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Improving glutathione extraction from crude yeast extracts by optimizing aqueous two-phase system composition and operation conditions

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Abstract−PEG-Dextran and PEG-salt aqueous two-phase systems (ATPS) have been applied to separate glutathione (GSH) from crude yeast extracts. Single-factor experiments were carried out to determine the important factors influencing the partition coefficient and extraction yield. The effect of PEG molecular weight, phase-forming components, PEG and Dextran concentration, pH value, and temperature on the GSH partitioning behavior in ATPS was investigated. Three factors, Dextran concentration, pH value, and temperature, were confirmed to have significant influence on the partition coefficient and extraction yield. These factors were further analyzed with the aid of central composite rotatable design and response surface methodology. The optimal conditions for GSH extraction in the PEG-Dextran system were determined, including PEG molecular weight 6,000, 10% PEG concentration, 14% Dextran concentration, pH 5.2, and temperature 32 °C. A high extraction yield (83.55%) of GSH from crude yeast extracts was achieved under these optimized conditions. This work is very helpful for developing one efficient and cost-effective process for the separation and purification of GSH from yeast broths.

Key words: Glutathione, Partition, Aqueous Two-phase Systems, Response Surface Analysis

INTRODUCTION

The tripeptide Glutathione (GSH, γ-glutamyl-L-cysteinylglycine) is the most abundant non-protein thiol compound in mammalian and other living organisms. Over 90% of the glutathione is present in the reduced form GSH, while the rest is in the form of oxidized state GSSG (glutathione disulfide), which can be reduced to GSH by glutathione reductase [1]. GSH has a great variety of clinical applications due to its significant physiological functions, such as an important antioxidant for protecting DNA, proteins, and other biomolecules against oxidative damage generated by reactive oxygen species, one of the most potent anti-viral agents in immune function, and an essential detoxifier in the presence of glutathione-S-transferase in higher eukaryotic organisms [2]. A number of diseases, such as HIV infection, liver cirrhosis, diabetes, and aging, may be closely related to GSH deficiency [3]. GSH is also gradually introduced into the food and cosmetic industries as food additives and cosmetic compositions [4].

GSH can be produced by chemical synthesis, enzymatic catalysis, microbial fermentation, or genetic/metabolic engineering. Although enzymatic production and genetic/metabolic pathway can lead to a high accumulation of GSH [5,6], the relatively high cost from three precursor amino acids limits their industrial application. GSH production with chemical method and microbial fermentation were commercialized in the 1950s and 1980s, respectively. Since only the L-form is physiologically active while chemically synthesized GSH is an optically inactive mixture of D- and L-isomers, the chemical method is gradually replaced by microbial fermentation [7]. The main problem in the production of GSH, however, lies in the separation of GSH from fermentation broth, which may limit the extensive applications of GSH. An advanced separation technique is essential to obtain highly-purified GSH with high recovery rate. Several methods have been suggested to separate GSH from fermentation broth, including copper-salt method, ion-exchange chromatography, and affinity chromatography [8-10]. The copper-salt method was gradually phased out because of its tremendous damage to the global environment caused by the huge amounts of H_2S in the separation process. The affinity separation of GSH with copper ion-containing resin is seriously restricted because of the relatively poor stability and high toxicity of copper ionic residue. Although ion-exchange technique has been applied to separate GSH from fermentation broth, the preparation of high-purity GSH is not cost-effective due to its inherent disadvantages, such as multi-steps, complexity, and being time-consuming. Besides, the GSH concentration in fermentation broth is usually very low, which will further restrict the practical application of ion-exchange chromatography in GSH extraction.

Aqueous two-phase systems (ATPS), which have been widely applied in the separation and purification of biomolecules, such as proteins, enzymes, and nucleic acids, is a promising alternative to many conventional processes because of its apparent advantages: simplicity (one-step separation), low energy consumption, ease of scale-up, and maintenance of biological activity [11,12]. ATPS is formed by mutual incompatibility of different polymers or polymer/salt in aqueous solutions above critical concentrations. Phase separation occurs over certain concentrations of the phases' components. However, reports on the application of ATPS for partitioning and purification of GSH from fermentation broth are scarce.

