Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 45 (2007) 480–486
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Physical–chemical characterization of binary systems of metformin hydrochloride with triacetyl-β-cyclodextrin

Giovanna Corti, Gaetano Capasso, Francesca Maestrelli, Marzia Cirri, Paola Mura *

Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Florence, via U.Schiff 6, 50019 Sesto Fiorentino, Florence, Italy

Received 16 June 2007; accepted 13 July 2007
Available online 20 July 2007

Abstract

Interaction products of metformin hydrochloride (MF·HCl), an oral anti-hyperglycaemic agent highly soluble in water, with triacetyl-β-cyclodextrin (TAβCyD), a hydrophobic CyD derivative practically insoluble in water, were prepared to evaluate their suitability for the development of a sustained-release dosage form of the drug. Equimolar MF·HCl–TAβCyD solid compounds were obtained by different techniques, i.e., physical mixing, kneading, co-grinding, sealed-heating, and spray-drying, in order to investigate and compare their effectiveness and influence on the physical–chemical properties of the final products. Differential scanning calorimetry, X-ray powder diffractometry, Fourier transform infrared spectroscopy and scanning electron microscopy were used for the solid-state characterization of the different MF·HCl–TAβCyD systems, whereas their *in vitro* dissolution properties were determined according to the dispersed amount method. According to the results of solid-state studies, the ability of the different preparation methods to promote effective interactions between drug and CyD varied in the order: spray-drying > co-grinding > kneading > sealed-heating ≈ physical mixing. The same effectiveness rank order was observed also in dissolution studies. In fact the time to dissolve 100% drug varied increased from 1 min, for pure drug, to 3, 7, 40, 120 up to 420 min for physically mixed, sealed-heated, kneaded, co-ground and spray-dried products, respectively. Thus the drug–TA(CyD products obtained by spray drying and co-grinding were selected as the best candidates for the future development of a suitable prolonged-release oral dosage form of MF·HCl.

Keywords: Triacetyl-β-cyclodextrin; Metformin hydrochloride; Differential scanning calorimetry; X-ray powder diffractometry; Fourier transform infrared spectroscopy; Scanning electron microscopy; Dissolution rate

1. Introduction

In the last years cyclodextrins (CyDs) received an increasing interest in the pharmaceutical field due to their ability to favourably modify physical, chemical and biological properties of drug molecules through the formation of inclusion complexes [1]. Recently, several kinds of chemically modified CyDs have been prepared in order to improve the physicochemical properties and inclusion abilities and extend the spectrum of the pharmaceutical applications of the parent molecules [2,3]. Among these, the hydrophilic CyDs have been extensively employed as helpful carriers to improve dissolution rate and bioavailability of poorly water-soluble drugs [4–7]. On the contrary, there are less data about the use of the hydrophobic CyD derivatives, such as the peracylated ones, which have been proposed as sustained-release carriers for highly soluble drugs with short biological half-lives, in virtue of the formation of poorly water-soluble complexes [8–10].

Metformin hydrochloride is an oral anti-hyperglycaemic agent highly water-soluble, whose low bioavailability and short and variable biological half-life (1.5–4.5 h) needs frequent administrations to maintain effective plasma concentrations, thus making the development of sustained-release forms desirable [11]. Moreover, the oral absorption of metformin is mainly confined to the upper part of the gastrointestinal tract, thus requiring the development of suitable delivery systems with a timely modulation of the drug release rate [12–14]. Thus, we considered it worthy of interest to evaluate the effectiveness

* Corresponding author. Tel.: +39 055 4573672; fax: +39 055 4573673.
E-mail address: mura@unifi.it (P. Mura).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.07.018

of triacetyl-β-cyclodextrin (TAβCyD), a hydrophobic CyD derivative practically insoluble in water, as a carrier for obtaining a slow-dissolving complex of the drug, to be used for the subsequent development of a well-timed sustained-release oral dosage form of metformin. It is known that different methods can be employed for preparing solid drug–cyclodextrin complexes, and the choice of the most efficacious one should be carefully evaluated case by case [15–17]. In particular, an in depth characterization of the solid-state properties of the obtained products is strongly advisable, since they can affect the drug–carrier interactions, which in turn influence the dissolution rate and drug stability [18,19].

Therefore, in the present work, equimolar drug–TAβCyD solid compounds were prepared by different methods, i.e., physical mixing, kneading, co-grinding, sealed-heating, and spray-drying and characterized by differential scanning calorimetry, X-ray powder diffractometry, Fourier transform infrared spectroscopy and scanning electron microscopy, in order to carefully investigate and compare the physical–chemical properties of the obtained products, for a rational selection of the best one. In addition, the *in vitro* dissolution behaviour of the different products was determined according to the dispersed amount method, with the aim of studying possible implications of the system preparation method on the dissolution properties of the drug.

