Effect of the Hydrophobic Nature of Triacetyl-β-cyclodextrin on the Complexation with Nicardipine Hydrochloride: Physicochemical and Dissolution Properties of the Kneaded and Spray-dried Complexes

Catarina Marques FERNANDES and Francisco José Baptista VEIGA*

Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra; Rua do Norte, 3000 Coimbra, Portugal. Received August 12, 2002; accepted September 24, 2002

The inclusion ability of triacetyl-β-cyclodextrin (TAβCD), a hydrophobic cyclodextrin (CD) derivative was examined, using nicardipine hydrochloride (NC) as model drug. The binary compounds were prepared in a 1 : 1 molar ratio by the kneading and the spray-drying techniques. In order to confirm the complexation between NC and TAβCD in the solid state, differential scanning calorimetry, X-ray diffractometry, Fourier transformation-infrared spectroscopy and scanning electron microscopy were carried out and the results were compared with the corresponding physical mixture in the same molar ratio. The kneaded product presented only slight modifications on the drug physicochemical and morphological properties, which could mean that no complex formation occurred during this process. In contrast, spray-drying was found to produce inclusion complexes with amorphous nature. *In vitro* dissolution studies were carried out in simulated gastric (pH 1.2) and intestinal (pH 6.8) fluids, according to the United States Pharmacopoeia (USP) basket method. The NC *in vitro* release from the kneaded and spray-dried products was markedly retarded in both dissolution media. However, this retarding effect was significantly more evident for the spray-dried compound. It was concluded that the formation of real inclusion complexes could only be achieved by the spray-drying method.

Key words triacetyl-β-cyclodextrin; sustained release; nicardipine; spray-drying; kneading

Cyclodextrins (CDs), cyclic oligosaccharides with a hydrophobic central cavity, have been widely applied as multifunctional pharmaceutical excipients due to their remarkable molecular complexation property with many drugs, modifying their physical, chemical and biological properties.[1—3] Recently, various kinds of chemically-derivated CDs have been prepared in order to extend the physicochemical properties and inclusion capacities of the parent βCD as multifunctional drug carriers.[3,4] For example, hydrophilic CD derivatives such methylated, hydroxyalkylated and branched CDs are now being widely applied to improve the low solubility, dissolution and bioavailability of poorly water-soluble.[3] On the contrary, there is little information concerning the hydrophobic CD derivatives, such acylated and ethylated CDs, which could act as slow or sustained-release carriers for drugs with short biological half-lives.[5,6]

Nicardipine hydrochloride (NC), a calcium channel-blocking agent, was used in this study as model drug (Fig. 1). NC is an effective drug in the management of mild to moderate hypertension, angina pectoris and cerebral disease. However, the drug biovailability is very limited (15—40%) and like other dihydropyridine derivatives, its standard formulation undergoes rapid absorption and extensive biotransformation in the liver, with short elimination half-life (about 1 h), which often results in significant fluctuations in plasma concentrations.[7] To attain a prolonged therapeutic effect and a reduced incidence of side effects, sustained or controlled release formulations of NC have been developed to maintain a suitable plasma level for a long period of time with minimal frequency of daily administration.[8—10]

The most applied preparation method of complexes with hydrophobic CDs is definitely the kneading technique. Thus, we decided to compare this method with the spray-drying one, which has not yet been used for this kind of complexes. Also, to our best knowledge, no characterization studies, like Fourier transformation-infrared spectroscopy and scanning electron microscopy, of inclusion complexes with hydrophobic CDs and particularly with triacetyl-β-cyclodextrin (TAβCD), have yet been published.

In previous studies the interaction between NC and TAβCD in solution was investigated by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR).[11] Thus, the main purpose of the present work was the preparation and characterization of the NC : TAβCD complexes in the solid state. The *in vitro* drug release behaviour from the TAβCD complexes was also investigated, anticipating their use as a novel sustained release drug carrier.

Experimental

Materials NC (MW=516) and TAβCD were purchased from Effechem SRL (Milan, Italy) and Aldrich (Steinheim, Germany) respectively. All other chemicals and solvents were of analytical reagent grade and deionised water (Millipore Elix 5 system) was used throughout the study.

Since NC is a light sensitive almost all experiments were carried out in a darkroom under yellow light (Philips Powertone SON E27), in order to avoid photodecomposition. When this photoprotection was impossible to achieve, all samples containing NC were protected from light by wrapping the vials with aluminium foil.

