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Solubility and phase separation of benzocaine and salicylic acid in 1,4-dioxane–water mixtures at several temperatures

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Abstract

The solubilities of benzocaine and salicylic acid were determined in water–dioxane mixtures at several temperatures (5–40 °C for benzocaine and 10–40 °C for salicylic acid). The solubility curves as a function of dioxane ratio showed a maximum at 90% dioxane at all temperatures. Above 25 °C, the homogeneous mixture splits into two liquid immiscible phases. For benzocaine, the initial dioxane concentration range at which phase separation takes place increased with temperature (50–60% at 25 °C, 50–70% at 30–35 °C and 40–70% at 40 °C). For salicylic acid, the dioxane concentration required for phase separation (40–60% dioxane) did not change with temperature. Phase separation was not related to solid phase changes (polymorphism or solvates). The phase composition and drug extraction at the drug-rich phase were determined. The apparent enthalpies of the solution process were a nonlinear function of the dioxane ratio for both drugs. The apparent enthalpy of solution of benzocaine was larger than that expected at the upper limit of phase separation (70% dioxane), whereas for salicylic acid the apparent enthalpy of solution decreased abruptly at the region corresponding to phase separation (40–70% dioxane). Both drugs showed a nonlinear pattern of enthalpy–entropy compensation.

Keywords: Phase separation; Benzocaine; Salicylic acid; Solubility; Solvent mixtures

1. Introduction

Phase separation of organic–water solvent mixtures induced by salts is well known and has been applied to the extraction of highly charged species. Takamuku et al. [1] studied the phase separation of 1,4-dioxane at 25 °C using several NaCl concentrations below saturation. On the other hand, liquid–liquid phase separation in aqueous-surfactant systems has been observed [2] However, to our knowledge no references are given in the literature concerning phase separation of organic–water mixtures induced by organic solutes, except for the work of Paruta et al. [3–5]. At saturation concentration, Paruta et al. [3–5] observed that salicylic acid and some paraben derivatives (ethyl, propyl and butyl paraben) formed a two-phase immiscible system in dioxane–water mixtures, a fact that was unprecedented in the literature. This effect was not found for the first member of the paraben series, methylparaben. Butyl paraben was the only derivative that induced phase separation in ethanol–water mixtures. These phases were found to be approximately invariant with respect to the concentration of the three components, a fact that was attributed to the formation of a solvate [6]. These studies were performed at a single temperature.

Solubility analysis of drug molecules in solvent mixtures is becoming increasingly important in the chemical industry of drug design and manufacture. However, the solubility data of drugs as a function of temperature are scarce, and in most

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instances the literature only provides water solubilities. The solubility range comprised between 20 and 40 °C is particularly important because it includes the physiological temperature and temperature changes that are most usual during storage to prevent precipitation of the solute. Dioxane–water mixtures have been used to study the solubility behavior in a relatively wide dielectric range and to determine the solubility parameters of solid solutes. The dioxane–water mixtures cover a wide range of the solubility parameter scale from dioxane (δ_T = 20.48 $MPa^{1/2}$) to water (δ_T = 47.97 $MPa^{1/2}$).

In this paper, induced phase separation of dioxane–water mixtures with benzocaine and salicylic acid has been studied. The solubilities of salcylic acid and benzocaine have been measured in the range 5–40 ± 0.1 °C in order to obtain an appropriate estimate of the real functional relationship between their solubilities and the volume ratio of dioxane in the solvent mixture and to test the influence of temperature in phase separation. Benzocaine is structurally related to parabens (it differs from ethylparaben in a $-NH_2$ instead of the –OH group attached to the benzene ring), and there are no data available on the solubility in dioxane-water at several temperatures. The effect of temperature on phase separation induced by these drugs was not reported previously. Ten cosolvent compositions have been selected covering 0–100% dioxane in water.

2. Experimental

Benzocaine, salicylic acid (Sigma Chemical, St. Louis, USA, 99+%) were used as received. Ten binary compositions were prepared by volume (0–100% dioxane) for each temperature using 1,4-dioxane (Panreac, Monplet, Barcelona, Spain, 99.5+%, anhydrous) and double-distilled water. The solubility measurements were performed at eight (benzocaine) and six (salicylic acid) temperatures between 5 and 40 °C in the dioxane–water mixtures. An excess of solute was added to the solvent mixtures and allowed to equilibrate at the highest temperature in a shaking constant-temperature bath (±0.1 °C). Attainment of equilibrium was tested from the dissolution curves of amount dissolved versus time. The temperature was then dropped and the samples were allowed to re-equilibrate at the desired temperature. Samples of the saturated solutions were filtered (Durapore membranes, 0.2 μm pore size) and diluted with ethanol 96% v/v. The concentrations were determined in a double beam spectrophotometer (Shimadzu UV-2101 PC, Japan) at 279 nm (*p*-aminobenzoic acid) and 298 nm (salicylic acid). For the dioxane-water ratios where phase separation occurs, the two liquid layers were previously separated using separatory funnel and transferred to volumetric flaks to re-equilibrate them at each temperature. Each phase was centrifuged for 15 min at 2500 rpm to eliminate the solid non-dissolved phase and the volume was measured in a graduated tube. Samples of each phase were filtered, diluted with ethanol and the concentration was determined spectrophotometrically. Except for three cases, the coefficient of variation of the drug concentration determined (standard deviation/mean expressed as percentage) was within 3%. The density of the saturated solutions was also measured to convert molar concentrations into mole fraction units, used in the phase diagram. All the concentrations and densities determined are the mean value of three replicated experiments.

