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Multiple Solubility Maxima of Oxolinic Acid in Mixed Solvents and a New Extension of Hildebrand Solubility Approach

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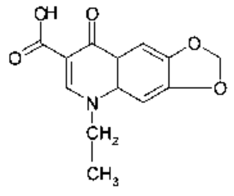
A new extension of the Hildebrand solubility approach which describes drug solubility in solvent mixtures showing multiple solubility peaks, the chameleonic effect, is proposed. The experimental solubilities of oxolinic acid were measured at 25 °C in solvent mixtures of ethanol–water and ethanol–ethyl acetate. A plot of the mole fraction of the drug against the solubility parameter (δ) of the solvent mixtures displays two peaks at δ=30.78 MPa$^{1/2}$ (80% v/v of ethanol in water) and at δ=20.90 MPa$^{1/2}$ (30% v/v of ethanol in ethyl acetate). The new extension proposed reproduces two solubility peaks. The thermograms of the solid phase before and after equilibration with the solvent mixtures did not show significant changes. The new extension was also tested with experimental data previously reported for drugs showing two solubility peaks of different height. The accuracy of other published models for describing two solubility maxima is also compared.

Key words multiple solubility maxima; hildebrand solubility approach; oxolinic acid; chameleonic effect; mixed solvent

Oxolinic acid possesses antibacterial effects against Gram-negative organisms. Its bactericidal action is similar to that of nalidixic acid. Oxolinic acid is used in the treatment of urinary tract infections due to Gram-negative organisms other than Pseudomonas species.[1)] Very low solubility of oxolinic acid restricts its therapeutic use. Its aqueous solubility at 20 °C is $10\pm0.5\,\text{mg}\,\text{l}^{-1}$ and the solubility of its salt form is $20.5\pm0.5\,\text{mg}\,\text{l}^{-1}$ at 20 °C.[2)]

Solubility determination of drugs in water–cosolvent mixtures provides useful data for better understanding of the solubility phenomenon in these media. In addition to the theoretical applications of the results of these determinations, the data can be employed for building the mathematical models and optimising the appropriate concentration of the pharmaceutical cosolvents for preparing liquid pharmaceutical preparations. In this study we focused on the theoretical applications of the cosolvency phenomenon.

The main aim of solubility data modelling is to provide a mathematical equation to represent the experimental results. By using these equations, it is possible 1) to report the data as a few model parameter values rather than presenting in a tabular form, 2) to screen the experimental data to detect the possible outliers, 3) to explore basic mechanisms of the solubility phenomenon and, finally, 4) to predict future results by the model parameters calculated based on training data set. In the pharmaceutical area, there has been considerable interest in forming a mathematical representation of solubility data in mixed solvents and several models have been presented over the past two decades.[3—6)] One of the most popular models for this purpose is the extended Hildebrand solubility approach (EHSA), which was proposed by Martin and co-workers. This model is able to calculate solubility data of drugs in water–cosolvent mixtures showing no peaks or a single maximum.[3,7)] However, EHSA cannot predict drug solubility in solvent mixtures displaying multiple solubility peaks.[8,9)]



Chemical structure of oxolinic acid

The aim of this paper is to propose a new extension of the Hildebrand solubility approach for calculating solubility in mixed solvents showing multiple peaks and to compare the accuracy of the proposed model with that of previously published models.

Theoretical Treatment The EHSA introduced an interaction term W to replace the product $\delta_1\delta_2$ of the Hildebrand equation[3,7)]:

$$-\ln X_2=-\ln X_2^{\mathrm{i}}+\frac{V_2\phi_1^2(\delta_1^2+\delta_2^2-2W)}{RT} \qquad (1)$$

where X_2 represents the mole fraction solubility of a drug in mixed solvent, X_2^{i} stands for the ideal solubility of the solute, V_2 is the molar volume of the solute, ϕ_1 denotes the volume fraction of the solvent, R is the molar gas constant, T is the absolute temperature, and δ_1 and δ_2 are the solubility parameters of the solvent and the solute. The term W is an adhesion parameter which represents solute–solvent interactions and is correlated by a power series of solubility parameters of the mixed solvent[3)]:

$$W=P_0+P_1\delta_1+P_2\delta_1^2+\ldots+P_n\delta_1^n \qquad (2)$$

The solubility parameters of the mixed solvent (δ_1) are calculated from:

$$\delta_1=\sum f_i\delta_i \qquad (3)$$

where δ_i is the solubility parameter of the pure solvents and f_i is their volume fraction in the mixture.

