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Research paper

Characterisation of nimesulide–betacyclodextrins systems prepared by supercritical fluid impregnation

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Abstract

The purpose of this study was to apply the supercritical CO_2 impregnation process for preparing solvent-free nimesulide (NMS)–betacyclodextrins (BCD) association systems with enhanced drug dissolution rate. Several drug-to-carrier molar ratios were tested (1:1; 1:2.5; 1:3.5) at different conditions of temperatures (40, 100, and 130 °C) and pressures (140, 190 or 220 bar). The physical and morphological characterisation of the systems using powder X-ray diffraction, thermal analysis, diffuse reflectance Fourier transform-infrared spectroscopy and scanning electron microscopy was carried out to understand the influence of this technological process on the physical status of single components and binary systems and to detect possible interactions between drug and carrier. These analyses provided no evidence of a complete inclusion of NMS in the carrier but the existence of interactions between drug and carrier together with a partial dehydration of the BCD and the formation of drug crystallites with lower melting point and heat of fusion than the native NMS. These phenomena were more intense when severe conditions of pressure and temperature (220 bar and 130 °C) were used during impregnation trials and when the amount of BCD augmented in the systems. These activated solid state of the impregnated systems promoted an enhancement of drug dissolution rate that, in keeping with the results of the physical characterisation, was function of the process conditions and BCD content.

Keywords: Supercritical CO_2 impregnation; Nimesulide; Betacyclodextrins; Physico-chemical characterisation; Dissolution enhancement

1. Introduction

The association between β-cyclodextrins (BCD) and drugs is known to improve some solution parameters of the drug such as dissolution rate and solubility in water. This improvement in hydrophilicity is obtained either through the formation of host–guest inclusion complexes or by means of highly homogeneous assemblies between the BCD and the drug in the solid state. To prepare a suitable BCD–drug association many techniques have been developed, e.g. grinding [1], kneading [2], spray drying [3], freeze-drying [4], roll mixing [5], ultrasound compaction [6], co-precipitation from various solvents [7]. Recently, technologies using supercritical fluids (SCFs), and in particular using supercritical carbon dioxide ($SCCO_2$) as a processing medium, have been proposed to prepare solvent-free BCD–drug association systems [8–10]. Following these experiences, in this research the good solvent power of CO_2 on nimesulide (NMS) has been appropriately manipulated over a wide range of temperatures and pressures to prepare BCD–drug association systems in different drug-to-carrier proportions. NMS is a poorly water soluble NSAID existing in at least two polymorphic forms [11], whose solubility has proved to be enhanced when included into BCD [12,13]. Hence, in this research the preparation of NMS–BCD association compounds using a supercritical process was considered with the aim of improving the in vitro drug dissolution rate. The binary systems were characterised with powder X-ray diffraction (XRD), differential scanning calorimetry (DSC), diffuse reflectance Fourier transform-infrared spectroscopy (DRIFT) and scanning electron microscopy (SEM), in order to understand the influence of the technological process and of considered parameters

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(temperature and pressure) on physico-chemical and morphological properties of the systems.

2. Materials and methods

2.1. Materials

NMS pharmaceutical grade and BCD with a 12% hydration water were both kindly donated by Eurand International (Trieste, Italy). Methanol analytical grade was purchased by Carlo Erba (Milan, Italy). CO_2 (purity 99.9%) was supplied by SIAD (Trieste, Italy).

2.2. Preparation of the samples

The experiments at 40 °C were performed heating the column with a thermo-stated water bath, while for the experiments conducted at 100 and 130 °C, the column was inserted in a oven. The two apparatus are very similar and sketched in Figs. 1 and 2, respectively.

NMS and BCD were firstly mixed in a mortar in different drug-to-carrier molar ratios, 1:1, 1:2.5, 1:3.5, obtaining the physical mixtures, PM (some samples of these mixtures where unprocessed as a comparison with the treated ones). Then, a stainless steel column (5.5 cm length and 1 cm diameter) was filled with about 2 g of a PM, pressurized with carbon dioxide at the required pressure (140, 190 or 220 bar) and left in a static mode for 6 h: this contact time was derived from a previous work [14]. At the end of the process depressurisation occurred almost instantaneously (in a few seconds). As a comparison, the single components were separately subjected to a treatment with $SCCO_2$ using the most severe conditions (130 °C and 220 bar). These samples were named NMS-CO_2 and BCD-CO_2.

2.3. Assay of the total drug content

Known amounts of the samples were dissolved in a 1:1 methanol:water (v/v) solution and then the drug content was evaluated spectrophotometrically at 297 nm (Mod. 552, Perkin Elmer, Norwalk, CT).