Preparation of Solid Binary Systems The preparation of NC : TAβCD solid binary systems was performed by kneading and spray-drying methods, which are described below in detail. The 1 : 1 guest/host stoichiometry of the complexes was estimated in the previous ^{1}H-NMR studies, by monitoring the chemical shift of NC protons in the presence of TAβCD.[11]

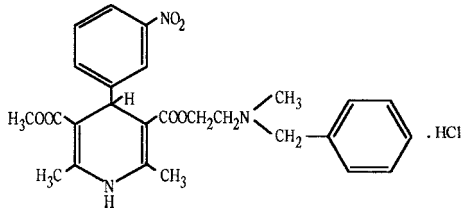

Fig. 1. Structural Formula of NC

* To whom correspondence should be addressed. e-mail: fveiga@ci.uc.pt

Physical Mixture: An equimolar physical mixture of NC and TAβCD was prepared by simple dry blending in a glass mortar of exactly weighed amounts of the 63—160 μm sieve granulometric fractions of the two components, until a homogeneous mixture was obtained. The mixing was performed adopting the geometric dilution method.

Kneading: TAβCD was wetted in a ceramic mortar with ethanol : water 50% (v/v) solution until a paste was obtained (about 30% of the total weight of CD and NC used). The required amount of NC was then added slowly whilst grinding, and the slurry was kneaded for about 45 min. During this process an appropriate quantity of solvent was added in order to maintain a suitable consistency. Further, the product was dried at 40 °C during 48 h. The dried solid was pulverized and the 63—160 μm sieve granulometric fraction was collected.

Spray-Drying: TAβCD was dissolved in a sufficient amount of ethanol : water 50% (v/v) solution and NC was added under continuous stirring. The resulting mixture was stirred for 24 h at room temperature and the obtained solution was subsequently spray-dried (LabPlant SD-05), under the following conditions: air flow rate – 50 m^3/h; atomising air pressure – 1×10^5 Pa; inlet temperature – 160 °C, outlet temperature – 85 °C; flow rate of the solution – 400 ml/h. The obtained product was sieved and the 63—160 μm granulometric sieve fraction was collected.

Differential Scanning Calorimetry (DSC) DSC measurements were performed on a Shimadzu DSC-50 differential scanning calorimeter with a thermal analyser. All accurately weighed samples (1 mg of NC or its equivalent) were placed in sealed aluminium pans, before being heated under nitrogen flow (20 ml/min) at a scanning rate of 10 °C min^{-1}, from 25 °C to 230 °C. An empty aluminium pan was used as reference. The equipment was periodically calibrated with indium (99.98%, mp 156.65 °C, Aldrich®, Milwaukee, U.S.A.). The thermal analysis was carried out past 400 °C during preliminary runs; however, the only thermal events observed past 230 °C were the decomposition of the materials.

X-Ray Diffractometry The powder X-ray diffraction patterns were recorded using a Philips X'Pert, model PW3040/00 diffractometer, with Co as anode material and a graphite monochromator, operated at a voltage of 40 kV and a current of 35 mA. The samples were analysed in the 2θ angle range of 5—50° and the process parameters were set as: scan step size of 0.025° (2θ), scan step time of 1.25 s and time of acquisition of 1 h.

Fourier Transformation-Infrared (FTIR) Spectroscopy Infrared spectra were obtained using a Philips PU9800 FTIR spectrometer. The samples were previously ground and mixed thoroughly with potassium bromide, an infrared transparent matrix, using a 1% (w/w) dilution. The KBr disks were prepared by compressing the powders, under force of 5×10^3 kg for 10 min and further 10×10^3 kg for 20 min, in a hydraulic press. Thirty scans were obtained at a resolution of 2 cm^{-1}, from 4500 to 400 cm^{-1}.

Scanning Electron Microscopy (SEM) The morphological features of the raw materials and the binary systems were examined by means of Jeol, JSM-5310 scanning electron microscope. The powders were previously fixed on a brass stub using double-sided adhesive tape and then were made electrically conductive by coating in a vacuum with a thin layer of gold (approximately 300 Å), for 30 s and at 30W. The pictures were taken at an excitation voltage of 15 kV and a magnification of 1500×.

Dissolution Studies The dissolution profiles were collected using a Vankel VK7000 apparatus, according to the USP rotating basket method. The dissolution media consisted of 1000 ml of enzyme-free simulated gastric (pH 1.2) and intestinal (pH 6.8) fluids (USP XXIV). Powdered samples containing 30 mg of NC or its equivalent in complexed or physically mixed form with TAβCD were used. The stirring speed was 100±2 rpm and the temperature was maintained at 37±0.2 °C. At settled time intervals for a period of 8 h, the concentration of dissolved drug was automatically determined by UV spectroscopy at 357 nm. Dissolution runs were performed 6 times for all samples.