Rearranging Eq. 1, $\ln \alpha_2/A$ can be also regressed against a polynomial in δ_1, where α is the activity coefficient defined as the ratio of the ideal to the experimental solubility (X_2^{i}/X_2), and A is $\frac{V_2\phi_1^2}{RT}$. Martin *et al.*[3)] also showed that $\ln X_2$ can be regressed on a polynomial in the volume fraction of the cosolvent. This was discussed in more detail by Barzegar-

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Jalali and Jouyban-Gharamaleki.[6)]

The extended Hildebrand method (Eqs. 1 and 2) is not able to reproduce two solubility peaks, the so-called chameleonic effect. Bustamante and co-workers[8,9)] described this effect for the first time in a quantitative way using partial solubility parameters related to Lewis acid–base interactions:

$$\ln X_2=C_0+C_1\delta_1+C_2\delta_1^2+C_3\delta_{1a}+C_4\delta_{1b}+C_5\delta_{1a}\delta_{1b} \tag{4}$$

where C_0—C_5 are the model constants, and δ_{1a} and δ_{1b} are the acidic and basic partial solubility parameters which are calculated from the volume fraction of the cosolvent as shown in Eq. 3. Equation 4 predicts two solubility peaks as found experimentally and explains the chameleonic effect in terms of differences of Lewis acid–base interactions of the solvent mixtures. Jouyban-Gharamaleki and Barzegar-Jalali[10)] then proposed a computer optimised model to calculate the solubility of solutes with two solubility peaks, using ratios of partial solubility parameters.

With the EHSA, one can predict the solubility of compounds showing multiple solubility maxima provided that some modifications are made. For two binary systems with a common solvent or for actual ternary systems, the δ_1 values in Eq. 2 are calculated by Eq. 5:

$$\delta_1=f_a\delta_{1(\text{solvent a})}+f_b\delta_{1(\text{solvent b})}+f_c\delta_{1(\text{solvent c})} \tag{5}$$

where $f_a+f_b+f_c=1$. By substitution of f_b with $1-f_a-f_c$ in Eq. 5:

$$\delta_1=f_a\delta_{1(\text{solvent a})}+(1-f_a-f_c)\,\delta_{1(\text{solvent b})}+f_c\delta_{1(\text{solvent c})} \tag{6}$$

Rearrangements in this equation yield:

$$\delta_1=f_a\delta_{1(\text{solvent a})}+\delta_{1(\text{solvent b})}-f_a\delta_{1(\text{solvent b})}-f_c\delta_{1(\text{solvent b})}+f_c\delta_{1(\text{solvent c})} \tag{7}$$

$$\delta_1=\delta_{1(\text{solvent b})}+f_a(\delta_{1(\text{solvent a})}-\delta_{1(\text{solvent b})})+f_c(\delta_{1(\text{solvent c})}-\delta_{1(\text{solvent b})}) \tag{8}$$

Substitution of δ_1 values from Eq. 8 into Eq. 2 yields:

$$\begin{aligned}W'=&P_0+P_1\{\delta_{1(\text{solvent b})}+f_a(\delta_{1(\text{solvent a})}-\delta_{1(\text{solvent b})})+f_c(\delta_{1(\text{solvent c})}\\&-\delta_{1(\text{solvent b})})\}+P_2\{[\delta_{1(\text{solvent b})}+f_a(\delta_{1(\text{solvent a})}-\delta_{1(\text{solvent b})})\\&+f_c(\delta_{1(\text{solvent c})}-\delta_{1(\text{solvent b})})]^2\}+\dots\end{aligned} \tag{9}$$

Appropriate rearrangements in this equation, considering δ_1 values for pure solvents as constant figures and deleting f_af_c terms yield:

$$W'=L_0+L_1f_a+L_2f_c+L_3f_a^2+L_4f_c^2+\dots \tag{10}$$

in which W' stands for the interaction term and L_0—L_4 are the model constants. In all solvent mixtures which exhibited multiple solubility peaks, the value of f_a or f_c is zero, thus all terms containing f_af_c are omitted from the equation. It should be noted that for 4 component solvent mixtures, *e.g.* water–ethanol, ethanol–ethyl acetate and ethyl acetate–hexane, the corresponding W' equation includes the additional terms f_d (volume fraction of hexane) and f_d^2.