2. Experimental

2.1. Materials

Metformin hydrochloride (MF·HCl) was kindly supplied by Menarini (Firenze, Italy). Triacetyl-β-cyclodextrin (TAβCyD) (Cavasol® W7 TA) was a kind gift of Wacker-Chemie (GmbH, Germany). All other chemicals and solvents were of analytical reagent grade.

2.2. Preparation of solid binary systems

MF·HCl–TAβCyD equimolar systems were obtained from the individual components previously sieved (75–150 μm): (a) by tumble mixing for 20 min with a turbula mixer (physical mixtures, PM); (b) by ball-milling physical mixtures in a high vibrational micro-mill for 30 min at 24 Hz (co-ground systems, GR); (c) by wetting physical mixtures in a mortar with the minimum volume of an ethanol–water 1:1 (v/v) solution and grinding thoroughly the slurry with a pestle to obtain a paste which was then dried under vacuum at 40 °C up to constant weight (kneaded systems, KN); (d) by heating physical mixtures in sealed containers at 90 °C for 2 h (sealed-heated systems, SH); (e) by dissolving physical mixtures in an ethanol:water 8:2 (v/v) solution and then spray-drying (IRA Mini Spray Ho, Italy) under the following conditions: inlet temperature, 120 °C; outlet temperature, 70 °C; flow rate of the solution, 13 mL min^{-1}; atomising air pressure, 3 kg/m^2; vacuum conditions of 70 mm H_2O (spray-dried systems, SP).

To exclude any effect of sample preparation method on the drug and carrier physicochemical characteristics, samples of pure MF·HCl and TAβCyD have been treated with the same techniques used for preparation of equimolar binary systems.

2.3. Differential scanning calorimetry (DSC)

DSC analysis was performed with a Mettler TA4000 Star^e system (Mettler Toledo, Switzerland) equipped with a DSC 25 cell. Samples of about 5–10 mg were accurately weighed (Mettler MX5 microbalance) in sealed aluminium pans with pierced lid and scanned at 10 K min^{-1}, under static air atmosphere, in the 30–200 °C temperature range. Measurements were carried out at least in triplicate. The instrument was calibrated using Indium as a standard (99.98% purity; melting point 156.61 °C; fusion enthalpy 28.71 J g^{-1}).

2.4. X-ray powder diffractometry (XRPD)

The powder X-ray diffraction patterns were taken at ambient temperature with a Brucker D8 apparatus (θ/θ geometry) using a Cu Kα radiation and a graphite monochromator. The samples were analysed in the 5–30° 2θ range at a scan rate of 0.05° s^{-1}.

2.5. Fourier transform infrared spectroscopy (FTIR)

Infrared spectra were recorded using a Perkin-Elmer Model 1600 spectrophotometer on KBr disks in the range between 4000 and 400 cm^{-1}.

2.6. Scanning electron microscopy (SEM)

Surface morphology of pure components and their equimolar binary systems obtained by different techniques was examined using a Philips XL-30 scanning electron microscope equipped with an image analysis system. Prior to examination, samples were sputter coated with gold–palladium under argon atmosphere (to render them electrically conductive) using a gold sputter module in a high vacuum evaporator.

2.7. Dissolution rate studies

In vitro dissolution rate studies of MF·HCl alone and from all the drug–carrier binary systems obtained with the different techniques were performed according to the dispersed amount method. Samples containing 50 mg of drug or its equivalent as binary system with TAβCyD were added in a 400 mL beaker containing 300 mL of intestinal artificial fluid (phosphate buffer at pH 6.5) at 37 ± 0.5 °C, and stirred at 100 rpm with a glass three-blade propeller (19 mm diameter) immersed in the beaker 25 mm from the bottom. At settled time intervals, samples were withdrawn with a syringe-filter (pore size 0.45 μm) and replaced with an equal volume of fresh medium. The drug concentration was spectrometrically determined (UV–vis 1600 Shimadzu spectrophotometer, Tokyo, Japan) at 232.2 nm. Each test was repeated three times (coefficient of variation < 5%). Dissolution efficiency (DE) was calculated from the area under the dissolution curve at time t and expressed as a percentage of the area of

the rectangle described by 100% dissolution in the same time [20].

3. Results and discussion

3.1. Solid-state studies

In order to correctly and accurately investigate drug–carrier solid-state interactions and exclude possible solid-state modifications due to the sample treatment, solid state studies were performed not only on the various MF·HCl–TAβCyD binary systems obtained with the different preparation techniques, but also on the pure components subjected to these same processes.

