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Optimization of bovine serum albumin sorption and recovery by hydrogels

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Abstract

Aqueous two-phase systems are composed of aqueous solutions of either two water-soluble polymers, usually polyethylene glycol (PEG) and dextran (Dx), or a polymer and a salt, usually PEG and phosphate or sulfate. Partitioning of proteins in such systems provides a powerful method for separating and purifying mixtures of biomolecules by extraction. If one of the phase forming polymers is a crosslinked gel, then the solution-controlled gel sorption may be considered as a modification of aqueous two-phase extraction. Since PEG/dextran systems are widely used in aqueous two-phase extraction and dextran gels (Sephadex) are common chromatographic media, we choose a PEG/dextran gel system as a model system in this study. The partitioning behavior of pure bovine serum albumin (BSA) in PEG/dextran gel systems is investigated to see the effects of variations in PEG and NaCl concentrations on the partition coefficient K. By making use of the Box–Wilson experimental design, K is shown to be maximized at 9.8 (%, w/w) PEG and 0.2 M NaCl concentrations, respectively, as 182.

Keywords: Box–Wilson experimental design; Aqueous two-phase system; Polyethylene glycol; Sephadex; Bovine serum albumin

1. Introduction

The advances in genetic engineering which are important for the production of many new proteins have resulted in Biotechnology to receive a lot of attention during the last decades. Compared to the technology for cloning genes for proteins, the technology for purification of the expressed gene product is improving rather slowly.

Aqueous two-phase systems have some advantages over the other commonly used separation and purification techniques such as high water content of both phases (70–80%, w/w) which means high biocompatibility and low interfacial tension minimizing degradation of biomolecules, good resolution and high yield, relatively high capacity, ease of scale-up, low material costs, and the possibility of polymer recycle [1–3].

However, aqueous two-phase extraction (ATPE) has certain drawbacks. For instance, it is necessary to use centrifugation in order to separate phases because of the low interfacial tension and small difference in densities between the phases. Also, separation of proteins from the phase-forming polymers may not be easy in some systems.

In 1991, Gehrke et al. [4] proposed solution-controlled gel sorption (SCGS) technique in which one of the phase-forming polymers is a crosslinked gel rather than a polymer solution. In this technique, the gel may selectively sorb protein when it gets contact with a solution of fermentation broth, polymer and appropriate salts. This allows the protein loaded gel to be separated from the rest of the solution by filtration or low speed centrifugation. Later, the protein can be desorped from the gel by changing the gel's environment. Clearly, this new method does not have the drawbacks mentioned above.

The concept of SCGS was previously demonstrated in polyethylene glycol (PEG)/dextran (Dx) systems using model proteins [4–6]. It was observed that the partition coefficient K (concentration of protein in gel/concentration of protein in solution) of cytochrome c and ovalbumin is directly proportional to the molecular weight of PEG. Experiments made with PEG and dextran gels showed that if the magnitude of charge on the protein and the ionic strength over a certain range are increased then the partitioning of ovalbumin into the dextran gels increases. The partition coefficient K was shown to be increasing as the salt concentration varies from 0.0045–0.09 M. Further, it was seen that K, as in the case of ATPE, is influenced by the concentrations and molecular

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weights of PEG, type and concentration of added salts, and pH.

The object of this work is to investigate the effects of PEG and NaCl salt concentrations on partitioning of bovine serum albumin (BSA) in PEG/dextran gel systems via Box–Wilson experimental design method. The classical one-variable at a time strategy usually fails, because maximizing value of one variable is assumed to be independent of the other, which is not usually true. Therefore, we propose Box–Wilson experimental design method as an alternative. Box–Wilson experimental design method belongs to a class of a more general method called a response surface methodology, a group of techniques used in the empirical study of relationships between one or more measured responses and a number of input variables. A detailed discussion on the Box–Wilson method can be found in [7].

The Box–Wilson experimental designs are of a general class of experiments that have been developed to efficiently serve as a basis for deriving the mathematical model of a physical process. Their usefulness is enhanced in the study of industrial applications because most physical situations can usually be approximated by a function over a reasonable range of variables.

