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MEASUREMENT AND MODELING SOLUBILITY OF BIOACTIVE COUMARIN AND ITS DERIVATIVES IN SUPERCRITICAL CARBON DIOXIDE

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Abstract – To design a supercritical fluid extraction process for the separation of bioactive substances from natural products, a quantitative knowledge of phase equilibria between target biosolutes and solvent is necessary. However, mostly no such information is available in literature to date. Thus in the present study, illustratively the solubility of bioactive coumarin and its various derivatives (i.e., hydroxy-, methyl-, and methoxy-derivatives) in supercritical CO_2 were measured at 308.15-328.15 K and 10-30 MPa. Also, the pure physical properties such as normal boiling point, critical constants, acentric factor, molar volume and standard vapor pressure for coumarin and its derivatives were estimated. By these estimated information, the measured solubilities were quantitatively correlated by an approximate lattice equation of state proposed recently by the present authors.

Key words: Measurement, Thermodynamic Modeling, Solubility, Supercritical Carbon Dioxide, Coumarin Derivatives

INTRODUCTION

Numerous efforts have been placed in recent years on the processing studies of supercritical fluid extraction (SFE) of bioactive substances from natural products [King and Bott, 1993; Rizvi, 1994; Burford, 1991; Ma et al., 1991; Liu et al., 1995; Modey et al., 1996; Bevan and Marshall, 1994; Joo et al., 1994; etc.]. However, major attention of the SFE studies in the field of pharmacognosy has been placed on the simple screening studies whether the SFE can be an alternative separation tool or not for the specific substances and natural products [Choi et al., 1996; Noh et al., 1997a, b].

For example, coumarin and its various derivatives are widely used for antibiotic and anticoagulation purposes. Also, these coumarin derivatives exist in many natural plants (i.e., *Angelica gigas, Angelica accutiloba, Citrus unshiu, Morus alba,* etc.). The chemical structure of some coumarin derivatives that we placed our attention in this work is shown in Fig. 1. However upon the present authors' literature survey, almost no quantitative experimental and thermodynamic modeling information of solubility behaviors of coumarin derivatives in supercritical fluids are available.

Furthermore, due to the complex nature of the coumarins, even the basic physical properties and constants for pure coumarin derivatives are not available. However, these properties are essential for the application of thermodynamic models [i.e., equation of state (EOS)] to perform necessary phase equilibrium calculation of such mixtures. Thus, we placed our efforts on the experimental measurement of solubility of coumarin derivatives, on the semi-empirical estimation of pure physical properties and on the application of an EOS to correlate the measured data. To model the measured solubility, we attempted to apply an EOS proposed recently by the present authors based on the two-fluid approximation of the nonrandom lattice-hole theory [Lee and Yoo, 1997]. We believe that the systematic methods presented in the present study can be reliably extended to other bioactive substances. Also, the results presented here can be directly applied to SFE process engineering of bioactive substances from natural resources.

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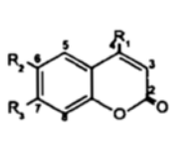
EXPERIMENTAL

1. Materials and Reagents

For the solubility measurement, reagent-grade coumarin and its derivatives (hydroxy-, methyl-, and methoxy-coumarins as shown in Fig. 1) were purchased from Sigma Co. (St. Louis, MO, USA). The 99.9 % CO_2 was purchased from Seoul Gas Co. (Seoul, Korea).

2. Solubility Measurement

As presented in detail elsewhere [Noh et al., 1997] a micro-scale flow-type apparatus was used for measuring solubility. The internal volume of the equilibrium cell was 60 ml. A syringe pump was used for feeding CO_2, and the pressure of the cell was measured by a Heise gauge. Temperature in the equilibrium cell was maintained by an air-bath and controlled within 0.1 K by a PID controller. Equilibrated effluent from the equilibrium cell was passed through a heating jacket,