Results and Discussion

DSC The solid binary systems were analysed by means of DSC to detect possible altered thermal properties with regard to the pure substances. When guest molecules are incorporated in the CD cavity their melting, boiling and sublimation points generally shift to a different temperature or disappear within the temperature range where the CD lattice is decomposed.[12)]

The DSC curves of NC, TAβCD and the respective drug-carrier equimolar combinations are shown in Fig. 2. The thermal curve of NC indicated its crystalline anhydrous state with a characteristic endothermic fusion peak at 174 °C. The TAβCD thermogram displayed a very broad endothermic effect with a maximum around 80 °C, which is attributed to a liberation of crystal water of this CD. The other endothermic peak at 192 °C observed in the DSC profile of TAβCD is due to the fusion process of this crystalline molecule.

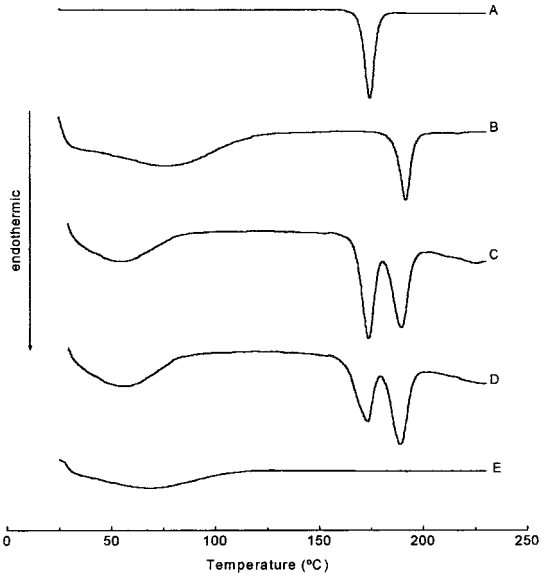

Fig. 2. DSC Thermograms of NC (A), TAβCD (B), NC : TAβCD Physical Mixture (C), NC : TAβCD Kneaded (D) and Spray-Dried (E) System

The appearance of three endothermic peaks corresponding to the fusion of both components and to the dehydration of TAβCD was also evident in the thermogram of the physical mixture, as if this DSC curve was the superposition of those of the components analysed separately. The shape and area of the NC melting peak was unaffected by the blending with TAβCD, and hence, the drug maintained its original crystallinity in the physical mixture. Considering the kneaded system, the three characteristic endothermic peaks of NC and TAβCD were also observed. However, the thermal profile of the kneaded product was slightly different from that obtained for the physical mixture, which was characterized by a sharp and definite endothermic effect in the region of the NC meting. Some reduction of area, broadening and small downshift of the peak temperature of NC melting endotherm (173 °C) was observed in the kneaded product. Since the kneaded product contains the same quantities of NC and TAβCD present in the physical mixture, the diminution of the drug melting peak is not a result of a dilution effect. That behaviour may be explained by a better dispersion of the NC microcrystals in the TAβCD[13)] or could be ascribed to some drug-CD interaction.[14,15)] Although the NC endothermic peak was partially reduced, the presence of this peak indicates that a true inclusion complex was not achieved by this preparation method.[16)]

The complete disappearance of the drug endothermic peak was found in the spray-dried preparation. The absence of the NC fusion peak indicated the existence of interactions between the drug and TAβCD in the solid state and may be considered as a strong indication for the formation of real in-

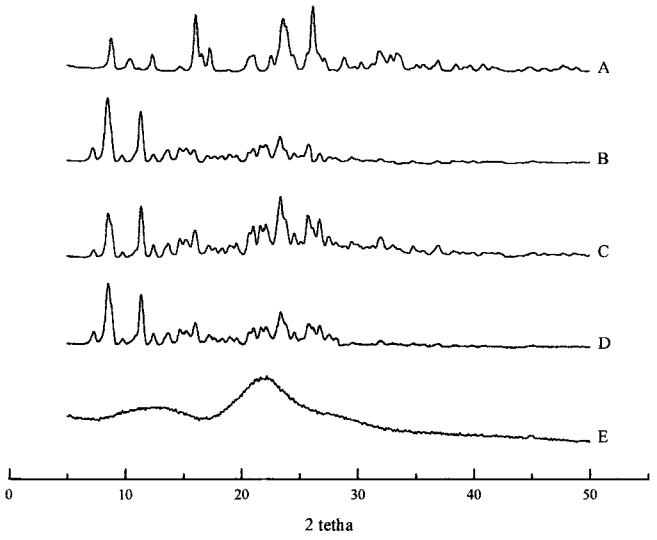

Fig. 3. X-Ray Diffractograms of NC (A), TAβCD (B), NC : TAβCD Physical Mixture (C), NC : TAβCD Kneaded (D) and Spray-Dried (E) System

clusion complexes.[12,13,17—19)]

