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Research Article

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Physicochemical Properties and Dissolution Studies of Dexamethasone Acetate-β-Cyclodextrin Inclusion Complexes Produced by Different Methods

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Abstract. Inclusion complexes between dexamethasone acetate (DMA), a poorly water soluble drug, and β-cyclodextrin (βCD) were obtained to improve the solubility and dissolution rate of this drug. Phase-solubility profile indicated that the solubility of DMA was significantly increased in the presence of βCD (33-fold) and was classified as A_L-type, indicating the 1:1 stoichiometric inclusion complexes. Solid complexes prepared by different methods (kneading, coevaporation, freeze drying) and physical mixture were characterized by differential scanning calorimetry, thermogravimetry, infrared absorption and optical microscopy. Preparation methods influenced the physicochemical properties of the products. The dissolution profiles of solid complexes were determined and compared with those DMA alone and their physical mixture, in three different mediums: simulated gastric fluid (pH 1.2), simulated intestinal fluid (pH 7.4) and distilled water. The dissolution studies showed that in all mediums DMA presented an incomplete dissolution even in four hours. In contrast, the complexes formed presented a higher dissolution rate in simulated gastric fluid (SGF pH 1.2), which indicate that these have different ionization characteristics. According to the results, the freeze–dried and kneaded products exhibited higher dissolution rates than the drug alone, in all the mediums.

KEY WORDS: β-cyclodextrin; dexamethasone acetate; dissolution rate; inclusion complexes; phase-solubility; physicochemical characterization.

INTRODUCTION

In recent years, the number of active agents having low aqueous solubility increased significantly. Oral delivery of poorly water-soluble drugs often results in low bioavailability since the rate-limiting step for absorption from the gastrointestinal tract is a significantly slower dissolution rate (1). Thus, many molecules that are biologically active *in vitro* are ineffective *in vivo* as a result of their limited solubility and slow rate of dissolution (2,3).

Several techniques have been used to improve the solubility/dissolution rate of poorly water soluble drugs and, among the possibilities, the cyclodextrin (CD) complexation is of particular interest (4,5). The CDs are cyclic oligosaccharides consisting of six, seven or eight D-glucopyranose units (α, β and γ CD) linked by (1–4) glycosidic bonds and arranged in a truncated cone shape structure (6,7). These agents exhibit a hydrophilic exterior and a hydrophobic internal cavity in which lipophilic soluble drugs may form inclusion complexes, being trapped entirely or at least partially (8–10). Several driving forces have been proposed for the inclusion of CDs with substrates: hydrogen binding, Van der Waals force, hydrophobic interaction and the release of "high energy water" molecules from the cavity, however, no covalent bonds exist between the CD and its guest (2). As a result of complex formation, the physicochemical properties of the guest molecules, such as solubility, thermal stability, melting point, chemical reactivity, spectroscopic and electrochemical properties will be changed. Out of three parent CDs, β-cyclodextrin (βCD) appears more useful as a pharmaceutical agent because of its complexing ability, cavity dimension, low cost, higher productive rate and other properties (11–13). The cavity size is suitable for common pharmaceutical drugs with molecular weights between 200 and 800 g mol^{-1} (3,14). Many drugs have been complexed with βCD, leading to a significantly increase in solubility of these molecules (2,4,15–18).

The dexamethasone acetate (DMA) is a synthetic glucocorticoid that is used clinically as an anti-inflammatory and immunosuppressive agent (19). In this study, investigations were performed on the possibility of complexation of DMA with βCD for improving the solubility and dissolution rate of this drug, as well as to characterize the physicochemical properties of formed complexes.

The complexes with βCD were prepared by different methods: kneading, coevaporation and freeze drying at

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stoichiometric ratios. The types and the stability constants of the complexation were established according to phase solubility studies. Differential scanning calorimetry, thermogravimetry, infrared spectroscopy and optical microscopy were used to characterize the solid state of all the products. The dissolution properties of the solid complexes were evaluated compared with those of DMA alone and of a physical mixture between DMA and βCD.

MATERIALS AND METHODS

Materials

The DMA was supplied by Laboratório Farmacêutico do Estado de Santa Catarina-LAFESC (Florianópolis, Brazil) and the βCD was obtained from Roquette (Genay, France).

