Optimization of the Phase System Composition of Aqueous Two-Phase System for Extraction of 2,3-Butanediol by Theoretical Formulation and Using Response Surface Methodology

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In recent days the aqueous two-phase extraction is attracting lot of attention in biotechnology. In this study theoretical exercise was taken leading to the development of biondal and tie-line equation, which eventually helped in calculation of total, top and bottom phase compositions theoretically for given pair of volume ratio and tie-line length and vice-versa. Experiments were done using response surface methodology to find the effects of volume ratio and tie-line lengths and, thereby, the phase composition of an aqueous two-phase system and their mutual interactions on (equilibrium) distribution coefficient of 2,3-butanediol and settling time, separately. A 2^2 full-factorial central composite experimental design was adopted and the data were analysed by statistical techniques. The optimum volume ratio and tie-line length were found to be 1.61 and $w = 22.84$ %, respectively, in the case when distribution coefficient was the response quantity and $w = 1.89$ and 22.22 %, respectively, when settling time was the response quantity. The final optimized phase compositions were found to be $w = 8.7\%$ dextran 40000 and $w = 9.0$ % polyethylene glycol 6000 in the case, where partition coefficient was the response parameter and the corresponding values were $w = 8.0\%$ and 9.2%, respectively, when settling time was the response quantity. The maximum distribution coefficient of 2,3-butanediol and the minimum settling time in the corresponding optimized phase composition were calculated to be 1.114 and 8.5 min from the respective equations.

Keywords:

Aqueous two-phase, binodal curve, statistical optimization techniques, separation, 2,3-butanediol

Introduction

Aqueous two-phase extraction has already been established as a powerful method for biomolecule purification.[1–3] It is increasingly gaining importance as an important tool for extractive fermentation process.[4–9] The aqueous two phases can be obtained by mixing two polymers (mutually incompatible) or a single polymer and a salt in water above a certain concentration. The most widely studied system is polyethylene glycol (PEG)-dextran-water system. By careful tailoring of the total composition of the polymers involved, dextran and polyethylene glycol, the product of the fermentation can be partitioned into one of the phases while the cells remain in the other.[10] The efficiency of separation of desired product depends upon its (equilibrium) distribution coefficient.[11] The (equilibrium) distribution coefficient (K) of a desired product in an aqueous two-phase system has been found to be a function of many structural properties of the product / biomolecules, such as electrochemical property, hydrophobic interaction, molecular mass dimension, biospecificity and conformation, as well as on the environmental properties.[1] One of the important properties is the phase system composition, which in turn governs the volume ratio (Ψ) and tie-line length (s_{TLL}), and vice-versa (tie-line length and volume ratio together, and not separately, fix a phase composition).

For continuous operation of extractive fermentation with cell recycle, the time required for the phases to separate (settling time), is an important factor which influences the dilution rate and thereby the productivity of the process. The settling time and its characteristic curve is also influenced by tie-line length and volume ratio and hence, by the phase composition.[12] It depends on the difference in density between the two phases and their viscosities. As the tie-line length increases the density difference between the two phases increases which may decrease the settling time. On the other hand, this may increase the viscosity of the bottom phase and there by hinder the phase separation. So, under the influence of two counteractive effects, there lie

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a moderate phase composition that balances these effects and gives the minimum settling time. When the tie-line comes close to plait point (where composition and the volumes of the two phases are theoretically equal) the density difference between the phases decreases and hence, time for phase separation increases.

In traditional single-factor optimization approach each quantity is considered to be independent on other process variables. In the statistical optimization process all the variables are considered simultaneously where different degree of interaction of the same variable and interaction among all different variables are also considered. This approach also decreases the number of experiments required and there by reduces the cost and labor. As the performance of aqueous two-phase systems characterized by distribution coefficient and settling time for phase separation depends on a number of physical and chemical properties, it may be worthwhile to study the system using statistical design experiments.

The present work of optimization of phase system for maximizing the extraction of 2,3-butanediol and minimizing the settling time of the phase system separately was a part of the bigger project for using the optimized phase system as means of extractive fermentation of 2,3-butanediol with cell recycle. A 2^2 full factorial central composite design using response surface methodology was employed for the purpose.[13–16] This paper describes the effects of the tie-line length and volume ratio on distribution coefficient and settling time, separately. This helped in fixing an optimal phase composition. This paper also comprises of theoretical formulations that help in getting equations of the binodal curve and tie-line and thereby enable calculation of the total mixture, top and bottom phase compositions, once the volume ratio and tie-line length is known for given two-polymer two-phase system and vice-versa.

Materials and methods

The chemicals used

The polymers used were Dextran 40 000 (dextran T40, Core health care Ltd., India) and Polyethylene glycol 6000 (PEG 6000, Sisco Research Laboratory Pvt. Ltd., Bombay, India). 2,3-butanediol was procured from Sigma chemicals Co (St. Louis, MO, USA).