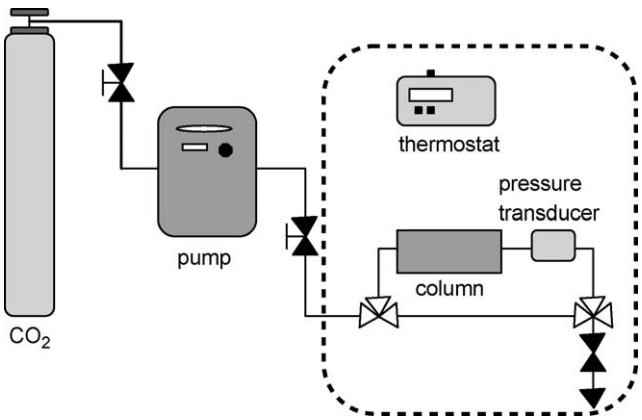

Fig. 1. Schematic drawing of the apparatus used for impregnation experiments at 40 °C.

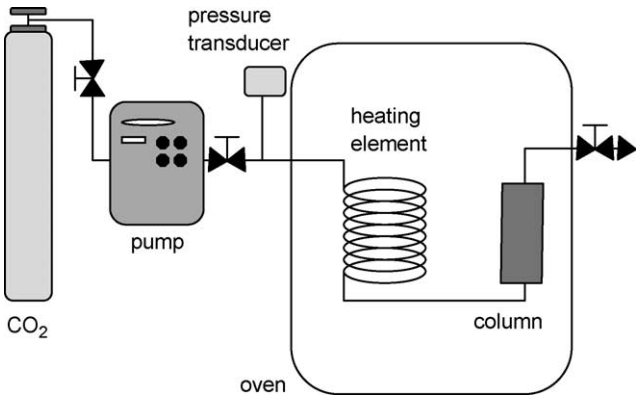

Fig. 2. Schematic drawing of the apparatus used for impregnation experiments at 100 and 130 °C.

2.4. Powder X-ray diffraction studies

Samples were studied by means of XRD technique using a STOE D500 (Siemens, Monaco, Germany) diffractometer with Cu Kα radiation, monochromatised by a secondary flat graphite crystal. The scanning angle ranged from 3 to 45° of 2θ, steps were of 0.1° of 2θ, and the counting time was of 2 s/step. The current used was 20 mA and the voltage 40 kV.

2.5. Differential scanning calorimetry

Calorimetric analyses were performed with a DSC mod. TA 4000 (Mettler, Greifensee, Switzerland), equipped with a measuring cell DSC 20. Samples, containing 0.6–0.8 mg of NMS were placed in pierced aluminium pans and heated at a scanning rate of 10 °C/min from 30 to 180 °C under air atmosphere.

2.6. Drift spectroscopy

Fourier transform-infrared spectra were obtained on a FT-IR spectrometer (FT-IR 200 Jasco, Tokyo, Japan) using the diffuse reflectance method (DRIFT). The samples, starting and treated materials, 1:1 (m/m) PM as it is, treated with relaxed conditions (40 °C and 140 bar) and with severe conditions (130 °C and 220 bar) were ground gently with anhydrous KBr, thus avoiding polymorphic transition possibly induced by extended grinding. The scanning range was 400–4000 cm^{-1} and the resolution was 4 cm^{-1}.

2.7. Scanning electron microscopy

The shape and surface characteristics of pure components and binary systems were observed by SEM. Samples were sputter-coated with Au/Pd using a vacuum evaporator (Edwards, Milano, Italy) and examined using

a scanning electron microscope (model 500, Philips, Eindhoven, The Netherlands) at 10 kV accelerating voltage using the secondary electron technique.

2.8. Determination of drug dissolution

NMS release profiles were obtained according to the USP 25 paddle method: 100 rpm, 900 ml of dissolution medium, $T = 37 \pm 0.1$ °C, sink conditions ($C < 0.2C_s$). The aqueous solution was filtered (0.45 μm porosity) and continuously pumped to a flow cell in a spectrophotometer and absorbances were recorded at 396 nm. The composition of the dissolution medium was 0.2 M KH_2PO_4/0.2 M NaOH (pH 7.4) according to USP 25, plus 0.5% w/v Tween 80. Experimental points were the average of at least three replicates, and standard deviations did not exceed 5% of mean value. Dissolution profiles were compared to that of the pure NMS and PM, at the same experimental conditions.

