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# SOLUBILITY OF A NEW ANTIRETROVIRAL DRUG (CRS 74) IN AQUEOUS ETHANOL MIXTURES

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**Abstract** - This study concerns a new antiretroviral drug named CRS 74, which has a limited bioavailability because of its low aqueous solubility and dissolution rate. To improve these properties, CRS 74 can be recrystallized by using Liquid Anti-Solvent (LAS) crystallization. Ethanol is chosen as the solvent under study for the molecule and water as the anti-solvent. Since solubility data is limited, it is necessary to collect experimental data for the molecule in relation to ethanol and water-ethanol mixtures at different temperatures in order to select suitable mixture compositions and temperature for LAS process design. In this work, the CRS 74 solubility measured in the temperature range 288.15 - 303.15 K in pure ethanol and in 95% water - 5% w/w ethanol mixtures, and the CRS 74 solubility measured at 303.15 K in water-ethanol mixtures containing from 30 to 70% w/w ethanol are presented. Measurements were performed using the shake-flask method for generating the saturated solutions followed by compositional analysis by HPLC of the solution. The experimental data showed that

the solubility of CRS 74 in binary hydroalcoholic mixtures increases upon increasing the temperature and mass fraction of ethanol.

In order to better understand the behavior of the system and to estimate supersaturation conditions for a larger range of CRS 74 crystallization conditions, two models have been chosen to describe the experimental data: UNIQUAC and Jouyban-Acree models. The modeling of experimental solid-liquid equilibrium data proved that both models could correlate satisfactorily the solubility of the studied drug. This study provided valuable data for the recrystallization of CRS 74 by using the Liquid Anti-Solvent (LAS) crystallization process.

**Keywords:** antiretroviral drug, solubility, UNIQUAC, aqueous ethanol mixture.

## INTRODUCTION

This study concerns a new compound, (2S, 3S, 5S)-2, -5 bis- [N-[N-[[N- methyl- N-[(2-isopropyl- 4- thiazolyl) methyl] amino] carbonyl] vanilyl] amino- 1,6- diphenyl- 3-hydroxyhexane, named CRS 74. This molecule has the activity of inhibiting human immunodeficiency virus (HIV) protease, an essential enzyme involved in the HIV replication process. Consequently, this new compound can be used for the treatment of HIV infections, alone or in combination with other anti-HIV medicines.

CRS 74 has a high biological activity as disclosed in PCT document WO 2005/111006 and US 7763733 (Bockelmann et al., 2005; Bockelmann et al., 2010) but its bioavailability is limited because of its low aqueous solubility and dissolution rate. Such properties imply difficulties not only in the design of pharmaceutical formulations, but may also result in biovariability.

Drug dissolution is a prerequisite for drug absorption and clinical response for almost all drugs administrated orally. Solid forms that have been investigated for drug

dissolution enhancement include salts, polymorphs and amorphous, among others. Amorphous formulations can achieve improved solubility, but the system is at serious risk of crystallizing to the thermodynamically stable form, even in the solid state (Yu, 2001; Rodríguez-Spong et al., 2004). Such transformations can compromise the performance of the formulation.

To save time and resources in product development, relatively simple approaches should be tried first like crystallization. Micro/nanonization during crystallization (Rasenack and Müller, 2002; Rasenack and Müller, 2004; Badawi et al., 2011; Viçosa et al., 2012), surface modification (Han et al., 2011) and crystal structure modification (Eerdenbrugh et al., 2009) may improve the dissolution rate of poorly water-soluble APIs (Active Pharmaceutical Ingredients).

The Liquid Anti-Solvent (LAS) crystallization process is an attractive method for CRS74 recrystallization, aiming at controlling its dissolution kinetics. It requires mild conditions (ambient temperature and atmospheric pressure) with no requirement for expensive equipment. In the LAS process, crystallization of the solute is achieved by decreasing the solubility of the solid in the system. This is done by addition of a non-solvent component for the solute, called the anti-solvent and miscible with the solvent.

The LAS crystallization process for production of ultra-fine particles has been widely researched over the last few decades (Thorat and Dalvi, 2012). One goal in this crystallization operation is the control of product properties, e.g., particle size and particle size distribution (Zhao et al., 2007; Zhang et al., 2009), purity, residual solvent content, crystallinity, polymorphic form (Balani et al., 2010) and dissolution rates in specific media during in vitro-tests (Viçosa et al., 2012; Plakkot et al., 2011) to a certain extent. The product properties can be manipulated by changing the process parameters and formulation.

