STUDY OF THE PHASE EQUILIBRIUM IN BINARY SYSTEMS: ANTICANCER DRUG - CARBON DIOXIDE

F.J. Ramos[a], R. Mazarro[b], A. de Lucas Martínez[a], J.F. Rodríguez*[a]
Chemical Engineering Department. University of Castilla-La Mancha
[a] *Faculty of Chemistry. Avenue Camilo José Cela, 12. 13071 Ciudad Real*
[b] *School of Industrial Engineers. University Campus s/n. 02071 Albacete*
e-mail: juan.rromero@uclm.es Fax: (+34) 926 295 242

Anticancer drugs are substances with a lot of medical interest nowadays. So, the obtaining of drug delivery systems containing anticancer drugs is a very important field of research in pharmacological industry. The implementation of supercritical technologies it would secure the quality of these products. Supercritical anti-solvent (SAS) processes are an election with important advantages to design clean particles. The synthesis of this kind of materials requires an important previous knowledge of the global system: drug-biopolymer substrate-solvent-supercritical fluid (carbon dioxide, normally). Consequently, one first step to create the final product could be the investigation of the binary system anticancer drug–supercritical carbon dioxide ($scCO_2$). The anticancer drugs selected have been two substances with available solubility data in literature: 5-fluorouracil and paclitaxel. A model based on different Equations of State (EOS), namely Peng-Robinson and Patel-Teja and using two $\alpha(T)$ (Soave and Aznar-Silva Telles) has been applied. Therefore, the most adequate pair of EOS and $\alpha(T)$ for each system has been selected by comparison of the theoretical results and the ones reported in the literature at different conditions. Ambrose and Joback methods have been used to estimate the unknown critical properties of the anticancer drugs. Finally, Aznar-Silva Telles parameters are reported for 5-FU and paclitaxel.

Key words: 5-fluorouracil, paclitaxel, supercritical CO_2, Equations of State, equilibrium.

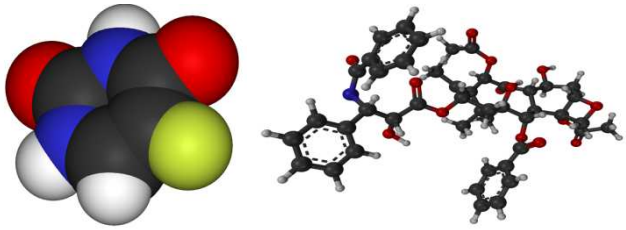
INTRODUCTION

The synthesis of new composites with pharmaceutical applications is a field with growing interest currently. These materials are called drug delivery systems. With regard to the structure of mentioned materials, two parts are well differentiated: the active ingredient and the biopolymeric cover or matrix [1].

Traditional methods to obtain these drugs, e.g. emulsion solvent evaporation techniques or spray-drying methods, require the utilization of organic solvents, frequently toxics. So, these techniques need an additional drying stage to preserve the patient health. On the other hand, supercritical technologies have been widely explored in particle design [2-4]. Considering the previous literature [5], the use of supercritical technologies to obtain the abovementioned biocomposites has several advantages, like the easy separation between the solvent and the drug, the purity of the synthesized product without solvent residues, the easy removal of the solvent, and the moderated operating temperatures.

This way, the final aim is the obtaining of a composite made of an anticancer drug and a biocompatible polymer using a supercritical technique. Consequently, the actives ingredients

selected are 5-fluorouracil (5-FU) and paclitaxel. The molecular structure of these drugs is shown in Figure 1.



a **b**

Figure 1. Molecules of 5-fluorouracil (5-FU) (**a**) and paclitaxel (**b**).

On the other hand, the polymer selected is poly(L-lactide) (PLLA), a biodegradable polymer with adequate properties for these systems and extendedly studied in literature [6].

Additionally, the supercritical technology is selected considering the previous literature [6]; in this case, the most adequate technology for the production of anticancer drugs + PLLA biocomposites is "supercritical anti-solvent process" (SAS). In these kind of processes the carbon dioxide works as anti-solvent and it is essential the selection of a solvent able to dissolve both polymer and drug and simultaneously miscible with CO_2. The solvents more employed in SAS processes are acetone, dichloromethane (DCM) and dimethyl sulfoxide (DMSO)[7]. All of them have adequate solubility properties for the studied system (anticancer drug + PLLA) and they are very soluble in carbon dioxide as well. Nevertheless, acetone seems to be the best selection for its low toxicity and cost. However, DCM and DMSO will be tested too. Finally, the chosen supercritical fluid is carbon dioxide due to it is innocuous and it has moderated critical constants.

