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Influence of relative humidity on the interaction between different aryl propionic acid derivatives and poly(vinylpyrrolydone) K30: Evaluation of the effect on drug bioavailability

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ABSTRACT

The present work assessed the physical interactions between several aryl propionic acid derivatives and polyvinyl(pyrrolidone) K30 (PVP), stored together at 298 ± 0.5 K at different relative humidities (RH 55, 75 and 86%). Results were compared to those obtained at low RH (22%), published in a previous paper. The water uptake percentage of binary mixtures were intermediate between that of pure PVP and pure drugs. By X-ray powder diffraction, for all the drugs, it was possible to note a marked decrease in crystallinity degree, in particular at highest RH%. The loss in crystallinity degree may be considered an evidence of the physicochemical interaction between the polymer and the drug, supporting the formation of a solid dispersion. By high-resolution ^{1}H solid-state NMR spectrometry, it was possible to observe an increase of drug–polymer interaction with aging, with the only exception of ibuprofen. Molecular docking proved the establishment of Van der Waals and electrostatic interactions for all the mixtures, and for mixtures with fenbufen and naproxen, also hydrogen bonds. The application of Gordon–Taylor rule to the thermal analysis revealed that the requirement of volume additivity of this rule was not fulfilled for any mixture, and a negative deviation from theoretical behaviour was always observed. The hydration of drug–PVP mixtures had important repercussion on drug solubility and intrinsic dissolution rate (IDR). In general, an increase in water solubility and consequently an increase in IDR were observed, with few exceptions, at highest RH%.

1. Introduction

Solid dispersion of a poorly soluble drug in a polymeric matrix has been extensively considered as an effective method to improve drug solubility and/or dissolution rate (Law et al., 2001; Urbanetz and Lippold, 2005; Leuner and Dressman, 2000). A solid dispersion is "the dispersion of one or more active ingredients in an inert carrier or matrix at solid state prepared by melting (fusion), solvent, or melting-solvent method" (Chiou and Riegelman, 1971).

The second generation of solid dispersions (Vasconcelos et al., 2007) consists in the dispersion of the insoluble drug in a hydrophilic polymer matrix (Chiou and Riegelman, 1971; Serajuddin, 1999).

The dispersion of a drug in a polymeric matrix may achieve a dual purpose, on the one hand improving drug solubility and/or dissolution rate, and on the other, preventing crystallization of drugs obtained in their amorphous state (Khougaz and Clas, 2000; Yoshioka et al., 1995; Simonelli et al., 1970; Sekikawa et al., 1978).

Water has been widely considered the main cause of the crystallization of amorphous solid dispersions because, in acting as plasticizer, it decreases the glass transition temperature of the system, improving molecule mobility (at temperatures near to room temperature) and thus favouring crystallization (Andronis et al., 1997; Schmitt et al., 1996; Shamblin and Zografi, 1999; Oksanen and Zografi, 2000).

Water may also affect other important properties of solid dispersions such as dissolution. Jørgensen and Torstenson (2008) reported an increase in diazepam dissolution rate from solid dispersions with PEG 3000 after storage at high RH% (60–75%). It was supposed that the carrier absorbs water during storage, decreasing the energy required to break up the intermolecular bonding of PEG, with a consequent increase in drug dissolution rate. Fitzpatrick et al. (2002) observed a change in PVP dissolution profile, consequent to the depression of the glass transition temperature of PVP favoured

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by the important moisture uptake observed in accelerated stability studies. Actually, exposure to elevated temperature and humidity resulted in a change in PVP from the glassy to the rubbery state.

The aryl propionic acid derivatives (APAD), belonging to the nonsteroidal anti-inflammatory drugs (NSAIDs), are "Class II" drugs in the biopharmaceutical classification system (Löbenberg and Amidon, 2000), since they posses high membrane permeability, but low water solubility, limiting their oral bioavalaibility.

In a previous paper, in spite of the great chemical similarity of ibuprofen, ketoprofen, naproxen, flurbiprofen and fenbufen, Gashi et al. (2009) demonstrated that only ibuprofen was able to spontaneously disperse in the PVP K30 when exposed to low relative humidity (22%), by forming partially amorphous mixtures. Ibuprofen's small molar volume, its high molecule flexibility and its weak crystalline structure may help in explaining the results and confirming the plasticizing effect of this drug when mixed with PVP.

The present study sought to demonstrate that APAD drugs can spontaneously interact with the amorphous polymer in the presence of water. Therefore, this study had a double objective:

a) Show the influence of water in favouring the molecular dispersion of the aryl propionic derivative drugs into PVP K30 and explain the reasons for it at the molecular level.
b) Evaluate the effect of drug molecular dispersion through the polymer on the solubility and the intrinsic dissolution rate of drugs.

For these purposes, drugs were mixed with the polymer in the ratio 50:50 (w:w) and the variation of the crystallinity degree of physical mixtures stored at 298 K at 86% RH was evaluated, and compared with data from storage at lower RHs (22, 55 and 75%).

2. Experimental

2.1. Materials

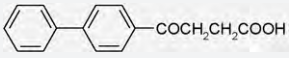
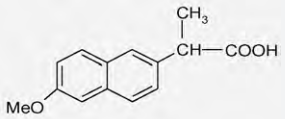
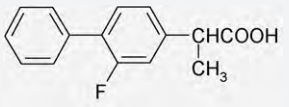
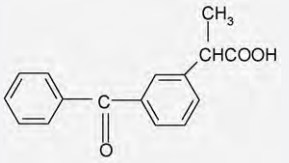
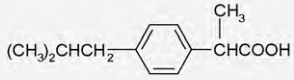
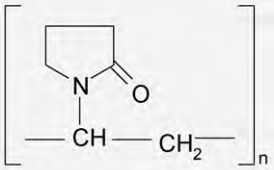
Fenbufen (FEN), naproxen (NAP), ibuprofen (IBP) (ACRAF, Ancona, Italy), flurbiprofen (FLU) (BASF, Aktiengesellschaft, Germany), ketoprofen (KET) (Rhône-Poulenc Rorer, Origgio, Italy), and PVP K30 (Plasdone K-30, BASF, Ludwigshafen, Germany) were used for this study. The molecular weights and structures of drugs and polymer are reported in Table 1. In order to avoid the influence of particle dimensions on particle–particle interaction and thus on particle reactivity, the same sieve fraction for all the drugs was selected. To this end, drug powders were sieved and the 50–100 μm sieve fraction was recovered and then conditioned at different relative humidities for the entire experiment length. Powders were also conditioned at the RH of 22% for comparison with the previous study (Gashi et al., 2009) and for the solubility and intrinsic dissolution experiments. In order to generate appropriate relative humidity percentage, several substances were used: potassium acetate, magnesium nitrate, sodium chloride, potassium chloride, potassium carbonate, and fructose (ACEF, Fiorenzuola D'Arda, Italy).

Drug powder densities were measured using a helium pychnometer (Accupyc 1330, Micromeritics, Norcross, GA, USA) equipped with a cell of 10 cm^3. Powders were first dried in a desiccator in the presence of P_2O_5 to remove water and promote the filling of void spaces by the helium.

2.2. Preparation of physical mixtures and storage

Physical mixtures (PMs) were prepared by gently mixing the drug with the polymer in appropriate amounts in a mortar and then

Table 1
Physicochemical properties of aryl propionic acid derivative drugs and PVP K30.

Substance	Molecular structure	Molecular weight (Dalton)	Powder density (g cm^{-3})	Glass transition T_g (K)
Fenbufen		254.29	1.2955 ± 0.0006	329.64[a]
Naproxen		230.26	1.2688 ± 0.0006	307.70[a]
Flurbiprofen		244.27	1.2961 ± 0.0011	269.12 ± 1.30
Ketoprofen		254.29	1.2888 ± 0.0007	271.08 ± 1.27
Ibuprofen		206.27	1.1206 ± 0.0003	250.18 ± 1.05
PVP K30		50,000.00	1.2263 ± 0.0002	450.09 ± 2.73

T_{onset}: onset melting temperature.
[a] T_g theoretical values.

in a V-shaped mixer for 10 min until homogeneity was obtained. The drug uniformity content of the mixtures was checked by UV-spectrophotometry at appropriate wavelengths, namely, 261, 264, 247, 284, and 230 nm, respectively, for KET, IBU, FLU, FEN, and NAP (Cary 1E UV–VIS, Varian, Palo Alto, CA, USA).

