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ABSTRACT

To predict partitioning of proteins between a temperature- and pH-sensitive hydrogel and its surrounding aqueous solution, it is appropriate to use Schnitzer's method for determining solute partitioning based on size exclusion and the cell model for polyelectrolyte solutions. The mean-pore size of the hydrogel is calculated using the method of Peppas et al. The charge of the network, which depends on pH and temperature, is calculated using weak-polyelectrolyte-titration theory. De Gennes' scaling relation and Odijk's wormlike model are used to determine the persistence length and the radius of gyration of the polyelectrolyte chain in the hydrogel. For N-isopropylacrylamide gels copolymerized with sodium acrylate or 2-dimethylaminoethyl methacrylate, predicted partition coefficients for cytochrome-C are in semiquantitative agreement with experiment.

(Keywords: temperature- and pH-sensitive hydrogel; polyelectrolyte; phase equilibrium)

INTRODUCTION

Much attention has been directed in recent years toward hydrogels which undergo large volume changes in response to small variations in solution conditions such as pH or

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temperature[1]. These pH- and temperature-sensitive hydrogels have been suggested for a variety of applications including controlled drug delivery[2], immobilized enzyme reactors[3], and separation of an aqueous proteins[4]. For these applications, it is useful to consider the thermodynamics of solutes partitioning between a hydrogel and its surrounding solution.

If there are no specific interactions between solutes and the polymer matrix, a size-exclusion model can be used to predict the distribution of a solute between the gel and its surrounding bath[5-6]. However, this method is insufficient for partitioning of proteins and other biomolecules between a polyelectrolyte gel and an aqueous solution. Recently, a molecular-thermodynamic method was developed to predict semi-quantitatively partitioning of proteins and small ions into a charged hydrogel[7-8]. This method couples a size-exclusion model with a cell model for polyelectrolyte solutions[9-10]. In this paper, we extend the previous method to analyze partition behavior of cytochrome-C into two types of weak-ionizable temperature-sensitive N-isopropylacrylamide (NIPA) hydrogels: a negatively-ionizable hydrogel synthesized from NIPA and sodium acrylate (SA); and a positively-ionizable hydrogel synthesized from NIPA and 2-dimethylaminoethyl methacrylate (DMA). The degree of ionization of the network is determined using polyelectrolyte titration theory. The size of a polyelectrolyte chain inside a hydrogel is found from the scaling theory of de Gennes[11] and from the wormlike model for polyelectrolyte solutions[12-13]. To determine the charge and pore size of the network, we assume that for the low degree of cross-linking considered here, the titration behavior and the configurational properties of the cross-linked polyelectrolyte chain are the same as those of a single chain in a solution.

PARTITION COEFFICIENT AND PHASE RQUILBRIUM

For a protein which distributes between a hydrogel and the surrounding solution, often called the bath, the equilibrium partition coefficient, K, is defined as

$$K = \frac{C_g}{C_b} \tag{1}$$

where C_g is the protein concentration in the hydrogel (based on swollen gel volume) and C_b is the protein concentration in the bath.

We assume that there are two independent additive contributions to $\log K$. Size-exclusion contribution, K_{se}, takes into account the influence of excluded volume of hydrogel and protein. Electrostatic interaction contribution K_0 takes into account electrostatic interactions between charged protein and charged polyelectrolyte hydrogel; it is calculated by the equilibrium distribution of the protein between the bath and a non-cross-linked polyelectrolyte solution which has the same concentration and composition as those of the hydrogel. The overall protein partition coefficient is

$$K = K_{se} K_0 \tag{2}$$

For the size-exclusion contribution, we use Schnitzer's uniform-pore model[5]:

$$K_{se} = (1-\phi_p)(1-\frac{r_s}{r_c})^2 \qquad r_s \le r_c$$

$$K_{se} = 0 \qquad r_s > r_c \tag{3}$$

where ϕ_p is the volume fraction of polymer in the gel, r_c is the mean pore radius of the hydrogel, and r_s is the radius of the protein solute. Later, we describe calculation of the mean pore radius of the hydrogel.