Despite the fact that the obtaining of anticancer drug–PLLA composites as particles using supercritical technologies with little toxic solvents is the final aim of the global study, the work here presented only covers the previous stage of the project: the study of the binary equilibrium anticancer drug-CO_2. After that, several studies will be carried out in order to develop the anticancer drug–PLLA biocomposites for pharmacological applications.

In literature, there are two works about the 5-fluorouracil solubility in supercritical carbon dioxide: Guney and Akgerman [8] and Suleiman et al. [9]. For the paclitaxel solubility in supercritical CO_2 there are three different studies in bibliography: Vandana and Teja [10], Nalesnik et al. [11] and the aforesaid Suleiman work [9].

The main problem is there are serious discrepancies between the different experimental data reported in literature. For paclitaxel, Vandana and Teja [10] and Suleiman et al. [9] reported similar experimental data; so Nalesnik et al. [11] data have been neglected. For 5-fluorouracil, Guney and Akgerman [8] and Suleiman et al. [9] experimental data are very different too.

Considering that the Suleiman data [9] have been contrasted before with paclitaxel, the selected data for 5-FU case have been those ones as well.

So, a theoretical model for vapour phase has been applied made using the solubility data selected (only Suleiman et al. [9] solubility data for 5-FU and only Vandana and Teja [10] and Suleiman et al. [9] solubility data for paclitaxel). The mentioned Equations of state have been used to apply this model.

The procedure has been developed according to the literature [12]. The process make it possible distinguish the more adequate EOS for each system in several conditions. Consequently, the theoretical model will be useful to estimate the optimal conditions to perform the future SAS experiments and to improve the knowledge of the global system.

METHODS

Before the model was applied, the knowledge of several parameters is necessary i.e. boiling point, critical properties and acentric factor.

In literature the boiling point of the paclitaxel is reported [13], but for 5-FU is not available. For this reason, the boiling point of 5-fluorouracil has been estimated using the Sanghvi and Yalkowsky groups contribution method [14], since it is known that the boiling point (T_b) of a compound is required to estimate its critical properties.

The critical properties of 5-FU and paclitaxel are not offered in bibliography either. This way, the critical properties: critical temperature (T_c), critical pressure (P_c), critical volume (V_c) and critical compressibility factor (z_c) are estimated too. Two different methods are used in this work to estimate the abovementioned critical properties: the Ambrose method [15,16], and the Joback method [17].

After that, the calculation of the compressibility factor (ω) is possible. To obtain ω, equations 1-2 are used, like is suggested in literature [18]:

$$\theta = \frac{T_b}{T_c} \tag{1}$$

$$\omega = \left(\frac{3}{7}\right)\left(\frac{\theta}{1-\theta}\right)\log(P_c) - 1 \tag{2}$$

When boiling points, critical properties and acentric factors for 5-fluorouracil and paclitaxel are kwon, applying the model is possible. Peng-Robinson [19] and Patel-Teja [20] are the Equations of State (EOS) selected to model the vapour phase of the anticancer drug-carbon dioxide systems. In this work will be study what is the most adequate EOS for these systems. Peng-Robinson EOS is shown in equations 3-4, while Patel Teja EOS is shown in equations 5-8. In these equations pressure (P), temperature (T) and molar volume (V) are in I.S. units (Pa, K and $m^3 \cdot mol^{-1}$ respectively). R is the ideal gas constant (R=8,314J·mol-1K^{-1}).

PENG-ROBINSON EOS

$$P = \frac{RT}{V-b} - \frac{a_c \alpha(T)}{V(V+b)+b(V-b)} \tag{3}$$

$$a_c = 0.45724 \frac{R^2 T_c^2}{P_c} \qquad b = 0.07780 \frac{RT_c}{P_c} \tag{4}$$