A technique for designing which experimental tests should be carried out to evaluate the coefficients of the model is the Box–Wilson composite rotatable design. For the purpose of the experiment, the independent variables are each specified at five levels. The specific values of these five levels for each variable depend on the number of variables included in the model and the range over which they are to be studied. The design principle includes three types of combinations, the axial, factorial, and center points. Axial points include each variable at its extreme level with the other variables at their center-point level. The center point is a test at the average level of each variable. Designs for any number of variables can be developed from these principles.

After the completion of experimental tests, a regression analysis is applied to estimate the coefficients in the proposed model. An analysis of variance by means of the F-test is then usually carried out to determine the significance of the model.

2. Materials and methods

2.1. Materials

PEG 20 000 (Lot #81300) was purchased from Fluka Company (Buchs, Switzerland), and Sephadex G-100 (Lot #30K1747) and BSA (Lot #79H082) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Crystallized and lyophilized BSA was used without purification to prepare a stock solution by weight with an accurately known concentration (10%). Concentrations of PEG stock solution was 25% polymer by weight.

2.2. Partition coefficient measurements

The dextran gels were pre-swollen in protein-free test solution (PEG, NaCl, 0.05 M phosphate buffer, pH = 7) before the partitioning experiments so that they would not absorb additional solution and salt, and thus alter PEG and salt concentrations during the partitioning experiments. Enough of the dried Sephadex gel beads were added to glass centrifugation tubes to obtain about 0.8 g of gel when swollen. Then 3 ml of test solution was added to each tube and 15 min were allowed to reach equilibrium. Afterwards, the tubes were centrifuged at 500 rpm for 60 min to separate the supernatant from the swollen beads. After the supernatant was removed from the centrifuge tube, 3 ml of solution with the same PEG and salt concentration as the pre-swelling solution, but including BSA protein was added to the tube. The tube content was then mixed by a vortex mixer and left in a water bath at 20 °C (Memmert, Germany) overnight. Finally, the supernatant was separated from the gel as before.

2.3. Assays

For the determination of BSA concentration in the PEG phase, a sample withdrawn from this phase was diluted with a known amount of distilled water, and its ultraviolet absorbance was measured at 280 nm (Hach DR/4000 UV-Vis, Loveland, CO, USA). An identically diluted solution of the corresponding phase from a system containing no BSA was used as a blank. The protein concentration in the gel phase was obtained by using the difference in protein concentration between the initial solution and the supernatant to complete the mass balance.

2.4. Experimental design

The Box–Wilson experimental design [7] is used in the optimization of partitioning coefficient K of BSA in aqueous two-phase systems. PEG (X_1, %) and NaCl (X_2, M) concentrations are independent variables in a series of repeated partitioning experiments, and partition coefficient of BSA (K) is the dependent output variable. In statistical calculations, the variables X_i are coded as x_i according to the equation

$$x_i = \frac{X_i - X_0}{\Delta X_i} \tag{1}$$

where X_0 is the value at the center point of the investigated area corresponding to i and ΔX_i is the step size. More precisely,

$$x_1 = \frac{X_1 - 7.0}{2.0} \tag{2}$$

and

$$x_2 = \frac{X_2 - 0.2}{0.1}. \tag{3}$$

Table 1
Real values of the independent variables in the experimental plan (X_1: PEG (%) and X_2: molarity of NaCl salt)

Real values	Coded values				
	−1.414	−1	0.0	1	1.414
X_1	4.2	5.0	7.0	9.0	9.8
X_2	0.06	0.1	0.2	0.3	0.34

Table 1 displays the coded values x_1 and x_2.

3. Results and discussion

A 2^2-factorial experimental design with four star points ($a = 1.414$) and six replicates at the center point were employed with a total of 14 experiments. To investigate the pattern of responses, the partition coefficient K is first fitted as a general fourth degree polynomial and then only the terms based on the Student's t-ratio test were kept. That is, we write

$$K = \sum_{m+n\leq 4} b_{mn} x_1^m x_2^n \tag{4}$$

where K is the predicted partition coefficient, b_{00} is the intercept term, b_{10} and b_{01} are coefficients of the linear terms, and b_{mn} when $m + n > 1$ are the coefficients of nonlinear terms.

