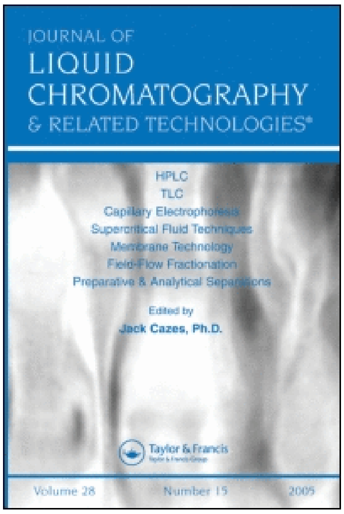

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Selection of Aqueous Two-Phase Solvent Systems in CCC

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ABSTRACT

Based on the theoretical models, including the electrolyte solution model and modified UNIFAC equation, program design for prediction of liquid–liquid equilibrium of two kinds of aqueous two-phase systems (ATPSs), including PEG/K_2HPO_4 and PEG/$(NH_4)_2SO_4$, is realized by using Visual Basic. The density calculation formula is an experimental one, resulting from the regression of many experimental data. In this program, according to thermodynamic criterion, it is determined whether there is a phase split in a solvent system with known total composition. If there is, the molar compositions, the mass compositions, and the volume of both phases are calculated. Results have been proven experimentally and by comparison with literature data. It is estimated that the program can

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shorten the experimental time and lead to the separation of substances such as proteins with ATPSs in countercurrent chromatography.

Key Words: Aqueous two-phase solvent system; Solvent selection; Countercurrent chromatography; Computer prediction; Modeling.

INTRODUCTION

Countercurrent chromatography (CCC) is a liquid–liquid partition chromatographic method which does not use a solid support in the separation column.[1] Thus, it offers many advantages, including high purity of fractions, high sample recovery, and large sample loading capacity. During the past decades, high-speed CCC has been widely used for preparative separation of many kinds of natural and synthetic substances.

Aqueous–aqueous polymer phase systems have been developed by Albertsson[2] and used for partitioning various macromolecules due to their characteristic of providing an ideal environment for biopolymers. Since the 1990s, using these solvent systems, CCC has been used for performing separation and purification of proteins.[3–5] Thus, the aqueous two-phase systems (ATPSs) present more common applications in CCC. During this type of operation, selection of solvent system is also a crucial point that can influence the separation result. But, in the past, it depended on experiences and practical operation to a great extent. Therefore, this led not only wasted time, but also caused the loss of solvent during experiments.

In this paper, by using Visual Basic, a program has been designed to predict the liquid–liquid equilibrium of two kinds of ATPSs, including PEG/K_2HPO_4 and PEG/$(NH_4)_2SO_4$, based on theoretical models. It can determine whether there is a phase split and the magnitude of molar compositions, mass compositions, and volumes of both phases. It also provides a convenient and user-friendly interface for calculating the compositions of the two phases and provides help for selection of the appropriate ATPS system.

THERMODYNAMIC MODEL AND CALCULATION OF RELEVANT PARAMETERS

Thermodynamic Model[6,7]

For PEG–K_2HPO_4–H_2O and PEG–$(NH_4)_2SO_4$–H_2O ATPSs, an extended UNIFAC equation with the Fowler-Guggenheim equation is used for prediction of the liquid–liquid equilibrium data due to the existence of electrolyte.

The working equation used is as follows:

$$\ln\gamma_i = \ln\gamma_i^{DH} + \ln\gamma_i^{UF} \tag{1}$$

The short-range interaction term takes the form of the UNIFAC equation:

$$\ln\gamma_i^{UF} = \ln\frac{\varphi_i}{x_i} + \frac{Z}{2}q_i\ln\frac{\theta_i}{\varphi_i} + l_i - \frac{\varphi_i}{x_i}\sum x_i l_i + \sum_k \upsilon_k^{(1)}[\ln\Gamma_k - \ln\Gamma_k^{(i)}] \tag{2}$$

