -CD (ranging from 0 to 232 mg/mL). The suspension was shaken for 72 h, after the steady state was reached. A portion of the sample was appropriately diluted with methanol and analyzed by an UV spectrophotometric method at 232 nm to assess the concentration of the Josamycin propionate dissolved according to European Pharmacopeia [13]. The phase solubility was constructed by plotting the total dissolved JMP concentration against the total γ -CD concentration. The stability constant (K_s) of the complex was calculated from

the slope of phase solubility diagram according to Higuchi and Connors equation:

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

where S_0 is the solubility of JMP in absence of γ -CD.

2.3. Preparation of Binary Mixtures. The complex of Josamycin propionate with γ -CD in 1:1 molar ratio was prepared by the coprecipitation method, by dissolving an appropriate amount of JMP (x mg) and γ -CD (y mg) in 25 mL of water ethanol (V/V). The solution was stirred for 10 h and the solvent was removed at 45°C . The physical mixture (noncomplexed drug) was obtained by mixing an exactly weighed (1:1 molar ratio) amounts of JMP and γ -CD powders in mortar with continuous stirring for 10 min.

2.4. Powder X-Ray Diffraction (XRD). XRD was used as another quantitative measure of crystallinity of JMP and developed to characterize the formation of the inclusion

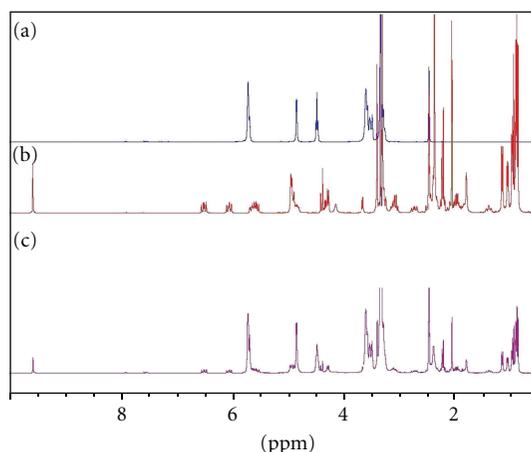


FIGURE 6: ^1H NMR spectra in DMSO-d_6 of pure γ -CD (a), JMP (b), and co-evaporated product (c).

complexation. The powder X-ray diffraction patterns of JMP, γ -CD, physical mixture, and corresponding complex were recorded using an automatic powder diffractometer (Philips X'Pert PRO). X-ray patterns were obtained in the angular range of $5\text{--}50^\circ 2\theta$, with 2 s fixed time for each 0.04° step.

2.5. FTIR Spectroscopy. The IR spectra of pure materials, physical mixture and corresponding complex were measured, using an FTIR spectrophotometer (TANSOR 27 Bruker ATR) using KBr pelleting. The scans were executed at a resolution of 8 cm^{-1} from 4000 cm^{-1} to 500 cm^{-1} .

2.6. ^1H NMR Spectroscopy. The ^1H NMR spectra of the samples were recorded at 25°C using the NMR spectrometer (AVANCE 300 Bruker) employing DMSO-d_6 as a solvent. The ^1H NMR chemical shift ($\Delta\delta$) caused upon complexation were measured only for both H_5 and H_3 which are located inside the cyclodextrin cavity, to confirm the inclusion complex of JMP and calculated according to the following formula:

$$\Delta\delta = \delta_{\text{complexed state}} - \delta_{\text{free state}} \quad (2)$$

3. Results and Discussion

3.1. Solubility Studies. The phase solubility diagram of JMP- γ -CD system was reported in (Figure 3). The solubility of JMP increases linearly as a function of γ -CD concentration ($0\text{--}250\text{ mg/mL}$). And the solubility curve can be classified as A_p type [2], according to the Higuchi and Connors classification. When there is a linear increase in guest molecule solubility with increase in cyclodextrin concentration, a cyclodextrin complex of the guest results from $1:2\text{ mol/mol}$ interactions. According with this theory, it is possible to assume that $1:2\text{ mol/mol}$ JMP/ γ -CD inclusion compound was formed. The solubility calculated for JMP in water was 0.35 mg/mL at 27°C ; this solubility increased linearly giving 4.60 mg/mL in the presence of 250 mg/mL γ -CD. The stability constant value calculated was 3060 M^{-1} , also the larger constant observed indicate that JMP interact strongly with

γ -CD. Furthermore the correlation between the solubility of JMP and the concentration of γ -CD suggested that water soluble complex was formed [14–16].

3.2. IR Spectra Studies. Figure 4 shows the infrared spectra of γ -CD, JMP, physical mixture, and corresponding complex. JMP has a carbonyl band of $1600\text{--}1750\text{ cm}^{-1}$, may be due to a ring lactone [17, 18], in the spectrum of the physical mixture there is no change. Whereas in the IR spectrum of the corresponding complex there is a significance decrease was observed in its intensity. IR spectrum of JMP contains a band with shoulders ($3000\text{--}3500\text{ cm}^{-1}$) due to hydrate form [19].

In the spectrum of γ -CD some bands in the range $1030\text{--}1160\text{ cm}^{-1}$ can be associated with the stretching frequency of primary and secondary C–OH groups [20], those bands disappearance in the complex.

The intensity and shape of bands between $800\text{--}1500\text{ cm}^{-1}$ changed dramatically for the inclusion compound as compared to those for pure JMP, γ -CD, and physical mixture. Thus indicating that the vibrating and bending of the guest molecule (JMP) was restricted due to the formation of inclusion complex [17, 20, 21].

