# Experimental Study and Mathematical Modeling of Partitioning of β-Amylase and Amyloglucosidase in PEG−Salt Aqueous Two-Phase Systems

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In this study, the partitioning of β-amylase in aqueous two-phase systems (ATPS) containing polyethylene glycol (PEG) with a molar mass of 6000 and 10 000 and $KH_2PO_4$ at $T$ = (301.65 and 304.65) K was experimentally studied. In addition, the partitioning of amyloglucosidase in ATPS containing polyethylene glycol with molar mass of 4000, 6000, and 10 000 in the presence of $Na_2SO_4$ at $T$ = (301.65 and 305.65) K was investigated. The effects of molar mass of PEG, temperature, mass fractions of salt, and the length of the tie line on partition coefficients of the enzymes were also studied. The experimental results showed that while the partition coefficients of enzymes decrease with increasing molar mass of polymer they increase with increasing tie line length as well as system temperature. Also, the salt concentrations in ATPS can significantly affect the partitioning of enzymes between the top and bottom phases. It was concluded that the partition coefficients of enzymes increase with an increase in the salt concentrations. The experimental data of the partition coefficients were correlated with the equation proposed by Diamond and Hsu. The results indicated that the Diamond and Hsu equation which is based on the lattice theory of Flory−Huggins can accurately correlate the experimental data collected in this work.

## Introduction

The hydrolyze enzymes group is composed of the enzymes β-amylase (EC 3.2.1.2, glucogenase) and amyloglucosidase (EC 3.2.1.3, 1,4-α-glucosidase) which break the starch into smaller molecules. The β-amylase and amyloglucosidase have many applications in food industries, pharmacy, medicine, nutrition, brewery, and bread making industries.[1] Therefore, the purification of such enzymes has been crucially important. To separate and purify these enzymes using the so-called aqueous two-phase systems (ATPS), the data on their partition coefficients are of central importance.[2]

Separation and purification of biomolecules in the downstream part is an important process in producing products for which almost two-thirds of the total cost of a process is devoted to this part. The final cost of the production depends on the selective methods.

It is well-known that the aqueous two-phase systems can be formed by two incompatible phases containing two hydrophilic polymers such as dextran (DEX) and polyethylene glycol (PEG) or a hydrophilic polymer in the presence of an electrolyte salt at high concentration. ATPSs containing polymer and salt, because of their low cost, low viscosity, greater density difference between phases, low interfacial tension, and short time for separation of phases, have received more attention than those of polymer−polymer.[3]

Beijerinck was the first researcher who formed the ATPSs by gelatin and agar in 1896.[4] Albertson during the mid-1950s used the aqueous two-phase systems for separation of animal and plant cells, micro-organisms, proteins, and nucleic acids.[5]

Furuya et al. measured and correlated partition coefficients of hydrolytic enzymes such as α-amylase, β-amylase, and glucoamylase in the ATPS PEG + DEX at 293 K.[6] They investigated the effects of different molar masses of PEG on partition coefficients and observed that the partition coefficients of α-amylase, β-amylase, and amyloglucosidase decrease by increasing the molar mass of PEG.

Mokhtarani et al. measured partioning of ciprofloxacin in aqueous two-phase systems of polyethylene glycol and sodium sulfate.[7] The influences of temperature, polymer, and salt concentrations and polymer molar mass on the partitioning of ciprofloxacin were studied. They represented that the partitioning of ciprofloxacin depends highly on salt concentration. Temperature and PEG concentration have a moderate effect on partitioning of ciprofloxacin, and the molar mass of polymer has no effect on partitioning of ciprofloxacin.

Pazuki et al. studied the partitioning process of penicillin G−asylase in aqueous two-phase systems containing polyethylene glycol 20 000 or 350 000 and $KH_2PO_4$/$Na_3$ citrate.[8] The experimental partition coefficients showed that partition coefficients of penicillin G depend on temperature, molar mass of polymer, mass fractions of salt and polymer, and salt concentrations. The concentration of salt has a large effect on partitioning of penicillin G, while the temperature has a small effect on partitioning of penicillin G.

Recently, Shahriari et al. investigated the partitioning process of β-amylase in aqueous two-phase systems PEG (4000, 6000, and 10 000) + $Na_2SO_4$ + water and amyloglucosidase in aqueous two-phase systems PEG (6000, 10 000) + $KH_2PO_4$ + water at different temperatures.[9] They showed that temperature has a small effect on partitioning of β-amylase and amyloglucosidase. Also, they concluded that partitioning of β-amylase

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**Table 1. Characterizations of Effective Variables on the Partitioning Process of Amylase Enzymes in the Aqueous Two-Phase Systems**

| enzyme | ATPS poly−poly/poly salt | range of PEG concentration | range of salt/DEX concentration | pH | $T$/K | $K_{enz.}$ | 100 TLL | ref |
|---|---|---|---|---|---|---|---|---|
| α-amylase | PEG4000−$K_2HPO_4$ | 8.88 to 12.29 | 8.49 to 10.47 | 7.1 | 298 | 1.089 to 1.468 | 14.205 to 22.289 | 11 |
| α-amylase | PEG4000−$Na_2SO_4$ | 8.86 to 10.92 | 7.06 to 8.10 | 5.4 | 298 | 0.659 to 0.765 | 13.615 to 21.344 | |
| α-amylase | PEG4000−$K_2HPO_4$ | 9.55 to 12.28 | 10.16 to 11.96 | 6.8 | 298 | 1.640 to 1.240 | - | |
| α-amylase | PEG4000−$K_2HPO_4$ | 10.93 to 13.64 | 7.59 to 9.42 | 9.1 | 298 | 2.910 to 1.570 | - | |
| α-amylase | PEG4000−DEX40 | 8.98 to 10.50 | 6.01 to 7.50 | - | 293 | 0.100 to 0.280 | 13.330 to 24.840 | 6 |
| α-amylase | PEG6000−DEX500 | 5.50 to 7.00 | 2.50 to 4.00 | - | 293 | 0.200 to 0.440 | 12.170 to 18.930 | |
| β-amylase | PEG4000−DEX70 | 8.53 to 10.02 | 5.49 to 7.00 | - | 293 | 0.110 to 0.380 | 14.970 to 25.250 | |
| glucoamylase | PEG4000−DEX500 | 7.31 to 9.51 | 4.28 to 6.00 | - | 293 | 0.310 to 0.690 | 11.940 to 21.990 | |
| β-amylase | PEG4000−$Na_2SO_4$ | 14.71 to 18.52 | 10.00 to 12.35 | 5.36 to 5.86 | 298.5 to 308 | 0.360 to 0.429 | 37.235 to 48.016 | 9 |
| β-amylase | PEG6000−$Na_2SO_4$ | 14.71 to 18.52 | 10.00 to 12.35 | 4.98 to 5.27 | 298.5 to 308 | 0.349 to 0.420 | 39.196 to 47.596 | |
| glucoamylase | PEG6000−$KH_2PO_4$ | 18.04 to 20.29 | 9.79 to 12.35 | 4.37 to 4.49 | 301.5 to 305 | 0.603 to 0.741 | 21.695 to 32.941 | |
| glucoamylase | PEG10000−$KH_2PO_4$ | 14.71 to 18.52 | 10.00 to 12.35 | 4.25 to 4.54 | 301.5 to 305 | 0.096 to 0.173 | 17.863 to 32.301 | |