Phase Solubility Studies

Solubility studies were carried out according to the method reported by Higuchi and Connors (20). DMA, in amounts that exceeded its solubility (16 mM), was added in distilled water containing increasing concentrations of βCD (3.2–16 mM), performing the following DMA:βCD molar ratios: 1:0, 1:0.2, 1:0.4, 1:0.6, 1:0.8, 1:1. At least three samples of each molar ratio were prepared. The flasks were sealed and shaken for 2 days at 25 °C, after which equilibrium was reached. Subsequently, the aliquots were filtered through 25 μm filter and appropriately diluted. A portion of the sample was analysed in spectrophotometer (23; Shimadzu-UV 1601PC, Japan) at 240 nm against blanks prepared in the same concentration of βCD in water, so as to cancel any absorbance that may be exhibited by the βCD. The apparent stability constant (K_c) of the complexes was calculated from the slope of the phase-solubility diagrams using the equation $K_c = \text{slope}/S_0(1 - \text{slope})$. The slope is obtained from the initial straight-line portion of the plot of DMA concentration against βCD concentration, and S_0 is the solubility of DMA in water, in absence of βCD.

Preparation of Physical Mixtures and Inclusion Complexes

The preparation of solid complexes of DMA and βCD were performed by different techniques, which are described below. Based on the results of the preliminaries phase solubility studies, the molar ratio was kept at 1:1 in all cases.

Physical mixture (PM): physical mixture was prepared by homogeneous blending of previously weighed DMA and βCD in a mortar for 10 min.

Kneading Method: kneaded (KN) product was obtained by adding small amount of water to βCD placed in a mortar and mixing to obtain a homogeneous paste. Then, DMA powder was slowly added and the mixture was kneaded for 60 min. During the process few drops of water were introduced to maintain a suitable consistency. The resulting paste was dried in an oven at 45 °C for 24 h. The dried complex was pulverized into a fine powder.

Coevaporation Method: coevaporated (CV) product was obtained by dissolving equimolar amount of βCD and DMA in suitable volumes of 50% ethanol. The solution was shaken for 7 days at 25 °C and the solvent was then removed in an oven at 45 °C for 24 h. The obtained solid was pulverized into a fine powder.

*Freeze Drying Method: f*reeze–dried (FD) product was prepared by dissolving the βCD in ethanol:NaOH 0,1M (1:3; V/V) solution and adding the stoichiometric amount of the drug. The suspension was shaken for 48 h at 25 °C, the resulting solution was frozen by keeping it in a repository at −20 °C and lyophilized in a freeze–dryer (Terroni Fauvel LT 1000/8, Brazil) for 24 h.

Differential Scanning Calorimetry (DSC)

The DSC curves were obtained in a Shimadzu DSC-60 cell using aluminum crucibles with about 2 mg of samples, under dynamic N_2 atmosphere (50 mL min^{-1}) and heating rate of 10 °C min^{-1} under a temperature range from 25 to 550 °C. The DSC cell was calibrated with indium (mp 156.6 °C; ΔH_{fus}=28.54 J g^{-1}), and zinc (mp 419.6 °C).

Thermogravimetric Analysis (TGA)

The TG curves were obtained with a thermobalance model Shimadzu TGA-50 under a temperature range 25–900 °C, using platinum crucibles with 4 mg of sample, under dynamic N_2 atmosphere (50 mL min^{-1}) and heating rate of 10 °C min^{-1}.

Infrared Spectroscopy (IR)

Complex formation was evaluated by comparing the IR spectra of the solid complexes and of the physical mixture containing the same amount of DMA assayed in all products. Blends corresponding to 4 mg of samples and 400 mg of KCl were produced, compressed and analysed on a spectrophotometer (Perkin-Elmer 1420, USA) in the region of 4,000–600 cm^{-1}.

Optical Microscopy

Microscopic observation of products was performed under a microscope (Zeiss Axiostar Plus, Japan). The samples were mounted on a glass slide, viewed under normal light and pictures were taken with a Zeiss MC80DX camera.

In Vitro Dissolution Studies

The dissolution behaviors of the DMA-βCD complexes were compared with those of pure DMA and physical mixture. The dissolution rate studies were performed according to the USP XXVI (21) rotating basket method in a dissolution tester (Nova Ética 299/6, Brazil). In order to maintain sink conditions, the samples, corresponding to 20 mg of DMA were placed into hard gelatin capsules. The products were analysed in three different mediums: 900 mL of simulated gastric fluid (SGF pH 1.2), simulated intestinal fluid (SIF pH 7.4) and distilled water (DW). The stirring speed was 100 rpm, and the temperature was maintained at 37 °C. The samples (10 mL) were withdrawn and analyzed by UV spectrophotometer at 240 nm. The same volume of fresh medium was replaced and the correction for the cumulative

dilution was calculated. Dissolution experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Phase Solubility Studies

The solubilization ability of CDs can be quantitatively evaluated by the phase solubility method developed by Higuchi and Connors (20). The phase solubility diagram at 25 °C was obtained by plotting the apparent equilibrium concentrations of the drug against βCD concentrations and is reported in Fig. 1. The apparent solubility of DMA increased linearly as function of βCD concentration (R^2=0.9946) up to 16 mM, corresponding to the aqueous solubility of βCD. The linear relation between DMA solubility and βCD concentration indicates a A_L-type phase-diagram, defined by Higuchi and Connors (20). This diagram is characteristic of 1:1 complexation and suggested that water soluble complex was formed in solution (11). Furthermore, the slope value was lower than one (0,088) indicating that inclusion complex in the molar ratio of 1:1 between the guest (DMA) and host (βCD) molecules was obtained. DMA solubility increased 33-fold when βCD was used in concentration of 16 mM. The apparent stability constant (K_c) of DMA-βCD complex (1:1) was calculated as 2285.7 M^{-1} from the linear plot of the phase solubility diagram.

