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Preformulation study of the inclusion complex warfarin-β-cyclodextrin

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Abstract

Inclusion complex between warfarin and β-cyclodextrin was obtained to improve the in vitro bioavailability of the drug in acidic media. Inclusion complexation in solution was studied by phase solubility technique. The apparent stability constant was influenced by the pH of the medium ranging from 633.26 M^{-1} (at pH 1.2, where the drug was in unionised form) to 99.81 M^{-1} (at pH 7.4, where the drug was in ionised form). Phase solubility study showed an A_L-type diagram indicating the formation of an inclusion complex in 1:1 molar ratio. Solid binary mixtures of the drug with β-cyclodextrin were prepared by several methods (physical mixing, kneading, co-evaporation, freeze-drying). Physicochemical characterizations were performed using differential scanning calorimetry, powder X-ray diffractometry and dissolution studies. Preparation method influenced the physicochemical properties of the binary mixtures. An inclusion complex was obtained by freeze-drying, and it showed a high solubility and drug dissolution rate. The physical stability of the complex was also studied. After one year storage in glass container at room temperature no significant changes were detected in the diffractogram, thermogram and dissolution profile of the freeze-dried product.

Keywords: β-Cyclodextrin; Warfarin; Inclusion complex; Differential scanning calorimetry; Powder X-ray diffractometry; Dissolution rate

1. Introduction

It is well known that many drugs show bioavailability problems due to their low water solubility, slow dissolution rate and instability in the gastrointestinal tract. In the last decades several methods were applied to overcome this problem. Among these methods cyclodextrins have been extensively studied to improve solubility (Hirayama and Uekama, 1999), dissolution (Montassier et al., 1997; Ficarra et al., 2000) and bioavailability (Wong and Yuen, 2001).

β-Cyclodextrin is cyclic oligosaccharide, containing seven D-glucopyranose units forming a ring with a relatively hydrophobic central cavity in which lipophilic water-insoluble drugs may form inclusion complexes (Loftsson and Brewster, 1996). Due to

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its price, availability and cavity dimension the β-cyclodextrin is widely used. The cavity size is suitable for common pharmaceutical drugs with molecular weights between 200 and 800 g/mol (Waleczek et al., 2003). The main problem is its low water solubility (18.5 g/l at 25 °C).

Warfarin is an acidic drug ($pK_a = 4.79$) widely used in the anticoagulant therapy. It is practically insoluble in water and acidic media in which presents also a very slow dissolution rate. Normally it is administered as sodium salt that may precipitate in the gastrointestinal tract leading erratic and interindividual variability in absorption. In an attempt to improve physicochemical properties of the drug at low pH value, drug complexation with β-cyclodextrin was carried out. In a previous paper (Lin and Yang, 1986), complexes between warfarin and α- or β-cyclodextrins were studied obtaining an increase in drug solubility and dissolution rate in water. In their work binary mixtures were prepared by freeze-drying method without investigating other complexation methods nor the influence of the pH of the medium on the complex formation. Furthermore, no data about physical stability of the complex were collected.

In our work inclusion complexes have been prepared in solution and in the solid state. Different methods were employed to prepare binary mixtures as kneading, co-evaporation and freeze-drying. Characterization of the binary mixtures was performed using phase solubility technique, differential scanning calorimetry and powder X-ray diffractometry. Solubility and dissolution measurements were also performed.

The aim of this work was to evaluate the effect of the different preparation methods on the physicochemical properties of warfarin in the binary mixtures. The role of the pH value in the interaction between drug and cyclodextrin was also investigated. Finally, a long term stability study was carried out to collect informations upon the stability of freeze-dried product.

2. Materials and methods

2.1. Materials

Warfarin, 3-(α-acetonylbenzyl)-4-hydroxycoumarin (MW = 308.3), was purchased from Sigma–Aldrich (Milan, Italy). Pharmaceutical grade β-cyclodextrin (Cawamax W7 Pharma, MW = 1135), containing 12% by weight of water, was supplied by Wacker–Chemie GmbH (Peschiera Borromeo, Italy). These chemicals were used as received without further purification. All other chemicals and solvents used were of analytical grade.

2.2. Preparation of binary mixtures

All the binary mixtures were prepared in the 1:1 molar ratio between drug and β-cyclodextrin on the basis of the results obtained from the preliminary phase solubility studies.