The δ_2 value is a characteristic of the solute, therefore omitting δ_2^2 from Eq. 1 and assuming the values of ϕ_1 to be equal to 1,[11)] the experimental values of W' can be calculated by Eq. 11. It should be noted that the solute solubility parameter is considered in the constant term of Eq. 10, *i.e.* L_0 term.

$$W'=\left[\frac{(\ln X_2-\ln X_2^i)RT}{2V_2}\right]+\left[\frac{\delta_1^2}{2}\right] \tag{11}$$

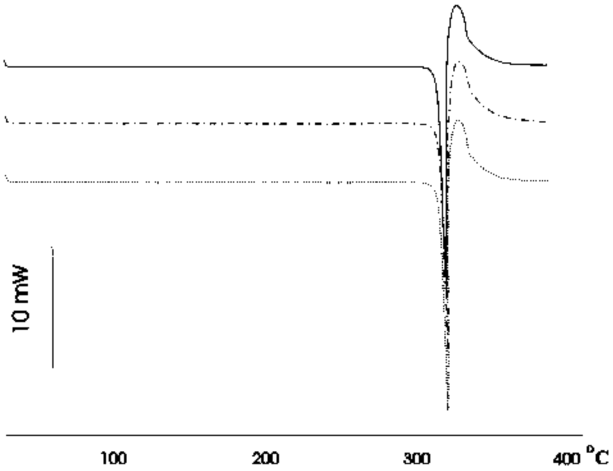

Fig. 1. DSC Profile of Oxolinic Acid, (—) Original Powder, Solid Phase after Equilibration with 50% v/v Ethanol in Water (-----) and 50% v/v Ethyl Acetate in Ethanol (······)

The term W' should not be confused with W. In the original EHSA, W is an adhesion term for the solute–solvent interaction whereas W' includes the adhesion term plus a cohesion term, the square of the solubility parameter of the solute, δ_2^2.

To compare the accuracy of the models, percent mean error, PME, was calculated using Eq. 12:

$$\text{PME}=\left(\frac{100}{N}\right)\sum_1^N\left(\frac{\left|X_m^{\text{calculated}}-X_m^{\text{observed}}\right|}{X_m^{\text{observed}}}\right) \tag{12}$$

where N denotes the number of experimental data points in each set. The average percent mean error (APME) was considered as an overall judgement criterion to compare the accuracy of the models.

Materials and Methods

Oxolinic acid was purchased from Sigma. The solvents used were ethyl acetate, ethanol (spectrophotometric grade, Panreac, Monplet and Esteban, Barcelona, Spain) and double distilled water.

Solubility Measurements Sealed flasks containing an excess of powder in the pure solvents and solvent mixtures were shaken at 25±0.1 °C in a temperature-controlled bath (Heto SH 02/100). The dissolution curves of drug dissolved *versus* time were studied. When the saturation concentration was attained, the solid phase was removed by filtration (Durapore membranes, 0.2 μm pore size). The drug did not significantly adsorb onto the membranes. Separate experiments (sedimentation, centrifugation) gave similar results to those obtained from filtration. The clear solutions were diluted with ethanol 96% v/v and assayed in a double beam spectrophotometer (Bausch Lomb 2000). The densities of the solutions were determined at 25+0.1 °C in a 10 ml pycnometer. All the experimental results are the average of at least three replicated experiments. The coefficient of variation (CV=S.D./mean×100) is within 2% among replicated samples.

Differential Scanning Calorimetry The thermograms of oxolinic acid were obtained with a differential scanning calorimeter (Mettler TA 4000). The melting point and the heat of fusion were measured in triplicate. Samples of 5—6 mg in perforated aluminium pans were heated at a rate of 5 °C/min under nitrogen purge. The temperature range studied was 30—350 degrees.

Thermograms of the solid phase after equilibration with the pure solvents and several solvent mixture ratios were also obtained to detect possible changes in the solid phase. The solvent excess was evaporated at room temperature until it reached a constant weight.

Results and Discussion

The molar enthalpy of fusion of oxolinic acid was $\Delta H^F=166.7$ J/g, and the temperature of fusion is $T^F=319.3$ °C.

Neither decomposition nor polymorphic changes was observed at the experimental temperature range. The thermograms did not vary after equilibration of the solid phase with the solvent systems (Fig. 1). Therefore, the presence of two solubility peaks could not be related to thermal changes of the solid phase and it can be attributed to the different hydrogen bonding ability of the two solvent mixtures.

