Drug Development and Industrial Pharmacy, 33:163–171, 2007

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040600832801

informa
healthcare

Investigation and Physicochemical Characterization of Clarithromycin–Citric Acid–Cyclodextrins Ternary Complexes

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ABSTRACT The purpose of this study was to investigate the effect of citric acid (CA) on the complexation of clarithromycin (CLM) with β-cyclodextrin (βCD) in aqueous solutions and in the solid state. A phase solubility study revealed a positive effect of CA on the drug solubility. A Bs-type solubility with an apparent stability constant (K_c) of 102.4 M^{-1} was obtained for CLM in βCD solution and 161.2 M^{-1} for CLM in 6 mM βCD solution. Solid ternary complexes were prepared by coevaporation and lyophilization. CLM–βCD–CA interactions were studied in the solid state by differential scanning calorimetry (DSC), infrared spectroscopy, scanning electron microscopy and X-ray diffractometry. A part of the guest molecule was located in the βCD host cavity. The results obtained suggest that the lyophilization method yields a higher degree of amorphous entity than coevaporation.

KEYWORDS Clarithromycin, β-Cyclodextrin, Citric acid, Solubility, Characterization

INTRODUCTION

Cyclodextrins (CDs) are very useful carriers for improving the therapeutic efficacy of drugs with poor solubility or stability problems owing to the formation of inclusion complexes (Loftsson et al., 1996; Rajewski et al., 1996; Stella et al., 1997). The βCD is widely used due to its low cost, availability and cavity dimension. The cavity size is suitable for common pharmaceutical drugs with molecular weights between 200 and 800 (Waleczek 2003). Unfortunately, the complexation efficiency of CDs is rather low and consequently a substantial amount of CDs is frequently needed to solubilize small amounts of a water-insoluble drug. Increasing the complexation and solubilization efficacy of CDs is one possible means of reducing the amount required for pharmaceutical formulations. Several techniques have been used in an effort to achieve this aim. For example, addition of organic solvents, such as ethanol (Piths et al., 1992) to the aqueous complexation media can increase the apparent intrinsic solubility (S_0) of the drug. However, organic solvent molecules can compete with drug molecules for a space in a CD cavity with subsequent decrease in K_c (Zia et al., 2001). Solvents such as acetone and various alcohols

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are frequently used in the production of complexation, but their complete removal from the product can be difficult. Thus, addition of organic solvents can result in an overall decrease in efficiency. It has been shown that for neutral CDs such as 2-hydroxypropyl-β-cyclodextrin (HPβCD) K_c decreases from 2- to 30-fold upon ionization of drug molecule (Mulski et al., 1995). However, it is sometimes possible to enhance CD solubilization of ionizable drugs by appropriate pH adjustments. Ionization of an ionizable drug molecule increases S_0. Although some decrease in K_c is generally observed (due to decreased affinity of the drug molecule for the lipophilic CD cavity), the increase in S_0 is frequently more than enough to compensate for the decrease, resulting in overall enhanced complexation. Addition of certain low molecular weight acids, such as acetic, citric, malic, or tartaric acid, to aqueous complexation media can markedly increase the solubilizing effect of cyclodextrin on basic drugs, as a result of the combined effect of salt formation and inclusion complexation (Barillaro et al., 2004; Mura et al., 2003, 2005; Redenti et al., 2000; Saetern et al., 2004). Similarly, the positive effects on drug solubility of ternary complexation involving an acidic drug, a basic additive have been reported (Redenti et al., 2001). Various pharmaceutical polymers, such as water-soluble polymers have a synergistic effect on the solubility-enhancing effect of the cyclodextrin (Jug et al., 2004; Aggarwal et al., 2002). It has been shown that such multicomponent complexes possess physicochemical properties distinct from those of individual CD molecules (Valero et al., 2004). CLM is a semisynthetic 14-member macrolide ($C_{38}H_{69}NO_{13}$, MW 747.9) exhibiting a broad in vitro antibacterial spectrum. Structurally, it differs from erythromycin only in the substitution of an O-methyl group for the hydroxyl group at position six of the lactone. It is a water-insoluble base ($pK_a = 8.76$) and its solubility is pH-dependent (Nakagawa et al., 1992). CLM has a bitter taste, which has been one of the obstacles to developing pediatric formulations containing it (Yajima et al., 2003). Several approaches have been adopted in order to overcome the solubility and bioavailability limitations of hydrophobic drugs and to guarantee drug effectiveness and safety. One of these is by enhancing solubility and hence, bioavailability, by complexing hydrophobic drugs with cyclodextrin. The efficacy of βCD-complexed CLM prepared in chloroform by co-evaporation against *Mycobacterium avium* complex infections in human macrophages had been investigated (Salem et al., 2003), but the solid states of the various CLM–CD complexes have not previously been characterized. In the present work, we performed a detailed investigation of the role of citric acid (CA) in enhancing the aqueous solubility of the complexes by adding small amount of the acid to the basic drug. A complexation technique was also used to mask the bitter taste of CLM. Two different techniques, coevaporation (COE) and lyophilization (LPh) were applied to obtain the ternary systems. Confirmation of the solid state interaction in ternary systems was performed by physicochemical characterization based on differential scanning calorimetry (DSC), infrared spectrometry (IR), scanning electron microscopy (SEM), X-ray diffractometry (XRD), and comparing these systems with the corresponding physical mixtures prepared in the same molar ratio.