Physical mixture was prepared by simple mixing, in a mortar with pestle for 10 min, the powders of both components previously sieved (granulometric fraction <250 μm was used).

Kneaded product was obtained by adding small amount of water to β-cyclodextrin placed in a mortar and mixing to obtain a homogeneous paste. Then, warfarin powder was slowly added and the mixture was kneaded for 45 min. During the kneading process few drops of water were introduced to maintain a suitable consistency. The resulting paste was dried in an oven at 45 °C for 48 h and the solid was finally ground and sieved through a 250 μm sieve.

Co-evaporated product was obtained by dissolving equimolar amount of β-cyclodextrin and warfarin in 250 ml of 50% aqueous ethanol. The solution was stirred up to complete dissolution of the powders and the solvent was then removed at reduced pressure using a rotary evaporator at 45 °C. The obtained solid was ground, sieved through a 250 μm sieve and placed in an oven at 45 °C for 48 h.

Freeze-dried product was prepared by dissolving the β-cyclodextrin in water and adding the stoichiometric amount of the drug. The suspension was left 48 h under stirring at room temperature and protected from the light. Filtration (0.45 μm membrane filter, Whatman) was performed to separate the undissolved drug and the resulting solution was frozen by immersion in acetone at −35 °C (Crycool Immersion cooler, mod. CC-65A, Neslab, The Netherland). The frozen solution was then freeze-dried over 48 h (freeze-dryer Hetossic, Mod. CD53-1, Heto, Sweden) at −45 °C and about 0.1 mPa. The obtained solid was ground, sieved through a 250 μm sieve and maintained in an air oven at 45 °C for 48 h.

2.3. Analysis of drug content in the binary mixtures

Samples of each binary mixture were assayed for drug content by dissolving weighed amounts in 10 ml of ethyl alcohol. The drug content was determined spectrophotometrically (Perkin Elmer, Mod 552) at 308 nm.

2.4. Phase solubility studies

Phase solubility diagrams were obtained at 37 °C in water and several buffer solutions (pH = 1.2, 5.0 and 7.4). An excess amount of warfarin (100 mg) was added to 20 ml of water or aqueous buffer solution containing increasing amounts of β-cyclodextrin (ranging from 0 to 0.02 M). The suspensions were shaken for 72 h after which the equilibrium was reached. Then they were filtered through 0.22 μm cellulose acetate membrane filter, appropriately diluted with their respective buffer and analysed by UV spectrophotometer at 308 nm.

The apparent stability constants (K_s) of the complexes were calculated from the slope of the phase solubility diagrams according to the following equation (Higuchi and Connors, 1965):

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

where S_0 was the solubility of warfarin at 37 °C at each pH value in absence of cyclodextrin.

2.5. Differential scanning calorimetry (DSC)

Differential scanning calorimetry analysis was performed with a Mettler TA 3000 apparatus equipped with a DSC 20 heat cell. Samples of drug, β-cyclodextrin and all the binary mixtures, containing 1.5 mg of the drug, were placed in sealed aluminium pans and heated at 10 °C/min in the 30–200 °C range, using an empty sealed pan as a reference. Temperature and heat flow calibrations were performed using indium as a standard.

2.6. X-ray diffractometry (XRD)

Diffraction patterns of raw materials and binary mixtures were obtained at room temperature with a Siemens powder diffractometer using a Cu Kα radiation (λ = 1.5418 Å), a current of 20 mA and a voltage of 40 kV. Powdered samples, ground in a mortar, were analysed over the 3–40 2θ range with a scan step size of 0.05 and time of acquisition of 2 s.

2.7. Drug solubility from binary mixtures

Drug solubilities from the different binary mixtures were determined by adding an excess amount of the mixture, corresponding to 40 mg of the drug, to 10 ml of water or buffer solution at pH 1.2 or 5.0. The suspensions were stirred for three days at 37 °C and filtered through 0.45 μm membrane filter, appropriately diluted and analysed by UV spectrophotometry. At least three measurements were performed on each sample.

