總 說
REVIEW

Affinity Chromatography 에서의 수학적 모델과 pH의 영향 분석

鄭 寅 載
한국과학원 화학공학과

Mathematical Modelling and Analysis of pH Effect on the Affinity Chromatography

In-Jae Chung
Department of Chemical Engineering
Korea Advanced Institute of Science, Seoul 131, Korea

要 約

Affinity Chromatography의 성질과 사용목적이 개략적으로 서술되였다. 또한 Chromatographic Column에 대한 수학적 모델을 세우고, Laplace변환에 의하여 구해진 moments를 이용하여 Breakthrough curve의 점근해를 구하는 방법이 설명되었다. Affinity Chromatography는 pH의 영향을 받는데 이를 설명하기 위하여 흡착현상의 모델을 설정하고 수학적 모델과의 상관관계를 설명하였다.

Abstract

Affinity chromatography is an effective method for the purification of biological material. A mathematical model has been set up by the material balance in the chromatographic column and the approximate solution has been obtained by the Gaussian expansion equation which expands in the derivatives of normal probability density function. The coefficients of the derivatives are functions of moments which are obtained from the Laplace transformation of the differential equations. Affinity chromatography is affected by changing of pH. Ionization model has been developed to explain the phenomena of pH effect on the affinity chromatography. The relation between the mathematical model and the ionization model is shown.

Introduction

The conventional purification procedures of enzyme, antigen or other biologically active compounds are usually long and require many different steps such as precipitation with salts or organic solvents, ion exchange chromatography, gel filtration, electrophoresis and so on. The multisteps may usually cause losses of biological material and its activity of reaction. These losses are due to the denaturation under the extreme conditions that are often used for isolation of a given material. Generally yields of material and activity are low when many steps are used for purification. Affinity chromatography is an effective and better method for the purification and isolation which does not need many steps[5,12-15].

The purification and isolation of biological macromolecules by affinity chromatography is based on the characteristic of specific binding[1,2,4,5,9-15]. One of the most noted characteristics of biological macromolecules is that they are capable of binding specifically and reversibly other molecules. For example, the first step in an enzymatic reaction is the binding of a specific substrate to the binding site of enzyme. Tyrosinase form a complex specifically with aminophenol.

The basic principle is to couple one of the componenets of the interacting system (e.g, substrate or aminophenol) to an insoluble porous support which can be used as a selective adsorbent for the affinity chromatography[3]. An ideal adsorbent must interact weakly with proteins or other impurities in general, in order to minimize the nonspecific adsorption of proteins or impurities. It should exhibit good flow properties. It must be mechanically and chemically stable to the varying conditions of pH, ionic strength, temperature, and presence of denaturants which may be needed for adsorption or elution. It should form a very loose, porous network which permits uniform and unimpaired entry and exit of macromolecules throughout the entire matrix.

In affinity chromatography, the biological material to be purified is passed through a column containing selective adsorbents. All impurities without substantial affinity to the adsorbents will pass directly through the column whereas one which binds specifically to the adsorbents will be retarded in proportion to its affinity constant. After the impurities are washed, elution is readily achieved by changing such parameters as salt concentration or pH.

As mentioned above, temperature, ionic strength and pH can be changed to give optimal conditions for adsorption as well as elution. In many cases, the complex is stable at neutral pH and unstable at acidic or alkaline pH, and so elution is induced by changing the pH, of the buffer[1,2,4]. Therefore, the effect of pH on the affinity chromatography should be considered. The research on the effect of ionic strength has been done[30] whereas it seems to me that nobody has tried to solve the pH effect on the affinity chromatography by showing a mechanism.

Mathematical Model

Chromatography refers to a process which consists of two phases: a stationary phase and a mobile phase percolating through the stationary phase by which different types of molecules are separated from each other by their different affinities to the stationary phase[31]. The stationary phase may be a solid or a liquid and the mobile phase may be a

liquid or a gas. Therefore, there are four possible systems: Gas-Solid chromatography, Liquid-Solid chromatography, Liquid-Liquid chromatography and Gas-Liquid chromatography[24]. Affinity chromatography may be classified as a Liquid-Solid chromatography.