$R_1 = R_2 = R_3 = H$	coumarin
$R_1 = OH$, $R_2 = R_3 = H$	4-hydroxy coumarin
$R_1 = R_2 = H$, $R_3 = OH$	7-hydroxy coumarin
$R_1 = H$, $R_2 = R_3 = OH$	6,7-dihydroxy coumarin
$R_1 = R_2 = H$, $R_3 = OCH_3$	7-methoxy coumarin
$R_1 = R_2 = H$, $R_3 = CH_3$	7-methyl coumarin
$R_1 = R_3 = H$, $R_2 = CH_3$	6-methyl coumarin
$R_1 = CH_2COOH$, $R_2 = H$, $R_3 = OH$	7-hydroxy coumarin 4-acetic acid
$R_1 = CH_2COOH$, $R_2 = H$, $R_3 = OCH_3$	7-methoxy coumarin 4-acetic acid

Fig. 1. Chemical structure of coumarin derivatives.

a metering valve, and it was collected by a two-step cold trap in which methanol was contained. For each 5 g sample, about 30 *l* of CO_2 at ambient condition was consumed. The experiment was performed at 308.15-328.15 K and 10-30 MPa.

3. Analysis of Solubility

For gas chromatographic (GC) analysis, a separated solute by evaporation from the methanol solution in the cold-trap was diluted by adding 1 ml/mg chloroform. Then, 1 ml/mg cholesterol was added to this diluted solution as an internal standard substance. Thus, for every chromatogram, the same magnitude of cholesterol peak appeared and it was used as a standard datum for compositional distribution of coumarins. The GC used was HP 5890 series II model. The column was HP-5 (5 % diphenylpolysiloxane, Hewlette Packard, USA) and FID detector was used. The column temperatures were in the range of 453.15 to 553.15 K, the temperature at the injector was 563.15 K, and at the detector was 573.15 K.

THERMODYNAMIC MODELING

1. Pure Physical Properties Estimation

For CO_2, necessary pure physical properties for phase equilibria calculation are available in literature. However for coumarin and its derivatives, almost no information of pure physical properties are reported in literature. Thus, the necessary pure physical properties of coumarin derivatives were estimated based on the semi-empirical methods described in a databook [Lyman et al., 1990]. The Lyderson-Forman-Thodos method for boiling point and critical temperature, and the Miller method for critical pressure and volume, were used. Estimated pure physical constants for coumarin derivatives are summarized in Table 1. Also, from these estimated data, molar volumes and vapor pressures was estimated by the Bhirud method and the Lee-Kestler method [Reid et al., 1987], respectively.

2. SCF Solubility Correlation by Lattice EOS

To model the solubility of coumarins in CO_2, a recent EOS formulated by the present authors based on nonrandom two-liquid approximation of the lattice-hole theory was illustratively applied [Lee and Yoo, 1997]. Omitting the details, the final expression of the lattice EOS is written for a general mixture by

$$P=\frac{1}{\beta V_H}\left\{\frac{z}{2}\ln\left[1+\left(\frac{q_M}{r_M}-1\right)\rho\right]-\ln(1-\rho)+\frac{z}{2}\sum_{i=1}^{c}\theta_i\left(\frac{\tau_{0i}}{\sum_{k=0}^{c}\theta_k\tau_{ki}}-1\right)\right\} \quad (1)$$

$$\tau_{ji}=\exp\left[\beta(\varepsilon_{ji}-\varepsilon_{ii})\right] \quad (2)$$

where, $q_M=\Sigma x_i q_i$, $r_M=\Sigma x_i r_i$, $\rho_i=N_i r_i/N_r$, $\rho=\Sigma\rho_i$ and x_j is the mole fraction of species i. We set the coordination number, z=10 and the unit lattice cell volume, V_H=9.75 cm^3mol^{-1}. Other expressions such as chemical potential necessary for phase equilibria calculation are omitted here [Lee and Yoo, 1997]. If we set the subscripts i=1 and j=0, Eq. (1) reduce to the EOS for pure fluids.

The two molecular parameters in the EOS for pure fluids; V_1^* and ε_{11} were related by the following temperature dependent correlations [Eq. (3) and (4)] and the coefficients were determined from the estimated pure properties data given in Table 1.

$$\varepsilon_{11}/k=E_a+E_b(T-T_0)+E_c(T\ln T_0/T+T-T_0) \quad (3)$$

$$V_1^*=V_a+V_b(T-T_0)+V_c(T\ln T_0/T+T-T_0) \quad (4)$$

where the reference temperature, T_0=273.15 K.