In this paper, direct extraction of GSH from crude yeast extracts

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by aqueous two-phase systems containing polymer-polymer or polymer-salt is described. The effect of PEG molecular weight, phase-forming compositions, PEG and Dextran concentration, pH value, and temperature on the extraction rate of GSH was investigated. Response surface analysis (RSA) is an effective statistical tool which has been widely used in process optimization, including experimental design, model fitting, validation, and condition optimization [13, 14]. In recent years, the application of RSA in the biological process optimization has gained great momentum and is becoming an innovative approach in many research studies [15-17]. Therefore, RSA was employed to further optimize the conditions for extraction of GSH from cell extracts in the present work.

MATERIALS AND METHODS

1. Materials

PEG with average molecular weight of 1,500, 2,000, 4,000, and 6,000, L-glutamate and ALLOXAN agent were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

2. Preparation of Aqueous Two-phase Systems

A system with final volume of 10 mL was prepared from stock solution of PEG (50%, w/w), Dextran 20, and phosphate (K_2HPO_4-KH_2PO_4, 23.5%, w/w), or PEG (50%, w/w), inorganic salt ($(NH_4)_2SO_4$, Na_2CO_3, or Na_2SO_4), and phosphate (K_2HPO_4-KH_2PO_4, 23.5%, w/w) in 10 mM phosphate buffer at appropriate pH and temperature.

3. Extraction of GSH from Crude Yeast Extracts by ATPS

A total volume of 1 mL crude yeast extracts containing 0.22 g/L glutathione was added into aqueous two-phase systems, and the final concentration of GSH in ATPS was 20 mg/L. The systems containing different salts were prepared by directly dissolving the salt powder into the system. The systems were mixed thoroughly and centrifuged at 3,000 rpm for 30 min to assist phase separation. The centrifuged systems were then allowed to settle for 5 min to separate into two clear phases. Samples from top and bottom phases were then carefully removed and assayed for glutathione concentration.

4. Experimental Design and Statistical Analysis

4-1. Single-factor Experiments

The effect of PEG molecular weight, phase-forming compositions, PEG and Dextran concentration, pH value, and temperature on the extraction yield of GSH was investigated. The average molecular weight of PEG was 1,500, 2,000, 4,000, 6,000, respectively. The phase-forming components included 10% PEG and 15% $(NH4)_2SO_4$, Dextran, Na_2CO_3, and Na_2SO_4, respectively, or 15% PEG and 10% $(NH4)_2SO_4$, Dextran, Na_2CO_3, and Na_2SO_4, respectively. The concentration of PEG and Dextran includes 8% PEG/16% Dextran, 10% PEG/14% Dextran, 12% PEG/10% Dextran, 14% PEG/10% Dextran, 16% PEG/8% Dextran, respectively. The pH value was set as 2.0, 4.0, 5.5, 8.0, 10.0. The temperature was set as 20 °C, 30 °C, 40 °C, 50 °C, 60 °C.

4-2. Response Surface Analysis

Central composite rotatable design (CCRD) is a response surface method employed in the optimization studies of biotechnological processes for the prediction and verification of model equation as well as the optimization of the response as the function of the independent parameters [17]. A second-order model is widely used in CCRD experimental design, as shown below:

Table 1. Design of response surface for three factors at three levels

Symbols	Factors	Levels and range		
		−1	0	+1
X_1	Dextran/%	12	14	16
X_2	Temperature/°C	25	30	35
X_3	pH value	4.5	5.5	6.5

Note: Y_t (%) was set as the reference index

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum\sum_{1<j} \beta_{ij} X_i X_j \quad (1)$$

Where β_0, β_j, β_{jj}, and β_{ij} are regression coefficients for the intercept, linear, quadratic and interaction coefficients, respectively, and X_i and X_j are the coded independent variables. Coded variables are expected to range from −1 to +1 for more even response and irrelevant parameters. The equation commonly used for coding is as below:

$$X = \frac{x - [x_{max} + x_{min}]/2}{[x_{max} - x_{min}]/2} \quad (2)$$

Where x is the natural variable, X is the coded variable and x_{max} and x_{min} are the maximum and minimum values of the natural variable.