So far affinity chromatography is not developed to the commercial scale, so its size is usually small and heat generation is negligible. It is considered as an isothermal operation.

The mathematical development to explain the phenomena in the chromatographic column has been done by various methods: rate theory which provides most information on the rate of adsorption or chemical reaction and longitudinal diffusion[17,32], plate theory which is characterized by the height equivalent to a theoretical plate in which the solution in the mobile phase is in equilibrium state with the concentration of the solute in the stationary phase[19,25,26,29], stochastic theory in which the rate of adsorption or desorption is related to the probability per unit time[16,18,23]. The rate theory is applied to the modelling of affinity chromatography[6].

An infinitely long column uniformly filled with porous spherical packing material of radius R is considered. The void volume fraction of the column is ε and the porosity of packed spheres is ε_s. The mobile phase is assumed to behave as plug flow with an average carrier velocity V. Dispersion of the solute in the mobile phase is assumed to be only longitudina; the dispersion coefficient, D, is assumed to be independent of concentration and a constant. Mass transfer of the solute into the stationary phase through the interphase layer is modeled with a constant mass transfer coefficient, H_c. The solute is then dispersed further into the interior of each spherical packing with a constant dispersion coefficient D_r. Finally, adsorption step is described for the solute component within the porous spherical packings. The material balance equations describing the above sorption process are

$$\frac{\partial C_m}{\partial t}+V\frac{\partial C_m}{\partial z}-D\frac{\partial^2 C_m}{\partial z^2}= -H_c(K_cC_m-C_s|_{r=R}) \tag{1}$$

for the mobile phase, and

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

for the stationary phase. The symbol C denotes the solute concentration and the subscripts m and s denote mobile and stationary phases respectively. n is the solute concentration adsorbed on the porous surface inside the spheres, K_c is the equilibrium constant between the concentration of solute outside and inside of particles at the interphase, and k_A and k_D are the rate constants for adsorption and desorption based on the total concentration of the solute respectively. If the solution is introduced at the entrance of the column as a step function, the initial and boundary conditions are:

$$C_m(z,t)=0 \quad \text{for } t\leq 0 \text{ and } z>0 \tag{3}$$
$$C_s(r,z,t)=0 \quad \text{for } t\leq 0 \text{ and } z>0 \tag{4}$$
$$n(z,r,t)=0 \quad \text{for } t\leq 0 \text{ and } z>0 \tag{5}$$
$$C_m(z,t)=C_i \quad \text{for } t\geq 0 \text{ and } z=0 \tag{6}$$
$$C_m(z,t)=0 \quad \text{for } t>0 \text{ and } z=\infty \tag{7}$$

At the surface of the particle,

$$H_c(K_cC_m-C_s|_{r=R})=-\frac{3(1-\varepsilon)}{R}\varepsilon_sD_r\frac{\partial C_s}{\partial r}|_{r=R} \quad \text{for } t>0 \text{ and } r=R \tag{8}$$

At the center of the particle, the concentration profile is symmetry around the center.

$$\frac{\partial C_s}{\partial r}|_{r=0}=0 \quad \text{for } t>0 \text{ and } r=0 \tag{9}$$

The expression $3(1-\varepsilon)\varepsilon_s/R$ gives the surface area of a spherical particle per unit volume of the column.

Solution

An exact solution for the mathematical model is almost impossible to obtain at this time. However, the solution may be characterized by statistical moments because generally the breakthrough curve at the exit of the chromatographic column is a monotonic increasing function such as the probability distribution function is. It was found that the statistical moments of the concentration profile of a solute zone obeying the material relations suggested by Lapidus and Amundson[28]. The usage of moments in chromatography gained popularity when Kubin and Kucera calculated them using the material balance equations[8, 20, 21, 27].

