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EFFECT OF pH ON POLYELECTROLYTES IN ADSORPTION CHROMATO-GRAPHY

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SUMMARY

An ionization model of polyelectrolytes in a mobile phase and in a stationary phase was used, together with step-input adsorption chromatography, in which longitudinal dispersion in the mobile phase, radial dispersion inside the porous spherical bed packing and sorption on the internal surface of the spherical bed packing are simultaneously taking place, for the analysis of the effect of pH. The variations of the effluent time-of-breakthrough curve of this coupled model were studied numerically for six typical cases of variation of pK values in each phase. It was found that the effluent time (the retention time) is related to the pK values in the mobile phase. A guide for choosing appropriate pH and pK values for the chromatography is suggested.

INTRODUCTION

Adsorption chromatography has proved to be an effective purification and isolation procedure for many enzymes, proteins and other natural and synthetic polymers[1-7]. It is a liquid–solid specific adsorption chromatographic method, primarily according to selective specific adsorbents. Factors responsible for the effect of pH on adsorption chromatography can be divided into two categories: those which influence the stability of solute materials in solution and those which influence the specific adsorption on the bed packing.

It is known that factors which influence the stability of an enzyme or a protein are those which affect the secondary, tertiary and/or quaternary structures of proteins. For example, most enzymes undergo irreversible denaturation in very acidic and very alkaline solutions. The pH at which this occurs varies with solute materials. The effect of pH on the adsorption or on the specific binding activity actually means the effect of pH on the ionization of prototropic groups (groups capable of ionization) involved in the active site of a solute material. Those prototropic groups are generally
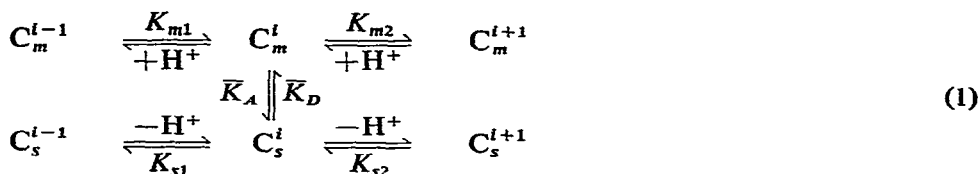
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located on the side-chains of the acidic and basic amino acid residues and may be involved (a) in maintaining the proper conformation of the active site, (b) in binding of solute material in particular polyampholytes or (c) in releasing solute material from the adsorbent for elution.

IONIZATION MODEL

In this paper, the following ionization model is used in the investigation of the pH effect on adsorption chromatography.

$$
\begin{array}{ccccc}
C_m^{i-1} & \underset{+\mathrm{H}^+}{\overset{K_{m1}}{\rightleftharpoons}} & C_m^i & \underset{+\mathrm{H}^+}{\overset{K_{m2}}{\rightleftharpoons}} & C_m^{i+1} \\[4pt]
 & & \overline{K}_A \Big\Updownarrow \overline{K}_D & & \\[4pt]
C_s^{i-1} & \underset{K_{s1}}{\overset{-\mathrm{H}^+}{\rightleftharpoons}} & C_s^i & \underset{K_{s2}}{\overset{-\mathrm{H}^+}{\rightleftharpoons}} & C_s^{i+1}
\end{array}
\tag{1}
$$

where the quantity C denotes the polyelectrolyte, subscripts m and s denote the mobile and stationary phase, respectively, and i is the number of negative charges. In this model, it is assumed that the solute C_m^i is adsorbed by a neutral ligand attached to the solid bed packing material to give C_s^i, and the ionizations between the hydronium ion and the solute in the mobile and stationary phases are so fast that equilibrium can be established instantaneously. The quantities $\overline{K}_A$ and $\overline{K}_D$ are the adsorption and the desorption rate constants between C_m^i and C_s^i, which control the overall reaction rates. The bar on $\overline{K}$ indicates that the values are independent of pH.

The equilibrium constant of the reaction in which the solute C_m^i adsorbed by a biospecific ligand A to form a complex A–C_m^i which is expressed as C_s^i is

$$
K_s = [C_s^i]/[A] \cdot [C_m^i]
\tag{2}
$$

where the square brackets [ ] represent concentrations.

The equilibrium constants for the ionization reaction in a mobile phase for the model given in eqn. 1 are

$$
K_{m1} = [C_m^i] \cdot [H^+]/[C_m^{i-1}]
\tag{3}
$$

$$
K_{m2} = [C_m^{i+1}] \cdot [H^+]/[C_m^i]
\tag{4}
$$

In a similar manner, the equilibrium constants for the ionization reaction in a stationary phase become

$$
K_{s1} = [C_s^i] \cdot [H^+]/[C_s^{i-1}]
\tag{5}
$$

$$
K_{s2} = [C_s^{i+1}] \cdot [H^+]/[C_s^i]
\tag{6}
$$

In a chromatographic operation, the equilibrium between an adsorption and a desorption process is based on the total concentrations in the mobile and the stationary phases:

$$[C_m]_T = [C_m^{i-1}] + [C_m^i] + [C_m^{i+1}] \tag{7}$$

$$[C_s]_T = [C_s^{i-1}] + [C_s^i] + [C_s^{i+1}] \tag{8}$$

Then, the equilibrium constant, K_e, based on the total concentrations is given by

$$K_e = [C_s]_T/[C_m]_T \tag{9}$$

On substitution of eqns. 3–6 into eqns. 7 and 8, the total concentration of each phase becomes

$$[C_m]_T = [C_m^i] \{1 + [H^+]/K_{m1} + K_{m2}/[H^+]\} \tag{10}$$

$$[C_s]_T = [C_s^i] \{1 + [H^+]/K_{s1} + K_{s2}/[H^+]\} \tag{11}$$

The equilibrium constant based on the total concentrations given by eqn. 9 becomes

$$K_e = K_s [A] \cdot \frac{1 + [H^+]/K_{s1} + K_{s2}/[H^+]}{1 + [H^+]/K_{m1} + K_{m2}/[H^+]} \tag{12}$$

In the derivation of eqn. 12, eqns. 10 and 11 together with eqn. 2 were substituted into eqn. 9.

The concentration of a ligand is much higher than that of the solute in the mobile phase. Therefore, in the analysis of chromatography, one can assume that [A] is a constant, so that $K_s[A] = \overline{K}_e = $ constant.

CHROMATOGRAPHIC MODEL

We consider an infinitely long column with a section uniformly filled with the porous spherical (radius R) bed-packing material which is a biospecific adsorbent (a ligand attached to the packing sphere) under isothermal conditions. The void volume fraction in the column is ε and the porosity of the packing material is ε_s. The velocity profile of the mobile phase is assumed to be a plug flow with an average carrier velocity V. The dispersion in the mobile phase is assumed to be in a longitudinal direction only and its dispersion coefficient, D, is also assumed to be a constant, independent of concentration. Then the solute is transferred into the stationary phase through the interphase layer by a mass transfer process with a constant mass transfer coefficient H_c. The solute dispersed further in the stationary phase into the interior of each spherical packing with a constant dispersion coefficient D_r. Finally, on those porous spherical packings, adsorption and desorption take place for the solute com-

ponent. The equations describing the above chromatographic process are the following mass balance equations:

$$\frac{\partial C_m}{\partial t} + V \frac{\partial C_m}{\partial z} - D \cdot \frac{\partial^2 C_m}{\partial z^2} = -H_c (K_c C_m - C_s |_{r=R}) \tag{13}$$

for the mobile phase and

$$\frac{\partial C_s}{\partial t} - D_r \left(\frac{\partial^2 C_s}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial C_s}{\partial r} \right) = -\frac{\partial n}{\partial t} \tag{14}$$

for the stationary phase. Assuming a finite rate of adsorption on the internal porous surface of a spherical bed packing with a linear isotherm, we have

$$\frac{\partial n}{\partial t} = k_A C_s - k_D n \tag{15}$$

for adsorption kinetics. All of the solute concentrations in the above equations are total concentrations, n is the concentration of solute adsorbed on the porous surface, K_c is the equilibrium constant between the concentration of solute outside and inside the particles at the interphase and k_A and k_D are the rate constants for adsorption and desorption, respectively. It is interesting to note that $K_D[C_s]_T = \overline{K}_D[C_s^i]$, which gives

$$K_D = \overline{K}_D \{ 1 + [H^+]/K_{s1} + K_{s2}/[H^+] \} \tag{16}$$

If a solute is introduced at the entrance of the column bed as a step function, the initial and boundary conditions are

$$C_m (z, T) = 0 \qquad \text{for } t \leqslant 0 \text{ and } z > 0 \tag{17}$$

$$C_s (r, z, t) = 0 \qquad \text{for } t \leqslant 0 \text{ and } z > 0 \tag{18}$$

$$n (r, z, t) = 0 \qquad \text{for } t \leqslant 0 \text{ and } z > 0 \tag{19}$$

$$C_m (z, t) = C_i \qquad \text{for } t \geqslant 0 \text{ and } z = 0 \tag{20}$$

$$C_m (z, t) = 0 \qquad \text{for } t \geqslant 0 \text{ and } z = \infty \tag{21}$$

$$\varepsilon H_c (K_c C_m - C_s |_{r=R}) = -\frac{3 (1 - \varepsilon)}{R} \cdot \varepsilon_s \cdot D_r \cdot \frac{\partial C_s}{\partial r} \Big|_{r=R} \quad \text{for } t > 0 \text{ and } r = R \tag{22}$$

$$\frac{\partial C_s}{\partial r} \Big|_{r=R} = 0 \text{ for } t > 0 \text{ and } r = 0 \tag{23}$$

The expression $3 (1 - \varepsilon) \cdot \varepsilon_s/R$ gives the surface area of a spherical particle per unit volume of the column. During the chromatographic operation, it is assumed that the

pH or the ionic strength throughout the bed is uniform. The binding of a solute with a ligand does not change the pH or the ionic strength. If one uses a suitable buffer solution in a system, this situation can be easily attained.