Based on the results of single-factor experiments, the PEG/Dextran system was chosen to be further studied in response surface analysis. Dextran concentration, pH value, and temperature were confirmed to have significant effects on distribution coefficient and extraction rate, and thus were set as significant factors in CCRD experimental design with extraction yield as the response of experiments. The PEG molecular weight and concentration was set at 6,000, and 10% respectively. The level and ranges for significant factors in CCRD are shown in Table 1.

5. Determination of GSH Content

The samples were centrifuged at 4,000 ×g for 5 min and filtered through 0.45 μm membrane before being subjected to HPLC analysis [18]. The C18 column (Hypersil BDS, 150×4.6 mm) was used. The mobile phase consists of methanol and 0.11% 1-heptanesulfonate in potassium phosphate buffer (50 mmol/L, pH 3.0) in the ratio of 5 : 95 (v/v). The detection wavelength was set at 210 nm. The influence of PEG and phosphate was avoided by analyzing samples against blanks containing the same phase composition in the absence of GSH. The extraction rate, Y_t, is related to the ratio of GSH content in the top and bottom phases.

6. Determination of Phase Ratio, Partition Coefficient, and Extraction Yield

The Phase ratio R was determined as

$$R = \frac{V_t}{V_b} \quad (3)$$

Where V_t and V_b are the volume of top phase and the volume of bottom phase, respectively.

The partition coefficient K was determined as

$$K = \frac{C_t}{C_b} \quad (4)$$

Where C_t (g/L) and C_b (g/L) are the concentration of top phase and

the concentration of bottom phase, respectively.

The extraction yield Y_t was determined as

$$Y_t = \frac{RK}{1+RK} \times 100\% \quad (5)$$

Thus, Y_t means the content of GSH in top phase (mg)/the content of GSH in fermentation broth (mg).

RESULTS AND DISCUSSION

1. Effect of PEG Molecular Weight on Partition Coefficient and Extraction Yield

Previous research work has demonstrated strong influence of polymer molecular weight on protein partitioning in ATPS [19,20]. In this study, the partition coefficient K and extraction yield Yt% was enhanced from 1.81 to 3.5 and from 50.64% to 80.23% with the increase of PEG molecular weight from 1,500 to 6,000, as shown in Fig. 1. The GSH content in top phase was enhanced with the increase of PEG molecular weight, so as the partition coefficient K. This may result from the easily-attached characteristics of GSH as a small molecule to high molecular weight polymers, a phenomenon opposite to previous experiments on protein partitioning using ATPS. The result indicated that better protein partitioning was achieved with lower molecular weight PEG.

Interfacial tension between the phases is one of the most important factors to determine the partitioning behavior of particles and cells [21]. PEG of low molecular weight is beneficial for protein partitioning since the breaking of interaction between phase components is required to facilitate the transfer of protein from one phase to the other. Besides, PEG of low molecular weight is inclined to interact strongly with proteins, while high molecular weight PEG is more likely to form intramolecular bonds, which further increases the interfacial tension [21]. Generally, increasing the molecular weight of a phase forming polymer will cause the accumulation of a protein in the opposite phase. Therefore, in a PEG/Dextran aqueous two-phase system, a protein's partition coefficient tends to decrease with the increase of PEG molecular weight since the protein will be enriched in the bottom phase. The extraction of GSH in ATPS, however, is completely different from that of protein since in GSH partitioning, the influence of adsorption to high molecular weight polymers is far more significant than the interfacial tension between phases. Thus, the partition coefficient will tend to be improved with increasing the molecular weight of PEG in GSH partitioning due to the stronger adsorbing ability of higher molecular weight polymers. Therefore, PEG with molecular weight 6,000 is suitable for the extraction of GSH in ATPS.