The mole fraction solubilities of oxolinic acid at 25 °C in the two solvent mixtures which cover a large range of the solubility parameter scale, from 18 to 48 $(\mathrm{MPa})^{1/2}$, are listed in Table 1. Figure 2 displays the experimental solubility (mole fraction units) against the solubility parameter of the solvent mixtures. Oxolinic acid shows chameleonic behavior, *i.e.* two solubility peaks. The solubility of the drug increases from 100% water ($\delta_1 = 47.97\ \mathrm{MPa}^{1/2}$) to reach a maximum at 80% v/v ethanol in water. Larger ethanol concentrations decrease the solubility. Addition of ethyl acetate to ethanol lowers the polarity of the solvent enhancing the solubility of oxolinic acid to reach a second maximum at 30% v/v ethanol in ethyl acetate ($\delta_1 = 20.9\ \mathrm{MPa}^{1/2}$) which is higher than that found in ethanol–water ($\delta_1 = 29.73\ \mathrm{MPa}^{1/2}$). A possible explanation for the two solubility peaks is that the possibility of dimerisation of the solute increases in less polar solvents. Therefore, we have two different solubility parameters for the solute. The solubility parameter comes from the Hildebrand solubility approach, *i.e.* Eq. 1 with $W = \delta_2 \delta_1$, and originally was developed for simple non-associated liquid mixtures. This approach has been applied for non-polar solids by assuming these chemicals as supercooled liquids. The solute's solubility parameters can be calculated using different methods including solubility data.[12)] Although different methods for determining the solute's solubility parameter give slightly different results, this parameter has been applied in various fields of pharmaceutical sciences such as solubility,[13)] drug binding to plasma proteins[14)] and tablet technology.[15)] More details of pharmaceutical applications of the solubility parameter were recently reviewed by Hancock *et al.*[16)]

Table 1. Experimental Solubility (Mole Fraction) of Oxolinic Acid in Ethanol–Water and Ethyl Acetate–Ethanol at 25 °C[a)]

	δ_1	δ_{1a}	δ_{1b}	X_2
% v/v Ethanol				
0	47.86	13.71	65.46	10785×10^{-6}
10	45.73	14.03	60.04	29211×10^{-6}
20	43.59	14.36	54.62	53991×10^{-6}
30	41.46	14.69	49.19	74102×10^{-6}
40	39.32	15.01	43.77	99686×10^{-6}
50	37.19	15.34	38.35	12658×10^{-5}
60	35.05	15.67	32.93	16336×10^{-5}
70	32.92	16.00	27.51	20166×10^{-5}
80	30.78	16.32	22.09	20990×10^{-5}
90	28.64	16.65	16.67	16879×10^{-5}
100	26.51	16.98	11.25	82357×10^{-6}
% v/v Ethyl acetate				
10	25.71	16.36	10.51	11781×10^{-5}
20	24.91	15.75	9.78	16534×10^{-5}
30	24.10	15.14	9.04	22560×10^{-5}
50	22.50	13.91	7.57	34887×10^{-5}
60	21.70	13.30	6.83	38134×10^{-5}
70	20.90	12.68	6.10	41588×10^{-5}
90	19.29	11.46	4.62	34276×10^{-5}
100	18.49	10.84	3.89	24502×10^{-5}

a) δ_1; total solubility parameter of the solvent mixtures, δ_{1a} and δ_{1b}; acidic and basic partial solubility parameters, X_2; the mole fraction solubility of the drug.

As for sulfonamides,[8,9)] the solubility curve is asymmetrical, the solvent power of the less polar mixture being larger. In contrast, the solubility peaks of paracetamol[17)] are of similar height in both mixtures. The location and height of the peaks could be related to the polarity of the solute. The highest ratio at the solubility maximum in ethanol–water to ethanol–ethyl acetate corresponds to paracetamol, which shows a solubility parameter larger than that for sulfonamides.

The extended Hildebrand method is applied to the experimental data of oxolinic acid (Eq. 2) and $\ln X_2$ is back calculated by Eq. 11. This method was not able to calculate the two solubility peaks, as shown in Fig. 2. In contrast, the modification proposed in this work (Eqs. 10 and 11) reproduces these two peaks (Fig. 2). The statistical significance of the curve-fit parameters (the *L* terms) was evaluated by using *t*-test and all parameters were significant ($p < 0.05$).