The heat and temperature of fusion were measured from differential scanning calorimetry, DSC, (Mettler TA 4000, Switzerland) at a heating rate of 5 °C/min. The DSC analysis was performed on the original powders and on each solid phase at equilibrium with all the cosolvent-water ratios at the lowest temperatures. This analysis was also applied to the solid phases where two liquid phases were observed, at 40 and 25 °C. The solid phases were placed on filter papers and the solvent excess was evaporated at room temperature until constant weight.

The water concentration in the lower phase was determined by the Karl Fisher rapid test (Merck, Germany) and that of the upper phase was calculated by subtracting the determined amount from the total amount of water.

3. Calculations

The apparent enthalpy of solution of the drugs was obtained from linear regressions of ln *M* (molar concentration) against $1/T - 1/T_m$ where T_m is the harmonic mean of the experimental temperatures:

$$\Delta H^S = -R \times \text{slope} \quad (1)$$

$$\Delta G^S_{hm} = RT_{hm} \times \text{intercept} \quad (2)$$

where *R* is the gas constant (8.3143 J/K mol).

From this regression the intercept gives the apparent free energy change ΔG^S_{hm} at the experimental harmonic mean temperature, T_{hm} [7]. Using this approach, Krug et al. [7] demonstrated that the errors of the slope and the intercept are not correlated and the calculated thermodynamic magnitudes can be used to detect a true chemical compensation relationship.

In the analysis of phase separation, the recovery (in percent) [2] of drug extracted, is obtained from the expression:

$$\text{Recovery} = \frac{D}{D + (\text{VUP}/\text{VLP})} \times 100 \quad (3)$$

where VUP and VLP are the volumes (*V*) of upper phase and lower phase (drug-rich phase) and *D* is the distribution coefficient, which describes the degree of solute partitioning between both phases and is defined as:

$$D = \frac{\text{MLP}}{\text{MUP}} \quad (4)$$

where MLP and MUP are the drug concentrations (*M*) in the lower phase (drug-rich phase) and the upper phase, respectively.

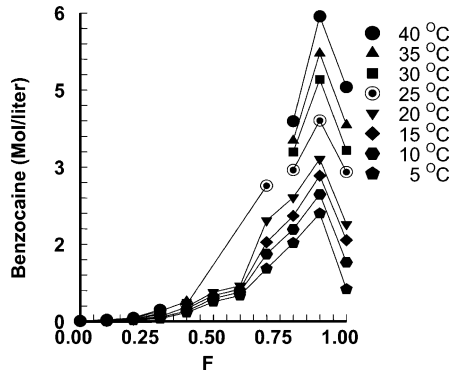

Fig. 1. Solubility of benzocaine at several temperatures vs. dioxane volume fraction (*F*).

4. Results and discussion

4.1. Benzocaine

From the DSC analysis, benzocaine showed an endothermic peak at 90.2 °C corresponding to the fusion (363.4 K, enthalpy of fusion ΔH^F = 21 kJ/mol) and a wide endotherm at 281.7 °C (554.9 K) possibly due to decomposition. The thermograms of the solid phase after equilibration with the saturated dioxane–water solutions did not differ from that found for the original powder, that is, the solvent mixtures do not induce solid phase changes such as polymorphism or solvates. The literature does not make reference to polymorphism for benzocaine. Two polymorphic forms were reported for the derivative, benzocaine picrate, having melting points of 129 °C (a metastable form) and 162 °C [8,9]. The stable form can be obtained drying the metastable form at 105 °C [8]. The polymorphic forms of benzocaine picrate melt between 129 and 132 °C y 162–163 °C [10,11].

Table 1 lists the solubilities (molar concentration) and coefficients of variation (CV = standard deviation/mean expressed as percentage) of benzocaine at the temperatures studied. Solubility increased with temperature and a maximum was obtained at 90% dioxane in water at all temperatures (Fig. 1). The solubility profile is quite smooth between 5 and 20 °C. However, at and above 25 °C, a separation into two liquid immiscible phases was observed for the mixtures containing 50–60% dioxane in water (25 °C), 50–70% dioxane (30–35 °C) and 40–70% dioxane (40 °C). The boundary between the two liquid phases contained oily drops that were more apparent as the temperature is raised.

