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Influence of solvent composition on the solid phase at equilibrium with saturated solutions of quinolones in different solvent mixtures

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Abstract

The dissolution profiles and solubilities of three quinolonic drugs (oxolinic, pipemidic, and nalidixic acids) in different solvent mixtures were studied. The behavior of the solid phase, during solubility experiments was in-depth investigated with the aim of detecting possible crystalline modifications, such as polymorphic transitions or solvate formations, that might modify drug stability and/or solubility properties. In order to test the influence of both the nature and polarity of the co-solvents, aqueous and non-aqueous binary mixtures have been prepared by using Lewis base (dioxane and ethyl acetate) and amphiprotic co-solvents (ethanol and water). Differential scanning calorimetry (DSC), hot stage microscopy, IR spectroscopy and X-ray powder diffraction were used in combination with solubility and dissolution studies to characterize and investigate the solid state properties of the original powders and the corresponding ones at equilibrium with the different pure solvents and solvent mixtures examined. The solid phases of nalidixic and oxolinic acids did not show any change after equilibration with the various pure solvents or binary solvent mixtures, regardless the chemical nature of the examined solvents. On the contrary, in the case of pipemidic acid, the different analytical techniques used to characterize the drug solid state enabled identification of a solvated form at equilibrium with pure dioxane and a trihydrated form in aqueous mixtures of water with both ethanol (amphiprotic) or dioxane (Lewis base) in a concentration range from 10 to 100% water.

Keywords: Oxolinic acid; Pipemidic acid; Nalidixic acid; Solubility profiles; Solvent mixtures; Equilibrium solid phase

1. Introduction

Many drugs are poorly soluble in the commonly used pharmaceutical solvents and the use of solvent mixtures is often exploited as a method to increase their solubility. Solubility mathematical models can

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be successfully developed for predicting drug solubility in such solvent mixtures, thus allowing reduction of the number of expensive and time-consuming experiments necessary during pre-formulation studies [1,2]. Reliable solubility values are of primary importance to test the effectiveness of such models. The importance of ensuring that saturation conditions are reached during the solubility experiments in order to obtain reliable solubility values determination is well recognized. However, little attention has been generally paid to the characteristics of the solid phase. On the contrary, it should be taken into account that the time required to attain equilibrium solubility may be very long for poorly soluble drugs and the solvents may induce solid phase crystalline modifications, such as polymorphic transitions or solvate formation, that could modify the solubility with respect to that of the original powder, thus leading to errors in the experimentally determined solubilities. In fact, it is known that different polymorphic forms of a same substance can have very different solubility and dissolution properties [3,4]. Analogously, the change in the thermodynamic activity of the drug due to solvation phenomena alters pharmaceutically important properties such as physical and chemical stability, as well as solubility and dissolution rate [5–7]. In addition, the use of a solvated or a metastable polymorphic form of a drug in pharmaceutical formulations could give rise to physical and/or chemical stability problems because the high-energy form of the drug can revert to the thermodynamically more stable one or decompose more rapidly than the stable form under processing and storage conditions.

In this work, we in-depth investigated the behavior of the solid phase, during solubility experiments in solvent mixtures, of three quinolonic anti-bacterial agents (nalidixic, oxolinic, and pipemidic acids) commonly used to treat and prevent infections in the urinary tract. Our aim was to detect possible changes of the solid phase that might give rise to changes in drug stability and/or solubility properties. Two polymorphic forms have been described for nalidixic acid that melted, respectively, at 228 (Form I) and 224 °C (Form II) [8]. The existence of three crystalline forms of the trihydrated pipemidic acid have been demonstrated, whose thermal curves were characterized by two endothermic peaks, corresponding, respectively, to the dehydration (which occurred between 102–120 °C, depending on the crystalline form) and to the anhydrous form melting (254 °C) [9]. No polymorphic forms have been reported for oxolinic acid.

In order to test the influence of both the nature and polarity of the co-solvents, different aqueous and non-aqueous binary mixtures have been prepared by using Lewis base (dioxane and ethyl acetate) and amphiprotic co-solvents (ethanol and water). Several techniques, such as differential scanning calorimetry (DSC), hot stage microscopy (HSM), IR spectroscopy and X-ray powder diffraction were used in combination with solubility and dissolution studies to characterize and investigate the solid state properties of the original powders and of the solid phases after equilibration with the different pure solvents and solvent mixtures examined.

2. Materials and methods

2.1. Materials

Nalidixic acid (1 ethyl-7-methyl-4 oxo-1,4-dihydro-1,8 naphtiridine-3-carboxilic acid) (lot 124H0145) and oxolinic acid (5 ethyl-5,8-dihydro-8 oxo-1,3-dioxole [2,3-γ] quinoline-7-carboxilic acid) (lot 20H0315) were purchased from Sigma (St. Louis, MO, USA). Pipemidic acid (8 ethyl-5,8-dihydro-5 oxo-2-(1-piperacynil) pirido [2,3-δ] pirimidin-6-carboxilic acid (lot LH 9311) was generously provided by Fournier laboratories. All these products were anhydrous, as tested with the Karl–Fisher method. The solvents used were 1,4-dioxane, ethyl acetate, ethanol (spectrophotometric grade, Pancreac, Monplet and Esteban, Barcelona, Spain) and double-distilled water. The different solvent binary mixtures were prepared by volume.