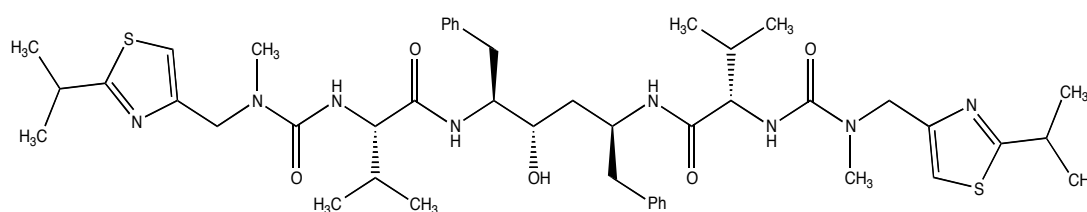
The solvent selection is one of the essential parameters to envisage any crystallization process. Therefore, the knowledge of the solubility of a target component in different solvents is required. In this work, the solubility of CRS 74 in pure ethanol and ethanol-water binary mixtures was measured in the temperature range of 288.15 K to 303.15 K at atmospheric pressure.

Although experimental data on solubility are essential to provide information about a system and help to understand its behaviour, correlations and prediction models are also required for the correct design of crystallization processes. Solid-liquid equilibria of ternary mixtures containing ethanol (solvent), water (anti-solvent) and the new antiretroviral drug were studied and the obtained solubility data were represented using two models: the UNIQUAC-based model (Walas, 1985) and the Jouyban-Acree model (Jouyban-Gharamaleki and Acree, 1998).

## EXPERIMENTAL SECTION

### Materials

The molecular formula of the active pharmaceutical ingredient (CRS 74) is  $C_{46}H_{66}N_8O_5S_2$  and its molar weight 875.2 g/mol. Its chemical structure is presented in Figure 1. It was provided with 99 % purity as a courtesy from Cristalia Ltda (Itapira, SP, Brazil). Ethanol (EtOH) (from Fluka Analytical, Sigma–Aldrich, France) and acetonitrile (ACN) high performance liquid chromatography (HPLC) grade (from Scharlau Chemie, Barcelona, Spain) were used as solvents with purity higher than 99%.



**Figure 1:** Chemical structure of CRS 74

## Methods

### *Solubility measurements*

The solubility of CRS 74 was determined by equilibrating an excess of CRS 74 in 5 g of water, ethanol and different ethanol/water combinations at a given temperature (from 298.15 to 303.15 K) in a temperature-controlled bath for 72 hours.

According to this method, the solid drug was added in excess to a certain medium, the flasks were sealed, and shaken at a predetermined time, 24, 48 and 72 hours. The saturation was confirmed by observation of the presence of un-dissolved material and measurements of dissolved drug concentration. The concentration was determined by removing the solid phase by filtration (0.22  $\mu\text{m}$  pore size, Pall 4506, France) and injection of the filtered solution onto the HPLC system with analysis at a wavelength of 210 nm. The HPLC system consisted of an Agilent Chromatograph (Model 1100 series) equipped with a UV-vis detector and a HPLC column ProntoSIL 300-5-ODSH 5 $\mu\text{m}$ , 250x4mm ID. The flow rate of the mobile phase (acetonitrile/water in the ratio of 70:30) was 1.0 mL/min.

### *Differential scanning calorimetric (DSC) analysis*

DSC measurement was carried out using a DSC-Q200 thermal analyzer (TA Instruments, France) in a temperature range of 293 to 483 K at a heating rate of 10° C/min under nitrogen atmosphere (50 mL min<sup>-1</sup>) in order to determine the onset of the melting point and the fusion enthalpy with about 3 mg of solid. The molecule was characterized by a melting point of 461.75 K and a melting enthalpy of 86.6 J/g.

### *Modelling*

Two models were chosen to describe experimental data: the UNIQUAC model (UNIversal QUASI Chemical) (Walas, 1985) and the Jouyban-Acree model (Jouyban-

Gharamaleki and Acree, 1998). The first model is based on the calculation of activity coefficients and the second is based on the mathematical representation of the solubility of drugs in mixed solvents with respect to solvent composition and temperature.