Pure crystalline drugs and PMs were placed in several boxes that were then set in an incubator (Velp Scientifica, FTC 90E, Usmate, Italy) and stored at 298 ± 0.5 K. In each box, different relative humidities (22, 55, 75 and 86%) were generated in presence of supersaturated salt solutions of potassium acetate, magnesium nitrate, sodium chloride, and potassium chloride, respectively, which generated controlled water vapour pressure. The experimental RH% was continuously checked using a thermo-hygrometer (Universal Enterprise Inc., Milano, Italy).

2.3. Water uptake experiments

Approximately 100 mg of powders were carefully weighed and stored at 298 K in dessicators containing saturated inorganic salt solutions giving various relative humidity percentages. The salts used were potassium acetate (22% RH), potassium carbonate (43% RH), magnesium nitrate (55% RH), fructose (64% RH), sodium chloride (75% RH), and potassium chloride (86% RH). Water uptake of powders stored in these conditions was followed by a discontinuous procedure, i.e. taking and weighing samples at regular intervals until equilibrium weight was reached (Kontny and Zografi, 1995). The water content of the powders was determined considering the initial water content and the change in weight induced by storing the samples under each relative humidity condition. The initial water content of the powders was determined by drying them at 120 °C for 5 h. Assays were carried out in triplicate.

2.4. Simultaneous thermal analysis (STA)

Simultaneous thermal analysis (STA) makes it possible to simultaneously analyse a sample for changes in weight (thermogravimetric analysis, TGA) and enthalpy flow (differential scanning calorimetry, DSC). In this text, the acronyms TGA–STA and DSC–STA will be used to refer to TGA and DSC obtained from STA. The analysis was performed with a simultaneous thermal analyser (STA 6000, Perkin Elmer, Inc., Waltham, MA, USA), under nitrogen atmosphere (20 ml/min) in 0.07 ml open aluminium oxide pans. The apparatus was calibrated for temperature and heat flow with three metal standards (tin, indium and zinc), taking into account their expected melting temperatures (505.08, 429.75, and 692.68 K, respectively), and also calibrated for weight with an external Perkin Elmer standard (calibration reference weight P/N N520-0042, material lot 91,101 GB, weight 55.98 mg, 01/23/08 VT). Calibration was repeatedly checked to assure deviation ≤ ±0.3 K. A scanning analysis was performed and approximately 10 mg of the samples were tested in quadruplicate by heating from 293 K to a temperature 10 K higher than drug melting temperature at a heating rate of 5 K min^{-1}.

2.5. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) analysis was also performed on a Pyris 1 (Perkin Elmer, Co. Norwalk, CT, USA) equipped with a cooling device (Intracooler 2P, Cooling Accessory, Perkin Elmer, Co. Norwalk, CT, USA). A purge of dry nitrogen gas (20 ml/min) was used for all runs. DSC was calibrated for temperature and heat flow using a pure sample of indium and zinc standards, respecting the same criteria previously described for STA. Sample mass was about 3–4 mg and aluminium perforated pans were used. Each run was performed in triplicate from 233 K to a temperature 10 K higher than the drug melting temperature at the heating rate of 10 K/min. To avoid confusion with DSC–STA results, this technique will be identified as conventional DSC.

2.6. X-ray powder diffraction (XRPD)

X-ray powder diffraction (XRPD) was carried out on pure drugs without grinding in order to prevent a change in the crystallinity degree. PMs were then analysed immediately after mixing to characterize their physical state and stability; analyses were repeated at regular intervals up to 24th months. A Philips PW 1730 (Philips Electronic Instruments Corp., Mahwan, NJ, USA) was used as X-ray generator for Cu Kα radiation (λ = 1.54178 Å). The experimental X-ray powder patterns were recorded on a Philips PH 8203. A Philips PW 1373 was used as the goniometer and a Philips PW 1390 for the channel control. Data were collected in the scan mode using a step size of 0.01° (2θ). The scanned range was 2–35° (2θ). The crystallinity degree of the mixtures was determined according to Gashi et al. (2009). Briefly, the crystallinity degree was evaluated by XRPD and calculated according to a calibration curve determined from physical mixtures of known crystallinity degree: pure drugs were considered as completely crystalline (100% crystalline) and pure polymer as completely amorphous (100% amorphous). The calibration curve was determined, in presence of an internal standard, by calculating the total area (A_{tot}) of the diffraction patterns (crystalline + amorphous) and the area (A_{cr}) of the crystalline part (the area over the peak baseline). The powder crystallinity degree was expressed according to the following Eq. (1):

$$\text{Crystallinity (\%)} = \frac{A_{cr}}{A_{tot}} \times 100 \quad (1)$$

Crystallinity degree represents the average value of three different measurements. The statistical significance was evaluated by a one-way ANOVA test for $\alpha = 0.05$.

2.7. High-resolution ^{1}H solid-state NMR spectrometry

^{1}H MAS spectra of the different samples were run on a Bruker Avance II instrument operating at 400.23 MHz for the ^{1}H nucleus. For all samples the magic angle was carefully adjusted from the ^{79}Br spectrum of KBr by minimising the linewidth of the spinning sideband satellite transitions. ^{1}H MAS spectra were run in a 2.5 mm probe at 32 kHz with the DEPTH sequence, in order to minimize the ^{1}H signal background, using a ^{1}H 90 degree pulse of 1.85 μs. 16 transitions were acquired with a relaxation delay of 10 s. ^{1}H CRAMPS spectra were acquired using a windowed-PMLG pulse sequence of dipolar decoupling at the spinning speed of 13 kHz, a relaxation delay of 10 s and a ^{1}H 90 degree pulse of 1.90 μs. The ^{1}H chemical shift scales were referenced to TMS at 0 ppm via secondary external adamantane (^{1}H signal at 1.63 ppm).

2.8. Molecular docking

Molecular docking was executed on a Silicon Graphics O_2 platform using the Builder, Docking, Discover and Ludi module of InsightII software (release 2005, Accelrys Ltd., Cambridge, UK).

Each drug molecule was built using the InsightII Builder module and minimized using the Discover module. The PVP was constructed by repeating ($n = 30$) the monomer-minimized structure. The raw linear polymer structure was used to generate a library of conformers, obtained with a given variation (30°) of each rotatable bond. All the conformers were submitted (using the Discover module) to at least 8 cycles of minimizations-dynamics (using consistent valence force field), conjugate gradients method to an RMS derivative of 0.001 kcal/mol, and $T = 350$ K for the dynamics (Dauber-Osguthorpe et al., 1988) until no further decrease in term of total energy was found. The conformer at the lowest energy was then chosen, and was used for the docking with each drug structure.

The docking and consequent energy minimization were carried out adding water molecules up to 86% relative humidity (taking into

account water experimentally absorbed at this RH%) and placing the drug at randomly chosen positions at 10 angstroms distance from the oxygen atom of the monomer ($n = 14$), using the Docking module and the same conditions described above. All molecules were treated as flexible during the docking.

All the results were obtained in terms of intermolecular energies ($E_{Total} = E_{Elect} + E_{VdW}$, where E_{VdW} and E_{Elect} are Van der Waals and electrostatic energy contributions, respectively) and predicted equilibrium dissociation constants derived from LUDI Score, using a standard equation (LUDI Score $= -100 \times \log_{10} K_d$) and Energy Estimate 2 as scoring function (Bohm, 1998). The output from InsightII and all modelling studies as well as images were rendered with PyMOL (2006, DeLano Scientific LLC, San Carlos, CA). PyMOL was also used to calculate the distances of hydrogen bonds as measured between the hydrogen and its assumed binding partner.

2.9. Equilibrium solubility

An excess of drug powder (50–100 μm sieve fraction) was added to 50 ml of ultrapure water (Gradient Milli-Q®, Millipore, Molsheim, France) and maintained at 298 ± 0.5 K under continuous stirring. Equilibrium was tested and judged to have been reached when three successive measurements differed by no more than 1%, generally after 24 h. After equilibrium was reached, aliquots were drawn at time intervals, filtered with a regenerated cellulose filter syringe of 0.45 mm pore size (Filalbet, Rossello, Barcelona, Spain) and, after an appropriate dilution, the concentration of the filtrate was determined by UV spectrophotometry (Cary 1E UV–VIS, Varian, Leinì, Italy). Assays were performed in triplicate.