The long-range interaction term takes the form of the Fowler-Guggenheim equation:

$$\frac{G_{DH}{}^{E}}{kT} = -\frac{V}{4\pi\alpha^3}\left[\ln(1+\kappa\alpha) - \kappa\alpha + \frac{(\kappa\alpha)^2}{2}\right] \tag{3}$$

For neutral molecules j:

$$\ln\gamma_j^{DH} = \frac{\bar{V}_j}{8\pi a^3 N_0}\left[1 + \kappa a - \frac{1}{1+\kappa a} - 2\ln(1+\kappa a)\right] \tag{4}$$

For ion I:

$$\ln\gamma_i^{DH} = -\frac{Z_i^2 e^2}{2DkT}\left(\frac{\kappa}{1+\kappa a}\right) \tag{5}$$

Discussion of Phase Split

The measurement and prediction of critical point are very difficult questions, especially for multi-component systems. For PEG–K_2HPO_4–H_2O and PEG–$(NH_4)_2SO_4$–H_2O systems, the thermodynamic stability conditions are as follows:

$$\frac{\partial\mu_i}{\partial n_i} > 0; \quad \begin{vmatrix} \frac{\partial\mu_1}{\partial n_1} & \frac{\partial\mu_1}{\partial n_2} \\ \frac{\partial\mu_2}{\partial n_1} & \frac{\partial\mu_2}{\partial n_2} \end{vmatrix} \geq 0 \tag{6}$$

When one of these conditions is not fulfilled, two phases will appear. So, according to the thermodynamic model, the activity coefficient of every component can be calculated and then the stability of the system can be judged through Eq. (6).

Calculation of Compositions Following Equilibrium

For the solvent system consisting of three solvents, the equilibrium equations are as follows:

$$x_i n_1 + x_i' n_1' = x_i{}^0 n_t \quad i = 1, 2, 3 \tag{7}$$

$$\sum_{i=1}^{3} x_i = 1, \quad \sum_{i=1}^{3} x_i' = 1 \tag{8}$$

where n_1, n_1' are the total molar numbers of two balanced phases, respectively. n_t is the total molar number of the system. x_i, $x_i{}'$ are the mole fractions of component i in upper and lower phases, respectively. $x_i{}^0$ is the total mole fraction of component i before equilibrium.

Meanwhile, the thermodynamic equilibrium equation is as follows:

$$x_i \gamma_i = x_i' \gamma_i' \tag{9}$$

where γ_i, $\gamma_i{}'$ are the activity coefficients of component i in two balanced phases, respectively, which can be solved by the extended UNIFAC equation. The required parameters during the solution process can be referred to the literature.[6,7]

Once the original composition of the system is given, the series of equations, including x_i, $x_i{}'$, n_1, n_1' which are unknown, can be solved and the compositions of two phases after equilibrium is obtained.

Calculation of Density

After different solvents are mixed, the total volume of solution will change, since the solvents are not ideal under actual circumstances. In order to obtain more precise results, the densities of all components, including PEG with various molecular weights, K_2HPO_4, and $(NH_4)_2SO_4$ are calculated through the regression of extensive experimental data in this paper. Meanwhile, the density formula of the mixture of PEG, salt, and water is also achieved by the same method. Then, it is found that the density of an individual component is linear with its mass percentage and the density of a mixture is in the order of two magnitudes to the mass percentage of the individual component made up in the mixture. Tables 1 and 2 show the data and their regression formulas.

Observing the density data for two kinds of salts, it is easily determined that their density changes with mass percent of the salt are approximate, so the salt, K_2HPO_4, is regarded as a deputy to mix with PEG for measuring the

Table 1. Calculated densities of the PEG, K_2HPO_4, $(NH_4)_2SO_4$ ATPS systems.