### UNIQUAC model

In the UNIQUAC model, the activity coefficient is calculated by assuming a combinatorial contribution, essentially due to the differences in size and shape of the molecules, and a residual contribution essentially due to energetic interactions. The expression for the activity coefficient of the component  $i$ ,  $\gamma_i$ , is described by a combinatorial and a residual contribution:

$$\ln(\gamma_i) = \ln(\gamma_i^C) + \ln(\gamma_i^R) \quad (1)$$

The combinatorial term  $\gamma_i^C$  is given by:

$$\ln(\gamma_i^C) = \ln\left(\frac{\phi_i}{x_i}\right) + \frac{z}{2} q_i \ln\left(\frac{\theta_i}{\phi_i}\right) + l_i - \frac{\phi_i}{x_i} \sum_j x_j l_j \quad (2)$$

where  $x_i$  is the mole fraction of component  $i$ ;  $z$  is the coordination number which is usually set to 10. The bulk factors  $l_i$  are given by:

$$l_i = \frac{z}{2} (r_i - q_i) - (r_i - 1) \quad (3)$$

$\theta_i$  and  $\phi_i$  are the molecular surface area and molecular volume of each component  $i$ , given by:

$$\phi_i = \frac{x_i r_i}{\sum_j x_j r_j} \quad (4)$$

$$\theta_i = \frac{x_i q_i}{\sum_j x_j q_j} \quad (5)$$

For pure components,  $r_i$  and  $q_i$  are calculated as the sum of the group-volume and group-area parameters,  $R_k$  and  $Q_k$ :

$$r_i = \sum_j v_i^k R_k \quad (6)$$

$$q_i = \sum_j v_i^k Q_k \quad (7)$$

where  $v_i^k$  (an integer) is the number of groups of type  $k$  in the molecule  $i$ .

The active molecule was decomposed into functional groups similarly to the group contribution UNIFAC method. The first parameters, volume and surface parameters ( $R_k$  and  $Q_k$ ), were estimated from the volume and surface area parameters of CRS 74 functional groups. The functional groups and sub-groups are reported in Table 1. The thiazole heterocycle ( $C_3HNS$ ) was replaced by the thiophene one ( $C_4H_2S$ ). The volume and surface area parameters  $r_i$  and  $q_i$  of the three components (water, ethanol and active molecule) are given in Table 2.

**Table 1:** UNIFAC Group Volume and Surface-Area parameters for groups of CRS 74.

Functional group	Subgroup	Number	$R_k$ (cm <sup>3</sup> /mol)	$Q_k$ (cm <sup>3</sup> /mol)
<b>CH<sub>2</sub></b>	CH <sub>3</sub>	8	0.9011	0.848
<b>CH<sub>2</sub></b>	CH <sub>2</sub>	2	0.6744	0.54
<b>CH<sub>2</sub></b>	CH	5	0.4469	0.228
<b>ACH</b>	ACH	10	0.5313	0.4
<b>ACCH<sub>2</sub></b>	ACCH <sub>2</sub>	2	1.0396	0.66
<b>CON (AM)</b>	AMHCH <sub>2</sub>	4	1.9637	1.488
<b>(C)<sub>3</sub>N</b>	CH <sub>3</sub> N	2	1.1865	0.94
<b>THIOPHEN</b>	C <sub>4</sub> H <sub>2</sub> S	2	2.5247	1.58
<b>OH</b>	OH	1	1	1.2



**Table 2:** UNIFAC Group Volume and Surface-Area,  $R_k$  and  $Q_k$  parameters for CRS 74.

Molecule i	Water (DIPPR)	Ethanol (DIPPR)	CRS 74
$r_i$ (cm <sup>3</sup> /mol)	0.9200	2.1055	34.4615
$q_i$ (cm <sup>3</sup> /mol)	1.4000	1.9720	26.5160

The expression of the residual term is given by:

$$\ln(\gamma_i^R) = q_i \left( 1 - \ln(\sum_j \theta_j \tau_{ij}) \right) - \sum_j \frac{\theta_j \tau_{ij}}{\theta_k \tau_{kj}} \quad (8)$$

In equation (8), the parameters  $\tau_{ij}$  are calculated from UNIQUAC binary interaction parameters  $u_{ij}$  as follows:

$$\tau_{ij} = \exp\left(-\frac{u_{ij} - u_{ii}}{RT}\right) \quad (9)$$

For a ternary system, twelve parameters are necessary: six parameters concerning the geometry of the molecules plus six binary interaction parameters. The binary interaction parameters ( $u_{ij}$ ) water/CRS 74 and ethanol/CRS 74 were identified from solubility measurements. Binary interaction parameters water/ethanol are available in DIPPR (Data Compilation Tables of Properties of Pure Compounds) and reported in Table 3.