Table 2
Coefficients of third-degree polynomials on the volume fraction of dioxane for benzocaine at several temperatures

T (°C)	C_0	C_1	C_2	C_3	R^2
5	−6.109	9.488	6.000	−9.609	0.989
10	−5.818	10.398	2.064	−6.341	0.990
15	−5.531	10.007	1.827	−5.711	0.994
20	−5.455	10.896	−0.213	−4.449	0.994
25	−5.215	10.482	1.566	−5.762	0.999
30	−4.936	9.834	1.886	−5.535	0.999
35	−4.666	9.658	1.266	−4.869	0.998
40	−4.592	10.412	−0.555	−3.701	0.998

The logarithm of the experimental solubilities obtained (ln *M*, molar concentration) could be fitted to a third-degree polynomial on the volume ratio of the cosolvent (dioxane) in the solvent mixture. These functions illustrate the relation between the benzocaine solubility and the dioxane ratio (v/v) in the solvent mixture. Table 2 includes the coefficients of the equations obtained at each temperature. The predicted values for these functions are in good agreement with the experimentally obtained solubility values. The slopes of the vant'Hoff relationships (ln *M* versus 1/*T*, Eq. (1)) were negative, meaning that the heat of solution is endothermic. The straights lines were not parallel indicating that the apparent heat of mixing ($\Delta H^M = \Delta H^S - \Delta H^F$) changed with dioxane ratio because the heat of fusion (ΔH^F) of the solid phase at equilibrium with the saturated solutions did not differ from the values obtained for the original powder.

The apparent enthalpy of solution decreased as dioxane ratio increased from 0 to 60% (Fig. 2). Since solubility increased at these cosolvent ratios (Fig. 1), the mechanism is enthalpy driven. In the range of 60–90%, the enthalpy of solution increases and opposes the solubility enhancement. This indicated that the dominant mechanism changes and

Table 1
Solubility of benzocaine in dioxane–water mixtures showing a single phase

Dioxane (%, v/v)	Solubility (*M*)							
	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C
0	0.002 (0.50)	0.003 (0.36)	0.004 (0.29)	0.0041 (1.55)	0.0056 (0.22)	0.007 (0.25)	0.0097 (1.15)	0.01 (1.05)
10	0.005 (4.37)	0.0057 (0.86)	0.009 (1.88)	0.013 (0.39)	0.015 (0.66)	0.019 (0.36)	0.02 (0)	0.03 (0.24)
20	0.02 (2.60)	0.031 (0.37)	0.032 (0.30)	0.038 (0.26)	0.043 (0.84)	0.051 (0.45)	0.06 (1.56)	0.07 (0.36)
30	0.05 (0.47)	0.07 (4.55)	0.077 (0.93)	0.09 (0.41)	0.13 (0.19)	0.15 (0.16)	0.20 (0.45)	0.23 (0.58)
40	0.17 (0.65)	0.20 (0.18)	0.25 (0.74)	0.28 (0.22)	0.33 (0.67)	0.35 (0.14)	0.39 (0.67)	*
50	0.38 (0.39)	0.44 (0.67)	0.50 (0.15)	0.56 (0.001)	*	*	*	*
60	0.50 (0.26)	0.56 (0.69)	0.63 (0.12)	0.69 (1.10)	*	*	*	*
70	1.02 (0.30)	1.31 (0.73)	1.54 (0.21)	1.95 (0.17)	2.64 (0.28)	*	*	*
80	1.53 (0.64)	1.79 (0.32)	2.05 (2.01)	2.40 (0.38)	2.95 (0.33)	3.30 (0.17)	3.52 (0.21)	3.89 (0.49)
90	2.10 (2.61)	2.47 (2.53)	2.83 (0)	3.16 (2.58)	3.91 (0)	4.70 (1.05)	5.22 (0.25)	5.93 (0.46)
100	0.62 (0.94)	1.15 (0.54)	1.58 (0.40)	1.89 (3.11)	2.91 (0.88)	3.33 (0.69)	3.82 (0.77)	4.55 (0.42)

* Two liquid immiscible phases. The values in parenthesis are the coefficients of variation (CV, %) of three replicate experiments.

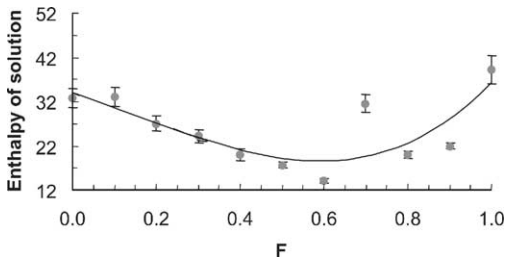

Fig. 2. Apparent molar enthalpy of solution vs. dioxane volume fraction (*F*, benzocaine).