PATEL-TEJA EOS

$$P = \frac{RT}{V-b} - \frac{a_c \alpha(T)}{V(V+b)+c(V-b)} \tag{5}$$

$$a_c = \Omega_a \frac{R^2 T_c^2}{P_c} \qquad b = \Omega_b \frac{RT_c}{P_c} \qquad c = \Omega_c \frac{RT_c}{P_c} \tag{6}$$

$$\begin{cases} \Omega_c = 1 - 3\eta \\ \Omega_b^3 + (2-3\eta)\Omega_b^2 + 3\eta^2\Omega_b - \eta^3 = 0 \ \rightarrow \ \Omega_b \text{ is the smallest positive real root} \\ \Omega_a = 3\eta^2 + 3(1-2\eta)\Omega_b + \Omega_b^2 + 1 - 3\eta \end{cases} \tag{7}$$

$$\eta = 0.329032 - 0.076799\omega + 0.0211947\omega^2 \tag{8}$$

For the calculation of the *α(T)* term, two expressions have been utilized: the classical Soave *α(T)* [21], shown in equations 9-11; and the Aznar-Silva Telles (A-ST) *α(T)* [22], shown in equations 12-13. The particularity of A-ST *α(T)* is that requires the correlation of the Aznar-Silva Telles parameters: *m*, *n* y *Γ*. So, the value of the Aznar-Silva Telles parameters will be reported in this work.

SOAVE *α(T)*

$$\alpha(T) = \left[1 + m\left(1 - \sqrt{T_r}\right)\right]^2 \tag{9}$$

$$m = 0.3476 + 1.5423\omega - 0.2699\omega^2 \tag{10}$$

$$T_r = T/T_c \tag{11}$$

AZNAR-SILVA TELLES *α(T)*

$$\alpha(T) = exp\left[m(1-T_r)|1-T_r|^{\Gamma-1} + n\left(\frac{1}{T_r} - 1\right)\right] \tag{12}$$

$$T_r = T/T_c \tag{13}$$

To apply the previous EOS, is necessary using a mixing rule. Van der Waals has been the employed mixing rule, because it does not require any additional parameter and it reproduces correctly simple systems [22]. Van der Waals mixing rule is explained in equations 14-17.

VAN DER WAALS MIXING RULE

$$a = \sum_{i=1}^{N} \sum_{j=1}^{N} y_i y_j \, a_{ij} \tag{14}$$

$$a_{ij} = \sqrt{a_i a_j}(1 - k_{ij}) \qquad k_{ij} = A + \frac{B}{T} \tag{15}$$

$$b = \sum_{i=1}^{N} \sum_{j=1}^{N} y_i y_j \, b_{ij} \cong \sum_{i=1}^{N} y_i b_i \tag{16}$$

$$b_{ij} = \frac{b_i + b_j}{2}\left(1 - l_{ij}\right) \cong \frac{b_i + b_j}{2} \tag{17}$$

The used criterion gives the subscript *1* to the anticancer drug and the subscript *2* to the carbon dioxide. So, the molar fraction of anticancer drug in vapour phase is y_1 and the molar fraction of carbon dioxide in vapour phase is y_2. For *a* parameter in the mixing, a geometric mean is used, but for *b* parameter in the mixing an arithmetic mean is employed with Van der Waals mixing rule. The binary interaction parameter l_{ij} is approximately zero for solids, so, equation 16 is simplified. The binary interaction parameter k_{ij} is obtained by adjust of *A* and *B* parameters.

So, four different sets of values for the modeled pressure of the system (*P*) are obtained, since there are four possible combinations of EOS with *α(T)*:

1. EOS: Peng-Robinson. *α(T)*: Soave
2. EOS: Peng-Robinson. *α(T)*: A-ST
3. EOS: Patel-Teja. *α(T)*: Soave
4. EOS: Patel-Teja. *α(T)*: A-ST

The accuracy for every pair of EOS-*α(T)* in anticancer drug – carbon dioxide systems is evaluated by two methods: the modeled pressure relative error in percentage, *ΔP(%)* (equation 18), and the coefficient of determination, r^2 (equations 19-20). The experimental and modeled pressure versus the molar fraction of anticancer drug (y_1) will be represented too.

$$\Delta P(\%) = \frac{|P_{exp} - P_{model}|}{P_{exp}} \cdot 100 \tag{18}$$

$$r^2 = 1 - \frac{SS_{error}}{SS_{Total}} \tag{19}$$

$$SS_{error} = \sum_i (P_{exp} - P_{model})^2 \qquad SS_{Total} = \sum_i (P_{exp} - \overline{P_{exp}})^2 \tag{20}$$

RESULTS AND DISCUSSION

The results of the estimated properties by group contributions methods for 5-fluorouracil (5-FU) and paclitaxel are shown in Table 1 and Table 2. The boiling point method used, the

critical properties estimation method employed, the boiling points, the critical properties (T_c, P_c, V_c and z_c) and the acentric factors are reported for the mentioned anticancer drugs.