Thermal Analysis

The thermal analysis has been reported as an important method to recognition and characterizes CDs complexes. When guest molecules were embedded in CDs cavities or in the crystal lattice, their melting point is generally shifted to a different temperature, and the intensity decrease or disappear (9,22). Therefore, the thermal behavior of DMA-βCD solid complexes and physical mixture was studied using DSC in order to characterize the complexation. The DSC curves of pure components and of the products are shown in Fig. 2. The

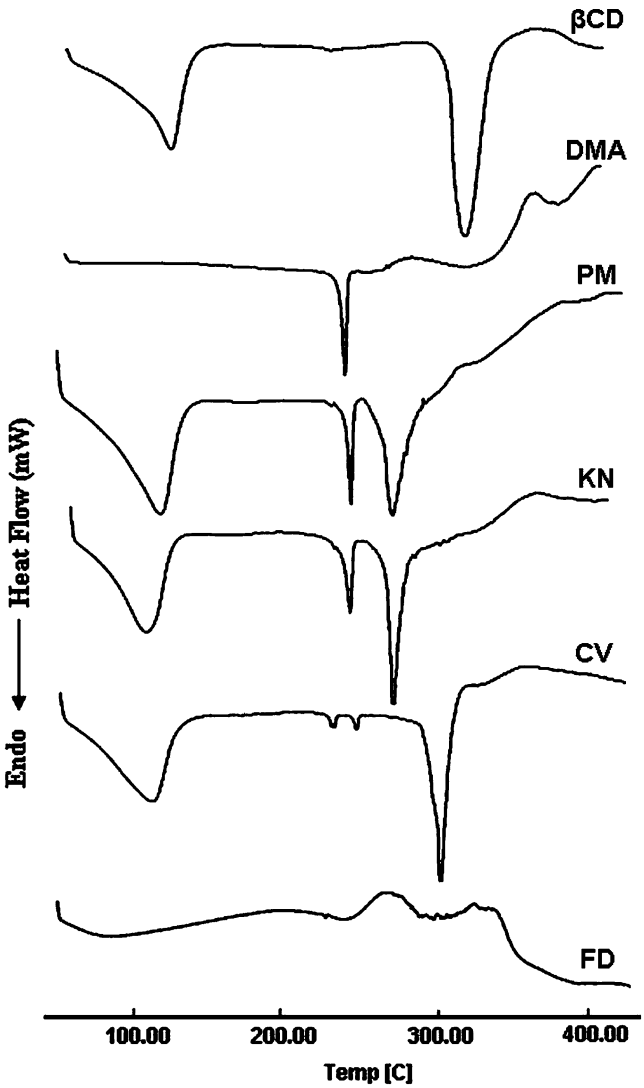

Fig. 2. DSC curves of βCD, DMA, physical mixture (PM), kneaded (KN), coevaporated (CV) and freeze-dried (FD) products

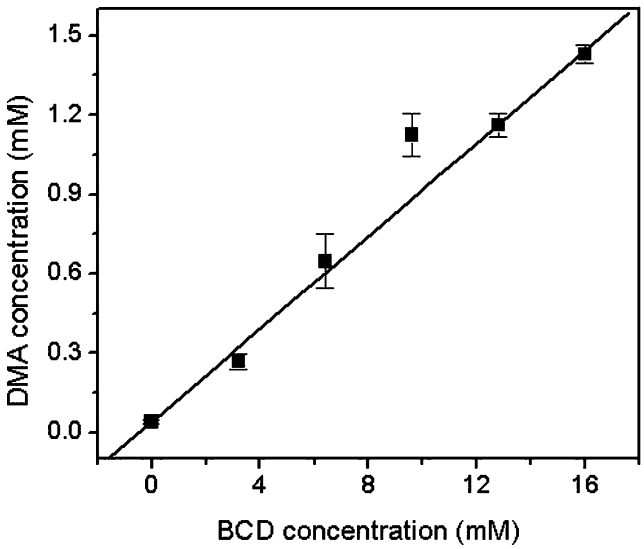

Fig. 1. Phase solubility diagram of the DMA in the presence of different concentrations of βCD at 25 °C

DSC curve of DMA was typical of a crystalline anhydrous substance with a sharp melting endotherm (T_{onset}=227.35 °C and ΔH_{fusion}=129.1 mJ), followed by two exothermic degradation events, confirmed by TGA/DTGA curves of drug. The DSC curve of βCD alone showed a very broad endothermic effect, between 51 °C and 138 °C, which attained a maximum at around 92 °C corresponding to dehydration process, followed by an irreversible solid phase transition (glass transition, T_g) at 215 °C. Finally, DSC curve showed an endothermic degradation process which took place at around 318 °C.