2.2. Solubility measurements

Sealed flasks containing an excess of drug powder in the presence of a fixed volume of pure solvent or solvent mixture were shaken in a temperature-controlled bath (25 ± 0.1 °C, Heto SH 02/100) until equilibrium. The dissolution curves versus time were studied in all the pure solvents and the different binary mixtures. The solid phase at equilibrium was removed by filtration (Durapore membranes, 0.2 μm

pore size). The drugs did not significantly adsorb onto the membranes. The samples were diluted with ethanol 96% (v/v) and spectrophotometrically assayed (Shimadzu UV-2001PC, Japan). The technique was validated for each drug. The ranges of linear response and the maximum absorption wavelengths used were: 1.0–8.5 μg/ml and 256 nm for nalidixic acid; 1.0–5.6 μg/ml and 260 nm for oxolinic acid; and 1.3–4.8 μg/ml and 278 nm for pipemidic acid. All the experimental results were the average of at least three replicated experiments (coefficient of variation <2%).

2.3. Differential scanning calorimetry (DSC)

The samples (5–6 mg) of the original powders were placed in perforated aluminum pans under nitrogen purge (Mettler TA 4000, Mettler-Toledo, Greifensee, Switzerland). A heating–cooling–heating cycle was performed between 30 °C and a temperature somewhat higher than the melting point of each drug. In a second series of experiments, a single heating cycle was carried out in a wider temperature range (30–350 °C) and at several heating rates (2.5, 5, 10, 20, and 40 °C/min). The thermal effects were measured at a rate of 5 °C/min. DSC analyses were also performed on samples of drug solid phases at equilibrium with saturated solutions in both pure solvents and solvent binary mixtures; the solid phases were removed by filtration and dried at room temperature, since more drastic treatment could eliminate solvent weakly bound to the crystal. A single heating cycle was used in the 30–350 °C temperature range at a heating rate of 5 °C/min.

2.4. Hot stage microscopy (HSM)

An Olimpus BX-50 microscope connected to a HFS 91 hot stage and a temperature controller was used to observe the solid phase behavior before and after equilibration with the saturated solutions under polarized light at a heating rate of 5 °C/min.

2.5. IR spectroscopy

A Perkin-Elmer 883 IR spectrophotometer was used. The samples were blended with KBr and then a disk was obtained (1 cm diameter and 1–2 mm thick).

2.6. X-ray powder diffraction

X-ray powder diffraction patterns were obtained with a Philips PW 1130 diffractometer (Co Kα radiation), at a scan rate of $2^\circ\,min^{-1}$ over the 10–40 2θ range.

3. Results and discussion

3.1. Characterization of the solid state and solubility properties of the original powders

3.1.1. DSC and HSM studies

The thermal curves of nalidixic and oxolinic acids, recorded during the first DSC heating, were indicative of their anhydrous crystalline state, only showing a single sharp endothermic effect, corresponding to their melting (Figs. 1 and 2), peaked at 228.7 and 319.3 °C, respectively. In the case of pipemidic acid, a small and broad endothermic effect was observed at 211.8 °C (Fig. 3), reasonably attributable to a sublimation phenomenon, followed by a sharp endothermic peak at 256.7 °C due to the drug melting.

During the cooling phase a single exothermic peak, due to the drug recrystallization, was observed for nalidixic acid, which in the second heating melted at about the same temperature observed during the first heating. On the contrary, no thermal effects were detected during cooling or second heating for both oxolinic and pipemidic acids, thus suggesting, for these drugs, possible heating-induced amorphization or decomposition phenomena consequent to the melting process.

In the experiments performed up to 350 °C, two broad overlapping endothermic effects appeared for nalidixic acid, probably due to decomposition phenomena, but they were well above the drug melting temperature (Fig. 1). In contrast, strong thermal effects appeared immediately after fusion in the cases of both oxolinic (exothermic) and pipemidic (endothermic) acids (Figs. 2 and 3), thus confirming the hypothesized decomposition phenomena following drug melting.

The heating rate did not influence the temperature and enthalpy of fusion of nalidixic acid, whereas for oxolinic and pipemidic acids, the higher was the heating rate, the higher the melting temperature (Table 1).

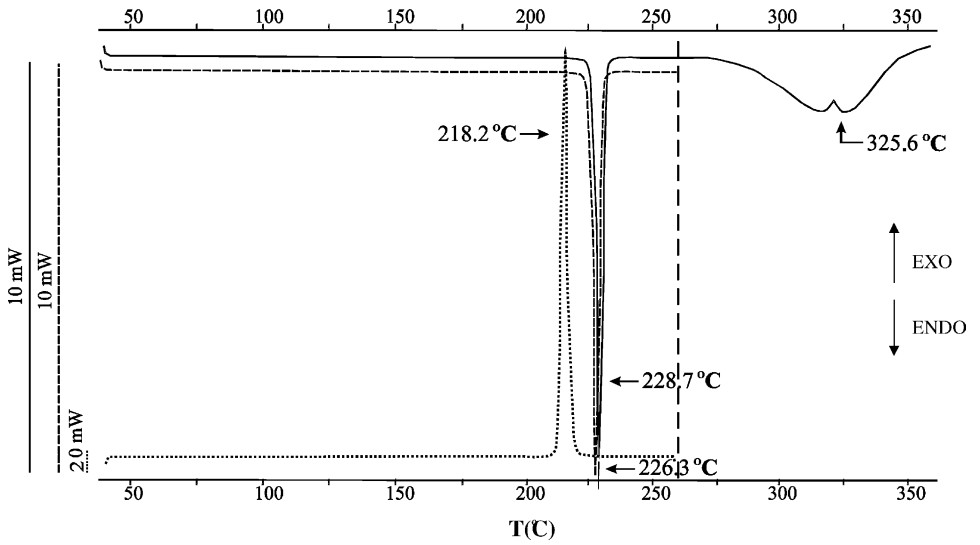

Fig. 1. Thermal curves of the original nalidixic acid. Key: (—) single heating, heating cycle: first heating; (· · ·) cooling and (---) second heating. The vertical broken line on the right shows the maximum temperature used in the heating–cooling–heating cycle.