Basically the temperature-dependent correlations defined by Eqs. (3) and (4) are the empirical ones for easy practice of the EOS over a wide range of system temperatures. The estimated values of coefficients for Eqs. (2) and (3) are summarized in Table 2 for CO_2 and for coumarin derivatives.

To apply the EOS to the solubility of coumarin derivatives in CO_2, we need a cross interaction energy, ε_{ij} which is writ-

Table 1. Estimated critical constants, acentric factor and molar volume of solid-phase coumarin and its derivatives

Species	Tc	Pc	ω	V_s
Coumarin	813.34	44.77	0.526	108.976
7-Hydroxycoumarin	906.35	50.56	0.7427	114.525
7-Methylcoumarin	812.15	37.86	0.5576	123.92
7-Methoxycoumarin	816.54	35.36	0.6347	129.45
4-Hydroxycoumarin	912.54	51.30	0.7578	114.51

Table 3. Best-fitted coefficients of temperature-dependent binary interaction energy parameter defined by Eq. (5)

	$\lambda_{12}^{(0)}$	$\lambda_{12}^{(1)}\times10^3$
CO_2/coumarin	−0.783116	2.83829
CO_2/7-hydroxycoumarin	−2.20586	3.69143
CO_2/7-methylcoumarin	0.0319718	0.2280
CO_2/7-methoxycoumarin	−1.66545	2.5707
CO_2/4-hydroxycoumarin	−1.76904	2.41686

Table 2. Estimated coefficients in energy and volume parameter correlation defined by Eq. (3) and (4)

	E_a	E_b	E_c	V_a	V_b	V_c
Carbon dioxide	85.91302	−.10298	−.36562	34.28608	.01428	−0.01304
Coumarin	183.40344	−.12944	−.15367	103.29127	.02854	−.02550
7-Hydroxycoumarin	89.13693	.42290	1.04999	38.35858	−.06517	−.59018
7-Methylcoumarin	158.64526	.52635	5.04721	118.49758	.05279	.20244
7-Methoxycoumarin	78.93190	.25310	.15909	51.69035	.01856	−.21231
4-Hydroxycoumarin	89.13118	.52506	2.05592	38.14928	−.11370	−1.06634

Table 4. Experimental solubility data of coumarin derivatives (y_2) in carbon dioxide