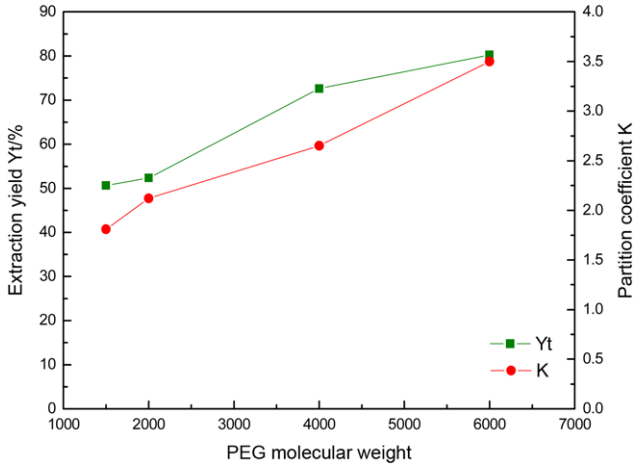

Fig. 1. Effect of PEG molecular weight on partition coefficient and extraction yield (PEG 10%, Dextran 15%, pH 5, temperature at 25 °C).

Table 2. Effect of phase compositions on partition coefficient and extraction yield (PEG with average molecular weight of 6,000, pH 5.0, and temperature at 25 °C)

Compositions		Extraction yield Y_t/%	Partition coefficient K
PEG/%	Salt/Dextran/%		
10	$(NH_4)_2SO_4$ 15	54.23	1.6
15	$(NH_4)_2SO_4$ 10	61.36	2.1
10	Dextran 15	80.95	3.5
15	Dextran 10	78.95	3.3
10	Na_2CO_3 15	68.48	2.3
15	Na_2CO_3 10	71.42	1.5
10	Na_2SO_4 15	70.38	2.6
15	Na_2SO_4 10	65.31	1.6

2. Effect of Phase Compositions on Partition Coefficient and Extraction Yield

Currently, the aqueous two-phase systems are mostly composed of either PEG-salts or PEG/Dextran, and the phase components would have some influence on the partition coefficient and extraction yield. The effect of phase compositions on partition coefficient and extraction yield in ATPS for GSH partitioning has been studied in this work. As shown in Table 2, the partition coefficient and extraction yield in ATPS containing PEG-salts ($(NH_4)_2SO_4$, Na_2CO_3, or Na_2SO_4) was below 3 and 72%, respectively. It was also found that $(NH_4)_2SO_4$ showed a lower partitioning efficiency (2.0) than Na_2CO_3 or Na_2SO_4. This result was consistent with the previous study on the effect of salts on partition coefficient in ATPS, which indicated that for negatively charged proteins in PEG/DEX systems, the partition coefficient would be successively decreased in the series as sulfate>fluoride>acetate>chloride>bromide>iodide and lithium>ammonium>sodium>potassium [22]. However, the partition coefficient and extraction yield in ATPS can reach 3.5 and 80%, respectively, with the PEG-Dextran system (Table 2), which was superior to PEG-salts system. Moreover, the PEG-Dextran system can avoid some disadvantages existing in the PEG-salts system, such as the economical and environmental problems associated with the large consumption of phase-forming chemicals, low solubility for amphiphilic proteins, and harm to the activity of targeted peptides and proteins [23,24]. Thus, only PEG-Dextran system was further investigated for more efficient separation of GSH from cell extracts.