Solutions

Ideally, one would like to obtain an exact solution of $C_m(z, t)$. The set of differential equations subjecting to these initial and boundary conditions is difficult to solve analytically. However, the chromatographic peak can be completely characterized by the statistical moments[6-14]. Two approximate solutions were obtained by Chung and Hsu[9] using the method of moments together with the Gaussian and the Poisson expansions. The normalized solution at the exit of the column, $C^*(\tau)\big|_{z^*=1}$ by the Gaussian expansion is obtained as

$$C^*(\tau) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\tau} e^{-x^2/2}\, dx - \frac{\gamma_3}{3!}\, \psi^{(2)}(\tau) + \frac{\gamma_4}{4!}\, \psi^{(3)}(\tau) + \frac{\gamma_3^2}{2!\,3!}\, \psi^{(5)}(\tau) -$$

$$\frac{\gamma_5}{3!}\, \psi^{(4)}(\tau) - \frac{\gamma_3\,\gamma_4}{3!\,4!}\, \psi^{(6)}(\tau) - \frac{\gamma_3^3}{(3!)}\, \psi^{(8)}(\tau) + \frac{\gamma_6}{6!}\, \psi^{(5)}(\tau) +$$

$$+ \frac{\gamma_3\,\gamma_5}{3!\,5!} + \frac{\gamma_4^2}{2!\,(4!)}\, \psi^{(7)}(\tau) + \frac{\gamma_3\,\gamma_4}{2\,(3!)^2\,(4!)^2}\, \psi^{(9)}(\tau) + \frac{\gamma_3^4}{4!\,(3!)^4}\, \psi^{(11)}(\tau) + \dots$$

$$(24)$$

in which

$$\gamma_3 = \mu_3/\sigma^3 \tag{25a}$$

$$\gamma_4 = (\mu_4/\sigma^4) - 3 \tag{25b}$$

$$\gamma_5 = (\mu_5/\sigma^5) - 10\,\mu_3/\sigma^3 \tag{25c}$$

$$\gamma_6 = (\mu_6/\sigma^6) - (15\,\mu_4/\sigma^4) - 10\,(\mu_3/\sigma^3)^2 + 30 \tag{25d}$$

where $\sigma = \sqrt{\mu_2}$ is a standard deviation and μ_k are the central moments. $\psi(\tau)$ is the Gaussian probability density function with a mean of zero and a standard deviation of 1, that is

$$\psi(\tau) = \frac{1}{\sqrt{2\pi}} \cdot e^{-\tau^2/2} \tag{26}$$

$$\psi^{(m)}(\tau) = (-1)^m\, \psi(\tau)\, H_m(\tau) \tag{27}$$

where $H_m(\tau)$, the Hermite polynomial, is denoted in the following form:

$$H_m(\tau) = \sum_{j=0}^{M} \frac{(-1)^j\, m!\, \tau^{m-2j}}{j!\,(m-2j)!\,2^j} \tag{28}$$

in which $M = m/2$ when m is an even number and $M = (m - 1)/2$ when m is an odd number.

The normalized solution by the Poisson expansion is obtained as

$$C^* (\tau) = \sum_{x=0}^{\tau} \psi (x) + \frac{\mu_2 - a_1}{2!} \cdot \sum_{x=2}^{\tau} \psi_2 (x) + \frac{\mu_3 - 3\mu_2 + 2a_1}{3!} \sum_{x=3}^{\tau} \psi_3 (x) +$$

$$+ \frac{\mu_4 - 6\mu_3 + 11\mu_2 - 6\mu_2 a_1 + 3 (a_1^2 - 2a_1)}{4!} \cdot \sum_{x=4}^{\tau} \psi_4 (x) + \dots \quad (29)$$

in which the Poisson probability density function has a mean but no variance in its form which is given as

$$\psi (\tau) = \frac{a_1^\tau}{\tau!} e^{-a_1} \qquad (\tau = 0, 1, 2, \dots) \quad (30)$$

The successive differences of $\psi_m (\tau)$ is defined as follows with the relation $\psi_0 (\tau) = \psi (\tau)$:

$$\psi_m (\tau) = -\psi_{m-1} (\tau) + \psi_m (\tau - 1) \quad (m = 1, 2, 3, \dots) \quad (31)$$

The successive differences also have the following relation:

$$\psi_m (\tau) = \psi_0 (\tau) p_m (\tau) \quad (32)$$

where $p_m (\tau) = \sum_{\nu=0}^{m} (-1)^{m-\nu} \binom{m}{\nu} \nu! \binom{\tau}{\nu} a_1^{-\nu} \quad (33)$

By using the orthogonality relation; $\sum_{\tau=0}^{\infty} p_k (\tau) \psi_m (\tau) = 0$ for $m \neq k$, and

$\sum_{\tau=0}^{\infty} p_k (\tau) \psi_m (\tau) = \frac{m!}{a_1^m}$, for $m = K$, the various moments at the exit of the column,