3.1.1. Differential scanning calorimetry (DSC)

The thermal curve of pure MF·HCl (Fig. 1A, curve a) indicated its crystalline anhydrous state and was characterized by a sharp endothermic fusion peak at 231.0 ± 0.6 °C with an associated fusion enthalpy of 292 ± 12 J/g. The thermal behaviour of TAβCyD (Fig. 1A, curve b) was instead more complex. The sample immediately started losing the weakly hydrogen-bonded water (as shown by the broad initial endothermic band), transforming into a lower melting anhydrous polymorph II which fuses at 191.8 ± 1.9 °C and then recrystallizes into a higher melting form, whose fusion endotherm peaked at 219.8 ± 2.0 °C. An analogous thermal behaviour has been described by Bettinetti et al. for commercial TAβCyD [21]. The thermal profile of the drug was almost unaffected by the different treatments, including spray drying (DSC curves not shown); on the contrary, in the case of TAβCyD this happened only for the sealed-heated product (Fig. 1B, curve b1). In fact, the DSC profiles of TAβCyD treated with both the kneading and co-grinding techniques were different from that of the original sample (Fig. 1B, curves b2 and b3). In particular, after the initial dehydration band, the appearance of a glass transition at about 135 °C was observed followed by an exothermic effect, peaking at 164.9 °C. This can be attributed to the recrystallization of an amorphous form, obtained during the mechanical treatment of the sample, into the higher melting crystalline form, characterized by a sharp fusion peak at 219.8 °C. A similar thermal behaviour was observed for X-ray amorphous TAαCyD and TAγCyD obtained, respectively, by microwave drying of a propanol–water solution or by spray drying of a water–acetone solution [21]. Finally, the spray-dried sample (Fig. 1B, curve b4) exhibited a flat profile with the complete disappearance of both exothermic and endothermic phenomena, suggesting the formation of a more stable amorphous form of the CyD. The thermal curve of the physical mixture (Fig. 1A, curve c) was practically the sum of those of pure components, showing an initial broad endothermic band, due to water evaporation, followed by three sharp endothermic peaks, due, respectively, to the melting of the two polymorphic forms of TAβCyD and

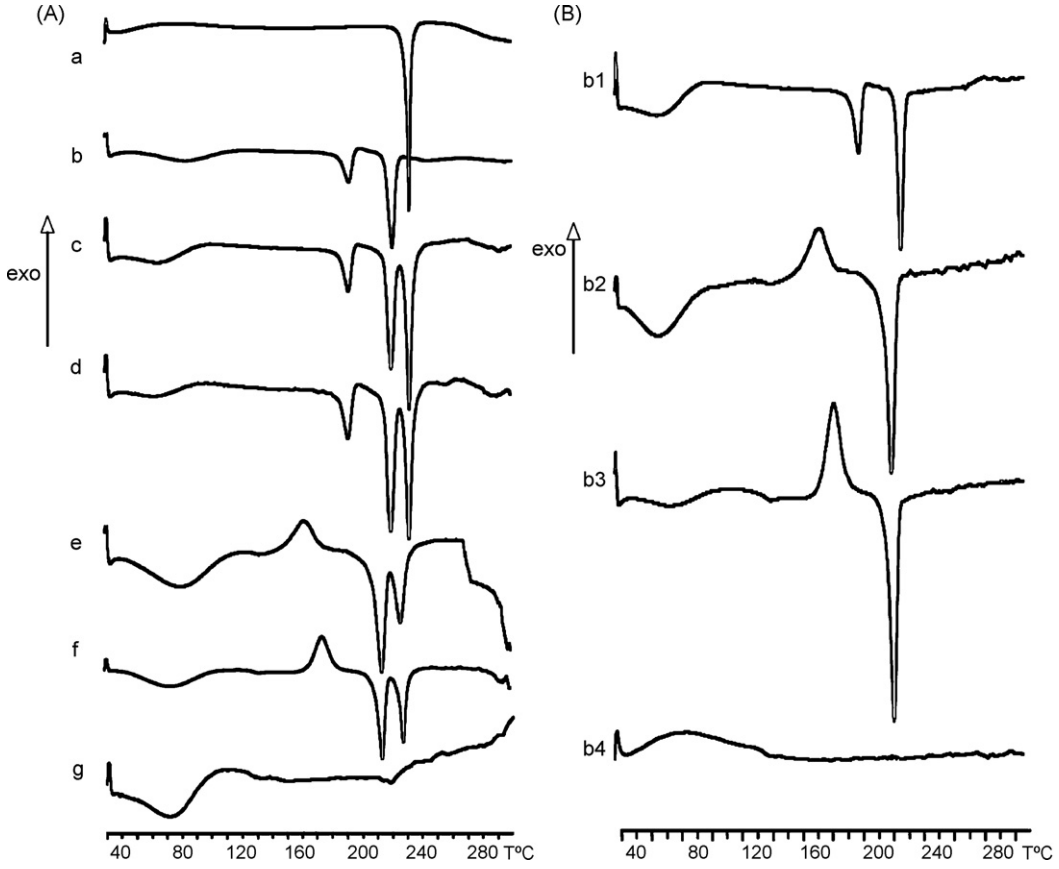

Fig. 1. DSC curves of (A) pure components and their equimolar systems obtained with the different techniques and (B) of TAβCyD after treatment with the same techniques. *Key*—A: (a) MF·HCl, (b) TAβCyD, (c) physical mixture, (d) sealed-heated, (e) kneaded, (f) co-ground, and (g) spray-dried products; B: TAβCyD after (b1) sealed-heating, (b2) kneading, (b3) grinding, or (b4) spray drying.