3. Results and discussion

The solubility of NMS in $SCCO_2$ has been determined in a previous work at 40 and 60 °C and at pressures ranging from 100 to 220 bar [15]. The drug solubility values expressed as molar fraction (ranging from 0.85 to 9.85×10^{-5}) revealed that NMS is a suitable candidate for supercritical impregnation experiments.

3.1. Drug content

The spectrophotometric evaluation of drug content in the samples revealed that the percentage of NMS in the samples was ranging from 83 to 100%, as reported in Table 1.

The effect of temperature, BCD molar content and pressure on drug loading is illustrated in Fig. 3a–c, respectively. As it can be seen, higher drug content in the binary system are obtained increasing the temperature or the amount of BCD or the pressure. A relationship between increase of temperature and improvement of drug content has been already noticed by Charoenchaitrakool et al. [9] in methyl-BCD/ibuprofen complexes prepared using $SCCO_2$, and by Nakai et al. [16] in cyclodextrins inclusion compounds prepared by several methods.

3.2. DSC, XRD and DRIFT analyses

For the sake of brevity, only the DSC curves of the 1:3.5 binary systems (impregnated systems and PM) are reported in Fig. 4 and compared to the single components whilst the thermal data of the other samples are listed in Table 1.

Table 1
Overview of the samples and relative drug content, preparation conditions and thermal data

Sample	Preparation of the samples				Thermal data			
	Drug content (%)	NMS:BCD molar ratio	T (°C)	P (bar)	ΔH H_2O (J/g)	T peak H_2O (°C)	ΔH NMS (J/g)	T peak NMS (°C)
BCD-CO_2			130	220	192.10	92.3		
BCD					262.00	69.8		
NMS-CO_2			130	220			109.30	149.6
NMS							127.40	150.4
F	91.7	1:1	40	190	255.98	97.8	120.50	150.4
G	83.3	1:1	40	140	270.25	97.0	143.00	150.3
T	100.0	1:1	40	220	262.04	101.5	96.38	150.1
H	96.6	1:1	100	190	280.20	91.2	88.39	149.7
I	100.0	1:1	100	140	235.29	86.3	106.13	150.3
S	93.3	1:1	130	220	201.50	94.3	102.73	149.4
B	96.0	1:2.5	40	190	264.83	109.9	97.58	149.7
C	96.0	1:2.5	40	140	200.51	100.9	93.90	149.6
D	90.0	1:2.5	100	190	134.55	95.3	87.54	149.5
E	96.7	1:2.5	100	140	192.21	103.4	91.20	150.3
R	100.0	1:2.5	130	220	144.67	98.6	83.49	149.3
Z	93.3	1:2.5	40	220	171.26	111.8	88.20	149.5
L	100.0	1:3.5	40	190	201.33	112.1	76.23	150.2
M	100.0	1:3.5	40	140	196.34	116.0	72.33	150.1
N	100.0	1:3.5	100	190	245.43	105.3	93.21	150.1
O	90.0	1:3.5	100	140	215.10	117.9	69.43	150.2
P	86.7	1:3.5	40	220	227.30	110.6	77.01	149.6
Q	100.0	1:3.5	130	220	141.70	99.2	66.09	148.8
PM		1:1			274.24	90.2	109.64	149.6
PM		1:2.5			220.50	87.5	100.00	149.5
PM		1:3.5			200.50	107.2	102.13	149.4