Binodal Curve and Phase Compositions

Several phase systems were prepared with different amounts of dextran and PEG mass fraction. The samples from the top and bottom phases were analysed for dextran and PEG fraction in the respective phases by measuring optical density and refractive index of the samples. The temperature of the phase system was maintained constant at 30 °C keeping the separating funnel in a temperature-controlled chamber during the phase separation. The phase compositions (top and bottom) were plotted on a graph to obtain the binodal curve. The Tie-line was obtained by joining top (T), total (M) and bottom (B) phase composition by a straight line and the volume ratio ($\Psi_{MB/MT}$) was also computed. The method is described in detail by *Albertsson*.[17]

Theoretical formulation for the Calculation of mixture composition for making two-phase system

The statistical design provides the tie-line length and volume ratio from which the corresponding total mixture compositions were calculated to get a phase system. The following mathematical formulation was employed for the purpose. The volume ratio of a two-phase system may be given by the Eq (1)[17] and is illustrated in Figure 1. The top, total mixture and bottom phase compositions, were indicated by the points T ($w_{D,t}$, $w_{P,t}$), M ($w_{D,m}$, $w_{P,m}$) and B ($w_{D,b}$, $w_{P,b}$) respectively (the coordinates of the corresponding points are given in brackets). Point P on the binodal curve (Figure 1) is known as the critical point (or plait point) at which the volumes and compositions of the top and bottom phases theoretically become equal.[17,18]

$$\Psi = \frac{V_t}{V_b} = \frac{\rho_b}{\rho_t} \cdot \frac{s_{MB}}{s_{MT}} \cong \frac{s_{MB}}{s_{MT}};$$

$$\text{considering } \frac{\rho_b}{\rho_t} \cong 1 \qquad (1)$$

For a given volume ratio, the total mixture composition may be calculated by the following formulae

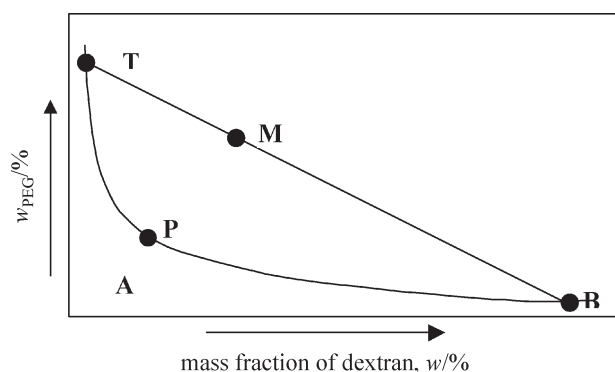$$w_{D,m} = \frac{\Psi w_{D,t} + w_{D,b}}{\Psi + 1} \qquad (2)$$

F i g. 1 – *Schematic phase diagram with tie-line*

$$w_{P,m} = \frac{\Psi\, w_{P,t} + w_{P,b}}{\Psi + 1} \qquad (3)$$

So, total mixture composition ($w_{D,m}$, $w_{P,m}$) may be known once the top ($w_{D,t}$, $w_{P,t}$) and bottom ($w_{D,b}$, $w_{P,b}$) phase compositions are known.

If two points on the binodal curve, that is top and bottom phase compositions for a particular mixture composition are known, the distance between them is the tie-line length and may be calculated using equation 4.

$$s_{TLL} = \sqrt{(w_{D,t} - w_{D,b})^2 + (w_{P,t} - w_{P,b})^2} \quad (4)$$

Diamond and *Hsu*[18] developed a correlation for aqueous polymer phase diagram behavior based on the Flory-Huggins theory from which following relationship may be obtained:

$$\ln\left|\frac{w_{P,t}}{w_{P,b}}\right| = \left(\left|\frac{w_{D,t}}{w_{D,b}}\right|\right)^{A} = \left(\left|\frac{w_{D,b}}{w_{D,t}}\right|\right)^{-A} \qquad (5)$$

which reduces to the form

$$\ln|w_{P,t} \cdot w_{D,t}^{-A}| = \ln|w_{P,b} \cdot w_{D,b}^{-A}| \qquad (6)$$

Equation (6) may be written as

$$w_{P,t} \cdot w_{D,t}^{-A} = w_{P,b} \cdot w_{D,b}^{-A} = g \qquad (7)$$

The general form of the eq (7) is,

$$w_{P} \cdot w_{D}^{-A} = g \qquad 8)$$

Where A and g are the constants for a given phase system at a certain temperature and may be calculated from the following rearranged form of the above equation (eq (8)).

$$\ln(w_{P}) = A \ln(w_{D}) + \ln(g) \qquad (9)$$

$\ln(w_{P})$ may be plotted against $\ln(w_{D})$ in a straight line to get *A* and *g*.