**Table 2. Parameters of Refractive Index Introduced into Equation 1**

| Component | $\alpha_0$ | $\alpha_1$ | $\alpha_2$ | 100 $\sigma^a$ |
|---|---|---|---|---|
| water | 1.3325 | | | |
| PEG4000 | | 0.1400 | | 0.004 |
| PEG6000 | | 0.1471 | | 0.005 |
| PEG10 000 | | 0.1484 | | 0.001 |
| $Na_2SO_4$ | | | 0.1485 | 0.008 |
| $KH_2PO_4$ | | | 0.1179 | 0.007 |

[a]

$$\sigma = \left[\sum_{i=1}^{N}\left(\frac{n_d^{calcd} - n_d^{exptl}}{n_d^{exptl}}\right)^2\right]^{0.5}$$

$N$ = number of experimental data points.

and amyloglucosidase depends on the mass fraction of salt in feed, and it decreases by increasing the molar mass of PEG.

The characterizations of effective variables on the partitioning process of amylase enzymes in the ATPSs are reported in Table 1.

In this work, the partition coefficients of β-amylase in ATPS PEG (6000, 10 000) + $KH_2PO_4$ and amyloglucosidase in ATPS PEG (4000, 6000, and 10 000) + $Na_2SO_4$ are measured at different temperatures. The effects of molar mass of PEG, temperature of the system, mass fractions of salt and polymer in feed, and length of tie line were considered on partitioning of β-amylase and amyloglucosidase enzymes.

The mathematical model proposed by Diamond and Hsu[10] was used to correlate the partition coefficients of β-amylase and amyloglucosidase in PEG + $Na_2SO_4$/$KH_2PO_4$ aqueous two-phase systems.

## Experimental Section

***Materials.*** Polyethylene glycol with various molar masses (number average molar mass, $M_r$ = 4000, 6000, and 10 000) and sodium sulfate ($Na_2SO_4$) with purity of 99 % and potasium dihydrogen phosphate ($KH_2PO_4$) with purity of 99.5 % were purchased from Merck (Germany, Darmstadt). β-Amylase ($M_r$ = 60 000) from barley (Art no. 10100) and amyloglucosidase ($M_r$ = 97 000) from *Aspergillus niger* (Art no. 10115) were provided by Fluka (Switzerland). Distilled deionized water with the conductivity of 0.055 $\mu s \cdot cm^{-1}$ was used to prepare the solutions under study.

***Methods.*** The experimental measurement of partition coefficients of biological molecules in ATPSs containing polymer and salt was extensively explained in the literature, and the method used in this work was similar to that elucidated in the previous work.[9] For each experiment, the aqueous two-phase systems were prepared by mixing deionized water, polymer, salt, and enzymes with specified weight percents in a double layer glass vessel with 800 $cm^3$ volume equipped with a recycling thermostat to keep the temperature of the mixture constant. To control the temperature of mixtures, a thermocouple with accuracy of ± 0.01 °C was used. Also, a magnetic stirrer was used to mix the ATPS. After 30 min, the mixtures were left without stirring for almost 24 h to make sure that the equilibrium condition was attained. The equilibrium condition was investigated by taking out a number of samples from the

**Table 3. Mass Fractions of PEG (1) + $KH_2PO_4$ (2) + β-Amylase (3) in the Top and Bottom Phases and Partition Coefficients of β-Amylase in ATPS**