3. Effect of PEG/Dextran Concentration on Partition Coefficient and Extraction Yield

The effect of polymer concentration on partition coefficient in ATPS can be described by phase diagrams, which indicated that the two polymers and water would be completely mixed with each other at low concentration under the critical point, while aqueous two-phase

Table 3. Effect of PEG/Dextran concentration on partition coefficient and extraction yield (PEG with average molecular weight of 6,000, pH 5.0, and temperature at 25 °C)

Phase components		Partition coefficient K	Extraction yield Y_t/%
PEG/%	Dextran/%		
8	16	2.19	72.53
10	14	3.31	83.17
12	12	2.96	78.56
14	10	2.54	73.62
16	8	1.87	68.77

systems would be formed as the polymer concentrations reached above the critical point. Consequently, the concentration of the phase-forming polymers would greatly influence the partitioning behavior of target products. Generally, the partition coefficient tends to become exceedingly higher or lower beyond the critical polymer concentration. However, it was also reported that the partition coefficient could increase at first, reach a peak value, and then decrease with the increase of concentration of phase-forming polymers.

The effect of concentration of phase-forming polymers (PEG and Dextran) on partition coefficient and extraction yield has been studied in this paper, as shown in Table 3. We can find that both the partition coefficient and extraction yield would reach the highest in aqueous two-phase system containing 10% PEG and 14% Dextran, while both would decrease when the concentration of PEG and Dextran was higher than 10% or lower than 14%. The ratio of phase-forming polymers in ATPS is an important factor to determine the partitioning behavior of systems, which may directly influence the partition coefficient. Table 3 shows that with the ratio of PEG to Dextran increasing from 1 : 2 to 2 : 1, the partition coefficient increased from 2.19 to 3.31, the peak value, and then reduced drastically to 1.87.

4. Effect of pH Value on Partition Coefficient and Extraction Yield

Charge is another important factor which could play a role in protein partitioning in ATPS, especially in polymer-polymer systems.

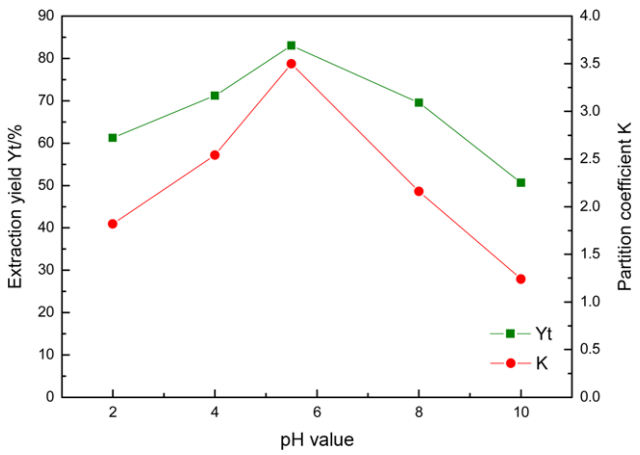

Fig. 2. Effect of pH value on partition coefficient and extraction yield (PEG 10%/Dextran 14%, PEG with average molecular weight of 6,000, temperature at 25 °C).

The effect of pH value on partition efficiency in PEG-Dextran systems for GSH extraction has been evaluated in this study. As shown in Fig. 2. The partition coefficient was improved from 1.82 to 3.5 by adjusting pH from 2 to 5.5, but it dropped drastically to 1.24 as the pH value was further increased up to 10. A similar trend was observed in extraction yield with the increase of pH value from 2 to 10, which was improved from 61.26 to 83.01, the peak value, and then dropped to 50.63.

