

CHROM. 10,299

CHROMATOGRAPHIC EVALUATION OF THE EQUILIBRIUM AND KINETIC CONSTANTS FOR REVERSIBLE COMPLEX FORMATION IN THE MOBILE PHASE

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(First received November 30th, 1976; revised manuscript received June 10th, 1977)

SUMMARY

A theoretical analysis is presented of chromatography with complex formation in the mobile phase: $A + B \xrightleftharpoons[k_{-1}]{k_1} AB$, where k_1 and k_{-1} are the corresponding rate constants. It is assumed that excess of a solute B is present in the column during the whole experiment, only solute A being sorbed by the stationary phase. The solution of the equation governing such a process allows us to determine the dissociation coefficient of the complex and the kinetic constants of the complex-formation reaction.

INTRODUCTION

The properties of such physiologically active substances as hormones, enzymes and antibiotics¹ can be modified by complex formation with soluble synthetic polymers. In recent years, methods have been developed for determining the constants of the complex-formation reaction, and the polymer-polymer complexes, the polymer-oligomer complexes or the polymer-low-molecular-weight organic ion complexes have been investigated by calorimetry and potentiometric titration²⁻⁴ and dynamic methods, including chromatography⁵. Elution chromatography with polymer-containing eluents has also been used successfully⁶⁻⁸. Calculation of the equilibrium constants of the complex-formation reaction is based on the assumption that both inter-phase and reaction equilibria are instantaneous, yet when experiments are made with substances of high molecular weight these conditions are usually not fulfilled. The aim of this work was to develop a theoretical approach to determination of the dissociation coefficient of the complex and the kinetic constants of the complex-formation reaction in the absence of both reaction and inter-phase equilibria.

THEORETICAL

The material balance for the reacting substances in the chromatographic column (including both mobile and stationary phases) is given by

$$\alpha \frac{\partial C_i}{\partial t} + (1 - \alpha) \frac{\partial a_i}{\partial t} = -v \frac{\partial C_i}{\partial x} + D^* \frac{\partial^2 C_i}{\partial x^2} + a_i l_i + (1 - \alpha) L_i \quad (1)$$

where α is the void fraction of the bed, t is the time, x is the distance from the inlet, C_i is the concentration in the mobile phase, a_i is the average concentration in the stationary phase (dependent on t and x), v is the average linear-flow velocity of the mobile phase, D^* is the axial dispersion coefficient, and l_i and L_i are the average rates of formation of substance i in the mobile and stationary phases, respectively.

Eqn. 1 can also be obtained from the macroscopic material balance⁹ for an arbitrary control volume of the column as follows:

$$\begin{aligned} \frac{\partial}{\partial t} \int_{\Delta\Omega'} C_{oi} d\Omega + \frac{\partial}{\partial t} \int_{\Delta\Omega''} a_{oi} d\Omega = \\ = - \int_{\Delta S} v C_{oi} n dS + \int_{\Delta S} D^* \nabla C_{oi} n dS + \int_{\Delta\Omega'} l_{oi} d\Omega + \int_{\Delta\Omega''} L_{oi} d\Omega \end{aligned} \quad (2)$$

where C_{oi} and a_{oi} are the local concentrations of the substances in the mobile and stationary phases, respectively, l_{oi} and L_{oi} are the rates of formation for substance i in both the mobile and stationary phases (the chemical sources). The term $\Delta\Omega$ represents the control volume, and $\Delta\Omega'$ and $\Delta\Omega''$ are the control volumes for the mobile and stationary phases, respectively; ΔS is the external surface of the control volume.

By comparing eqn. 1 with eqn. 2, we may define more exactly the meanings of the variables and the parameters of eqn. 1. The void fraction of the bed (α) is $\frac{\Delta\Omega'}{\Delta\Omega}$, the average concentration (C_i) of substance i is $\int_{\Delta\Omega'} C_{oi} d\Omega / \Delta\Omega'$, the average concentration (a_i) is $\int_{\Delta\Omega''} a_{oi} d\Omega / \Delta\Omega''$, and the average rates of formation of substance i are $l_i = \int_{\Delta\Omega'} l_{oi} d\Omega / \Delta\Omega'$ and $L_i = \int_{\Delta\Omega''} L_{oi} d\Omega / \Delta\Omega''$. Thus, eqn. 1 can be obtained by dividing eqn. 2 by $\Delta\Omega$.