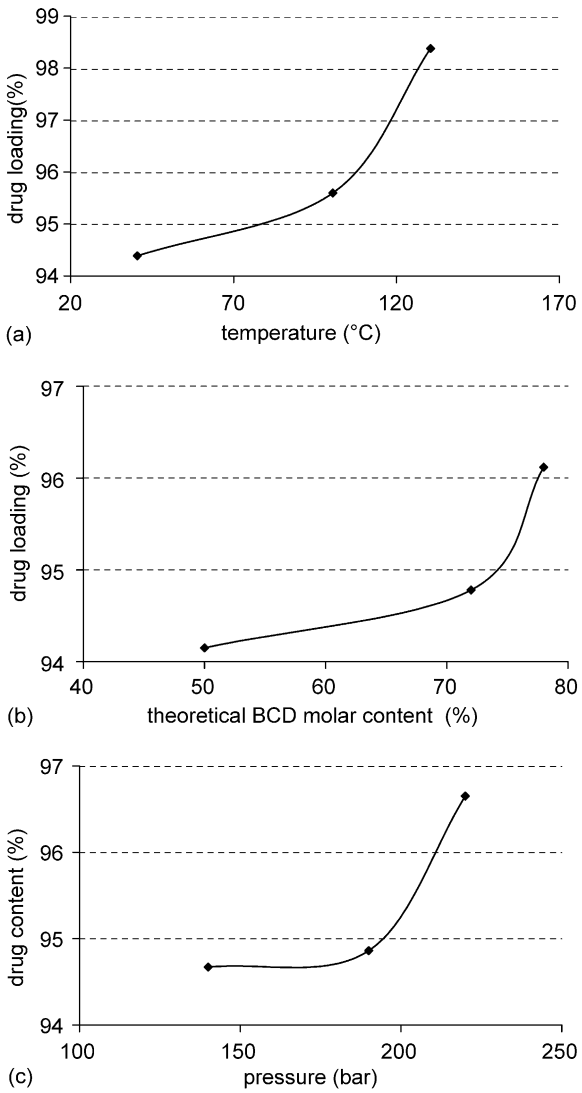

Fig. 3. Effect on drug loading of (a) the temperature; (b) BCD molar content; (c) the pressure.

The DSC of pure BCD curve has a large endothermic peak, centred between 110 and 120 °C that is related to the loss of hydration water of the starting material. BCD decomposes at about 300 °C, so there is no trace of melting peak of BCD in the chosen temperature range.

The DSC curve of NMS showed a sharp endothermic peak at 150.4 °C (127.4 J/g), according to previous literature data [13].

As a comparison, the single components were separately treated with $SCCO_2$ and their DSC curves are depicted in Fig. 4. The thermal analysis of NMS-CO_2 revealed a certain diminution of the fusion peak together with a broadening of its shape. These facts indicate the formation of a nanocrystalline structure [17] and suggested that NMS partially converted in a different polymorphic form. As regards to BCD-CO_2, the dehydration enthalpy decreased in comparison to starting BCD indicating a reduction of water content in the BCD. The partial removal of water could

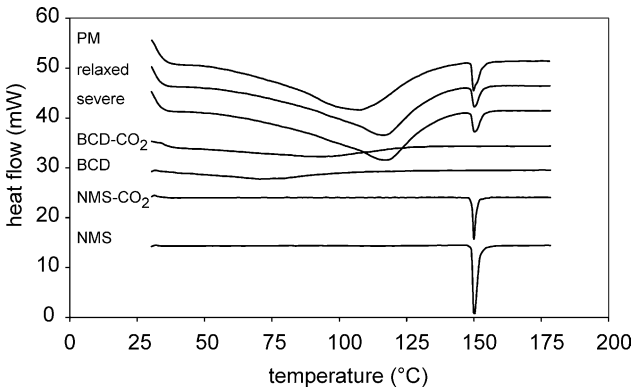

Fig. 4. DSC curves of the 1:3.5 systems impregnated with different conditions compared to the corresponding PM and the single components.

favour the formation of a complex between BCD and drug [8]. In fact, since the cavity is partially free the exchange between water and drug might be promoted.

In the CO_2-binary systems a remarkable reduction of NMS melting temperature and enthalpy was noticed. This phenomenon became more intense as the pressure and the temperature of impregnation experiments and the BCD content increased. Further, NMS peak lost his sharp appearance and broadened. In comparison to the impregnated systems, the PM showed only a scarce lowering of drug melting temperature and a slight reduction of its enthalpy, with a trend analogous to that of the samples impregnated with relaxed conditions (40 °C and 140 bar).

In conclusion, on the basis of these results the presence of some interactions between drug and carrier was found, whose intensity increased when severe impregnation conditions (130 °C and 220 bar) were used. However, the detection of drug melting peak in all the performed systems denied the existence of a complete inclusion of the drug in the BCD cavity [18].

The diffractograms of the single components before and after treatment are compared in Fig. 5a. The XRD patterns of native and NMS-CO_2 are substantially superimposable in the range 3–25° of 2θ, where only little variation in the intensity of the signals was noticed. Conversely, in the range between 25 and 30 of 2θ significant differences in shape and position of the signal can be observed, suggesting, in agreement to previous thermal data, a probable partial reorganisation in a different solid phase. The differences between the diffractograms of the BCD before and after treatment are more significant. The two high intensity peaks originally present at 12.6 and 13.2° of 2θ assumed the shape of a doublet (12.4–12.9) and a signal at 13.5° of 2θ, respectively. Further differences can be noticed in the range between 18 and 20° 2θ (e.g. the signals at 18, 18.6, 18.9 and 19.5 shifted to 18.1, 19.1, 19.3 of 2θ).