Differentiating equation 8, we get

$$\Delta w_{P} w_{D}^{-A} - A w_{P} w_{D}^{-(A+1)} \Delta w_{D} = 0 \qquad (10)$$

which on rearrangement gives

$$\frac{\Delta w_{P}}{\Delta w_{D}} = A \frac{w_{P}}{w_{D}} \qquad (11)$$

Applying limiting concept,

$$\lim_{\Delta D \to 0} \frac{\Delta w_{P}}{\Delta w_{D}} = \frac{dw_{P}}{dw_{D}} = A \frac{w_{P}}{w_{D}}$$

it is clear that the curvature of the binodal curve changes as the mass fraction of PEG and dextran change. So, the slope at the plait point may be calculated as follows:

$$\frac{dw_{P}}{dw_{D,p}} = A \frac{w_{P,p}}{w_{D,p}} \qquad (12)$$

$w_{D,p}$ and $w_{P,p}$ may be found experimentally. Hence, the general equations of tie-lines (which are parallel) for given mixture compositions ($w_{D,m}$, $w_{P,m}$) may be obtained as

$$w_{P} - w_{P,m} = A \frac{w_{P}}{w_{D}}(w_{D} - w_{D,m}) \qquad (13)$$

Which may be linearized to the form

$$w_{P} = A \frac{w_{P,p}}{w_{D,p}} \cdot w_{D} + \left(w_{P,m} - A \frac{w_{P,p}}{w_{D,p}} \cdot w_{D,m}\right)(14)$$

For all the tie-lines the slope $\left(m = A \dfrac{w_{P,p}}{w_{D,p}}\right)$ will remain constant and the intercept

$$\left(w_{C} = w_{P,m} - A \frac{w_{P,p}}{w_{D,m}} \cdot w_{D,m}\right)$$

on PEG mass fraction axis will change depending on the mixture composition.

Hence, the equation of tie-line (Eq. (14)) may be written as

$$w_{P} = m w_{D} + w_{C} \qquad (15)$$

Using equation 4 and 15 we get,

$$s_{TLL} = (w_{D,b} - w_{D,t})\sqrt{m^2 + 1} \qquad (16)$$

From 7 we write

$$w_{P,t} = g\, w_{d,t}^{A} \quad \text{and} \quad w_{P,b} = g\, w_{d,b}^{A} \qquad (17)$$

Upon subtracting we get,

$$w_{P,t} - w_{P,b} = g\,(w_{D,t}^{A} - w_{D,b}^{A}) \qquad (18)$$

Equation (15) and (18) may be combined to get

$$m(w_{D,t} - w_{D,b}) = g\,(w_{D,t}^{A} - w_{D,b}^{A}) \qquad (19)$$

Combining equations (16) and (19) we get

$$\frac{m\, s_{TLL}}{g\sqrt{m^2 + 1}} = w_{D,b}^{A} - w_{D,t}^{A} \qquad (20)$$

Equation (16) and (20) may be simultaneously solved to get $w_{D,t}$ and $w_{D,b}$, which may be put in the eq (8) to get $w_{P,t}$ and $w_{P,b}$ respectively. The total mixture composition, that is mass fraction dextran ($w_{D,m}$) and fraction PEG ($w_{P,m}$) required to get respective phase system for a given tie-line length and volume ratio, can then be calculated using the equations 2 and 3. It may be noted that for a known binodal curve, values of A, g and plait point mass fraction ($w_{D,p}$, $w_{P,p}$) are known.

Statistical optimization method

A full-factorial design,[13,15] which includes all possible combinations for each of the factors, is a powerful tool for understanding complex processes. The full-factorial central composite design consists of 1) a complete 2^k factorial design, where k (= 2) is the number of test variables, 2) n_0 center points ($n_0 > 1$) and 3) two axial points on the axis of each design variables at a distance of α ($\alpha = 2^k/4$, = 1.414 for $k = 2$) from the design center.[14] Hence, the total number of design point is $N = 2^k + 2k + n_0$ and these data are fitted in a second order polynomial model. An orthogonal 2^2 full-factorial central composite design[19] with five replicates ($n_0 = 5$) at the central point, all in duplicates, resulting in total of 13 experiments in each case, were used to optimize the chosen key variables for maximizing distribution coefficient and minimizing settling time. The levels of parameters that have effect on the partition coefficient and settling time are given in Table 1 for both cases. The variables are coded according to the Eq (21).

$$X_i = \frac{x_i - x_0}{\Delta x_i} \qquad (21)$$

The behavior of this system was explained by the following second-degree polynomial equation:

$$Y = \beta_0 + \Sigma \beta_i X_i + \Sigma \beta_{ii} X_i^2 + \Sigma \beta_{ij} X_i X_j \quad (22)$$

A statistical program package, Design Expert (Start-Ease Inc., Minneapolis, MN) was used for regression analysis of the data obtained and to estimate the coefficient of multivariate equation. The graphical representation of these equations are called response surfaces, which was used to describe the individual and cumulative effects of the test-variables on the response, and to determine the mutual interactions between the test variables and their subsequent effect on the response.[14] The correlation measures for the estimation of the regression equations are the multiple correlation coefficients R and the determination coefficient R^2. ANOVA (Analysis of Variance) was used to test the significance and adequacy of the model.

