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Research paper

Raman and thermal analysis of indomethacin/PVP solid dispersion enteric microparticles

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Abstract

Indomethacin (IMC) and three types of poly-(vinylpyrrolidone) (PVP 12PF, PVP K30 and PVP K90) were studied in the form of solid dispersion, prepared with the solvent evaporation method, by spectroscopic (Raman, FT-IR, X-ray diffraction), thermal (differential scanning calorimetry, thermogravimetry, hot-stage microscopy), fractal and image analysis. Raman and FT-IR micro-spectroscopy indicated the occurrence of drug/polymer interaction and the presence of an amorphous form of IMC, as also resulting from X-ray diffractometry. Hot-stage microscopy suggested that the interaction between IMC and the polymer occurring on heating of a physical mixture, is common to other acidic compounds and causes a depression of the temperature of the appearance of a molten phase. Co-evaporated particles were coated by spray-congealing process with molten stearic acid for gastroprotection, but also for stabilization of the amorphous structure of the drug: the final particles were spherically shaped. Dissolution tests carried out on the final microparticles showed that the coating with stearic acid prevents IMC release at acidic pH and also protects against recovery of the IMC crystallinity, at least after 9 months of aging: the extent and mode of the release, before and after aging, overlap perfectly. The test revealed a notable improvement of the drug release rate from the solid dispersion at suitable pH, with respect to pure IMC. The comparison of the present solid dispersion with IMC/PVP (surface) solid dispersion obtained by freeze-drying of an aqueous suspension, where IMC maintained its crystalline state, revealed that there was no difference concerning the release rate, but suggested a superior quality of this last process as a mean of improving IMC availability for the easiness of preparation and stability, due to the absence of the amorphous state of the drug, as a possible instability source of the system. Finally, the coating with stearic acid is discussed as a determining process for the practical application of solid dispersions.

Keywords: Indomethacin/poly-(vinylpyrrolidone) co-evaporated particles; Raman and FT-IR micro-spectroscopy; Thermal analysis; Co-evaporated and freeze-dried system comparison

1. Introduction

The improvement of the release rate of poorly soluble drugs represents an important challenge for researchers, who have designed a variety of formulations for this purpose. Poorly soluble drugs may benefit from formulation approaches that overcome poor solubility and/or a dissolution rate-limited bioavailability and, for this purpose, solid dispersions are frequently proposed. The main objective of the preparation of solid dispersions is to enhance drug dissolution rates via dispersion of the drug within water-soluble polymer matrices: in fact, because the crystallinity of drugs is often responsible for their low solubility in water, crystallization of drugs embedded in a polymer matrix should be avoided, since it has a favourable impact on the dissolution rate and the therapeutic value of such products is consequently affected. However, challenges and opportunities in the preparation of solid dispersions of poorly water-soluble drugs have recently been critically

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revised and discussed in some detail and several limitations to their commercial diffusion have been outlined [1].

These systems can be prepared with a variety of methods (solvent evaporation, melt extrusion and mechanical treatment of the mixtures (co-grinding) [2] and materials (polymers and lipid substances).

As a consequence of the important results that can be obtained with these systems, it could be rather interesting to carefully evaluate the preparative aspects of these formulations and to suggest variations to the proposed methods with a view to promoting their practical and commercial applications. We therefore compared two methods for obtaining a solid dispersion, employing indomethacin (IMC) and poly-(vinylpyrrolidone) (PVP), and evaluated the differences but also the advantages of each process, also discussing a process enabling stabilization of the solid dispersions. IMC represents a model drug practically insoluble in water and PVP, a soluble hydrophilic polymer able to form a complex and drive dissolution of the drug.

In this paper IMC and PVP were closely associated by the preparation of a solid dispersion, dissolving both drug and polymer in a common solvent (ethanol), and leading to a homogeneous system after the evaporation of the solvent. The weight ratio of the IMC/PVP physical mixtures was kept constant to 1:1 and three types of PVP were employed: 12PF, K30 and K90, differing for their MW and solubility in ethanol. The IMC/PVP solid dispersion system obtained by the solvent method is referred to as *co-evaporated* and was analyzed by a variety of techniques from Raman and ATR FT-IR micro-spectroscopy to differential scanning calorimetry (DSC), thermogravimetry (TG) and hot-stage microscopy (HSM) analysis, from SEM to X-ray diffractometry.

In a previous paper [3] we proposed a different method to couple IMC and PVP in a solid dispersion: an aqueous suspension where IMC, very poorly soluble in water, represented the solid phase dispersed, while hydrosoluble PVP was present as a solute in the dispersing medium. The final system represented a surface solid dispersion and was obtained by freeze-drying, where PVP covers the IMC crystals as a fine precipitate and is referred to as *freeze-dried*. This mode of preparation does not involve a change to the IMC phase that remains crystalline inside the solid dispersion. As a consequence it should display a notable stability, higher than that containing an amorphous phase inside the dispersion.

Powders of the two different systems were finally processed by a spray-congealing apparatus, where the starting particles of both types were coated by a thin film of stearic acid and are referred to as *co-evaporated* and *freeze-dried microparticles*.

2. Experimental part

2.1. Materials

Indomethacin (IMC) was a commercial sample of pharmaceutical grade (Sigma Chemical Company, St. Louis, MO) with a melting point of 161 °C (pure gamma form); IMC was sieved and only the <200 μm fraction was used. PVP 12PF, K30 and K90 of different molecular weights (2.5×10^2, 5×10^4, 1×10^6) were gifts from BASF (Ludwigshafen, Germany) and were used as received. Stearic acid (SA) of pharmaceutical grade (69–71 °C) was purchased from Fluka (Buchs, Switzerland). Other solvents were commercial samples of reagent grade.

2.2. Co-evaporated particles

IMC/PVP solid dispersion was prepared using the solvent evaporation technique. Equi-ponderal amounts (5 g) of IMC and PVP were dissolved in 95% v/v ethanol at 65 °C and filtered, obtaining a yellow solution. The solvent was then removed at reduced pressure (14 mm Hg) at 50 °C, using a rotary evaporator. The final residue was kept at reduced pressure (1 mm Hg) for 24 h for complete elimination of the solvent. It was then milled and the size fraction $100 > \times > 200$ μm, used for further tests, was stored in a desiccator over silica gel to prevent humidity absorption.

2.3. Spray-congealing

The microparticles were produced using an ultrasonic atomizer [3]: the sonotrode was a type UIP 250 (Hielscher, Berlin, Germany), the frequency was 25 kHz and the power output was 2.2 kW. A sample of stearic acid (SA), melted and kept at 60 °C, was added to an amount of IMC/PVP co-evaporated particles up to a 20% w/w SA. The suspension turned to a pale yellow due to partial dissolution of the IMC in the SA. The suspension was poured into a thermostated reservoir (preset at 80 °C) surrounding the sonotrode. The reservoir was not stirred, because the US cavitation effect prevented the settling of the suspended material. The mixture, dropped on the sonotrode plate vibrating at US frequency, was fragmented into small droplets and atomized by US energy, without acquiring kinetic energy. Falling for 1.5 m, the droplets solidified at room temperature and were collected in a cylindrical chamber and stored in a vacuum desiccator, over silica gel at room temperature. Each formulation was produced in duplicated experiments.

The particles displayed an evident spherical shape and showed a pale yellow colour. According to accepted definitions, the final particles can be referred to as microcapsules, since the external coating and the nucleus are chemically different. Using the same method, we also prepared microparticles containing only IMC/SA, without PVP, for comparison.