This work deals with the simplest case of a complex-forming reversible reaction, $A + B \xrightleftharpoons[k_{-1}]{k_1} AB$, in which A and B are the substances that form the complex AB, and k_1 and k_{-1} are the rate constants of the forward and reverse reactions, respectively. Suppose that only one substance (A) is sorbed, then, if B and the complex do not penetrate into the sorbent and B is continuously fed (in excess) with the eluent into the column during the experiment, C_B can be considered as constant and the reaction is pseudo first-order. If we neglect axial dispersion, we obtain the following equations:

$$\alpha \frac{\partial C_A}{\partial t} + (1 - \alpha) \frac{\partial a_A}{\partial t} = -v \frac{\partial C_A}{\partial x} + \alpha (k_{-1} C_{AB} - k_1' C_A) \quad (3)$$

$$\alpha \frac{\partial C_{AB}}{\partial t} = -v \frac{\partial C_{AB}}{\partial x} - \alpha (k_{-1} C_{AB} - k_1' C_A)$$

where $k_1 = k_1 C_B$

Let us assume that Henry's law is applicable to substance A, that is, $a_A^* = k_d C_A$. Then the grain-average concentration (a_A) of substance A is defined by eqn. 4, which is obtained by solving the problem of diffusion of A into the sorbent with the intraparticle diffusion coefficient (\bar{D}) as:

$$a_A = \frac{6\bar{D}}{R^2} \int_0^\infty \sum_{n=1}^{\infty} e^{-n^2\pi^2 \frac{\bar{D}}{R^2}(t-\xi)} a_A^*(x, \xi) d\xi \quad (4)$$

where a_A^* is the concentration in equilibrium with the mobile-phase concentration C_A , R is the particle radius and K_d is the sorption coefficient.

Eqns. 3, 4 and 5 must be solved at the initial conditions [$C_i(x,0) = 0$; $x > 0$; $i = A, AB$]. The boundary conditions corresponding to the introduction of an infinitely narrow zone of sample are given by:

$$C_A(0, t) = \frac{Q_1}{\alpha v S} \delta(t) = N_1 \delta(t); C_{AB}(0, t) = \frac{Q_2}{\alpha v S} \delta(t) = N_2 \delta(t) \quad (5)$$

where Q_1 and Q_2 are the amounts of substances introduced into the column, S is the total area of the column cross-section and $\delta(t)$ is the "delta-Dirac" function.

If equilibrium occurs instantaneously at the inlet of the column, then:

$$\frac{C_A}{C_{AB}} = \frac{k_{-1}}{k_1} = K = \frac{N_1}{N_2} = \frac{Q_1}{Q_2} = \frac{K'}{C_B} \quad (6)$$

where K' is the dissociation coefficient of the complex.

It is convenient to use the following parameters:

$$\tau = \frac{\bar{D}}{R^2} \left(t - \frac{\alpha x}{v} \right); \lambda = 3(1-\alpha) K_d \frac{\bar{D}x}{R^2 v}; \quad (7)$$

$$K = \frac{k_1 C_B R^2 \alpha}{3(1-\alpha) \bar{D} K_d}; r = \frac{k_{-1} R^2 \alpha}{3(1-\alpha) \bar{D} K_d}$$

where τ is the generalized time, λ is the generalized column length, and k and r are the normalized rate constants of the forward and reverse reactions, respectively.