The F value (Fisher's variance ratio, Sr^2/Se^2) was calculated from ANOVA. F values much higher than unity indicate, that the factors explain adequately the variation in the data about its mean and effects of estimated factors are true.

The Student-t-distribution and the corresponding probability values (P values) indicate the significance of each of the coefficient, which in turn governs the patterns of interactions between the variables. The smaller the value of P the more significant is the corresponding coefficient.[14]

Estimation of distribution coefficient of 2,3-butanediol

The phase systems were prepared in a separating funnel. A pure (analytical grade) 2,3-butanediol was added (10 mg g^{-1} of total phase system) while making the aqueous two-phase preparation. The pH of the phase system was adjusted to 7.0 using 0.1 mol l^{-1} phosphate buffer and temperature was maintained at 30 °C, keeping the separating funnel in a temperature controlled chamber. The samples were then drawn from both phases and analysed for 2,3-butanediol by gas chromatography (Nucon Gas Chromatograph, India) method. Nitrogen gas ($Q = 35$ ml min^{-1}) was used as the carrier gas. The Flame Ionization Detector (FID) and Poropak~q column were used for analysis (Oven Temperature: 200 °C, Injector Temperature: 300 °C, Detector Temperature: 300 °C). The distribution coefficient of 2,3-butanediol for each system was calculated from the concentration ratio of the top phase to that of the bottom phase and is given by Nernst's equation (Eq. (23)).

$$K = \frac{c_t}{c_b} \qquad (23)$$

Estimation of settling time

The settling time for each of the phase system was obtained by disturbing the phase system fully and allowing it to settle in a graduated cylinder. The volume settled was then observed at one minute interval. Though, theoretically a phase system takes infinite time to settle, practically settling time is the time when no further significant increment in lower phase volume is observed.

T a b l e 1 – *Experimental range and levels of the independent variables (volume ratio and tie-line length) for each of the responses (distribution coefficient of 2,3-butanediol and settling time)*

Dependent variable	$-\alpha$	-1	0	1	α
Volume ratio (Ψ)	0.086	0.5	1.5	2.5	2.914
Tie-line length (s)	5.86	10	20	30	34.14

Results

To construct phase separation curve (binodal curve) for dextran T40 / PEG 6000 / water system, a number of different mixture compositions were taken: {(mass fraction dextran w_{T40}/% and PEG 6000 w_P/%): (6.0, 6.5), (6.5, 7.0), (7.0, 7.5), (7.5, 8.0), (8.0, 8.5)}. Corresponding to each phase composition (total mixture composition) a pair of top and bottom phase composition was obtained. To get the theoretical equation of the binodal curve, the constants (A and g) of the equation 8 were obtained by plotting the top and bottom phase compositions thus obtained, in the form given in equation 9 (Figure 2). The values A and g were calculated as –0.645 and 16.24 respectively for Dextran T40 / PEG 6000 / water two-phase system. Hence, the equation 8 takes the following shape, which is the equation of the binodal curve for this given pair of polymer system.
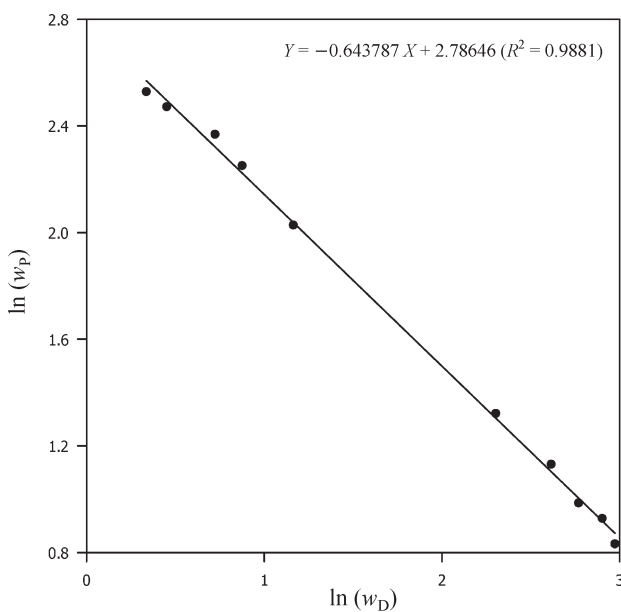

$$Y = -0.643787\,X + 2.78646\ (R^2 = 0.9881)$$

F i g . 2 – *Correlation of aqueous polymer phase diagram data for dextran T40 / PEG 6000 / water system using Eq. (9)*