2.4. SEM analysis

The morphological examination (shape and surface characteristics) of each formulation was performed by scanning electron microscopy (SEM, Philips XL-4) after gold coating (10 nm) of the particles. The following three parameters, which are all normalised (defined to have values lying in the range 0–1), were determined to quantify the different aspects of particle shape: circularity, elongation, and fractal dimension of the particle contour.

Sphericity or “circularity” of particles ranges between 0 and 1, with 1 representing a perfect circle. It is determined from measured area A and perimeter P, using the equation $4\pi A/P^2$. A perfect circle has a sphericity of 1, while a very ‘spiky’ or irregular object has a circularity closer to 0.

Elongation is a measurement of the length/width ratio: it is defined as 1 – [width/length]. Shapes symmetrical in all axes, such as circles or squares, have similar length and width (elongation close to 0).

Fractal dimension of the particle contour can be obtained by SEM by means of suitable programs: it ranges from 1, for the regular contour of a Euclidean geometrical object, to 2 for a very irregular perimeter of a particle.

3. Thermal analysis

3.1. Differential scanning calorimetry (DSC) analysis

Thermal characteristics of the pure materials, the physical mixtures and solid dispersions were determined by an automatic thermal analyzer system (Mettler 821^e). The data processing system (Mettler 821^e/500/847 thermo-cryostat) was connected to the thermal analyzer. Holed aluminum pans were used for the measurements of all the samples. Temperature calibrations were made using indium as standard. The thermograms were run at a scanning of 10 °C/min, from 30 to 200 °C.

3.2. Thermogravimetric analysis (TGA)

Thermogravimetric analysis was performed with a Mettler Toledo automatic thermal analyzer system TGA/SDTA851^e/SF/1100). Open alumina crucibles were used for analysis in the temperature range 30–300 °C at 10 °C/min scanning rate, under nitrogen stream.

3.3. Hot stage microscopy (HSM)

Thermomicroscopy employed a hot-stage Mettler Toledo p90 coupled to an optical microscope Nikon Eclipse E400, equipped with Nikon PU 100 Digital Net colour camera to acquire images. The different samples were observed under the microscope by using a scanning speed of 1 °C/min. Changes in sample morphology were noted as a function of temperature. Data were imported into a computer for real-time observation and storage.

4. Spectroscopic analysis

4.1. Raman micro-spectroscopy

Spectra were recorded by means of a Renishaw Raman Invia interfaced with a Leica DMLM microscope (maximum spatial resolution: 1 μm^2). Experimental details: sources: Laser Ar$^+$ (514.5 nm), Diode Laser (780.0 nm); monochromator: 1800 and 1200 lines/mm; detector: CCD (Charge-Coupled Device) (cooled at 203 K); spectral resolution: 2 cm^{-1}; power on the sample 0.03–3 mW; accumulation time: 20 s; scan number = 4.

4.2. Micro-FT-IR

ATR and near-normal reflection–absorption spectra were recorded by a Nicolet FT-IR Nexus 470 connected to a Nicolet Continuum microscope. Experimental details: source globar (SiC candle); beam splitter m-IR: KBr; detector: MCT (CdTe, doped by Hg); spectral window: 4000–650 cm^{-1}; lateral resolution: 7–80 μm; spectral resolution: 4 cm^{-1}.

4.3. X-ray diffractometric analysis (XRD)

To perform X-ray diffractometric analysis a Philips PW 3719 diffractometer was used, controlled by a computer. Experimental conditions were as follows: Cu Kα radiation ($\lambda = 1.78896$ Å); 40 kV and 30 mA. Scanning interval: 5°–50° 2θ; Time for step: 1 s; Graphite monochromator on the diffracted beam.

4.4. Dissolution study

The final particles were sieved and different fractions were tested for in vitro IMC release using a USP paddle apparatus with 1000 ml of pH 2.0 ± 0.1 buffer solution (artificial gastric juice) as the dissolution medium at 37 °C and at 100 rpm; and with pH 7.2 ± 0.1 buffer (artificial intestinal juice) in similar experimental conditions.

5. Discussion

5.1. IMC/PVP systems

The IMC/PVP system was studied in the form of solid dispersion prepared with the solvent method. The two components were dissolved in ethanol, as a common and safe solvent. Due to the different solubility of the three polymers, the volume of the solvent needed to provide complete PVP dissolution increased in the order 12PF < K30 < K90: complete dissolution of the IMC/PVP system is required to achieve a homogeneous distribution of the drug inside the various size fractions of the co-evaporated material. After evaporation of the solvent, a monophasic solid system was thus obtained, where the drug is dispersed as a solute inside the polymer that acts as a (solid) solvent at molecular level. The possibility of obtaining an amorphous and hydrophilic final material allows the prediction of a high dissolution rate and thus availability of the drug. The presence of PVP, with a high glass transition temperature, increases that of the whole system, since it decreases the phase mobility and reduces the tendency of the drug to crystallize, and this fact is known to guarantee a certain level of stabilization.

The same IMC/PVP association was proposed in a previous paper [3], obtained by freeze-drying an aqueous suspension: the final system consisted of IMC crystals covered by a PVP layer, since the starting system actually contained IMC particles in the dispersing aqueous medium formed by a PVP solution. On sublimation of the ice from the frozen sample, the solute PVP deposited on the surface of the insoluble IMC crystal surface: the result was a surface solid dispersion of PVP onto IMC crystals. In this case a close association between PVP and IMC was obtained where each component retained its physical phase, as in a physical mixture; in this solid dispersion, however, the two components are no longer spatially separated, but are closely contiguous, since the polymer coats the drug crystals as a film. Due to the absence of an amorphous phase of the drug, the dissolution rate could be expected to be lower, but the stability to be decisively higher, than that of the previous system.

5.2. *Effect of the PVP type*

To study the effect of the polymer chemistry on the stability of solid dispersion three types of PVP were chosen to prepare the solid dispersion of different molecular weight grades (K90, K30 and 12PF) and different glass transition temperatures (T_g): PVP K90 and PVP K30 have high T_g values (176 °C and 171.5 °C, respectively, for the dry material), while PVP 12PF has a T_g value around 100 °C [4,5].

In the light of the obtained results, the nature of the polymers seems to have a minor role on the overall behaviour of both systems: this agrees with what was reported [6], that it, is not the PVP type that inhibits the crystallization process of IMC during dissolution but its content in the mixture. Elsewhere it was also reported [7] that the major basis for the crystal inhibition of amorphous IMC in molecular dispersions with PVP is not related to the polymer molecular weight, but rather to the ability of the polymer to form a hydrogen bond with IMC, inhibiting the formation of IMC dimers of the crystalline form [8]. It is evident that, where crystalline IMC is present, PVP plays a role only with its hydrophilicity improving the release of the drug.

5.3. *SEM analysis of co-evaporated particles*

SEM offers images of the particle shape of the different materials employed. PVP, due to the process employed for its polymerization, presents particles as irregularly rounded spheroids which on fracture reveal empty and rich in cracks and fissures, at least for polymers of lower MW, (PVP 12PF and K30) (Fig. 1A and B), while PVP K90 particles are irregularly shaped (Fig. 1C). IMC is formed by plate crystals with smooth borders, but irregularly shaped and this morphology is retained also at low size (Fig. 1D).

The particles of the present study are formed by multiple stratified thin layers stacked on each other, as a result of evaporation of the solvent: this is particularly evident in the case of the sample containing PVP K90 (Fig. 1E). XRD analysis (see below) suggests that co-evaporated microparticles are dominantly amorphous. Co-freeze-dried particles recall the plated IMC morphology: the IMC crystal surface, during the freeze-drying process, is simply coated by PVP film deposited on it (Fig. 1F).