K_0 can be written as

$$K_0 = \frac{C_g^{'}}{C_b} \tag{4}$$

where $C_g^{'}$ is the concentration of protein in the non-cross-linked polyelectrolyte solution; $C_g^{'}$ differs from C_g, the equilibrium protein concentration inside the hydrogel, because $C_g^{'}$ does not consider size-exclusion. When the non-cross-linked polyelectrolyte solution is in equilibrium with the bath, the chemical potential of protein salt (i.e. protein ion coupled with its counterion) must be the same in each phase.

$$\mu^{pe} = \mu^{bath} \tag{5}$$

where μ^{pe} is the chemical potential of the protein salt in the polyelectrolyte solution and μ^{bath} is the chemical potential of the protein salt in the bath. In the calculation of μ^{pe}, we assume that the solution containing buffer and salt is equivalent to a simple monovalent salt solution.

At constant temperature, the chemical potential of the protein salt in the non-cross-linked polyelectrolyte solution is related to concentration $C_g^{'}$ by

$$\mu^{pe} = \mu^0(P+\Pi) + RT\ln C_g^{'}\gamma_{\pm} \tag{6}$$

where $\mu^0(P+\Pi)$ is the chemical potential of the protein salt in a hypothetical ideal dilute solution when $C_g^{'} = 1M$ at system temperature T and pressure $P+\Pi$; P is the system pressure and Π is the elastic pressure of the hydrogel; R is the gas constant. The mean ionic activity coefficient of protein salt is designated by $\gamma_{\pm}$.

The elastic pressure of the hydrogel is calculated using the phantom model of gel elasticity[14]

$$\Pi = -\frac{1}{2}(\frac{\psi}{V_0})RT\left(\frac{\phi_2}{\phi_0}\right)^{1/3} \tag{7}$$

where V_0 is the volume of hydrogel at preparation; ψ is the moles of chains in the gel; ϕ_2 is the volume fraction of polymer in the swollen gel and ϕ_0 is the volume fraction of polymer at preparation.

The mean activity coefficient of the protein salt is defined by

$$\gamma_{\pm} = (\gamma_p^{z_c}\gamma_c^{z_p})^{1/(z_p+z_c)} \tag{8}$$

where γ_p is the activity coefficient of charged protein; γ_c is the activity coefficient of its counterion; z_p is the number of charges on one protein molecule; z_c is the number of charges on one counterion, here equal to unity. Activity coefficients γ_p and γ_c are calculated using the cell model of Gueron and Weisbuch[15]

$$\gamma_{counterion} = \frac{0.7X/(\xi z_{cn})+1}{X+1} \tag{9}$$

$$\gamma_{coion} = \frac{0.7X/(\xi z_{co})+1}{0.53X/(\xi z_{co})+1} \tag{10}$$

where $X = C_m / C_s$, the concentration of polymer (as monomer) divided by the salt concentration in the hydrogel; $\xi = l_B / b$, where b is the length of one charged monomer, and l_B is the Bjerrum length, defined by

$$l_B = e^2/(4\pi\varepsilon_0 DkT) \tag{11}$$

where e is electron charge; ε_0 is the permittivity of free space; k is Boltzmann's constant; D is the dielectric constant of water; z_{cn} and z_{co} are the valences of counterion and coion. Here counterion means the ion which has a charge opposite to that of the hydrogel; coion means the ion which has a charge identical to that of the hydrogel. Therefore, when the charge of a protein is opposite to that of the hydrogel, Eq.(9) is used to calculate γ_p; otherwise, Eq.(10) is used.