The thermal behavior observed by HSM analysis was in agreement with the DSC results, as can be seen when comparing the data reported in Table 2 with those in Table 1 related to the same heating rate (5 °C/min).

3.1.2. IR spectroscopy

The IR spectra of the original samples of nalidixic and oxolinic acids are shown in Fig. 4. Both spectra were characterized by wide and weak bands in the 3300–2500 cm^{-1} range corresponding to the

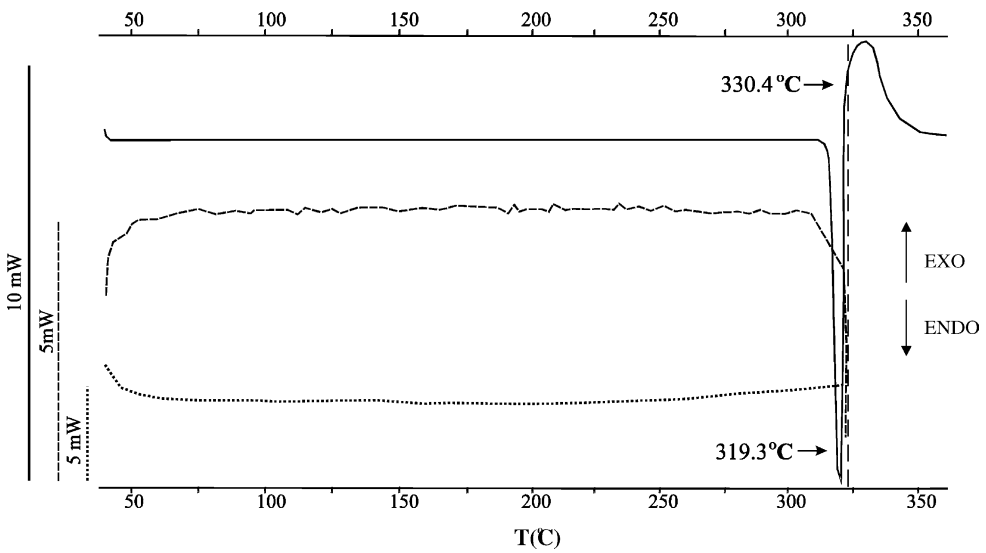

Fig. 2. Thermal curves of the original oxolinic acid. Key: (—) single heating, heating cycle: first heating; (· · ·) cooling and (---) second heating. The vertical broken line on the right shows the maximum temperature used in the heating–cooling–heating cycle.

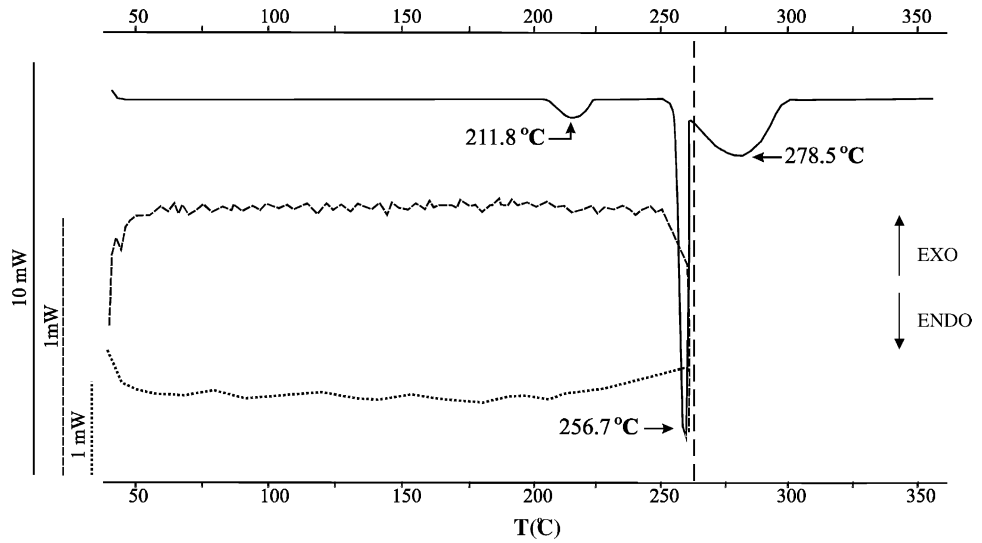

Fig. 3. Thermal curves of the original pipemidic acid. Key: (—) single heating, heating cycle: first heating; (· · ·) cooling and (---) second heating. The vertical broken line on the right shows the maximum temperature used in the heating–cooling–heating cycle.

carboxilic OH, indicative of possible hydrogen bonding with the carbonyl group, and by an intense peak at 1715 cm^{-1} is probably due to the stretching vibration of the carboxilic C=O group. Moreover, the peak at 1620 cm^{-1} observed in the IR pattern of nalidixic acid is attributable to the stretching of the C=O group of C-4 or the C-2 and C-3 groups conjugated to the carbonyl group or to a combination of both effects [10]. The IR spectrum of pipemidic acid (Fig. 5) exhibited typical bands corresponding exactly to those described for its anhydrous form [11].

