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Partitioning of bovine serum albumin in an aqueous two-phase system: optimization of partition coefficient

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Abstract

Partitioning of proteins in aqueous two-phase systems has been shown to provide a powerful method for separating and purifying mixtures of biomolecules by extraction. There are many factors which influence the partition coefficient *K*, the ratio of biomolecule concentration in top phase to that in the bottom phase, in aqueous two-phase systems. In this work, the partition behavior of pure bovine serum albumin in aqueous two-phase systems was investigated in order to see the effects of changes in phase properties on the partition coefficient *K*. pH and concentration of NaCl salt were found to be the factors having influence on *K*. Optimal conditions of these factors were obtained using the Box–Wilson experimental design. The optimum value of *K* was found as 0.018 when NaCl concentration, and pH were 0.0195 *M* and 8.9, respectively, for a phase system composed of 8% (w/w) polyethylene glycol 3350–6% (w/w) dextran 37 500–0.05 *M* phosphate at 20°C.

Keywords: Aqueous two-phase systems; Partition coefficient; Bovine serum albumin

1. Introduction

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. The value of the partition coefficient relies on the physico–chemical properties of the target biomolecule and other molecules and their interactions with those of the chosen system.

Aqueous two-phase systems have a number of advantages when compared with other commonly used separation and purification techniques. Some advantages can be summarized as follows [1]: the 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.

The general properties of the aqueous two-phase systems have been studied by several researchers [1–3]. However, the mechanism governing the partition of biological materials is still not well understood. The observed partition coefficient is a result of van der Waals, hydrophobic, hydrogen bond, and ionic interactions of the biomolecules with the surrounding phase. Therefore, the partition coefficient is influenced by many factors, including the concentrations and molecular masses of PEG and dextran, type and concentration of added salts, temperature and pH.

As it has been observed by some researchers [1,4], many salts are slightly partitioned into the dextran-rich bottom phase in the PEG–dextran two-phase system. This results in an electrostatic potential

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difference between the two phases even though each phase is electrically neutral. As a result of such a potential, negatively charged substances are likely to be partitioned into the top phase and positively charged substances into the bottom phase.

It appears likely that the specific interactions between the salts and proteins, in addition to the potential difference created by the salt, are responsible for the effect of different salts on protein partitioning. Moreover, different salts affect the water structure and hydrophobic interactions differently, and as salt concentration increases, the partition coefficient of a biomolecule with large hydrophobic region or surface in its structure will change due to its interaction with the surrounding phases [5].

The object of this work is to investigate the effects of pH and NaCl salt concentration on partitioning of bovine serum albumin (BSA) in aqueous PEG–dx two-phase systems via Box–Wilson experimental design method. The classical one-variable at a time strategy usually fails, because it tacitly assumes that minimizing value of one variable is independent of the other, which is not usually true. Therefore, we use the Box–Wilson experimental design method as an alternative. The 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 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 single test at the average level of each variable. Designs for any number of variables can be developed from these principles.

When the experimental tests have been completed, a regression analysis is carried out to determine the coefficients in the assumed model. An analysis of variance by means of the F-test is usually carried out after the model has been developed to determine its significance.

2. Experimental

2.1. Materials

Dextran (M_r 37 500, Lot No. 44H0568), PEG (M_r 3350, Lot No. 44H0122) and BSA (Lot No. 57H1090) were purchased from Sigma (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 and dextran stock solutions were 25% (w/w) polymer. Different stock solutions of K_2HPO_4 and KH_2PO_4 were used to prepare the aqueous two-phase systems at different pH levels.

2.2. Partition coefficient measurements

Aqueous two-phase partitioning experiments were performed at 20°C by weighing and mixing the phase forming polymer stock solutions with stock solutions of BSA, buffer, and salts. BSA was added to a final concentration of 0.1% (w/w). Distilled water was then added to obtain the final weight (10 g). The systems were well mixed by a vortex mixer and left in a water bath (Memmert, Germany) overnight. Samples were carefully withdrawn from the top phase and to take samples from the bottom phase the bottom of the tubes were pinched. For the determination of BSA concentration, a sample withdrawn from each phase was diluted with a known amount of distilled water, and its ultraviolet absorbance was measured in a dual-beam spectrophotometer (Hach DR/4000 UV–Vis, Loveland, CO, USA) at 280 nm. An identically diluted solution of the corresponding phase from a system containing no BSA was used as a blank.

2.3. Experimental design

The Box–Wilson experimental design [6] was used in the optimization of partitioning of BSA in aqueous two-phase systems. pH (X_1) and NaCl concentration (X_2, molarity) were chosen as indepen-

Table 1
Real values of the independent variables in the experimental plan (X_1: pH 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

dent variables in a series of repeated partitioning experiments, and partition coefficient of BSA (K) was the dependent output variable. For statistical calculations the variables X_i were coded as x_i according to the equation,

$$x_i = (X_i - X_0)/\Delta X_i \quad (1)$$

where x_i is the coded value of the variable i, X_0 is the value of the variable i at the center point of the investigated area, and ΔX_i is the step size. More specifically, we set

$$x_1 = (X_1 - 7.0)/2.0 \quad (2)$$

$$x_2 = (X_2 - 0.2)/0.1 \quad (3)$$

In Table 1, the values of these quantities are displayed.