5.4. *Thermal analysis*

The association between the drug and the polymer operates through a hydrogen bond formed between the PVP amide carbonyl and the IMC carboxylic acid hydroxyl, thus disrupting the IMC dimers in the solid state, responsible for the low solubility and dissolution rate of the drug. Occurrence of the interaction, previously examined by FT-IR and Raman spectra [9], was also demonstrated here by thermal analysis. The IMC thermogram shows a unique narrow and symmetric melting endotherm, as a consequence of the crystalline nature of the drug. It was reported [3] that a physical mixture or a solid dispersion displays a common thermal behaviour since their thermograms recall that of pure PVP considered, irrespectively of MW, without any melting endotherm of IMC. This can be explained either by amorphization of the drug during the preparation of the solid dispersion or by its dissolution into the carrier as temperature scans in the case of heterogeneous systems. The PVP thermogram shows only a broad and asymmetric endotherm associated with a weight loss of about 6% in the temperature range 74–110 °C, recorded by TGA: this can be attributed to dehydration of the polymer, since PVP is a hygroscopic material and, likewise many polymers, is reported not to melt. However, HSM reveals an unexpected aspect (Fig. 2), since starting from 100 °C (Fig. 2B and C) vesicles of homogeneous molten material are present on the observation plate. For comparison we examined two other NSAIDs different from IMC. In the presence of an acidic, either lower or higher melting point, drug (e.g., ibuprofen or diclofenac, respectively), either in the physical form of a mixture or as a solid dispersion, droplets of molten materials appear at a lower temperature than that of the melting of the drug. In the case of ibuprofen, melting at 78 °C, PVP particles appear to dissolve into molten ibuprofen, while in the case of diclofenac, vesicles of molten material are again visible at temperatures lower than that of diclofenac (m.p. 179 °C). These findings support the idea that the interaction described for PVP and IMC could actually be general for acidic drugs: the hydrogen bond interaction, observed in the case of PVP systems, inhibits the preferential formation of carboxylic acid dimers, which represent the usual solid state structure for these acidic drugs. The “solution” nature [9] of the amorphous state produced during the preparation of a solid dispersion between PVP and IMC was thus confirmed. The same situation, occurring in the formation of a solid dispersion by the solvent method, can be reached simply by heating the two components together: the presence of melting material

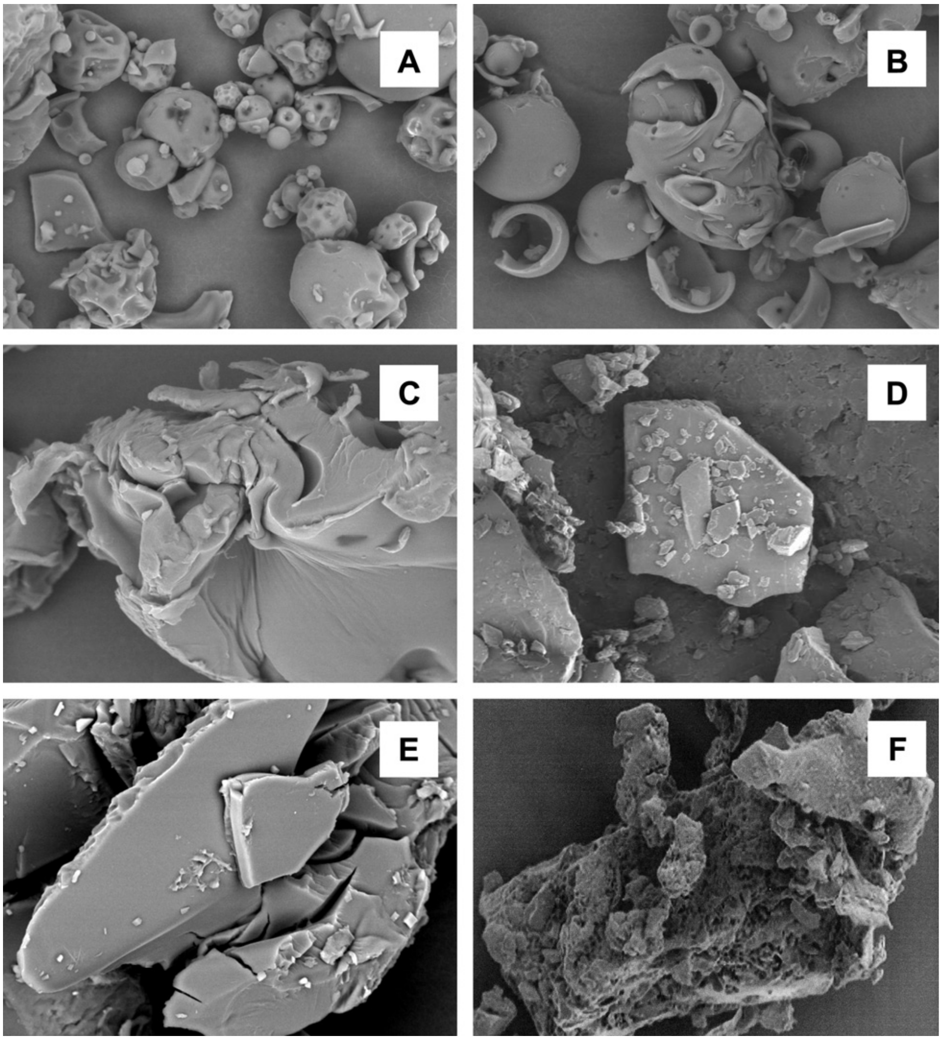

Fig. 1. SEM micrographs of the starting materials used to prepare the co-evaporated system. (A) PVP 12PF; (B) PVP K30; (C) PVP K90; (D) indomethacin; (E) IMC/PVP K90 co-evaporated sample; (F) IMC/PVP K90 co-freeze-dried sample.

(Fig. 2D) supports the occurrence of a strong interaction between PVP and an acidic compound, such as IMC, favouring the depression of the melting point.

5.5. Raman analysis

Vibrational spectroscopy provides an excellent method for probing solid state interactions between molecules, and the use of IR micro-spectroscopy for this purpose is widely documented, while Raman micro-spectroscopy is less frequently employed in the field of pharmaceutical research [10–13]. Moreover, modern equipment provides a very narrow spatial resolution and this aspect can be self-defeating when mixtures are analyzed, since the resulting spectrum depends on the position of the beam; but at the same time, this fact can offer evidence of heterogeneity. In this way, spectroscopy can be employed as a tool to describe a mixture and reveal the situation of the components (e.g., amorphous or crystalline; free or bonded) [5,14].

Fig. 3A shows the Raman spectra of pure gamma-IMC and pure PVP together with that of the co-evaporated system. From the comparison of the three spectra it emerged that the frequencies in the range 1500–1700 cm^{-1} are useful to evaluate changes. The IMC molecule contains two carbonyl groups of the benzoyl and carboxyl moieties. Taylor and Zografi [9] also suggested that the peaks present in this range must be attributed to the asymmetric benzoyl stretching, while the peaks associated with the symmetrical stretching vibrations of the cyclic dimer formed by the carboxyl groups are not active in the Raman spectra. There is a very narrow and symmetric peak at 1700 cm^{-1}: the same authors provided an explanation for the higher frequency with respect to common amide groups. Three other peaks of lower intensity are observed at lower frequencies: 1580, 1590 and 1621 cm^{-1}. This is clearly visible in Fig. 3B where a detailed and magnified portion of the spectrum is shown.