It is also important to remark that the disappearance of the NC melting peak in the spray-dried product was associated to a smaller peak for the TAβCD water evaporation. These results are also in favour of the occurrence of inclusion complexation, indicating that the drug penetrated into the TAβCD cavity, replacing some water molecules.[20,21)]

X-Ray Diffractometry X-Ray diffraction studies were performed to examine the crystallinity and provide further evidence of complex formation. The analysis of the X-ray powder diffraction patterns of CD inclusion compounds is a powerful and very well assessed method for the characterization of complexes in the solid state.[22)] Significantly different X-ray diffraction patterns are to be expected if an inclusion complex is formed, since crystal structure will change.[23)]

The X-ray diffraction patterns of NC, TAβCD and the corresponding binary systems are represented in Fig. 3. The diffractograms of NC and TAβCD exhibited a series of sharp and intense diffraction peaks, which are indicative of their cristallinity. The diffraction pattern of the physical mixture was found to correspond exactly to the simple sum of the raw materials diffractograms, indicating the presence of NC in the crystalline state. The kneaded product showed with respect to the diffraction patterns of the starting materials, the broadening and the disappearance or intensity diminution of some NC diffraction peaks, especially those situated between 30 and 40° (2θ). These findings suggest the presence of a new solid phase with a lower degree of cristallinity, which could be originated by the molecular interaction of the host TAβCD and the guest NC.[24,25)]

The spray-dried compound presented a diffraction pattern completely diffused, with the disappearance of the characteristic peaks of NC and TAβCD, reflecting the amorphous nature of this binary system. This is a direct proof of the formation of a new solid phase and can be considered as a very probable indication of the inclusion complex formation between NC and TAβCD in the solid state.[18,25—27)]

FTIR Spectroscopy Although the above-mentioned results show a strong interaction between the drug and the TAβCD, suggesting the formation of a real complex by the spray-drying method, FTIR spectroscopy was used in order to confirm this assumption. The 3800—2200 cm^{-1} and 2000—500 cm^{-1} FTIR spectra of NC, TAβCD and NC : TAβCD binary systems are shown in Figs. 4 and 5, respectively. The FTIR spectrum of the physical mixture can be considered as the result of the addition of NC and TAβCD spectra. Also, no significant shifts or reductions of intensity of the FTIR bands of NC were observed in the kneaded product. On the contrary, the FTIR spectrum of the spray-dried

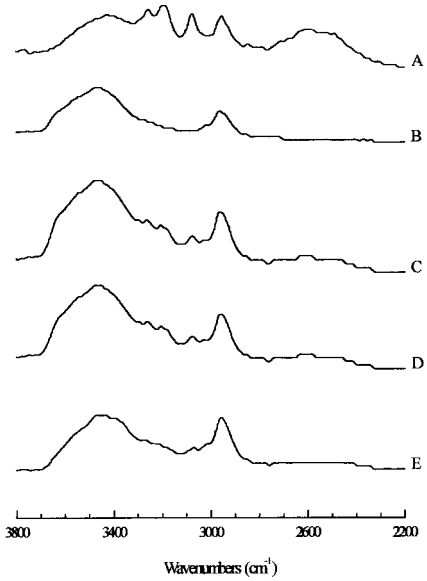

Fig. 4. 3800—2200 cm^{-1} FTIR Spectra (in Absorbance) of NC (A), TAβCD (B), NC : TAβCD Physical Mixture (C) and NC : TAbCD Kneaded (D) and Spray-Dried System (E)

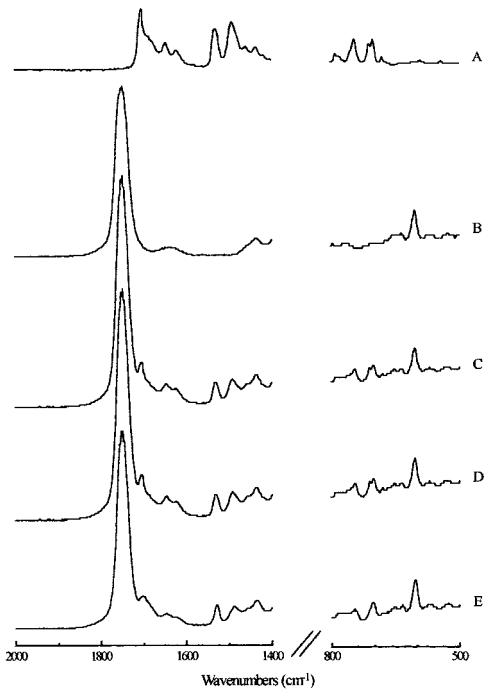

Fig. 5. 2000—500 cm^{-1} FTIR Spectra (in Absorbance) of NC (A), TAβCD (B), NC : TAβCD Physical Mixture (C) and NC : TAβCD Kneaded (D) and Spray-Dried System (E)

system exhibited some significant differences (shifts, broadenings or attenuations) in the characteristic NC bands, revealing a modification of the drug environment.