2.10. Intrinsic dissolution study (IDR)

The dissolution study was carried out by the rotating disk method (Banakar, 1992). 13 mm diameter tablets were obtained by compressing 300 mg of powder in a Perkin Elmer hydraulic press, at a force of 15 kN/cm for 10 min. This yielded tablets with a surface area of 132.73 mm that would not disintegrate during the test. Tablets were inserted into a stainless steel holder, so that only one face was exposed to the dissolution medium. The holder was then connected to a stirring motor, centrally immersed in a 1000-ml beaker containing 900 ml of ultrapure water (Gradient Milli-Q®, Millipore, Molsheim, France) at 37 °C and rotated at 50 rpm. Suitable aliquots were withdrawn with a regenerated cellulose filter syringe at specified times and assayed spectrophotometrically for drug content at appropriate wavelength. A correction was calculated for cumulative dilution caused by replacement of the sample with an equal volume of original medium. Each test was repeated six times. Low standard deviations were obtained, indicating the good reproducibility of this technique. The intrinsic dissolution rates (IDR) were calculated from the slope of the straight line of cumulative drug release.

3. Results and discussion

3.1. Water uptake and water content

In Fig. 1a, the water uptake of the polymer and that of the drug–PVP 50:50 (w:w) physical mixes exposed at different RH% at 298 K are indicated. Pure PVP exhibited the most significant water uptake because of its hydrophilic properties and amorphous nature (Hamaura and Newton, 1999). Amorphous materials such as PVP showed high hygroscopicity, since water can be adsorbed not only at the surface but also into their internal structure (Zografi, 1988; Ahlneck and Zografi, 1990; Crowley and Zografi, 2002). Pure crystalline drugs were not indicated in the graph because the water uptake was less than 1% (w/w) for any substance, confirming the hydrophobic character of this class II drugs. As it would be confirmed by XRPD, drug exposure to different RH% did not promote change in the crystalline state, not even at the highest RH (86%), indicating that water does not favour loss of crystallinity or formation of hydrates on the drugs under study. The water uptake of physical mixtures was intermediate between that of the pure polymer and that of pure drugs, increasing with increased relative humidity %. Differences were negligible till a RH% of 64%. Up to RH levels higher than 64%, the highest water uptake was observed for KET–PVP and IBU–PVP physical mixtures, while the lowest was noted for FLU–PVP and FEN–PVP physical mixtures. These results may be undoubtedly correlated with the wettability of these drugs. Anyway, their water solubility may influence this behaviour. This aspect will be evaluated together to the analysis of solubility results.

Fig. 1b expresses the weight loss % determined by TGA–STA of the physical mixtures at different ratios of drug–PVP versus drug amount. The weight loss % decreased for any drug–polymer mixture in relation to increases in drug amount, confirming that the polymer absorbs water better than the crystalline drug.

3.2. X-ray powder diffraction

PMs were analysed by XRPD immediately after mixing, and again after exposure at an RH of 86% for 3 months. In order to compare the effect of exposure to high RH% with that of exposure to lower RH%, PMs were also exposed at RH of 55 and 75%. In Fig. 2, the XRPD patterns of PMs stored for 3 months at different RH% are reported and compared to those of PMs at time 0 (immediately after mixing) and those of pure substances (PVP and the corresponding drug). In Fig. 3, one can follow the change in drug crystallinity degree with time of drug–PVP physical mixes 50–50 during storage at 298 K at different RH%.

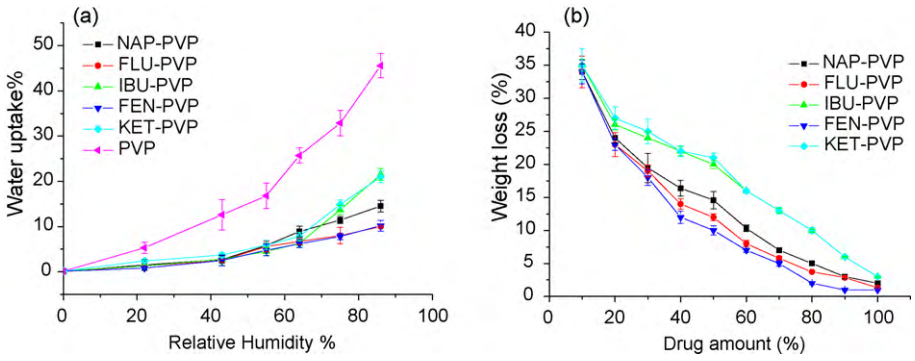


Fig. 1. (a) Water uptake of drug–PVP 50:50 (w:w) physical mixes at 298 K expressed versus RH%. (b) Weight loss % expressed versus drug amount of drug–PVP physical mixes stored at 298 K at 86% RH up to the equilibrium and determined by TGA–STA.

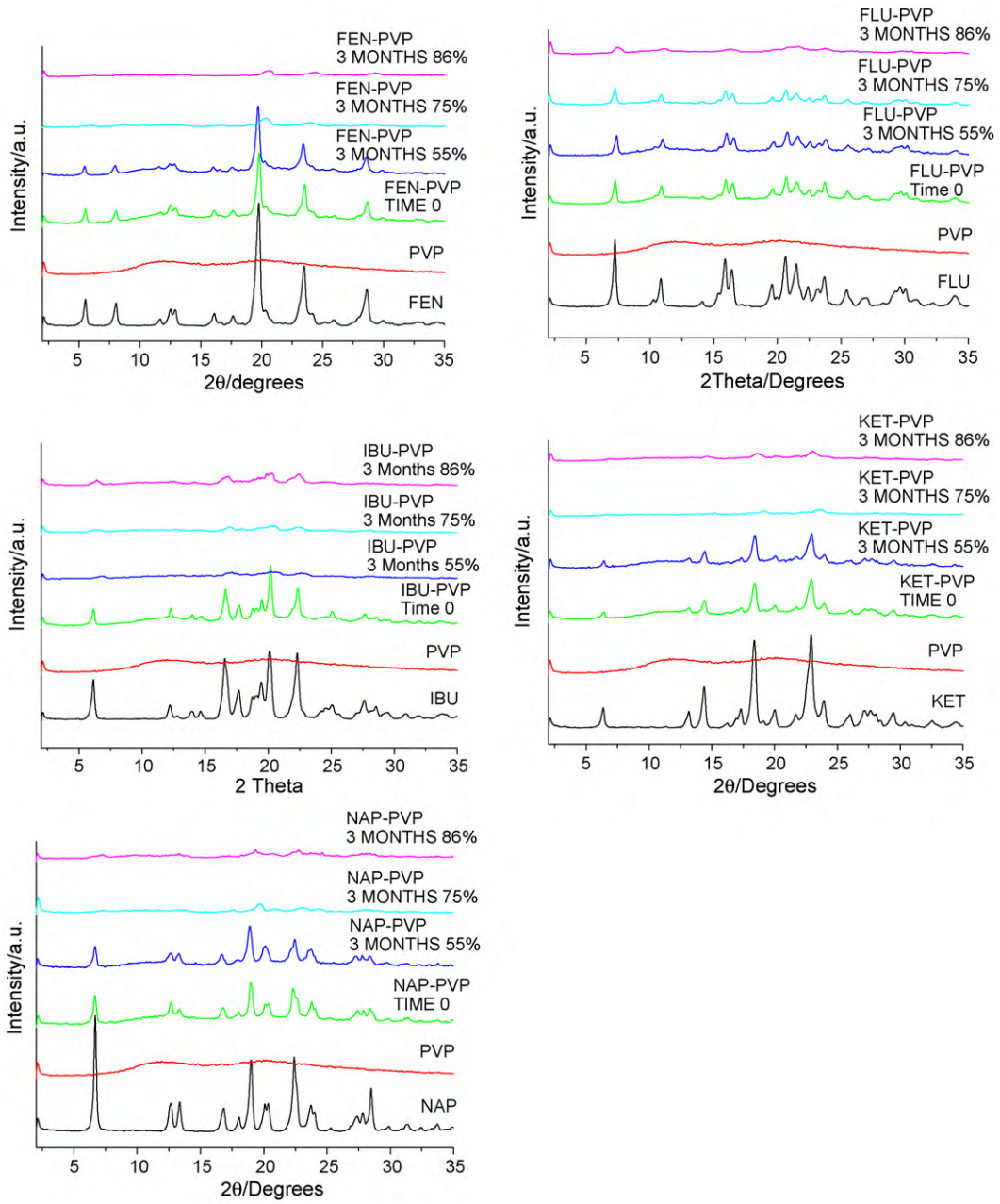


Fig. 2. XRPD of drug–PVP physical mixtures (50:50, w:w) (FEN–PVP; FLU–PVP; IBU–PVP; KET–PVP; NAP–PVP) exposed at different RH (55, 75, 86%). Spectra are compared to those of drug–PVP physical mixtures immediately after mixing and to those of pure compounds (drug and PVP).