The results obtained from the DSC, HSM and IR analyses made it possible to conclude that the original powder of crystalline nalidixic acid was in the Form I, and that of pipemidic acid was in the anhydrous form.

3.1.3. Solubility in pure solvents

Figs. 6–8 show the saturation solubility curves of the three drugs in pure water, ethanol, ethyl acetate, and dioxane at 40 °C. The saturation curves of nalidixic and oxolinic acids in all the examined solvents (Figs. 6 and 7) exhibited an initial ascendant region where

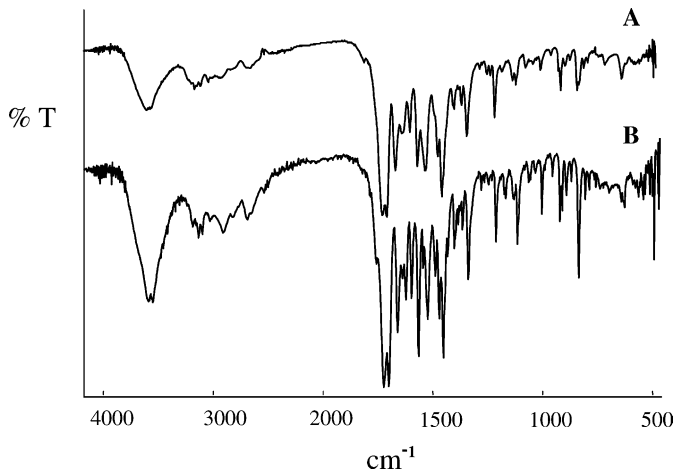

Fig. 4. Infrared spectra of the original nalidixic acid (A) and oxolinic acid (B).

Table 1
Temperatures and enthalpies of fusion (T_F, ΔH_F) and sublimation (T_T, ΔH_T) of nalidixic, oxolinic and pipemidic acids at different heating rates

Heating rate (°C/min)	Nalidixic acid		Oxolinic acid		Pipemidic acid			
	ΔH_F (KJ/mol)	T_F (°C)	ΔH_F (KJ/mol)	T_F (°C)	ΔH_{sub} (KJ/mol)	T_{sub} (°C)	ΔH_F (KJ/mol)	T_F (°C)
2.5	34.23	228.6	42.17	319.3	0.02	211.0	32.80	256.7
5	35.92	228.7	43.59	319.3	0.02	211.8	32.85	256.7
10	34.57	228.6	42.95	321.2	0.02	211.8	32.92	259.6
20	34.95	228.4	43.93	323.1	0.02	211.6	32.96	261.7
40	35.28	228.2	42.58	323.5	0.02	212.0	32.97	265.4

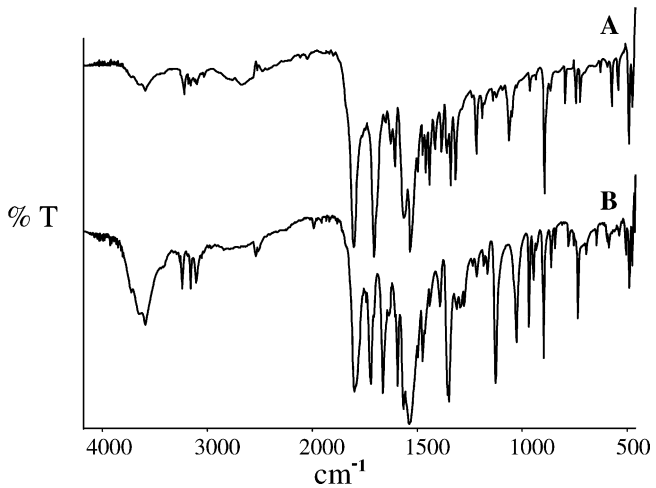

Fig. 5. Infrared spectra of the original pipemidic acid (A) and of the solid phase of pipemidic acid after equilibration with dioxane (B).

the concentration of the dissolved drug increased with time and a second region where the concentration leveled-off. The time required to attain equilibrium solubility depended on the polarity of both the solvent and the drug. In general, longer times were required to reach equilibrium in water (3 days for oxolinic acid and 5 days for nalidixic acid). The dissolution was faster in less polar solvents with lower solubility parameter values, according to that previously observed for mefenamic acid [12].