The binary interaction parameters of the UNIQUAC model were identified in two steps. Initially, solubility data for the binary ethanol/CRS 74 as a function of temperature were considered to estimate the two binary interaction parameters ethanol/CRS 74 from the activity coefficient of the solute calculated by equation (10).

The temperature dependent experimental activity coefficient of the solute CRS 74,  $\gamma_{CRS74\ solute}$ , can be estimated from the melting enthalpy,  $\Delta h_m$ , its melting temperature  $T_m$  and the mole fraction at saturation,  $x_{CRS74\ solute}$ , at a given solution temperature  $T$ :

$$\ln(\gamma_{CRS74\ solute} x_{CRS74\ solute}) = -\frac{\Delta h_m}{R} \left( \frac{1}{T} - \frac{1}{T_m} \right) \quad (10)$$

**Table 3:** Binary interaction parameters for the UNIQUAC model.

Binary Systems	i-j	$u_{ij}$ (cal/mol)
Water-CRS 74	1-3	-546.14
	3-1	23 809.35
Ethanol-CRS 74	2-3	-494.45
	3-2	1 999.09
Water-Ethanol (database Simulis)	1-2	$-96.4730 + 0.6843 * T(K)$
	2-1	$31.6290 + 0.4759 * T(K)$

In a second stage, the binary interaction parameters of water/CRS 74 were calculated from experimental data for the ternary water/ethanol/CRS 74 with a mass ratio ethanol/(ethanol-water) of 5 % and variable temperature. A ternary mixture was chosen because the solubility data in water as a function of temperature are not available.

In both cases, the objective function  $C$  of the curve fitting method used to estimate the binary interaction parameters was the weighted root mean square error, given by:

$$C = \frac{2}{n_{exp}} \sqrt{\sum_i^{n_{exp}} \frac{\left( \gamma_{CRS74\ solute}^{exp_i} - \gamma_{CRS74\ solute}^{cal}(T_i) \right)^2}{\left( \gamma_{CRS74\ solute}^{exp_i} + \gamma_{CRS74\ solute}^{cal}(T_i) \right)^2}} \quad (11)$$

in which  $n_{exp}$  is the number of measurements; each one corresponds to an experiment done at a temperature  $T_i$ .  $\gamma_{CRS74\ solute}^{exp_i}$  is estimated by equation (10) and  $\gamma_{CRS74\ solute}^{cal}(T_i)$  is calculated with the UNIQUAC model in which the unknown parameters are obtained at the minimum of  $C$ .

### Jouyban-Acree model

The Jouyban-Acree model is represented by Equation 12:

$$\ln(w_{m,T}^*) = z_1 \ln(w_{1,T}^*) + z_2 \ln(w_{2,T}^*) + \left[ \frac{z_1 z_2}{T} \sum_{i=0}^2 J_i (z_1 - z_2)^i \right] \quad (12)$$

where  $w_{m,T}^*$  is the solute mass fraction solubility in the solvent mixtures at temperature  $T$ ;  $z_1$  and  $z_2$  are the mass fractions of solvents 1 and 2 in the absence of a solute;  $w_{1,T}^*$  and  $w_{2,T}^*$  denote the mass fraction solubility of the solute in the mono-solvents 1 and 2, respectively. The terms  $J_i$  are constants of the model and are computed by regressing  $\ln(w_{m,T}^*) - z_1 \ln(w_{1,T}^*) - z_2 \ln(w_{2,T}^*)$  against  $\frac{z_1 z_2}{T}$ ,  $\frac{z_1 z_2}{T} (z_1 - z_2)$ , and  $\frac{z_1 z_2}{T} (z_1 - z_2)^2$  using a no-intercept least square analysis (Jouyban-Gharamaleki and Hanaee, 1997).