In this same Fig. 3, results obtained at 22% and presented in a previous paper are also given for comparison (Gashi et al., 2009). For all the drugs, it is possible to note a marked decrease in crystallinity degree for all the substances, in particular those at the highest RH%. At the RH of 55%, the loss in crystallinity degree was still poor and only in the case of IBU was an appreciable decrease in crystallinity degree evident. At the RH of 75%, there was a relevant decrease in the crystallinity degree for all drugs. The only exception was FLU, which still exhibited 45.35 ± 1.23 of crystallinity degree after 3 months at an RH of 75%. The crystallinity degree was low for all the binary mixes at the highest RH under study (86%), with the exception of IBU that shows a characteristic behaviour: the crystallinity degree significantly decreases during the first 2 months, but after this period of time it seems that a slight crystallization of the drug occurs, so that after 3 months the crystallinity degree is higher than that of mixtures stored at RH of 55 and 75%. This behaviour may be related to the ability of water to favour the crystallization of amorphous mixtures as also explained in Section 1 (Andronis et al., 1997; Schmitt et al., 1996; Shamblin and Zografi, 1999; Oksanen and Zografi, 2000).

The loss in crystallinity degree has been previously considered evidence of a physicochemical interaction between the polymer and the drug that may support the formation of a solid dispersion (Gashi et al., 2009; Bogdanova et al., 2005). For example, Sekizaki et al. (1995) explained that PVP and IBU yield a solid dispersion because a hydrogen bond forms between the drug and the polymer. A frequent explanation offered for the formation of solid dispersions has been that the drug and the polymer establish physical interactions, demonstrated by more or less weak interactions, in particular through hydrogen bond formation. When the polymer is amorphous, the interaction with the drug frequently leads to amorphization of the binary mixture. However, we would argue that amorphization of a binary mix involves a more complex scenario, as outlined in the following paragraphs, and cannot be simply

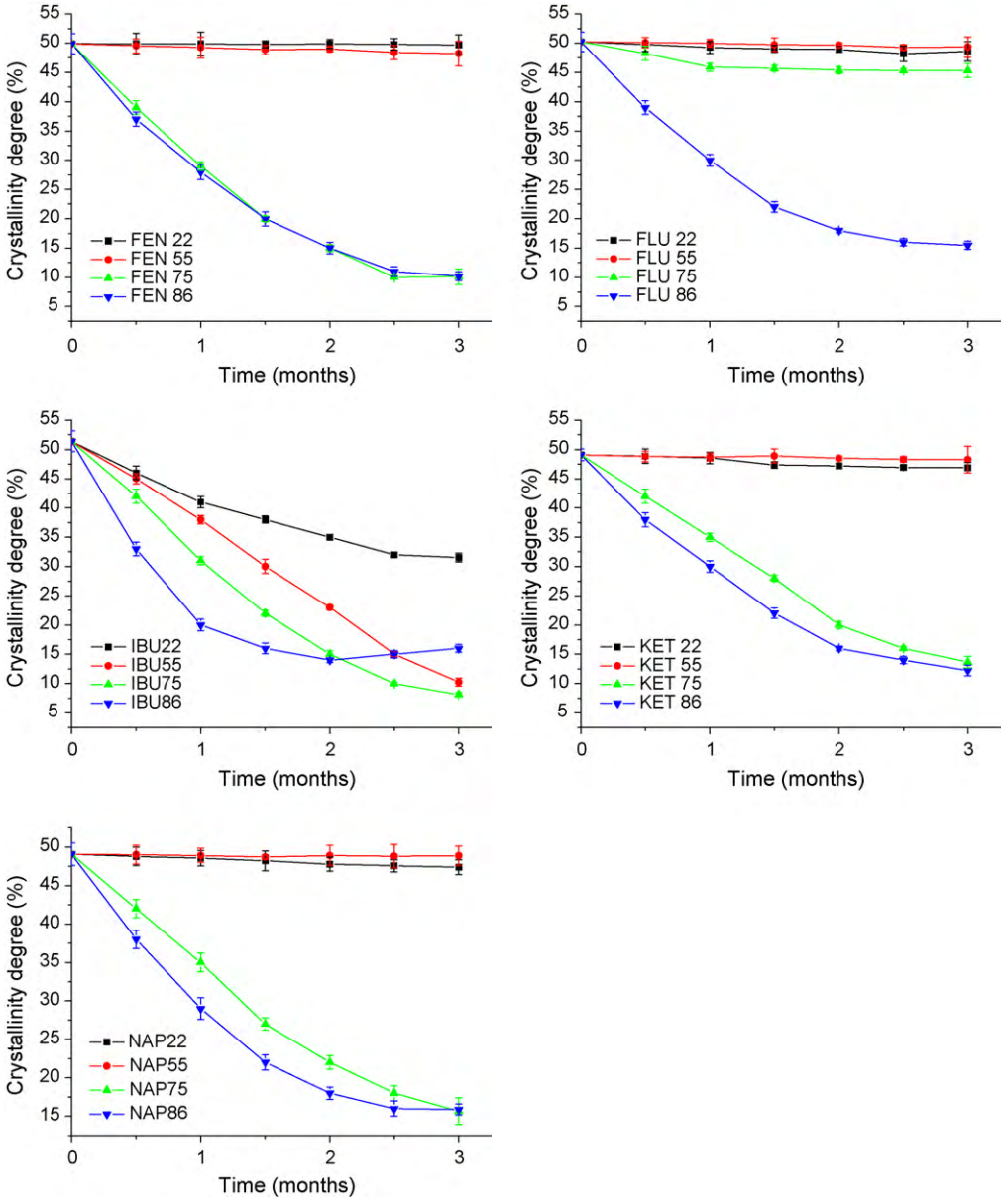


Fig. 3. Evolution of crystallinity degree with time of drug–PVP physical mixtures 50:50 (w:w) during storage at 298 K at different RH%. Results of 22% RH refer to previous paper (Gashi et al., 2009).

ascribed to the establishment of hydrogen bonds between the drug and the polymer.