Finally, in the range 20–40° of 2θ the relative intensity of the peaks is remarkably different. In the whole spectrum of BCD-CO_2 a remarkable scattering phenomenon is also evident, indicating a certain percentage of amorphous solid

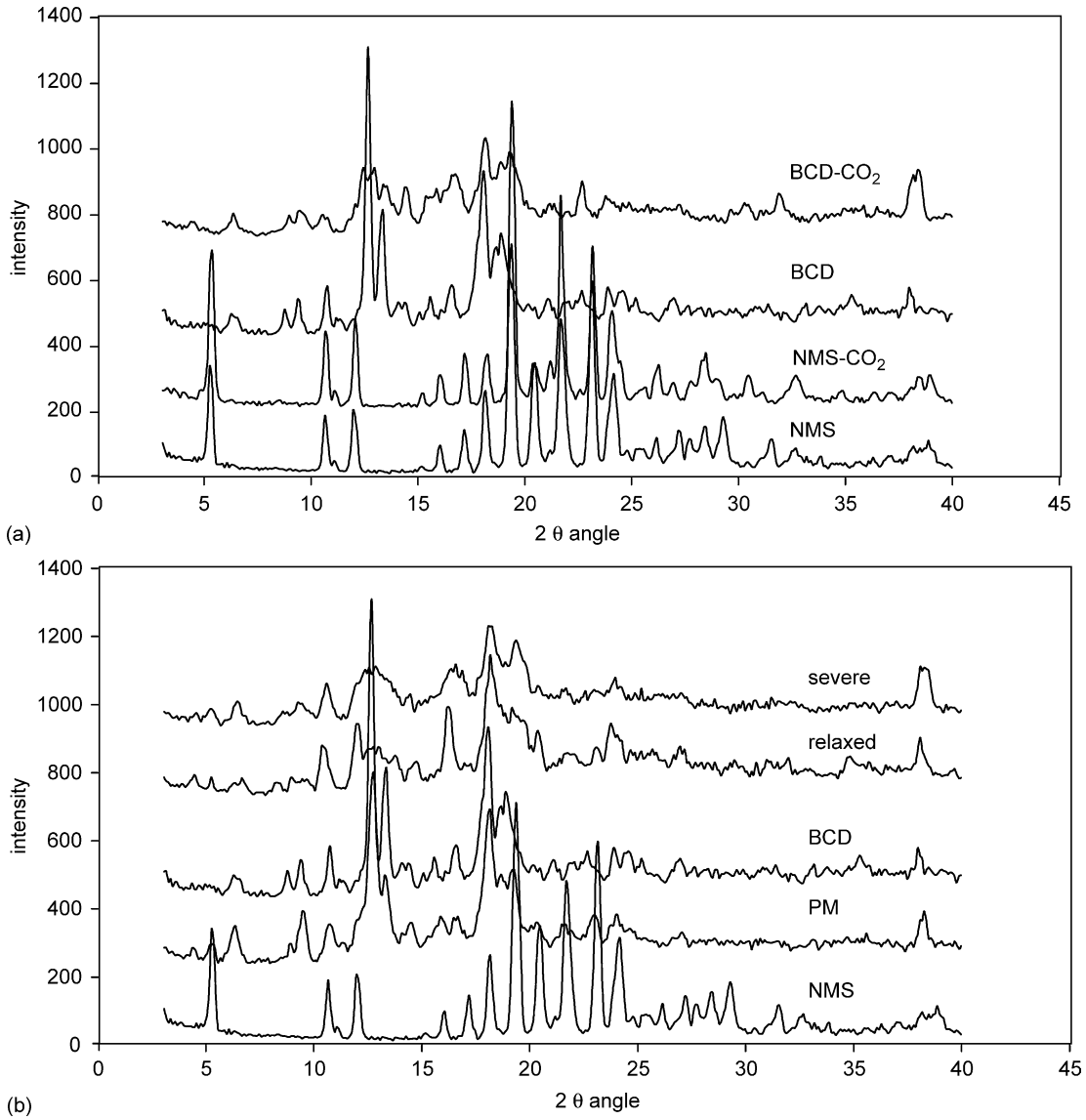

Fig. 5. XRD patterns of (a) NMS, NMS-CO_2, BCD, BCD-CO_2, and the (b) 1:3.5 drug-to-carrier molar ratios systems compared to the starting materials.

probably due to a partial dehydration of the BCD. A similar phenomenon was previously found by other authors in BCD mechanically activated [1,17,19].