The DSC thermograms of the physical mixture, kneaded and coevaporated products showed the persistence of the endothermic phenomenon due to loss of water, characteristic of βCD, and the melting peak described for the drug slightly shifted to higher temperatures. However, a new endothermic peak can be observed, with T_{onset} at 263, 261 and 291 °C for the physical mixture, kneaded and coevaporated products, respectively. The TGA curves of products (Fig. 3) revealed that these peaks are not attributed to the degradation, because no weight loss is observed at these temperatures.

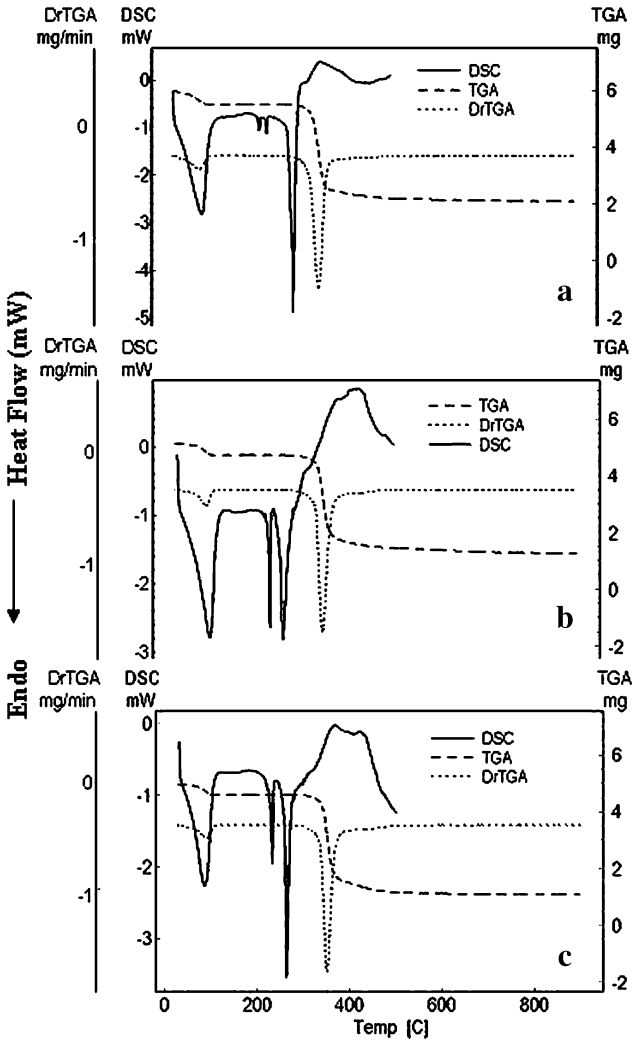

Fig. 3. DSC and TGA/DTGA curves of coevaporated product (**a**); physical mixture (**b**) and kneaded (**c**) products

The appearance of DMA melting peak in the physical mixture, kneaded and coevaporated thermograms is indicative of the drug alone. It can be suggested that the drug melting leads to solubilization of βCD and possibly the complexation *in situ* with DMA in the physical mixture and kneaded product. However, for the kneaded product the DMA melting peak was smaller and the new melting peak was higher and sharper than the physical mixture, indicating that the complexation had already occurred. In case of the coevaporated product, the quantity of non-complexed drug is significantly smaller, and melting peak was sharper, suggesting a better complexation between DMA and βCD, as well the complex formation more crystalline when this method was employed. Thus, these thermal behaviours changes show a interaction between DMA and CD and indicate the possibility of inclusion complex formed by these techniques.

The thermal analysis of freeze–dried systems revealed the disappearance of the endothermic peak of DMA at 227.4 °C. Furthermore, no endothermal event, other that one indicating the water loss from sample, was noticed for this product. This phenomenon it may be indicative of complete inclusion complex formed without alone DMA, drug amorphization, stronger interaction in the solid state between drug and CD and/or amorphous complex formation.