3.3. High-resolution ^1H solid-state NMR spectrometry

Valuable information on the interaction and mixing degree of drug–excipient mixtures with aging can be obtained by solid-state NMR studies. This is particularly true in samples lacking homogeneity where the drug nuclei experience a number of different environments in the dispersion process. Even small changes in structural properties in the passage from pure drug to drug–excipient mixture in principle affect NMR spectral properties such as chemical shift, linewidth, signal intensity, etc. Particular chemical features of drug–excipient interaction and their modification with aging can be achieved by simple analysis of the isotropic ^1H and ^{13}C chemical shifts. All solid-state ^1H NMR spectra of 50:50 (w:w) NAP–PVP K30, 50:50 (w:w) FLU–PVP K30, 50:50 (w:w) FEN–PVP K30, 50:50 (w:w) KET–PVP K30, and 50:50 (w:w) IBU–PVP K30 with values of chemical shift on hydrogen bonding interaction are given in Fig. 4, which records the spectra of the same mixtures at time 0 (t_0), after 3 months at 86% RH, after 6 months at 86% RH, and after 10 months at 86% RH (a, b, c, and d, respectively). ^1H MAS spectra of 50:50 (w:w) NAP–PVP K30, 50:50 (w:w) FLU–PVP K30, 50:50 (w:w) FEN–PVP K30, and 50:50 (w:w) KET–PVP K30 mixtures at time 0 (t_0) and 3 months show no differences in the signal attributed to the hydrogen bond interaction, and the chemical shift value is the same as that of free drug, meaning that there is no interaction between drug and polymer matrix. Conversely, the same mixtures at aging times of 6 and 10 months had a small shift towards lower values passing from pure drug to solid dispersion. In all four mixtures, this shift is of the order of 0.5 ppm. We can suppose that with aging, the drug increases its interaction with the polymer matrix. For IBU, it was necessary to use the ^1H CRAMPS technique, which makes it possible to record proton spectrums even at rotational speeds of the order of 11–13 kHz, because the IBU–PVP mixtures at a rate of 32 kHz become viscous fluids, and thus all information about hydrogen bonding interactions are totally lost. ^1H CRAMPS spectra of IBU–PVP mixtures at different

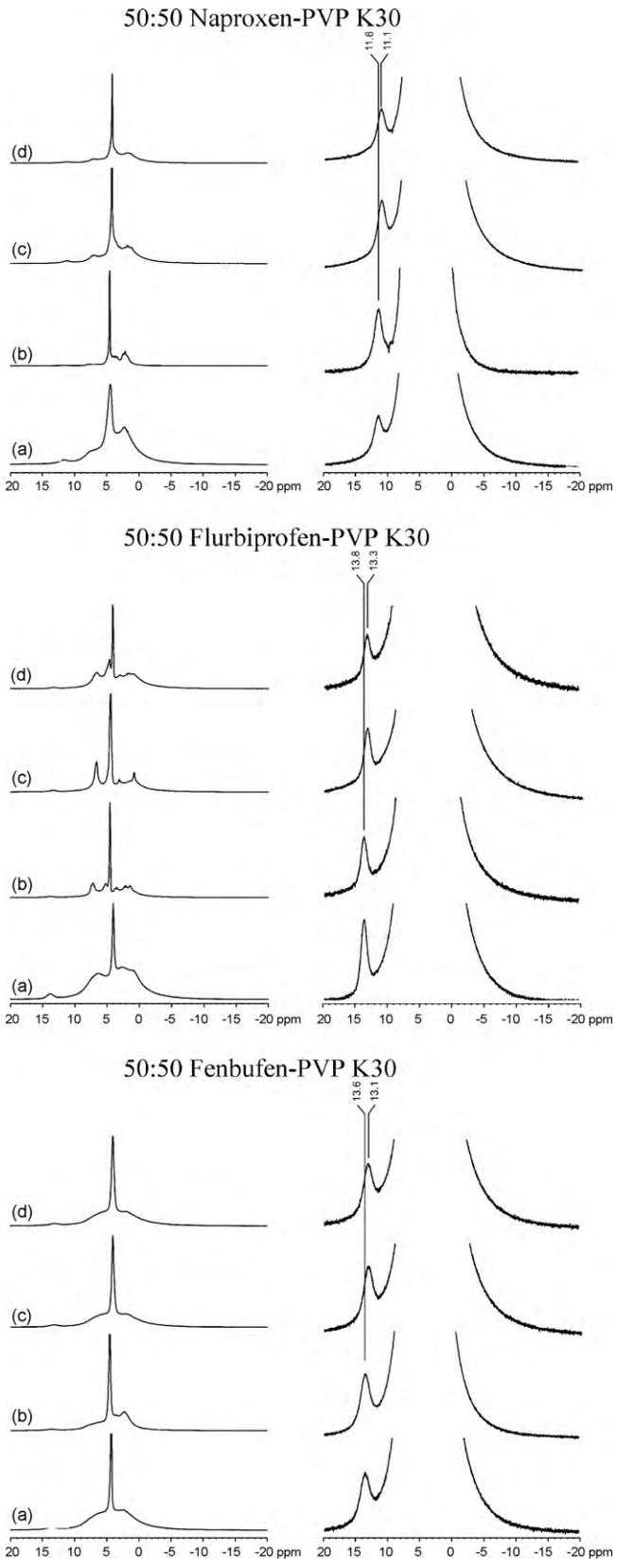


Fig. 4. ^{1}H CRAMPS spectrum of the 50:50 (w:w) drug–PVP K30 mixture recorded at 13 kHz MAS (a) time 0, (b) after 3 months at 86% RH, (c) after 6 months at 86% RH and (d) after 10 months at 86% RH.

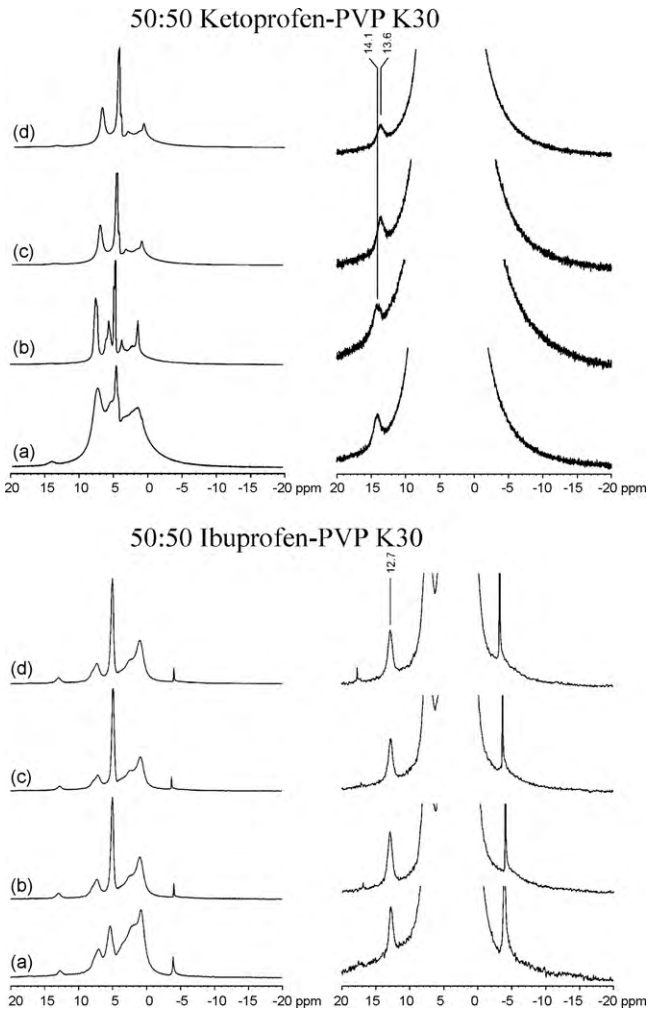


Fig. 4. (*Continued*)

aging times did not show significant differences and in particular, the chemical shift related to the hydrogen bond interaction (12.7 ppm) was just the same of that of the pure drug. In summary, during the same period in which amorphization occurred for any drug–PVP physical mixture (as indicated by XRPD), no hydrogen bond was formed.

3.4. Molecular docking

The drug positioning within the major groove of hydrated PVP and the modelled structure of hydrated PVP (compared to anhydrous PVP) are shown in Fig. 5. Experimentally, PVP exhibits high water vapour absorption and a strong water–pyrrolidone hydrogen bond (Taylor et al., 2001). In this molecular modelling study, it is very interesting to note the significant change in PVP chain conformation after hydration. Compared to the non-hydrated structure, the PVP chain was longer and the pyrrolidone group took on a conformation perpendicular to the main chain, while in the non-hydrated polymer, this group was folded on the main chain. The transversal section showed a more expanded spiral conformation in the case of the hydrated polymer. Table 2 reports the intermolecular energies, the predicted dissociation constants of the drug–polymer complexes K_d^{pred} and the H-bond lengths formed within the complexes. A previous study carried out at low RH% showed the establishment of hydrogen bonds between all the drugs under study and the PVP chain. The hydrogen bonds were

Table 2
Total intermolecular energy (with Van der Waals (E_{VdW}) and electrostatic (E_{Elect}) contributions), predicted equilibrium dissociation constants K_d^{pred} (derived from LUDI Score) and H-bond length (formed between the COOH group of the drug and the CO group of the polymer) of the drug/polymer complexes.

