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ORIGINAL ARTICLE

Preparation and physicochemical characterization of omeprazole:methyl-beta-cyclodextrin inclusion complex in solid state

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Abstract In this work, we illustrate the usefulness of cyclodextrins, namely, methyl-β-cyclodextrin (MβCD), an amorphous, methylated derivative of the natural β-cyclodextrin (βCD), as a tool to form an inclusion complex with omeprazole (OME), a poorly water soluble drug. Solid binary systems between OME and MβCD were prepared experimentally in a stoichiometry 1:1 by different techniques (physical mixing, kneading, spray-drying and freeze-drying). Afterward these products were characterized by Fourier transform-infrared spectroscopy (FTIR); X-ray diffractometry (XRPD) and scanning electron microscopy (SEM). The results obtained suggest that spray-drying and freeze-drying methods yield a higher degree of amorphous entities suggesting the formation of inclusion complexes between OME and MβCD.

Keywords Inclusion complex · Freeze-drying · Methyl-β-cyclodextrin · Omeprazole · Spray-drying

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Introduction

The ultimate mediator of acid secretion is the H^+/K^+-ATPase. This pump is unique to the parietal cells and a number of specific inhibitors of it have, therefore, been developed. The available compounds belong to the family of substituted benzimidazoles: omeprazole (OME), lansoprazole, pantoprazole and rabeprazole [1]. In the acid space of the secreting parietal cells these compounds are converted to thiophilic sulfonamide or sulfenic acid which reacts mainly with the Cys-813 residue in the catalytic subunit of the H^+/K^+-ATPase, which is critical for enzyme inactivation [2]. OME, the primary member of the proton pump inhibitors, has been extensively used to control acid disorders. However, the main pharmaceutical drawbacks are mainly related to the physicochemical instability to heat, light and acidic media, even with coated formulations. Moreover the low aqueous solubility of OME (~0.4% at 25 °C) is responsible for small dissolution rates and hence low bioavailability [3]. In recent years, to overcome the drugs stability and solubility limitations, several approaches have been investigated. Cyclodextrins (CDs) have been extensively used as complexing agents to improve solubility and stability of a variety of poorly soluble and labile drugs. Natural CDs have limited water solubility that negatively influences water solubility of the formed complex. To overcome this problem alkyl moiety, such as hydroxyalkyl or methyl, on free hydroxyl groups of βCD were introduced. The complexing ability of CD derivatives was significantly modified in respect to the parents [4].

Therefore the purpose of this work was to investigate the ability of MβCD to include OME and to

perform the physicochemical characterization of the inclusion complex formed in solid state by Fourier transform-infrared spectroscopy (FTIR), X-ray diffractometry (XRPD) and scanning electron microscopy (SEM).

Materials and methods

Chemicals

Methyl-beta-cyclodextrin, MβCD (Lot 768240; MW = 1190 and an average degree of substitution, DS = 0.5) was a gift from Roquette (Lestrem, France) and OME (Lot 18104; MW = 345.42) was kindly donated by Belmac Laboratory, S.A. (Barcelona, Spain). All other chemicals and solvents were of analytical grade.

Preparation of solid binary systems

The preparation of OME:MβCD solid systems was performed in 1:1 molar ratio by kneading, spray-drying and freeze-drying. Physical mixtures were prepared as reference to perform comparative evaluations with solid inclusion complexes.

Physical binary mixture (PM)

PM was prepared by homogeneous blending in a mortar of previously sieved (63–160 μm sieve granulometric fraction) and weighted OME and MβCD.

Kneaded binary product (KN)

MβCD was wetted in a ceramic mortar with a basic aqueous solution with pH of 10 ± 0.1 until a paste was obtained (about 30% of the total weight). The required amount of OME was then slowly added and the slurry was kneaded for about 45 min. During this process an appropriate volume of the basic aqueous solution was added in order to maintain a suitable consistency and to avoid OME degradation. The final product was then allowed to equilibrate at room temperature for 48 h protected from light.

Spray-dried binary product (SD)

Equimolar amounts of OME and MβCD were dissolved in ethanol and basic aqueous solution (pH = 10 ± 0.1), respectively. Both solutions were mixed and stirred for 24/48 h, being the pH of the solutions adjusted during the process to avoid the possible OME degradation. Solutions were subsequently spray dried in a LabPlant SD-05, under the following conditions: inlet temperature 102 °C, outlet temperature 60–65 °C, flow rate of the solution 400 ml/h, airflow rate 40–50 m^3/h and atomizing air pressure 1.0–1.1 bar. [5].