TGA were performed to investigate the thermal stability of physical mixture and DMA-βCD solid complexes prepared. A systemic analysis on the TGA curves shows that DMA begin it degradation at 272 °C in two exothermic events, while βCD begin at 319 °C in a single endothermic event. The physical mixture, kneaded and coevaporated products shows different thermal stabilities, with degradations that occurred at higher temperature than that for the DMA alone (T_{onset} of degradation 294, 278 and 287 °C, respectively). These data indicated that the thermal stability of drug was improved when it was complexed. The physical mixture was

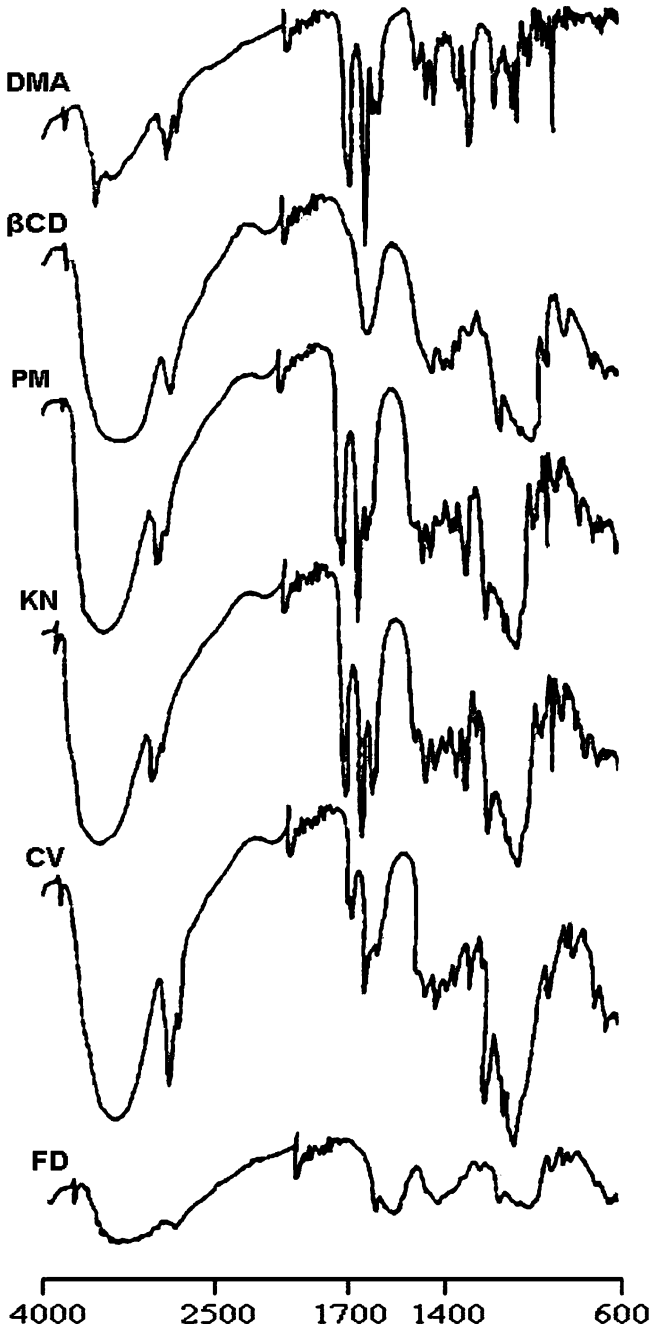

Fig. 4. IR spectra of DMA, βCD, physical mixture (PM), kneaded (KN), coevaporated (CV) and freeze–dried (FD) products