Due to a considerable intensity diminution, it was difficult to observe in the spray-dried product (Fig. 4E) the NC bands at 3254 and 3184 cm^{-1}, probably assigned to N–H and C–H stretching vibration, respectively.

As shown in the Fig. 5A, a strong band of NC may be observed at 1703 cm^{-1}, due to the C=O vibration of the carbonyl group. In this spectral region, TAβCD displayed a very strong band at 1751 cm^{-1}, due to C=O vibration of the acetyl group. The NC carbonyl band profile was broader and less intense with a shift to lower frequency (1699 cm^{-1}) in the spectrum of the spray-dried compound than that of the physical mixture and the kneaded system. Similar effects were observed and explained previously[28] for the complexes between NC and β-CD or hydroxypropyl-β-CD, suggesting the formation of intermolecular hydrogen bonds between the drug carbonyl group and the hydroxyl groups of the host cavity, during inclusion complexation.[29—31] In this case, since TAβCD does not have primary or secondary OH groups, which were replaced by $-OCOCH_3$ groups, these hydrogen bonds may be exist between NC and the water molecules present in the TAβCD host cavity. In fact, when a carbonyl group is joined to a hydroxylic compound by hydrogen bonds, the stretching band is displaced to lower frequency due to a weakening of the carbonyl radical double bond.[32]

The phenyl group vibration (ring carbon–carbon stretching) appears at 1620 cm^{-1} in the spectrum of NC, but it diminishes of intensity in the spectrum of the spray-dried complex. The band at 1491 cm^{-1} is probably due to the phenyl stretching vibration. In the spray-dried system, this band was shifted to lower frequency (1487 cm^{-1}) with concomitant intensity reduction and broadening. The observed decreases in the intensities of these bands may be due to the restriction of the phenyl groups within the TAβCD cavity. These results are in agreement with previous ^{1}H-NMR studies, which defined the NC aromatic rings as the most probable groups of inclusion inside the TAβCD cavity.[11]

Finally, the doublet band at 700 and 709 cm^{-1} in NC, physical mixture and kneaded become a broad band centred at 702 cm^{-1} in the spray-dried compound spectrum.

Moreover, in the different spectra of the binary systems no new peaks appear, which indicate that no chemical bonds were created in the formed compounds.[33]

From the FTIR spectral analysis of the spray-dried compound it can be concluded that the functional groups mainly involved in the complexation process were the carbonyl and the phenyl ones, although the NH group was also affected.

Since the kneaded product FTIR spectrum showed no significant band modifications, we could confirmed the idea that this method does not give a true encapsulation of NC.

SEM The shape and surface morphology of NC, TAβCD and the binary systems are presented in the Fig. 6. NC and TAβCD have quite different morphological characteristics. NC appeared as irregular and three-dimensional crystals with smooth surfaces and homogeneous size. The TAβCD SEM analysis revealed the presence of rhomboidal shape crystals with different dimensions.