Density component	Mass percent 5%	10%	15%	20%	Density formula (10^3 kg/m^3 or g/cm^3)
$(NH_4)_2SO_4$	1.026	1.053	1.080	1.104	$d = 0.520m + 1.001$
K_2HPO_4	1.024	1.056	1.088	1.116	$d = 0.610m + 0.995$
PEG					
$M = 1{,}000$	1.005	1.013	1.023	1.031	$d = 0.175m + 0.996$
$M = 2{,}000$	1.005	1.015	1.023	1.032	$d = 0.174m + 0.997$
$M = 4{,}000$	1.006	1.015	1.024	1.034	$d = 0.180m + 0.997$
$M = 6{,}000$	1.003	1.012	1.022	1.031	$d = 0.182m + 0.996$

mixed density of the ATPS. Similarly, the densities of PEG with different molecular weights are also approximate, except for the one of PEG whose molecular weight is 6000; so, PEG whose molecular weight is 4000 is selected to measure the mixed density of the solvent system.

Calculation of Volumes of Two Phases

In this paper, the volume of each phase is calculated on the basis of density and mass fraction of each component; the working equation is as follows:

$$V_i = \frac{\left(\sum_{i=1}^{3} x_i n_{st} M_i\right)}{d_m} \tag{10}$$

where V_i and n_{st} stand for the volume and the total mole number of one phase, x_i and M_i is the mole fraction and molecular weight of component i in this phase.

Table 2. Calculated densities for PEG($M = 4000$) + K_2HPO_4 + H_2O mixtures with various mass percent of polymer and/or salt.

W% (K_2HPO_4)	1.99	3.05	3.12	3.86	3.96	4.92	5.28
W% (PEG)	25.9	21.9	19.9	18.21	16.20	14.02	11.83
d_m(10^3 kg/m^3)	1.055	1.055	1.052	1.054	1.052	1.053	1.052
W% (K_2HPO_4)	4.93	6.03	6.22	8.16	8.89	12.4	
W% (PEG)	10.00	7.95	3.92	6.01	3.98	1.94	
d_m (10^3 kg/m^3)	1.047	1.050	1.045	1.061	1.062	1.082	

$$d_m = 0.998 + 0.636x_{\mathrm{KHP}} + 0.172x_{\mathrm{PEG}} + 0.143x_{\mathrm{KHP}}^2 - 0.0055x_{\mathrm{PEG}}^2$$

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RESULTS AND DISCUSSION

Judgment of Stability of Solvent System

For PEG/K_2HPO_4 ATPS, the stability is investigated and the result is shown in Fig. 1. It can be seen that most fitted values approach the original experiment results. That is, the judgment about stability of system is feasible on the whole once the experiment point leaves the critical line.

Prediction and Comparison of Constituents of Two Balanced Phases

For PEG–K_2HPO_4–H_2O and PEG–$(NH_4)_2SO_4$–H_2O ATPSs, the mole fraction and mass percent of each component after phase splitting are predicted by this program and compared with the experimental results. Then, the error between prediction (p) and experiment (e) is presented in terms of the root-mean-square error

$$\left[\delta = \sqrt{\frac{1}{n}\sum_i (x_{i,\mathrm{e}} - x_{i,\mathrm{p}})^2}\right] \tag{11}$$

Table 3 shows the predicted and experiment values.

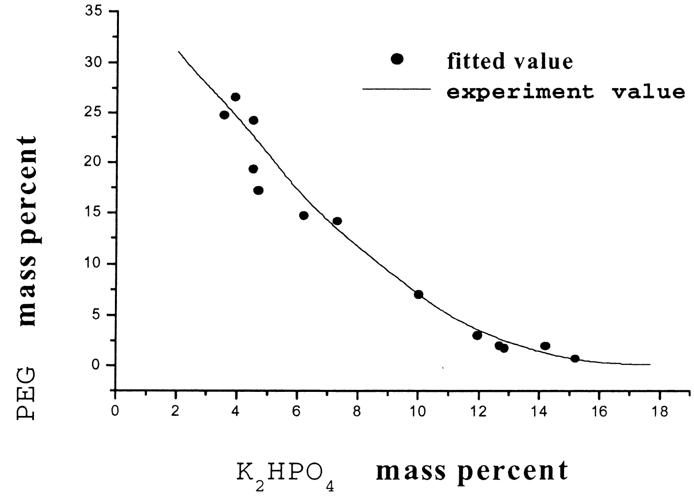

Figure 1. Comparison between fitted and experiment values.