Drug	H-bond length (Å)	E_{VdW} – VdW energy (kcal/mol)	E_{Elect} – electrostatic energy (kcal/mol)	Total energy (kcal/mol)	K_d^{pred} (mM)
Fenbufen	2.03	−13.06	−4.98	−18.03	60.26
Flurbiprofen	–	−19.45	−1.69	−21.14	23.44
Ibuprofen	–	−10.37	−2.96	−13.33	323.6
Ketoprofen	–	−6.06	0.11	−5.95	190.6
Naproxen	2.07	−16.76	−3.08	−19.84	51.29

able to stabilize the binary system, as shown by the low K_d^{pred}, ranging between 7.94 and 24.54 mM for all the complexes (Gashi et al., 2009). In this study, FLU–PVP resulted in the most stable complex in terms of total intermolecular energy and predicted dissociation constant. Of considerable interest is the fact that IBU–PVP gave the least stable complex, exhibiting a very high K_d^{pred}. According to our predicted docking model, only FEN and NAP should be able to form an H-bond with the oxygen of PVP, although this ability should not affect the affinity of PVP for the ligands. In fact, total intermolecular energy was mainly due to the contribution of Van der Waals, and was related to the surface contact area between the drug and PVP in the complex thus formed. However, both these surface contact areas and the H-bond lengths were lower compared to previously analysed anhydrous drug–PVP complexes (Gashi et al., 2009), indicating the role of water molecules. In particular, water molecules, determining a relative humidity of 86% and inducing a less organized structure compared to anhydrous PVP favour a greater exposure of oxygen atoms (Fig. 5).

3.5. Glass transition temperature of ternary mixes

In order to determine the influence of water on the T_g of the drug–polymer physical mixtures, all the mixtures in all the proportions (from 10–90 to 90–10) were heated beyond the drug melting temperature in the DSC apparatus. Complete melting was checked by thermogram. The liquid was then quench cooled in the DSC by spontaneous cooling. Each drug was cooled to a minimal temperature that was 20 K lower than its T_g ($T_g - 20$ K). Once this temperature was reached, the mixture was immediately transferred to a box and exposed in a well-controlled environment at an RH of 86% and a temperature of 298.0 K. After equilibrium was reached, a second DSC heating was programmed in order to determine the T_g of the hydrated mixture. The time required to reach equilibrium was determined after several preliminary checks in which the change in T_g was lower than 0.3 K. During the second heating, the glass transition temperature (T_g) was determined in correspondence to the variation in specific heat (ΔCp), which can be defined as the heat amount absorbed by a material of m mass, when heated from T_1 to T_2.

Drug–polymer co-melting can be considered a means to promote the interaction between the drug and the polymer (Gashi et al., 2009; Di Martino et al., 2004): from a theoretical point of view, once the polymer softens, overcoming the T_g, and once the drug melts, the latter can interact with the polymer and diffuse through it by forming a solid dispersion. The measured T_g is a function of the mixture composition and the ratio of the two components. The Gordon–Taylor equation has been extensively used to predict the interaction between two compounds by evaluating deviations from an ideal behaviour (Van den Mooter et al., 2001; Van Drooge et al., 2006). Theoretical T_g values for each mixture can be calculated by the Gordon–Taylor equation according to the Eq. (2) (Gordon and Taylor, 1952):

$$T_{g1,2} = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2} \quad (2)$$

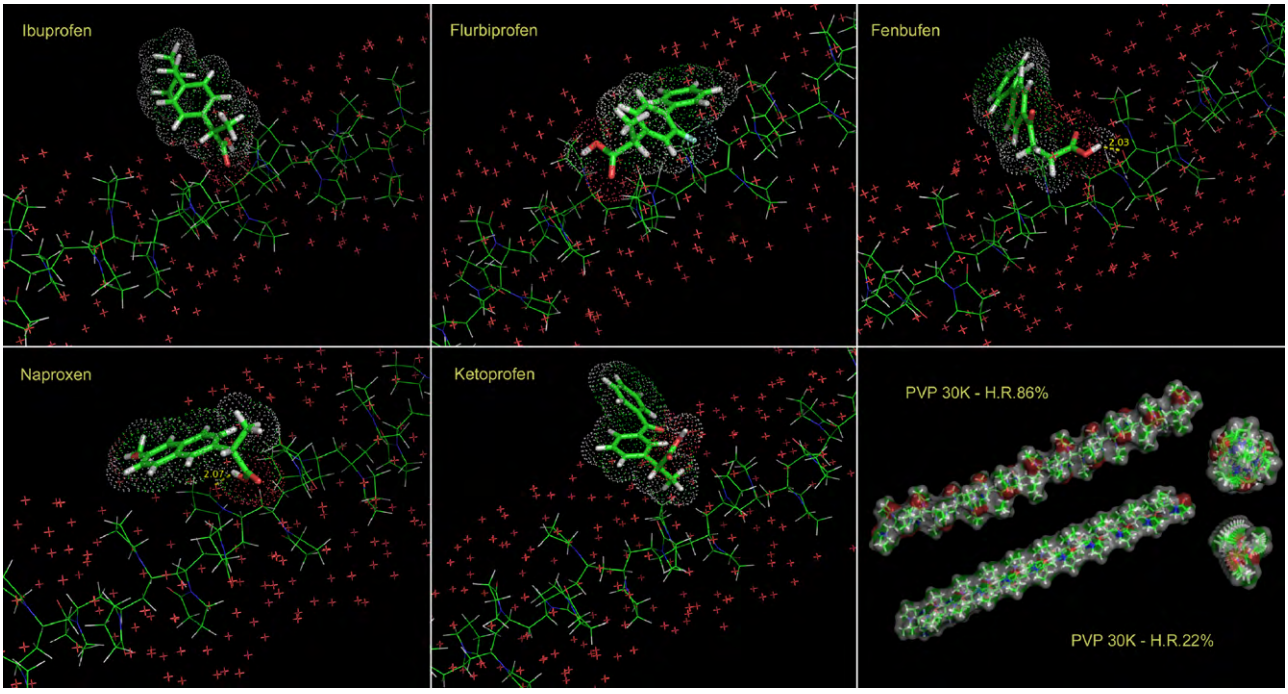


Fig. 5. Molecular docking showing the interaction between all tested drugs and the major groove of PVP.