The physically mixed and the kneaded systems were char-

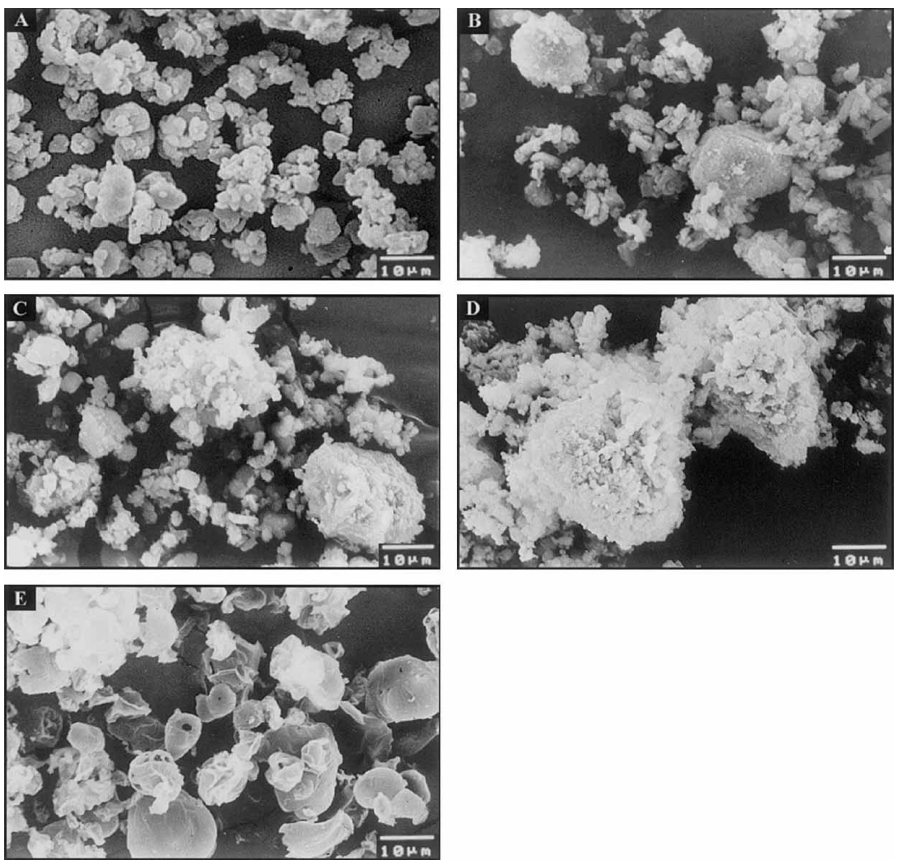

Fig. 6. SEM Photographs of NC (A), TAβCD (B), NC : TAβCD Physical Mixture (C), NC : TAβCD Kneaded (D) and Spray-Dried (E) System

acterized by the presence of unmodified particles of TAβCD, which were mechanically covered by few crystals of drug. This phenomenon was more evident in the kneading system, where it was more difficult to detect the features of the crystals of both components, appearing as only one type of granules. This behaviour could be explained by the partial solubilization of TAβCD, which improves the adhesion of NC crystals to its surface.

A remarkable change in the morphology of the materials was shown in the spray-dried system. The spray-drying technique yielded products of amorphous appearance, with the presence of particles, partially broken, of a typical spherical shape and a smooth surface with some fissures in the coating layer. The formation of aggregates of these spherical particles was also observed.

In fact, the physical appearance, morphology and size of the spray-dried product were completely different from those of the mother products, and it was not possible to differentiate the distinctive crystals of NC and TAβCD. These observations, although scarcely conclusive, lead us to estimate the existence of a single phase in the spray-dried preparation[34] and, consequently, the formation of an inclusion complex.

Dissolution Studies The dissolution profiles of NC and NC : TAβCD binary systems in simulated gastric (pH 1.2) and simulated intestinal (pH 6.8) fluids are presented in Figs. 7 and 8, respectively. The aqueous solubility of NC is drastically pH dependent. Since NC is a weak basic drug, the solubility and dissolution rate of this drug at acidic pH values are very high, which difficult control release in stomach. As it can be seen in Fig. 7, the physical mixture showed approximately the same dissolution profile of the NC. On contrary, the drug dissolution rate was markedly retarded from both the kneaded and spray-dried products. This indicated that the kneading compound is not merely a physical mixture of the individual constituents, as it was evidenced by the previous physicochemical characterization. However, the retarding effect on NC dissolution was dramatically more evident for the spray-dried system. After 8 h, the percentage of dissolved drug from the spray-dried complex was only nearly 60% in opposite to the complete dissolution from the kneaded product. In addition, it was clearly observed that the drug release profile from the spray-dried complex consisted of a very slow release in the first stage (up to 1 h) and a faster release in the second phase. In the first stage the NC seems to be released according to zero-order kinetics.

As expected, at pH 6.8 the dissolution profile of plain NC presented lower percentage of dissolved drug (pK_a=7.2). All the three binary systems exhibited an initial sustained effect on the NC dissolution, presenting the spray-dried product the most prominent one. This retardation on the drug dissolution rate when physically mixed or kneaded with TAβCD may be attributed to "*in situ*" formation of complexes in the dissolution medium. To our best knowledge, no comparisons between the drug dissolution profiles from physical mixtures with acylated CDs and from the respective complexes were presented in the few articles available about this subject, which difficult additional interpretations and the possibility to cross the results.

The drug release from the spray-dried complex occurred again in two stages, being the first one (up to 1 h) dramatically slower. The NC seems to be released from the spray-dried complex according to zero-order kinetics.