PVP shows only a broad and rounded peak (centred at 1666 cm^{-1}) in this range, attributed to the amide carbonyl of the PVP units. The three types of PVP employed to pre-

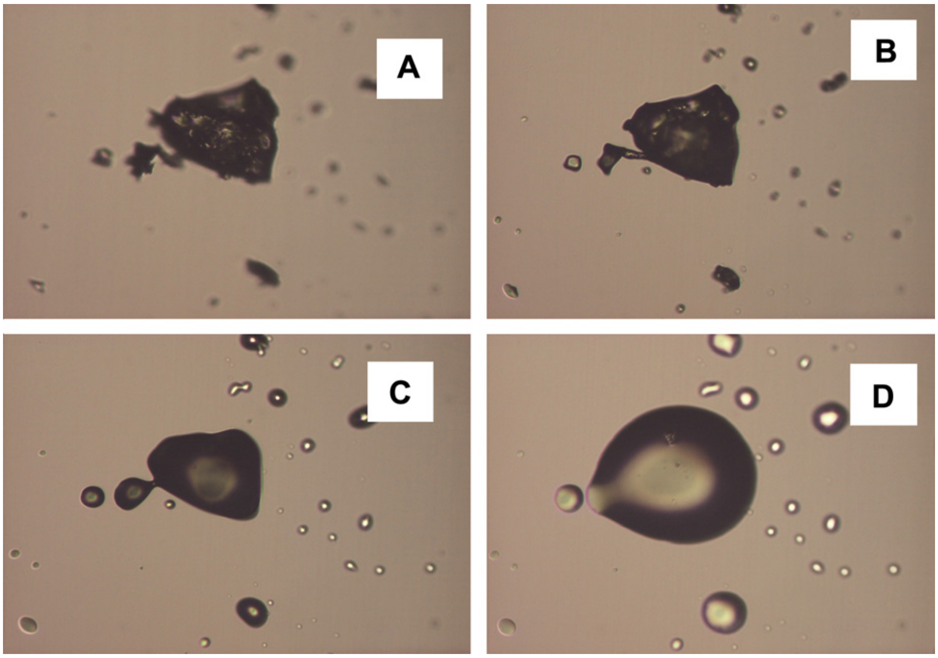

Fig. 2. Hot Stage Micrographs of IMC/PVP K30 co-evaporated particles at different temperatures: (A) 37 °C; (B) 99 °C; (C) 107 °C; (D) 130 °C.

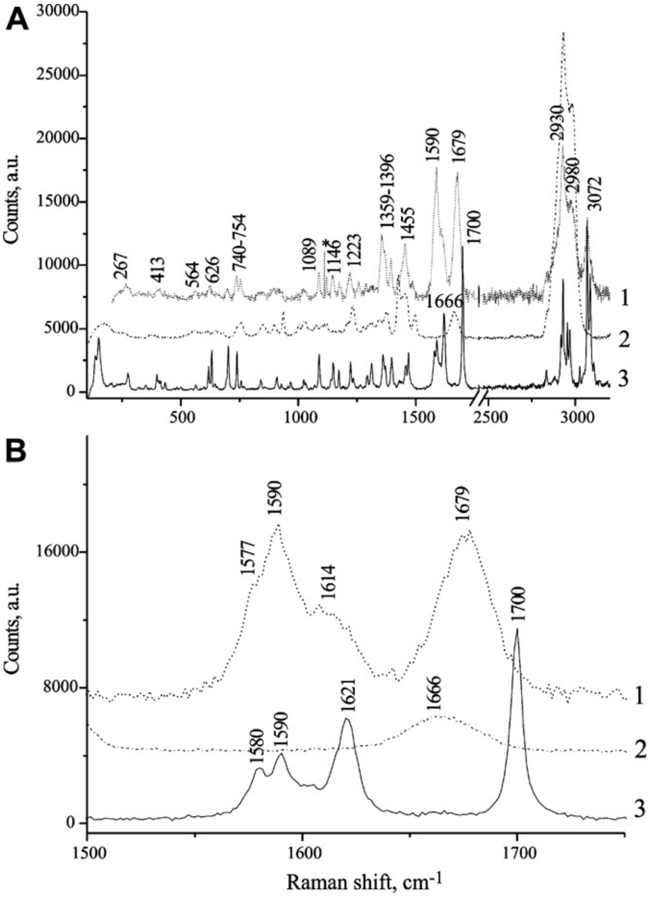

Fig. 3. Raman spectra of co-evaporated samples. (A) – (1) IMC/PVP K30 co-evaporated sample; (2) PVP K30; (3) indomethacin; (B) details of the three spectra of (A) in the range 1500–1800 cm^{-1}.

pare solid dispersions, PVP 12PF, PVP K30, PVP K90, differing in MW, cannot be distinguished by Raman spectra, because the monomer units are identical and have identical backbone, differing only in chain length. It was also reported [9] that the frequency (933 cm^{-1}) can be associated with the ring breathing of the pyrrolidone function. Both peaks appear of low intensity and not useful for the present purposes.

All IMC peaks in the co-evaporated system are red-shifted (1679, 1614, 1590 and 1577 cm^{-1}, respectively) and the Raman spectrum appears simplified, since only two peaks appear well defined and are attributed to the presence of amorphous IMC. This fact outlines the change undergone by IMC when a solid dispersion is prepared by the solvent method and the different frequencies of the two peaks at 1700 cm^{-1} and 1679 cm^{-1} can be useful to distinguish the presence of the solid dispersion of crystalline or amorphous IMC inside the sample.

Since it is well known [5] that the interaction of PVP with water molecules absorbed by the polymer can also be responsible for the shifts observed in Raman spectra, we measured the content of water in the present samples. As a consequence of the preparation of both systems, water is present, but at very low levels (as documented by TGA profiles of both materials) and cannot be considered as the origin of the observed shifts. This fact outlines the changes that occurred to IMC when a solid dispersion was prepared by the solvent method, whereas the origin of these changes can be attributed to the hydrogen bond formed between the amide carbonyl of PVP and the hydroxyl group of the IMC carboxyl, as previously described [9].

In this paper we used these shifts differently. In fact, the different frequencies of the two bands at 1700 cm^{-1} and 1679 cm^{-1} proved useful to distinguish the presence of IMC in the solid dispersion (as amorphous) or of the pure IMC (as crystalline) inside the samples: in other words, sites where starting molecules were found unchanged. A number of spectra in different portions of the samples were registered, paying attention to the presence of these bands: only very few cases were encountered of crystalline IMC, always together with amorphous IMC. This last form represents as a consequence practically the unique one present in the co-evaporated sample.