Table 1. Estimated boiling points for 5-FU and paclitaxel.

Compound	Boiling point Method	T_b (K)
5-FU	Sanghvi and Yalkowsky	703.59
Paclitaxel	Literature [13]	491- 495

Table 2. Estimated critical properties and acentric factors for 5-FU and paclitaxel.

Compound	Critical properties Method	T_c (K)	P_c (bar)	V_c (cm^3/mol)	z_c	θ	ω
5-FU	Ambrose	1056.17	58.59	248.0	0.1655	0.6662	0.5071
	Joback	1032.45	62.20	285.5	0.2069	0.6815	0.6395
Paclitaxel	Ambrose	562.91	9.19	2198.1	0.4319	0.8758	1.8947
	Joback	827.05	7.44	2321.5	0.2512	0.5961	-0.4523

For 5-FU, both methods offer very similar results for critical properties and acentric factors. Using the Ambrose or the Joback method is indifferent, and both results are coherent. However, in the case of paclitaxel, the selected method is a very important step, because Joback method gives impossible values for acentric factor (ω<0); only Ambrose method is correct for paclitaxel. This behavior is expected because Joback method is unsuitable with big molecules [23]. To compare both compounds in the same conditions, the critical properties estimated by Ambrose method have been used in 5-FU case too.

Subsequently, the study of the most accurate pair of EOS- $\alpha(T)$ is developed. In Table 3 the modeled pressure relative error (ΔP) and the coefficient of determination (r^2) are reported for the four possible combinations of EOS-$\alpha(T)$. In Figure 2, the experimental (P_{exp}) and the modeled pressure (P_{model}) versus the molar fraction of anticancer drug (y_1) are shown for some temperatures. In both cases, selecting the most correct pair of EOS-$\alpha(T)$ is the final purpose.

Table 3. Modeled pressure relative error in percentage, $\Delta P(\%)$, and coefficient of determination, r^2, with the different models selected in this work.

Compound	Equation of State (EOS)	$\alpha(T)$	ΔP (%)	r^2
5-FU	Peng-Robinson	Soave	5.99	0.91661
		Aznar-Silva Telles	3.90	0.94009
	Patel-Teja	Soave	11.99	0.78712
		Aznar-Silva Telles	6.38	0.90270
Paclitaxel	Peng-Robinson	Soave	6.51	0.91559
		Aznar-Silva Telles	5.65	0.86699
	Patel-Teja	Soave	10.73	0.88752
		Aznar-Silva Telles	6.05	0.94263