The approximate solution is obtained as follows:

$$C_m(t)=\frac{1}{\sqrt{2\pi}}\int_{-\infty}^{t} e^{-x^2/2}dx+a_1\Psi^{(2)}(t)+a_2\Psi^{(3)}(t)+\cdots\cdots \quad (11)$$

where $\Psi(t)=\frac{1}{\sqrt{2\pi}}e^{t^2/2}$ is normal probability density function and $\Psi^{(m)}(t)$ is the mth derivative of normal probability density function; by successive differentiations its recurrence relation is

$$\Psi^{(m)}(t)=(-1)^m\Psi(t)H_m(t) \quad (12)$$

where the $H_m(t)$'s are the Hermite polynomials defined by

$$H_m(t)=\sum_{j=0}^{M}\frac{(-1)^j m!\, t^{m-2j}}{j!\,(m-2j)!\,2^j} \quad (13)$$

where $M=m/2$ when m is an even number and $m(m-1)/2$ when m is an odd number. In the equation (11), the values of a_1, a_2 and so on are determined by the moments[8].

Absolute kth order moment is defined as follows:

$$\alpha_k=\int_0^\infty t^k\frac{dC_m}{dt}dt=\lim_{s\to 0}\int_0^\infty e^{-st}t^k\frac{dC_m}{dt}dt$$
$$=\lim_{s\to 0}(-1)^k\frac{d^k}{ds^k}\int_0^\infty e^{-st}\frac{dC_m}{dt}dt$$
$$=\lim_{s\to 0}(-1)^k\frac{d^k}{ds^k}sC_m \quad (14)$$

The solution of Laplace formed equations of Eq. (1) through (8) gives the value of absolute kth order moment by the equation (14).

The central moments are obtained from the absolute moments:

$$\mu_k=\int_0^\infty(t-\alpha_1)^k dC_m=\sum_{i=1}^{k}\binom{k}{i}(-\alpha_1)^{k-1}\alpha_i \quad (15)$$

where $\binom{k}{i}$ is a combination.

The first order absolute moment and the second order central moment are calculated from the equation (1) through (8) in the following form:

$$\alpha_1=\frac{L}{V}(1+\phi K_c(1+K_e)) \quad (16)$$

$$\mu_2=\frac{2D^2}{V^3}\gamma_1^2+\frac{L}{V}\gamma_2 \quad (17)$$

where L is the length of the column, $\phi=(1-\varepsilon)\varepsilon_s/\varepsilon$, $K_e=k_A/k_D$ equilibrium constant of reaction, $\gamma_1=1+\phi K_c(1+K_e)$ and

$$\gamma_2=\frac{2\phi^2K_c(1+K_e)^2}{H_c}+\frac{2\phi K_cR^2(1+K_e)}{15D_r}+\frac{2\phi K_cK_e}{k_D}$$

Since the coefficients of the mth derivatives of normal probability density function are calculated from the moments, the breakthrough curve at the exit of the chromatographic column is obtained using equation (11).

HETP

Since Martin and Synge developed the height equivalent to a theoretical plate (HETP) concept[27], it has become a widly accepted tool in various ways for chromatographic analysis. This plate model, although some-

what ambiguous in the case of continuous operations, is a useful parameter for the evaluation of system performance in terms of zone spreading. Resolution is useful in describing the system efficiency in separating two components, and can be related to the plate number. The dependence of resolution on plate height is to be expected since, for a given difference in retention time, resolution between two consecutive components will increase with a decrease in the width of the concentration zone, that is, with an increase in the plate number. The plate number is the length of column divided by the plate height. The first moment, as described by Grushka[21], is the retention time, t_R, of the solute, which is

$$t_R=1+\phi K_c(1+K_e) \tag{18}$$

The second moment leads to a direct expression of the plate height, or the height equivalent to a theoretical plate[22], as

$$\text{HETP}=H=\frac{L\mu_2}{\alpha_1^2}=\frac{2D}{V}+\frac{V}{(1+\phi K_c(1+K_c))^2}\left[\frac{2\phi^2K_c(1+K_e)^2}{H_c}+\frac{2\phi K_cR^2(1+K_e)}{15D_r}+\frac{2\phi K_cK_e}{k_D}\right] \tag{19}$$

The first term on the right-hand side of equation (19) is the usual solute diffusion in mobile phase, the second is the resistance to mass transfer at the surface of the particle, the third is the diffusion in the pore of the particle and the last term is due to the adsorption and desorption rates within the pore.