Substance, y_2\ P [MPa]	8.5	10.0	15.0	20.0	25.0
			308.15 K		
Coumarin, $y_2\times10^3$	0.996±0.035	1.183±0.117	1.430±0.196	1.790±0.194	2.119±0.137
4-Hydroxycoumarin, $y_2\times10^6$	0.093±0.093	0.168±0.028	0.337±0.032	0.411±0.069	0.454±0.042
7-Hydroxycoumarin, $y_2\times10^6$	0.223±0.012	0.358±0.013	0.677±0.096	1.029±0.213	1.472±0.239
7-Methoxycoumarin, $y_2\times10^4$	0.165±0.058	0.880±0.269	1.288±0.019	2.946±0.794	4.099±0.024
7-Methylcoumarin, $y_2\times10^3$	0.368±0.042	0.691±0.129	1.131±0.069	1.217±0.074	1.396±0.159
6-Methylcoumarin, $y_2\times10^3$	1.167±0.011	3.097±0.096	7.814±0.217	11.68±0.389	10.65±0.706
7-Hydroxy-4-methylcoumarin, $y_2\times10^6$	0.852±0.248	1.781±0.199	3.336±0.907	3.912±0.249	5.171±1.980
			313.15 K		
Coumarin, $y_2\times10^3$	0.452±0.112	1.532±0.007	1.683±0.193	1.873±0.288	2.659±0.284
4-Hydroxycoumarin, $y_2\times10^6$	0.022±0.008	0.128±0.035	0.324±0.006	0.487±0.066	0.598±0.017
7-Hydroxycoumarin, $y_2\times10^6$	0.033±0.019	0.366±0.028	0.808±0.065	1.254±0.054	1.517±0.034
7-Methoxycoumarin, $y_2\times10^4$	0.051±0.011	0.557±0.085	1.631±0.212	3.091±0.182	4.004±0.618
7-Methylcoumarin, $y_2\times10^3$	0.103±0.020	0.656±0.211	1.317±0.069	1.606±0.041	1.793±0.066
6-Methylcoumarin, $y_2\times10^3$	0.745±0.139	2.597±0.605	6.300±0.455	8.109±0.249	10.65±0.615
7-Hydroxy-4-methylcoumarin, $y_2\times10^6$	0.367±0.088	1.594±0.410	4.409±0.753	4.696±1.147	5.811±2.350
			323.15 K		
Coumarin, $y_2\times10^3$	0.062±0.009	0.231±0.061	0.457±0.064	0.756±0.048	0.902±0.075
4-Hydroxycoumarin, $y_2\times10^6$	0.012±0.007	0.079±0.002	0.413±0.034	0.877±0.023	1.021±0.007
7-Hydroxycoumarin, $y_2\times10^6$	0.015±0.003	0.134±0.036	1.032±0.210	1.659±0.079	2.270±0.095
7-Methoxycoumarin, $y_2\times10^4$	0.005±0.003	0.457±0.056	2.092±0.035	3.931±1.610	4.546±0.098
7-Methylcoumarin, $y_2\times10^3$	0.033±0.012	0.163±0.034	2.165±0.046	1.719±0.048	2.165±0.089
6-Methylcoumarin, $y_2\times10^3$	0.600±0.036	1.291±0.015	8.343±0.733	15.38±1.370	5.061±0.370
7-Hydroxy-4-methylcoumarin, $y_2\times10^6$	0.057±0.013	0.699±0.069	5.216±1.751	8.525±3.555	12.65±0.023

ten by the following mixing rule with introducing temperature-dependent binary interaction energy parameter, $\lambda_{ij}(T)$ by

$$\varepsilon_{ij} = (\varepsilon_{ii}\varepsilon_{jj})^{0.5}[1-(\lambda_{ij}^{(0)}-\lambda_{ij}^{(1)}T)] \quad (5)$$

where the binary coefficient $\lambda_{ij}^{(0)}$ and $\lambda_{ij}^{(1)}$ were fitted by the experimental data obtained in the present study and they were summarized in Table 3.

RESULTS AND DISCUSSION

The measured solubility of coumarin and its derivatives (i.e., 7-hydroxy-, 7-methyl-, 7-methoxy-, 4-hydroxy-coumarin, 6,7-dihydroxycoumarin, 7-hydroxycoumarin-4-acetic acid and 7-methoxy-coumarin-4-acetic acid, etc.) in supercritical CO_2 were summarized in Table 4.

In Fig. 2, the effect of temperature (i.e., 308.15, 313.15

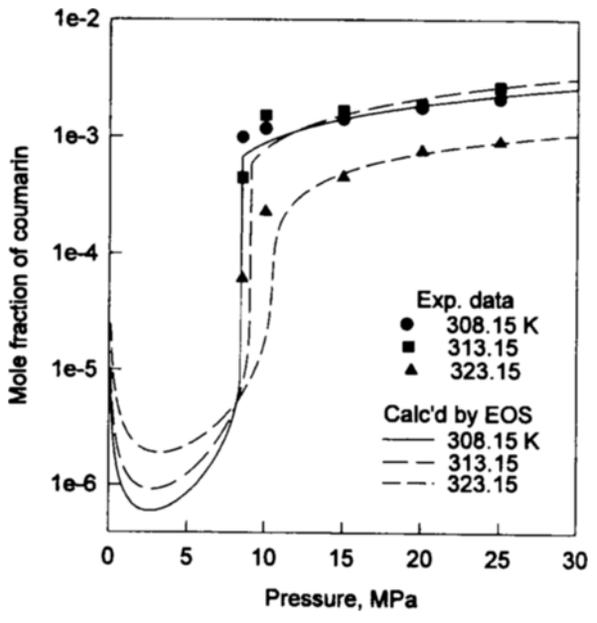


Fig. 2. Measured and calculated solubility of coumarin in supercritical CO_2.