Combination of the Jouyban-Acree and the Van't Hoff model enables prediction of drug solubility in mixed solvents at different temperatures after the training process by using a minimum number of solubility data points, e.g., at the lowest and highest temperatures (Jouyban et al., 2012; Sardari and Jouyban, 2013). In the training process, the model constants are computed using measured solubility data, then the solubility values at other solvent compositions and temperatures could be predicted using simple algebraic operations. The combined version could be represented as

$$\ln(w_{m,T}^*) = z_1 \left( A_1 + \frac{B_1}{T} \right) + z_2 \left( A_2 + \frac{B_2}{T} \right) + \left[ \frac{z_1 z_2}{T} \sum_{i=0}^2 J_i (z_1 - z_2)^i \right] \quad (13)$$

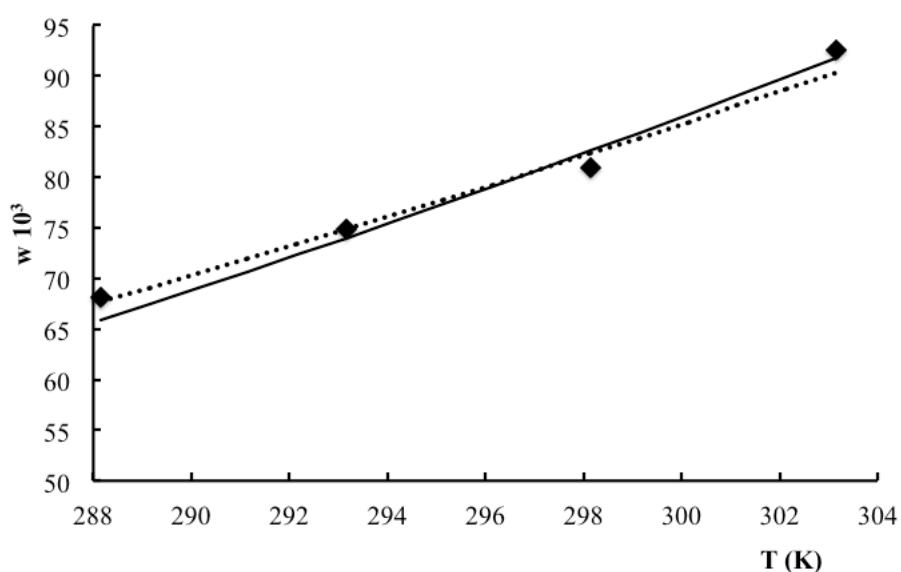
where the  $A_1$ ,  $A_2$ ,  $B_1$ ,  $B_2$  and  $J_i$  terms are the model constants.

## RESULTS AND DISCUSSION

### CRS 74 solubility in pure ethanol : effect of temperature

First, the drug solubility in pure ethanol was experimentally measured within the temperature range of 288.15 to 303.15 K.

From data obtained from 24 to 72 h, it was concluded that the liquid-solid system reached equilibrium after 24 h of stirring at a controlled temperature  $\pm 0.5$  K. Thereafter the saturation mass fraction  $\bar{w}$  was taken as the average of the three readings (Table 4 and Figure 2). The standard deviation obtained ( $\leq 4.2$  %) is in part due to evaporation losses of solvent after sampling and, in part, closely related to systematic errors linked to analytical methods, like dilution and calibration curve.



**Figure 2:** Experimental (symbols) and calculated CRS 74 solubilities (solid line for the UNIQUAC model and dashed line for the Jouyban-Acree model) expressed in mass fraction in ethanol at different temperatures

**Table 4:** Experimental and ideal solubilities of CRS 74 expressed in mass fraction in pure ethanol as a function of temperature

T (K)	Experimental data $\bar{w}^*$ $10^3$	Experimental data SD** $10^3$	$\bar{w}_{ideal}^{***}$ $10^3$	Calculated data by UNIQUAC $\bar{w}^*$ $10^3$	Calculated data by Jouyban-Acree Model $\bar{w}^*$ $10^3$
288.15	68.1	4.2	0.13	65.8	67.7
293.15	74.8	2.9	0.22	74.0	74.8
298.15	80.9	2.3	0.38	82.6	82.3
303.15	92.6	1.9	0.62	91.7	90.3

\* mean mass fraction of solute, \*\* Standard deviation, \*\*\* calculated with an activity coefficient of solute equal to 1 (equation (10)).

The results revealed that this molecule exhibited a solubility in pure ethanol increasing from  $68.1 \pm 4.2 \cdot 10^{-3} \text{ g/g}_{\text{solution}}$  at 288.15 K to  $92.6 \pm 1.9 \cdot 10^{-3} \text{ g/g}_{\text{solution}}$  at 303.15 K.