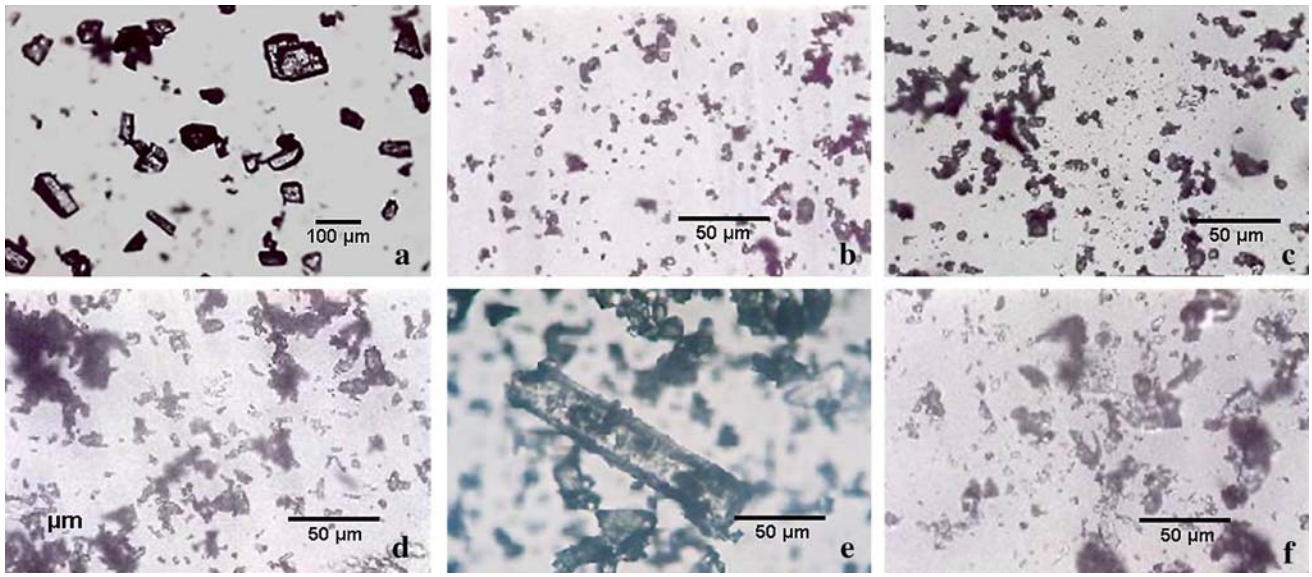

Fig. 5. Optical microscopy of βCD (**a**) at magnification of ×100 and DMA (**b**), physical mixture (**c**), kneaded (**d**), coevaporated (**e**) and freeze–dried (**f**) products at magnification of ×400

more stable than the others products. On the other hand, for the freeze–dried product, there can be seen a decrease in the thermal stability of DMA (T_{onset} of degradation 233 °C), since the degradation process occurs at lower temperature than that for the drug alone. This phenomenon may be consequence of the material amorphization process.

Infrared Spectroscopy

Fig. 4 shows IR spectra of DMA, βCD, physical mixture and DMA-βCD solid complexes formed by kneading, coevaporation and freeze drying techniques. In spectrum 4A characteristic bands of DMA are observed at 1,663 cm^{-1} (C=O vibration), 1,622 and 1,603 cm^{-1} (range of C=C and C=O vibration). βCD spectrum (Fig. 4b) presents a large band and a peak in the region of 2,900–3,800 cm^{-1}, a shorter band between 1,600 and 1,700 cm^{-1}, and a large region which displays distinct peaks in the region of 900–1,400 cm^{-1}. The physical mixture and kneaded exhibited spectrum corresponding to a superposition of their parent components (Fig. 4c and d, respectively), however, for the kneaded product the drug characteristics peaks were smaller. Whereas in the IR spectra of coevaporated product, there is a change in the characteristic bands absorption of carbonyl and alkenes groups of DMA. The freeze–dried spectra also showed differences, with absence of these absorption bands.

Table I. Dissolution Efficiency (DE)[a], Percent of Dissolved DMA at 10 (Q_{10}) And 60 min (Q_{60}), $t_{50\%}$[b] and Relative Dissolution Rate at 20 min (rdr 20')[c] of DMA, Physical Mixture (PM), Kneaded (KN), Coevaporated (CV) and Freeze–Dried (FD) Products in Three Different Mediums[d]

Products	Medium	DE (%)	Q_{10} (%)	Q_{60} (%)	$t_{50\%}$ (min)	rdr 20'
DMA	DW	25.6±4.3	2.5±0.2	22.2±5.3	393[e]	1
	SGF	28.9±4.8	6.5±1.2	24.4±3.2	286[e]	1
	SIF	48.5±6.9	2.8±1.1	44.1±5.2	90	1
PM	DW	30.6±1.6	2.2±0.1	23.8±1.1	247[e]	2.12
	SGF	37.5±14.5	8.8±4.8	28.2±13.4	180	1.01
	SIF	34.9±6.2	5.4±3.7	25.4±6.6	200[e]	1.91
KN	DW	81.3±2.8	2.6±3.7	77.5±1.1	19	13.38
	SGF	84.6±7.9	41.1±9.3	29.1±11.7	14	3.37
	SIF	71.2±7.6	29.1±6.4	65.8±11.5	21	8.18
CV	DW	23.8±0.8	15.0±0.8	21.7±0.4	670[e]	4.39
	SGF	19.5±0.5	11.1±0.6	17.4±0.3	932[e]	0.76
	SIF	22.5±1.2	13.9±1.2	20.8±1.2	872[e]	2.86
FD	DW	94.7±4.5	47.5±40.8	108.2±0.3	9	26.37
	SGF	97.2±3.1	104.9±1.9	107.4±1.5	3	6.17
	SIF	95.8±3.3	94.5±8.2	105.4±0.8	4	17.23

[a] Calculated from the area under the dissolution curve at 240 min
[b] Time in which 50% of DMA is dissolved
[c] Ratio between amount of drug dissolved from a product and dissolved from drug alone at 20 min
[d] Distilled water (DW), simulated gastric fluid pH 1.2 (SGF) and simulated intestinal fluid pH 7.4 (SIF).
[e] $t_{50\%}$ was esteemed through of curve linearization, with linear correlation coefficient superior to 0.9800 (CI=95%).