It turned out after making use of the Student's t-ratio test that (4) takes the form

$$K = b_{00} + b_{30}x_1^3 + b_{40}x_1^4 + b_{02}x_2^2 + b_{03}x_2^3 + b_{04}x_2^4 + b_{11}x_1x_2 \tag{5}$$

where

$$b_{00} = 52.62, \quad b_{30} = 25.83, \quad b_{40} = 13.9,$$
$$b_{02} = -54.21, \quad b_{03} = 8.63, \quad b_{04} = 27.17, \quad b_{11} = 7.20.$$

The parameter estimates and the analysis of variance are given in Tables 2 and 3, respectively. Since the regression coefficient R^2 is very close to 1, $R^2 = 0.997$, we may deduce that the model (5) is quite satisfactory. The goodness of fit can be easily seen from Table 4 as well.

Table 2
Parameter estimates

Term	Estimate	Standard error	Asymptotic 95% confidence interval	
			Lower	Upper
Intercept	52.62	1.313	49.51	55.72
x_1^3	25.83	0.71	24.12	27.53
x_1^4	13.94	0.65	12.38	15.49
x_2^2	−54.20	3.83	−63.26	−45.14
x_2^3	8.63	0.71	6.93	10.33
x_2^4	27.16	2.07	22.25	32.08
x_1x_2	7.20	1.60	3.39	11.00

Table 3
Analysis of variance

Source	d.f.	Sum of squares	Mean square	F ratio
Model	7	66960.2	9565.7	92.3
Error	7	72.5	10.36	
Total	13	67032.7		

Table 5 displays the regression coefficients calculated by setting b_{mn} equal to zero one at a time in (5) so as to determine their contributions. Rankwise, the important terms, in order, are obtained as b_{30}, b_{40}, b_{02}, b_{04}, b_{03}, and b_{11}.

We note that b_{10} and b_{01} do not enter in (5), meaning that K does not depend significantly on the linear terms. Also, the mixed terms (interaction terms) except x_1x_2 are also absent in our model, which make it easy to determine the critical points of the partition coefficient K. One can easily see that the critical points of K in the rectangle $[-1.414, 1.414] \times [-1.414, 1.414]$ are $(-1.384, -0.091)$, $(-1.308, -1.084)$, $(-0.377, -1.114)$, $(-0.006, -0.0004)$, $(0.0, 0.0)$, and $(0.295, -1.134)$. Thus, the function $K(x_1, x_2)$ attains the local extremum values 35.74, 8.65, 17.18, 52.62, 52.62, and 13.61, respectively, at these points. The value 52.62 of K may or may not be the absolute maximum of K, depending on whether or not the function K takes on a larger value at a boundary point. Indeed, a larger value of K is obtained along the boundary $x_1 = 1.414$ and $x_2 \in [-1.414, 1.414]$. We have seen that the partition coefficient K takes on its maximum value at (1.414, 0.0) as 181.39, agreeing with the experimental result, 182.70, displayed in Table 4.

The response surface $K = K(x_1, x_2)$ of the model which is given in Fig. 1 is plotted by using the Mathematica software. It is seen that the partition coefficient of BSA assumes larger values at high levels of PEG concentration for any fixed value of NaCl concentration. At the boundary point (1.414, 0.0) at which the partition coefficient K takes on its maximum value, PEG and NaCl concentrations are 9.8% and 0.2 M, respectively.