$$w_P\, w_D^{0.645} = 16.24 \qquad (24)$$

The phase system becomes more and more sensitive as the tie-line length becomes shorter and shorter and it is highly sensitive for a phase composition close to plait point and experimental determination of the plait point almost impossible (1). However, this was obtained by extrapolating the curve joining the middle points of the experimental tie-lines (Figure 3). The binodal curve shown in Figure 3 is the logarithmic fit of the experimental points of the top and bottom phase compositions for
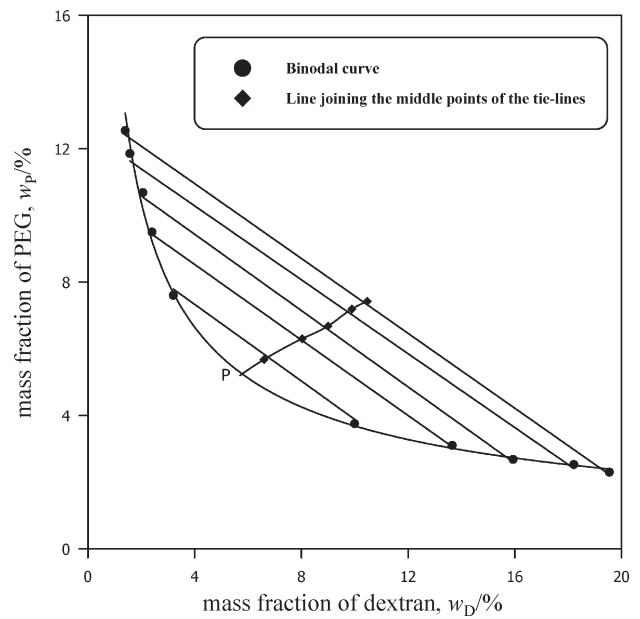

F i g . 3 – *Determination of the plait point for dextran T40 / PEG 6000 binodal curve. Temperature 30 °C, pH 7.0 in 0.1 mol dm^{-3} phosphate buffer. The total mixture compositions for the tie-lines were {in increasing order of tie-line length ($w_{dextran\ T40}$, $w_{PEG\ 6000}$): (6.0, 6.5), (6.5, 7.0), (7.0. 7.5), (7.5, 8.0), (8.0, 8.5)}*

each total mixture compositions used for Figure 2. The plait point P, as shown in Figure 3, composition were found to be $w_{D,p}$ = 5.7 % dextran and $w_{P,p}$ = 5.2 % PEG. Hence, the general equation of the tie-line (Eq. (15)) was found as follows (Eq. (25)), where the value of w_C depends on total mixture composition, $w_{D,m}$ and $w_{P,m}$ (Eq. (14)).

$$w_P = -0.5875\,w_D + w_C \qquad (25)$$

Two separate sets of experiments were carried out using two different response parameters for optimizing the phase composition. The combinations of variables and the experimental results for each case are presented in coded and uncoded form in Table 2. The partition coefficient was higher and settling time was lower at the phase composition near to center point position. In both the cases the experimental data were analysed by employing statistical method. The following two equations were obtained independently, one for the optimization of distribution coefficient (Eq. (26)) and the other for the optimization of settling time (Eq. (27)) by regression analysis using the experimental data.

$$Y_1 = 1.112 + 0.001768 X_1 + 0.0145 X_2 - 0.014126 X_1^2 -$$

$$0.026630 X_2^2 + 0.005 X_1 X_2 \qquad (26)$$

$$Y_1 = 8.90048 - 1.28919 X_1 - 2.00905 X_2 + 1.54963 X_1^2 +$$

$$+ 4.30046 X_2^2 + 0.25 X_1 X_2 \qquad (27)$$

T a b l e 2 – *Coded and uncoded full factorial central mass fraction design for the test variables (volume ratio and tie-line length) for both the responses (distribution coefficient of 2,3-butanediol and settling time)*

Obser-vation number	Volume ratio Ψ		Tie-line length s_{TLL}		Distribution coefficient K	Settling time t/min
	Code	Value	Code	Value % w		
1	−1	0.5	−1	10.0	1.06	15.0
2	1	2.5	−1	10.0	1.05	15.0
3	−1	0.5	1	30.0	1.08	10.0
4	1	2.5	1	30.0	1.09	11.0
5	−1.414	0.086	0	20.0	1.08	18.0
6	1.414	2.914	0	20.0	1.09	10.0
7	0	1.5	−1.414	5.86	1.04	22.0
8	0	1.5	1.414	34.14	1.08	17.0
9	0	1.5	0	20.0	1.1	8.0
10	0	1.5	0	20.0	1.13	9.0
11	0	1.5	0	20.0	1.12	9.5
12	0	1.5	0	20.0	1.11	9.0
13	0	1.5	0	20.0	1.10	9.0

Tables 3 and 4 show the results of the quadratic response-surface model fitting for both cases, in the form of analysis of variance (ANOVA). The mean square values for model and error using the different response parameters are given in the tables mentioned above. The F values for models and for each of the response variables were calculated by dividing the mean square due to model variance by that due to error variance. The F

T a b l e 3 – *Analysis of variance (ANOVA) when distribution coefficient was the response quantity*

Source of variation	Sum of square ΣR^2	Degree of free-dom	Mean square R^2	F value	Probabi-lity > F
Model	0.007558	5	0.001512	14.72	0.0013
Error	0.000719	7	0.000103	–	–
Corrected total	0.008277	12	–	–	–

T a b l e 4 – *Analysis of variance (ANOVA) when settling time was the response quantity*

Source of variation	Sum of quare ΣR^2	Degree of free-dom	Mean square R^2	F value	Probabi-lity > F
Model	181.3514	5	36.27028	4.822	0.0314
Error	52.6486	7	7.52123	–	–
Corrected total	234.0	12	–	–	–

values and corresponding P values which give the significance of the model is presented in Tables 3 and 4.