Table 2
Temperature of fusion (T_F) of nalidixic, oxolinic and pipemidic acids obtained from HSM analysis (heating rate 5 °C/min)

Drug	T_F (°C)
Nalidixic acid	229
Oxolinic acid	318
Pipemidic acid	255

Different dissolution profiles were instead observed for pipemidic acid (Fig. 8). The saturation curves in water, ethanol and ethyl acetate were characterized by a concentration maximum obtained at an earlier stage (less than 90 min) followed by a solubility decrease according to an apparent first order kinetics. Finally, an asymptotic region was obtained after 50–100 h, depending on the polarity of the solvent. In dioxane, after the initial concentration increase, a plateau region was maintained up to about 200 h. Then the concentration gradually decreased and reached an asymptotic region after about 14 days. Analogous results have been obtained in studies on crystalline solvated and non-solvated forms of some pharmaceuticals by Shefter and Higuchi [13] who attributed the initial maximum concentration to a supersaturation phenomenon due to the presence of metastable forms with respect to the stable one. Thus, the formation of

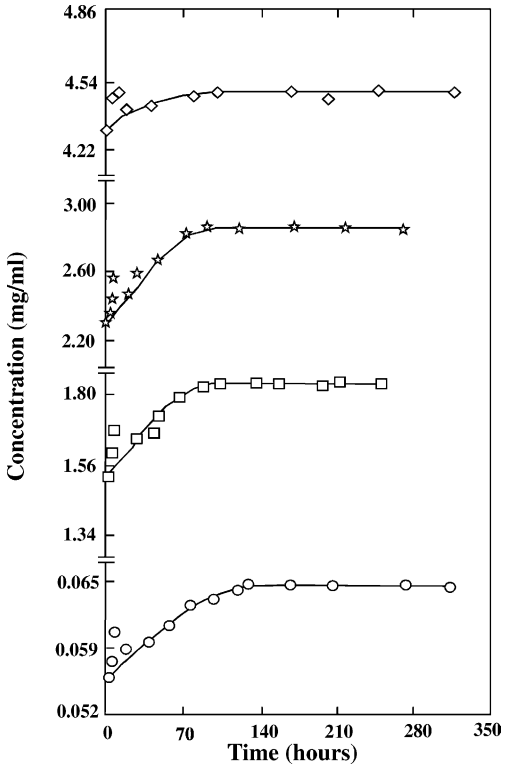

Fig. 6. Saturation curves of nalidixic acid at 40 °C in water (○), ethanol (□), ethyl acetate (☆) and dioxane (◇).

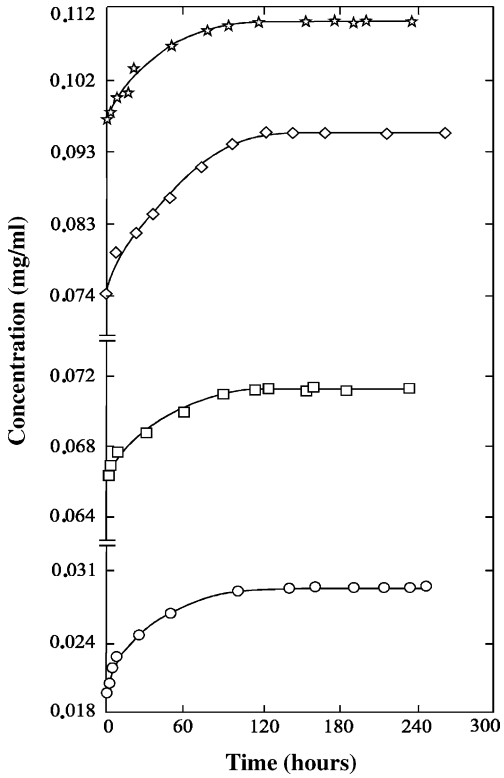

Fig. 7. Saturation curves of oxolinic acid at 40 °C in water (○), ethanol (□), ethyl acetate (☆) and dioxane (◇).

transitory metastable more soluble forms of pipemidic acid in the presence of dioxane has been hypothesized, which was converted into the stable, less soluble form at the asymptotic region.

3.2. Effect of solvent mixtures on the solid phase at equilibrium with saturated solutions

3.2.1. Dioxane–water mixtures

The results of IR spectroscopy, DSC and HSM analyses indicated that the solid phases of nalidixic and oxolinic acids after equilibration with the saturated solutions in the different dioxane–water mixtures remained unchanged. The absence of hydrates formation was also confirmed by Karl–Fischer analysis. The largest variation of the fusion temperature was 0.8 °C for nalidixic acid and about 2.2 °C for oxolinic acid (Table 3).

In contrast, the thermal curves of residual pipemidic acid solid phases at equilibrium revealed possible

Table 3
Temperature (T_F) and enthalpy (ΔH_F) of fusion of the solid phase of nalidixic and oxolinic acids at equilibrium with dioxane–water saturated solutions (heating rate 5 °C/min)

Dioxane (%)	Nalidixic acid		Oxolinic acid	
	T_F (°C)	ΔH_F (KJ/mol)	T_F (°C)	ΔH_F (KJ/mol)
0	229.3	35.90	317.4	44.73
10	228.7	35.09	319.3	42.85
20	229.0	34.85	319.4	42.83
30	229.4	34.62	319.6	41.63
40	229.3	35.45	319.3	42.27
50	229.4	34.90	319,5	43.10
60	229.5	35.09	319.1	43.39
70	229.3	34.57	318.9	44.43
80	229.1	34.53	319.0	43.15
85	229.3	34.09	–	–
90	229.0	34.89	318.3	41.89
100	228.9	35.94	318.4	42.58