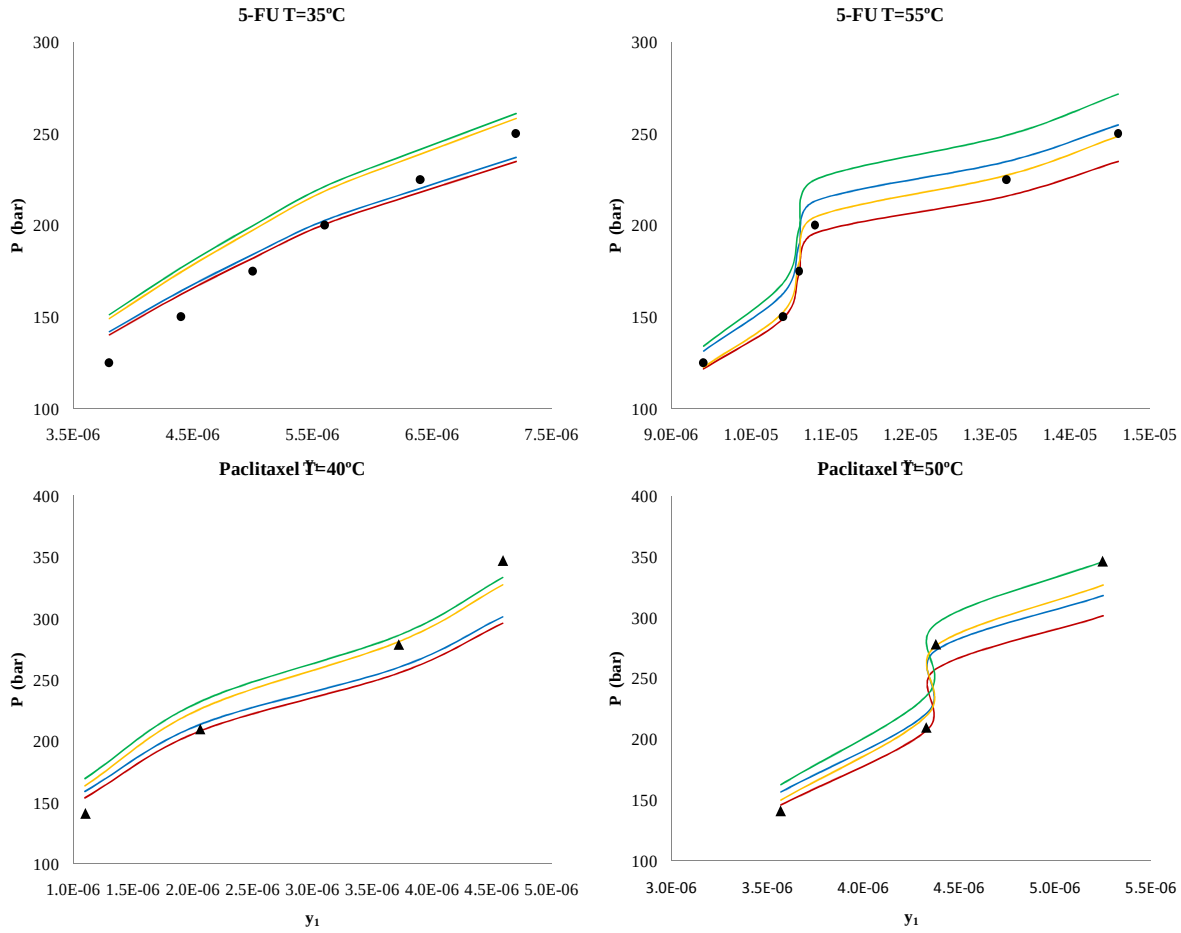


Figure 2. Experimental data and graphic representations of the models obtained for 5-FU (*T*= 35 and 55ºC) and paclitaxel (*T*=40ºC and 50ºC).
●: Experimental data from Suleiman et al. [9]. ▲: Experimental data from Vandana and Teja [10].
EOS: Peng-Robinson. *α(T)*: Soave. EOS: Peng-Robinson. *α(T)*: Aznar-Silva Telles.
EOS: Patel-Teja. *α(T)*: Soave. EOS: Patel-Teja. *α(T)*: Aznar-Silva Telles.

Generally speaking, with the introduction of the Aznar–Silva Telles *α(T)* an improvement is revealed. The Aznar-Silva Telles term offers better fitted models than the Soave term (except for paclitaxel with Peng-Robinson). With the mentioned case exception, the Aznar–Silva Telles option decreases the *ΔP(%)* and increases the r^2. In this exception the *ΔP(%)* is decreased but the r^2 is not increased.

At low pressures (< 200bar), Peng–Robinson Equation of State is more accurate than Patel-Teja Equation of State. Nevertheless, the contrary phenomenon is observed at high pressures (> 250bar).

On the one hand, the best pair of EOS-*α(T)* for 5-FU is Peng-Robinson with Aznar-Silva Telles ($\Delta P(\%)$=3.90 and r^2=0.94009).

On the other hand, the best pair of EOS-$\alpha(T)$ for paclitaxel is Patel-Teja with Aznar-Silva Telles too ($\Delta P(\%)$=6.05 r^2=0.94263). Although Peng-Robinson with Aznar-Silva Telles has got less pressure relative error in percentage ($\Delta P(\%)$=5.65), the coefficient of determination is too bad (r^2=0.86699).

Independently of the employed model, as temperature increases the errors increases too, since experimental data fit worse at high temperatures.

Finally, the Aznar-Silva Telles parameters obtained by correlation for each compound are shown in Table 4, both Peng-Robinson and Patel-Teja EOS.

Table 4. Aznar-Silva Telles parameters (*m*, *n* and *Γ*) obtained by adjust for 5-FU and paclitaxel with the different EOS.

Compound	Equation of State (EOS)	Aznar-Silva Telles parameters		
		m	***n***	***Γ***
5-FU	Peng-Robinson	0.8199	-0.5731	-0.0057
	Patel-Teja	0.8091	-0.5655	-0.0025
Paclitaxel	Peng-Robinson	1.2516	-0.7579	-0.3097
	Patel-Teja	1.0394	-0.5981	-0.3931

It is revealed that Aznar-Silva Telles parameters reported in this work for 5-FU and paclitaxel are independent of the Equation of State utilized.