The diffractograms of the 1:1, 1:2.5, 1:3.5 impregnated systems using relaxed (40 °C and 140 bar) and severe conditions (130 °C and 220 bar) were compared to the corresponding PM and native materials. Though the remarkable superimposition of the signals due to the drug and the carrier making difficult the interpretation of the data, in all the impregnated systems at least 3 peaks attributable to the NMS can be distinguished. These peaks are resolved in the PM whereas confused in the impregnated systems, especially in the systems treated with severe conditions. Further, in the CO_2-processed systems, analogously to the BCD alone, a significant scattering phenomenon was observed, which intensified with BCD high content and with high pressures and temperatures, indicating a higher amorphous content and

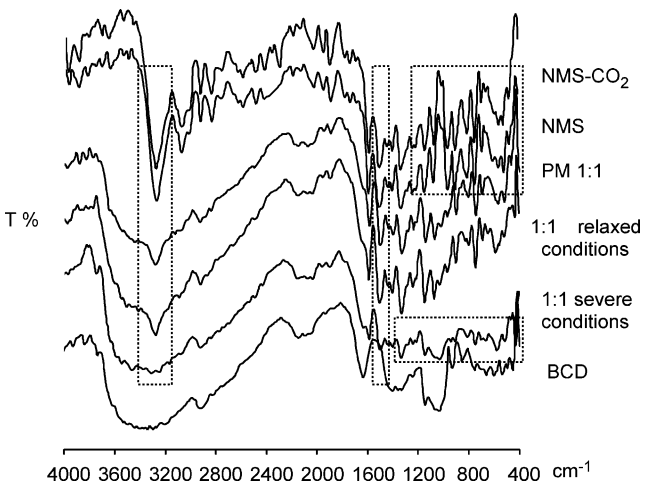

Fig. 6. DRIFT spectra of 1:1 systems impregnated with relaxed and severe conditions compared to the corresponding PM and the single components.

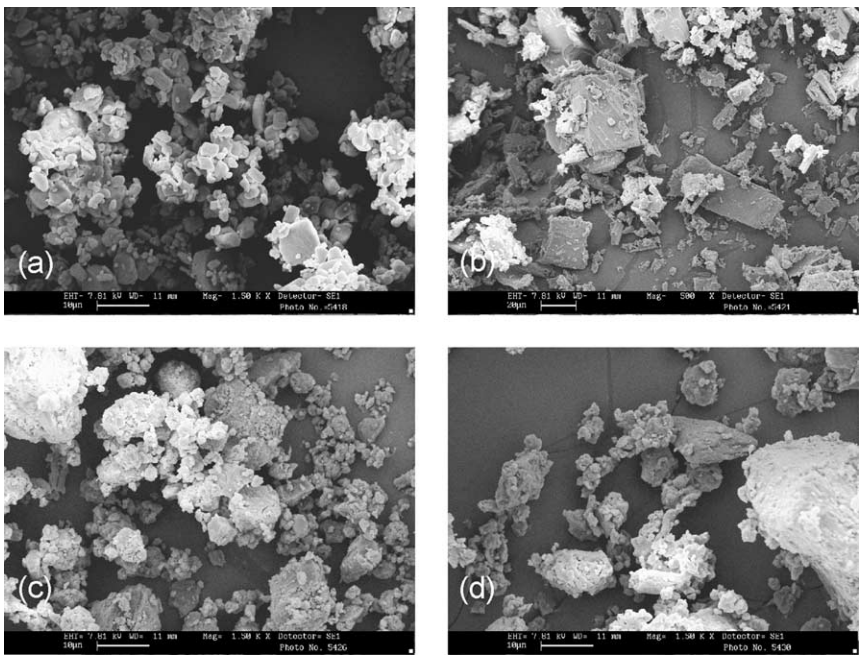

Fig. 7. SEM images of (a) NMS; (b) NMS-CO_2; (c) BCD; (d) BCD-CO_2.