3.3. X-Ray Diffraction Analysis. The XRD pattern of pure drug presented several diffraction peaks indicating the crystalline nature of the drug (Figure 5), the γ -CD also exhibited a typical crystalline diffraction pattern. Among the physical mixture showed several peaks attributable both to the crystalline drug and γ -CD. The coevaporated product showed a single very broad band in which the diffraction peaks of drug and γ -CD disappeared. This phenomenon confirmed that an inclusion complex between drug and γ -CD was formed and which indicates the formation of a new crystalline phase [21–26].

3.4. ^1H NMR Spectroscopy. Cyclodextrin has six identifiable protons in the NMR spectrum: protons H_1 , H_2 , H_4 being at the outer surface of CD, while protons H_3 and H_5 sit in the

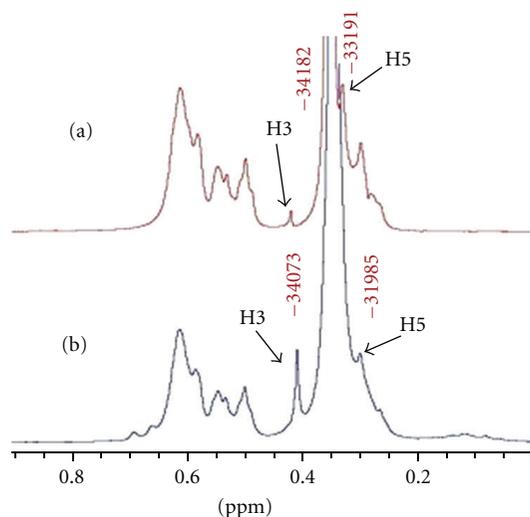


FIGURE 7: Partial ¹H NMR spectra in DMSO-d₆ of pure γ -CD (a), and Co-evaporated product (b).

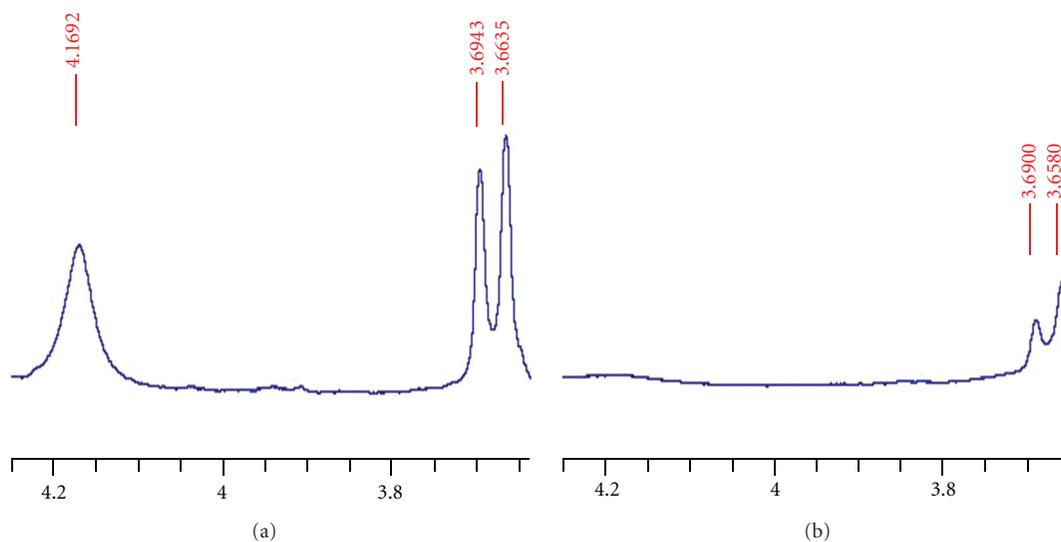


FIGURE 8: Partial ¹H NMR spectra in DMSO-d₆ of pure JMP (a), and co-evaporated product (b).

cavity and are very important for the study of the interaction of guest molecules with cyclodextrins.

The NMR spectra (Figure 6) and the partial ¹H NMR spectra in DMSO-d₆ of pure γ -CD and co-evaporated product (Figure 7) show that the inclusion of JMP into the γ -CD induces upfield changes in the ¹H NMR chemical shift values for both the protons H3 and H5 with ($\Delta\delta = 0.0306$ ppm and $\Delta\delta = 0.0106$ ppm), respectively. It is noteworthy that NMR allows clear distinction between inclusion and any other possible external interaction processes, with large effects observed on the proton located in the hydrophobic cavity (H3 and H5), clearly proving inclusion in aqueous medium. On the other hand, protons located on the outside experience no or very small shift [27, 28].

As shown in (Figure 7) and in the partial ¹H NMR spectra in DMSO-d₆ of pure JMP and coevaporated product (Figure 8), the singlet peak at the value of the chemical shift of 4.16 ppm which can be attributed to the methyl group

belonging to the portion of the conjugated carbon chain of pure JMP disappears in the association complex.

However, it is probably that ($-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}-\text{CH}_3$) part of JMP structure, which is highly hydrophobic, was completely embedded inside the lipophilic core of γ -cyclodextrin.

4. Conclusion

The results of this study clearly evidence that the Josamycin propionate can be efficiently complexed with γ -cyclodextrin in a relatively high proportion forming an inclusion complex.

The properties of complex were characterized by phase-solubility techniques, X-ray diffraction, FTIR spectroscopy, and ¹H NMR spectroscopy. Thus, the pharmaceutical property of aqueous solubility of Josamycin propionate can be improved.

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