CONCLUSIONS

The critical properties estimation methods of Ambrose and Joback are suitable to obtain critical temperature, pressure, volume and compressibility factor for not very big molecules (like 5-FU). However, for more complex molecules (like paclitaxel), only Ambrose method works, like is predicted in previous literature [23].

In general, the introduction of the Aznar-Silva Telles $\alpha(T)$ improves the precision of the model with Peng-Robinson and Patel-Teja Equations of State in the studied systems: anticancer drug (5-FU or paclitaxel) – supercritical carbon dioxide. At low pressures (<200bar), the accuracy of Peng-Robinson EOS is better; but, at high pressures (>250bar) the opposite fact is observed: Patel-Teja is more adequate. In this work, all EOS have problems to explain the behavior of the systems at high temperatures.

Regarding the Aznar-Silva Telles parameters obtained, it is worthy to say that the value of them is independent of the Equation of State used.

REFERENCES

[1] BOZKIR, A. and SAKA, O.M. Il Farmaco, Vol.60, **2005**, p. 840.

[2] DATEA, A.A., PATRAVALE, V. B. Curr. Opin. Colloid Interface Sci., Vol. 9, **2004**, p. 222.

[3] REVERCHON, E. J. Supercritical Fluids, Vol. 15, **1999**, p.1.

[4] YORK, P. PSTT, Vol. 2, **1999**, p. 430.

[5] COCERO, M.J., MARTÍN, A., MATTEA, F., VARONA, S. J. of Supercritical Fluids, Vol. 47, **2009**, p. 546.

[6] CHEN, A.Z., PU, X.M., KANG, Y.Q., LIAO, L., YAO, Y.D., YIN, G.F. Macromolecular Rapid Communications, Vol. 27, **2006**, p. 1254.

[7] JUNG, J. and PERRUT, M. J. of supercritical fluids, Vol.20, **2001**, p. 179.

[8] GUNEY, O. and AKGERMAN, A. J. Chem. Eng. Data Vol. 45, **2000**, p. 1049.

[9] SULEIMAN, D., ESTÉVEZ, L.A., PULIDO, J.C., GARCÍA, J.E., MOJICA, C. J. Chem. Eng. Data, Vol. 50, **2005**, p. 1234.

[10] VANDANA, V. and TEJA, A.S. Fluid Phase Equilibria, Vol. 135, **1997**, p. 83.

[11] NALESNIK, C.A., HANSEN, B.N., HSU, J.T. *Fluid Phase Equilibria*, Vol. 146, **1998**, p. 315.

[12] ŠKERGET, M., NOVAK-PINTARIC, Z., KNEZ, Z., KRAVANJA, Z. Fluid Phase Equilibria, Vol. 203, **2002**, p. 111.

[13] HANMI PHARM. CO., LTD. WIPO Patent: WO2008/75834, Patent kind code: A1, **2008.**

[14] SANGHVI, R. and YALKOWSKY, S.H. Industrial Engineering Chemical Research, Vol. 45, **2006**, p. 2856.

[15] AMBROSE, D. NPL Technical Report. Chem., Vol. 92 (Nat. Physical Lab., Teddington, UK), **1978**.

[16] AMBROSE, D. NPL Technical Report Chem. Vol. 92 (Nat. Physical Lab., Teddington, UK), **1979**.

[17] JOBACK, K.G. and REID, R.C. Chemical Engineering Communications, Vol. 57, **1987**, p. 233.

[18] REID, R.C., PRAUSNITZ, J.M., POLING, B.E. The Properties of gases and liquids, 4th Edition, McGraw-Hill, **1987**, Ch. 2.

[19] PENG, D.Y. and ROBINSON, D.B. Industrial Engineering Chemical Fundamentals, Vol. 15, **1976**, p. 59.

[20] PATEL, N.C. and TEJA, A.S. Chemical Engineering Science, Vol. 37, **1982**, p. 463.

[21] SOAVE, G. Chemical Engineering Science. Vol. 27, **1972**, p. 1197.

[22] AZNAR, M., SILVA TELLES, A., VALDERRAMA, J.O. Chemical Engineering Communications, Vol. 190, **2003**, p. 1411.

[23] KONTOGEORGIS, G.M. and TASSIOS, D.P. Chemical Engineering Journal, Vol. 66, **1997**, p. 35.