With the above dimensionless members, double-Laplace transform of eqn. 3 yields:

$$\begin{cases} qz - N_1 = -G(p)Z + ry - Kz \\ qy - N_2 = -ry + kz \end{cases} \quad (8)$$

where z and y are the images of C_A and C_{AB} in the Laplace domain, respectively, q and p are the transformed values of λ and τ , and

$$G(p) = 2 \sum_{n=1}^{\infty} \frac{p}{p + n^2\pi^2} = \sqrt{p} \operatorname{th} \sqrt{p} - 1.$$

The solution of eqn. 8 in the Laplace domain is given by:

$$\frac{z + y}{N_1 + N_2} = \frac{k}{(q+r)(k+r)} + \frac{\frac{r}{k+r} \left(1 + \frac{k}{q+r} \right)}{q \left(1 + \frac{k}{q+r} \right) + G(p)} \quad (9)$$

and this can be used for determining the moments of the concentration distribution. It can be shown that the moment of any order is given by:

$$\mu_n = (-1)^n \lim_{p \rightarrow 0} \frac{\partial^n F(q, p)}{\partial p^n} \quad (10)$$

The first moment determines the position of the centre of gravity of the distribution $C(\lambda, \tau)$.

From the right-hand side of eqn. 9, by using eqn. 10 and taking into account that $G_{(p)/p \rightarrow 0} \rightarrow 0$, $G'_{(p)/p \rightarrow 0} \rightarrow 1/3$, we deduce that

$$\mu_1 = \frac{K}{K+1} \cdot \frac{\lambda}{3} \quad (11)$$

where

$$K = \frac{r}{k} = \frac{K'}{C_B}$$

Taking the variables V and x we calculate that:

$$\frac{v(t_c - t_0)}{(1 - \alpha) K_d x} = \frac{K}{K+1} \quad (12)$$

where $t_c = V_c/vS$ and $t_0 = V_0/vS$

After solving the eqn. 12 with respect to K we obtain:

$$K = \frac{V_c - V_0}{V_0 - V_c + (1 - \alpha) K_d V_K} \quad (13)$$

where V_0 is the void volume, and V_K the total volume, of the column and V_c is the centre of gravity of the zone (expressed in units of volume).

The centre of gravity of the zone is derived from the equation:

$$V_c = \mu_1^v \approx \frac{\sum_{i=1}^n V_i C_i \Delta V_i}{\sum_{i=1}^n C_i \Delta V_i} \quad (14)$$

where ΔV_i is the elution-volume increment. From V_c we can calculate the value K according to eqn. 13.

The magnitude of the rate constants of the complex-formation reaction can be evaluated by two methods. The second moment can be obtained from eqns. 9 and 10. On the other hand, the second central moment is derived from the first and the second moments as follows:

$$\sigma^2 = M_2 = \mu_2 - \mu_1^2 \quad (15)$$

In this event

$$\sigma^2 = \frac{K}{K+1} \left[\frac{2 \exp(-k(K+1)\lambda)}{9k^2(K+1)^3} + \left(\frac{2}{4S} + \frac{2}{9k(K+1)^2} \right)^2 - \frac{2}{9k^2(K+1)^3} \right] \quad (16)$$

The exponential term can be written in power-series form as:

$$\sigma^2 = \frac{K}{K+1} \left\{ \frac{2}{4S} \lambda + \frac{2}{9(K+1)} \left[\frac{\lambda^2}{2!} - k(K+1) \frac{\lambda^3}{3!} + k^2(K+1)^2 \frac{\lambda^4}{4!} - k^3(K+1)^3 \frac{\lambda^5}{5!} \dots \right] \right\} \quad (17)$$

Using the first three terms of this series, we obtain:

$$\sigma^2 = \frac{K}{K+1} \left\{ \frac{2}{4S} \lambda + \frac{2}{9(K+1)} \left[\frac{\lambda^2}{2} - k(K+1) \frac{\lambda^3}{6} \right] \right\} \quad (18)$$

Solving eqn. 18 relative to rate constant k we obtain:

$$k = \frac{\left[\sigma^2 - \frac{2K\lambda}{4S(K+1)} - \frac{K\lambda^2}{9(K+1)^2} \right] \cdot 6}{K\lambda^3} \quad (19)$$

The accuracy of evaluating of rate constant k is better than 10%, if $\lambda \leq 0.7/\sqrt{k(K+1)}$. The dispersion of the experimentally obtained zone is determined:

$$\sigma^2 \approx \frac{\sum_{i=1}^n V_i^2 C_i \Delta V_i}{\sum_{i=1}^n C_i \Delta V_i} - \left(\frac{\sum_{i=1}^n V_i C_i \Delta V_i}{\sum_{i=1}^n C_i \Delta V_i} \right)^2 \quad (20)$$

By substituting for σ^2 and λ in eqn. 19 and using K obtained from eqn. 13 we can calculate the value of the rate constant k .