2.8. Dissolution studies

The USP XXIV rotating paddle apparatus (Erweka, mod DT-1, Germany) was used with a stirring rate of 100 rpm. Dissolution medium (water or aqueous buffer solution at pH 1.2 or 5.0) was thermostated at 37 °C. Powdered samples (granulometric fraction < 250 μm) of pure drug or different binary mixtures, containing suitable amount of warfarin for sink condition ($C < 0.2C_s$), were added over the surface of 900 ml of the dissolution medium. The aqueous solution was filtered and continuously pumped to a flow cell in a spectrophotometer and absorbance values were recorded at 308 nm. The time necessary to obtain 50% of drug dissolution (t_{50}) from each sample was determined together with the dissolution efficiency after 10 and 60 min (DE_{10} and DE_{60}, respectively). Dissolution tests were performed at least three times for each sample.

2.9. Physical stability test

Stability studies of the binary mixtures were performed storing the solid sample in glass containers with a screw cap, protected from the light, at room temperature and pressure for 12 months. After this period DSC, X-ray and dissolution studies were repeated.

Table 1
Drug content in binary mixtures (% molar ratio ± S.D.)

	Theoretical (%)	Actual (%)
Physical mixture	50	49.1 ± 0.66
Kneaded product	50	49.3 ± 0.51
Co-evaporated product	50	48.7 ± 0.11
Freeze-dried product	50	10.2 ± 1.10

3. Results and discussion

3.1. Drug content in the binary mixtures

First of all, the actual drug content in each binary mixture was determined. The results are reported in Table 1. As can be seen, physical mixture, kneaded and co-evaporated products showed a good agreement between theoretical and actual drug content. In the case of freeze-dried product, due to poor solubility, the drug was not completely dissolved in water and the actual drug content was consequently lower than theoretical one.

3.2. Phase solubility studies

The phase solubility diagrams at 37 °C were obtained by plotting the apparent equilibrium concentrations of the drug against β-cyclodextrin concentrations and are reported in Fig. 1. It can be observed that the apparent solubility of warfarin increased linearly as a function of β-cyclodextrin concentration over the entire concentration range studied. The same behavior was observed in water and in all pH values. Linearity was characteristic of A_L-type system (Higuchi and Connors, 1965) and suggested that water soluble complex was formed in solution. Furthermore, the slope values were always lower than one indicating that inclusion complex in the molar ratio of 1:1 between the guest (warfarin) and host (β-cyclodextrin) molecules was obtained irrespective of pH values.

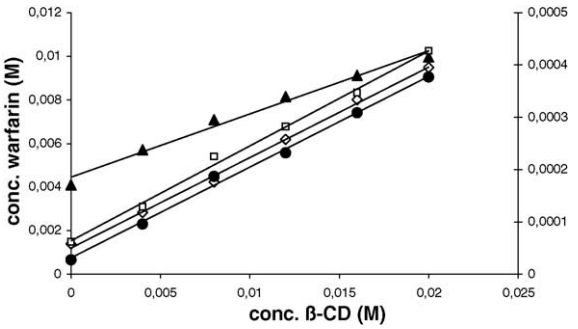

Fig. 1. Phase solubility diagrams of warfarin-β-cyclodextrin systems at pH 1.2 (●), pH 5.0 (◯), water (□), (left *y*-axis), and pH 7.4 (▲) (right *y*-axis). Each point is the mean of three measurements. Error bars were omitted for clarity.

The apparent stability constants K_s of the 1:1 complexes at each pH value were calculated from the slopes of the phase solubility diagrams and the respective S_0 values (Table 2). A decrease of the K_s values increasing the pH of the solvent has been observed. This phenomenon could be due to the ionisation of the acidic warfarin ($pK_a = 4.79$, Okimoto et al., 1996) at $pH > pK_a$. Ionic form of the drug showed lower hydrophobicity and weaker interactions with the hydrophobic cavity of β-cyclodextrin than the unionised drug (Loftsson and Brewster, 1996; Okimoto et al., 1996; Fernandes et al., 2002). It can be concluded that pH played an important role in determining the strength of complexation between warfarin and cyclodextrin. By plotting K_s values against pH values a linear relationship was observed (Fig. 2).