$z^* = 1$, are calculated, which yield

$$a_1 = 1 + \varphi K_c (1 + K_e) \quad (34)$$

$$\mu_2 = \frac{2}{Pe_c} \cdot a_1^3 + \gamma_2 \quad (35)$$

$$\mu_3 = \frac{12}{Pe_c^2} \cdot a_1 + \frac{6}{Pe_c} \cdot a_1 \gamma_2 + \gamma_3 \quad (36)$$

$$\mu_4 = \left(\frac{120}{Pe_c^3} + \frac{12}{Pe_c^2}\right) a_1^4 + \left(\frac{72}{Pe_c^2} + \frac{12}{Pe_c}\right) a_1^2 \gamma_2 + \frac{8}{Pe_c} \cdot a_1 \gamma_3 +$$

$$+ \left(\frac{6}{Pe_c} + 3\right) \gamma_2^2 + \gamma_4 \qquad (37)$$

$$\mu_5 = \left(\frac{1680}{Pe_c^4} + \frac{240}{Pe_c^3}\right) a_1^5 + \left(\frac{1200}{Pe_c^3} + \frac{240}{Pe_c^2}\right) a_1^3 \gamma_2 + \left(\frac{120}{Pe_c^2} + \frac{20}{Pe_c}\right) a_1^2 \gamma_3 +$$

$$+ \left(\frac{180}{Pe_c^2} + \frac{60}{Pe_c}\right) a_1 \gamma_2^2 + \left(\frac{20}{Pe_c} + 10\right) \gamma_2 \gamma_3 + \frac{10}{Pe_c} a_1 \gamma_4 + \gamma_5 \qquad (38)$$

where

$$\varphi = (1 - \varepsilon) \, \varepsilon_s / \varepsilon \text{ (overall porosity in column)} \qquad (39)$$

$$K_e = k_A / k_D \text{ (adsorption equilibrium constant)} \qquad (40)$$

$$\gamma_2 = \frac{2\varphi^2 K_c (1 + K_e)^2}{H_c^*} + \frac{2\varphi K_c Pe_p (1 + K_e)^2}{15} + \frac{2 K_c K_e}{k_D^*} \qquad (41)$$

$$\gamma_3 = \frac{6\varphi^3 K_c (1 + K_e)^3}{H_c^{*2}} + \frac{4\varphi^2 K_c Pe_p (1 + K_e)^3}{5 H_c^*} + \frac{4\varphi K_c Pe_p^2 (1 + K_e)^3}{105} +$$

$$+ \frac{12\varphi^2 K_c K_e (1 + K_e)}{H_c^* k_D^*} + \frac{4\varphi K_c Pe_p K_e (1 + K_e)}{5 k_D^*} + \frac{6\varphi K_c K_e}{k_D^{*2}} \qquad (42)$$

$$\gamma_4 = \frac{24\varphi^4 K_c (1 + K_e)^4}{H_c^{*3}} + \frac{24\varphi^3 K_c Pe_p (1 + K_e)^4}{5 H_c^{*2}} + \frac{72\varphi^2 K_c Pe_p^2 (1 + K_e)^4}{175 H_c^*} +$$

$$+ \frac{8\varphi K_c Pe_p^3 (1 + K_e)^4}{525} + \frac{72\varphi^3 K_c K_e (1 + K_e)^2}{H_c^{*2} k_D^*} + \frac{48\varphi^2 K_c Pe_p K_e (1 + K_e)^2}{5 H_c^* k_D^*} +$$

$$+ \frac{16\varphi K_c Pe_p^2 K_e (1 + K_e)^2}{35 k_D^*} + \frac{24\varphi^2 K_c K_e (2 + 3K_e)}{H_c^* k_D^{*2}} + \frac{8\varphi K_c Pe_p K_e (2 + 3K_e)}{5 k_D^{*2}} +$$

$$+ \frac{24\varphi K_c K_e}{k_D^{*3}} \qquad (43)$$

$$
\gamma_S = \frac{120\varphi^5 K_c (1 + K_e)^5}{H_c^{*4}} + \frac{32\varphi^4 K_c Pe_p (1 + K_e)^5}{H_c^{*3}} + \frac{136\varphi^3 K_c Pe_p^2 (1 + K_e)^5}{35 H_c^{*2}} +
$$

$$
+ \frac{16\,^2K_c Pe_p^3 (1 + K_e)^5}{63 H_c^{*}} + \frac{16 K_c Pe_p^4 (1 + K_e)^5}{2079} + \frac{480\,^4K_c K_e (1 + K_e)^3}{H_c^{*3} k_D^{*}} +
$$

$$
+ \frac{96\varphi^3 K_c Pe_p K_e (1+K_e)^3}{H_c^{*2} k_D^{*}} + \frac{288\varphi^2 K_c Pe_p^2 K_e (1+K_e)^3}{105 k_D^{*}} + \frac{32\varphi K_c Pe_p^3 K_e (1+K_e)^3}{105 k_D^{*}} +
$$