MATERIALS AND METHODS

Materials

Clarithromycin was procured from Jinhua Lixin Pharma Chemical Co., Ltd (Jinhua, China). βCD was procured from Maxdragon International Corporation (Guangzhou, China). Methanol was of HPLC grade. All other chemicals were of analytical grade and were used without further purification, and deionized double-distilled water was used throughout the study.

Phase Solubility Studies

Phase solubility studies were performed using the method that was previously reported by Higuchi & Connors (1965). In this method, 50 mg CLM was added to a series of 6.0 mL βCD solutions from 0.001 to 0.010 mol/L. In addition, 100 mg CLM was added to a series of 6.0 mL of βCD solutions in 6 mM CA solution from 0.001 to 0.010 mol/L. Flasks were sealed to avoid changes caused by evaporation and the suspensions were vigorously shaken at 25°C in a thermostat shaking bath for 3 days. The 3-day equilibration was considered sufficient. The samples were clarified by passing them through 0.45 μm pore-diameter membranes. The CLM concentrations were determined by an HPLC-UV method as described below. All assays were conducted in triplicate. K_c of the

complexes was determined from the slope of the ascending portion of the straight line of the phase solubility diagram according to the equation of Higuchi & Connors (1965).

Coevaporated Ternary Products

The amount of CLM and βCD was a molar ratio of 1:1. βCD was dissolved in water maintained at 40°C and gave a clear solution. CLM was dissolved in an aqueous solution containing 0.5% (w/v) CA. The resulting mixture was stirred on a magnetic stirrer at 40°C for 3 hr to give a clear solution. CLM were dissolved in aqueous containing 0.5% (w/v) CA. The resulting mixture was stirred at 40°C for 4 hr, and the clear solution obtained was evaporated under a vacuum at 50°C in a rotary evaporator. The solid residue was further dried at 40°C for 24 hr.

Lyophilized Ternary Products

The amount of CLM and βCD was a molar ratio of 1:1. βCD was dissolved in water maintained at 40°C and gave a clear solution. CLM was dissolved in an aqueous solution containing 0.5% (w/v) of CA. The resulting mixture was stirred on a magnetic stirrer at 40°C for 3 hr to give a clear solution. The resulting solution was frozen and then lyophilized in a freeze-dryer for 48 hr.

For comparison, physical mixtures (PM) were prepared in the same molar ratio as the complexes. All resultant dried COE and LPh were sieved and collected for further studies.

Drug Content

To ensure no loss and degradation of CLM during the preparation of CLM–βCD ternary systems, accurately weighed samples of ternary products were dissolved in a known amount of mobile phase. After suitable dilution of the samples, the concentration of CLM in the solution was determined by HPLC method as described below. The drug content was calculated from the following equation: Percentage drug content (%) = (practical drug content/theoretical drug content) × 100. The determinations were performed in triplicate. The accurate determination of CLM concentrations for the solubility study was performed in triplicate using a high-performance-liquid chromatographic (HPLC) equipment (HP1100, Agilent). The mobile phase consisted of 65% methanol and 35% (v/v) 0.05 M monobasic sodium phosphate buffer. The pH was adjusted to 4.0 using orthophosphoric acid. A Zirchrom Kromasil C_{18}, 5 μm, 150 × 4.6 mm column was used for separation at a flow rate of 1.0 mL/min. Concentrations were determined using UV detector set at 210 nm. Method linearity was established over concentrations of 25–1000 μg/mL with a regression coefficient of 0.9998. The method was proved to be sensitive and specific.