| | | feed | | | top phase | | | bottom phase | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $T$/K | pH | 100 $w_1$ | 100 $w_2$ | 100 $w_3$ | 100 $w_1$ | 100 $w_2$ | 100 $w_3$ | 100 $w_1$ | 100 $w_2$ | 100 $w_3$ | 100 TLL | $K$ |
| | | | | | PEG6000 + $KH_2PO_4$ | | | | | | | |
| 301.65 | 4.36 | 18.523 | 10.009 | 0.0279 | 25.592 | 8.358 | 0.014 | 2.209 | 17.940 | 0.060 | 25.270 | 0.233 |
| 301.65 | 4.37 | 18.52 | 10.009 | 0.0136 | 25.079 | 8.999 | 0.008 | 2.772 | 18.000 | 0.028 | 24.054 | 0.276 |
| 301.65 | 4.34 | 18.04 | 12.358 | 0.0272 | 32.583 | 7.270 | 0.020 | 2.396 | 19.657 | 0.035 | 32.630 | 0.555 |
| 301.65 | 4.21 | 14.71 | 10.477 | 0.0292 | 24.885 | 7.967 | 0.013 | 2.454 | 17.973 | 0.048 | 24.562 | 0.275 |
| 304.65 | 4.42 | 18.523 | 10.009 | 0.0279 | 25.926 | 6.669 | 0.015 | 1.996 | 18.544 | 0.056 | 26.715 | 0.276 |
| 304.65 | 4.42 | 18.52 | 10.009 | 0.0136 | 25.400 | 6.054 | 0.008 | 1.913 | 18.648 | 0.026 | 26.651 | 0.319 |
| 304.65 | 4.46 | 18.04 | 12.358 | 0.0272 | 31.877 | 4.419 | 0.019 | 1.477 | 20.464 | 0.037 | 34.374 | 0.510 |
| 304.65 | 4.53 | 14.71 | 10.477 | 0.0292 | 27.991 | 7.910 | 0.013 | 1.133 | 19.367 | 0.046 | 29.200 | 0.293 |
| | | | | | PEG10000 + $KH_2PO_4$ | | | | | | | |
| 301.65 | 4.36 | 18.523 | 10.009 | 0.0279 | 24.558 | 7.680 | 0.015 | 1.428 | 18.050 | 0.063 | 25.349 | 0.243 |
| 301.65 | 4.39 | 18.52 | 10.009 | 0.0136 | 26.957 | 6.782 | 0.007 | 1.977 | 17.952 | 0.027 | 27.363 | 0.262 |
| 301.65 | 4.47 | 18.04 | 12.358 | 0.0272 | 31.694 | 6.248 | 0.015 | 2.349 | 19.350 | 0.042 | 32.136 | 0.354 |
| 301.65 | 4.35 | 14.71 | 10.477 | 0.0292 | 19.194 | 8.326 | 0.013 | 1.882 | 16.546 | 0.075 | 19.164 | 0.178 |
| 304.65 | 4.54 | 18.523 | 10.009 | 0.0279 | 26.942 | 6.632 | 0.015 | 1.419 | 18.146 | 0.055 | 27.999 | 0.269 |
| 304.65 | 4.56 | 18.52 | 10.009 | 0.0136 | 26.220 | 6.946 | 0.008 | 1.595 | 18.094 | 0.027 | 27.031 | 0.288 |
| 304.65 | 4.38 | 18.04 | 12.358 | 0.0272 | 32.292 | 6.004 | 0.016 | 1.624 | 19.839 | 0.040 | 33.644 | 0.403 |
| 304.65 | 4.33 | 14.71 | 10.477 | 0.0292 | 20.279 | 7.724 | 0.013 | 1.417 | 16.622 | 0.067 | 20.856 | 0.198 |

**Table 4. Mass Fractions of PEG (1) + $Na_2SO_4$ (2) + Amyloglucosidase (3) in the Top and Bottom Phases and Partition Coefficients of Amyloglucosidase in ATPS**

| | | feed | | | top phase | | | bottom phase | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *T*/K | pH | 100 $w_1$ | 100 $w_2$ | 100 $w_3$ | 100 $w_1$ | 100 $w_2$ | 100 $w_3$ | 100 $w_1$ | 100 $w_2$ | 100 $w_3$ | 100TLL | *K* |
| | | | | | PEG4000 + $Na_2SO_4$ | | | | | | | |
| 301.65 | 5.73 | 18.523 | 10.009 | 0.0279 | 43.185 | 1.643 | 0.028 | 2.125 | 16.044 | 0.028 | 43.512 | 1.028 |
| 301.65 | 5.5 | 18.52 | 10.009 | 0.0136 | 42.339 | 1.700 | 0.020 | 1.934 | 16.022 | 0.009 | 42.869 | 2.260 |
| 301.65 | 5.45 | 18.04 | 12.358 | 0.0272 | 47.343 | 1.226 | 0.051 | 3.916 | 16.712 | 0.016 | 46.105 | 3.185 |
| 301.65 | 5.37 | 14.71 | 10.477 | 0.0292 | 39.772 | 2.168 | 0.051 | 1.113 | 15.584 | 0.018 | 40.921 | 2.883 |
| 305.65 | 5.6 | 18.523 | 10.009 | 0.0279 | 44.366 | 1.608 | 0.030 | 1.987 | 16.106 | 0.026 | 44.790 | 1.155 |
| 305.65 | 5.43 | 18.52 | 10.009 | 0.0136 | 43.427 | 1.685 | 0.022 | 1.865 | 16.154 | 0.008 | 44.008 | 2.859 |
| 305.65 | 5.42 | 18.04 | 12.358 | 0.0272 | 49.268 | 1.026 | 0.052 | 2.983 | 17.322 | 0.015 | 49.070 | 3.353 |
| 305.65 | 5.77 | 14.71 | 10.477 | 0.0292 | 40.738 | 1.930 | 0.052 | 0.758 | 15.649 | 0.017 | 42.268 | 3.010 |
| | | | | | PEG6000 + $Na_2SO_4$ | | | | | | | |
| 301.65 | 5.18 | 18.523 | 10.009 | 0.0279 | 40.354 | 1.575 | 0.028 | 2.243 | 15.825 | 0.028 | 40.688 | 1.029 |
| 301.65 | 5.32 | 18.52 | 10.009 | 0.0136 | 40.892 | 1.581 | 0.018 | 1.923 | 16.007 | 0.011 | 41.554 | 1.682 |
| 301.65 | 5.12 | 18.04 | 12.358 | 0.0272 | 44.575 | 1.232 | 0.045 | 3.670 | 16.701 | 0.018 | 43.732 | 2.505 |
| 301.65 | 5.75 | 14.71 | 10.477 | 0.0292 | 38.476 | 1.954 | 0.035 | 1.212 | 15.163 | 0.026 | 39.536 | 1.382 |
| 305.65 | 5.45 | 18.523 | 10.009 | 0.0279 | 42.291 | 1.542 | 0.038 | 1.621 | 15.903 | 0.020 | 43.131 | 1.890 |
| 305.65 | 5.05 | 18.52 | 10.009 | 0.0136 | 41.001 | 1.541 | 0.020 | 1.188 | 16.264 | 0.008 | 42.447 | 2.393 |
| 305.65 | 5.24 | 18.04 | 12.358 | 0.0272 | 46.484 | 1.092 | 0.048 | 3.173 | 17.126 | 0.017 | 46.184 | 2.879 |
| 305.65 | 5.33 | 14.71 | 10.477 | 0.0292 | 38.984 | 1.923 | 0.036 | 0.966 | 15.205 | 0.025 | 40.271 | 1.454 |
| | | | | | PEG10000 + $Na_2SO_4$ | | | | | | | |
| 301.65 | 5.04 | 18.523 | 10.009 | 0.0279 | 39.931 | 1.510 | 0.029 | 2.109 | 15.333 | 0.027 | 40.269 | 1.097 |
| 301.65 | 5.06 | 18.52 | 10.009 | 0.0136 | 40.462 | 1.519 | 0.020 | 1.931 | 15.310 | 0.009 | 40.924 | 2.365 |
| 301.65 | 4.66 | 18.04 | 12.358 | 0.0272 | 44.258 | 1.159 | 0.027 | 3.515 | 16.353 | 0.027 | 43.484 | 1.008 |
| 301.65 | 4.74 | 14.71 | 10.477 | 0.0292 | 37.737 | 1.884 | 0.029 | 0.946 | 15.081 | 0.029 | 39.086 | 1.010 |
| 305.65 | 4.56 | 18.523 | 10.009 | 0.0279 | 43.877 | 1.135 | 0.033 | 1.982 | 15.460 | 0.024 | 44.276 | 1.376 |
| 305.65 | 4.47 | 18.52 | 10.009 | 0.0136 | 43.603 | 1.073 | 0.021 | 1.773 | 15.400 | 0.008 | 44.216 | 2.509 |
| 305.65 | 4.76 | 18.04 | 12.358 | 0.0272 | 47.124 | 0.921 | 0.032 | 3.522 | 16.413 | 0.025 | 46.273 | 1.318 |
| 305.65 | 4.9 | 14.71 | 10.477 | 0.0292 | 40.602 | 1.580 | 0.036 | 0.740 | 15.355 | 0.025 | 42.176 | 1.442 |