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Table 3. Comparison between computed predictions and experimental results.

				$x_{i,\mathrm{e}}$ (experimental, mol/mol)			$x_{i,\mathrm{p}}$ (calculated, mol/mol)			
System	$x_{\mathrm{PEG}}{}^0$	M_{PEG}	$x_{\mathrm{K_2HPO_4}}{}^0$	PEG	K_2HPO_4	H_2O	PEG	K_2HPO_4	H_2O	δ
1[a]	0.00334	1,000	0.0184	0.00854	0.00719	0.9843	0.00821	0.00758	0.9842	0.000301
				0.000312	0.0253	0.9743	0.000274	0.0253	0.9744	0.000062
2[a]	0.00105	2,000	0.0133	0.00185	0.0103	0.9878	0.00164	0.0116	0.9868	0.000955
				0.000505	0.0164	0.9831	0.000614	0.0146	0.9848	0.00143
3[a]	0.00048	4,000	0.0119	0.000829	0.00971	0.9895	0.000580	0.0106	0.9888	0.000669
				0.000331	0.0140	0.9857	0.000232	0.0152	0.9846	0.000942
4[a]	0.00028	6,000	0.0113	0.000555	0.00771	0.9917	0.000399	0.00988	0.9897	0.00171
				0.000117	0.0133	0.9866	0.000200	0.0124	0.9874	0.00700
5[b]	0.00094	4,000	0.0188	0.00218	0.00887	0.9890	0.00202	0.0102	0.9878	0.00104
				0.000106	0.0327	0.9672	0.000134	0.0254	0.9745	0.00596
6[b]	0.00067	6,000	0.0152	0.00118	0.00620	0.9926	0.00135	0.00720	0.9914	0.000907
				0.000041	0.0251	0.9748	0.000039	0.0225	0.9775	0.00216

(*continued*)

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Table 3. Continued.

System	$x_{PEG}{}^0$	M_{PEG}	$X_{salt}{}^0$	$x_{i,e}$ (experimental, mol/mol)			$x_{i,p}$ (calculated, mol/mol)			δ
				PEG	$(NH_4)_2SO_4$	H_2O	PEG	$(NH_4)_2SO_4$	H_2O	
7[a]	0.00415	1,000	0.0306	0.00543	0.0193	0.9753	0.00734	0.0165	0.9762	0.00202
				0.00196	0.0290	0.9690	0.00213	0.0276	0.9703	0.00111
8[a]	0.00185	2,000	0.0237	0.00453	0.0101	0.9854	0.00454	0.0135	0.9820	0.00278
				0.000133	0.0328	0.9671	0.000378	0.0293	0.9703	0.00274
9[a]	0.00053	4,000	0.0192	0.00153	0.0109	0.9876	0.00162	0.0104	0.9880	0.00037
				0.000090	0.0235	0.9764	0.000096	0.0227	0.9772	0.00065
10[b]	0.00055	4,000	0.0250	0.00234	0.00812	0.9895	0.00379	0.0122	0.9840	0.00289
				0.000159	0.0325	0.9673	0.000223	0.0264	0.9734	0.00500
11[b]	0.00077	6,000	0.0174	0.000883	0.00535	0.9938	0.00099	0.00750	0.9915	0.00182
				0.000077	0.0304	0.9695	0.000066	0.0481	0.9518	0.0144

Note: For each system, the first row corresponds to the upper phase (PEG rich), the second row is that of the lower phase (salt rich).