The Student-t-test and the corresponding P values, along with the parameter estimates are given in Table 5 when distribution coefficient was response parameter, and in Table 6 when settling time was the response quantity. The graphical representation

T a b l e 5 – *The least-square fit and the parameter estimation when distribution coefficient was the response quantity*

Variables	Parameter estimate	Standard error	t value	Probability > |t|
Intercept	1.112	0.004532	245.4	–
X_1	0.001768	0.003583	0.4934	0.6369
X_2	0.014572	0.003583	4.067	0.0048
$X_1 \times X_1$	−0.014126	0.003843	−3.676	0.0079
$X_2 \times X_2$	−0.026630	0.003843	−6.93	0.0002
$X_1 \times X_2$	0.005	0.005067	0.9868	0.3566

The standard error of the mean is 0.002811.

T a b l e 6 – *The least-square fit and the parameter estimation when settling time was the response quantity*

Variables	Parameter estimate	Standard error	t value	Probability > |t|
Intercept	8.90048	1.22648	7.257	–
X_1	−1.28919	0.96969	−1.329	0.2254
X_2	−2.00905	0.96969	−2.027	0.077
$X_1 \times X_1$	1.54963	1.04002	1.49	0.1798
$X_2 \times X_2$	4.30046	1.04002	4.135	0.0044
$X_1 \times X_2$	0.25	1.37124	0.1823	0.8605

The standard error of the mean is 0.76063.

of regression equations (26 and 27), called the response surface curves, was obtained using the same software package and are represented in Figure 4 (when responses were measured in terms of distribution coefficient) and Figure 5 (when responses were measured in terms of settling time). From the contour plots this is clear that the optimum values of the volume ratio and the tie-line length lie in the range of $w = 1.5$–1.7 and 22.0–23.5 %, respectively, when distribution coefficient was the response quantity. Whereas, the same value lie in the range of $w = 1.8$–1.95 and 21.80–22.35 %, respectively, when settling time was the response quantity. The

regression equations (26 and 27) were solved independently for both cases by multistage Montecarlo optimization method. The optimum values (coded form) of the test variables were $X_1 = 0.1126606$ and $X_2 = 0.2841979$ in case of first set of experiments when distribution coefficient was the response quantity. Whereas, when settling time was the response parameter they were $X_1 = 0.3980582$ and $X_2 = 0.222013$. The corresponding uncoded values were $w_1 = 1.61$ and $w_2 = 22.84$ % in the first set of experiments and $w_1 = 1.89$ and $w_2 = 22.22$ % in the second set of experiments. When the optimum coded values were substituted in equation 26 the optimum value of the partition coefficient was obtained as 1.12. The minimum value of settling time was found to be 8.5 min when Eq. (27) was solved with the coded values of the second set of experiments.

The optimized volume ratio and tie-line length in each case fixed the phase composition for the corresponding response quantity. In the first case this was estimated to be $w_D = 8.7$ % dextran and $w_{PEG} = 8.9$ % PEG. When settling time was the response distribution the phase composition was estimated to be $w_D = 8.0$ % dextran and $w_{PEG} = 9.2$ % PEG. Table 7 gives corresponding top and bottom phase compositions (experimentally obtained) for both cases. Figure 6 shows the experimental settling time characteristic curve corresponding to the optimized phase composition.
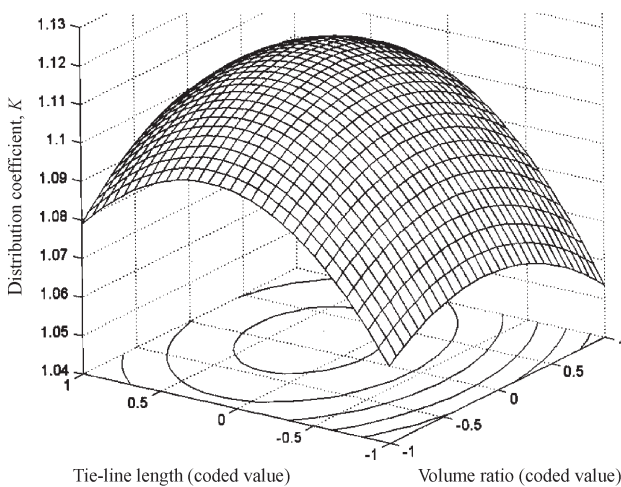