IR Spectroscopic Analysis

The IR spectra of the pure components, the inclusion complexes, and the physical mixtures were recorded on an IR spectrophotometer (Bruker, Switzerland) using the KBr disk technique. The scanning range was 400–4000 cm^{-1}.

Differential Scanning Calorimetry Analysis

The DSC curves of pure materials and ternary systems were measured by a differential scanning calorimeter (Shimadzu, Kyoto, Japan). The thermal behavior was studied by heating 1–5 mg samples in a sealed aluminum pan under nitrogen gas flow, using an empty sealed pan as reference, over the temperature range 30–400°C, at a scan rate of 10°C/min.

Scanning Electron Microscopy

The surface morphology of the raw materials and ternary complex was examined using a scanning electron microscope (SHIMADZU SSX-550, Japan). The samples were fixed on a brass stub using double-sided tape and then made electrically conductive by coating in a vacuum with a thin layer of gold. Photographs were taken at an excitation voltage of 10 kV and appropriate magnifications.

X-ray Diffractometry

Power XRD patterns of CLM, CA, βCD, and ternary systems (physical mixture and complexes) were analyzed at room temperature using a voltage of 56 kV and a current of 35 mA in an X-ray diffractometer (D/MAX-3C, Riguku Co., Japan). Samples were finely

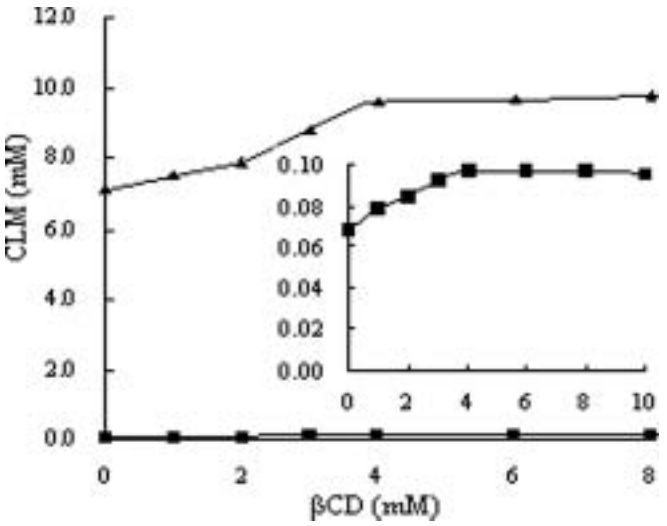

FIGURE 1 **Phase Solubility Profile of CLM-βCD at 25°C in 6 mM Citric Acid Solution (▲) and in Water (■).**

ground in an agate mortar. The XRD traces of all raw materials and ternary systems were compared with regard to the peak position and relative intensity, the presence and/or absence of peaks in certain regions of 2θ values. Crystallinity was determined by comparing some representative peak heights in the diffraction patterns of the ternary systems with a reference. The relationship used for the calculation of the crystallinity was the relative degree of crystallinity (RDC) = I_{SA}/I_{REF}, where I_{SA} is the peak height of the sample under investigation and I_{REF} is the peak height of the same angle for the reference with the highest intensity. To identify the possible interactions among CLM, CA, and βCD, CLM was used as a reference sample for calculating the RDC values of all ternary systems.

RESULTS AND DISCUSSION

Phase Solubility Studies

The phase solubility diagrams of CLM in aqueous solutions of different concentrations of βCD alone and in the presence of CA were obtained by plotting the changes in CLM solubility as a function of βCD. As shown in Fig. 1, the solubility curves can be classified as B_s-type according to Higuchi & Connors (1965). As the slopes of these solubility diagrams were all less than 1, it was possible to assess a 1:1 stoichiometry and calculate K_c using the equation of Higuchi & Connors. K_c values depended on the initial solubility of the drug and the pH of the medium because CLM is weak base.