then of the drug. The binary product obtained by sealed-heating (Fig. 1A, curve d) displayed a very similar behaviour to that of the physical mixture, accounting for the absence of apparent solid-state interactions between drug and CyD. On the other hand, the thermal profiles of both the binary kneaded and co-ground products (Fig. 1A, curves e and f) showed the presence of an additional exothermal effect, followed by the fusion peak of the higher melting polymorphic form of TAβCyD and then of the drug. DSC analysis of pure components made it possible to exclude drug–carrier interactions as being responsible for such exothermal phenomenon and to correctly attribute it to the presence of a TAβCyD unstable amorphous form, obtained during kneading or grinding process, which, during the DSC heating, recrystallizes into the more stable higher melting crystalline form (Fig. 1B, curves b2 and b3). Some reduction of fusion enthalpy and lowering of melting temperature of MF·HCl, observed in the binary kneaded and, particularly, in the co-ground products, can be ascribed to some drug–CyD interactions occurring during sample preparation [15,16]. The DSC curve of the spray-dried binary product (Fig. 1A, curve g) showed the complete disappearance of all melting peaks corresponding to both components, indicating total system amorphization as a consequence of strong drug–carrier interactions and/or drug inclusion complexation. In fact, the absence of the drug melting peak in this system is not attributable to the spray-drying process, which does not substantially affect the solid-state properties of MF·HCl, since the thermal behaviour of the spray-dried drug alone was very similar to that of the untreated sample (curve not shown).

3.1.2. X-ray powder diffractometry (XRPD)

The X-ray diffraction patterns of MF·HCl, TAβCyD, and their respective equimolar binary systems obtained with the different techniques are shown in Fig. 2A, whereas representative spectra of pure components after the different treatments are presented in Fig. 2B. A series of sharp and intense typical diffraction peaks indicated the crystalline state of pure MF·HCl. Also the TAβCyD diffraction pattern was characterized by the presence of several sharp peaks indicative of its crystallinity. The diffraction pattern of the physical mixture was simply the superimposition of those of pure components (Fig. 2A, curve c), indicating the presence of both MF·HCl and TAβCyD in the crystalline state. The diffraction characteristics of the individual components were maintained also in the binary product obtained by sealed-heating (Fig. 2A, curve d), confirming the ineffectiveness of this technique in establishing solid-state drug–CyD interactions, in agreement with the results of DSC analysis. The loss of crystallinity observed in the kneaded product (Fig. 2A, curve e), and even more in the co-ground product (Fig. 2A, curve f), can be considered as a consequence of drug–carrier interactions brought about by the mechanical treatment. In fact, the kneading and co-grinding processes caused almost complete amorphization of pure TAβCyD (Fig. 2B, curves b2 and b3), whereas it did not markedly reduced drug crystallinity (Fig. 2B, curves a1 and a2). On the other hand, the spray-dried compound, according to DSC analysis results, presented a completely amorphous diffraction pattern, with the disappearance