$$
+ \frac{72\varphi^3 K_c K_e (1 + K_e)(7 + 13K_e)}{H_c^{*2} k_D^{*2}} + \frac{48\varphi^2 K_c Pe_p K_e (1 + K_e)(7 + 13K_e)}{5 H_c^{*} k_D^{*2}} +
$$

$$
+ \frac{16\varphi K_c Pe_p K_e (1 + K_e)(7 + 13K_e)}{35 k_D^{*2}} + \frac{48\varphi^2 K_c K_e (9 + 19K_e)}{H_c^{*} k_D^{*3}} +
$$

$$
+ \frac{16\varphi K_c Pe_p K_e (9 + 19K_e)}{5 k_D^{*3}} + \frac{120\varphi K_c K_e}{k_D^{*4}} \qquad (44)
$$

The various reduced variables in the above equations are defined as follows:

$$
C^* = \frac{C_m}{C_i}; \qquad C_s^* = \frac{C_s}{C_i}; \qquad n^* = \frac{n}{C_i} \qquad (45a,b,c)
$$

$$
r^* = \frac{r}{R}; \qquad z^* = z/L; \qquad \tau = t L/V \qquad (46a,b,c)
$$

$$
k_A^* = k_A L/V; \qquad k_D^* = k_D L/V; \qquad H_c^* = H_c L/V \qquad (47a,b,c)
$$

$$
Pe_c = V L/D = \text{the Peclet number of the column bed} \qquad (48)
$$

$$
Pe_p = V R^2/D_r L = \text{the Peclet number of the particle} \qquad (49)
$$

NUMERICAL RESULTS

The ionization model on the adsorbent surface is incorporated into the chromatographic model for investigation of the effects of pH on adsorption chromatography characterized by the effluent time-of-breakthrough curve. The effluent times as a function of pH at various concentration levels were calculated by the Gaussian expansion formula. The calculation procedures and the computer programming were described in detail by Chung[10]. Ten terms in the Gaussian expansion equation

TABLE I

pK VALUES USED FOR CALCULATION OF THE EFFLUENT TIME-OF-BREAKTHROUGH CURVES FOR THE IONIZATION MODEL DESCRIBED IN EQN. 1

| Case	Coefficients	pK_{m1}	pK_{m2}	pK_{s1}	pK_{s2}
I	$pK_{m1} < pK_{m2} < pK_{s1} < pK_{s2}$	7.0	7.5	7.6	8.0
II	$pK_{s1} < pK_{s2} < pK_{m1} < pK_{m2}$	2.1	2.5	1.0	2.0
III	$pK_{m1} < pK_{s1} < pK_{m2} < pK_{s2}$	5.0	9.0	5.3	11.0
IV	$pK_{s1} < pK_{m1} < pK_{s2} < pK_{m2}$	5.0	9.0	4.7	8.7
V	$pK_{m1} < pK_{s1} < pK_{s2} < pK_{m2}$	5.0	9.0	5.2	8.8
VI	$pK_{s1} < pK_{m1} < pK_{m2} < pK_{s2}$	5.0	9.0	3.0	11.0
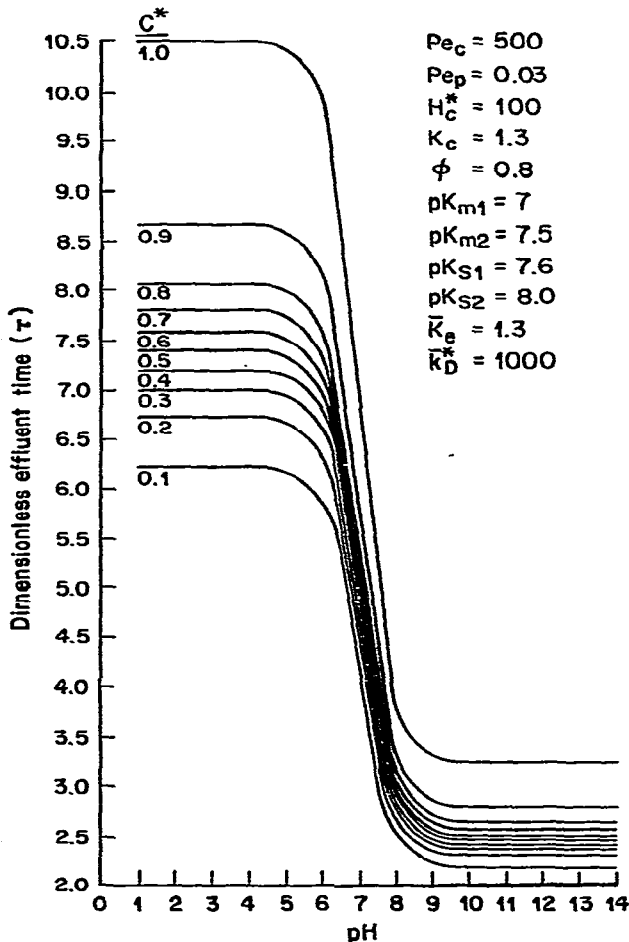
Fig. 1. Effluent time of breakthrough as a function of pH calculated by the ionization model described in eqn. 1 for case I, where $pK_{m1} < pK_{m2} < pK_{s1} < pK_{s2}$.

and four successive differences in the Poisson expansion equation were used in the calculation. For both cases the convergence was found to be sufficient by using those terms. The results obtained by the two expansion formulae were almost identical, and therefore only the results by the Gaussian formula are presented here.