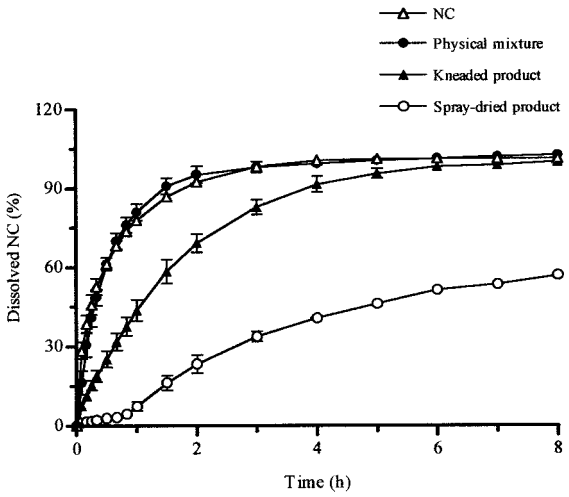

Fig. 7. Dissolution Profiles of NC and NC : TAβCD Binary Systems in Simulated Gastric Fluid (pH 1.2)

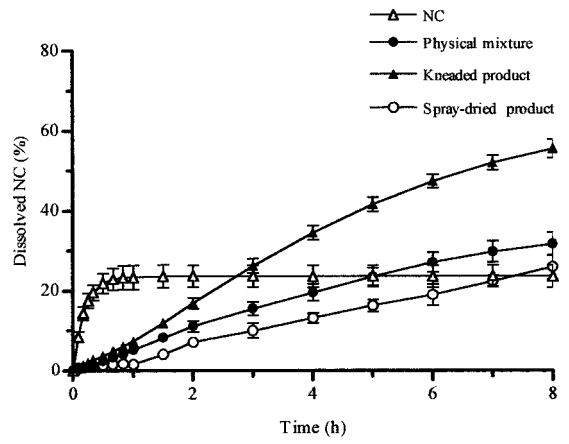

Fig. 8. Dissolution Profiles of NC and NC : TAβCD Binary Systems in Simulated Intestinal Fluid (pH 6.8)

Interestingly, a trend to increase the total percentage of dissolved NC from all the binary systems could be noticed. This unexpected behaviour could be explained on the basis of a local action of the carrier, operating in the microenvironment on the hydrodynamic layer surrounding the drug particles, which improves the NC wettability. Although TAβCD is practically insoluble in aqueous solutions, we observed that this CD presents a much better wettability than NC. Also the equilibrium established in solution between the free and the complexed drug and the modification in NC crystallinity during the kneading and spray-drying processes could contribute to the appearance of this kind of dissolution profiles.

The dissolution rate of the spray-dried complex was slower compared with the kneaded product, which was attributed to the formation of inclusion complexes in the former. For this reason, the increase in the drug solubility after 8 h from the kneaded product was higher than that observed to the spray-dried complex.

Although the kneaded product displayed apparently better dissolution properties at pH 6.8 with respect to spray-dried complex, we select the later for further studies. Considering the mean residence time of drug formulations in stomach

(about 1.5 h) and analysing again the NC dissolution profile at pH 1.2 from the kneaded product it could be easy concluded that this compound could not be used in the design of drug sustained or controlled release formulations.

In a previous paper,[28] we reported that the NC solubility and dissolution rate in the simulated intestinal fluid (pH 6.8) could be improved by complexation with hydrophilic βCDs. The critical combination of NC/TAβCDβCD complexes, as a slow release fraction, and NC/hydrophilic βCDs complexes, as a fast-releasing fraction could be a promising drug delivery system, with a prolonged therapeutic effect. The behaviour of these combinations, in different mixing ratios, is under investigation.

Conclusions

The characterization of NC/TAβCD systems showed that the preparation technique deeply influences the physicochemical and morphological properties and the drug *in vitro* dissolution behaviour. Under the present conditions, the spray-drying technique was more appropriate than kneading to achieve complexation between NC and TAβCD. The results indicated that the compound obtained by the kneading method did not seem to be a true inclusion complex. The kneading method could only have interest if the objective is a slight retardation in the drug solubility or dissolution rate, without requiring the formation of true inclusion complexes.