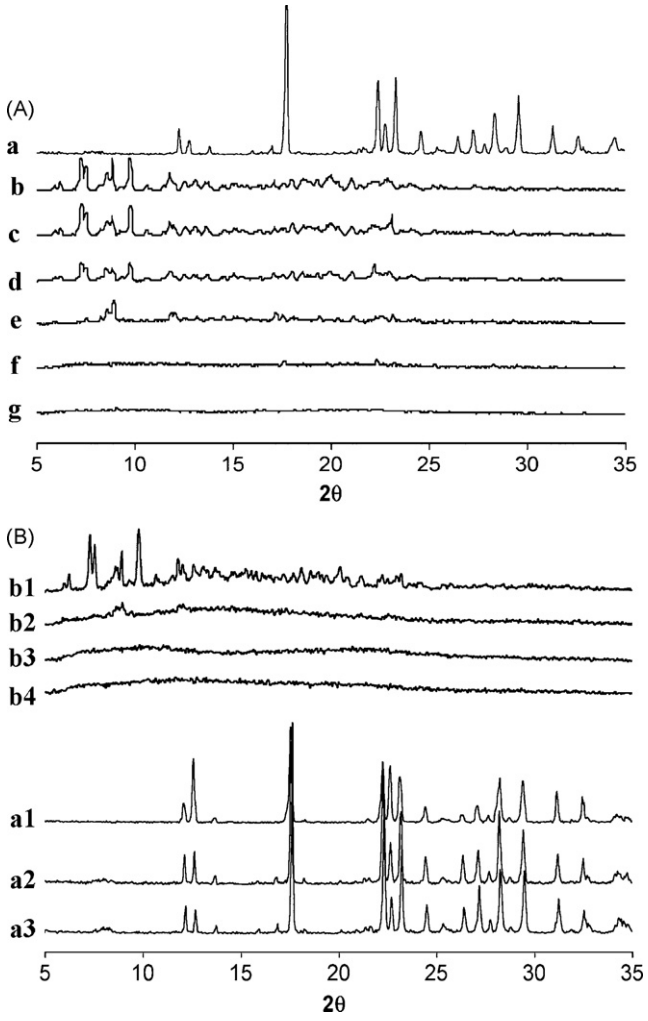

Fig. 2. X-ray powder diffraction patterns of (A) pure components and their equimolar systems obtained with the different techniques and (B) of pure components after treatment with the same techniques. *Key*—A: (a) MF·HCl; (b) TAβCyD, (c) physical mixture, (d) sealed-heated, (e) kneaded, (f) co-ground, (g) spray-dried product; B: MF·HCl after (a1) kneading, (a2) grinding or (a3) spray drying and TAβCyD after (b1) sealed-heating, (b2) kneading, (b3) grinding or (b4) spray drying.

of the characteristic crystallinity peaks of both MF·HCl and TA(CyD (Fig. 2A, curve g). Considering that the spray-drying process caused amorphization of pure carrier (Fig. 2B, curve b4) but, in agreement with DSC results, it did not cause an appreciable reduction of crystallinity of pure MF·HCl (Fig. 2B, curve a3), the result obtained for the binary spray-dried product could be imputable to the formation of strong interactions between drug and TAβCyD and/or to the possible drug inclusion complexation.

3.1.3. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of MF·HCl, TAβCyD, and their respective equimolar binary systems in the 4000–3000 and 2000–1500 cm^{-1} regions (selected as the most interesting ones to point out eventual drug–carrier solid-state interactions) are shown in Fig. 3. The FTIR spectrum of pure MF·HCl showed two typical bands at 3369 and 3294 cm^{-1} (Fig. 3A, a) relative to