Lyophilized binary product (LPh)

An appropriate amount of MβCD was dissolved to a basic aqueous solutions (pH = 10 ± 0.1). After that, OME was added to this solution under stirring, according to the stoichiometry 1:1. The solution stirring was maintained for 24/48 h. Furthermore, the resultant clear solution was frozen by immersion in an ethanol-bath at –50 °C (Shell Freezer, Labconco, Freezone® model 79490) and then the frozen solution was lyophilized in a freeze-dryer (Lyph-lock 6 apparatus, Labconco) for 72 h.

The obtained powders were sieved (63–160 μm) and their drug content was determined by UV assay at 305.5 nm.

X-ray powder diffraction (XRPD)

X-ray powder diffraction patterns of OME, MβCD and binary systems (PM and inclusion complexes) were obtained at room temperature with a Philips X'Pert, model PW 3040/00 diffractometer system equipped with Co as anode material and a graphite monochromator using a voltage of 40 kV and a current of 35 mA. The difractograms were recorded in the 2θ angle range between 5 and 50° and the process parameters were set at: scan step size of 0.025 (2θ); scan step time of 1.25 s; and acquisition time of 1 h.

Scanning electron microscopy (SEM)

The surface morphology of the raw materials and binary systems (PM and inclusion complexes) were examined by means of a scanning electron microscope (Jeol, JSM 5310, Tokyo, Japan). The samples were fixed on a brass stub using double-sided tape and then made electrically conductive by coating in vacuum with thin layer of copper. The photographs were taken with a Pentax (model MZ-10) camera at an excitation voltage of 10 KV and magnifications factors of 500 and 3500.

Fourier transform infrared spectroscopy (FTIR)

OME, MβCD, PM and inclusion complexes spectra were recorded using a Jasco FT/IR-420 spectrometer associated with an ATR horizontal reflexion (Miracle™, PIKE Technologies). Spectra acquisitions were per-

formed directly in powder samples with the application of 16 scans at a resolution of 4 cm^{-1} over the range 4000–400 cm^{-1}.

Results

X-ray powder diffraction (XRPD)

The X-ray powder diffractograms of the pure components (OME and MβCD), PM and inclusion complexes, are shown in Fig. 1 and peak intensities are presented in Table 1. The XRPD patterns of OME revealed several diffraction peaks which are indicative of its crystalline character, while a hollow pattern was recorded for MβCD which comprove its amorphous state. Comparing the diffraction patterns of pure components with PM it was possible to observe that diffractograms of PM resulted of the combination of the components analyzed separately, with a marked decrease in the intensity of the diffraction peaks, which can be attributed to the amorphous character of this CD and the reduction in particle size during the preparation of the PM. The KN system presented a diffraction pattern quite similar to that of PM. However, a lower intensity of it diffraction peaks and overlapping between some OME and MβCD peaks was also observed. This fact can be explained by the presence of reciprocal interactions in the solid state between host and guest. OME:MβCD SD and LPh systems exhibited a complete amorphous character, showing a typical flat behaviour that confirms the strong ability of the amorphous carrier MβCD to induce drug amorphization [6]. On the other hand, the loss of the crystallinity state can be a consequence of the lyophilization process and because of that X-ray data could not discriminate whether the LPh product obtained was a true inclusion complex or a homogeneous dispersed mixture of the amorphous components [7]. However, in the case of OME:MβCD SD system we could observe a diffraction pattern completely diffuse, which revealed its amorphousness. This behaviour could be attributed to an interaction between OME and MβCD showing the presence of a new solid phase where a possible formation of an inclusion compound was contemplated [7]. Finally, it was possible to observe that peaks intensities decrease in the following order: PM > KN > SD > LPh systems corroborating with the results previously report.