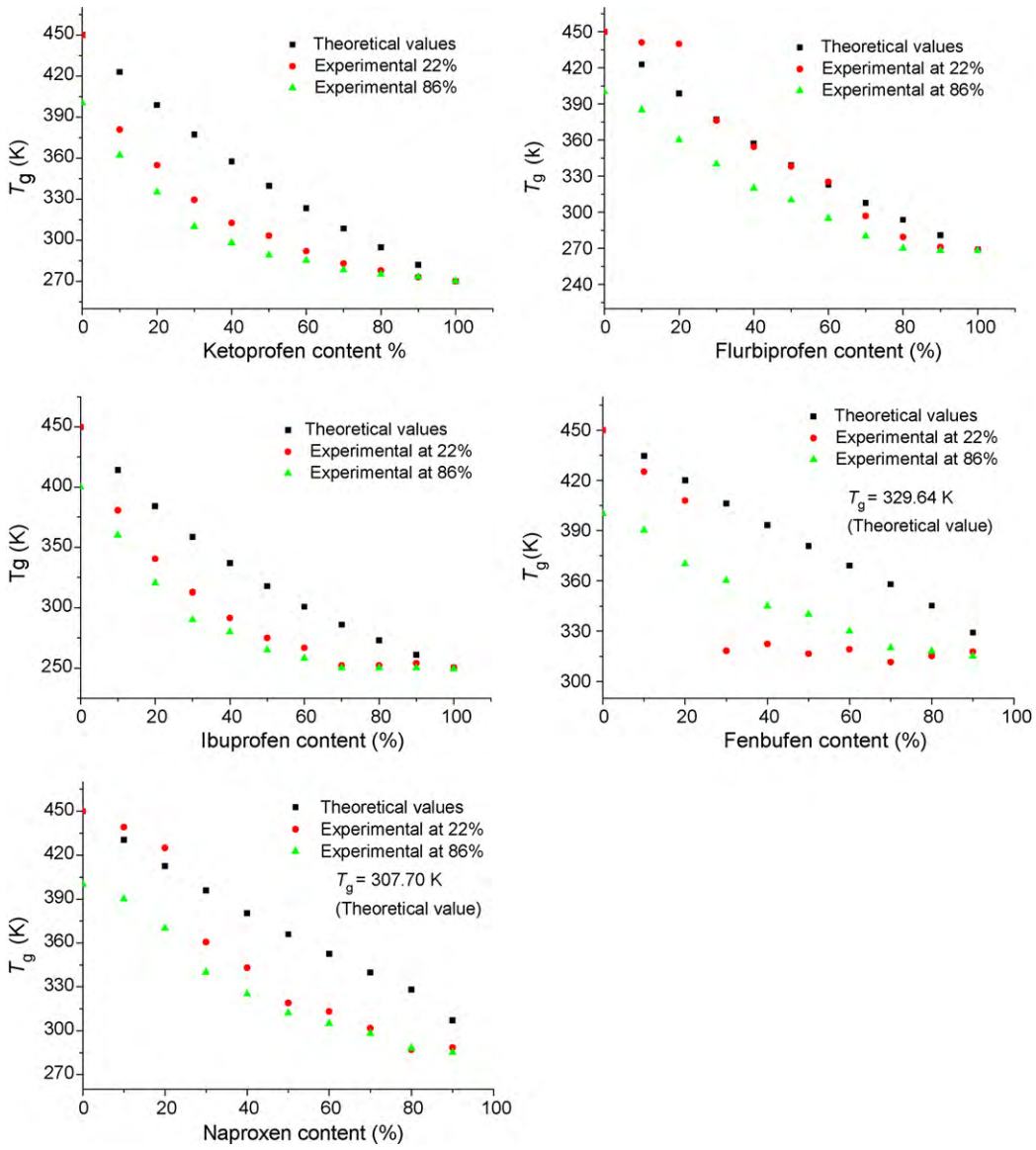


Fig. 6. T_g of drug–PVP mixtures expressed versus the drug %. (Small square symbols: theoretical T_g values predicted by the Gordon–Taylor equation; spotted symbols: T_g values experimentally determined by DSC for mixtures stored at 22% of RH; triangle symbols: T_g values experimentally determined by DSC for mixtures stored at 86% of RH.)

where $T_{g1,2}$ is the glass transition temperature of PMs in presence of water, w_1 and w_2 are the weight fractions, and T_{g1}, T_{g2} are the glass transition temperatures (in Kelvin) of drug and polymer, respectively.

The constant K_1, which is a measure of the interaction between the components, can be approximated using the following equation (Simha and Bayer, 1962):

$$K_1 = \frac{\rho_1 T_{g1}}{\rho_2 T_{g2}} \tag{3}$$

where ρ_1 and ρ_2 are the powder densities of the components (Table 1).

Results of dependence of predicted and experimental T_g values versus the drug content % are given in Fig. 6.

The T_g of pure hydrated PVP K30 was lower than that of the anhydrous polymer because water acts as plasticizer for PVP, and the experimental value was in good agreement with literature for the specific water content (Hamaura and Newton, 1999). Before commenting on the results obtained, it must be pointed out that for both of the pure drugs FEN and NAP, it was not possible to determine the experimental T_g value as explained by Gashi et al. (2009), and thus we used the theoretical T_g value provided in their paper (Table 1). Contrary to the previous results of Gashi et al. (2009), obtained after complete dehydration of drug–polymer mixtures, the experimental T_g was always lower than the theoretical ones for any drug at any drug–polymer ratio. According to Nair et al. (2001), the T_g of the blends is usually lower when adhesion forces between two components exceed the cohesion forces between the molecules of the same substance. More precisely, Schneider (1989) demonstrated that negative deviations from the predicted ideal behaviours might be caused by hydrogen bonding between drug and polymer, resulting in a change of free volume of the system. Generally speaking, the requirement of volume additivity of the Gordon–Taylor rule is not fulfilled.

In the case of the mixtures considered in this study, the hydration of the polymer may play a major role, as seen through molecular docking and now through the influence of water on its T_g. In fact, polymer may absorb a high quantity of water because of its hydrophilicity and amorphous state, and thus modify the PVP chain conformation. Among other things, the cohesion forces among the PVP chains are reduced because of the presence of the water layer. In addition, water acts as plasticizer for the polymer, increasing

Table 3
Water solubility of drug–polymer physical mixes 50:50 (w:w) stored for 3 months at 298 K at two different RH% and compared to those of pure drugs and of physical mixes immediately after mixing (t_0).

	Pure drug (g/ml)	Physical mixes drug–PVP K30		
		t_0 (g/ml)	RH 22% (g/ml)	RH 86% (g/ml)
Ketoprofen	0.032 ± 0.002	0.121 ± 0.005	0.365 ± 0.012	0.590 ± 0.003
Ibuprofen	0.082 ± 0.004	1.232 ± 0.025	2.710 ± 0.035	1.987 ± 0.014
Flurbiprofen	0.002 ± 0.001	0.022 ± 0.007	0.092 ± 0.015	0.179 ± 0.021
Fenbufen	0.001 ± 0.001	0.003 ± 0.001	0.034 ± 0.008	0.043 ± 0.008
Naproxen	0.022 ± 0.002	0.058 ± 0.005	0.161 ± 0.010	0.205 ± 0.007

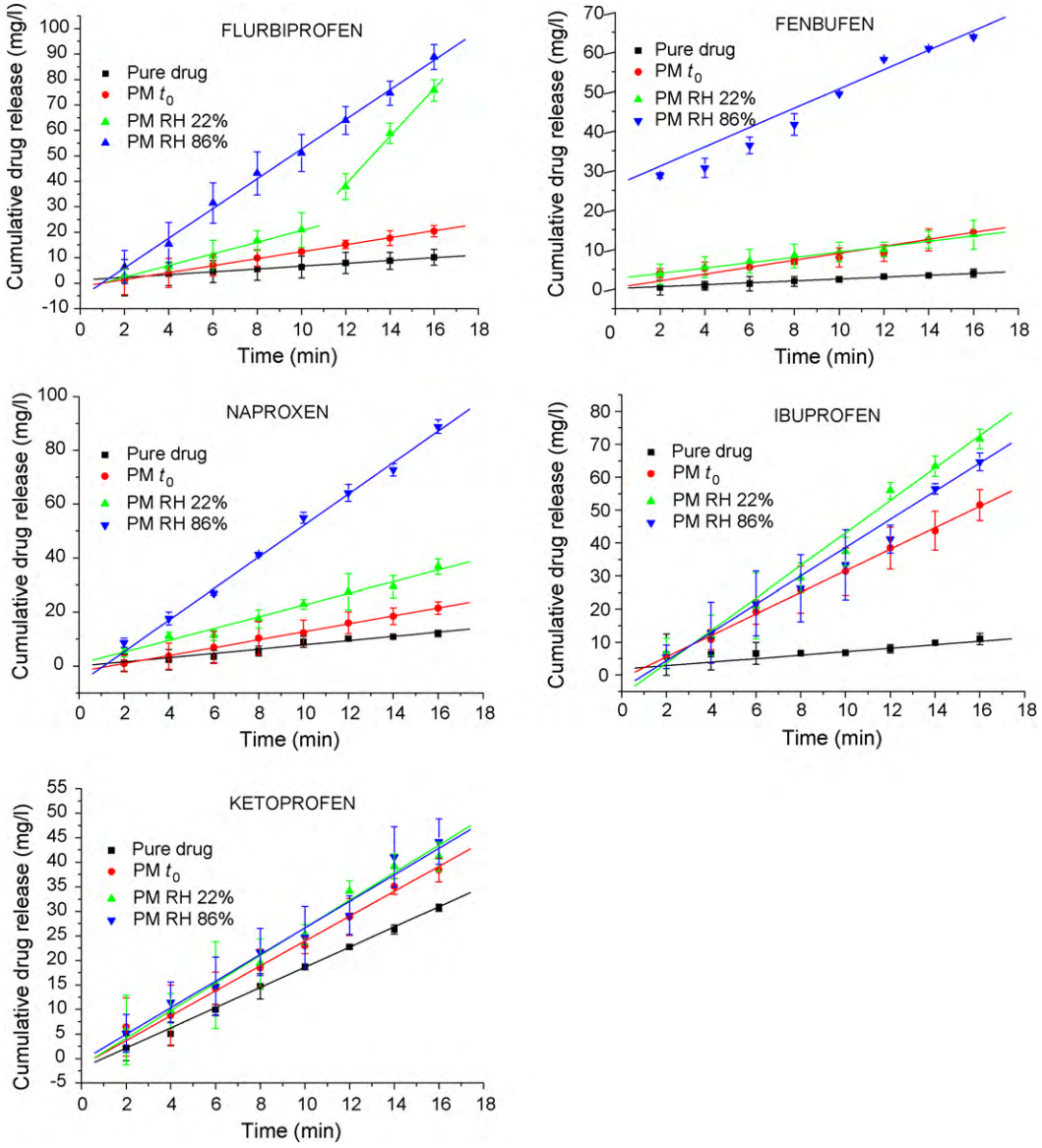


Fig. 7. Cumulative drug release in water measured by the rotating disk method of pure drugs, mixtures immediately after mixing (t_0) and after 3 months at two different RH (22 and 86%).