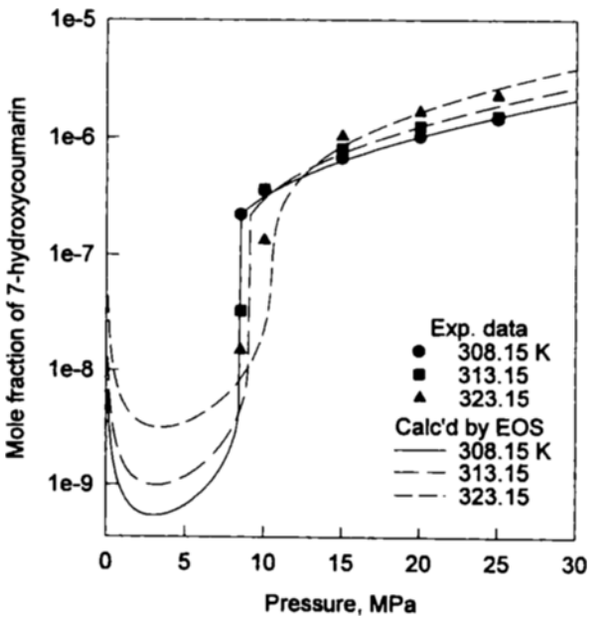


Fig. 3. Measured and calculated solubility of 7-hydroxycoumarin in supercritical CO_2.

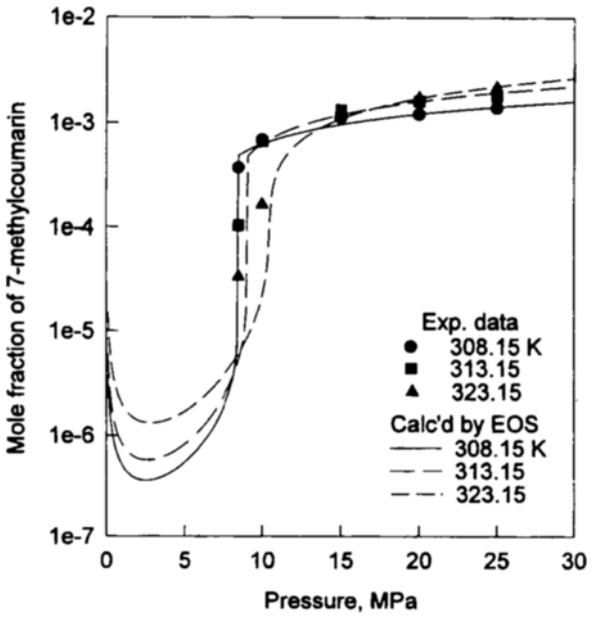


Fig. 4. Measured and calculated solubility of 7-methylcoumarin in supercritical CO_2.

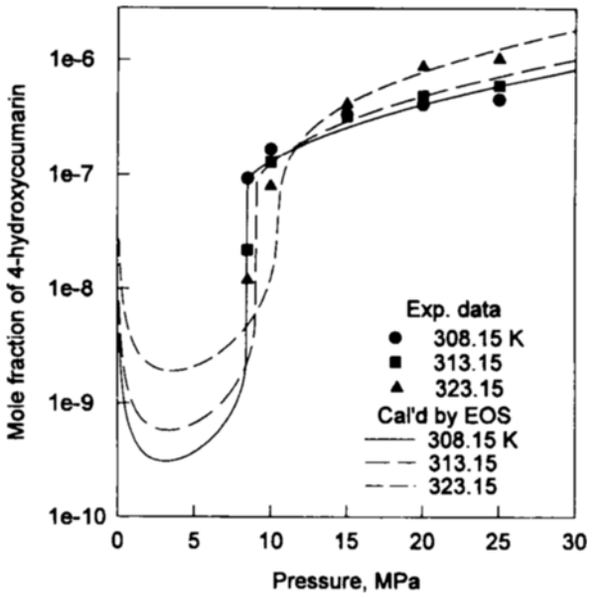


Fig. 6. Measured and calculated solubility of 4-hydroxycoumarin in supercritical CO_2.