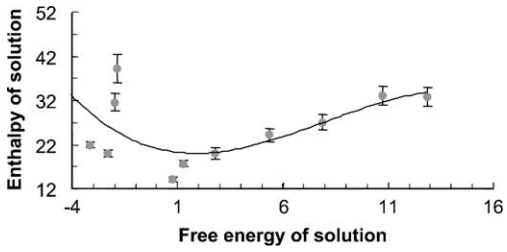

Fig. 3. Enthalpy–entropy compensation plot (benzocaine).

entropy decrease favored solubility. This fact was corroborated from the nonlinear enthalpy–entropy compensation pattern obtained (Fig. 3). The change of slope from negative to positive indicates two different dominant mechanisms that control solubility changes. Pure dioxane and 70% dioxane did not fit the general pattern showing ΔH^S values larger than expected. In addition, the 70% ratio was the upper limit of phase separation above 25 °C (Table 1). Then, between 60 and 70% dioxane, there were a strong solubility increase, and the solubility-temperature dependence was much larger at this ratio than for the remaining solvent compositions (Fig. 1). At the temperatures where no phase separation exits, the solubility increased by two- to three-fold from 60 to 70% dioxane.

Table 3 summarizes the composition of the upper and lower phases at each temperature. It includes the temperature, initial dioxane ratios (v/v) in the solvent mixtures, volume of the phases (ml), dioxane concentration (percentage)

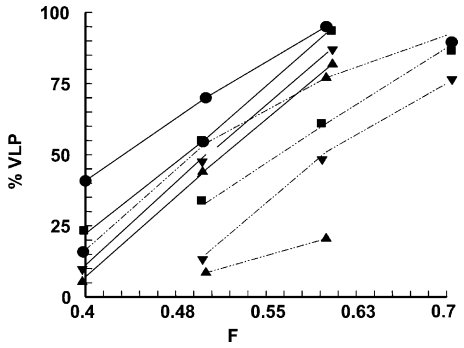

Fig. 4. Percent volume of the lower phase (VLP) against initial dioxane volume fraction (*F*). Key: - - - benzocaine, — salicylic acid at 25 °C (▲), 30 °C (▼), 35 °C (■) and 40 °C (●).

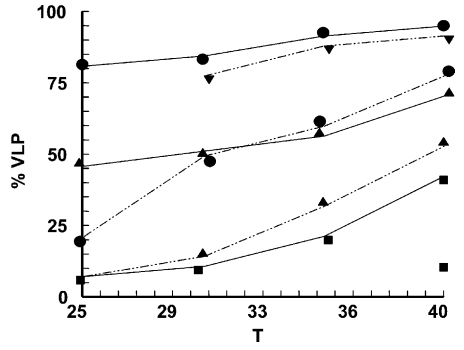

Fig. 5. Percent volume of the lower phase (VLP) vs. temperature. Key: - - - benzocaine and — salicylic acid at 40% (■), 50% (▲), 60% (●) and 70% (▼) initial volume fraction (*F*).

in the solvent mixture at each phase and the molar concentrations of benzocaine found at each phase. The less dense upper phase is water rich (37–50% v/v dioxane) whereas the lower, more dense phase, is dioxane and benzocaine rich (70–80% and 2.75–5.28 M, respectively).

Fig. 4 and Fig. 5 show that the volume percent of the lower phase (VLP) of benzocaine increased as the initial dioxane concentration and temperature increased. The upper phase showed the opposite trend. At constant temperature, both the dioxane ratio (v/v) in the mixtures and the concentration of

Table 3
Composition of the upper and lower phases of the benzocaine saturated solutions

T (°C)	Initial dioxane (%, v/v)	Phase volume (ml)		Dioxane (%, v/v) at each phase		Concentration of benzocaine (*M*)	
		Upper	Lower	Upper	Lower	Upper	Lower
25	50	43	4	48	79	0.13 (18.46)	2.95 (16.74)
25	60	31	8	58	70	0.14 (1.34)	2.75 (1.28)
30	50	36	0.5	47	73	0.15 (0.002)	3.07 (0.001)
30	60	22	23	50	72	0.17 (0.27)	2.93 (0.001)
30	70	10	45	62	73	0.22 (13.77)	3.31 (0.004)
35	50	32	16	41	78	0.24 (0.20)	3.52 (1.05)
35	60	20	31	43	78	0.25 (0.18)	3.12 (0.31)
35	70	10	70	46	77	0.29 (0.16)	3.57 (0.27)
40	40	35.5	7	38	75	0.22 (0.21)	4.40 (0.01)
40	50	28	33	39	72	0.27 (0.17)	4.21 (0.01)
40	60	15	50	38	72	0.32 (0.40)	4.11 (0.03)
40	70	6	70	37	71	0.36 (0.13)	5.28 (0.01)

The values in parenthesis are the coefficients of variation (CV, %) of three replicated experiments.

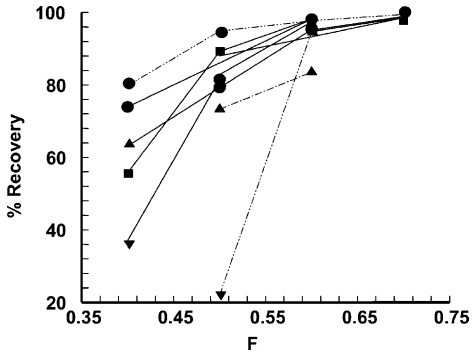

Fig. 6. Drug recovery vs. initial dioxane volume fraction (*F*). Key: - - - benzocaine, — salicylic acid at 25 °C (▲), 30 °C (▼), 35 °C (■) and 40 °C (●).