F i g . 4 – *Response surface curve for the mutual effect of volume ratio and tie-line length when responses were measured in terms of distribution coefficient of 2,3-butanediol (based on Eq. (26))*
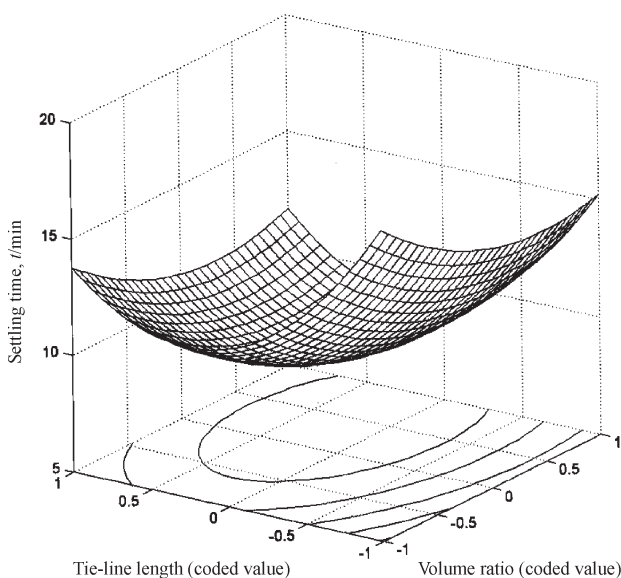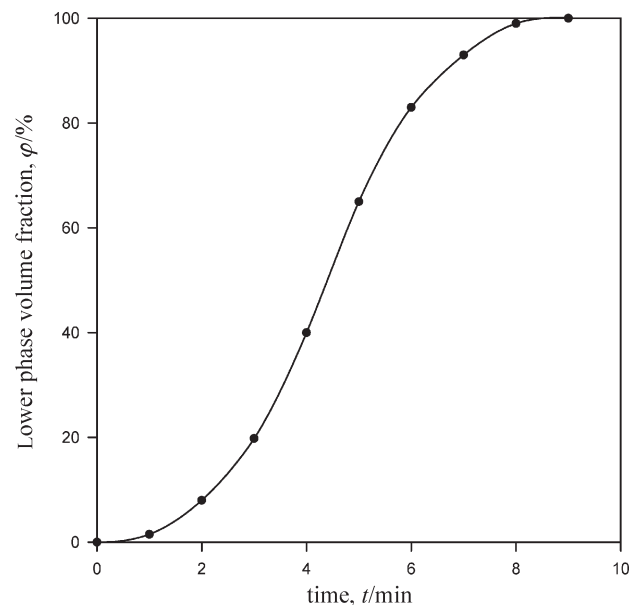

F i g . 5 – *Response surface curve for the mutual effect of volume ratio and tie-line length when responses were measured in terms of time required to separate the phases (based on Eq. (27))*

F i g . 6 – *Settling time characteristics curve for the optimized mixture composition (w = 8.0 % dextran and w = 9.2 % PEG) obtained from the second model calculation. The phase system was made at 30 °C temperature, pH 7.0 in 0.1 mol dm⁻³ Phosphate buffer.*

T a b l e 7 – *Optimum phase compositions for both the models considering distribution coefficient and settling time as the response quantity independently*

Response parameter	Mixture composition $w/\%$			Top phase composition $w_t/\%$			Bottom phase composition $w_b/\%$		
	Dextran	PEG	Water	Dextran	PEG	Water	Dextran	PEG	Water
distribution coefficient	8.7	8.9	82.4	1.38	13.13	85.49	20.83	2.26	76.91
settling time	8.0	9.2	82.8	1.39	12.8	14.19	20.28	2.28	77.44

Discussion

The theoretical formulation for translating the information of a given pair of volume ratio and tie-line length into the corresponding total mixture composition ($w_D/\%$, $w_{PEG}/\%$) which will eventually give the same volume ratio and tie-line length, was successful. This exercise also led to the development of theoretical equation of binodal curve and tie-line. The mathematical exercise can also be used to calculate the volume ratio and tie-line length theoretically if the total mixture composition is known.