In the cell model of Gueron and Weisbuch[15], every monomer of polyelectrolyte has a fixed charge; the distance between nearest fixed charges is equal to the monomer length. For the polymer chain inside a weakly-ionizable hydrogel, only some of the monomers in the polymer chain are charged. We assume that the existence of neutral monomers does not affect the distribution of mobile ions around the fixed charges and that this distribution is described by the cell model with a characteristic distance equal to the monomer length (i.e., each charged monomer defines a unit cell). Therefore, we use monomer length b to calculate ξ in Eqs (9) and (10).

In Eq(9) and Eq (10), interactions between mobile ions are neglected. For consistency. these interactions are also neglected in the bath. Therefore, the bath becomes an ideal solution: and μ^{bath} is given by

$$\mu^{bath} = \mu^0(P,T) + RT \ln C_b \tag{12}$$

$\mu^0(P,T)$ is the chemical potential of the protein salt in a hypothetical ideal dilution solution when $C_b = 1M$ at system temperature T and pressure P.

From thermodynamics

$$\mu^0(P+\Pi,T) - \mu^0(P,T) = \Pi\overline{V} \tag{13}$$

where $\overline{V}$ is the partial molar volume of protein salt assumed to be independent of pressure. With Equations (5)-(13), we calculate K_0, the equilibrium distribution of the protein between the bath and a non-cross-linked polyelectrolyte solution.

CHARACTERIZATION OF THE HYDROGEL

To calculate the protein partition coefficient, we must determine two fundamental parameters of the hydrogel: the concentration of charges and the mean pore size. We now discuss calculation of these parameters.

Degree of ionization of a weak-ionizable hydrogel

Unlike simple weak acids or bases, where the dissociation constants depend only on temperature, the dissociation of ionizable monomers in a hydrogel is also a function of interactions between fixed charges. Here we assume that the titration behavior of a hydrogel is the same as that of a polyelectrolyte chain in solution.

The dissociation constant of a polyelectrolyte is a function of the degree of ionization and the ionic strength of solution. The negative logarithm of an apparent dissociation constant pK_{app} is used to describe the polyelectrolyte titration behavior. For a polyacid, it is defined as

$$pK_{app} = pH + \log\frac{1-\alpha}{\alpha} \tag{14}$$

Similarly, the apparent dissociation constant for a polybase is defined as

$$pK_{app} = pH + log\frac{\beta}{1-\beta} \tag{15}$$

where α, β are degree of ionization for a polyacid and a polybase, respectively.

Two groups of analytic theories are available to calculate pK_{app}. One group uses Manning's counterion condensation model[16] and its later development[17-18]. The other group uses models obtained from solution to the Poisson-Boltzmann (PB) equation[19]. Because no analytical solution to the PB equation is available, we use Manning's method.

For $\alpha \geq \bar{\xi}^{-1}$

$$pK_{app} = pK_0 + 0.434 - \log A^2 + 2\log(\alpha\bar{\xi}) - 0.434(\alpha\bar{\xi})^{-1} - \log C_s \tag{16}$$

where $\bar{\xi} = l_B/(\alpha b)$; A is a constant defined by

$$A^2 = (8\pi)\times 10^3 N_{av} {l_B}^3 \tag{17}$$

where N_{av} is the Avogadro constant.

For $\alpha < \bar{\xi}^{-1}$

$$pK_{app} = pK_0 - 0.434\{2\alpha\bar{\xi}\ln\left[1-\exp(-A\alpha^{-1}\bar{\xi}^{-1}C_s)\right]$$

$$-AC_s^{1/2}\left[\exp(A\alpha^{-1}\bar{\xi}^{-1}C_s^{1/2})-1\right]^{-1}\} \quad (18)$$

We assume that for a weakly ionizable gel, the dependence of the degree of ionization on pH can be related to the effect of pH on its swelling behavior. For a weakly-acidic polyelectrolyte hydrogel at low pH, the ionizable groups in the hydrogel remain neutral, and the swelling ratio of hydrogel is small; however, at high pH, where all ionizable groups dissociate, the swelling ratio of the hydrogel is large because of repulsive interactions between the charged groups. When the hydrogel is half swollen, we postulate that half of the ionizable groups are charged. The pH at this mid-point is taken as the intrinsic dissociation constant pK_0. *Figure 1* shows the dependence of the degree of ionization on solution pH for a weakly-acidic NIPA/SA gel and for a weakly-basic NIPA/DMA gel at 22.2 and 36.4 ^{o}C.