top and bottom phases, and measurement of the amount of components in both phases confirmed the equilibrium conditions. A plastic syringe was used for sampling from the top and bottom phases.

***Quantitative Analysis.*** Flame photometry (Sherwood 410, from Cambridge, England) was used to determine the mass fraction of $Na_2SO_4$ and $KH_2PO_4$.

The mass fraction of PEG was determined by refractive index measurements using a digital ABBE refractometer (model DR-A1, from Atago, Japan). The correlation between the refractive index ($n_d$) and the mass fraction of polymer ($w_p$) and salt ($w_s$) can be written as[8]

$$n_d = a_0 + a_1 w_p + a_2 w_s \quad (1)$$

The parameters of eq 1 and standard deviations are reported in Table 2 for different PEG + $Na_2SO_4$/$KH_2PO_4$ aqueous two-phase systems.

The mass fractions of $\beta$-amylase and amyloglucosidase in the top and bottom phases were measured by using a UV/V spectrophotometer (model: Cary300, Varian, USA). The concentration of $\beta$-amylase and amyloglucosidase was determined at 280 nm against the blanks with the same compositions as the samples, but without any enzyme, to avoid the interface of PEG and salt.

A pH meter (model: 744, Metrohm, Switzerland) was used to measure the pH of aqueous solution studied in this work.

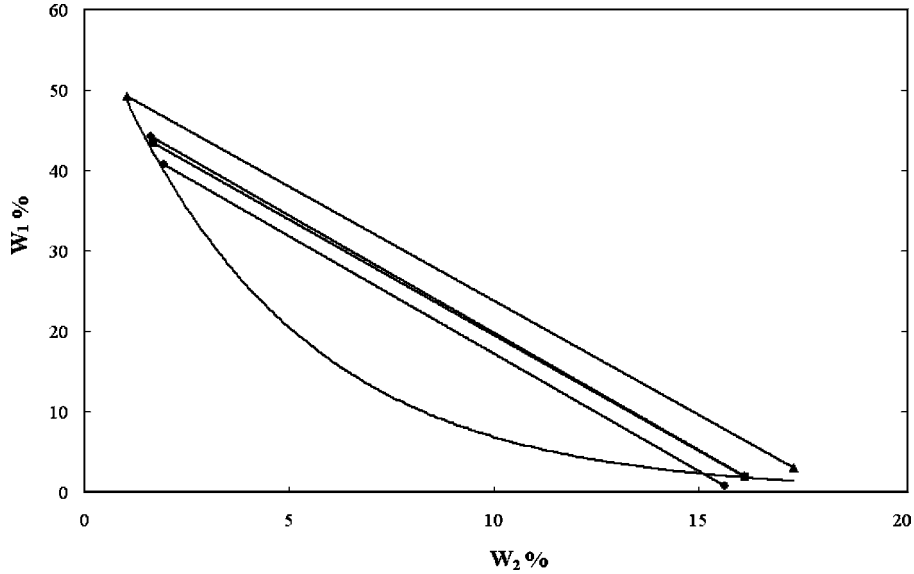


**Figure 1.** Binodal curve and tie lines of the PEG4000 (1) + $Na_2SO_4$ (2) aqueous two-phase system at 305.65 K.

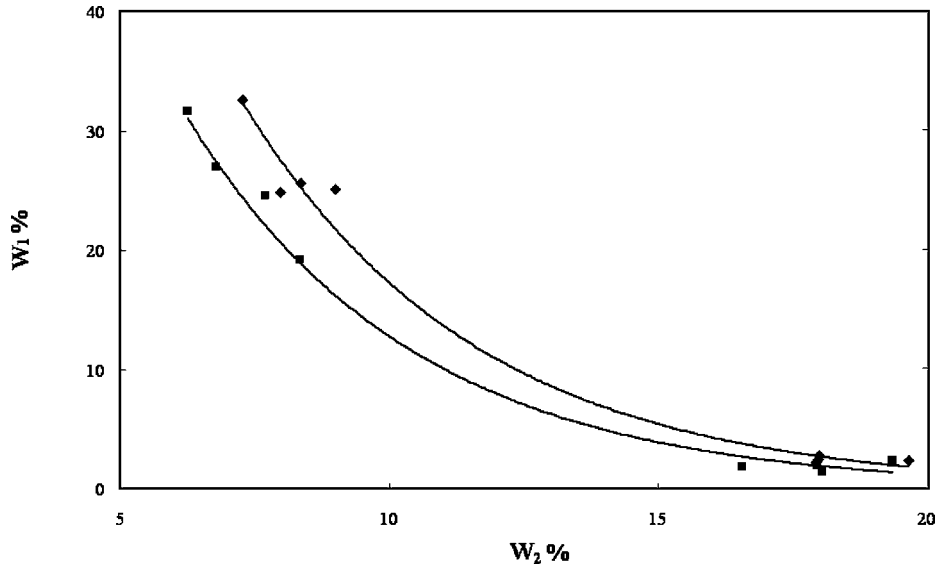


**Figure 2.** Effect of PEG molar mass on two-phase separation in PEG (1) + $KH_2PO_4$ (2): ◆, PEG6000; ■, PEG10000; −, binodal curves.