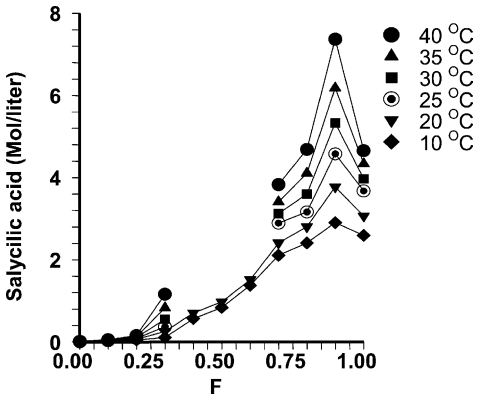

Fig. 7. Solubility of salicylic acid at several temperatures vs. dioxane volume fraction (*F*).

benzocaine dissolved tend to increase at the upper phase as the initial dioxane concentration increased (Table 3). These trends agree with the fact that solubility increased with dioxane ratio for the solutions were no phase separation exists (Fig. 1). The drug recovery (Eq. (3)) in the lower, drug-rich phase ranges from 73 to 99.5% and increases as the initial dioxane ratio becomes larger (Fig. 6). The only exception was observed for the 50% initial dioxane concentration at 30 °C where the recovery value obtained was lower (22%), possibly due to the small volume of the lower phase (Table 3). Temperature does not much influence the recovery value of benzocaine.

4.2. Salicylic acid

The DSC profile of salicylic acid showed two thermal effects. A first endotherm at 159.3 °C corresponding to the fusion (ΔH^F = 23.06 kJ/mol), and a second wide endotherm at 225.7 °C due possibly to decomposition. As found for benzocaine, the thermograms of the solid phase of salicylic acid at equilibrium with the dioxane-water saturated solutions did not change suggesting that the solvent mixtures did not induce polymorphic changes.

Table 4 lists the experimental solubilities of salicylic acid at several temperatures. The solubility profile was quite smooth below 25 °C (Fig. 7) but at and above 25 °C, it showed a break between 40 and 60% (v/v) dioxane due to the separation into two liquid immiscible phases. A solubility maximum at 90% dioxane in water was obtained at all temperatures. Paruta et al. [3] studied the solubility of salicylic acid at a single temperature (30 °C) as a function of the dielectric constant in several binary solvents systems. The cosolvent ratio at the maximum corresponds to a particular dielectric requirement whereas the magnitude of the peak depends on the nature of the solvent mixture. Paruta also observed phase separation at 30 °C in a dioxane–water mixture with salicylic acid dissolved. However, Gadalla et al. [12] did not describe this effect. The results obtained in the present paper confirm the Paruta findings and show that phase separation occurs at and above 25 °C at dioxane ratios in the range 40–60%. The boundary at the liquid phases showed oily droplets of smaller size than those observed for benzocaine. As for benzocaine this phase separation is not due to any solid phase change (polymorphism or solvates) because the thermograms of the solid phase after contact with the saturated solutions did not change. Therefore, phase separation was due to solute–solvent or solvent–solvent interactions. As contrasted with benzocaine, the initial dioxane concentration at which phase separation takes place did not

Table 4
Solubility of salicylic acid in dioxane–water mixtures showing a single phase

Dioxane (%, v/v)	Solubility (*M*)					
	10 °C	20 °C	25 °C	30 °C	35 °C	40 °C
0	0.008 (2.18)	0.011 (1.60)	0.012 (2.19)	0.015 (2.38)	0.017 (1.77)	0.020 (1.00)
10	0.021 (2.97)	0.029 (1.68)	0.039 (1.23)	0.041 (0.19)	0.047 (0)	0.058 (0.27)
20	0.049 (0.70)	0.071 (1.16)	0.087 (0.62)	0.107 (0.77)	0.127 (1.39)	0.158 (1.11)
30	0.111 (0.002)	0.258 (1.55)	0.373 (0.27)	0.559 (0.36)	0.832 (0.70)	1.166 (0.02)
40	0.570 (0.29)	0.706 (0.40)	*	*	*	*
50	0.841 (0.34)	0.976 (0.30)	*	*	*	*
60	1.381 (0.25)	1.517 (0.19)	*	*	*	*
70	2.111 (0.40)	2.411 (0.63)	2.896 (1.54)	3.127 (0.44)	3.412 (1.14)	3.837 (0.25)
80	2.411 (0.55)	2.808 (0.24)	3.168 (0.79)	3.605 (0.001)	4.109 (0.18)	4.689 (0.16)
90	2.906 (0.91)	3.779 (0.73)	4.582 (1.90)	5.332 (1.49)	6.183 (0.98)	7.374 (0.54)
100	2.596 (0.002)	3.065 (0.004)	3.680 (0.44)	3.974 (0.27)	4.340 (0.29)	4.659 (0.41)

* Two liquid immiscible phases. The values in parenthesis are the coefficients of variation (CV, %) of three replicated experiments.