Mean pore radius of a hydrogel

According to Peppas et al[20], the mean pore radius of a gel is given by

$$r_c = \frac{1}{2}\phi_p^{-1/3} < R_{ee}^2 >^{1/2} \quad (19)$$

where $< R_{ee}^2 >$, the mean-square end-to-end distance of a polymer chain, is calculated using Yamakawa's formula[21]

$$< R_{ee}^2 >= 2p^2(\frac{l}{p} - 1 + e^{-l/p}) \quad (20)$$

where p is persistence length, and l is contour length

$$l = Nb \quad (21)$$

where N is the number of monomers per polymer, and b is the length of one monomer.

The persistence length of a polyelectrolyte chain in solution can be calculated using the wormlike model of Fixman[12] or Odijk[13]. Because the former model neglects excluded-volume effects, it is more suitable for a stiff polyelectrolyte chain. We prefer Odijk's model that takes into account excluded volume effects; the persistence length of a polyelectrolyte chain is given by

$$p = p_0 + \frac{1}{12} l_B N_c^2 h(\kappa l) \quad (22)$$

where p_0 is the intrinsic persistence length of the polyelectrolyte; N_c is the number of charges per polymer chain; κ is the reciprocal Debye screening length defined by

$$\kappa^2 = l_B \sum_i \rho_i z_i^2 \tag{23}$$

where ρ_i is the number density of mobile ion i in solution; z_i is the number of charges on ion i. Function h is given by

$$h(y) = e^{-y}(e^{-y} + 5y^{-2} + 8y^{-3}) + 3y^{-2} - 8y^{-3} \tag{24}$$

We assume that p_0 is equal to the persistence length of an aqueous neutral NIPA polymer which has the same number of monomers. The persistence length of a neutral polymer in solution can be related to its mean-square radius of gyration by[21]

$$< S^2 >^{1/2} = \frac{lp_0}{3} - p_0^2 + 2p_o^3 / l - 2p_0^4 l^{-2}\left[1 - \exp(-l / p_0)\right] \tag{25}$$

Therefore, we can obtain p_0 if we know $< S^2 >$ and l. To estimate $< S^2 >$, we use light-scattering data for the mean-square radius for gyration of NIPA polymer in an aqueous solution[22]. The dependence of the mean-square radius of gyration on the number of monomers can be obtained using the scaling law of de Gennes[11]. For temperatures below the polymer collapse temperature, (36.4 °C for NIPA polymer), the root-mean-square radius of gyration scales as

$$< S^2 >^{1/2} \sim N_0^{0.6} \tag{26}$$

At the collapse temperature,

$$< S^2 >^{1/2} \sim N_0^{0.5} \tag{27}$$

For temperatures higher than the collapse temperature

$$< S^2 >^{1/2} \sim N_0^{1/3} \tag{28}$$

From Eq.(21), we find l when we assume that the length of a monomer is 2.52 Å, which corresponds to the distance between alternate carbons in a polymer chain. With Eqs (19)-(28). we can calculate the mean-pore radius of a polyelectrolyte hydrogel. Table 1 presents initial parameters for these gels of interest here. Table 2 presents concentration of fixed charges in the gels and Table 3 gives mean-pore radii as a function of pH.