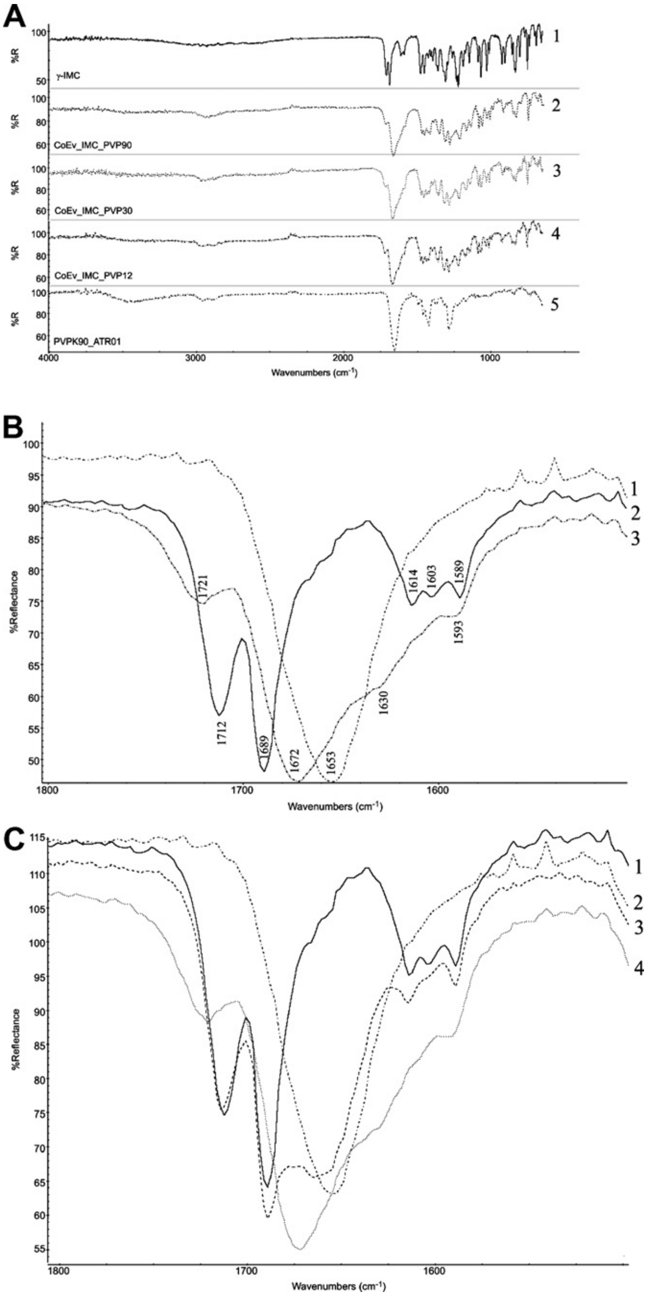

Fig. 4. ATR spectra of (A) – (1) indomethacin; (2) IMC/PVP K90 co-evaporated sample; (3) IMC/PVP K30 co-evaporated sample; (4) IMC/PVP K12 co-evaporated sample; (5) PVP K90. (B) Details of (1) PVP K90; (2) indomethacin; (3) IMC/PVP K90 co-evaporated sample, in the range 1600–1800 cm^{-1}. (C) Details of (1) indomethacin; (2) PVP K90; (3) IMC/PVP K90 co-freeze-dried sample; (4) IMC/PVP K90 co-evaporated sample, in the range 1600–1800 cm^{-1}.

5.6. ATR analysis

Fig. 4A shows the ATR spectra of IMC, PVP K90 and the three co-evaporates samples with the different PVP: the frequency range 1600–1750 cm^{-1} is again useful for comparison. Fig. 4B shows a detail of the spectrum in this wave number range: IMC shows peaks at 1712, 1689, 1614, 1603 and 1589 cm^{-1}; unfortunately also PVP spectrum shows a broad band, peaking at 1653 cm^{-1}, which could interfere. Even in this case a notable shift of IMC frequencies can be appreciated, even though the resulting broad peak at 1672 cm^{-1} in the co-evaporated samples masks possible single peaks. The changes in the carbonyl spectral region are very important for the elucidation of the molecular state of the drug. A marked change in the region of PVP K90 carbonyl can be observed with the appearance of a weak band (a shoulder) at 1630.5 cm^{-1} shifted to lower wave numbers, compared to the carbonyl band of starting PVP at 1653 cm^{-1}: this change was interpreted as due to the carbonyl group of PVP H-bonded with the hydroxy of the carboxyl group of the drug. The carbonyl of the drug embedded into PVP was found at 1721 cm^{-1}, shifted to the high wave number region compared to the corresponding band of pure IMC (at 1712 cm^{-1}). This indicates the breakage of the IMC–IMC interactions, characteristic of the solid form of this drug, after the binding of IMC molecules to PVP chains in the solid dispersion. Interaction of the proton donor groups with the carbonyl oxygen via H-bonding decreases the frequency of the carbonyl vibration mode due to the weakening of the carbonyl bond and stabilizes the amorphous state.

Similar observations of the shifts to the high wave number region compared to the band of the crystalline ibuprofen have been reported for solid dispersions prepared by supercritical fluid or via conventional routes [15–17].

The case of IMC in the sample obtained by freeze-drying (Fig. 4C) is different: it is in fact easy to find points of the sample that give spectra of pure PVP and IMC, suggesting the absence of an interaction between the two components of the solid dispersion. What is evident from Fig. 4B and C is sufficient to distinguish the different states of IMC in the two types of solid dispersion. These results suggest the presence of amorphous IMC in the co-evaporated sample, with the formation of a chain of IMC molecules interacting with PVP through the acid moiety; on the contrary, the presence of unchanged gamma-IMC in the freeze-dried sample was thus confirmed.

5.7. XRD analysis

Solid dispersions are expected to produce highly dispersed amorphous drug/polymer systems: this was clearly demonstrated by X-ray diffractograms (Fig. 5). While IMC shows a diffractogram typical of crystalline material, rich in narrow and symmetric peaks (Fig. 5A), PVP on the contrary is dominantly amorphous in nature (Fig. 5B) and forces the IMC contained in the co-evaporated to also become amorphous (Fig. 5C): these materials exhibit diffused spectra typical of amorphous structures. On the contrary the system previously studied was prepared by freeze-drying a suspension of IMC crystals (insoluble in water) in an aqueous solution of PVP: the final microparticles therefore actually consisted of crystalline IMC covered by PVP multiple layers. Diffractograms of this last system show the patterns of crystalline IMC, even though of reduced intensity due to the composition of the system [3]. The presence of an amorphous phase in the co-evaporated system introduces a source of instability, even though stabilized by the presence of PVP: as a consequence, co-freeze-dried particles, containing the crystalline phase of the drug, can be considered a more stable formulation.

6. Microcapsules

6.1. Spray-congealing

Particles obtained by the two methods of preparing solid dispersion were suspended in molten SA and spray-congealed obtaining a coating with a film of stearic acid. Fig. 5D suggests the microcapsule nature of the particle obtained by spray-congealing process: the external coating is formed by crystalline stearic acid, without any peaks of different substances. The final particles have a spherical shape, with a not perfectly smooth surface (Fig. 6): the presence of IMC complexed by PVP seems to hinder smoothness, since IMC/SA microparticles, obtained at the same weight ratio and experimental conditions, are smoother. Possibly the mutual solubility of SA and IMC in the molten phase forces the solid mixture to a less crystalline structure. In some cases, the SA coating reveals the difficulty to obtain a perfect shape (Fig. 6A and B). Moreover, SA crystallization does not leave the particle surface perfectly smooth (Fig. 6C) like those prepared by a solid dispersion formed by IMC and SA in the absence of PVP: in this last case the mutual solubility of IMC and SA in the molten phase allows the formation of smooth and perfectly shaped microspheres (Fig. 6D).