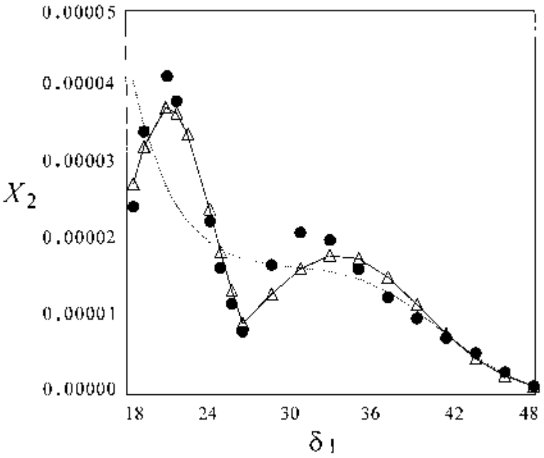

Fig. 2. Experimental Solubility (Mole Fraction) of Oxolinic Acid in Ethanol–Water and Ethyl Acetate–Ethanol Mixtures at 25 °C

(●) Experimental solubility values. Calculated solubility values: (······) with Eq. 2, (△) with Eq. 4 and (—) with Eq. 10.

Table 2. The Model Constants and Statistical Parameters for Eqs. 10 and 11

No.[a)]	L_0[b)]	L_1[b)]	L_2[b)]	L_3[b)]	L_4[b)]	r^2[c)]	F[d)]	PME[e)]	N[f)]
1	341.896	595.283	−170.467	169.773	−7.540	1.000	344383	7.75	21
2	345.674	605.847	−174.145	142.195	—[g)]	1.000	331830	9.23	24
3	336.890	590.174	−177.820	175.494	—[g)]	1.000	276070	13.69	26
4	359.4063	581.178	−183.731	168.665	−17.780	1.000	105238	12.73	25
5	332.454	596.615	−181.959	182.067	9.649	1.000	304421	11.87	19

a) Numbers 1—5 represent sulphanilamide,[9)] sulphamethazine,[9)] sulphamethoxypyridazine,[8)] paracetamol[17)] and oxolinic acid dissolved in water–ethanol and ethanol–ethyl acetate mixtures. *b*) The numerical values of the curve-fit parameters (L_0—L_4) were computed by fitting the experimental values of W' using Eq. 10. *c*) r^2 is the coefficient of determination and $r^2 = 1.000$ means $r^2 > 0.999$. *d*) F is the calculated value of F-test which was used to assess the significance of the correlation. *e*) PME is the percent mean error which is calculated by Eq. 12. *f*) N is the number of data points in each data set. *g*) These parameters are not significant ($p > 0.05$).

Table 3. The Percent Mean Error (PME) and Average Percent Mean Error (APME) Values for Different Models

No.[a]	Eq. 4	Eq. 10	EFE	CNIBS/R-K
1	8.39[b]	7.75	2.65[c]	2.56[c]
2	11.29[b]	9.23	7.30[c]	4.53[c]
3	18.19[b]	13.69	2.42[c]	2.47[c]
4	12.31[b]	12.73	8.92[c]	5.32[c]
5	11.81	11.87	8.05	1.03
APME	12.40	11.05	5.87	3.18
No. of constant terms	6	6	7	9

a) The key to the drugs is the same as Table 2. *b*) PME values are calculated by employing the model constants taken from the references.[8,9,17] *c*) PME values are taken from the references.[18,19]

In addition to the data of oxolinic acid, four solubility data sets showing two maxima of different heights cited in the literature[8,9,17] are fitted to Eqs. 10 and 11. The coefficients and PME values obtained are shown in Table 2. In all cases, these equations reproduce the two solubility peaks. As mentioned in the theoretical section, one can use another version of the proposed extension, *i.e.* Eq. 10, for correlating solute solubility in higher order multicomponent mixed solvents. In an earlier paper, Escalera *et al.*[8] reported a wider solvent polarity range using water–ethanol, ethanol–ethyl acetate and ethyl acetate–hexane mixtures. This mixture provides a solvent media covering the solubility parameter ranging from 14.9 to 47.8 MPa$^{1/2}$. The corresponding equation for expressing the W' term for the solubility of sulphamethoxypyridazine in the solvent mixture is:

$$W' = 336.879 + 590.229 f_a - 177.772 f_c - 283.945 f_d + 175.452 f_a^2 - 16.411 f_d^2 \quad (13)$$

$$r^2 = 1.000,\ F = 189949,\ p < 0.0005,\ \mathrm{PME} = 13.79,\ N = 29$$

All the model constants in Eq. 13 are statistically significant at $p < 0.004$.