In order to simplify the numerical calculation, the following constants were used for a chromatographic model: $Pe_c = 500$, $Pe_p = 0.03$, $H_c^* = 100$, $\varphi = 0.8$, $K_c = 1.3$, $\overline{K}_e = 1.3$ and $k_D = 1000$.

For the ionization model, six cases were studied. The pK values for these six cases used in the calculations are summarized in Table I. It can easily be shown that pK_{m2} is greater than pK_{m1} and pK_{s2} is greater than pK_{s1}.

Case I ($pK_{m1} < pK_{m2} < pk_{s1} < pK_{s2}$)

The pK values of the stationary phase are greater than those of the mobile phase. The effluent time-of-breakthrough curves as a function of pH at various concentration levels are shown in Fig. 1. The effluent time of breakthrough decreases with increase in pH from 5 to 9. In the low pH region (<5) the effluent time remains constant and is higher than those in the high pH region (>9). The bandwidth in the
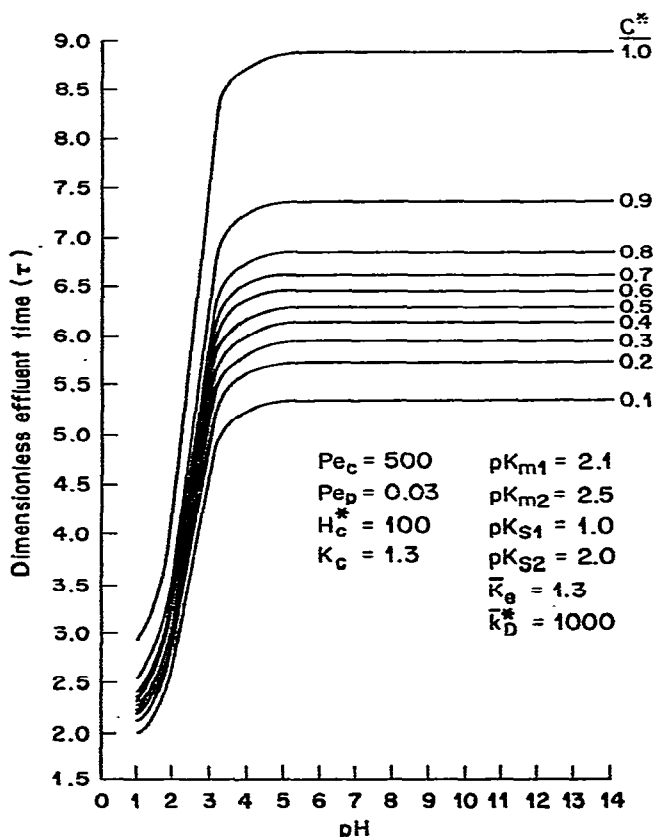
Fig. 2. Effluent time of breakthrough as a function of pH calculated by the ionization model described in eqn. 1 for case II, where $pK_{s1} < pK_{s2} < pK_{m1} < pK_{m2}$.

low pH region is wider than that in the high pH region. The spreading of solute in the low pH region is greater than that in the high pH region.

Case II ($pK_{s1} < pK_{s2} < pK_{m1} < pK_{m2}$)

The pK values of the mobile phase are greater than those of the stationary phase. The effluent time-of-breakthrough curves as a function of pH are shown in Fig. 2. Owing to the small pK values assigned in the calculation, the changes take place in the low pH region. The effluent time of breakthrough increases with increasing pH up to pH 5, then becomes independent of pH in the region pH > 5 for all concentration levels. The band spreading is higher in the high pH region (>5).

Case III ($pK_{m1} < pK_{s1} < pK_{m2} < pK_{s2}$)