From Table 3, we can see that the error for prediction of the PEG–K_2HPO_4–H_2O system is smaller than the error for the PEG–$(NH_4)_2SO_4$–H_2O system on the whole. The main reason is, perhaps, that the parameters used in the extended UNIFAC equation for the PEG/$(NH_4)_2SO_4$ system are unsafe, compared with those of the PEG/K_2HPO_4 system. But, the error between prediction and experiment is acceptable due to the accuracy of the UNIFAC equation itself. So, the results of calculation can be used to design the actual experiments and provide general information about the ATPS.

Prediction and Comparison of the Volumes of Two Balanced Phases

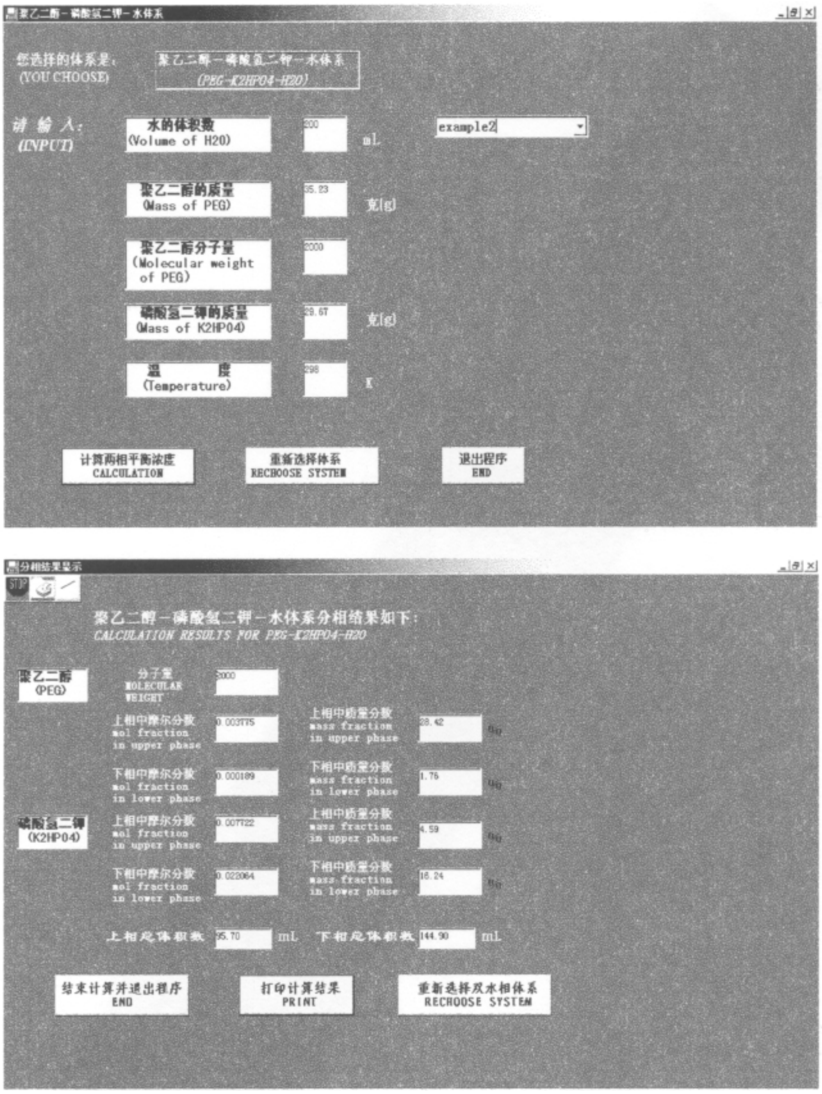
Table 4 shows the comparative predicted and experimental results; the error between prediction (p) and experiment (e) is presented in terms of the relative error

$$\left[\delta = \frac{|\mathrm{pre} - \exp|}{\exp}\right] \tag{12}$$

Table 4 shows the predicted and experimental values of two phases' volumes.

Table 4. Experimental (e) and predicted (p) values for the phase volumes of PEG–phosphate ATPS systems.