An electrical double layer can be formed near the interface between two phases due to the unequal distribution of cations and anions in the polymer-polymer aqueous systems, which could influence the partitioning behavior of charged particles, according to the sign and magnitude of their net charge. Consequently, a change of pH value in ATPS may lead to a change of the charge distribution of the systems and a variation of the ratio of the charged species present. Generally, the protein is more negatively charged at higher pH, which may result in an increase of partition coefficient due to electrostatic interactions between the protein and PEG units. Previous research work has reported the change of pH value in solution and thereby the change of partitioning behavior of proteins in ATPS, which indicated that negatively charged proteins were more likely to be enriched in the top phase while positively charged proteins to the bottom phase [25, 26]. Thus, the protein prefers to accumulate in PEG phase rather than the Dextran phase in a pH value above its isoelectric point. The pK_a values of various groups in GSH are present below: NH_3 8.66, SH 9.2, $COOH_1$ 3.53, $COOH_2$ 2.12 [27]. In addition, the partitioning behavior of small molecules in ATPS will also be greatly influenced by the interfacial potential. In this work, the concentration of HPO_4^{2-} will be enhanced with the increase of pH value, which may lead to the improvement of partition coefficient because of the reduction of interface potential. The partition coefficient however, will be reduced eventually with the pH value continuing to increase to 8 and 10 because of the enhancement of OH^- concentration and thus the interface potential. In our experiment, the suitable pH is 5.5 for the ATPS operation; however, the most suitable pH value should be determined by testing different pH values in the range of pH 5.5 to 8.0.

5. Effect of Temperature on Partition Coefficient and Extraction Yield

Temperature is an important factor in protein partitioning in ATPS, although its influence has not yet been thoroughly investigated. The effect of temperature on partition coefficient and extraction yield in PEG-Dextran aqueous systems has been evaluated in this work. As shown in Fig. 3, the partition coefficient was improved dramatically from 0.81 to 3.5 as the temperature increased from 20 °C to 30 °C, while it was reduced to 1.25 with the temperature increased to 60 °C. A similar trend was observed in extraction yield, which was improved from 51.63 to 82.74 when the temperature was increased from 20 °C to 30 °C, while dropping to 57.72 as the temperature was increased to 60 °C.

The effect of temperature on partition behavior of proteins in ATPS was studied by various research groups previously. It was demonstrated that the partition coefficient decreased with increase in temperature (20 °C, 30 °C, 40 °C) of PEG-PAA aqueous system for partitioning of ovalbumin and myoglobin [28]. Although the mechanism of the temperature influence on partitioning behavior in ATPS for protein partition is unclear, it has been suggested that the physi-

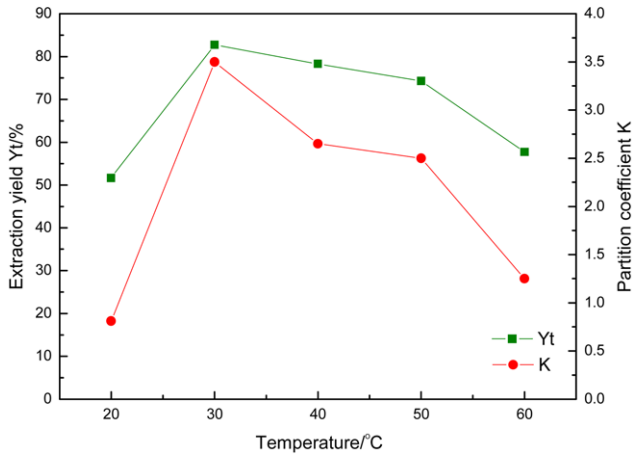

Fig. 3. Effect of temperature on partition coefficient and extraction yield (PEG 10%/Dextran 14%, PEG with average molecular weight of 6,000, pH 5.5).

Table 4. Experimental scheme and results

Sequence	X_1	X_2	X_3	Y_t/%
1	0	−1	−1	63.14
2	0	−1	1	78.32
3	0	1	−1	83.56
4	0	1	1	62.31
5	−1	0	−1	70.13
6	−1	0	1	75.94
7	1	0	−1	79.16
8	1	0	1	65.03
9	−1	−1	0	71.58
10	−1	1	0	78.37
11	1	−1	0	77.06
12	1	1	0	65.15
13	0	0	0	82.74
14	0	0	0	84.36
15	0	0	0	83.28

Note: X_1, Dextran concentration; X_2, temperature; X_3, pH value; Y_t, extraction yield

cal properties of phase-forming compositions in ATPS, such as viscosity and density, will be greatly influenced by temperature, which may affect the distribution of GSH in the top and bottom phases. Usually, the partition coefficient will mostly be influenced by temperature when it reaches the critical point since the phase diagram will then be effective to the largest extent at this time. Besides, it has been reported that the partition coefficient would be decreased due to the reduction of preferential interaction between PEG and proteins as the temperature increases [29].