Table 5
Coefficients of second-degree polynomials on the volume fraction of dioxane for salicylic acid at several temperatures

T (°C)	C_0	C_1	C_2	R^2
10	−5.085	13.164	−7.053	0.989
20	−4.671	12.892	−7.066	0.995
25	−4.504	12.891	−7.052	0.995
30	−4.313	13.250	−7.553	0.991
35	−4.146	13.600	−8.01	0.983
40	−3.960	13.933	−8.460	0.977

vary with temperature. Salicylic acid interfered with the Karl Fisher reactant giving a pink color that makes it difficult to observe the color change as the reactant is added to the samples. Therefore, the water content could not be determined with precision from this technique. However, an approximate solvent composition range could be estimated from the solubilities experimentally obtained for salicylic acid in each phase.

To obtain the functional relationship between the salicylic acid solubility and the dioxane ratio (v/v), the experimental solubility data (logarithm of molar concentration) were fitted on the volume ratio of dioxane in the mixtures. Table 5 lists the coefficients of the best equation, a quadratic expression, at each temperature.

As for benzocaine, the slopes obtained from Eq. (1) are negative indicating endothermic enthalpies of solutions. When the enthalpy of fusion is subtracted from the enthalpy of solution some of the resulting enthalpies of mixing are exothermic due to favorable solute–solvent interactions (possibly hydrogen bonding in these mixtures). The high magnitude of the enthalpy of fusion is responsible for the endothermic heats of solution obtained. Fig. 8 shows the apparent enthalpy of solution changes as a function of dioxane ratio. The values ranged from 57.94 to 6.44 kJ/mol. There is a large enthalpy increase from 23.43 kJ/mol (water) to 57.94 kJ/mol (30% dioxane). At the region corresponding to phase separation (40–60% dioxane), the sign of the slope changed and the enthalpy decreased abruptly (from 57.94 to 14.93 kJ/mol). The slope was again positive (enthalpy increase) above 70% dioxane where a single phase exists.

The enthalpy–entropy compensation plot also shows a complex pattern with a change of slope from positive to negative (enthalpy decreases as free energy increases) at the region where the liquid phase separation occurred (Fig. 9). The positive slopes below 30% and above 60% dioxane (the region of a single phase) agree with the increasing free-energy values (unfavorable). However, at the phase separation region the increase of free energy is accompanied by an enthalpy decrease (favorable). This means that the solubility process is enthalpy controlled at the region of a single phase and entropy controlled at the region of phase separation. Hydrophobic effect may be related to the phase separation process.

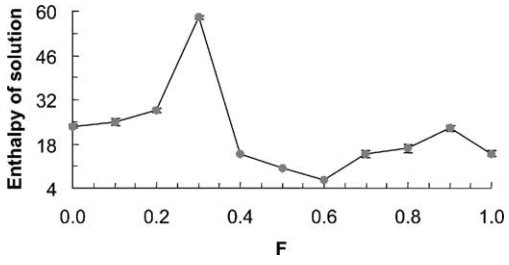

Fig. 8. Apparent molar enthalpy of solution vs. dioxane volume fraction (F, salicylic acid).

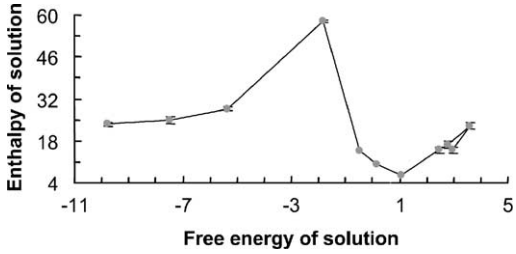

Fig. 9. Salicylic acid. Enthalpy–entropy compensation plot.

Table 6 gives the composition of the liquid phases for salicylic acid. The lower denser phase was both dioxane and drug rich whereas the upper less dense phase was water rich. As for benzocaine, the percent volume of the lower phase tends to increase with both temperature and initial dioxane ratio (Figs. 4 and 5) and those of the upper phase showed the opposite trends. The concentrations of salicylic acid dissolved at each phase did not much differ with respect to the initial dioxane ratio and temperature (Table 6). The drug extraction recovery (Eq. (3)) in the lower, rich-drug phase varied between 63 and 97% and increased as the initial dioxane ratio became larger, except for the 40% initial dioxane ratio at 30 °C where the recovery value is 37% (Fig. 6). As was also found for benzocaine, most of drug is extracted in the lower phase. Temperature does significantly influence the recovery value of salicylic acid.