The complex exhibits higher solubility than the guest molecule, but its limit is reached within the tested CD concentration range. Increasing the amount of available CD-molecules does not lead to a rise in solubility, indicating that all guest molecules have been converted into a less soluble inclusion complex, which denotes an initial rise in the solubility of the solute followed by a plateau because of the limited solubility of the complexes. K_c of the complexes was 102.4 M^{-1} and 161.2 M^{-1} determined in water and in 6 mM CA solution, respectively at 25°C. The K_c values described in literature on drug–CD complexation are normally between 100 and 20,000 M^{-1} (Stella et al., 1997). The CLM solubilization efficiency was improved nearly 104-fold in aqueous 6 mM CA in comparison with CLM intrinsic solubility. The marked increase in solubility of the hydrophobic drug when the multicomponent complex is dissolved in water can be explained by the mutual interaction among the components. The pH measured was about 4.5–5.0 and the ternary system solubility at this pH was higher than the theoretical one calculated by adding the CD or CA at the same pH. Presence of CA, which acts as a counter-ion, may result in an amphipathic property characterized by a hydrophobic portion and a hydrophilic polar head. Not much increase in the apparent stability was explained by the higher initial drug solubility because of an increased ionization of CLM in the presence of CA. This is in agreement with other studies in which tartaric acid was used as an acidifier in the complexation process (Ribeiro et al., 2003, 2005). In preparing ternary systems, an organic solvent was not used to give a clear solution. This prevented any environmental pollution, and a better complexation achieved. Indeed, βCD and CA had a synergistic effect on CLM solubility, allowing better solubility results to be achieved with a reduction in the amount of βCD required to dissolve the CLM. The acidic microenvironment created by the complex could facilitate the dissolution of CLM at higher pH (5–6) and a bioavailability improvement may be realized.

Drug Content

The HPLC analysis performed on the prepared ternary systems showed a drug content of 101.6%, based on the theoretical composition. No new elution peaks appeared in the chromatograms of the CLM-βCD-CA ternary system.

IR Spectroscopic Analysis

Supporting evidence for complexation of a guest molecule with βCD can be obtained by IR spectroscopy. Inclusion complex formation might be proved by IR spectrometry because bands caused by the included part of the guest molecule are generally shifted or their intensities altered. The IR spectra of the complexes were similar to the corresponding pure CD and dissimilar to CLM and a physical mixture as shown in Fig. 2. The IR