6. Response Surface Analysis

Based on the single-factor experiments, the partitioning behavior of GSH in PEG-Dextran aqueous systems was significantly influenced by Dextran concentration, pH value, and temperature. Therefore, these factors were analyzed in detail with the aid of central composite rotatable design and response surface methodology. The molecular weight and concentration of PEG were fixed at a con-

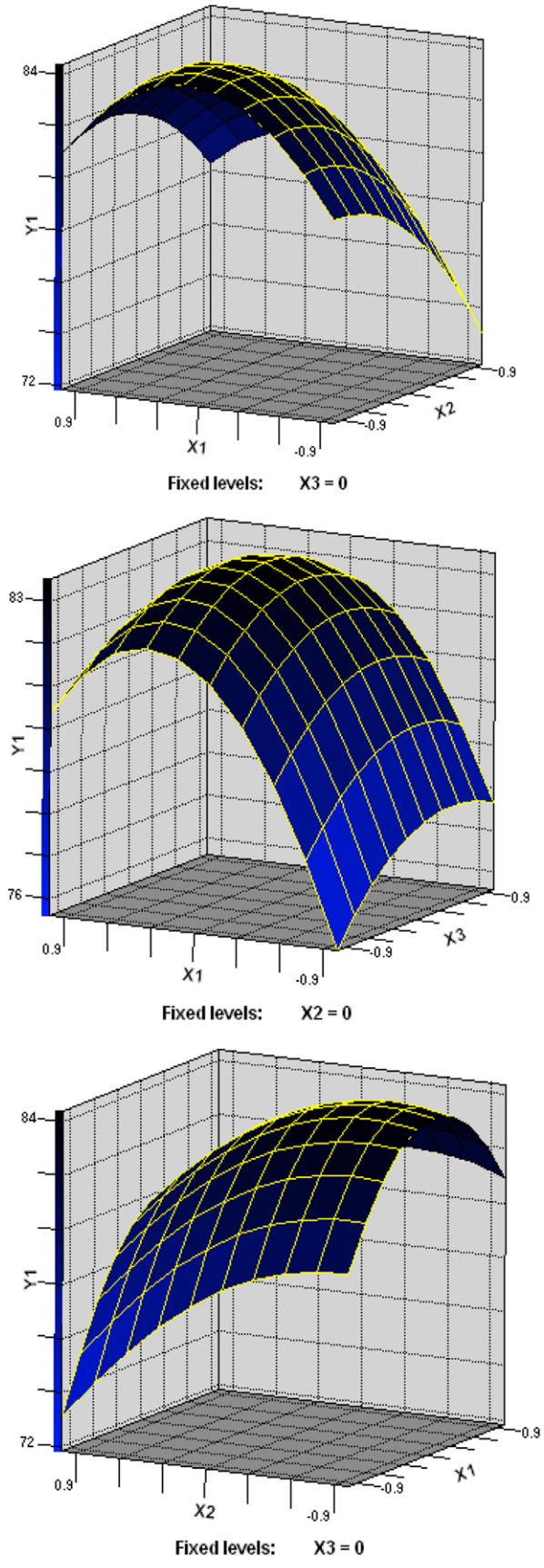

Fig. 4. 3D graph of response value Y (extraction yield) and response surface of each factor (X_1: Dextran concentration, X_2: temperature, X_3: pH value).

stant value, as 6,000 and 10%, respectively. The conditions for extraction of GSH from the cell extracts in PEG/Dextran system were optimized by response surface analysis (RSA) using Minitab15 with the Dextran concentration, pH value, and temperature as the independent variables (designed by Box-Behnken), and the extraction yield of GSH as the response value. The results are shown in Table 4. Fig. 4 shows the results of response surface analysis with the help of Statistica, based on the results of Table 4. A regression analysis was performed to predict the outcome of extraction yield of GSH in PEG-Dextran systems with a simple equation by fitting and regressing experimental data in CCRD, which is expressed below:

$$Y=83.46-1.20X_1-0.09X_2-1.80X_3-4.84X_1^2-4.68X_1X_2 -5.58X_2^2-4.99X_1X_3-9.11X_2X_3-6.05X_3^2 \quad (6)$$

Where Y is the extraction yield set as the response value, and X_1, X_2, and X_3 are the Dextran concentration, temperature, and pH value, respectively, set as the independent variables.