6.2. Image analysis of microcapsules

Image analysis, carried out by means of SEM, extracts information contained in the microscopic observation by means of digital image processing analysis and presentation of the shape and size parameters of the particles. The following three parameters, which are all normalised

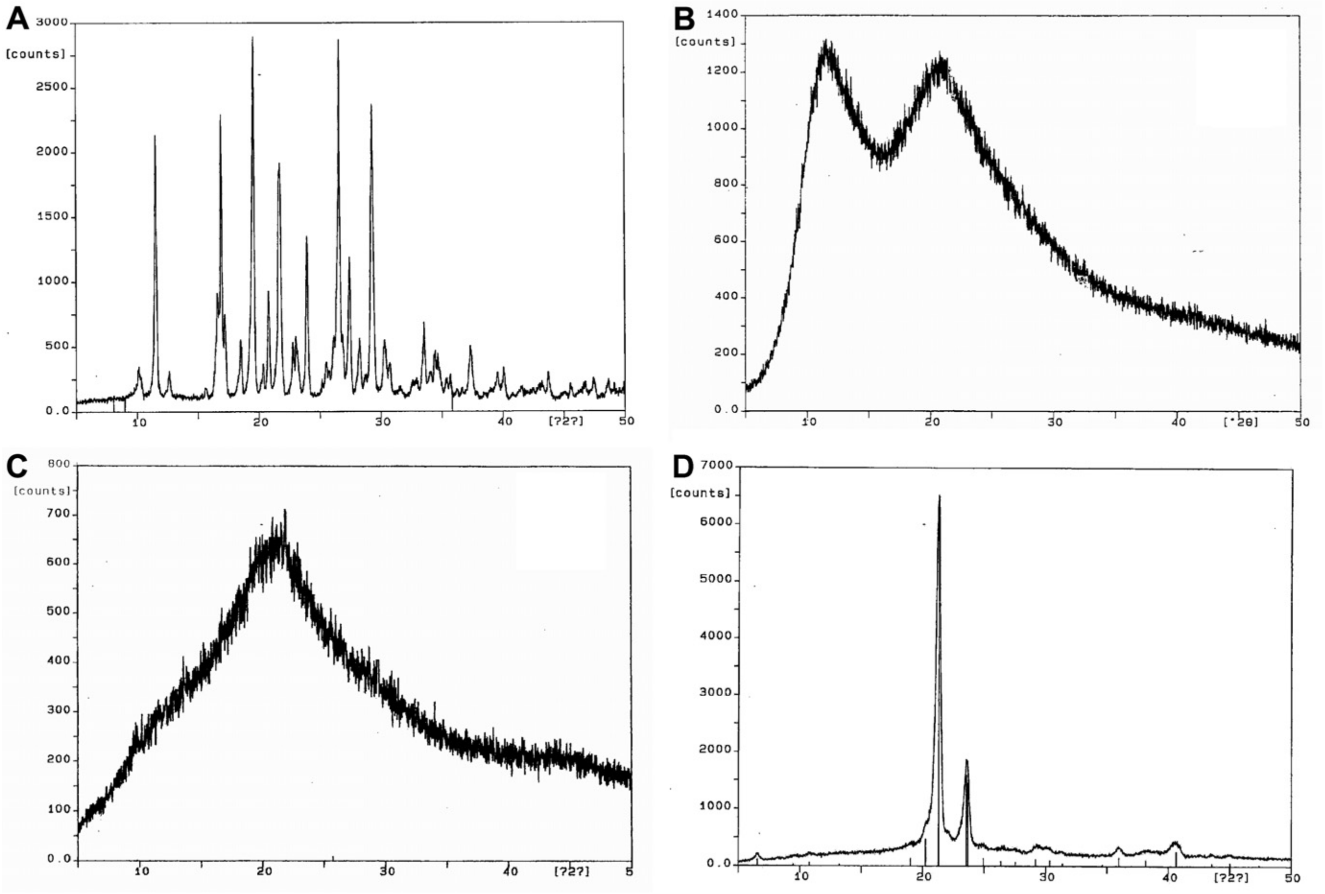

Fig. 5. X-ray diffractograms. (A) Indomethacin; (B) PVP K30; (C) IMC/PVP K30 co-evaporated sample; (D) particle obtained by ultrasound spray congealing of IMC/PVP K30 co-evaporated sample (stearic acid of the surface).

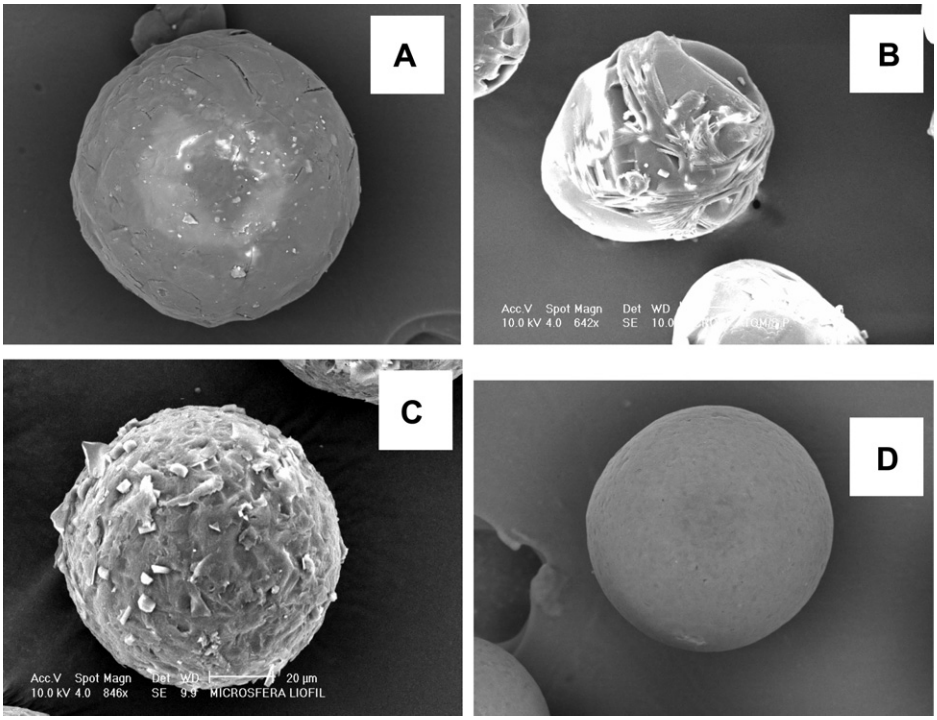

Fig. 6. SEM micrographs of the microparticles obtained by spray-congealing process. (A) IMC/PVP K30 co-evaporated sample; (B) IMC/PVP K30 co-freeze-dried sample (first); (C) IMC/PVP K30 co-freeze-dried sample (second). (D) IMC/SA solid dispersion sample obtained by melting method and then spray-congealed.

(defined to have values lying in the range 0–1) were determined: circularity, elongation, and fractal dimension of the particle contour. Particles of both types, after US spray-congealing process, display a sphericity value higher than 0.90, in accordance with the images shown in Fig. 6. Elongation, which is a measurement of the length/width ratio, is defined as 1 – [width/length]: shapes symmetrical in all axes, such as circles or squares, have similar lengths and widths (elongation close to 0): final particles show very low elongation values indicating a notable symmetry, independently of the size and external shape, in accordance with the sphericity value.

The value of the fractal dimension of the final microparticles was found to be 1.16, low and close to 1 which is the reference value for a regular contour.

Pharmaceutical particles, as obtained by technological processes, are not exactly fractal objects: however, careful examination makes it possible to state that they behave as ‘‘statistical’’ fractal objects, and fractal analysis can be applied to these systems to measure the irregularity of their profile. The microsphere surface appears not perfectly smooth due to the presence of minute flakes or platelets emerging from it (Fig. 6C): this makes the contour that can be appreciated on the projection of the particle of the microscope (and thus the microsphere surface) somewhat irregular, and justifies the slight difference from the reference value (Fig. 6C). However, the almost perfectly spherical shape and the sufficiently smooth surface gave the final microspheres obtained by US spray-congealing optimum technological properties for practical applications.

Coating of the solid dispersion particles with a film of stearic acid, obtained by spray-congealing treatment, can represent a key process for a practical application of the solid dispersion technique. With this method it is not the material deriving from the different methods of preparing solid dispersion that is handled, but spherically shaped particles, with excellent technological properties of hardness, stability and flowability suitable for manufacturing of the final formulation [19].