its molecular mobility. The change in polymer conformation and molecule cohesiveness and the increase in molecular mobility may influence the behaviour of the drugs under study. Drugs that in normal conditions are unable to diffuse into the system are now able to diffuse through the polymeric chains. This behaviour may be due to several concurrent factors: the polymeric chain flexibility may favour the diffusion of the drug, and, as the drug interacts with the polymer it loses its crystallinity. With their higher molecular mobility and small molar volume, the drug molecules may easily diffuse through the flexible polymeric network.

3.6. Equilibrium solubility and intrinsic dissolution rate (IDR)

The effect of physical interactions between the drug and the polymer and thus the progressive loss of crystallinity degree of physical mixes were evaluated in terms of drug solubility and dissolution. In the interests of clarity, Table 3 only presents the results obtained at the two extreme RH% conditions (22 and 85%). For intermediate RH% values, intermediate behaviours were observed, though for the sake of simplicity, the results are not reported here. Table 3 reports the water equilibrium solubilities of the drugs in

Table 4
Intrinsic dissolution rates (IDR) of drug–polymer physical mixes 50:50 (w:w) stored for 3 months at 298 K at two different relative humidities and compared to those of pure drugs and of physical mixes immediately after mixing (t_0).

			Slope (mg/l/min)	R	IDR (mmol/min/mm^2)
Ketoprofen	Pure drug		2.0591	0.9990	6.1007E-05
	Physical	t_0	2.5377	0.9943	7.5187E-05
	mix	3 months RH 22%	2.8127	0.9867	8.3335E-05
		3 months RH 86%	2.7097	0.9907	8.0283E-05
Fenbufen	Pure drug		0.2397	0.9984	7.1018E-06
	Physical	t_0	0.8793	0.9979	2.6052E-05
	mix	3 months RH 22%	0.6768	0.9884	2.005E-05
		3 months RH 86%	2.4475	0.9792	7.2514E-05
Flurbiprofen	Pure drug		0.5514	0.9977	1.7007E-05
	Physical	t_0	1.3701	0.9992	4.2258E-05
	mix	3 months RH 22%	2.2898	0.9966	7.0625E-05
			9.4020	0.9982	2.900E-03
		3 months RH 86%	5.8332	0.9982	1.7992E-03
Ibuprofen	Pure drug		0.5292	0.8951	1.9329E-05
	Physical	t_0	3.2652	0.9990	0.1193E-03
	mix	3 months RH 22%	4.9365	0.9920	0,1803E-03
		3 months RH 86%	4.2897	0.9943	0.1567E-03
Naproxen	Pure drug		0.7919	0.9726	2.5911E-05
	Physical	t_0	1.4711	0.9992	4.8134E-05
	mix	3 months RH 22%	2.1717	0.9891	7.1058E-05
		3 months RH 86%	5.8472	0.9975	0.1913E-03

mixture with the polymer stored for 3 months at 298 K at the two different RH% of 22 and 86%. These solubilities were compared to those of pure drugs and physical mixes immediately after preparation. The water solubility of pure drugs was very low, in particular for FEN and FLU, confirming their hydrophobic character. The water solubility of physical mixes at time 0 (t_0) was slightly higher than that of pure drugs, probably because the polymer increased the drug wettability. Actually, in Fig. 1b it was demonstrated that the weight loss determined by TGA (and thus conversely the water uptake) for drug–PVP mixtures increased for higher PVP content. In any case, solubility remained very poor for FEN. The water solubility considerably increased for the IBU–PVP mix immediately after mixing and after 3 months at 22 RH%. This result is in agreement with the fact that IBU was the only drug able to easily diffuse through the polymer even stored at low RH%, as demonstrated by the Gashi et al. (2009) work. At 86% RH, the solubility was still high, but lower than that at 22%. The reason for this behaviour may be undoubtedly correlated with the results of XRPD presented in this study, that showed an increasing of crystallinity degree for IBU–PVP mixture stored for 3 months at the highest RH%. Water solubility considerably increased at 86% RH for KET, FLU, and NAP. Even though the water solubility of FEN increased compared to that of pure drug and PM at time 0, it nonetheless exhibited the lowest increase of the drugs studied. The solubility results well correlate with data presented in Fig. 1a, which showed the different susceptibility of binary mixtures to undergo water uptake at increased RH%. These results prove that the drug dispersion into the hydrophilic polymer favoured by polymer water hydration increases drug solubility.

Fig. 7 provides data on cumulative drug release in water of pure drugs, drug–polymer mixes immediately after mixing (t_0), and after 3 months, at two different RH (22 and 86%). In Table 4, the IDRs are indicated together with the slopes of the curves of cumulative drug release.

IBU which showed the best wettability (Fig. 1a) and solubility (Table 3) showed a significant increase in IDR from pure drug to PMs even at t_0. The IDR reflects the solubility, and the IDR of PM stored at a RH of 22% is higher than that of PM stored at a RH of 86%, confirming that drug solubility and IDR are influenced by the crystallinity degree of the drug. For FEN and FLU, which exhibited the lowest wettability and solubility, the IDR significantly increases for PM stored at a RH of 86%. Even, their dissolution behaviour is characteristic: the cumulative drug release of FLU stored at a RH of 22% show a significant change in the slope; the cumulative drug release of FEN stored at a RH of 86% shows a burst effect. These two behaviours may be related to a marked increase in drug wettability favoured by the presence of the polymer that significantly improves the drug release. In spite of its relatively better wettability and solubility, KET showed no significant differences among cumulative drug releases of pure drug and PMs. Finally, NAP showed significant increase in cumulative drug release and IDR when stored at a RH of 86%.

As a general consideration, the interactions between the drug and PVP, also affected by the presence of water, may strongly influence the ability of drug molecules to interact with the solvent, and thus the solubility. As a consequence of an increased solubility, the dissolution rate is going to be modified; in particular, in the amorphous state, the dissolution rate will be bigger than in the crystalline state, because the bonds between the drug molecules are wicker than in the crystalline state.

4. Conclusion

Drug–PVP physical mixtures exposed to high RH until equilibrium could be considered as a ternary system of very complex interrelationships among drug, PVP and water. Drugs act as plasticizers and decrease PVP T_g. The adsorbed water has also a pronounced plasticizing effect on the hydrophilic polymer PVP leading to increase of drug mobility and drugs' crystallization. PVP, however, having a high T_g when is mixed with drugs will raise the T_g of the physical mixture (compared to T_g of each drug), that is, it will decrease drug mobility and drug crystallization. According to Taylor and Zografi (1997), the inhibition of drugs' crystallization could not be explained solely by the antiplasticizing effect of PVP because drug–PVP interactions play very important role in process of drug amorphization. Moreover, PVP is able to molecularly disperse a variety of compounds capable of forming hydrogen bonding with PVP. It is well known that water provokes drug crystallization in amorphous solid dispersions provided no drug–polymer interactions take place. On the other hand, the character of these interactions is usually very complex because different mechanisms contribute, such as hydrogen bonding, Van der Waals interactions, charge transfer, aromatic, hydrophobic interactions, etc.

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