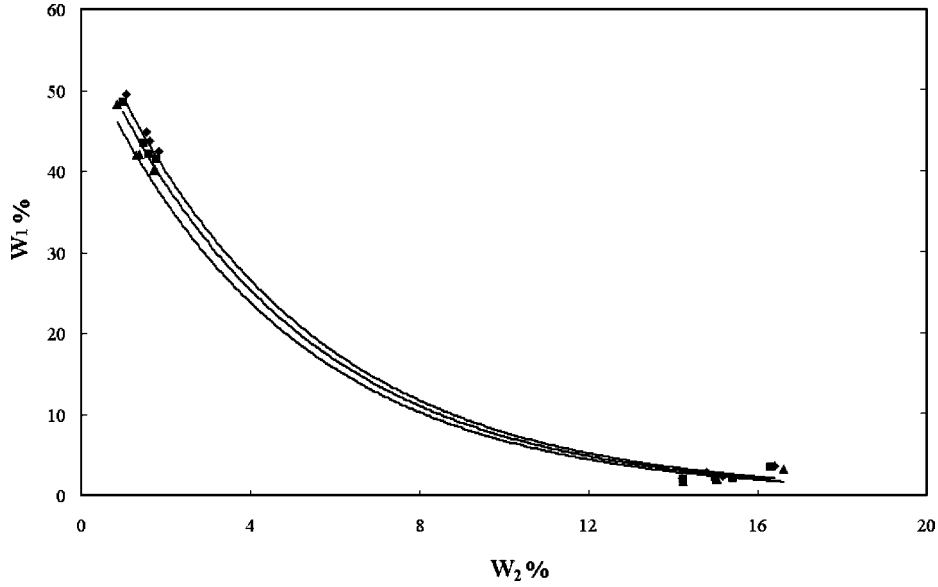


**Figure 3.** Effect of PEG molar mass on two-phase separation in PEG (1) + $Na_2SO_4$ (2): ◆, PEG4000; ■, PEG6000; ▲, PEG10000; −, binodal curves.

The relative uncertainty in the mass fractions of salt and polymer is less than 3.9 %. The relative experimental uncertainty for enzyme is less than 8 %.

***Modeling.*** As expounded earlier, the experimental data of the partition coefficients generated in this work were correlated with the equation proposed by Diamond and Hsu.[10] The equation is based on the lattice theory of Flory−Huggins and could be used to accurately correlate the experimental data on the partition coefficients.

The partition coefficient ($K_{enz}$) of $\beta$-amylase and amyloglucosidase in an ATPS can be written as[9]

$$K_{enz} = \frac{w_{enz}^{top}}{w_{enz}^{bottom}} \tag{2}$$

where in eq 2 $w_{enz}$ is the mass fraction of enzyme.

A linear correlation between the natural logarithm of the partition coefficients and the difference between mass fractions of polymers in the two phases was first presented by the Diamond and Hsu equation based on the lattice theory of Flory−Huggins.[10] The equation takes the following form

$$\frac{\ln K_{enz}}{\Delta W(\mathrm{PEG})} = A + B\cdot\Delta W(\mathrm{PEG}) \tag{3}$$

Using a linear regression between the experimental partition coefficients and those obtained from eq 3, the adjustable parameters, $A$ and $B$, introduced by eq 3 can be obtained.

The following objective function was used to obtain the parameters of eq 3

$$\mathrm{rmsd} = \frac{\sqrt{\sum_{i=1}^{N}(K_{enz}^{exptl} - K_{enz}^{calcd})_i^2}}{N} \tag{4}$$

Notably, in eq 4, $N$ is the number of tie lines.

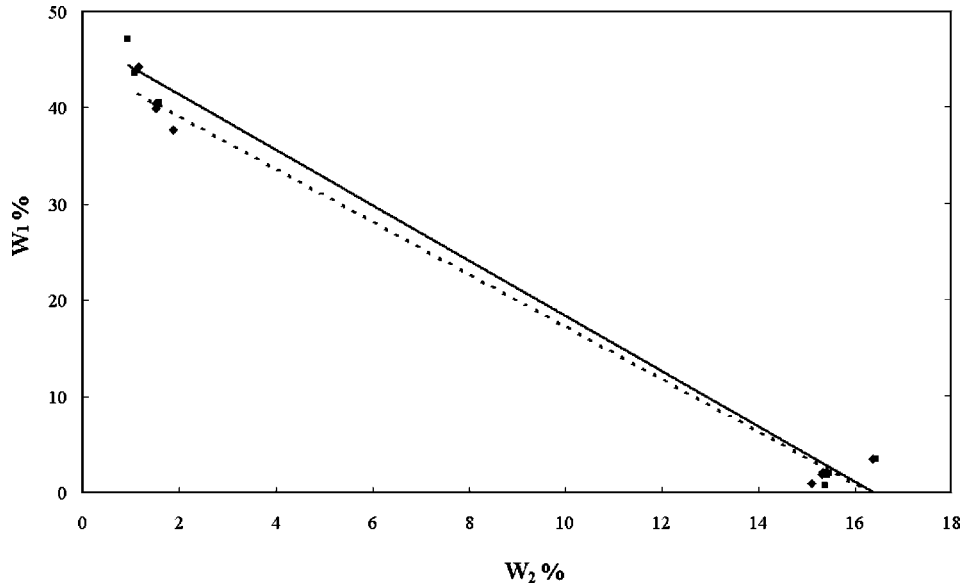


**Figure 4.** Effect of temperature on two-phase separation in PEG10000 (1) + $Na_2SO_4$ (2) aqueous two-phase systems: ◆, $T = 301.65$ K; ■, $T = 305.65$ K; −, binodal curves.