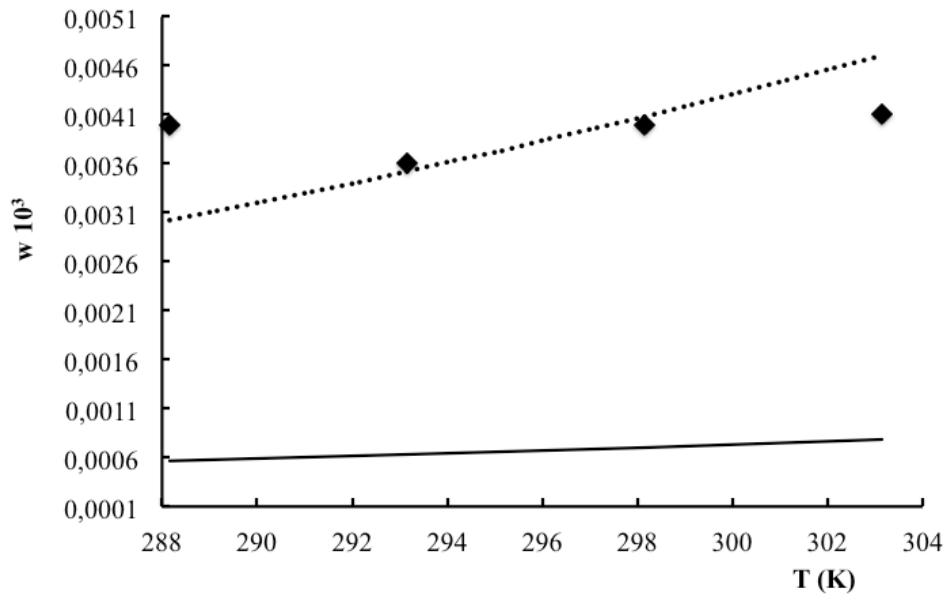
#### **CRS 74 experimental solubility in aqueous ethanol mixture containing 95% water and 5% ethanol : effect of temperature**

The results revealed that this molecule exhibited a very poor solubility in pure water (not detected by HPLC analysis). So, the drug solubility in a binary mixture containing 95 % water and 5 % ethanol was experimentally measured within the temperature range of 288.15 to 303.15 K. Table 5 and Figure 3 show the CRS 74 mass fraction as a function of temperature. It can be seen that the solubility in this mixed aqueous system was not affected by the temperature in the studied range.

**Table 5:** Experimental and calculated solubility of CRS 74 expressed as mass fraction in 95% (w/w) water - 5% (w/w) ethanol mixture as a function of temperature.

T (K)	Experimental data $\bar{w}^*$ $10^3$	Experimental data SD** $10^3$	Calculated data by UNIQUAC $\bar{w}^*$ $10^3$	Calculated data by Jouyban-Acree Model $\bar{w}^*$ $10^3$
288.15	0.0040	0.0002	0.0006	0.0030
293.15	0.0036	0.0001	0.0006	0.0035
298.15	0.0040	0.0001	0.0007	0.0041
303.15	0.0041	0.0001	0.0008	0.0047

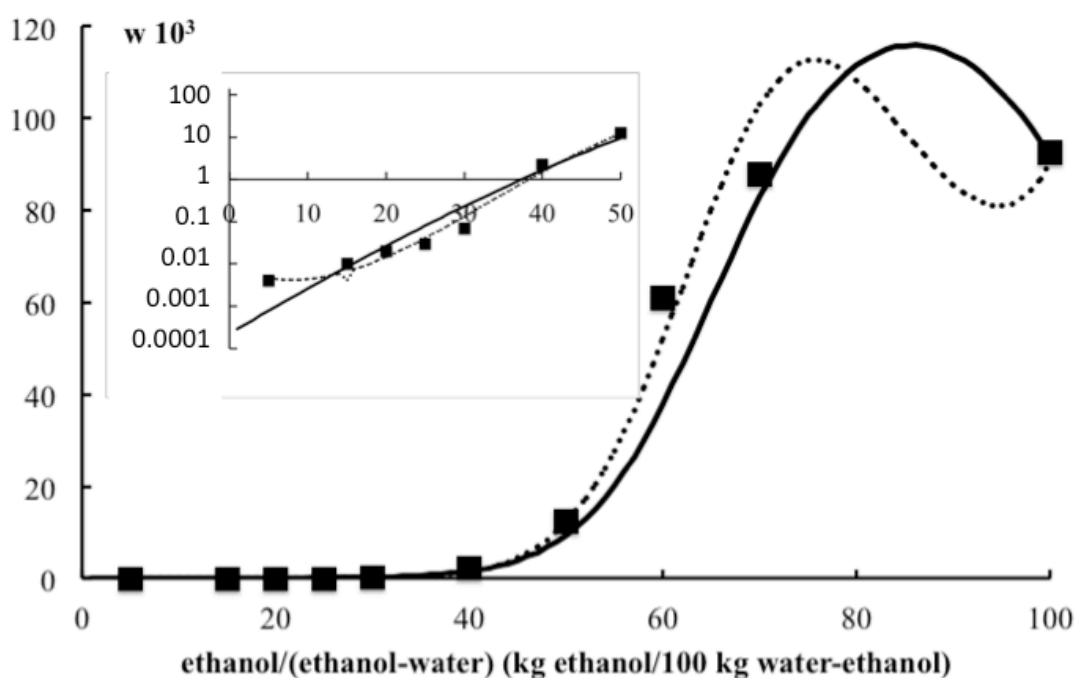
\*mean mass fraction of solute, \*\* Standard deviation