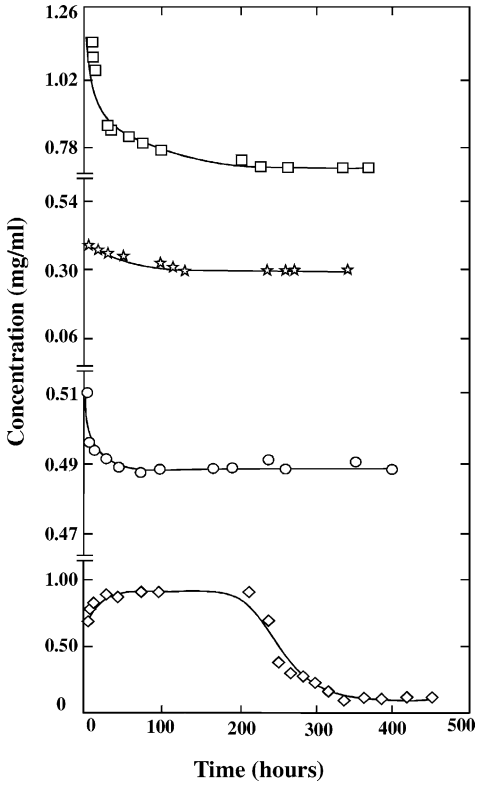

Fig. 8. Saturation curves of pipemidic acid at 40 °C in water (○), ethanol (□), ethyl acetate (☆) and dioxane (◇).

phase changes (Fig. 9) when compared with that of the original powder. In fact, besides the two endothermal effects at 195.6–211.9 °C (sublimation) and 258–260 °C (melting) (Table 4), an additional third endothermic peak at lower temperatures was observed, due to desolvation of a solvate formed in the presence of the saturated solutions (Fig. 9). In the case of the solid phase at equilibrium with saturated solutions in 100% dioxane, the desolvation endotherm occurred at 158 °C and was due to dioxane removal (Fig. 9, curve B). In the case of the solid phases at equilibrium with the different dioxane–water solvent mixtures (10–90% dioxane) the peak between 102–109 °C (Table 4 and Fig. 9, curve C) could be attributed to dehydration because the onset temperature (79.6 °C) is close to that of dehydration of hydrated caffeine (71–73 °C) [14]. Additional techniques such as thermomicroscopy, IR spectroscopy and Karl–Fisher method were used to further corroborate this result.

HSM analyses enabled to observe solvent release, as indicated by the formation of bubbles. This confirmed that the additional peaks at 102–109 °C and 158 °C (Table 4) were not due to any polymorphic change of the solid phase but, respectively, to the desolvation of the hydrated and dioxane–solvate forms.

Table 4 lists the enthalpy values obtained for the three endothermic effects (desolvation, sublimation and fusion) and the respective temperatures.

The IR spectrum of pipemidic acid after equilibration with 100% dioxane solution (Fig. 5, curve B) was clearly different from that of the original anhydrous sample, showing evident modifications in the shape and/or variations in the relative intensities of some of the major characteristic bands in the region between 1400–1700 cm^{-1}, as well as in the range between 2500–3200 cm^{-1}. On the other hand, the IR

Table 4
Temperature and enthalpy of desolvation (T_{des}, ΔH_{des}), sublimation (T_{sub}, ΔH_{sub}) and fusion (T_F, ΔH_F) of the solid phase of pipemidic acid at equilibration with dioxane–water saturated solutions (heating rate 5 °C/min)

Dioxane (%)	T_{des} (°C)	ΔH_{des} (KJ/mol)	T_{sub} (°C)	ΔH_{sub} (KJ/mol)	T_F (°C)	ΔH_F (KJ/mol)
0	109.3	120.1	195.6	4.43	260.3	30.9
10	106.9	120.1	195.8	5.25	261.2	30.6
20	109.8	120.5	196.8	6.22	260.1	30.4
30	106.3	119.8	195.6	5.31	259.8	30.4
40	105.2	120.1	195.8	5.40	259.7	30.3
50	107.9	120.2	198.8	5.46	260.1	31.2
60	102.3	119.9	197.2	5.52	258.2	31.0
70	103.4	119.9	197.5	5.43	258.0	30.0
80	101.9	120.6	198.0	5.58	258.7	29.1
90	106.0	119.7	197.9	5.58	257.6	29.4
100	158.1	26.3	211.9	5.76	257.3	30.0

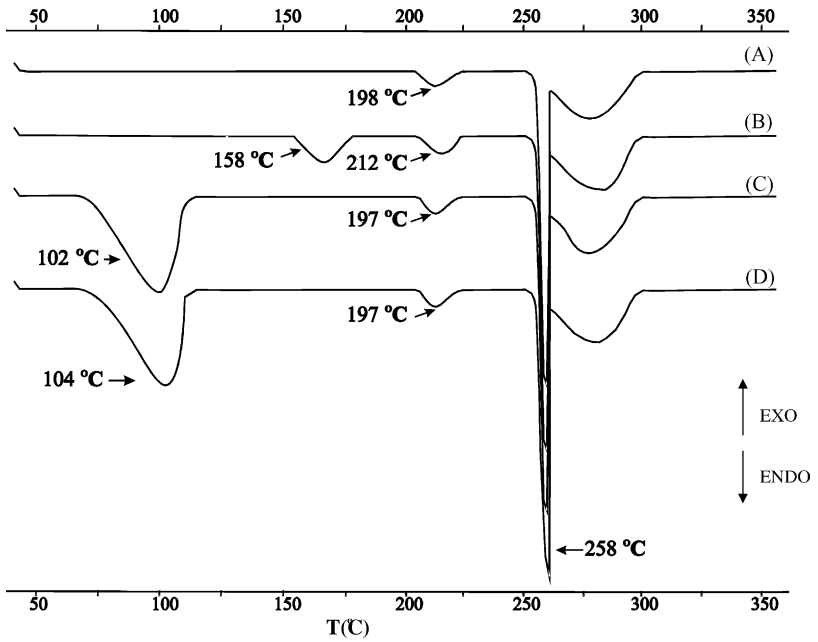

Fig. 9. Thermal curves of the solid phases of pipemidic acid at equilibrium with the different saturated solutions. Key: (A) pure ethanol and ethanol–ethyl acetate mixtures; (B) 100% dioxane; (C) 10–100% water in dioxane; (D) 10–100% water in ethanol.