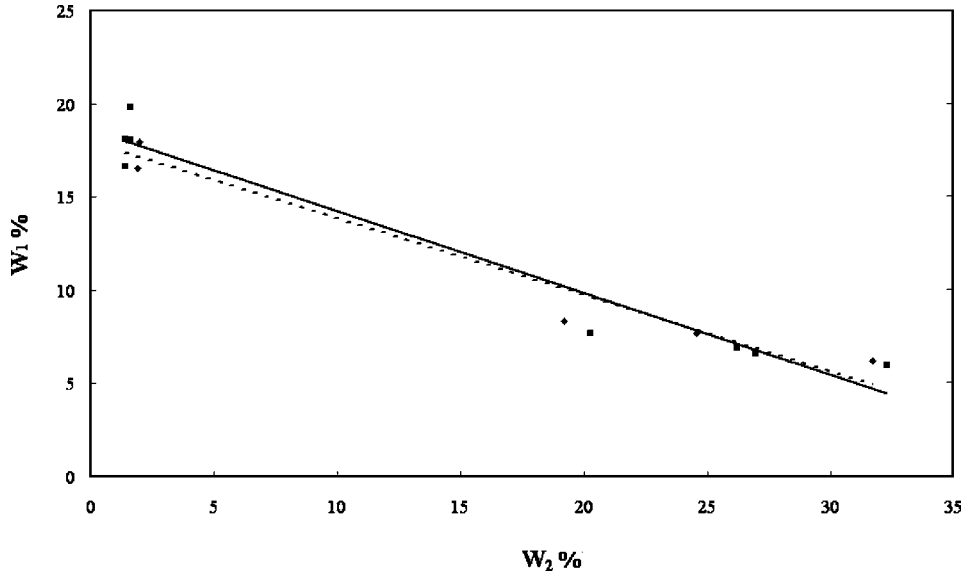


**Figure 5.** Effect of temperature on two-phase separation in PEG10000 (1) + $KH_2PO_4$ (2) aqueous two-phase systems: ◆, $T = 301.65$ K; ■, $T = 304.65$ K; −, binodal curves.

## Results and Discussion

***Experimental.*** The experimental results for partition coefficients of $\beta$-amylase and the mass fractions of salt, polymer, and enzyme in the top and bottom phases of the system of PEG + $KH_2PO_4$ + water at temperatures $T = (301.65$ and $304.65)$ K are presented in Table 3. As observed, the mass fraction of $\beta$-amylase in the bottom phase, i.e., salt-rich phase, is more than that of $\beta$-amylase in the top phase or polymer-rich phase. Therefore, the partition coefficient of $\beta$-amylase is less than unity in such systems.

Also, the experimental results for partition coefficients of amyloglucosidase with certain mass fractions of salt, polymer, and enzyme in the top and bottom phases in the systems of PEG + $Na_2SO_4$ + water at temperatures $T = (301.65,305.65)$ K are reported in Table 4. Going through Table 4, it can be found that amyloglucosidase tends toward the top phase which is the polymer-rich phase, and thus, the partition coefficients are greater than unity. Such an observation may occur because the net charge of the biomolecules is changed in the presence of salt in the ATPS. Therefore, the enzyme molecule with positive charge prefers to stay in the salt-rich phase, bottom phase, and conversely the enzyme molecule with negative charge prefers to stay in the polymer-rich phase, i.e., the top phase.[11]

Also, from the experimental results reported in Tables 3 and 4, it could be inferred that the mass fractions of compounds in feed have a significant effect on the partitioning of $\beta$-amylase and amyloglucosidase. Hence, it can be concluded that increasing the molar mass of PEG can decrease the partition coefficients of $\beta$-amylase and amyloglucosidase. The reason for such a decrease in partition coefficients would be attributed to two different factors such as increasing hydrophobicity of the PEG-rich phase and decreasing free volume available for enzyme molecules in phase.[9]

The phase diagrams of the PEG4000 + $Na_2SO_4$ + water system at 305.65 K are shown in Figure 1. Figures 2 and 3 show the effect of molar mass of PEG on the separation of aqueous two-phase systems containing PEG + $Na_2SO_4$/$KH_2PO_4$

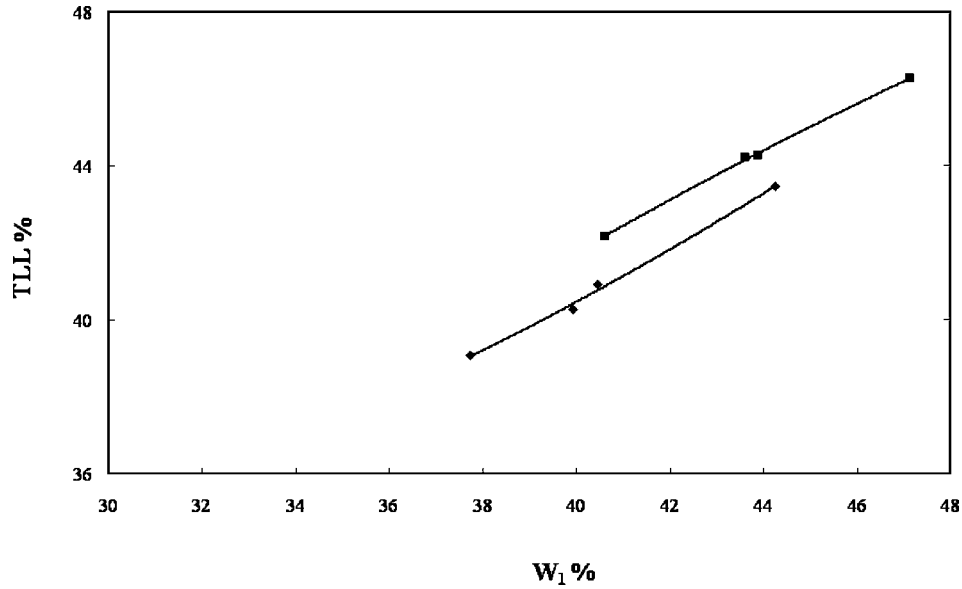


**Figure 6.** Effect of temperature on the tie line length (TLL %) in PEG10000 (1) + $Na_2SO_4$ (2): ◆, $T = 301.65$ K; ■, $T = 305.65$ K.