**Figure 3:** Experimental (symbols) and calculated CRS 74 solubilities (solid line for UNIQUAC model and dashed line for the Jouyban-Acree model) expressed as mass fraction in ethanol-water mixture containing 95% (w/w) water and 5% (w/w) ethanol at different temperatures

### CRS 74 experimental solubility in aqueous ethanol mixture at 303.15 K: effect of the binary mixture composition

The solubility of CRS 74 in different water-ethanol mixtures at 303.15 K is listed in Table 6 and plotted in Figure 4. On the one hand, the solubility of CRS 74 increases in the aqueous ethanol mixtures with the ethanol proportion. It is well-known that the addition of an organic co-solvent to water can dramatically change the solubility of drugs (Yalkowsky and Roseman, 1981) as observed here for more than 40 % ethanol. On the other hand, combinations containing more than 60% water as an anti-solvent are favourable for further crystallization studies.



**Figure 4:** Experimental (symbols) and calculated CRS 74 solubilities (solid line for UNIQUAC model and dashed line for the Jouyban-Acree model) expressed as mass fraction in different ethanol-water mixtures at 303.15 K

**Table 6:** Solubility of CRS 74 expressed as mass fraction in ethanol/water mixtures at 303.15 K.

Solvent	Mean mass fraction of solute $\bar{w} \pm SD 10^3$	Calculated data by UNIQUAC $\bar{w}^* 10^3$	Calculated data by Jouyban-Acree Model $\bar{w}^* 10^3$
Water	not detected by HPLC analysis	-	-
95%(w/w) water - 5%(w/w) ethanol	0.004±0.0001	0.001	0.005
85%(w/w) water - 15%(w/w) ethanol	0.010±0.0004	0.009	0.007
80%(w/w) water - 20%(w/w) ethanol	0.020±0.0020	0.027	0.015
75%(w/w) water - 25%(w/w) ethanol	0.030±0.0020	0.080	0.041
70%(w/w) water - 30%(w/w) ethanol	0.070±0.0030	0.230	0.134
60%(w/w) water - 40%(w/w) ethanol	2.290±0.0900	1.658	1.515
50%(w/w) water - 50%(w/w) ethanol	12.300±1.4100	9.511	12.342
40%(w/w) water - 60%(w/w) ethanol	60.960±5.2400	37.85	52.106
30%(w/w) water - 70%(w/w) ethanol	87.890±4.0000	82.997	103.071
Ethanol	92.600±1.9000	91.742	90.329



## Correlation of solubility data by UNIQUAC and Jouyban-Acree models

*UNIQUAC model.* The experimental and calculated activity coefficients ( $\gamma_{CRS74\ solute}^{exp}$  and  $\gamma_{CRS74\ solute}^{calc}$ ) for the two systems are reported in Table 7. The root mean square error  $C$  (see Equation (11)) for the binary system ethanol/CRS 74 and for the ternary system water/ethanol/CRS 74 are respectively 0.013 and 0.058.

**Table 7:** Experimental and calculated activity coefficients with UNIQUAC model for ethanol/CRS 74 and ethanol/water/CRS 74.