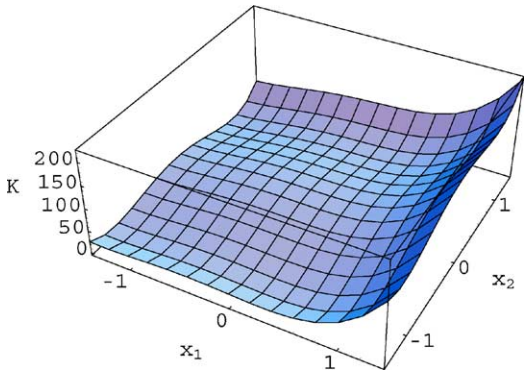

Fig. 1. Response surface of BSA partition coefficient.

Table 4
A comparison of experimental and predicted partition coefficients of BSA

Experiment no.	x_1	x_2	X_1 (PEG (%), w/w)	X_2 (NaCl, M)	$K^{experimental}$	$K^{predicted}$
1	+1	+1	9.0	0.3	78.34	81.19
2	−1	+1	5.0	0.3	16.04	15.12
3	+1	−1	9.0	0.1	48.60	49.52
4	−1	−1	5.0	0.1	15.11	12.26
5	+1.414	0	9.8	0.2	182.70	181.39
6	−1.414	0	4.2	0.2	34.00	35.33
7	0	+1.414	7.0	0.34	77.93	77.25
8	0	−1.414	7.0	0.06	27.75	28.43
9	0	0	7.0	0.2	54.62	52.62
10	0	0	7.0	0.2	50.05	52.62
11	0	0	7.0	0.2	49.47	52.62
12	0	0	7.0	0.2	52.38	52.62
13	0	0	7.0	0.2	51.30	52.62
14	0	0	7.0	0.2	57.89	52.62

Table 5
R^2 values after modification

Model	R^2
$K = 52.62 + 13.94x_1^4 - 54.21x_2^2 + 8.63x_2^3 + 27.17x_2^4 + 7.20x_1x_2$	0.384
$K = 66.55 + 25.83x_1^3 - 47.22x_2^2 + 8.63x_2^3 + 20.18x_2^4 + 7.20x_1x_2$	0.782
$K = 46.25 + 25.83x_1^3 + 13.14x_1^4 + 8.63x_2^3 - 0.74x_2^4 + 7.20x_1x_2$	0.901
$K = 49.91 + 25.83x_1^3 + 12.58x_1^4 - 6.68x_2^2 + 8.63x_2^3 + 7.20x_1x_2$	0.915
$K = 52.62 + 25.83x_1^3 + 13.94x_1^4 - 54.21x_2^2 + 27.17x_2^4 + 7.20x_1x_2$	0.928
$K = 52.62 + 25.83x_1^3 + 13.94x_1^4 - 54.21x_2^2 + 8.63x_2^3 + 27.17x_2^4$	0.987

As a result, we can conclude that the Box–Wilson method is applicable to determine the polymer–gel system conditions for optimizing protein partitioning by altering PEG and salt concentrations. In our earlier work [8], we applied this method to the aqueous two-phase system (PEG 3350, 8% + dextran 37,500, 9% + NaCl, 0.2 M; pH = 7 and T = 20 °C) and found out that the partition coefficient of BSA is 0.017. It is remarkable that the value $1/0.017 = 58.82$ is comparable with the experimental result $K = 54.62$ (PEG 20,000, 7% + NaCl, 0.2 M; pH = 7 and T = 20 °C) obtained in this study.

Partitioning and diffusion of large molecules in fibrous structures are important in several applications such as gel permeation chromatography, controlled drug release, ultrafiltration and electrophoresis. Partition and diffusion coefficients of proteins in gels can be measured by various methods, among them are ultrafiltration, fluorescence recovery after photobleaching and gel permeation chromatography. Bosma and Wesselingh [9] reported the partition coefficient of BSA in an ion-exchange gel at pH 4.4 and acetate concentration of 1 mol/l as 0.59 by gel permeation chromatography technique. Karlsson et al. [10] used Electronic Speckle Pattern Interferometry technique and determined the partition coefficient of BSA in 4% agarose gel at pH 5.6 and 0.1 M NaCl as 0.44.

As a last remark, we may note that optimization of protein separation is easier with the technique used in this study (SCGS) than with other techniques because the conditions for a separation can be determined in small test tubes.

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