3.3. Differential scanning calorimetry

The DSC curves for raw materials and all the binary mixtures studied were reported in Fig. 3. Warfarin showed a typical behavior of anhydrous crystalline drug with a well defined melting peak at 167.6 °C ($\Delta H = 136.4$ J/g). In the considered temperature range,

Table 2
Warfarin solubility (S_0), slope, K_s and correlation coefficient (r^2) from phase solubility diagrams

Solvent	$S_0 \pm$ S.D. (10^{-5} M)	Slope	K_s (M^{-1})	r^2
Water (unbuffered pH 5.5)	6.24 ± 0.28	0.018	295.41	0.996
Buffer solution pH 1.2	2.78 ± 0.43	0.017	633.26	0.997
Buffer solution pH 5.0	5.75 ± 0.14	0.017	306.17	0.997
Buffer solution pH 7.4	409.4 ± 1.61	0.290	99.81	0.990

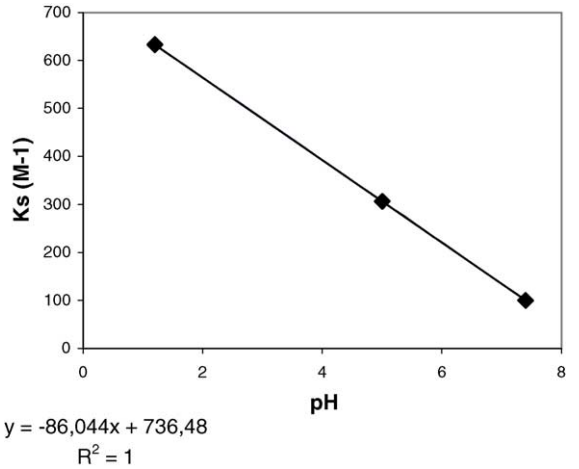

Fig. 2. Linear correlation between calculated K_S and pH values.

DSC curve of β-cyclodextrin exhibited a very broad endothermal phenomenon between 60 and 120 °C due to loss of water (Hassan et al., 1990). The warfarin melting peak was observed in the physical mixture, kneaded and co-evaporated products even if the ΔH values were slightly lower than that of the pure drug, as reported in Table 3. Only weak interactions can be postulated in the case of kneaded and co-evaporated products. No endothermal peak, other that indicating the water loss from sample, was noted in the freeze-dried product suggesting that the complete inclusion complex without free warfarin was formed. These results were in good agreement with those previously reported (Lin and Yang, 1986; Choi et al., 2003).

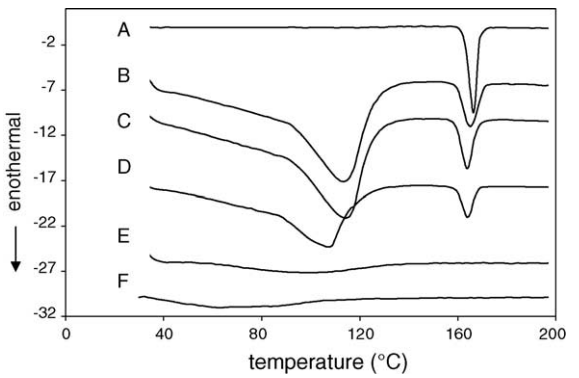

Fig. 3. DSC thermograms of warfarin (A), physical mixture (B), kneaded product (C), co-evaporated product (D), freeze-dried product (E) and β-cyclodextrin (F).

Table 3
Differential scanning calorimetric data of the examined samples

	T_{onset} (°C)	T_{peak} (°C)	ΔH (J/g)
Warfarin	160.0	167.6	136.4
Physical mixture	153.9	165.1	132.4
Kneaded product	156.2	163.4	126.6
Co-evaporated product	150.0	160.7	111.2
Freeze-dried product	–	–	–

3.4. X-ray diffraction analysis

The XRD pattern of pure drug presented several diffraction peaks indicating the crystalline nature of the drug (Fig. 4). The β-cyclodextrin also exhibited a typical crystalline diffraction pattern. Among the binary mixtures, freeze-dried product showed a single very broad band in which the diffraction peaks of the drug and excipient disappeared. This phenomenon confirmed that an inclusion complex between drug and cyclodextrin was formed. The other binary mixtures showed several peaks attributable both to crystalline drug and excipient. Only a slight decrease in peak intensity was noted. No inclusion complex was obtained by physical mixtures while kneaded and co-evaporated products showed weak interactions confirming the DSC results.