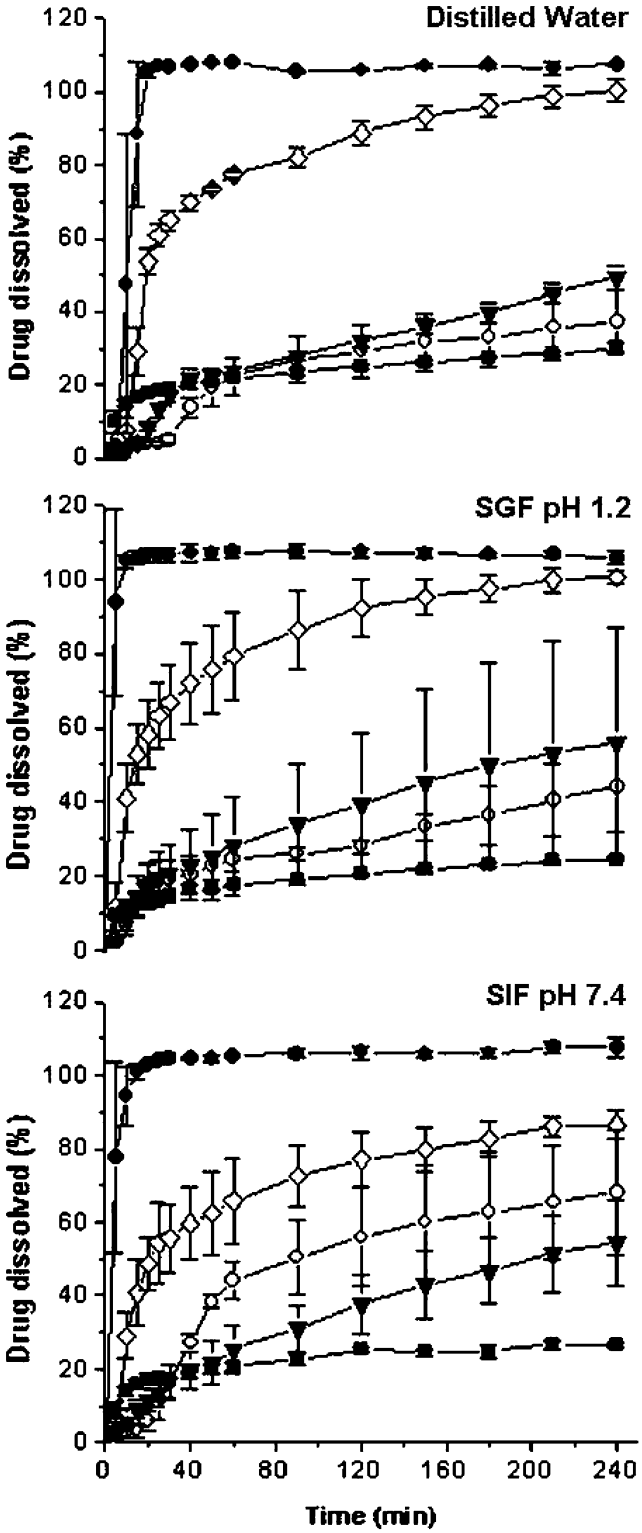

Fig. 6. Dissolution curves of complexes, drug alone and physical mixture between DMA and βCD in distilled water, SGF pH 1.2 and SIF pH 7.4. Key: (*empty circles*) DMA; (*filled triangles*) physical mixture; (*empty diamonds*) kneaded, (*filled squares*) coevaporated and (*filled circles*) freeze–dried products

These spectral changes suggest that carbonyl group of A ring is probably enclosed in the βCD cavity and confirm the published by Vianna *et al.* (9).

Optical Microscopy

In order to investigate the influence of complexation on morphology of particles, optical microscopy was performed for the DMA, βCD, physical mixture and DMA-βCD solid complexes. The photomicrographs of samples are show in Fig. 5. The images of βCD (Fig. 5a) and DMA (Fig. 5b) indicate that these powders are preferably crystalline, and the size of βCD crystals is larger than DMA crystals. This difference can be visualized in the photomicrography of physical mixture (Fig. 5c), where both DMA and βCD crystals can be detected. The kneaded product shows a crystalline powder with particles distinct morphologically of DMA (Fig. 5d). This difference in the morphology of particles also occurred in coevaporated product, however, this material presented a very superior size, indicating the formation of a new crystal (Fig. 5e). It confirms the results of DSC curves, where a new fusion event appears in the temperature above to the DMA melting. Furthermore, the presence of some amorphous particles could be evidenciated between the crystal particles of coevaporated product. On the other hand, the freeze–dried product showed the presence of amorphous particles (Fig. 5f), characteristic of products prepared by freeze drying process.