The experimental results showed good partitioning of the product 2,3-butanediol between the two aqueous phases. The relatively short settling time indicates good separation characteristics of the aqueous two-phase system. The very high F values and low P values of the two models signify that both the models are highly significant. However, in the first case where distribution coefficient was the response parameter, the F value (14.72) is higher then that in the second case (4.82). Very low probability value (P value, the probability of failure of the model) for both the cases enforce the same conclusion. But, the failure probability of the second model is more than the first signifying that the settling time is more sensitive to changes in volume ratio and tie-line length compared to partition coefficient. The correlation measures R and R^2 for partition coefficient were 0.9232 and 0.8511, respectively. The corresponding values for settling time were 0.775 and 0.6143, respectively. R^2 value of the first set indicates that only 15 % of the data were left unexplained by the model, whereas, 39 % remained unexplained in the second case. The values of coefficient of variation (Ψ) for the two models, 0.93 % and 21.94 %, respectively, also show the relative reliabilities of the two models. From the Student-t-test and P values (Table 5) it was evident that the coefficient of quadratic effect of tie-line length was the most significant coefficient for the equation 26. The coefficients for linear effect of the tie-line length and quadratic effect of volume ratio also appear to be significant. The other coefficients seem to be insignificant. Similarly, for Eq. (27), the model for the settling time (Table 6), the coefficient for the quadratic effect of tie-line length was highly significant, and the linear effect of tie-line length was also significant. The other coefficients were less significant. The elliptical nature of the contour plots, Figures 4 & 5, suggested that both the response quantities were highly correlated with the variables and thereby, the polymer fraction. Experimentally obtained top and bottom phase compositions for respective optimized systems can be taken from Table 7 to calculate the volume ratios and tie-line lengths using equations 1 and 4 respectively for both the systems. In the first case, where distribution coefficient was taken as the response quantity, these values were found to be w = 1.64 and 22.28 %, respectively and in the second case, where settling time was used as the response quantity, they were w = 1.87 and 21.62 %, respectively. In both cases the values are in good agreement to that obtained from the respective models. Interestingly, the optimum values of phase compositions from both the models are almost the same, and so, any one of these compositions can be considered for the purpose of continuous extractive fermentation. The volume ratios from the two independent models were computed to be 1.61 (first model) and 1.89 (second model) and tie-line lengths were 22.84 % and 22.22 % respectively. The settling time was much reduced to 8.5 min compared to very high values (15 min to 22 min) in unoptimized phase compositions. But, the same degree of improvement was not observed in the case of distribution coefficient, which may be attributed to the structural properties of the molecule separated. 2,3-butanediol is a very simple, low molecular mass molecule compared to other biomolecules such as proteins, vitamines, antibiotics. Factors such as electrochemical properties, hydrophobicity, biospecificity are not playing any role in separation. It has been well reported in the literature that generally, low molecular mass compounds partition more evenly between the phases (1). The distribution coefficient of 2,3-butanediol and the settling time at the respective optimum phase compositions were experimentally found to be 1.15 and 9.0 min (Figure 5) respectively. These values are in very good agreement to those calculated (1.114 and 8.5 min respectively) from the corresponding model equations.

Conclusion

The results of the present study proves undoubtedly that the both response parameters, distribution coefficient and settling time, are strongly related to the test variables (the volume ratio and tie-line length). The distribution coefficient is more strongly related to these test variables than the settling time. The tie-line length has more influence upon the both responses than the volume ratio, signifying that the total polymer fraction (top and bottom together) in the phase system plays a vital role in determining the distribution coefficient of 2,3-butanediol and phase separation time. The closeness of the experimental results and theoretically predicted values from the models demonstrate that the statistical design technique what is employed here is much more successful in predicting the behavior of the aqueous two-phase systems. The theoretical exercise that led to the development of binodal curve and tie-line equation, that eventually led to the calculation of total phase compositions from given pairs of volume ratio and tie-line lengths, was of immense help for the models to be successful,

Notation

χ_b – molar fraction of 2,3-butanediol in the bottom phase

χ_t – molar fraction of 2,3-butanediol in the top phase

ρ_b – density of the bottom phase

ρ_t – density of the top phase

$w_{D,b}$ – mass fraction of dextran in the bottom phase and the abscissa of the point B, %

$w_{D,m}$ – mass fraction of dextran in the total mixture and the abscissa of the point M, %

$w_{D,t}$ – mass fraction of dextran in the top phase and the abscissa of the point T, %

K – distribution coefficient of 2,3-butanediol

s_{TLL} – tie-line length or the length of the line segment between the points T and B

s_{MB} – length of the line segment between the points M and B

s_{MT} – length of the line segment between the points M and T

w_b – mass fraction of PEG in the bottom phase and the ordinate of the point B, %

w_m – mass fraction of PEG in the total mixture and the ordinate of the point M, %

w_t – mass fraction of PEG in the top phase and the ordinate of the point T, %

Ψ – volume ratio

V_b – volume of the of the bottom phase

V_t – volume of the of the top phase

x_i – uncoded value of the ith test variable

x_0 – value of the ith test variable at the center point

X_1 – coded value of volume ratio

X_2 – coded value of tie-line length

X_i – coded value for the variable i (dimensionless)

X_j – coded value (dimensionless) of another variable j, when i is not equal to j

Y – predicted response

Y_1 – predicted response of partition coefficient

Y_2 – predicted response of settling time

β_i – coefficient of linear effect

β_{ii} – coefficient of quadratic effect

β_{ij} – coefficient of interaction effect

β_0 – offset term

Δx_i – step change value

Q – gas flow rate

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