Eq. (6) indicates that the effect of temperature on partition coefficient is much gentler than that of polymer concentration and pH value, while the partition coefficient is significantly influenced by the interactions of these factors, whether the interactions of polymer concentration and temperature, or that of pH value and temperature, or that of polymer concentration and pH value. For protein partitioning in polymer-polymer systems, the combination of hydrophobic interactions and electrostatic interactions plays a key role in determining the partition behavior, which is a function of the polymer concentration and solution pH value [28]. As a small molecule, the partition behavior of GSH in polymer-polymer systems is also significantly influenced by the adsorption interaction and interfacial potential, which is also a function of the polymer concentration and solution pH. Consequently, a suitable Dextran concentration and pH value should be controlled in PEG-Dextran systems for GSH extraction, in order to achieve a high extraction yield.

The analysis of the regression equation is shown in Table 5, with a linear correlation coefficient as 0.98, indicating that this model could truly express the effect of Dextran concentration, pH value, and temperature on the partitioning behavior of GSH in PEG-Dextran systems. With the aid of CCRD and RSM, the optimized conditions for GSH extraction in PEG-Dextran systems were obtained, which are shown below: Dextran concentration 13.8%, pH 5.2, and 31.6 °C, while the molecular weight and concentration of PEG was maintained at 6,000 and 10%, respectively. The predictive value of extraction yield for GSH partition in PEG-Dextran system under the optimized conditions is 83.82%. Experiments were carried out according to the optimized conditions: PEG molecular weight 6,000, PEG concentration 10%, Dextran concentration 14%, solution pH 5.2, and temperature 32 °C. An extraction yield of 83.55% was achieved, which is consistent with the predictive value of the regression model. Besides, the ratio of solid impurities in the upper phase and those in the lower phase was about 1/5 after separation, which indicates that most of the solid impurities can be separated from the upper phase by extraction as well.

CONCLUSIONS

The partition of GSH in aqueous two-phase systems containing polymer-polymer or polymer-salt has been studied in this work, and PEG-Dextran systems have proven to be an ideal environment for GSH extraction, with a higher partition coefficient and extraction yield compared with PEG-salt systems. Different from protein partition in PEG-Dextran systems, in which the partitioning behavior of proteins is determined by the combination of hydrophobic interactions and electrostatic interactions, GSH partition is also greatly influenced by adsorption interactions and interfacial potential since GSH is a small biomolecule. It was found after single-factor experiments that three factors (Dextran concentration, pH value, and temperature) were significant to influence the partition coefficient of GSH in the PEG-Dextran systems. Central composite rotatable methodology has been applied to improve the separation efficiency of GSH. The results showed that a highest extraction yield (83.55%) of GSH was achieved with the optimal ATPS conditions (Dextran concentration 14%, solution pH 5.2, and temperature 32 °C). This ATPS work will be very helpful to develop one cost-effective and high-efficient process for the separation and purification of GSH from yeast broth.

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Table 5. Analysis of square deviation (SD) of regression equation

S. SD	D.F.	S.S.	M. SD	F	Pr>F
Regression	9	848.473898	9.595439	26.72	0.0010
Residual	5	17.638675	3.527735		
Total deviation	14	866.077655			
Linear correlation coefficient	0.9796				

Note: S. SD: Source of SD, D.F.: Degree of Freedom, S.S.: Sum of Square, M. SD: Mean of SD, F: F value

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