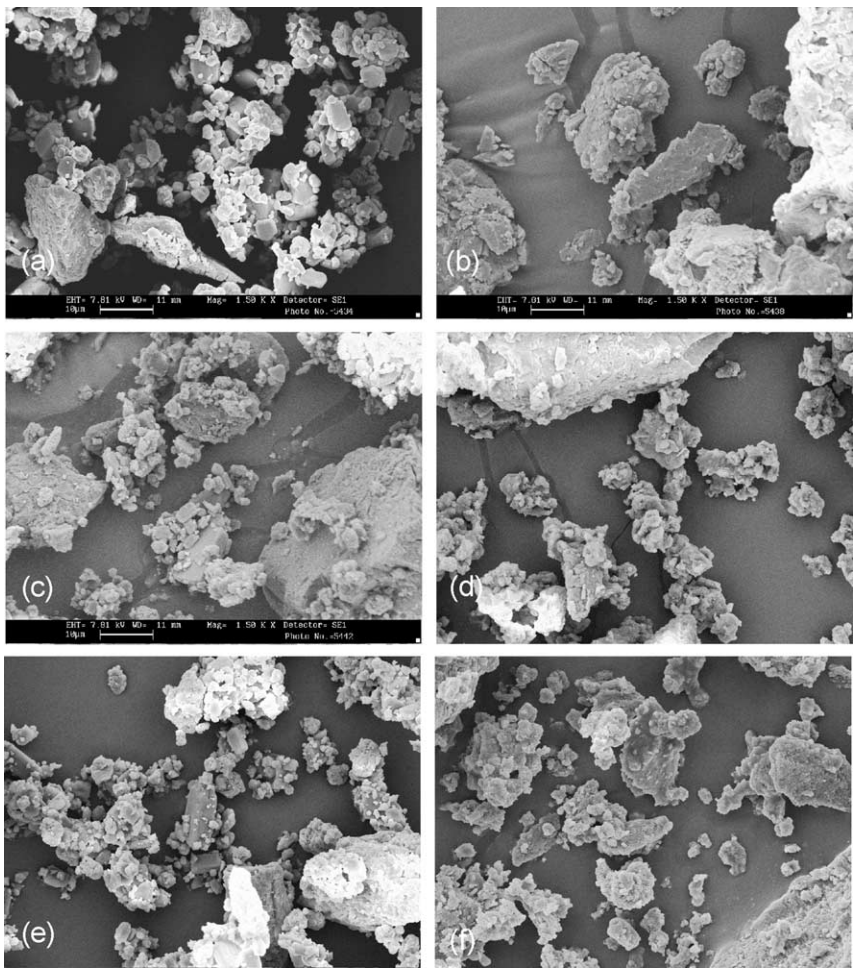

Fig. 8. SEM images of 1:1 systems impregnated with (a) relaxed and (b) severe conditions; 1:2.5 systems impregnated with (c) relaxed and (d) severe conditions; 1:3.5 systems impregnated with (e) relaxed and (f) severe conditions.

a partial dehydration of the carrier in these systems. Hence, for brevity, only the 1:3.5 systems have been reported (Fig. 5b) were the above-mentioned phenomena resulted more evident.

As it can be observed in Fig. 6, the comparison between native and NMS-CO_2 revealed substantially superimposable DRIFT spectra, and scarce differences were noticed only in the finger print region. Analogously, BCD-CO_2 (data not shown) and the starting BCD showed only little variations of the shape of the spectral bands.

In agreement with previous physical characterisations, this analysis reveals a substantial correspondence between the PM and the system prepared with relaxed conditions, whilst several remarkable differences with the 'severe' system. In particular, the band corresponding to the –NH stretching (about 3283 cm^{-1}) is present in both PM and 'relaxed' system but it is absent in the severe system. The $-NO_2$ stretching shifted by 5 cm^{-1} towards lower frequencies (from 1510 in the PM and relaxed system to 1505 cm^{-1} in the severe system). In the finger print region of severe system spectra little shifts of the bands can be observed and a broadening of the bands.

3.3. SEM studies

The observation by SEM of NMS particles (Fig. 7a) revealed that the drug was originally formed by almost spherical particles with various dimensions, whilst after treatment with $SCCO_2$ it assumed the shape of prisms having higher dimensions (Fig. 7b). Starting and treated BCD (Fig. 7c and d, respectively) appeared quite similar.

In the 1:1 system (Fig. 8a) impregnated with relaxed conditions, NMS having an aspect analogous to that of commercial sample was detectable. In the 1:2.5 relaxed system (Fig. 8c) NMS was positioned on the top of BCD agglomerates while in the 1:3.5 (Fig. 8e) the drug was scarcely distinguishable. On the other hand, in the severe systems (Fig. 8b, d, f), having and aspect independently from the drug to carrier ratio very similar to the BCD-CO_2, NMS was scarcely visible.