Ionization Model

As mentioned earlier, affinity chromatography is affected by the change of pH in the solution. Although many people recognized the pH effect on the affinity chromatography experimentally, nobody has tried to explain it by the reaction mechanism.

It is known that factors which influence the stability of an enzyme or a protein are those which affect the secondary, tertiary and/or qunternary 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) involves in the active site of a solute material. Those prototropic groups are generally 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[7,32,34].

The following ionization model is used in the investigation of the pH effect on adsorption chromatography.

$$\begin{array}{ccccc} C_m^{i-1} & \underset{+H^+}{\overset{K_{m1}}{\rightleftharpoons}} & C_m^i & \underset{+H^+}{\overset{K_{m2}}{\rightleftharpoons}} & C_m^{i+1} \\ & & \Big\| k_A \quad k_D & & \\ C_s^{i-1} & \underset{K_{s1}}{\overset{-H^+}{\rightleftharpoons}} & C_s^i & \underset{K_{s2}}{\overset{-H^+}{\rightleftharpoons}} & C_s^{i+1} \end{array} \tag{20}$$

where the symbol 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 packing material to give C_s^i, and the ionizations between the hydronium ion and the solute in mobile and stationary phases are so

fast that equilibrium can be established instantaneously. The quantities k_A and 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 k indicates that the values are independent of pH.

The equilibrium constants for the ionization reaction in a mobile phase for the model given in equation (20) are

$$K_{m1}=[C_m^i][H^+]/[C_m^{i-1}] \quad (21)$$

$$K_{m2}=[C_m^{i+1}][H^+]/[C_m^i] \quad (22)$$

where the square brackets [ ] represent concentration.

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

$$K_{s1}=[C_s^i][H^+]/[C_s^{i-1}] \quad (23)$$

$$K_{s2}=[C_s^{i+1}][H^+]/[C_s^i] \quad (24)$$

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

$$C_s=[C_m^{i-1}]+[C_m^i]+[C_m^{i+1}] \quad (25)$$

$$n=[C_s^{i-1}]+[C_s^i]+[C_s^{i+1}] \quad (26)$$

where the symbols of C_s and n are the same as those in the equation (2). Since $k_AC_s=\bar{k}_A[C_m^i]$ and $k_Dn=\bar{k}_D[C_s^i]$,

$$K_e=\bar{K}_e\frac{1+[H^+]/K_{s1}+K_{s2}/[H^+]}{1+[H^+]/K_{m1}+K_{m2}/[H^+]} \quad (27)$$

and

$$k_D=\bar{k}_D(1+[H^+]/K_{s1}+K_{s2}/[H^+]) \quad (28)$$

where $\bar{K}_e=\bar{k}_A/\bar{k}_D$.

For the ionization model, there are only six cases when all constants except the values of $pK(pK=-\log_{10}K)$ are fixed, because the value of pK_{m2} is higher than the value of pK_{m1} and the value of pK_{s2} is higher than that of pK_{s1}. The change of pH affects the overall equilibrium constant of reaction and the overall desorption rate constant as shown in equations (27) and (28). Although the reaction mechanism is not explained, the significance of the equilibrium constant for the successful purification by the affinity chromatography is emphasized[31].

Concluding Remark

It has been pointed out that although the affinity chromatography is effective method for the purification of a biological material when it is compared with conventional method, it is not developed for the mass production of purified material. It may be a task which should be solved in near future.

Generally there are three approaches to derive the equation for the chromatography: rate theory, plate theory and stochastic theory. The solution of the mathematical model in this paper is obtained approximately by the Gaussian expansion equation, which indicates the equation (11).

Although the mechanism to explain the pH effect on the affinity chromatography can not be completely understood, the modelling may be a starting point for a long march to search the true mechanism.

References

1. N. G. Anderson et al., *Analytical Biochemistry*, **66** (1975), 159.
2. N. G. Anderson et al., *Analytical Biochemistry*, **68** (1975), 371.
3. R. Axen, J. Porath and S. Ernback, *Nature*, **214** (1967), 1302.
4. P. H. Campbell, E. Luescher and L.S. Lerman, *Proc. Nat.* Acad. Sci., U.S.A., **37** (1951), 575.