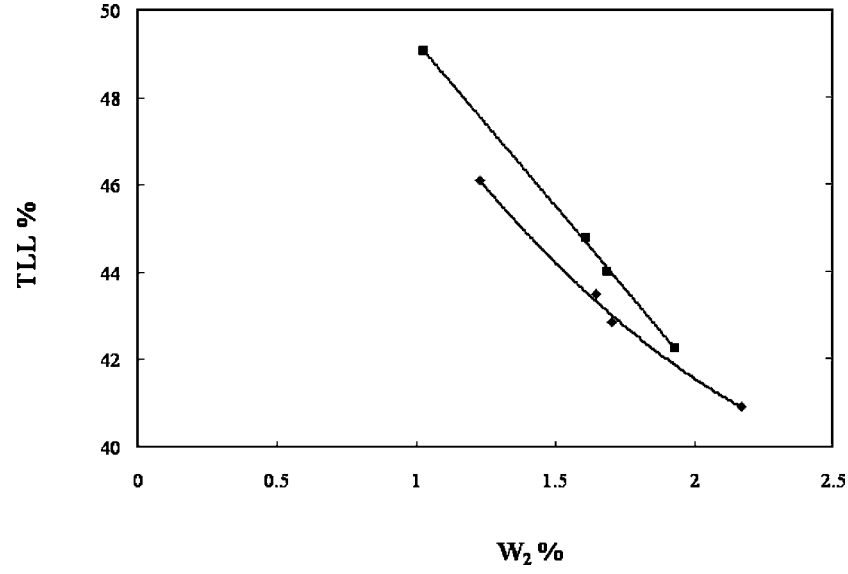


**Figure 7.** Effect of temperature on the tie line length (TLL %) in PEG4000 (1) + $Na_2SO_4$ (2): ◆, $T = 301.65$ K; ■, $T = 305.65$ K.

+ water. As observed by increasing the PEG molar mass, the binodal curves shift toward a lower mass fraction of PEG and salt.

Figures 4 and 5 indicate the effect of temperature on the binodal curves for the ATPS of PEG + $Na_2SO_4$ or $KH_2PO_4$ + water. As shown in Figures 4 and 5, the slope of equilibrium tie lines increases with an increase in temperature. It is worthwhile to mention that this behavior has been observed previously by Voros et al. for similar aqueous two-phase systems.[12] Also, it should be stated that temperature can change the mass fractions of each component in the top and bottom phases. Therefore, the slope of the tie line can change with temperature.

Figure 6 gives the variations of the mass fraction of polymer in the top phase in connection to the variations in the tie line length. As seen, the mass fraction of polymer increases with an increase in temperature and, in turn, decreases in the bottom phase.[12]

Figure 7 shows the mass fraction of salt in the top phase on the basis of tie line length. As observed, the mass fraction of salt decreases in both the top and bottom phases as temperature increases.[12]

Figures 8 and 9 show the variation of partition coefficients versus the tie line length (TLL). It should be noted that the tie line length can be given according to the following relation

$$\mathrm{TLL} = \sqrt{(W_1^{\mathrm{top}} - W_1^{\mathrm{bottom}})^2 + (W_2^{\mathrm{top}} - W_2^{\mathrm{bottom}})^2} \quad (5)$$

where subscripts 1 and 2 refer to polymer and salt, respectively. As observed from these figures by increasing the tie line length, the partition coefficient increases. With increasing tie line length, the two-phase region widens such that the recovery of enzyme could increase.

***Modeling.*** In this research, the experimental data of partition coefficients of $\beta$-amylase and amyloglucosidase were correlated by using eq 3. Parameters $A$ and $B$ in eq 3 were determined by

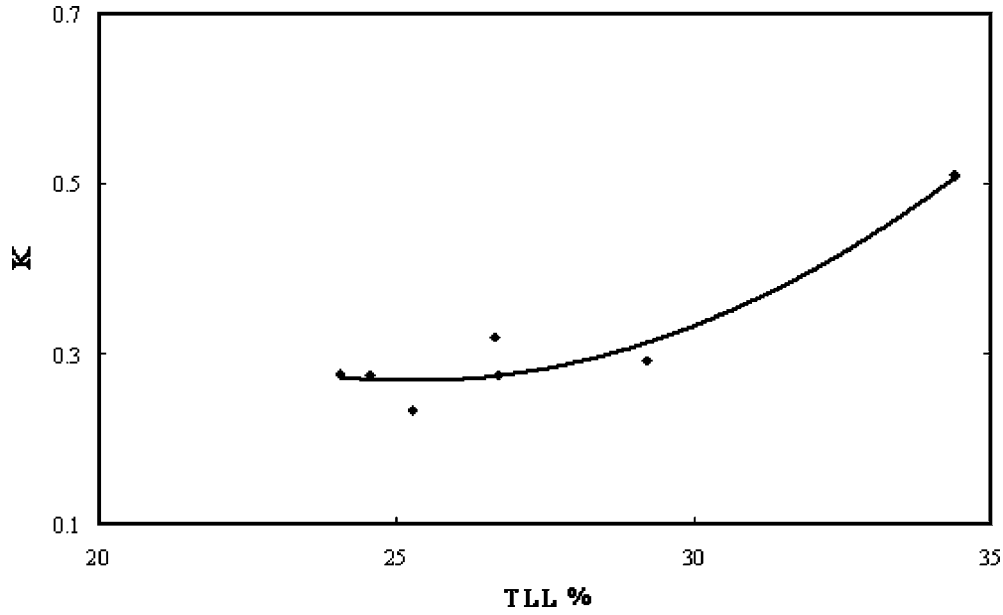


**Figure 8.** Partitioning of $\beta$-amylase in aqueous two-phase systems containing PEG10000 + $KH_2PO_4$ versus the tie line length.