The second method of evaluating the rate constants of the complex-forming reaction requires the solution of the double-Laplace transforms of eqn. 3 given by eqn. 8. The inverse transforms yield the solution

$$\begin{aligned} \frac{C_A}{N_1} = & e^{-k\lambda} F(\lambda, \tau) + ke^{-r\lambda} \int_0^\lambda e^{-(k-r)\xi} I_0(\eta) F(\xi, \tau) d\xi + \\ & + \sqrt{kr} e^{-r\lambda} \int_0^\lambda \sqrt{\frac{\xi}{\lambda - \xi}} e^{-(k-r)\xi} I_1(\eta) F(\xi, \tau) d\xi \quad (21) \end{aligned}$$

$$\begin{aligned} \frac{C_{AB}}{N_2} = & e^{-r\lambda} \delta(\tau) + re^{-r\lambda} \int_0^\lambda e^{-(k-r)\xi} I_0(\eta) F(\xi, \tau) d\xi + \\ & + \sqrt{kr} e^{-r\lambda} \int_0^\lambda \sqrt{\frac{\lambda - \xi}{\xi}} e^{-(k-r)\xi} I_1(\eta) F(\xi, \tau) d\xi \end{aligned}$$

where $\eta = 2\sqrt{kr(\lambda - \xi)\xi}$, and I_0 and I_1 are the Bessel function of zero-th and first orders, respectively. $F(\lambda, \tau)$ is the instantaneous concentration distribution inside the particle due to particle diffusion. As a first approximation, we assume that the sorption process is at equilibrium. In that case, $F(\lambda, \tau) = 3\delta(\lambda - 3\tau)$ and eqn. 21 becomes:

$$\begin{aligned} \frac{C_A}{N_1} = & 3e^{-k\lambda} \delta(\lambda - 3\tau) + 3e^{-r(\lambda - 3\tau) - 3k\tau} [kI_0(\eta) + \sqrt{\frac{3kr\tau}{\lambda - 3\tau}} I_1(\eta)] \\ \frac{C_{AB}}{N_2} = & e^{-r\lambda} \delta(\tau) + 3e^{-r(\lambda - 3\tau) - 3k\tau} [rI_0(\eta) + \sqrt{\frac{kr(\lambda - 3\tau)}{3\tau}} I_1(\eta)] \quad (22) \end{aligned}$$

RESULTS

The concentration distributions were calculated by computer for different values of k , r and λ ; they agreed very well with the results obtained statistically by

Keller and Giddings¹⁰. We also found that, when the generalized column is small, the mixture is divided into two zones, the first dominated by B and the other by A. When λ becomes sufficiently large, the zones merge. If we call λ_m the maximum value of λ at which the mixture is divided into two zones, then analysis of eqn. 22 gives the correlation between K , r and λ_m . The zone becomes much more symmetrical as λ increases. The limiting position of the zone maximum agrees very well with that predicted by the theory developed earlier⁸.

The dependence of K on r at different values of λ_m is shown in Fig. 1. In order to determine r the value of K must be derived from experimental data by using eqn. 13. Then the maximum value ($\lambda = \lambda_m$) at which separation of the zone takes place must be defined experimentally.

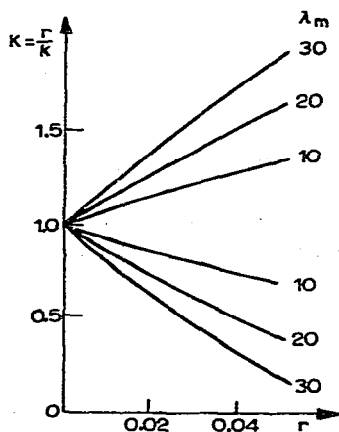


Fig. 1. Graphs showing dependence of the concentration ratio, K , on r at different values of λ_m .

From a knowledge of these two values (K and λ_m), we can determine the value r from Fig. 1. This, the result of theoretical analysis enables us to calculate both the equilibrium and kinetic constants of the first-order complex-formation reaction by using the results of two chromatographic experiments.

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