References

1) Szejtli J. (ed.), "Cyclodextrin Inclusion Complexes," Kluwer Academic Publishers, Dordrecht, 1988, pp. 79—170.
2) Bekers O., Uijtendall E., Beijnen J., Buit A., Underberg W., *Drug Dev. Ind. Pharm.*, **17**, 1503—1549 (1991).
3) Uekema K., Hirayama F., Irie T., *Chem. Rev.*, **98**, 2045—2076 (1998).
4) Szejtli J. (ed.), "Cyclodextrin Derivatives," Kluwer Academic Publishers, Dordrecht, 1994, pp. 19—32.
5) Ikeda Y., Kimura K., Hirayama F., Arima H., Uekama K., *J. Control. Release*, **66**, 271—280 (2000).
6) Lemesle-Lamache V., Wouessidjewe D., Chéron M., Duchêne D., *Int. J. Pharmaceut.*, **141**, 117—124 (1996).
7) Sorkin E., Clissold S., *Drugs*, **33**, 296—345 (1987).
8) Yüksel N., Tinçer T., Baykara T., *Int. J. Pharmaceut.*, **140**, 145—154 (1996).
9) Yüksel N., Dinç E., Onur F., Baykara T., *Pharm. Dev. Techn.*, **3**, 115—121 (1998).
10) Özyazici M., Sevgi F., Ertan G., *Drug Dev. Ind. Pharm.*, **23**, 761—770 (1997).
11) Fernandes C. M., Carvalho R. A., Pereira da Costa S., Veiga F. J. B., Put information in detail, if already published.
12) Cabral-Marques H. M., Hadgraft J., Kellaway I. W., *Int. J. Pharmaceut.*, **63**, 259—266 (1990).
13) Esclusa-Díaz M. T., Guimaraens-Méndez M., Pérez-Marcos M. B., Vila-Jato J. L., Torres-Labandeira J. J., *Int. J. Pharmaceut.*, **143**, 203—210 (1996).
14) Mura P., Adragna E., Rabasco A. M., Moyano J. R., Pérez-Martinez J. I., Arias M. J., Ginés J. M., *Drug Dev. Ind. Pharm.*, **25**, 279—287 (1999).
15) Erden N., Celebi N., *Int. J. Pharmaceut.*, **48**, 83—89 (1988).
16) Castillo J. A., Palomo-Canales J., Garcia J. J., Lastres J. L., Bolas F., Torrado J. J., *Drug Dev. Ind. Pharm.*, **25**, 1241—1248 (1999).
17) Özdemir N., Ordu S., *Drug Dev. Ind. Pharm.*, **24**, 19—25 (1998).
18) Hassan M. A., Suleiman M. S., Najib N. M., *Int. J. Pharmaceut.*, **58**, 19—24 (1990).
19) Williams III R. O., Mahaguna V., Sriwongjanya M., *Eur. J. Pharm. Biopharm.*, **46**, 355—360 (1998).
20) Singh U. V., Aithal K. S., Udupa N., *Pharmazie*, **53**, 208—210 (1998).
21) Montassier P., Duchêne D., Poelman M., *Int. J. Pharmaceut.*, **153**, 199—209 (1997).
22) Saenger W., *Angew. Chem. Int. Ed. Eng.*, **19**, 344—362 (1980).
23) Green A. R., Miller E. S., Guillorry J. K., *J. Pharm. Sci.*, **80**, 186—189 (1991).
24) Caccia F., Dispenza R., Fronza G., Fuganti C., Malpezzi L., Mele A., *J. Agric. Food Chem.*, **46**, 1500—1505 (1998).
25) Moyano J. R., Arias-Blanco M. J., Gines J. M., Giordano F., *Int. J. Pharmaceut.*, **148**, 211—217 (1997).
26) Sanghavi N. M., Venkatesh H., Tandel V., *Drug Dev. Ind. Pharm.*, **20**, 1275—1283 (1994).
27) Kedzierewicz F., Hoffman M., Maincent P., *Int. J. Pharmaceut.*, **58**, 221—227 (1990).
28) Fernandes C. M., Vieira M. T., Veiga F. J. B., *Eur. J. Pharm. Sci.*, **15**, 79—88 (2002).
29) Vila-Jato J. L., Blanco J., Torres J. J., *S.T.P. Pharma*, **3**, 28—32 (1987).
30) Lin S., Kao Y., *Int. J. Pharmaceut.*, **56**, 249—259 (1989).
31) El-Nahhas S. A., *Pharmazie*, **51**, 960—963 (1996).
32) Otero-Espinar F. J., Anguiano-Igea S., García-González N., Vila-Jato J. L., Blanco-Méndez J., *Int. J. Pharmaceut.*, **79**, 149—157 (1992).
33) Ammar H. O., Ghoras M., El-Nahhas S. A., Emara L. H., Makram T. S., *Pharmazie*, **54**, 142—144 (1999).
34) Moyano J. R., Arias M. J., Gines J. M., Pérez J. I., Rabasco A. M., *Drug Dev. Ind. Pharm.*, **23**, 379—385 (1997).