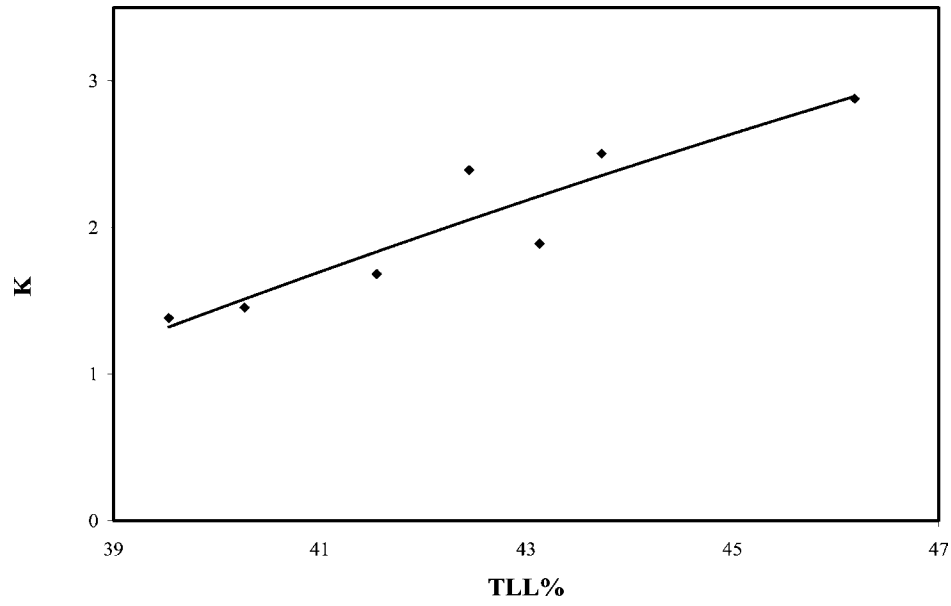


**Figure 9.** Partitioning of amyloglucosidase in aqueous two-phase systems containing PEG6000 + $Na_2SO_4$ versus the tie line length.

**Table 5. Values for the Parameter *A* and *B* Constants Introduced in Equation 3 Along with the rmsd of the Model from the Experimental Partition Coefficient Data**

| system | $A \pm 0.001$ | $B \pm 0.001$ | rmsd |
|---|---|---|---|
| PEG4000 + $Na_2SO_4$ | 1.494 | 1.131 | 0.299 |
| PEG6000 + $Na_2SO_4$ | −11.929 | 33.817 | 0.109 |
| PEG10000 + $Na_2SO_4$ | −2.101 | 7.427 | 0.196 |
| PEG6000 + $KH_2PO_4$ | −16.461 | 46.777 | 0.024 |
| PEG10000 + $KH_2PO_4$ | −18.246 | 51.038 | 0.009 |

using experimental data of partition coefficients. The values of parameters *A* and *B* and root-mean-square deviation were reported in Table 5. The results of Table 5 show that the proposed model can correlate the partition coefficients of $\beta$-amylase and amyloglucosidase enzymes with good accuracy.

## Conclusions

In this work, partitioning of $\beta$-amylase and amyloglucosidase was studied in polymer−salt aqueous two -phase systems containing PEG with different molar mass in the presence of $Na_2SO_4$ or $KH_2PO_4$ at various temperatures using an equilibrium cell equipped with a temperature control system. The experimental results showed that the partition coefficients of $\beta$-amylase and amyloglucosidase decrease by increasing the molar mass of PEG. Also, the type of salt and mass fraction of salt in initial feed have a significant effect on partitioning of the enzyme. Also, the partition coefficients of $\beta$-amylase and amyloglucosidase increase by increasing the length of the tie line. The experimental data collected in this work were modeled using the equation proposed by Diamond and Hsu. The results showed that the model can correlate the partition coefficients of $\beta$-amylase and amyloglucosidase with good accuracy.

## Literature Cited

(1) Smith, A. *Oxford dictionary of biochemistry and molecular biology*; Oxford University press: New York, 1997.
(2) Walter, H.; Brook, D. E.; Fisher, D. *Partitioning in aqueous two phase systems: Theory, Method, Uses and Applications to Biotechnology*; Academic Press: London, 1985.
(3) Mattiasson, B. Applications of aqueous two phase systems in biotechnology. *Trends Biotechnol.* **1983**, *1*, 16–20.

(4) Beijernick, M. W. Qualitative und quantitative microbiologische analyse. *Zentralbl. Bakteriol. Parasitenkd.* **1896**, *2*, 627–698.
(5) Albertson, P. A. *Partition of cell particles and macro molecules*; Wiley-Interscience: New York, 1986.
(6) Furuya, T.; Iwai, Y.; Tanaka, Y.; Uchida, H.; Yamada, S.; Arai, Y. Measurement and correlation of partition coefficients of hydrolytic enzymes for dextran + poly ethylene glycol + water aqueous two-phase systems at 20 °C. *Fluid Phase Equilib.* **1995**, *110*, 115–128.
(7) Mokhtarani, B.; Karimzadeh, R.; Amini, M. H.; Darvish Manesh, S. Partitioning of Ciprofloxacin in aqueous two phase system of poly ethylene glycol and sodium sulphate. *Biochem. Eng. J.* **2008**, *38*, 241–247.
(8) Pazuki, G. R.; Taghikhani, V.; Vossoughi, M. Partitioning of penicillin G-asylase in aqueous two-phase systems of poly ethylene glycol 20000, 35000 and potassium di-hydrogen phosphate, sodium citrate. *J. Chem. Eng. Data* **2010**, *55*, 243.
(9) Shahriari, Sh.; Taghikhani, V.; Vossoughi, M.; Safekordi, A. A.; Alemzadeh, I.; Pazuki, G. R. Measurement of partition coefficients of $\beta$-amylase and amyloglucosidase enzymes in aqueous two-phase systems containing poly ethylene glycol and $Na_2SO_4/KH_2PO_4$ at different temperatures. *Fluid Phase Equilib.* **2010**, *292*, 80–86.
(10) Diamond, A. D.; Hsu, J. T. Fundamental studies of biomolecule partitioning in aqueous two-phase systems. *Biotechnol. Bioeng.* **1989**, *34*, 1000–1014.
(11) Haghtalab, A.; Mokhtarani, B.; Maurer, G. Experimental results and Thermodynamic modeling of the partitioning of lysozyme, Bovin serum albumin, and α-Amylase in Aqueouse two-phase systems of PEG and ($K_2HPO_4$ or $Na_2SO_4$). *J. Chem. Eng. Data* **2003**, *48*, 1170–1177.
(12) Voros, N.; Proust, P.; Fredenslund, A. Liquid-Liquid phase equilibria of aqueous two-phase systems containing salts and poly ethylene glycol. *Fluid Phase Equilib.* **1993**, *90*, 333–353.

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