The process of spray-congealing to obtain microspheres that follows the preparation of both types of solid dispersions also displays important functions in terms of practical applications of solid dispersions, since it overcomes many of the limitations discussed by Serajuddin [1].

First, any possible remaining traces of solvent, employed in the solvent method of preparation of solid dispersion, could be eliminated during the mixing with molten SA; second, the temperature of molten SA is relatively low and does not compromise the thermal stability of the drug too much, which however is complexed with the polymer and somehow, at least partially, stabilized; third, the coating material is not a low-melting mixture and does not display any difficulty in solidifying after the spray-congealing process; fourth, a lipid material protects the core containing the drug from moisture, air and light, possible responsible for the crystallization of the amorphous form [18]; fifth, ultrasound (when present) provides stirring of the mass to be spray-congealed, preventing agglomeration of the suspended material; last, but not least, the final physical form of the spherical microcapsules overcomes problems of preparing the final formulation in terms, e.g., of direct hard gelatine capsule filling.

Finally, the drug solubility and miscibility in melted lipid, which determine the loading capacity of drug in the lipid particles, are less important in the present case, since the lipid material represents only the encapsulation layer external to the preformed IMC/PVP nuclei. Solid lipid particles, composed of a physiological and well-tolerated lipid, combine several advantages: the possibility of controlled drug release and drug targeting; the protection of incorporated active compounds against changes or degradation and possibly of re-crystallization of the amorphous phase including, by contact with moisture and with gastric environment, by enzymes or acidic fluids.

However, an analysis of the final material reveals that there are still some problems. Fig. 7 shows the Raman spectra of materials present on the microsphere surface obtained for both types of materials. Obviously, the most frequently encountered material is SA, whose spectrum is shown in Fig. 7A. The two samples can be distinguished by the presence of IMC peaks that refer either to the crystalline (Fig. 7B) or amorphous structure (Fig. 7C) of IMC, according to the preparation method of the solid dispersion. Both IMC forms could be detected in some instances also in the freeze-dried sample (Fig. 7D), looking at the presence of typical bands of one or of the other type of IMC: it can be hypothesized that IMC particles, not completely covered by PVP during the freeze-drying, could dissolve into the molten SA, originating a limited amount of amorphous portion. IMC particles could be observed at the microcapsule surface as distinct crystals also in the co-evaporated samples. This can be a consequence of the instability of the amorphous phase during the spray-congealing process of the formation of droplets. In fact, solid dispersion in the present study contains IMC and PVP at 50/50 w/w ratio: according to calculated values [9], this ratio corresponds to a 1:2 mole ratio IMC/PVP (monomers), which is exceedingly high and guarantees hydrogen binding of all the IMC molecules present in PVP chains, in other words to ensure complete amorphousness of the system. However, a low glass transition temperature for IMC was reported ($T_g = 42.3$ °C) [20]: in the presence of

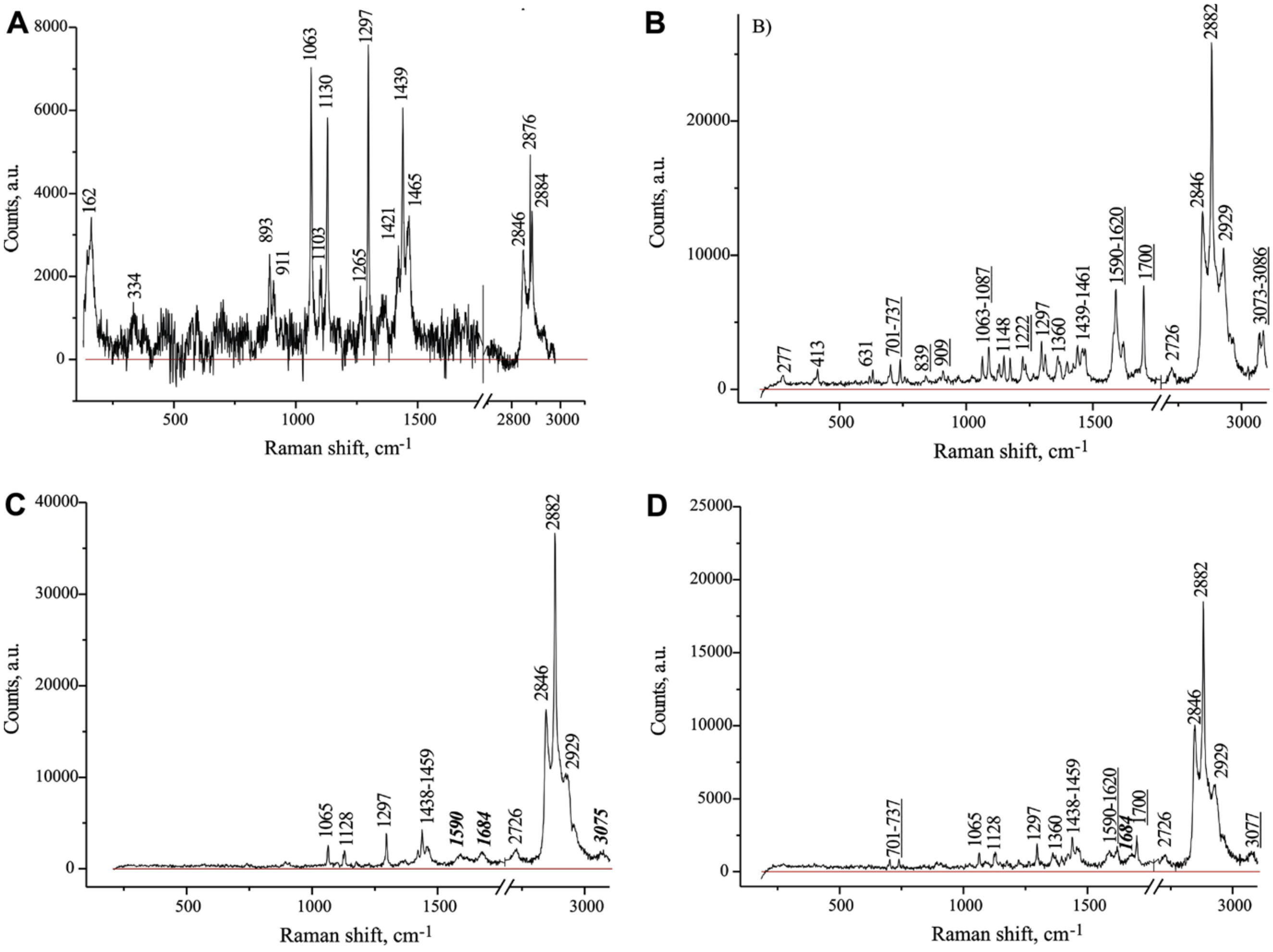

Fig. 7. Raman spectra of microparticles where the following substances were detected: (A) stearic acid; (B) stearic acid and crystalline IMC (underlined peaks); (C) stearic acid and amorphous IMC (italic bold peaks); (D) stearic acid, crystalline IMC (underlined peaks) and amorphous IMC (italic bold peaks).

an IMC/PVP binary mixture this value increases up to 78.1 °C (for PVP K30 and possibly this could be practically the same for other PVP types). As a consequence, this value could easily be overcome during the US atomization process, since the solid dispersion particles are in contact with molten SA to produce final microspheres: at the working temperature for this process (80 °C), stabilization of the amorphous state can no longer be assured, even by the presence of PVP. This can account for the presence of crystalline IMC on the surface of the final microcapsules, after the coating with a film of stearic acid.