RESULTS AND DISCUSSION

Figure 2 shows predicted and experimental[23] partition coefficients as a function of pH for cytochrome-C in a NIPA/SA gel. Swelling-ratio data for these gels at different temperature and pH are obtained from reference 23. For comparison, the result calculated by ideal Donnan equilibrium is also shown; in that calculation, the mean ionic activity coefficient of the protein salt is equal to unity. The partition coefficients calculated by the cell model approximate the experimental data; however, the ideal Donnan equilibrium fails at high pH because it neglects interactions between the protein and fixed charges, and because the charge density of the hydrogel increases with pH. Figure 3 shows cytochrome-C partitioning when the gel collapses. Both figures show that the predicted results fall below those measured, probably because the calculations neglect adsorption of protein on the gel surface.

Figure 4 shows partitioning of cytochrome-C in a NIPA/DMA gel. In the range of pH considered, both the protein and the hydrogel are positively charged. We expected a very low partition coefficient of protein in this gel. The predicted results confirm our expectation. However, the experimental partition coefficients are larger than expected. The observed partition behavior is unexpectedly confirmed with ideal Donnan equilibrium. Figure 5 also shows that results from ideal Donnan equilibrium are closer to experiment than those from the cell model. perhaps because repulsive electrostatic interactions between protein and network compensate for effects of adsorption. The hydrogel in Figure 5 is in a collapsed state. When pH rises, the charge density inside the gel decreases and the gel collapses further. In the collapsed state. adsorption of protein on the surface become more important. Therefore, predicted results from both methods are smaller than those from experiment.

Figure 6 shows partitioning of cytochrome-C into swollen and collapsed states of neutral NIPA gel. Only size exclusion and elastic pressure are taken into account here. The partition coefficients are independent of solution pH; predicted results follow those from experiment except at pH 8.0 at 36.4 C, perhaps because of partial denaturation of the protein at these conditions.

CONCLUSIONS

Calculation of cytochrome-C partitioning indicates that a cell model, combined with a size-exclusion model, can provide a first estimate of the partition coefficient. To improve prediction of solute partitioning between a charged hydrogel and the bath solution, we need an improved polyelectrolyte solution theory which can also take adsorption into account when the gel is not highly swollen.

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Table 1 Initial parameters for these gels

Gels	%T	%C	N	ϕ_0	ψ / V_0
NIPA/10%SA	15	1	49.5	0.125	0.027
NIPA/10%DMA	15	1	49.5	0.127	0.025
NIPA	15	1	49.5	0.125	0.026

%T=100×mass of all monomers (g) /volume of water (ml)

%C=mole percent of crosslinkers in initial monomer mixture

%SA(DMA)=mole percent of SA(DMA) in initial monomer mixture

Table 2 Concentration of fixed charges in swollen hydrogels (mol/l)

pH	5	6	7	8
NIPA/10%SA(22.2 °C)	0.002	0.012	0.027	0.035
NIPA/10%SA(36.4 °C)	0.047	0.048	0.053	0.159
NIPA/10%DMA(22.2 °C)	0.027	0.036	0.036	0.035
NIPA/10%DMA(36.4 °C)	0.074	0.082	0.095	0.099

Table 3 Mean-pore radius of swollen hydrogels (Å)

pH	5	6	7	8
NIPA/10%SA(22.2 °C)	59.4	63.	65.	65.3
NIPA/10%SA(36.4 °C)	21.6	43.8	49.0	49.8
NIPA/10%DMA(22.2 °C)	67.7	66.7	65.7	63.9
NIPA/10%DMA(36.4 °C)	43.0	41.4	36.9	26.8
NIPA(22.2 °C)	50.3	50.4	51.3	51.3
NIPA(36.4 °C)	25.3	24.8	25.1	25.3

Figure captions

Figure 1 Degree of ionization of weakly-ionizable monomers in a polyelectrolyte hydrogel vs. pH of aqueous solution. Salt concentration C_S=0.1 M. (1. SA at 22.2 °C; 2. SA at 36.4 °C; 3. DMA at 22.2 °C; 4. DMA at 36.4 °C.)