The pK values of the stationary phase are greater than those of the mobile phase, as for case I, but the lower pK values in the stationary phase and the higher
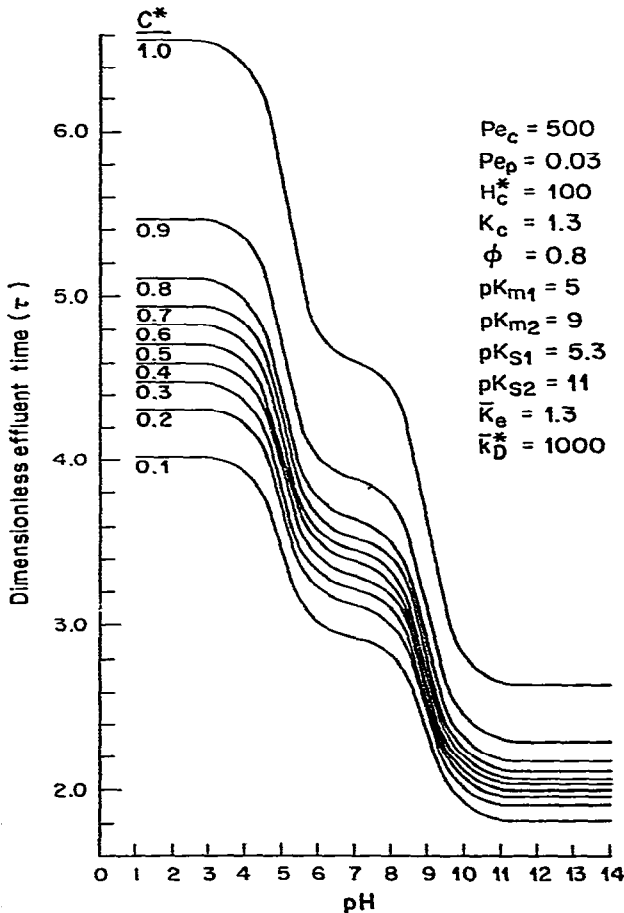

Fig. 3. Effluent time of breakthrough as a function of pH calculated by the ionization model described in eqn. 1 for case III, where $pK_{m1} < pK_{s1} < pK_{m2} < pK_{s2}$.

pK values in the mobile phase overlap. The calculated results of the effluent time of breakthrough as a function of pH are shown in Fig. 3. Almost all of the characteristics in Fig. 3 are the same as those in Fig. 1, except in the transition region where the the curves for various concentration levels show a point of inflection rather than the smooth variations for case I.

Case IV ($pK_{s1} < pK_{m1} < pK_{s2} < pK_{m2}$)

This case is similar to case III, with overlapping of the lower pK values of mobile phase with the higher pK values of the stationary phase. The calculated results are shown in Fig. 4. Almost all of the characteristics are the same as those in Fig. 2 and are exactly the same as those in Fig. 3, all the curves showing a point of inflection in the transition region.

Case V ($pK_{m1} < pK_{s1} < pK_{s2} < pK_{m2}$)

The pK values of the stationary phase lie in between those of the mobile phase. The calculated results are presented in Fig. 5. It is interesting that the effluent time
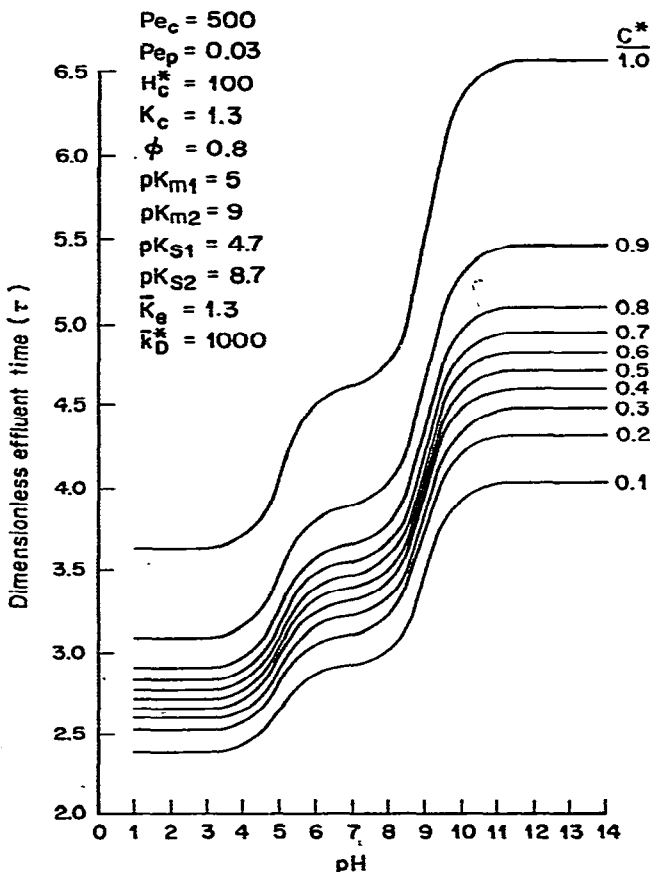