System	$x_{PEG}{}^0$	M_{PEG}	$x_{K_2HPO_4}{}^0$	Volume ratio (e)	Volume ratio (p)	δ
1	0.000665	4,000	0.0162	0.67	0.52	0.22
2	0.000294	6,000	0.0125	0.91	0.76	0.16
3	0.000226	6,000	0.0171	0.56	0.75	0.34
System	$x_{PEG}{}^0$	M_{PEG}	$x_{(NH_4)_2SO_4}{}^0$	Volume ratio (e)	Volume ratio (p)	δ
4	0.000708	4,000	0.0178	2.99	2.71	0.09
5	0.000485	4,000	0.0208	0.46	0.28	0.39
6	0.00077	6,000	0.0174	2.52	2.89	0.15



聚乙二醇－磷酸氢二钾－水体系

您选择的体系是：(YOU CHOOSE) 聚乙二醇－磷酸氢二钾－水体系 (PEG-K2HPO4-H2O)

请 输 入：(INPUT)

水的体积数 (Volume of H2O): 200 mL

聚乙二醇的质量 (Mass of PEG): 35.23 克(g)

聚乙二醇分子量 (Molecular weight of PEG): 2000

磷酸氢二钾的质量 (Mass of K2HPO4): 29.67 克(g)

温 度 (Temperature): 298 K

example2

分相结果显示

聚乙二醇－磷酸氢二钾－水体系分相结果如下：
CALCULATION RESULTS FOR PEG-K2HPO4-H2O

聚乙二醇 (PEG)

分子量 MOLECULAR WEIGHT: 2000

上相中摩尔分数 mol fraction in upper phase: 0.003775 上相中质量分数 mass fraction in upper phase: 28.42 %

下相中摩尔分数 mol fraction in lower phase: 0.000189 下相中质量分数 mass fraction in lower phase: 1.76 %

磷酸氢二钾 (K2HPO4)

上相中摩尔分数 mol fraction in upper phase: 0.007722 上相中质量分数 mass fraction in upper phase: 4.59 %

下相中摩尔分数 mol fraction in lower phase: 0.022064 下相中质量分数 mass fraction in lower phase: 16.24 %

上相总体积数 95.70 mL 下相总体积数 144.90 mL

Figure 2. The screen interface of the calculation program. Top: input of data; bottom: results displayed.

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Examining Table 4, we can see that the relative errors are generally large. The main reasons that should be considered are as follows. On one hand, the density formulas used in this program are only valid in the single-phase zone, so they are perhaps not fitted for the calculation in a biphasic zone. On the other hand, the mixed density formula is related to the mole fractions of PEG and salt; therefore, the mole fraction of each component should influence the volume value to a great extent. Because the calculation produces results with a significant error, the mixed density of one phase will appear with a significant deviation and then the volume value will have a large error. This is contained in Eqs. (10) and (11).

Brief Introduction to the Program

In the program, the required original data include the volume of water and the masses of PEG and salt, besides the temperature and molecular weight of PEG. After the solvent system is chosen, the data are input and the button for calculation is pressed; the results of two phases will be presented. Certainly, if the solvent system with the input data has not phase split, a message box will appear with a warning. Figure 2 shows the calculation interface in the program.

CONCLUSION

Based on the theoretical models, including the extended UNIFAC equation and the Fowler-Guggenheim equation, a program is designed to calculate and predict the liquid–liquid equilibrium of PEG–salt–H_2O ATPSs. In order to obtain more precise results, the calculation formula of density of solvent system is regressed through numerous experiments.

In this program, a judgment is made about whether there is a phase split or not, firstly, then the mole fraction and mass percent of each component is calculated if there is phase split. Meanwhile, the volumes of the two phases are provided. A convenient and user-friendly interface is presented so that the man–machine interaction becomes more comfortable. Finally, results have been proven by the experiments and by comparison with literature data. Due to the accuracy of UNIFAC equation, the prediction results are generally acceptable.

Through this program, it becomes easier to choose the solvent system while separating protein by using an ATPS in CCC. Moreover, it can also save the experimental time and the solvent system itself.

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