and 323.15 K) and pressure (i.e., 8.5, 10, 15, 20 and 25 MPa) on the solubility of the basic coumarin in CO_2 were shown. The solubilities of coumarin at 323.15 K show noticeably lower values than those at 308.15 and 313.15 K. The phenomenon of crossover of solubility in the high pressure region was detected. Also, in this figure, a calculated results by the EOS were also shown in which the binary interaction energy parameter, λ_{12} (T) was made as the function of temperature [Eq. (5) and Table 3]. Next, in Figs. 3-6, the measured and calculated solubility of 7-hydroxycoumarin, 7-methylcoumarin, 7-methoxycoumarin, and 4-hydroxy-coumarin in CO_2 was summarized. For every systems, there were an effect of crossover of solubility above the critical region of CO_2. In general, the EOS fit the data reasonably with a single set of λ_{12} (T) given by Table 3.

Finally, the effect of different functional groups in position 4 and 7, on their respective solubility at 308.15, 313.15 and 323.15 K were shown in Figs. 7-9. We found that the basic coumarin, 7-methylcoumarin and 7-methoxycoumarin showed significantly higher solubility than the cases of 7-hydroxycoumarin and 4-hydroxycoumarin. Similar trend of solubility were detected for other isotherms (i.e., 308.15 and 313.15 K). Also, in general coumarin derivatives with hydroxy group show lower solubility than the cases of methyl- and methoxy-group substituted coumarin derivatives.

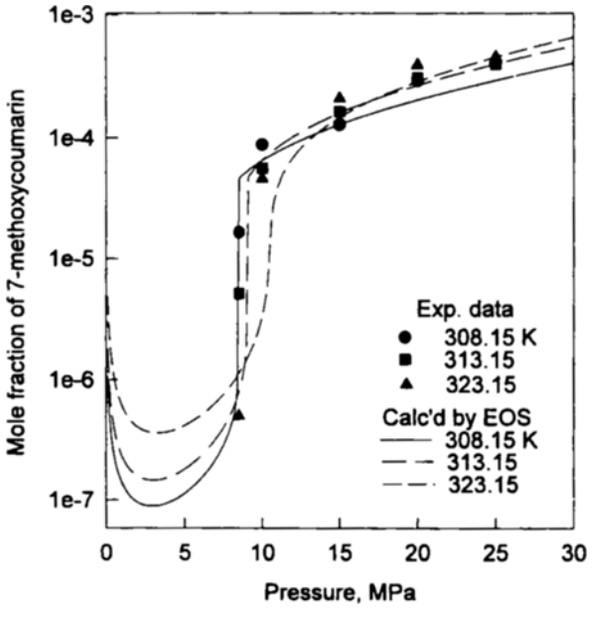


Fig. 5. Measured and calculated solubility of 7-methoxycoumarin in supercritical CO_2.

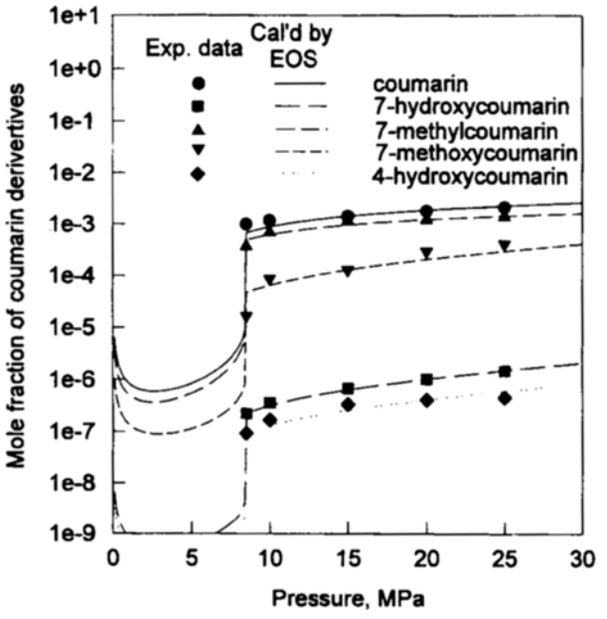


Fig. 7. Effect of functional groups on the solubility of coumarin derivatives at 308.15 K.