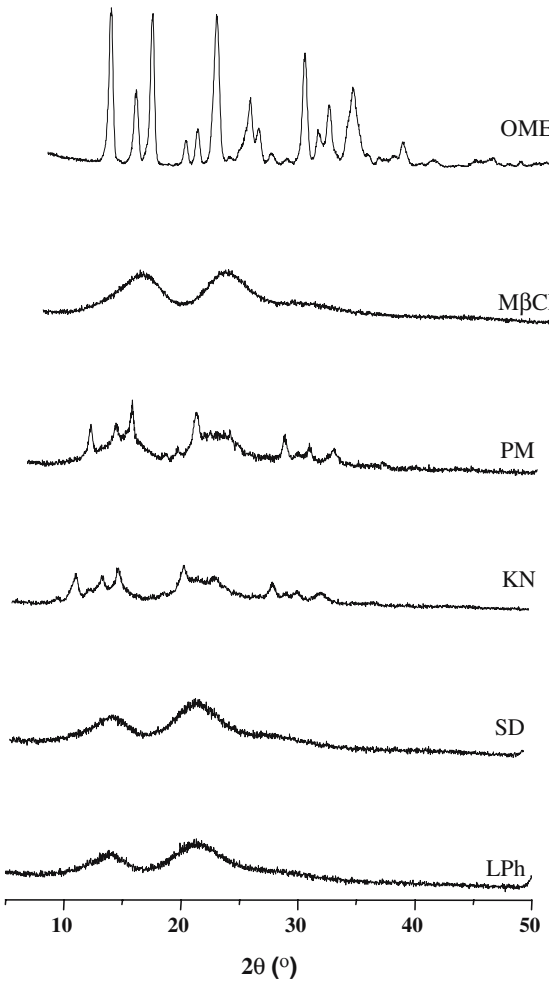

Fig. 1 X-ray diffractograms of OME:MβCD inclusion complexes. Omeprazole (OME), methyl-beta-cyclodextrin (MβCD), physical mixture (PM), kneaded (KN), spray dried (SD) and lyophilized (LPh) systems

Scanning electron microscopy (SEM)

SEM photomicrographs of OME, MβCD and OME:MβCD systems (PM and inclusion complexes) are reported in Fig. 2. OME is characterized by regular shaped crystals while MβCD is composed of amorphous spherical particles [8]. OME:MβCD PM showed the presence of OME small crystals adhered on the surface of CD particles. This fact demonstrated the inexistence of apparent interactions between both species in the solid state. In KN product, it was possible to distinguish OME crystals agglomerated on the surface of CD particles that had lost their original shapes and a drastic change in the aspect of MβCD particles were observed. SD sample showed the typical morphology of preparations generally obtained by this method, very small size spherical particles with high tendency to aggregation [3], which were different from those of raw materials. Finally, LPh product appeared to have a less crystalline structure with a soft, fluffy appearance and again, the crystals of single

Table 1 Peak intensities of OME in the XRPD patterns of OME-MβCD binary systems

2θ	OME	OME:MβCD			
		MF	MLX	SD	LPh
12,813	1785	691	669	470	393
14,288	3429	842	790	500	417
22,912	1608	604	581	556	469
27,713	2573	553	538	339	266
29,838	1449	408	398	305	269

Omeprazole (OME), methyl-beta-cyclodextrin (MβCD), physical mixture (PM), kneaded (KN), spray dried (SD) and lyophilized (LPh) systems

components were still not distinguishable [9]. We can conclude that the changes of KN product when compared with PM was due to the effect of the preparation method in MβCD particles and not due to an interaction between both components [10]. On the other hand, the drastic change of the particle shape and aspect in SD and LPh products, which corroborates the formation of a new solid phase, could be the simple consequence of a crystalline habitus change in those systems or it may support the evidence of the existence of a single phase [5]. Although this technique is not conclusive for assessing the existence of a true inclusion compound in the solid state, it can be useful to prove the homogeneity of the solid phases [3].

Fourier-transform infrared spectroscopy (FTIR)

The infrared spectra of different samples (OME, MβCD, OME:MβCD PM and inclusion complexes) are presented in Fig. 3. In FTIR spectra, C = C–N and S–C = N stretching link vibrations (1626.7 cm^{-1}) and Ar–C–O–CH_3 vibration (1203.4 cm^{-1}) accompanied by the resonance band at 1075 cm^{-1} were used to assess the interaction between MβCD and guest molecule (OME) in the solid state [3]. We can observe that the intensity of both bands is decreased when comparing spectra recorded on the OME:MβCD complexes (KN, SD and LPh) with those of the PM product. The intensity and amplitude of the shifts verified on these bands were in the following range: PM > KN > SD ≈ LPh. In SD and LPh systems, it can be observed that these bands almost disappear, probably owing to a restriction of the vibration related to the complexation process. The band