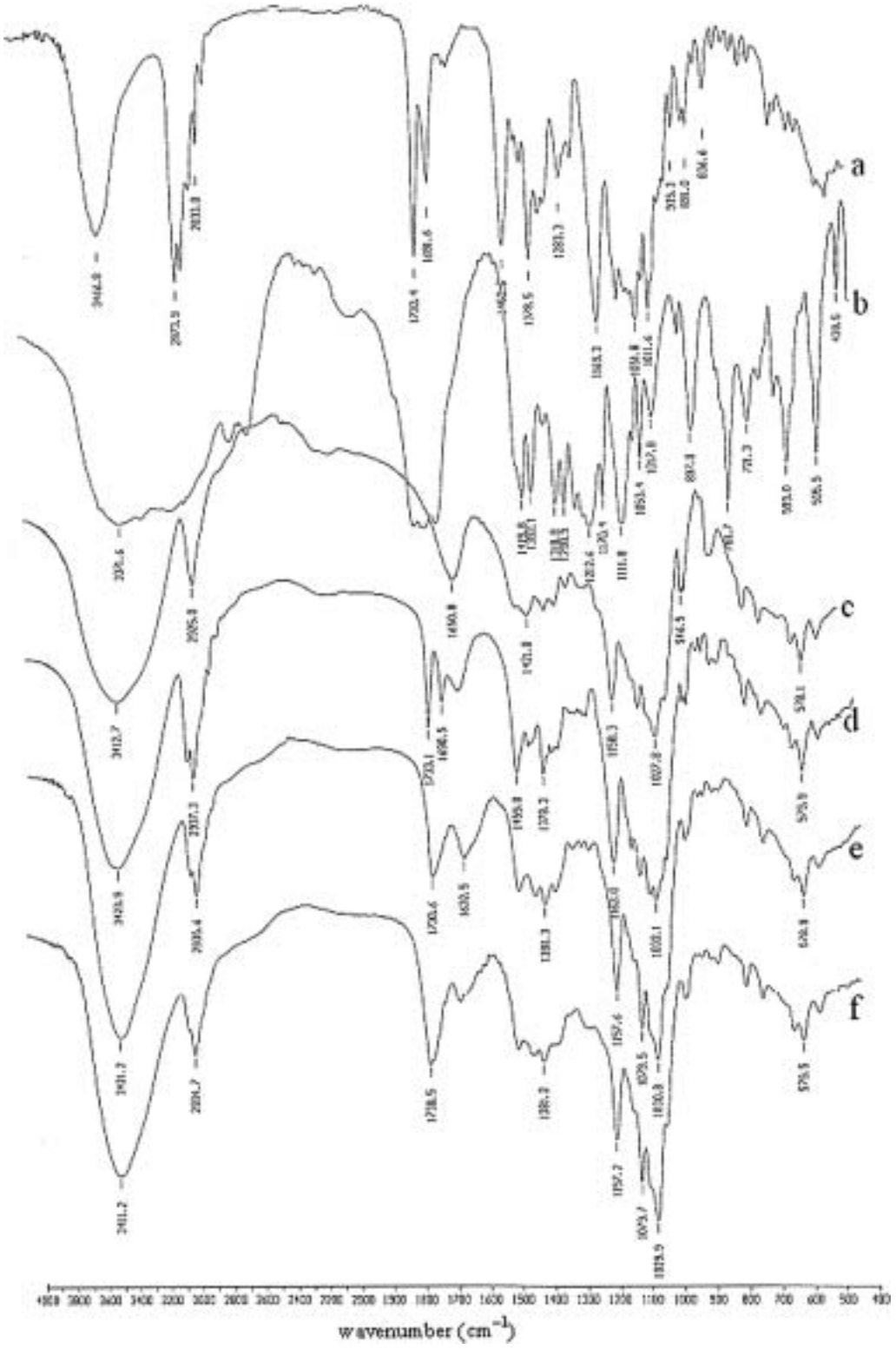

FIGURE 2 IR Spectra of Individual Components and Ternary Systems, CLM (a), βCD (b), CA (c), PM (d), COE (e), LPh (f).

spectrum of the physical mixtures differed from those of the single components to some extent because of a synergistic effect. The complex spectrum showed peaks at 1733, 1079, 1378, 1157 cm^{-1}, which were attributed to C=O, C–O–C, $–CH_3$, and N–C of CLM, suggesting that these groups were not included fully within the βCD cavity. Disappearance of peaks at 1691 cm^{-1} and 1459 cm^{-1} which were attributed to another C=O and $–CH_3$ of the guest also confirms an interaction between the drug, CA, and βCD, whereas the bands of CLM located at 2973, 1733, 1378, 1169, 1051, and 1011 cm^{-1} had shifted. The broadening of the peaks of the guest also confirms an interaction between the drug and βCD molecules. The results showed that C=O of the CLM was partly embedded in the cavity of βCD.

Differential Scanning Calorimetry Analysis

Thermal methods are widely used in the assessment of solid phases. In this study, DSC was used to characterize CLM complexes in the solid state and to obtain further supporting evidence of complex formation.

The thermograms of pure material and ternary systems in the melting range of the drug and dehydration of the carrier are presented in Fig. 3. The thermal curve of pure CLM was typical of a crystalline anhydrous substance with a sharp endothermic peak at 228.90°C corresponding to the melting point of the drug. A broader endothermal effect was recorded for amorphous βCD as a consequence of water loss.

Both characteristic peaks of CLM and βCD (water loss) were clearly distinguishable in the ternary PM. The intensity of the peak was reduced in the PM. The slight change relative to the peak of pure CLM suggests a weak interaction between the pure CLM with CA during the mixing or heating required for DSC scanning.

The disappearance of the CLM endothermic peak in the ternary COE and LPh products is a strong evidence of the formation of amorphous entities and/or inclusion complexes. These results suggest that only the COE and LPh products can be considered as true inclusion complexes, differing from simple PM.