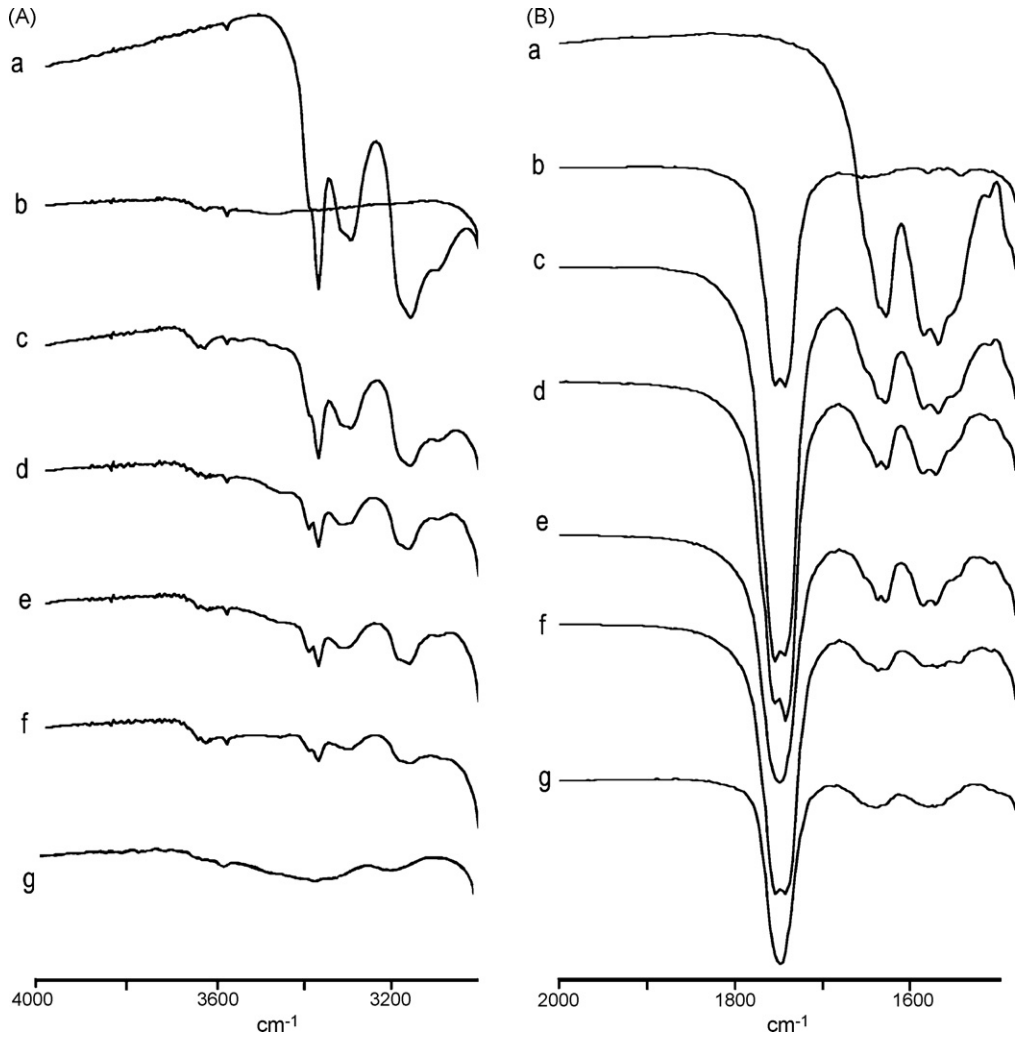

Fig. 3. FTIR spectra of pure components and their equimolar systems obtained with the different techniques in the 3000–4000 cm^{-1} (A) and 1500–2000 cm^{-1} (B) regions. Key: (a) MF·HCl, (b) TAβCyD, (c) physical mixture, (d) sealed-heated, (e) kneaded, (f) co-ground and (g) spray-dried products.

the N–H primary stretching vibration and a band at 3155 cm^{-1} due to the N–H secondary stretching, and characteristic bands at 1626 and 1567 cm^{-1} (Fig. 3B, a) assigned to C=N stretching. TAβCyD displayed a very strong band at 1741 cm^{-1} due to the C=O vibration of the acetyl group (Fig. 3B, b). The physical mixture spectrum (Fig. 3A and B, c) can be considered as the sum of pure MF·HCl and TAβCyD spectra. No significant shifts or reduction in intensity of the FTIR bands of MF·HCl were observed in the binary sealed-heated product (Fig. 3A and B, d). On the contrary, the FTIR spectra of the binary kneaded (Fig. 3A and B, e) and even more so of the co-ground (Fig. 3A and B, f) products presented appreciable shifts and reduction in intensity of the characteristic MF·HCl bands, evidencing the presence of more or less intense solid-state interactions between the components. The FTIR spectrum of the spray-dried compound, on the other hand, showed a strong reduction (Fig. 3B, g) or the complete disappearance (Fig. 3A, g) of the characteristic MF·HCl bands, indicative of strong drug–carrier interactions and, possibly, inclusion complexation of the drug, thus substantially confirming the results previously obtained by DSC and X-ray diffraction analysis.

3.1.4. Scanning electron microscopy (SEM) studies

SEM analyses were performed on pure MF·HCl and TAβCyD samples and on their equimolar combinations obtained by different preparation methods, in order to gain insight about the possible morphological changes caused by the different treatments. MF·HCl particles appeared as lamellar, rather irregular-sized, crystals, with a tendency to self-agglomerate (Fig. 4A); on the contrary TAβCyD consisted of homogeneous small crystals (Fig. 4B). The micrographs of the drug–carrier equimolar physical mixture and sealed-heated product (not shown) clearly displayed MF·HCl crystals dispersed on the surface of the almost unmodified carrier particles. The kneaded and co-ground products presented instead a different morphology, showing a uniform, finely dispersed, powder with an evident particle size reduction and loss of crystallinity with respect to the original components (Fig. 4C). However, the most marked change in morphology was undoubtedly observed for the spray-dried product, which appeared formed by amorphous round particles of very homogeneous and small dimensions (2–5 μm) (Fig. 4D). These findings were consistent with the above results of solid-state studies, confirming complete system amorphiza-