Fig. 4. Effluent time of breakthrough as a function of pH calculated by the ionization model described in eqn. 1 for case IV, where $pK_{s1} < pK_{m1} < pK_{s2} < pK_{m2}$.
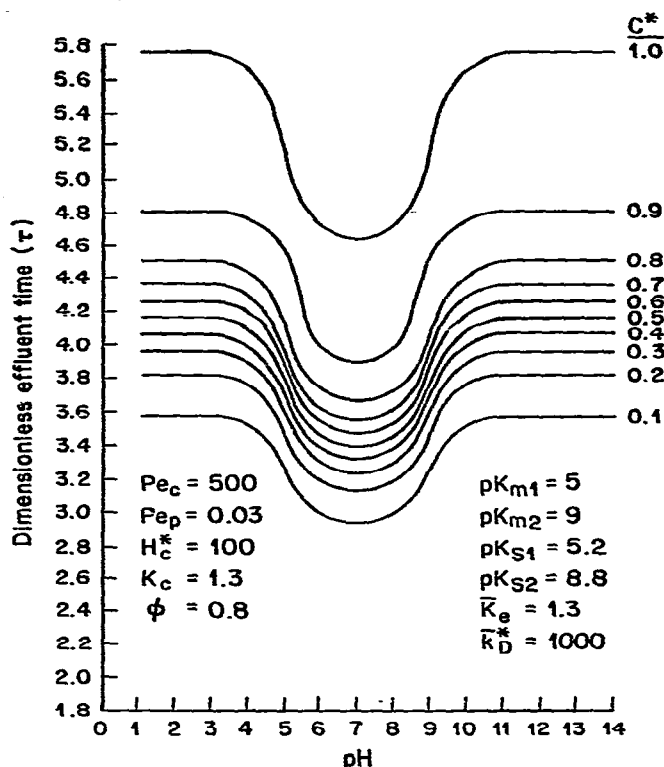
Fig. 5. Effluent time of breakthrough as a function of pH calculated by the ionization model described in eqn. 1 for case V, where $pK_{m1} < pK_{s1} < pK_{s2} < pK_{m2}$.

of breakthrough as a function of pH at various concentration levels shows a minimum at pH 7 and variations take place in the region pH 4–10. Outside this pH region the effluent times of breakthrough at all concentration levels are independent of pH. The bandwidth in the pH-dependent region is narrower than that of the pH-independent region.

Case VI ($pK_{s1} < pK_{m1} < pK_{m2} < pK_{s2}$)

The pK values of the mobile phase lie in between the two pK values in the stationary phase. The calculated results are shown in Fig. 6. The effluent time of breakthrough as a function of pH varies only in the region pH 4–10 and shows a maximum at pH 7. Outside this region the effluent time of breakthrough is independent of pH. The bandwidth in the pH-dependent region is, contrary to case V, wider than that of the pH-independent region.

DISCUSSION

It is clear that the effluent time-of-breakthrough curves and the bandwidths of the solute components are greatly affected by both the pH and pK values in the mobile and stationary phases. In separation or purification processes, one is always
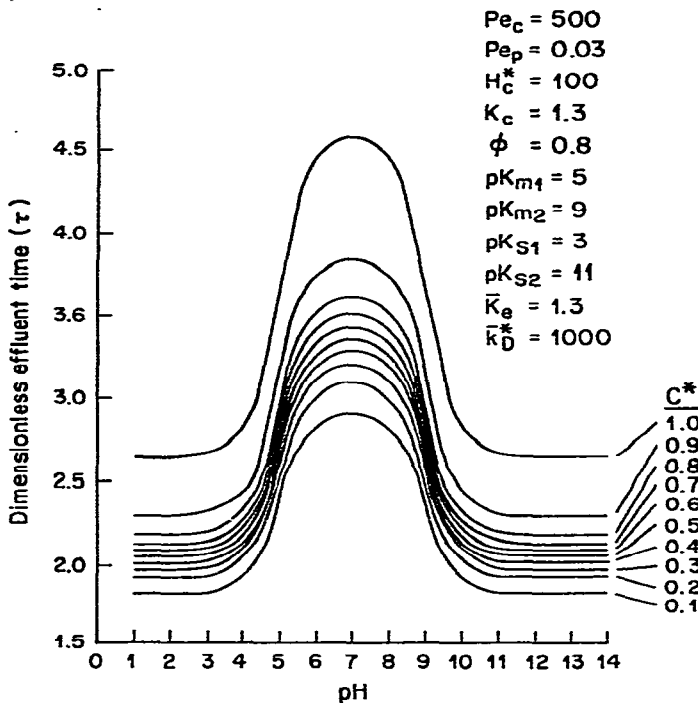
Fig. 6. Effluent time of breakthrough as a function of pH calculated by the ionization model described in eqn. 1 for case VI, where $pK_{s1} < pK_{m1} < pK_{m2} < pK_{s2}$.

interested in achieving longer retention times. Therefore, it is concluded that the retention time or the effluent time-of-breakthrough curve increases with an increase in the difference between the pK values of the stationary and mobile phases. The longer the retention time, the wider is the bandwidth spreading that also takes place. The choice of a phase with a higher pK value is entirely dependent on the solute material and whether its stability and activity are favorable in a low or a high pH region. If one prefers to effect the operation close to the neutral region and to obtain a longer effluent time of breakthrough, one can choose a larger difference in the pK values of the stationary and mobile phases, such as that shown in case VI, but not *vice versa*, as shown in case V.

In general, it can be concluded that the effluent time of breakthrough is proportional to the pK value in the mobile phase. The effluent time of breakthrough at a high pH increases with an increase in pK. The six cases considered here can be used in the selection of suitable pH and pK values for adsorption chromatography, particularly for polyampholytes. It can also be used as a general approach to describe the distribution of polyampholytes between the phases.

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