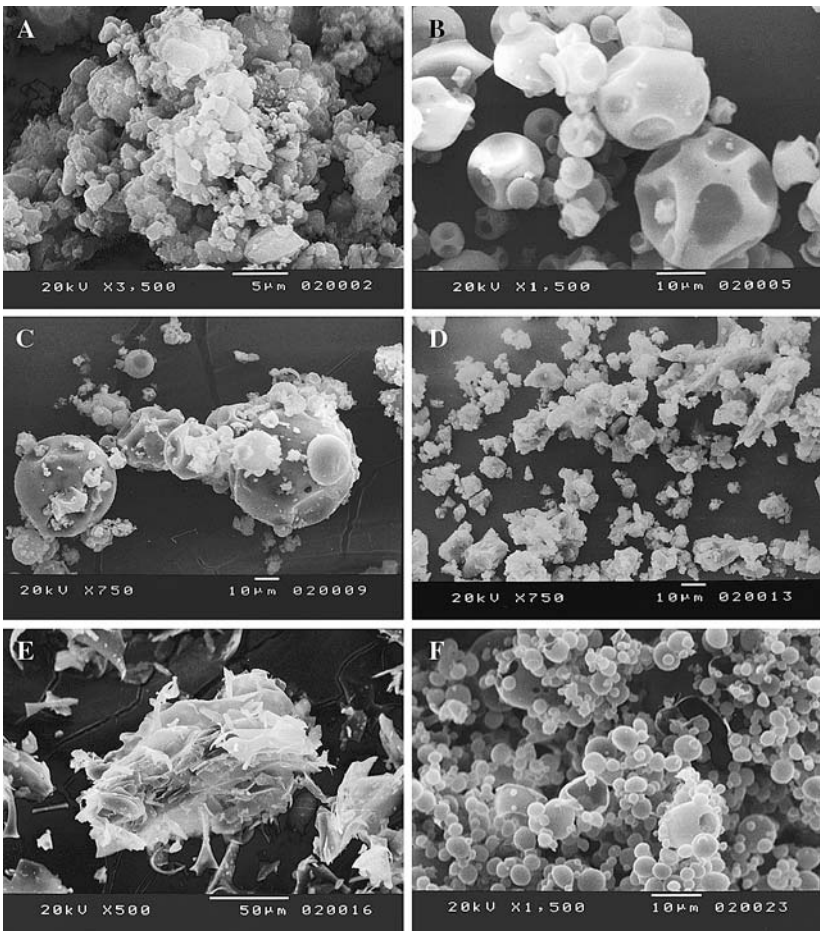

Fig. 2 Scanning electron microphotographs of omeprazole (**A**), methyl-beta-cyclodextrin (**B**), OME:MβCD physical mixture (**C**), OME:MβCD kneaded (**D**), lyophilized (**E**) and spray dried (**F**) systems

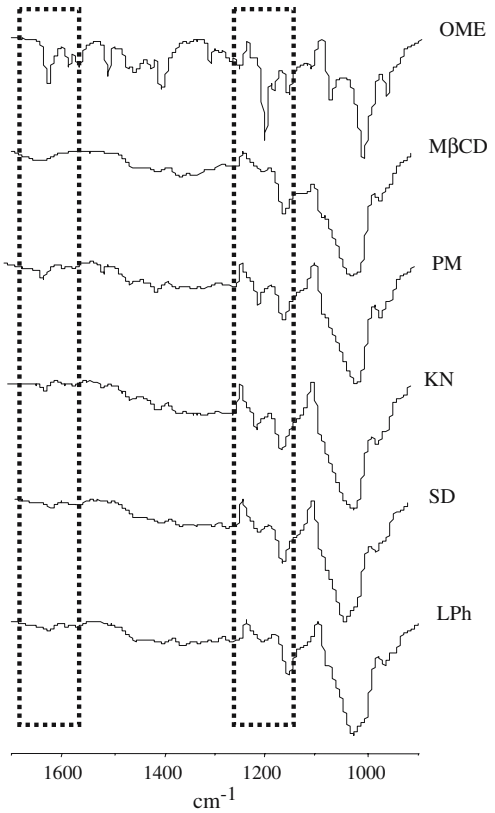

Fig. 3 FTIR spectra (in absorbance) of OME:MβCD inclusion complex. Omeprazole (OME), methyl-beta-cyclodextrin (MβCD), physical mixture (PM), kneaded (KN), spray dried (SD) and lyophilized (LPh) systems

positioned at 1203.4 cm^{-1} is related to bending vibrations of the methoxyl groups of OME and its behaviour may be understood by the occurrence of a restriction due to the inclusion within the cavity of MβCD. These results were also corroborated by ^{1}H-NMR experiments under publication.

Conclusions

SEM, FTIR spectroscopy and XRPD diffractometry have been used to study the inclusion complexes formed between OME and MβCD in solid phase. Remarkable changes were detected in all characterization methods, indicating the promising formation of new solid phases, some of them in amorphous state, allowing to the conclusion of strong evidences of binary inclusion complex formation between OME and MβCD, particularly for SD and LPh systems.

Taking in account these results, we can conclude that the interaction of OME with MβCD, through the formation of an inclusion complex, can lead to important modifications on the physicochemical properties (solubility, stability and consequently bioavailability) of the guest molecule (OME). Those modifications may have a significant impact on the biological effects of the drug. Therefore, studies are being performed to evaluate the potential biopharmaceutical effects of OME:MβCD product on a new drug delivery system.

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