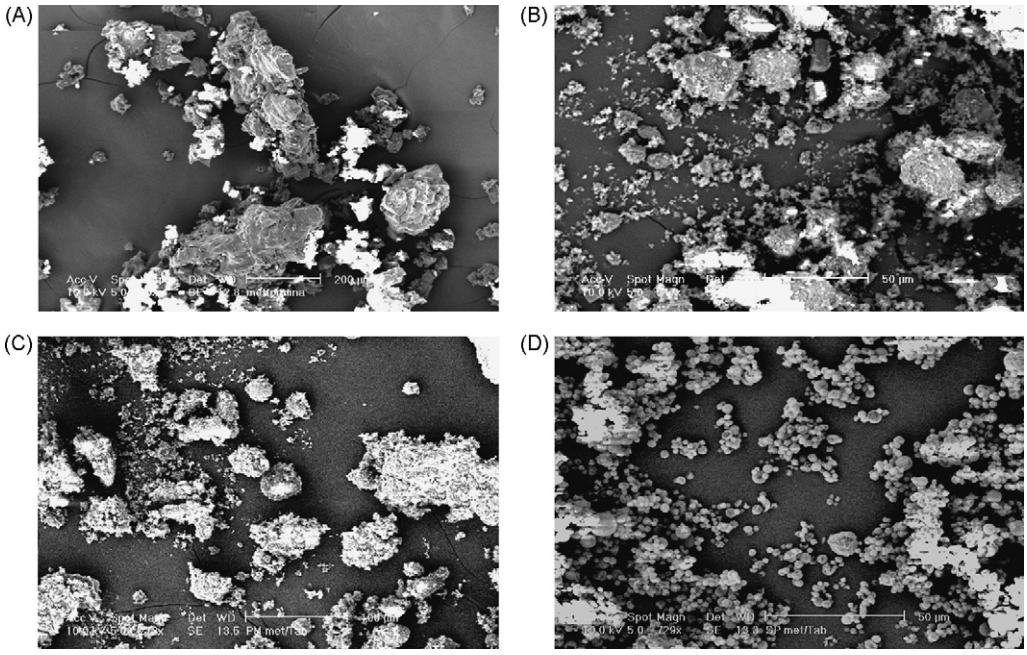

Fig. 4. Scanning electron micrographs of pure metformin·HCl (A) and TAβCyD (B) and of their equimolar co-ground (C) and spray-dried (D) products.

Table 1
Percent dissolved (PD) and dissolution efficiency (DE) values at different times (min) of metformin hydrochloride (MF·HCl) alone and from its equimolar binary systems with TAβCyD obtained by physical mixing (PM), sealed-heating (SH), kneading (KN), co-grinding (GR), and spray-drying (SP)

Sample	PD (10 min)	PD (40 min)	PD (120 min)	PD (420 min)	DE (10 min)	DE (40 min)	DE (120 min)	DE (420 min)
MF·HCl	100				90			
PM	100				83			
SH	100				79			
KN	60	97			38	70		
GR	45	65	100		23	44	74	
SP	8	13	33	100	5	9	17	68

tion and very intimate interaction between the components brought about by the spray-drying process of the drug–carrier mixture.

3.2. Dissolution studies

The dissolution profiles of MF·HCl alone and from its different binary systems with TAβCyD in simulated intestinal fluid (pH 6.5) are shown in Fig. 5, whereas the related dissolution parameters, expressed as percent drug dissolved, and dissolution efficiency values at various times are presented in Table 1. MF·HCl completely dissolved within a few minutes, reflecting its high aqueous solubility. The dissolution from the physical mixture showed approximately the same behaviour of pure MF·HCl, with only a very slight initial slowing down of the drug dissolution rate, due to the presence of the hydrophobic cyclodextrin, which reduces the drug wettability. The sealed-heated product presented a dissolution profile similar to that of the physical mixture, reaching 100% dissolved drug within less than 10 min, thus further confirming the incapability of this technique to promote formation of effective drug–carrier interactions. On the contrary, the MF·HCl dissolution rate from kneaded and even more from co-ground products was significantly retarded, reaching 100% of dissolved drug after about 40 min and 2 h, respectively. The observed significant slowing of drug dissolution rate can be attributed to the interactions between the drug and the hydrophobic carrier established during the sam-

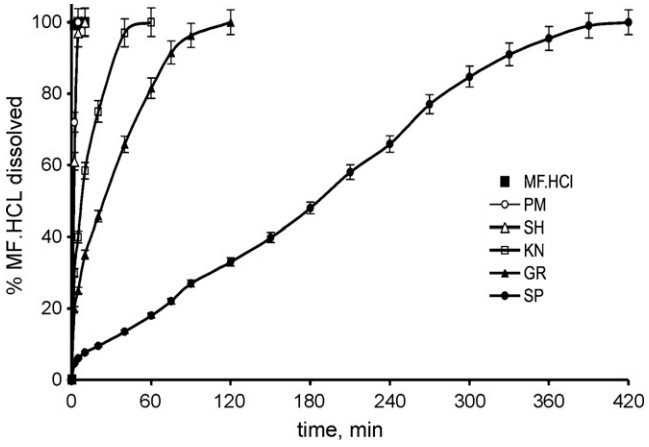

Fig. 5. Dissolution curves of metformin HCl (MF·HCl) alone or from its equimolar physical mixture (PM), sealed-heated (SH), kneaded (KN), co-ground (GR), and spray-dried (SP) products with TAβCyD.

ple treatment, which were more or less intense, depending on the different conditions used for the kneading and co-grinding methods, respectively. Finally, the clearly greater effectiveness of the spray-drying method in inducing powerful drug–CyD interactions, which has already emerged from solid-state studies, was further confirmed from the results of dissolution tests. In fact, the spray-dried systems showed the greatest retarding effect on the dissolution rate of MF·HCl, and allowed obtainment of an almost linear slow-dissolving profile, reaching 100% of dissolved drug after only about 7 h.