Dissolution Studies

The results of the dissolution studies are summarized in Table I in terms of dissolution efficiency (DE), percent of dissolved DMA at 10 (Q_{10}) and 60 min (Q_{60}), $t_{50\%}$ and relative dissolution rate at 20 min (rdr). In the cases in which the percent of dissolved drug has not reached 50% until 240 min, $t_{50\%}$ was presumed through curve linearization, considering a linear correlation coefficient superior to 0.9800 (CI=95%). The dissolution profiles were drawn as the percentage of drug dissolved *versus* time in Fig. 6. In all mediums DMA showed an incomplete dissolution even in four hours. Nevertheless, the rate dissolution of DMA in simulated intestinal fluid (SIF pH 7.4) was increased slightly due to the acidic characteristic of molecule, being significant statistically of other mediums ($p<0.01$). In contrast, the complexes formed presented a higher dissolution rate in simulated gastric fluid (SGF pH 1.2), which indicate that these have different ionization characteristics. According to the results, it is evident that the freeze–dried and kneaded products exhibited higher dissolution rates than the drug alone, in all the mediums. The extent of the enhancement of the dissolution rate was found to be dependent on the preparation method, since physical mixture and coevaporated showed smallest dissolution rates.

As shown in Table I, the freeze–dried product presented larger effectiveness in terms of DE, Q_{10}, Q_{60}, $t_{50\%}$ and rdr 20'. The rdr 20'was statistically different ($p=0.0085$), following the rank order of freeze–dried > kneaded > coevaporated > physical mixture (except SGF, in which coevaporated product shows a smaller quantity of dissolved drug compared to physical mixture). As example, when the medium used was the distilled water, the freeze–dried product increased 26.4-fold the dissolution rate of DMA. The same results were obtained in SGF and SIF without statistic difference ($p>0.05$). The great increase of the drug dissolution rate observed

in the freeze–dried product was probably due to amorphisation of the drug or formation of DMA-βCD complex by the freeze drying process. These results confirm the finding in DSC and optical microscopy analysis for this product. The complexes prepared by kneading technique also appears an improvement in drug dissolution properties, exhibiting a dissolution rate up to 41% of DMA in 10 min, and up to 77% in 60 min, whereas DMA alone exhibited the dissolution of 6,47% at 10 min and not more than 24% at 60 min, except in SIF. According to the results found through physicochemical characterizations, this increase in dissolution rate could be attributed to the inclusion of DMA into the hydrophobic cavity of the βCD and formation of a complex more soluble that the drug alone. Furthermore, the solubility of non-complexed drug could be increased due to the solubilizing effect of the βCD and the dissolution medium can be propitiated the complexation *in situ*. The dissolution profiles of kneaded product in the three different mediums showed statistic difference ($p<0,05$). Considering the DE values of kneaded product and DMA, the increase in dissolution rate was more significantly in SGF and distilled water than in SIF, since that drug alone had better solubility in this medium.

The physical mixture showed a slight increase in the dissolution rate and in the parameters exhibited in Table I in distilled water and SGF. Considering the results obtained by DSC, IR and optical microscopy, the complexation between DMA and βCD is difficult when this technique is employed. Thus, the improvement of dissolution obtained with physical mixture is probably attributed to both, improved drug wettability due to presence of hydrophilic CD, which can reduce the interfacial tension between poorly soluble drug and dissolution medium, as well the formation of readily soluble complexes *in situ*. The percentile difference of DMA dissolved from physical mixture in the three mediums analysed was statistically significant ($p<0.001$) and suggest that the complexation *in situ* could be dependent of dissolution medium. Although the thermal analysis, IR and optical microscopy of coevaporated product demonstrate the complex formation, the dissolution of these product was incomplete and smaller than others products. These results may be explained by the formation of a compound highly crystalline between DMA and βCD, with higher stability and smaller free surface energy than drug alone. Furthermore, the optical microscopy indicates that the particle size of coevaporated product is superior to the drug alone, which results in a smaller surface contact with mediums. Probably, all these factors could actuate together resulting in solubility decrease and dissolution properties of coevaporated product. However, the rdr 20' presumed for this product was higher than rdr of physical mixture, and this fact may be attributed to the presence of amorphous particles (confirmed by photomicrographs) that propitiated a fast dissolution in the first moment, followed of slow dissolution in consequence of the low solubility of the crystalline complex formed. For this product, the percent of drug dissolved also demonstrate statistics difference between the three mediums analyzed ($p<0.001$).

CONCLUSION

The present study shows that DMA can form inclusion complexes with βCD in the stoichiometric ratio of 1:1, resulting in a A_L-type phase-diagram. It was proven that the physicochemical properties of the products were different in relation to drug, which indicates the complex formation of DMA with βCD. Furthermore, the complexation methods influenced the thermal stability, infrared absorption and particle form of products, demonstrating that the interaction between the drug and the CD is dependent of the technique used.

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