Salicylic acid and benzocaine are not structurally related but both drugs have relatively low melting points that, in addition to solute–solvent interactions, may be related with their much higher solubilities in dioxane–water mixtures when compared with other organic drug molecules [13]. The information on phase separation of dioxane–water mixtures produced by organic solutes is limited. Takamuku et al. [1] reported that phase separation of dioxane–water mixtures was induced by NaCl in a range of $0.1 < X_{\text{diox}} \leq 0.70$ 1,4-dioxane (mole fraction units). Using large-angle X-ray scattering, mass spectrometry and NMR relaxation studied, three types of structures of neat dioxane–water mixtures (without solute dissolved) were proposed [1]:

(1) at $0.3 \leq X_{\text{diox}} < 1$, the neat structure of 1,4-dioxane remains, and water molecules are possibly involved in the structure by hydrogen bonding;
(2) at $0.15 \leq X_{\text{diox}} < 0.2$, both structures of water and 1,4-dioxane are disrupted to form small binary aggregates of one or two 1,4-dioxane molecules and several water molecules;

Table 6
Composition of the upper and lower phases of the salicylic acid saturated solutions

T (°C)	Initial dioxane (%, v/v)	Phase volume (ml)		Dioxane (%, v/v) at each phase		Concentration (M) of salicylic acid	
		Upper	Lower	Upper*	Lower*	Upper	Lower
25	40	45	15	20–30	70–80	0.54 (0.16)	2.77 (0.14)
25	50	35	28	20–30	70–80	0.54 (0)	2.58 (0.34)
25	60	8	35	20–30	70–80	0.54 (0.41)	2.49 (0.72)
30	40	40	5	20–30	70–80	0.54 (0.46)	2.54 (0.15)
30	50	25	25	20–30	70–80	0.54 (0.09)	2.38 (0.16)
30	60	8	40	20–30	70–80	0.57 (0.18)	2.42 (0.16)
35	40	35	10	20–30	70–80	0.54 (0)	2.38 (0)
35	50	20	35	20–30	70–80	0.55 (0.37)	2.60 (0.16)
35	60	4	50	20–30	70–80	0.56 (0.09)	2.40 (0.17)
40	40	32	22	30–40	70–80	0.55 (0)	2.27 (0.14)
40	50	30	30	30–40	70–80	0.56 (0.09)	2.71 (0.16)
40	60	6	52	30–40	70–80	0.56 (0.004)	2.4 (0.16)

* An approximate dioxane concentration (v/v) range in the solvent mixture at each phase was estimated from the concentration of salicylic acid dissolved. The values in parenthesis are the coefficients of variation (CV, %) of three replicated experiments.

(3) at $X_{diox} \leq 0.1$, the hydrogen-bonded network of water is dominant.

Fig. 10 shows, as a sample, the ternary phase diagram of benzocaine at 20, 30 and 40 °C, expressed as percent mole fraction of the three components. The solid line limits the two-phase region (at the right and above the line) that occurs at temperatures above 20 °C. At and below this temperature (bottom and left par of the diagram), a single phase remains. The broken lines joint the solubilities of benzocaine (single phase region), that increase from 20 to 40 °C. Table 7 includes the dioxane mole fractions at which phase separation took place for benzocaine and salicylic acid. At these ranges, type (2) structures of the solvent mixture are dominant, as was

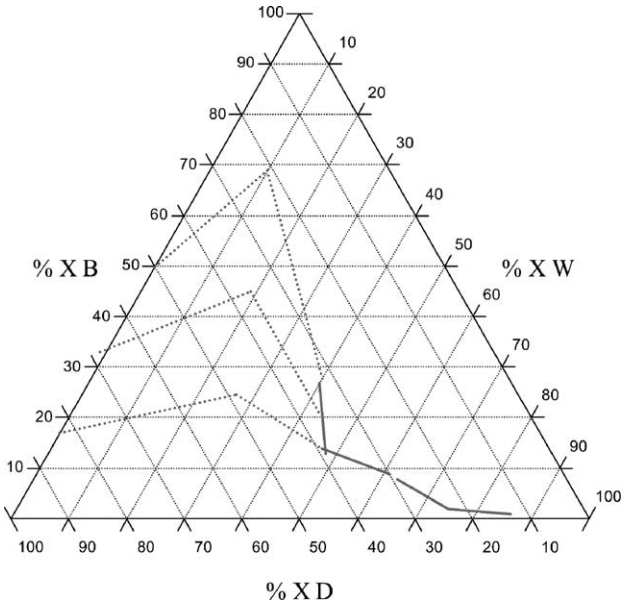

Fig. 10. Phase diagram for the ternary system benzocaine (B) dioxane (D) and water (W) (percent mole fraction X). The broken lines represent the solubility of benzocaine at (■) 20 °C, (◯)30 °C and (□) 40 °C. The solid line limits the region of phase separation.