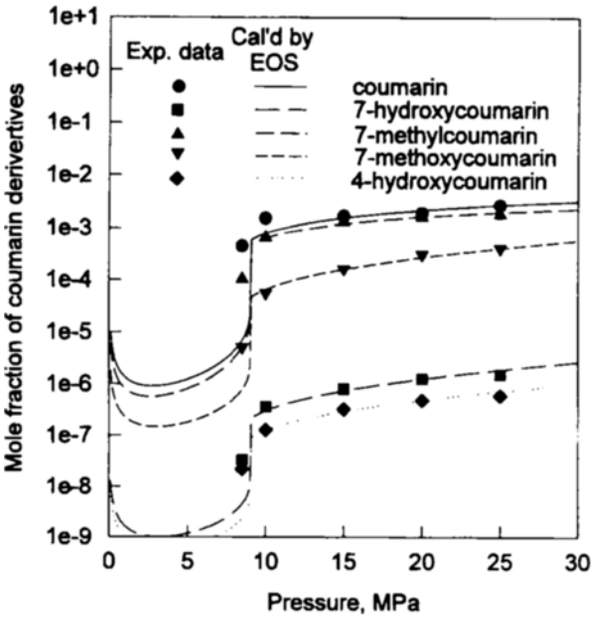


Fig. 8. Effect of functional groups on the solubility of coumarin derivatives at 313.15 K.

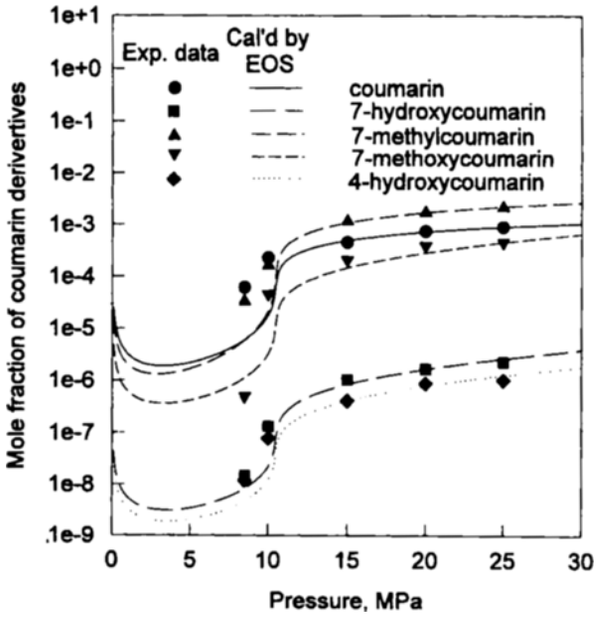


Fig. 9. Effect of functional groups on the solubility of coumarin derivatives at 323.15 K.

Furthermore derivatives made up by the substitution of two functional groups such as 6,7-dihydroxycoumarin, 7-hydroxycoumarin-4-acetic acid and 7-methoxycoumarin-4-acetic acid showed almost no solubility (y_2 were lower than 10^{-8}) and we omit here further discussion on them.

CONCLUSIONS

For economic design of SFE process for processing natural products, experimental and thermodynamic information for systems containing biosolutes and supercritical fluids are necessary. However, no such data are available in literature. Thus, in the present study, a systematic thermodynamic procedure was presented for illustratively coumarin and its various derivatives. We could draw the following results:

1. A quantitative measurement of solubility of coumarin and its derivatives was performed. We found that basic coumarin, 7-methylcoumarin and 7-methoxycoumarin showed significantly higher solubility than 7-hydroxycoumarin and 4-hydroxycoumarin.

2. Like coumarin derivatives, for systems where no pure physical properties are available, a semi-empirical estimation methods [Lyman et al., 1990] can be a valuable tool. Also, from these estimated pure property data, an EOS parameters can be estimated, and subsequently the EOS can quantitatively applied to the phase equilibria calculation of systems comprised complex biosolutes and supercritical solvent.

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