3.4. Dissolution test

The in vitro dissolution rate of all impregnated systems (Fig. 9) was increased compared to the drug alone. On one hand, this enhancement can be attributed to the higher hydrophilic character of the systems due to the presence of the carrier, which can reduce the interfacial tension between the poorly soluble drug and the dissolution medium [1]. Moreover, in the case of BCD, in the early stage of dissolution process, the carrier dissolves more rapidly than the drug. Hence, it can act on the hydrodynamic layer surrounding the particles of the drug, resulting in an 'in situ' inclusion process that improves the dissolution of the drug [1]. In fact, the systems containing the higher amount of BCD showed a quicker drug dissolution. On the other hand, it must be pointed out that the impregnated systems showed an improved drug dissolution with respect to the corresponding PM. This better performance can be explained in the light of the above-mentioned results of the physical characterisation, and thus it can be ascribed to the activated solid state of

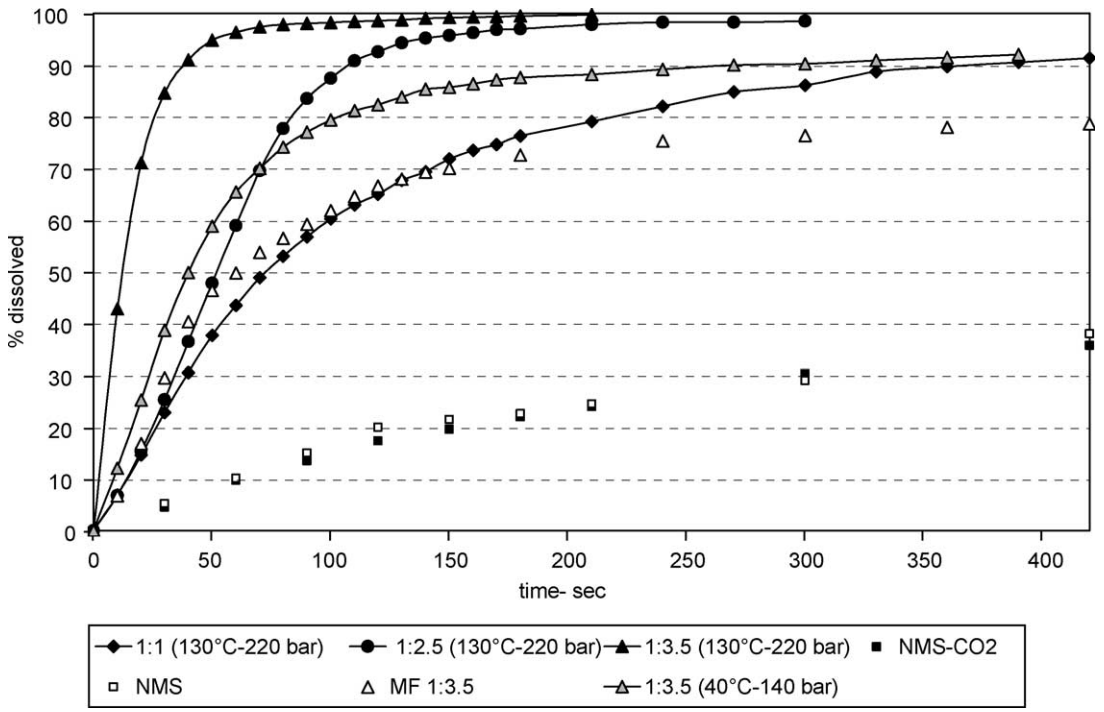

Fig. 9. In vitro dissolution profiles of the systems impregnated with severe conditions compared to the corresponding PM, the 1:3.5 system impregnated with relaxed conditions, the commercial and the CO_2-treated NMS.

the drug, due to the nanocrystalline NMS, the interactions with BCD and the partial dehydration of the BCD. In particular, the partial loss of water causes structural changes in the BCD that might have a significant influence on their pharmaceutical properties. In fact, the lack of water in the crystalline structure leads to an increased affinity towards water, due to their higher wettability and higher rate of solubilisation [19]. In fact, systems containing partially dehydrated BCD were found to have improved dissolution characteristics compared to the corresponding hydrate BCD [1].

As a confirmation of these assumptions the systems providing the fastest dissolution rate are the binary systems prepared with the most severe conditions, where the previous DSC, XRD and DRIFT analyses have demonstrated an intensification of solid state activation.

4. Conclusions

The solid state analysis of the binary systems prepared with the supercritical impregnation technology indicated the absence of a complete inclusion of NMS into the BCD. However, the physico-chemical characterisation pointed to the existence of interactions between drug and carrier that provided the activation of both carrier and drug that was favourable for the in vitro drug dissolution. The entity of these interactions was found to be strictly related to the conditions adopted during the impregnation trials and to the amount of BCD in the systems. Work is in progress to investigate the nature of these interactions, also including solid state NMR studies.

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