Scanning Electron Microscopy

Figure 4 shows the results of SEM analysis. It can be seen that pure drug particles appeared as small

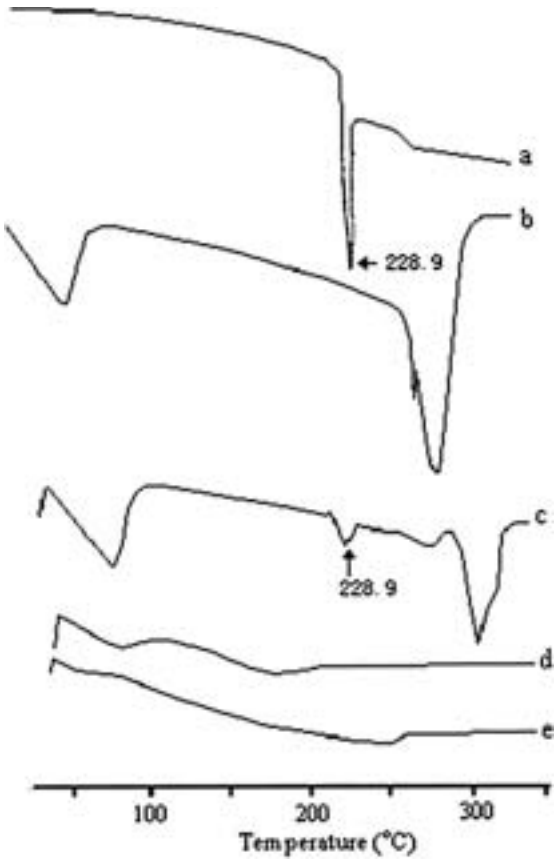

FIGURE 3 DSC Curves of Individual Components and Ternary Systems, CLM (a), βCD (b), PM (c), COE (d), LPh (e).

crystals regular in shape and homogeneous in size (Fig. 4a) whereas hollow spherical particles with a large size distribution were evident in the βCD microphotographs (Fig. 4b). The PM showed particles of βCD embedded with CLM particles and a comparable morphology with pure components occurring separately, revealing no apparent interaction between both species in the solid state (Fig. 4c). On the contrary, a drastic change in the original morphology and shape of both CLM and βCD particles (Fig. 4d and e) was observed in ternary products. The morphology of COE and LPh products was quite similar, showing the loss of column aspect crystal form, smooth surfaced with a tendency to form tiny aggregates of amorphous pieces, clearly different from those of the raw materials attributed to the total solubilization of the raw materials in the course of their preparation. This drastic change in the particle shape, aspect, and size, led us to estimate the existence of a single phase in COE and LPh preparations (Moyano et al, 1997).

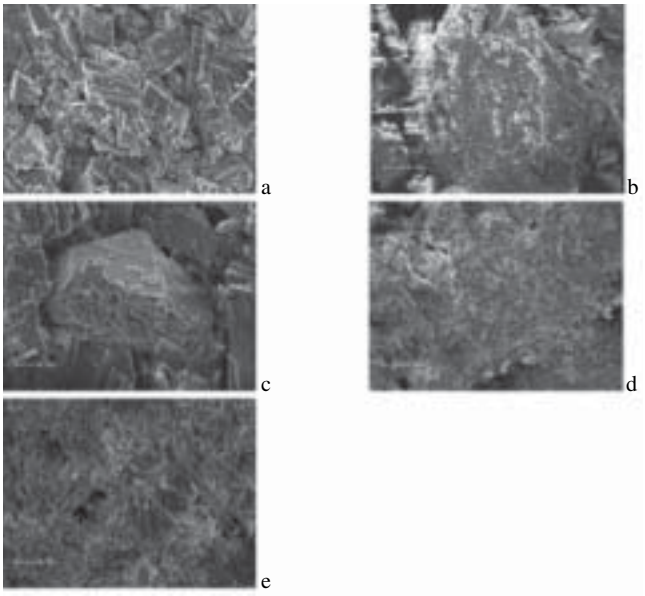

FIGURE 4 SEM of Individual Components and Ternary Systems, CLM (a), βCD (b), PM (c), COE (d), LPh (e).

X-ray Diffractometry

Powder XRD is a useful method for the detection of CD complexation on powder or microcrystalline states. The diffraction pattern of the complex should be clearly distinct from those of the superposition of each component if a true inclusion complex has been formed. The inclusion process may increase the amorphous character and this can be explained by procedure used to obtain the complex.