3.5. Solubility and dissolution rate

The solubilities of the drug alone and from the binary mixtures in acidic media (pH = 1.2 and 5.0) were

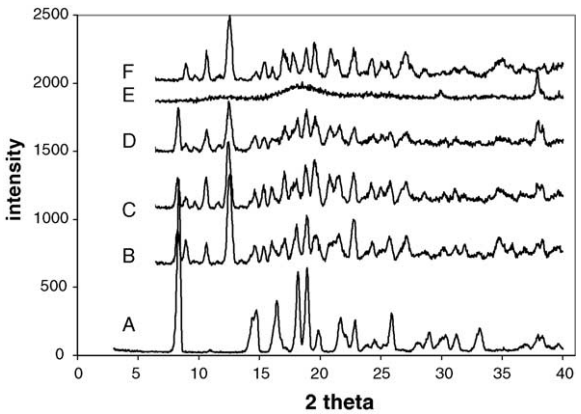

Fig. 4. X-ray diffraction patterns of warfarin (A), physical mixture (B), kneaded product (C), co-evaporated product (D), freeze-dried product (E) and β-cyclodextrin (F).

Table 4
Drug solubility (mg/100 ml) from prepared binary systems at pH 1.2 and 5.0

	pH 1.2	pH 5.0
Warfarin	0.86 ± 0.13	1.77 ± 0.35
Physical mixture	6.56 ± 0.41	10.18 ± 1.35
Kneaded product	13.98 ± 0.12	16.45 ± 1.22
Co-evaporated product	14.87 ± 0.21	18.75 ± 1.78
Freeze-dried product	24.8 ± 0.65	34.5 ± 0.04

reported in Table 4. Warfarin, as an acidic drug showed a very low solubility at pH 1.2 (0.86×10^{-3} g/100 ml). It can be seen that an increase of the solubility values was obtained from all the binary mixtures. This was probably due to the presence of hydrophylic cyclodextrin and a better wettability of the drug. Freeze-dried product showed a 30-fold increase of the solubility attributable to the formation of inclusion complex.

The dissolution profiles of the raw materials and their binary mixtures at pH 1.2 have been reported in Fig. 5. Commercial warfarin showed a very slow dissolution rate due to its acidic nature. The dissolution was incomplete even after three hours. The physical mixture showed a slight increase of the dissolution rate. This result was due to the solubilizing effect of the cyclodextrin and also to improve wettability of the drug (Damian et al., 2000). In situ formation of readily soluble complexes was also possible (Moyano et al., 1997). The warfarin dissolution rate from kneaded product was similar to warfarin dissolution rate from co-evaporated product, as expected from physicochemical characterizations. A very high increase of the drug

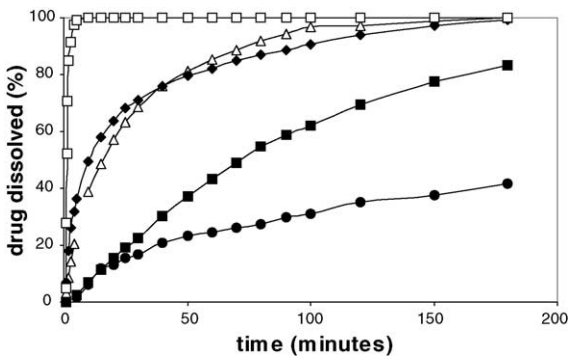

Fig. 5. Dissolution profiles at pH 1.2 of pure warfarin (●), physical mixture (■), kneaded product (△), co-evaporated product (♦) and freeze-dried product (□).

Table 5
Dissolution efficiencies (DE_{10} and DE_{60}) and t_{50} of warfarin and its binary mixtures at pH 1.2 and 5.0

	DE_{10} (%)	DE_{60} (%)	t_{50} (min)
pH 1.2			
Warfarin	6.09	24.68	>180
Physical mixture	6.78	43.14	72
Kneaded product	38.91	85.15	12
Co-evaporated product	40.24	83.78	11
Freeze-dried product	100	100	5
pH 5.0			
Warfarin	11.38	40.57	100
Physical mixture	14.02	65.02	42
Kneaded product	65.77	91.6	6
Co-evaporated product	68.10	92.5	6.5
Freeze-dried product	100	100	<2

dissolution rate was found in the case of freeze-dried product probably due to several reasons: the formation of soluble inclusion complex, amorphisation of the drug and consequently solubility increase, better wettability and reduction of particle size (Veiga et al., 2001). Same results were obtained at pH 5 and in water (dissolution profiles not shown) even if the dissolution rates were faster at this pH value due to higher solubility of the drug. Dissolution efficiencies and t_{50} values at pH 1.2 and 5.0 were reported in Table 5. Considering the DE values, the dissolution rate of warfarin was increased in the order: drug < physical mixture < kneaded product ≈ co-evaporated product < freeze-dried product suggesting that dissolution rate was influenced by the preparation method of the binary mixtures.