4. Conclusions

The present work has demonstrated that the actual effectiveness of the hydrophobic cyclodextrin-derivative TAβCyD as a carrier for obtaining a slow-dissolving form of MF·HCl is strongly dependent on the preparation technique used for obtaining the drug–carrier product. In fact, the results have pointed out the fundamental role played by the preparation method in promoting efficacious interactions between the components, able to adequately modify the drug dissolution behaviour.

In particular, results of solid state studies were all consistent in indicating that the most evident drug–carrier solid-state interactions occurred in the MF·HCl–TAβCyD system obtained by spray-drying, followed by those prepared by co-grinding and then by kneading.

The spray-dried product also gave rise to the most intense effect on the drug dissolution rate, as clearly indicated by the time to dissolve 100% MF·HCl, which varied from less than 10 min for sealed-heated systems, to about 40, 120 and 420 min for kneaded, co-ground and spray-dried products, respectively. Therefore, the MF·HCl–TAβCyD spray-dried and co-ground products were selected as the most effective candidates for the subsequent development of a well-timed sustained-release dosage form of the drug.

Acknowledgement

Financial support from MUR is gratefully acknowledged.

References

[1] F. Hirayama, K. Uekama, Adv. Drug Delivery Rev. 36 (1999) 125–141.
[2] K. Uekama, F. Hirayama, T. Irie, Chem. Rev. 98 (1998) 2045–2076.
[3] T. Loftsson, D. Duchêne, Int. J. Pharm. 329 (2007) 1–11.
[4] T. Loftsson, D. Hreinsdóttir, M. Másson, Int. J. Pharm. 302 (2005) 18–28.
[5] P. Mura, S. Furlanetto, M. Cirri, F. Maestrelli, G. Corti, S. Pinzauti, J. Pharm. Biomed. Anal. 37 (2005) 987–994.
[6] L.M.A. Pinto, L. Fernandes Fraceto, M.H.A. Santana, T.A. Pertinhez, S. Oyama Jr., E. de Paula, J. Pharm. Biomed. Anal. 39 (2005) 956–963.
[7] L. Liu, S. Zhu, J. Pharm. Biomed. Anal. 40 (2006) 122–127.
[8] K. Nakanishi, T. Masukawa, T. Nadai, K. Yoshii, S. Okada, K. Miyajima, Biol. Pharm. Bull. 20 (1997) 66–70.
[9] C.M. Fernandes, F.J.B. Veiga, Chem. Pharm. Bull. 50 (2002) 1597–1602.
[10] C. Marques Fernandes, P. Ramos, A. Celta Falcão, F.J.B. Veiga, J. Control. Release 88 (2003) 127–134.
[11] P. Marchetti, R. Navalesi, Clin. Pharmacokinet. 16 (1989) 100–128.
[12] A.J. Scheen, Clin. Pharmacokinet. 30 (1996) 359–371.
[13] N. Vidon, S. Chaussade, M. Noel, C. Franchisseur, B. Huchet, J.J. Bernier, Diab. Res. Clin. Pract. 4 (1988) 223–229.
[14] P.H. Marathe, Y. Wen, J. Norton, D.S. Greene, T.F. Barbhaiya, I.R. Wilding, Br. J. Clin. Pharmacol. 50 (2000) 325–332.
[15] P. Mura, E. Adragna, A.M. Rabasco, J.R. Moyano, J.I. Pérez-Martìnez, M.J. Arias, J.M. Ginés, Drug Dev. Ind. Pharm. 25 (1999) 279–287.
[16] P. Mura, M.T. Faucci, P.L. Parrini, S. Furlanetto, S. Pinzauti, Int. J. Pharm. 179 (1999) 117–128.
[17] S. Junco, T. Casimiro, M. Ribeiro, M. Nunes Da Ponte, H. Cabral Marques, J. Incl. Phenom. 44 (2002) 117–121.
[18] G.P. Bettinetti, M. Sorrenti, S. Rossi, F. Ferrari, P. Mura, M.T. Faucci, J. Pharm. Biomed. Anal. 30 (2002) 1173–1179.
[19] G.P. Bettinetti, P. Mura, M.T. Faucci, M. Sorrenti, M. Setti, Eur. J. Pharm. Sci. 15 (2002) 21–29.
[20] K.A. Khan, J. Pharm. Pharmacol. 27 (1975) 48–49.
[21] G.P. Bettinetti, M. Sorrenti, L. Catenacci, F. Ferrari, S. Rossi, J. Pharm. Biomed. Anal. 41 (2006) 1121–1205.