3. Results and discussion

A 2^2-factorial experimental design with four star points ($a = 1.414$) and six replicates at the center point with a total of 14 experiments was employed. For studying the pattern of responses, a second-degree polynomial was fitted and only the terms based on Student's t-ratio test were kept. The model of regression has the form

$$K = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{12}x_1x_2 + b_{22}x_2^2 \quad (4)$$

where K is the predicted response, b_0 is the intercept term, b_i values are coefficients of the linear terms and b_{ij} values are coefficients of the quadratic terms.

Using Eq. (4) we obtained the relationship for predicting the partition coefficient (K) as follows

$$K = 0.0215 - 0.0066x_1 + 0.0035x_1^2 + 0.0075x_2^2 \quad (5)$$

The parameter estimates and analysis of variance are given in Tables 2 and 3, respectively. The high value of the R^2, $R^2 = 0.979$, indicates the efficiency of the fitted model. The goodness of fit can be observed from Table 4. The model as fitted was able to explain 97.9% of the variation in the partitioning of BSA in the aqueous two-phase systems studied. The regression coefficients were ranked for their importance. Rankwise, the important terms, in order, were x_2^2, x_1 and x_1^2 as can be seen from Table 5. From our model we see that pH (x_1) has a bigger effect on K in a linear way, but not in a non-linear form (x_1^2). On the other hand, we see that NaCl concentration (x_2) does not have an effect on K in a linear form, but it affects in a non-linear form (x_2^2).

To understand our model (Eq. (5)) mathematically better, let us rewrite it in a general form

$$K = a + bx_1 + cx_1^2 + dx_2^2. \quad (6)$$

Using some Calculus we can easily show that the function K given in Eq. (6) attains a local minimum value at

$$(x_1, x_2) = \left(-\tfrac{b}{2c}, 0\right)$$

Table 3
Analysis of variance

Source	DF	Sum of squares	Mean square	F Ratio
Model	3	0.00083145	0.000277	132.59
Error	10	0.00002090	0.000002	
Total	13	0.00085236		

Table 2
Parameter estimates

Term	Estimate	Standard error	t Ratio	Prob.>|t|
Intercept	0.0215	0.00059	36.43	0.0000
x_1	−0.0066	0.00051	−12.99	0.0000
x_1^2	0.0035	0.00053	6.58	0.0001
x_2^2	0.0075	0.00053	14.10	0.0000

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

Exp. No	x_1	x_2	$K^{\text{experimental}}$	$K^{\text{predicted}}$
1	+1	+1	0.026	0.025
2	−1	+1	0.037	0.039
3	+1	−1	0.027	0.026
4	−1	−1	0.038	0.039
5	+1.414	0	0.018	0.019
6	−1.414	0	0.040	0.038
7	0	+1.414	0.036	0.037
8	0	−1.414	0.038	0.037
9	0	0	0.021	0.022
10	0	0	0.022	0.022
11	0	0	0.023	0.022
12	0	0	0.020	0.022
13	0	0	0.022	0.022
14	0	0	0.021	0.022

Table 5
R^2 values after modification

Model	R^2
$K=0.0215-0.0066x_1+0.0035x_1^2$	0.49
$K=0.0215+0.0035x_1^2+0.0075x_2^2$	0.56
$K=0.0215-0.0066x_1+0.0075x_2^2$	0.87

which may or may not be the global minimum of K depending on the domain of interest. In our case, by considering the boundary points, we also see that this minimum value is the global minimum of K defined by Eq. (5).

The response surface of the model obtained by using the Mathematica software is given in Fig. 1. It is seen that the partition coefficient of BSA gets higher at both low and high levels of NaCl concentration at any fixed value of pH. Around the central area, NaCl concentration and pH are 0.195 *M* and 8.9, respectively. The partition coefficient K defined by Eq. (5) takes on its minimum value 0.018 at this point.

As a result, we can conclude that the Box–Wilson method is applicable to determine aqueous two-phase system conditions for optimizing BSA partitioning by altering pH and salt concentration.

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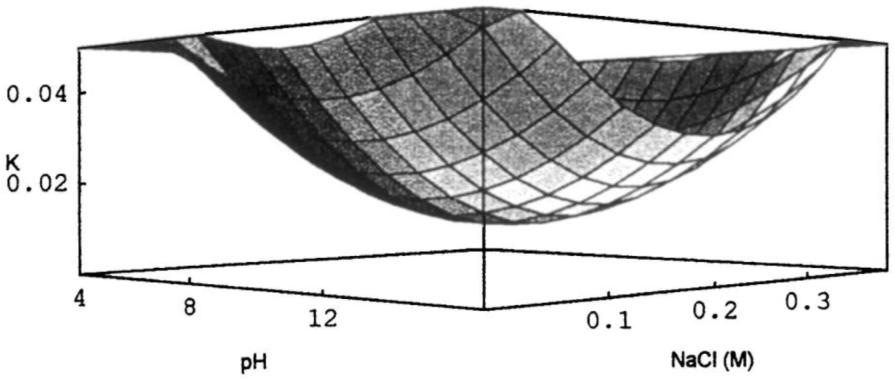

Fig. 1. Response surface of BSA partition coefficient.