absorption pattern of the pipemidic acid sample after equilibration with water–dioxane mixtures ranging from 10 to 100% water (not shown), was consistent with that obtained by Pierini et al. [11] for the trihydrated form of the drug.

The formation of a hydrated form of pipemidic acid in dioxane–water mixtures was further confirmed using Karl–Fischer titrimetry. Table 5 shows that the water content was practically the same in all the samples equilibrated with the saturated solutions containing 10–100% water in dioxane. The amount of determined water corresponded to a stoichiometry of three water molecules per pipemidic acid molecule. Therefore, the initial anhydrous product converted into a trihydrated one in all the mixtures containing 10–100% water in dioxane. A trihydrated form of pipemidic acid has been previously described also by Yates et al. [9]. The method of Khankari et al. [15,16] was also employed to calculate the hydrate stoichiometry, using the DSC transition enthalpies (J/g) and the enthalpy of vaporization of water (2261 J/g). The results well agreed with those obtained from the Karl–Fisher method, as shown in Table 5.

The pipemidic acid trihydrate form was also characterized by X-ray powder diffraction analysis. As be can see in Fig. 10, the pattern of the hydrated form exhibited a clear loss of crystallinity with respect to that of the original anhydrous one, but did not show any appearance of new peaks or disappearance or shifts of

Table 5
Percentage water (w/w) obtained with the Karl–Fisher method and number of water molecules obtained from this method (n^a) and from Khankari's method [15] (n^b) for pipemidic acid after equilibration with dioxane–water saturated solutions

Dioxane (%)	Water (%)	n^a	n^b
0	15.17	3.15	3.07
10	15.25	3.22	3.08
20	15.03	3.05	3.18
30	15.30	3.25	3.02
40	15.30	3.25	3.10
50	15.12	3.13	3.11
60	15.22	3.20	3.05
70	15.03	3.05	3.02
80	15.12	3.13	3.21
90	15.05	3.07	2.97
100	3.5	0	–

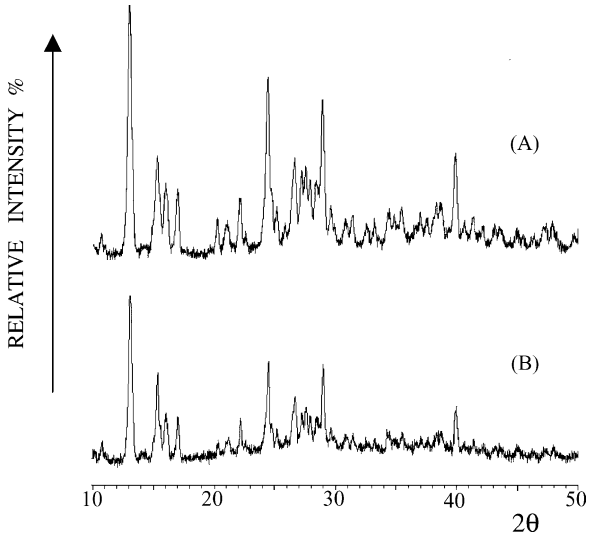

Fig. 10. X-ray powder diffraction patterns of original anhydrous pipemidic acid (A) and of its solid phase after equilibration with dioxane–water saturated solutions (0–90% dioxane) (B).

typical diffraction peaks, indicating that the incorporation of water molecules did not substantially modify the drug crystal lattice.

3.2.2. Amphiprotic mixtures: ethanol–water and ethanol–ethyl acetate

The solid phases of nalidixic and oxolinic acids did not change after equilibration with saturated solutions of both the aqueous and non-aqueous amphiprotic mixtures (Table 6) as was previously found in dioxane–water mixtures (Table 3). This was also confirmed by IR analysis and thermomicroscopy. Moreover, their water content was equal to that found for the original powders.

In contrast, the DSC curves of pipemidic acid after equilibration with the saturated solutions revealed solid phase changes. Fig. 9 shows the different DSC profiles obtained for the solid phase after equilibration with ethanol or ethanol–ethyl acetate mixtures (curve A) and with ethanol–water mixtures containing 10–100% water (curve D). In both cases, the sublimation and fusion peaks occurred near to the temperatures found for the original powder (Table 7 and Fig. 3). On the other hand, the additional endothermic effect present in the solid phase after equilibration in the 10-100% water–in ethanol mixtures (curve D) seems to be due to the desolvation of the trihydrated

Table 6
Temperature (T_F) and enthalpy (ΔH_F) of fusion of nalidixic and oxolinic acids after equilibration with ethanol–water and ethanol–ethyl acetate saturated solutions (heating rate 5 °C/min)