T (K)	System ethanol/CRS 74 $C^* = 0.013$		System water/ethanol/CRS 74 Ethanol/(Ethanol+water) = 0.05 $C^* = 0.058$	
	$\gamma_{CRS\ 74\ solute}^{exp}$ $10^2$	$\gamma_{CRS\ 74\ solute}^{calc}$ $10^2$	$\gamma_{CRS\ 74\ solute}^{exp}$ $10^{-2}$	$\gamma_{CRS\ 74\ solute}^{calc}$ $10^{-2}$
288.15	0.1851	0.185	0.804	0.973
293.15	0.277	0.283	1.532	1.488
298.15	0.429	0.411	2.348	2.241
303.15	0.612	0.625	3.762	3.330

\*objective function calculated with equation (11)

*Jouyban-Acree model.* The model parameter values were assessed by nonlinear curve fitting based on the least mean square method. The trained form of the Jouyban-Acree model for prediction of CRS 74 solubility in ethanol and water mixtures at specific temperature  $T$  is given in equation 14:

$$\ln(w_{m,T}^*) = z_1 \left( 10.032 + \frac{1675.976}{T} \right) + z_2 \left( 2.775 - \frac{2248.868}{T} \right) + \frac{z_1 z_2}{T} [3132.043 + 5395.102 (z_1 - z_2) - 7114.846 (z_1 - z_2)^2] \quad (14)$$

where  $w_{m,T}^*$  denotes the solute solubility in the binary solvent mixtures at temperature  $T$  (K) expressed in g/kg. This trained form of the model may be used to explore the predictability of the Jouyban–Acree model for the solubility of the tested drug in ethanol + water mixtures at various temperatures and solvent compositions. In addition, it may be used to predict the aqueous solubility of CRS 74 at various temperatures by considering  $z_1=0.0$  and  $z_2=1.0$ .

Table 4 and Figure 2 and Table 5 and Figure 3 give respectively the solubilities calculated in pure ethanol and in 5% ethanol - 95% water mixture as a function of temperature with the two models. In pure ethanol, the calculated concentrations are in good agreement with experimental values. In 5% ethanol - 95% water mixture, the UNIQUAC model underestimates the solubility of the active molecule. The Jouyban-Acree model seems to provide more accurate predictions.

Figure 4 and Table 6 present the calculated solubility at 303.15 K as a function of the ethanol mass proportion in the mixture ethanol-water with the two models. The data calculated with UNIQUAC model showed good agreement with experimental results. The two models revealed a maximum solubility of CRS 74 for an ethanol mass fraction in the mixture ethanol-water between 0.70 and 1. The maximum solubility for alcohol-water mixtures was predicted by the models but still not confirmed experimentally. A maximum solubility for a solute in a mixed solvent system has been observed experimentally for other systems (water/acetone/ketoprofen (Espitalier et al., 1995); water/ethanol/paracetamol and water/dioxane/phenacetin (Ruckestein et al., 2003); water/ethanol/hydrocortisone (Ali et al., 2009) and n-heptane/ethanol/eflucimide (Teychene and Biscans, 2011)). Different studies with the Hidelbrand solubility approach have shown that the location and the height of the peaks could be linked with the polarity of the solute (Jouyban-Gharamaleki, 2000; Peña et al., 2006). Hydroxyl and

amine groups of the solute give a polar character to this molecule that could explain the maximum of solubility calculated for a high ratio of ethanol in the mixture.

Indeed, the Jouyban-Acree model predicts a local minimum for the mixture 95% ethanol-5% water. To the best of our knowledge, this behaviour has not yet been described in the literature and should be treated with caution.

## CONCLUSIONS

CRS 74 is soluble in ethanol (92.6 mg/g<sub>solution</sub> at 303.15 K). To the best of our knowledge, there are no other published data on the solubility of the new antiretroviral molecule (CRS 74) in pure ethanol and in ethanol-water mixtures.

The data generated in the present work represent a useful tool to define the mass proportion between solvent (ethanol) and anti-solvent (water) for our ongoing research on LAS recrystallization of CRS 74, in an attempt to improve its dissolution properties. The models demonstrate the capability to predict the solubility trend and magnitude, predicting a maximum in solubility in ethanol-water mixtures of around 115 mg/g solution as determined using the UNIQUAC-based model, or 112 mg/g solution using the Jouyban-Acree correlation. The data could be used for pharmaceutical purposes to explore a larger range of operating conditions for drug recrystallization, requiring less experimental measurements.

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