The XRD patterns of CLM, CA, βCD, the physical mixture, and the ternary system are shown in Fig. 5 and the RDC values and peak intensities of CLM–βCD–CA are presented in Tables 1 and 2. The complexation products were identified by comparing their diffractograms with those of pure CLM, βCD and PM. The XRD pattern of the PM contains the principal diffraction peaks of CLM and βCD with a marked reduction in the intensity of the diffraction peaks of CA. This can be attributed to the reduction in particle size as a consequence of the preparation method and to the dilution of the drug in the PM. The XRD pattern of COE showed several peaks attributable to both the crystalline drug and excipient. A reduced number of signals were noticeable in the complexes, with a markedly reduced intensity, demonstrating the nature of the inclusion compounds, compared with the free molecules. This observation was also in agreement with the results of the IR and DSC studies. The degree of amorphous entity formation in the various ternary systems can be ranked in the following order: PM < COE < LPh. The extent of the formation of amorphous species was found to be dependent on the selected method of preparation.

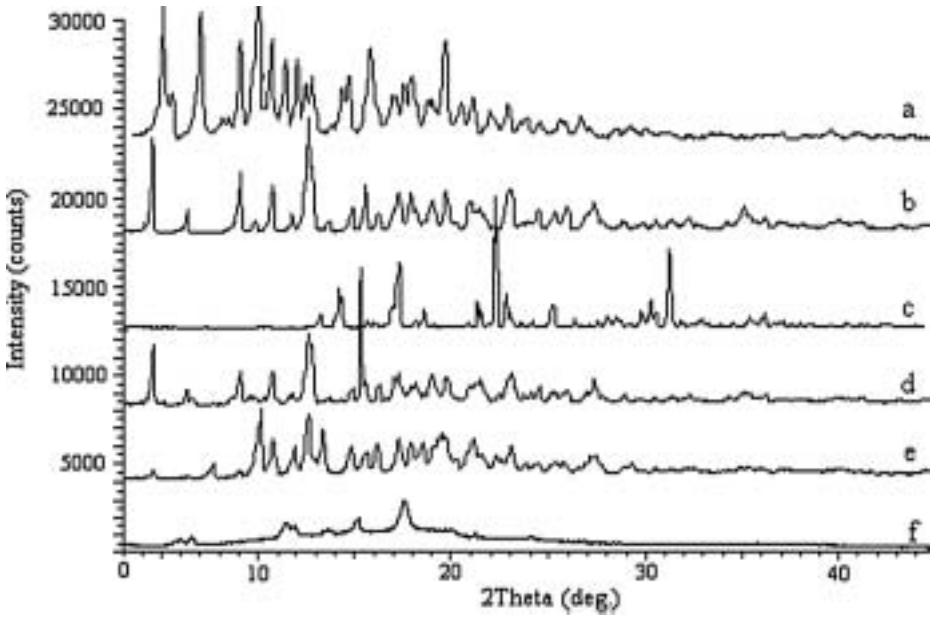

FIGURE 5 XRD Spectra of Individual Components and Ternary Systems, CLM (a), βCD (b), CA (c), PM (d), COE (e), LPh (f).

TABLE 1 **RDC of CLM-βCD-CA Ternary Systems**

Ternary Systems	PM	COE	LPh	COE	LPh
RDC	0.570	0.101	0.051	0.131	0.097
Reference used	CLM	CLM	CLM	PM	PM

TABLE 2 **Peak Intensities of CLM in the XRD Patterns of Ternary Systems**

Peak position (2θ°)	CLM	PM	COE	LPh
4.550	7600	2800	450	295
6.450	7250	600	240	720
8.461	5670	3100	400	380
9.459	8870	5050	900	450
10.055	5820	3200	1100	650
10.760	4770	1200	850	770
11.393	4750	580	800	400
15.153	5300	6700	880	650
18.975	5670	2230	2200	1120

CONCLUSIONS

The physicochemical solid state characterization of the suggested new solid phases had been formed, some of them amorphous, and provided strong evidence of the formation of ternary inclusion complexes between CLM, βCD, and CA, particularly for LPh ternary products.

Based on these results, we believe that the interaction of CLM with βCD and CA, through the formation of ternary inclusion complexes, produces important modifications in the physicochemical properties of the drug, which could have an important pharmaceutical potential use in the development of a suitable oral formulation.

ACKNOWLEDGMENT

This work was financially supported by Grant 2003AA2Z347C of the 863 Program of China.

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