Table 7
Mole fraction dioxane ranges in the solvent mixtures where phase separation is produced

Temperature (°C)	Mole fraction dioxane intervals	
	Benzocaine	Salicylic acid
25	$0.17 \leq X_{diox} \leq 0.24$	$0.12 \leq X_{diox} \leq 0.17$
30	$0.17 \leq X_{diox} \leq 0.33$	$0.12 \leq X_{diox} \leq 0.17$
35	$0.17 \leq X_{diox} \leq 0.33$	$0.12 \leq X_{diox} \leq 0.17$
40	$0.12 \leq X_{diox} \leq 0.33$	$0.12 \leq X_{diox} \leq 0.17$

also found in the NaCl-induced phase separation [1]. This suggests that the solvent structure plays an important role in the phase separation phenomena induced by both NaCl and organic molecules, although the inorganic and organic solutes differ in the affinity by the water-rich and dioxane-rich phases. Thus solute–solvent interactions are larger between water and NaCl, and the denser phase at the bottom is both NaCl- and water-rich. On the contrary, the affinity of benzocaine and salicylic acid by the organic solvent (dioxane) is higher and the denser, lower phase, is both drug- and dioxane-rich. Among the intermolecular interactions, hydrogen bonding may play an important role in the phase separation phenomena. Salicylic acid has two possible hydrogen bonding groups (COOH and OH) whereas benzocaine has the amine NH_2 group available for hydrogen bonding.

At dioxane mole fractions below 0.1, the type (3) structure of the solvent mixture is dominant, and the highly ordered water structure is responsible for the low solubility of both drugs in water. In mole fraction units, X_{drug} = 0.001–0.002 for benzocaine and X_{drug} = 0.001–0.003 for salicylic acid, from the lower to the highest temperature. As dioxane is added, the water structure becomes less ordered favoring drug-solvent hydrogen bonding that increases solubility. At the phase separation region ($0.12 \leq X_{diox} < 0.36$, Table 7) the drug–dioxane interactions overcome the solvent–solvent interactions between the small type (2) water–dioxane aggregates, disrupting the solvent structure. Water is squeezed out and a denser phase, dioxane-rich and drug-rich separates at

the bottom. The disruption of the solvent structure requires heat and the process is favored by increasing temperature ($T \geq 25^{\circ}$). At dioxane mole fractions larger than 0.33 (Table 7), a single phase appears again. Although more drug molecules are present (the solubility of both drugs increases), the neat structure of dioxane remains (type 3 structure) and the water molecules, being less ordered, can accommodate in the drug–dioxane hydrogen bonding structures forming again a single phase. The small differences between the dioxane ratios at which phase separation appears may be related to the different hydrogen bonding ability of the functional groups of each drug. Benzocaine has a single hydrogen-bonding group and the heat evolved after hydrogen bonding with the solvent should be smaller, requiring more heat to disrupt the solvent structure, than for salicylic acid which has two hydrogen bonding groups. Thus, for benzocaine, the dioxane range for phase separation increases with temperature, a fact that is not observed for salicylic acid.

5. Conclusion

The recovery achieved in the lower phase is of about 97 and 99% for salicylic acid and benzocaine, respectively, for initial dioxane ratios between 60 and 70%. The pre-concentration factors are high for both drugs being of about three and eight times at 25 °C and 30 °C (Table 6) for salicylic acid at 40% initial dioxane ratio. In the case of benzocaine, the pre-concentration factor is approximately 11 times that at 25 °C and 50% of the initial dioxane ratio (Table 3). This, together with recovery values near 100%, allows us to suggest that this phase separation could be applicable to purify and concentrate these products in a previous stage before analytical determination.

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References

[1] T. Takamuku, A. Yamaguchi, D. Matsuo, M. Tabata, T. Yamaguchi, T. Otomo, T. Adachi, J. Phys. Chem. B 105 (2001) 10101–10110.
[2] D. Sicilia, S. Rubio, D. Pérez-Bendito, Anal. Chim. Acta 460 (2002) 13–22.
[3] A.N. Paruta, B.J. Sciarrone, N.G. Lordi, J. Pharm. Sci. 53 (1964) 1349–1353.
[4] A.N. Paruta, B.J. Sciarrone, N.G. Lordi, J. Pharm. Sci. 54 (1965) 838–841.
[5] A.N. Paruta, J. Pharm. Sci. 58 (1969) 204–206.
[6] A.N. Paruta, J. Pharm. Sci. 58 (1969) 364–366.
[7] R.R. Krug, W.G. Hunter, R.A. Grieger, J. Phys. Chem. 80 (1976) 2341–2351.
[8] L. Borka, Acta Pharm. Suec. 11 (1974) 295–303.
[9] K.C. Lee, J.A. Hersey, Acta Pharm. Suec. 4 (1977) 213–216.
[10] T.K. Nielsen, L. Borka, Acta Pharm. Suec. 9 (1972) 503–512.
[11] L. Borka, L.y.M. Kuhnert-Brandstäter, Arch. Pharm. 307 (1974) 377–385.
[12] M.A.F. Gadalla, G.M. Gaily, W. Samara, Int. J. Pharm. 38 (1987) 71–78.
[13] P. Bustamante, S. Romero, A. Peña, B. Escalera, A. Reíllo, J. Pharm. Sci. 40 (1989) 265–271.