Two theoretical cosolvency models for reproducing multiple solubility maxima have been shown in recent papers.[18,19] Williams and Amidon[4] proposed an excess free energy (EFE) approach to estimate solute solubility in ternary solvent mixtures. This model was applied with modifications for reproducing multiple solubility peaks in mixed solvents.[18] An extension of the combined nearly ideal binary solvent/Redlich-Kister (CNIBS/R-K) was also employed to describe the solubility profile of solvent mixtures showing two solubility peaks.[19] Table 3 summarises the PME values and the number of constant terms of the models describing two solubility maxima in solvent mixtures. The CNIBS/R-K model produced the most accurate results. Our recent study showed that this is also the most accurate model for calculating solute solubility data in binary mixtures.[20]

Whilst considering the number of constant terms, as shown in Table 3, the more constant the terms the more accurate the predictions. However, in terms of Eqs. 4 and 10 containing an equal number of constant terms, the order of accuracy is Eq. 10>Eq. 4.

Although the original EHSA equation cannot reproduce the solute solubility data in mixed solvents showing two maxima (Fig. 1), the results show that the modified EHSA is able to produce two solubility peaks. Of the theoretical models, EFE and CNIBS/R-K, produced the most accurate results, although the intercept is not significant statistically in the case of oxolinic acid.

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References

1) Martindale the Extra Pharmacopoeia 28th ed. by Reynolds E. F., Prasad A.B., The Pharmaceutical Press, London, 1982, pp. 1052—1053.
2) Brazier M., Julien R., Ceolin R., Robert H., Khodadad P., *Pharm. Acta Helv.*, **66**, 226—229 (1991).
3) Martin A., Wu P. L., Adjei A., Lindstrom R. E., Elworthy P. H., *J. Pharm. Sci.*, **71**, 849—856 (1982).
4) Williams N. A., Amidon G. L., *J. Pharm. Sci.*, **73**, 9—13 (1984).
5) Acree W. E., Jr., *Thermochim. Acta*, **198**, 71—79 (1992).
6) Barzegar-Jalali M., Jouyban-Gharamaleki A., *Int. J. Pharm.*, **152**, 247—250 (1997).
7) Martin A., Wu P. L., Velasquez T., *J. Pharm. Sci.*, **74**, 277—282 (1985).
8) Escalera J. B., Bustamante P., Martin A., *J. Pharm. Pharmcol.*, **46**, 172—176 (1994).
9) Bustamante P., Ochoa R., Reillo A., Escalera J. B., *Chem. Pharm. Bull.*, **42**, 1129—1133 (1994).
10) Jouyban-Gharamaleki A., Barzegar-Jalali M., *Pharm. Sci.*, **2**, 559—560 (1996).
11) Yalkowsky S. H., Amidon G. L., Zografi G., Flynn G. L., *J. Pharm. Sci.*, **64**, 48—52 (1975).
12) Cave G., Kothari R., Poisieux G., Martin A. N., Carstensen J. T., *Int. J. Pharm.*, **5**, 267—272 (1980).
13) Bustamante P., Escalera B., Martin A., Selles E., *J. Pharm. Sci.*, **78**, 567—573 (1989).
14) Bustamante P., Selles E., *J. Pharm. Sci.*, **75**, 639—643 (1986).
15) Rowe C. E., *Int. J. Pharm.*, **41**, 219—222 (1988).
16) Hancock B. C., York P., Rowe R. C., *Int. J. Pharm.*, **148**, 1—21 (1997).
17) Romero S., Reillo A., Escalera B., Bustamante P., *Chem. Pharm. Bull.*, **44**, 1061—1064 (1996).
18) Jouyban-Gharamaleki A., *Chem. Pharm. Bull.*, **45**, 1383—1384 (1997).
19) Jouyban-Gharamaleki A., Acree W. E., Jr., *Int. J. Pharm.*, **167**, 177—182 (1998).
20) Jouyban-Gharamaleki A., Valaee L., Barzegar-Jalali M., Clark B. J., Acree W. E., Jr., *Int. J. Pharm.*, **177**, 92—101 (1999).