Figure 2 Partition coefficient of cytochrome-C between a weakly-ionizable hydrogel of NIPA/SA and an aqueous solution at 22.2 °C. Solution ionic strength I=0.1 M (a, ideal Donnan equilibrium; b, cell method; c, experimental data)

Figure 3 Partition coefficient of cytochrome-C between a weakly-ionizable hydrogel of NIPA/SA and an aqueous solution at 36.4 °C. Solution ionic strength is 0.1 M. (a, ideal Donnan equilibrium; b, cell method; c, experimental data)

Figure 4 Partition coefficient of cytochrome-C between a weakly-ionizable hydrogel of NIPA/DMA and an aqueous solution at 22.2 °C. Solution ionic strength is 0.1 M. (a, ideal Donnan equilibrium; b, cell method; c, experimental data)

Figure 5 Partition coefficient of cytochrome-C between a weakly-ionizable hydrogel of NIPA/DMA and an aqueous solution at 36.4 °C. Solution ionic strength is 0.1 M. (a, ideal Donnan equilibrium; b, cell method; c, experimental data)

Figure 6 Partition coefficient of cytochrome-C between a neutral NIPA hydrogel and an aqueous solution. Solution ionic strength is 0.1 M.

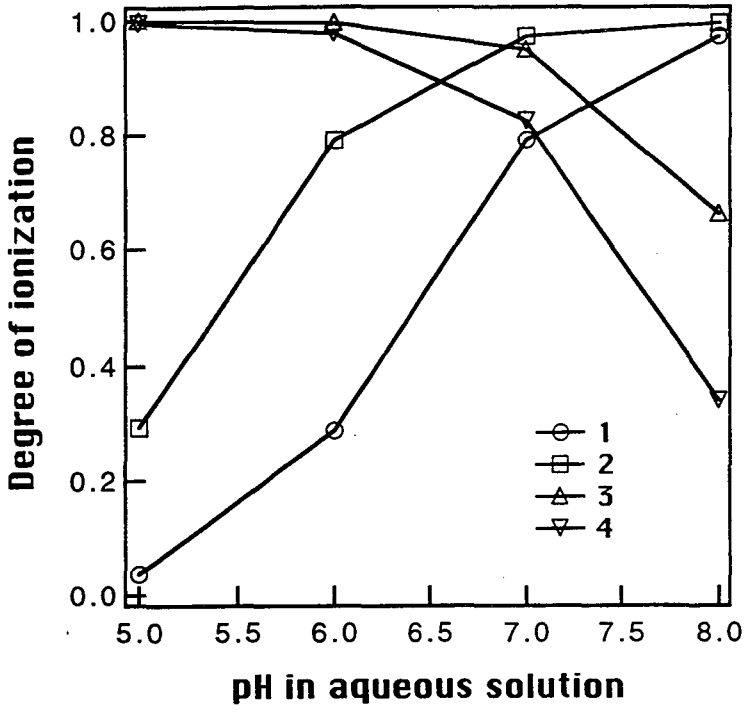

Figure 1

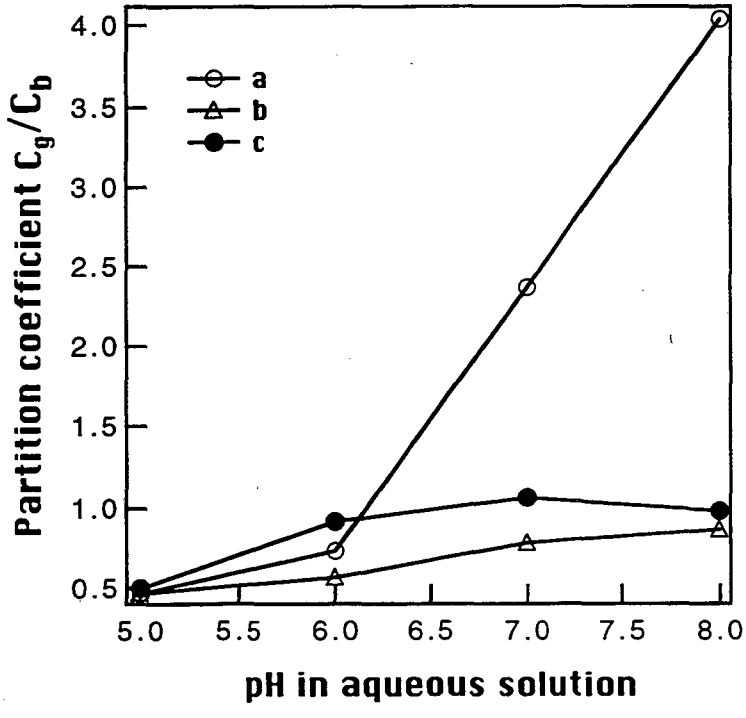

Figure 2

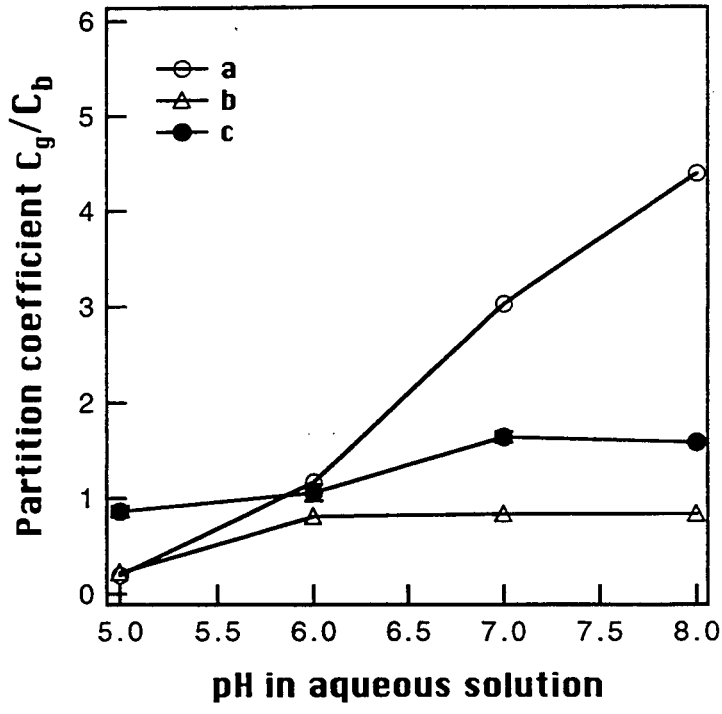

Figure 3

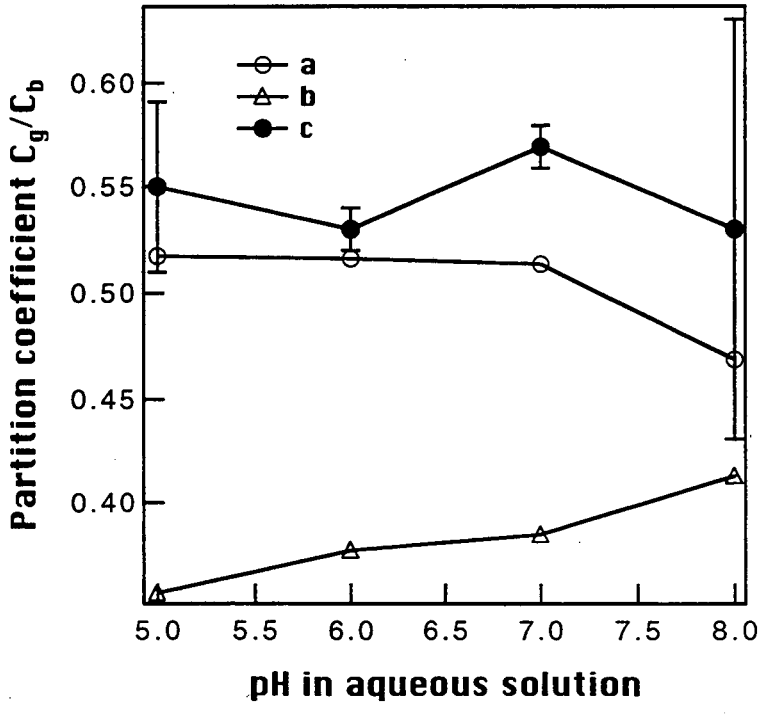

Figure 4

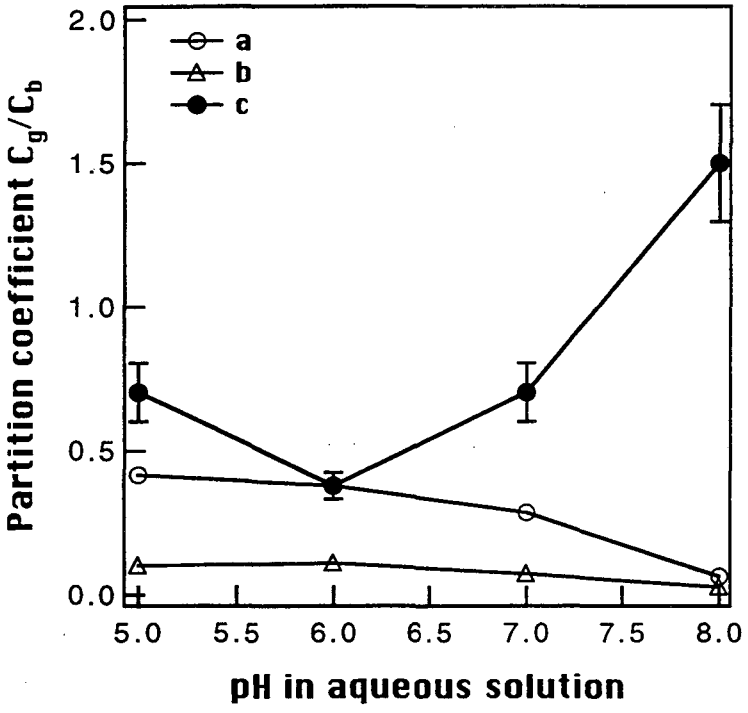

Figure 5

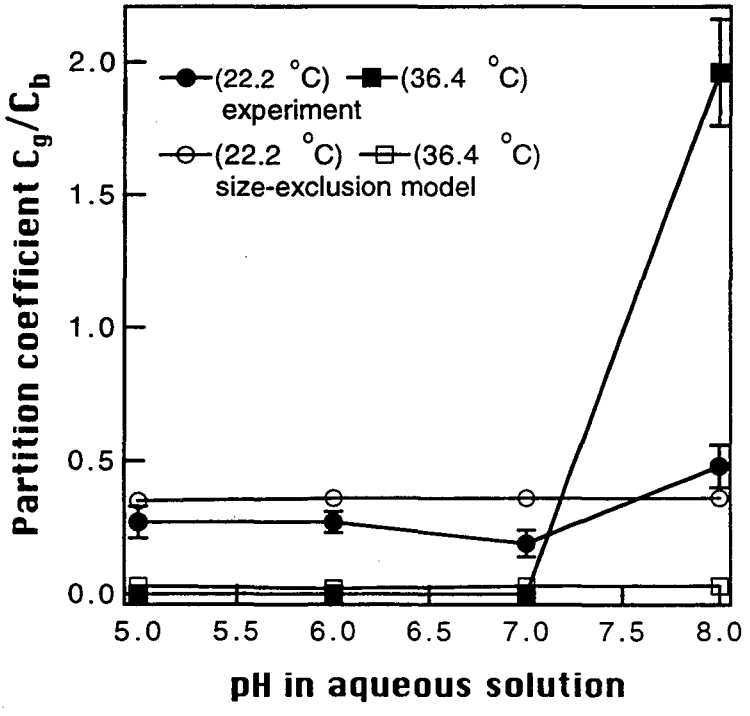

Figure 6

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