5. W.C. Chan and M. Takahashi, *Biochem. Biophys. Res. Commun.*, **37** (1969), 272.
6. I. J. Chung and H.W. Hsu, *Chem. Eng. Sci.*, **33** (1978), 399.
7. E. J. Cohn and J. T. Edsall, Protein, Amino Acids Peptides, Reinhold Publishing Co., New York, (1948).
8. H. Cramer, Mathematical Methods of Statistics, Princeton Princeton University Press, Princeton, (1946).
9. P. Cuatrecasas, *J. of Biological Chemistry*, **245** (1970), 3059.
10. P. Cuatrecasas, in Biochemical Aspects of Reaction on Solid Supports, ed. George R. Stark, Acad. Press, New York, (1971).
11. P. Cuatrecasas and C.B. Anfinsen, *Ann. Rov. Biochem.*, **40** (1971), 259.
12. P. Cuatrecasas and C.B. Anfinsen, in Methods in Enzymology, Vol. XXII, ed. W.B. Jakoby, Acad. Press, New York, (1971), 345.
13. P. Cuatrecasas and Meir Wilchek, *Biochem. Biophys. Res. Commun.*, **33** (1968), 235.
14. P. Cuatrecasas, M. Wilchek and C.B. Anfinsen, *Proc. Nat. Acad. Sci.*, U.S.A., **61** (1968), 636.
15. G. Feinstein, *Die Natur.*, **58** (1971), 389.
16. J. C. Giddings, *J. of Chem. Phys.*, **26** (1957), 169.
17. J. C. Giddings, *J. of Chromatography*, **5** (1961), 46.
18. J. C. Giddings and Henry Eyring, *J. of Phys. Chem.*, **59** (1955), 416.
19. E. Glueckauf, Trans. *Faraday Soc.*, **51** (1955), 34.
20. O. Grubner, *Advan. Chromatogr.*, **6** (1968), 173.
21. E. Grushka, J. Phys. Chem., **76** (1972), 2586.
22. H. W. Hsu and I. J. Chung, J. of Chromatogr., **138** (1977), 267.
23. R. A. Keller and J. C. Giddings, *J. of Chromatogr.*, **3** (1960), 205.
24. A.I.M. Keulemans, Gas Chromatography, Reinhold Publishing Co., New York, (1959).
25. R. Kieselback, *Analytical Chemistry*, **33** (1961), 23.
26. A. Klinkenberg and F. Szenitzer, *Chem. Eng. Sci.*, **5** (1956), 258.
27. E. Kucera, *J. of Chromatogr.*, **19** (1965), 237.
28. L. Lapidus and N. R. Amundson, *J. Phys. Chem.*, **56** (1952), 984.
29. A. J. Martin and R.L.M. Synge, *Biochem. J.*, **35** (1941), 1358.
30. R. V. Metha, R.L. Merson and B.J. Mc Coy, *AIChE J.*, **19** (1973), 1068.
31. G. H. Reiner and A. Walch, *Chromatographia*, **4** (1971), 578.
32. I. H. Segel, Enzyme Kinetics, John Wiley & Sons, New York, (1975).
33. J. J. Van Deemer, F. J. Zuiderwerg and A. Klinkenberg, *Chem. Eng. Sci.*, **5** (1956), 271.
34. J. R. Whitaker, Principles of Enzymology for the Food Sciences, Marcel Dekker, New York, (1972).

저 자 약 력

정인재 박사는 한양대학교 화학공학과를 1968년에 졸업하고, 그 이듬해 도미하여 황산공정 최적화에 관해 석사학위를 받고, affinity chromatography의 이론적 분석으로 1976년 University of Tennessee에서 박사학위를 수여 받았다.

졸업후 동대학의 연구원으로 재직하다가 1977년 8월에 한국과학원 화학공학과에 부임했으며, 연구계획은 폐수처리와 고분자의 Rheology 등에 관한 것이다.