These facts again outline the potential instability of the amorphous phase and the great importance of Raman and FT-IR micro-analysis in exploring even small particles to gather information on the structure of the sample and the possible interactions existing among the components of a given formulation. It is worth noting that the results of the Raman analysis of the sample surface have statistical value since only a limited number of points can be examined.

6.3. Dissolution test

Fig. 8 shows the release profiles of the drug from microspheres.

Beside inhibiting the contact with water, SA coating offers protection against the gastric pH: dissolution at low pH was observed to a very low extent (lower than 5% after 2 h, with the exception of the smallest size fraction considered) (Fig. 8A). Differences appear marked only when the first moments of the dissolution are considered or when pH increased up to that of the intestinal environment: in this last case, release is complete in few minutes (Fig. 8B). Behaviour as a function of time is more important: aging appears not to have any influence on the release and the dissolution profiles readily overlap after 1 week and after 9 months (Fig. 8B). These facts show the importance of the protection by stearic acid that stabilizes the amorphous phase formed by the solvent evaporation method against the absorption of water as plasticizer.

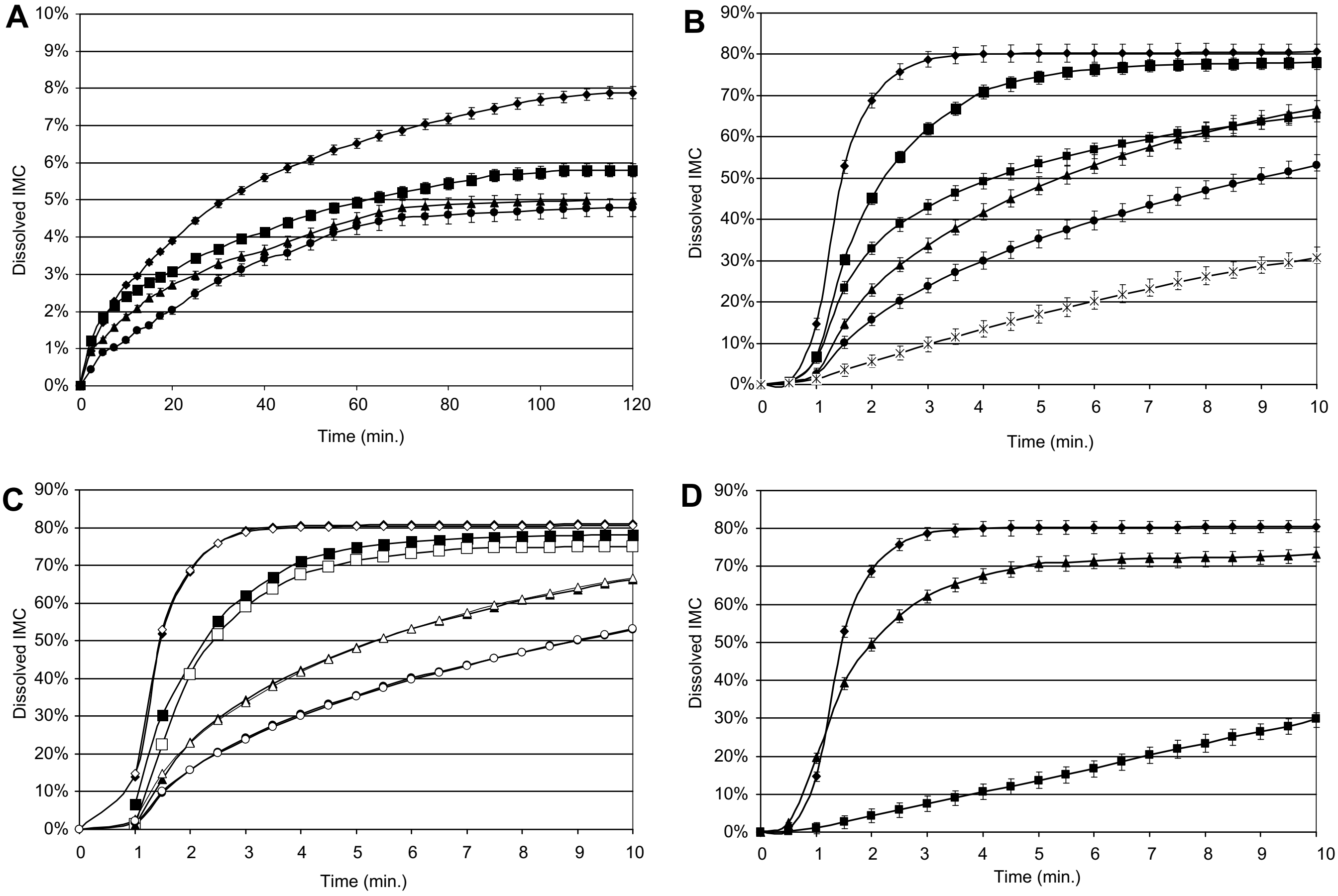

Fig. 8. Dissolution profiles. (A) Microparticles obtained by spray-congealing a suspension of IMC/PVP K30 particles with molten stearic acid: effect of the particle size at pH 2.0. ♦: $50 < x < 100$ μm; ■: $100 < x < 200$ μm; ▲: $200 < x < 355$ μm; ●: $355 < x < 500$ μm. (B) Microparticles obtained by spray-congealing a suspension of IMC/PVP K30 particles with molten stearic acid: effect of the particle size at pH 7.4 ♦: $50 < x < 100$ μm; ■: $100 < x < 200$ μm; ▲: $200 < x < 355$ μm; ●: $355 < x < 500$ μm; ■: unsieved; ×: pure indomethacin <200 μm. (C) Microparticles obtained by spray-congealing a suspension of IMC/PVP K30 particles with molten stearic acid: effect of the particle size at pH 7.4 and of aging: after a week (black symbols) and after 9 months (open symbols) ♦ ◇: $50 < x < 100$ μm; ■ □: $100 < x < 200$ μm; ▲ △: $200 < x < 355$ μm; ● ○: $355 < x < 500$ μm. (D) Comparison of IMC dissolution profiles between microparticles ($100 < x < 200$ μm) obtained by spray-congealing a suspension of IMC/PVP K30 particles with molten stearic acid at pH 7.4 ♦: co-freeze-dried sample; ▲: co-evaporated sample; ■: pure IMC $100 < x < 200$ μm (for comparison).

Finally, the comparison between the release profiles of both systems reveals practically no difference and this suggests that the two methods of association between IMC and PVP behave in the same way to improve the release of the drug and that the choice between the two methods must be made taking into account the parameters other than release, such as stability of the final system and easiness of the process (Fig. 8C).

7. Conclusions

Although solid dispersions offer a significant improvement in drug release with respect to a neat amorphous state, there is still a potential risk of crystallization of these systems, when an amorphous phase is formed. As an alternative, the solid dispersion obtained by co-freeze-drying allows IMC to be associated with the hydrophilic PVP, though maintaining its crystalline phase and avoiding any risk of changes on aging. Despite this, the formulation greatly improves the availability of the anti-inflammatory drug through rapid dissolution. This process can be proposed as a first and general choice for other anti-inflammatory drugs that, like IMC, are poorly soluble in water: the process is quite simple and suitable for practical applications, since it does not require organic solvents, heating or complex apparatus, but only a water-soluble polymer. Finally, particles of the solid dispersion, protected by a stearic acid coating, are stabilized against possible degradation caused by the external environment. This process can also be suggested as a general way of stabilizing an amorphous phase that could be protected against the recovery of the crystalline state, maintaining the advantages of amorphization obtained with both solvent and melting methods to prepare solid dispersions.

Acknowledgement

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