	Nalidixic acid		Oxolinic acid	
	T_F (°C)	ΔH_F (KJ/mol)	T_F (°C)	ΔH_F (KJ/mol)
Ethanol (%)				
0	229.3	35.90	317.4	44.73
10	229.6	34.90	–	–
20	228.7	34.78	317.7	41.84
30	228.8	35.29	–	–
40	229.5	34.53	318.3	41.81
50	229.1	34.39	317.1	43.66
60	228.7	34.16	–	–
70	228.8	34.50	–	–
80	228.7	34.69	317.9	44.94
85	228.8	34.62	–	–
90	229.2	34.67	319.0	44.38
95	229.3	34.25	–	–
100	229.3	34.20	319.1	43.00
Ethyl acetate (%)				
10	229.0	34.30	319.2	39.01
20	–	–	319.9	41.65
30	229.1	34.78	319.6	43.46
50	229.6	34.99	319.7	42.71
60	–	–	319.3	41.75
70	228.9	34.81	319.0	44.67
80	229.4	34.74	–	–
90	229.0	34.34	319.6	42.57
100	229.1	35.16	318.2	42.50

form, as previously found in dioxane–water mixtures (curve C), because, as it is evident by comparing data in Tables 4 and 7, both the temperatures and enthalpies of desolvation were very similar.

These results were also confirmed by thermomicroscopy, IR spectrometry and Karl–Fisher titrimetry. The stoichiometry of samples after equilibration in ethanol–water mixtures containing 10–100% water was three water molecules for pipemidic acid molecule (Table 8). Therefore, the anhydrous solid converted into the trihydrated form in the aqueous solvent mixtures whereas the initial crystalline form remained unchanged in the pure solvents, ethanol and ethyl acetate, and in the non-aqueous ethanol–ethyl acetate mixtures (Fig. 9, curve A). Table 8 also shows that the stoichiometry determined by the method of Khankari et al. [15,16] provided results similar to those obtained for the solid phase residual after equilibration in dioxane–water mixtures containing 0–90% dioxane (Table 5).

Table 7
Temperature and enthalpy of desolvation (T_{des}, ΔH_{des}), sublimation (T_{sub}, ΔH_{sub}) and fusion (T_F, ΔH_F) of pipemidic acid solid phase at equilibrium with ethanol–water and ethanol–ethyl acetate saturated solutions (heating rate 5 °C/min)

	T_{dehydr} (°C)	ΔH_{dehydr} (KJ/mol)	T_{sub} (°C)	ΔH_{sub} (KJ/mol)	T_F (°C)	ΔH_F (KJ/mol)
Ethanol (%)						
0	109.3	120.1	195.6	4.43	260.3	30.88
10	106.9	120.1	195.8	6.22	258.0	30.39
20	109.2	120.1	196.8	5.31	258.7	30.30
30	106.3	119.9	195.6	5.46	259.8	30.61
40	106.9	120.1	195.8	5.25	259.7	30.30
50	107.9	120.2	198.8	5.43	260.1	30.05
60	103.8	119.9	197.2	5.58	258.2	31.00
70	103.4	119.9	197.5	5.76	259.0	20.00
80	106.3	120.6	198.0	5.52	258.7	29.09
90	104.9	119.7	197.9	5.40	258.6	29.36
100	–	–	198.2	5.58	261.2	29.09
Ethyl acetate (%)						
10	–	–	196.5	5.40	260.1	30.61
30	–	–	196.8	5.25	259.8	30.39
50	–	–	196.8	5.58	259.7	29.09
60	–	–	198.8	5.46	260.1	29.36
70	–	–	197.5	5.46	260.1	30.45
80	–	–	197.2	4.43	258.7	30.30
90	–	–	198.2	5.76	258.7	30.00
100	–	–	197.9	5.25	258.6	30.03

Table 8
Percentage water (w/w) obtained with the Karl–Fisher method and number of water molecules obtained from this method (n^a) and from Khankari's method [15] (n^b) for pipemidic acid after equilibration with ethanol–water saturated solutions

	Water (%)	n^a	n^b
Ethanol (%)			
0	15.17	3.15	3.07
10	15.30	3.25	3.10
20	15.25	3.22	3.08
30	15.30	3.25	3.10
40	15.03	3.05	3.02
50	15.12	3.13	3.21
60	15.02	3.05	3.05
70	15.03	3.05	3.02
80	15.12	3.13	3.21
90	15.12	3.13	3.21
100	3.4	0	–

4. Conclusion

Nalidixic and oxolinic acids showed similar trends in their solubility profiles in the different solvents, all characterized by an initial rising curve followed by a plateau. The solid phases of nalidixic and oxolinic acids did not experience any change after equilibration with the different pure solvents or the various binary solvent mixtures examined, regardless of the different chemical nature of the examined solvents or the solvent mixture composition.

In contrast, the solubility profiles of pipemidic acid showed an initial maximum of concentration immediately followed by a decrease phase and by a final plateau. Moreover, interestingly, in the presence of dioxane, an initial plateau was observed before the concentration decrease and the final plateau phases. Solid-state studies showed that pipemidic acid formed solvated forms in pure dioxane and trihydrated forms in aqueous mixtures of water with both ethanol (amphiprotic) or dioxane (Lewis base) in a concentration range from 10 to 100% water. No solvate formation or polymorphic transitions were instead found for the pipemidic acid solid phase after equilibration with both pure ethyl acetate and ethanol or in non-aqueous mixtures of ethanol–ethyl acetate.

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