3.6. Physical stability test

During storage, stability problems might affect the physicochemical and pharmaceutical properties of a dosage form. Recrystallization, changes in polymorphic form of the drug and interaction between components may negatively influence solubility and dissolution rate of a drug. DSC, XRD and dissolution tests were used to assess the physical stability of the warfarin-β-cyclodextrin freeze-dried product, in which an amorphous inclusion complex of the drug was found. In Fig. 6A–C the X-ray diffraction patterns, DSC thermograms and drug dissolutions in acidic medium (pH 1.2) were shown for the freshly

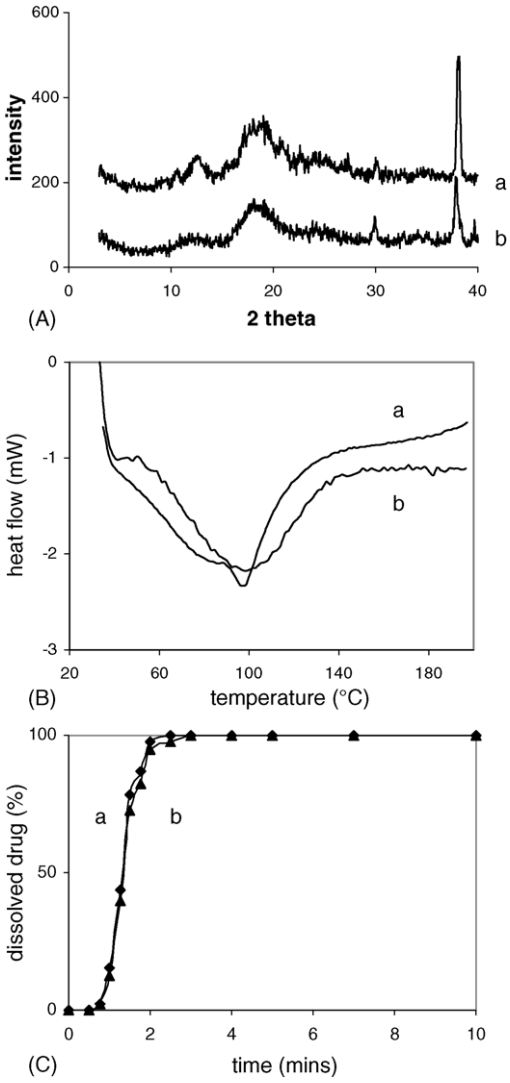

Fig. 6. X-ray diffractograms (A), DSC thermograms (B) and dissolution profiles (C) of freshly prepared (a) and after 12 months storage (b) freeze-dried product.

prepared freeze-dried product and after 12 months storage in glass containers at ambient temperature and protected from the light. Initially, as showed above, no endothermal event was observed in DSC thermogram, no diffraction peaks were detected by X-ray analysis and very high dissolution rate was obtained by freeze-drying. Analyses performed 12 months later indicated that no recrystallization process had occurred and also the dissolution profile was superimposable to that of freshly prepared product. From DSC thermogram, only a slight increase in absorbed water after 12 months was observed, attributable to the hygroscopic nature of β-cyclodextrin. These data suggested a high physical stability of the inclusion complex in the freeze-dried product.

4. Conclusions

β-Cyclodextrin has shown to form inclusion complex with warfarin. Preparation method and pH strongly influenced the ability of cyclodextrin to include the drug. Ionic form of the drug showed decrease in inclusion complex formation and lower stability constant value due to weaker interaction with the hydrophobic cavity of the cyclodextrin than neutral form of the drug. Freeze-drying was the best method to obtain the highest dissolution rate of the drug and higher solubility also suggesting possible improvement of oral warfarin base bioavailability.

Interesting results were obtained in stability tests in which no drug physical changes neither different dissolution profiles of the warfarin were detected after 12 months of storage at ambient temperature.

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