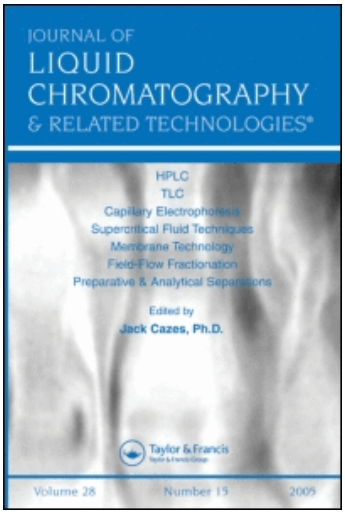

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Theory of Open Capillary Chrcmatography Using Electrical Diffuse Layer as the Virtual Stationary Phase for Charged Macro Mxecular Separations

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THEORY OF OPEN CAPILLARY CHROMATOGRAPHY

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ABSTRACT

Electrical diffuse layers established by arrangement of ions along a metal capillary column can be used as the virtual "stationary phase" for charged macromolecular separations and determinations. Preliminary studies have indicated that resolution depends on solvent flow rate, pH, column diameter, etc., as well as the applied potential. In this communication we report a theory which underlies the mechanism of retention of charged macro-molecules, e.g., proteins, on the column. The utility and difficulties of this novel fractionation technique will also be discussed.

INTRODUCTION

of exclusion methods. Molecules are sorted according to their sizes or mass by the pores of the preselected gels. Molecules having sizes greater than the pores will be excluded and eluted with the same velocity as the solvent. On the other hand, molecules having smaller sizes than the pores will be retained in the pores until they are replaced by the solvent molecules. Resolution of these molecules is frequently not possible; thus, the usefulness of the column is limited to a narrow molecular weight or size range by the

pore size distribution of the packing material.

Charged particles, e.g., proteins, enzymes, etc., can also be separated by electrophoresis, affinity chromatography, isoelectric focusing, or the combination of these techniques. Some of these methods are very specific and selective, while others are of more general use but subject to the similar limitations as the exclusion method. Moreover, these are usually slow methods. Although they may be adoptable for the analysis of many samples, each separation requires hours for completion. In addition, many exhibit serious cross sample interferences (memory effect). The column can only be used once (e.g., gel electrophoresis), or has to be regenerated prior to the next analysis (e.g., affinity chromatography). Some methods require immobilization of a specific chemical entity on the column and others need specific development methods. These requirements and drawbacks disqualify the above techniques to be universal methods for macromolecular separations and determinations.

Giddings (1) has mentioned many advantages of field-flow-fractionation using open-bed channels over the conventional packed column chromatographic technique. These include better resolution in the separtion of very high molecular weights, greater number of theoretical plates, and greater versatility in separations. However, the requirement of an external cross flow field and the need of semipermeable membranes impose quite serious experimental problems. Other people (2 - 14) have reported some success in using treated capillary columns for separation. Most of them deal with small molecules, for which the application of this technique is less promising because of the technical difficulties in using capillaries of extremely small diameter (1 - 10 um).

We have suggested the use of electric multiple layers (diffuse double layer) as the virtual "stationary phase" for separation (15). These layers were established from physical arrangement of ions under an applied potential. Their thickness and polarity can be easily varied. Difficulties such as column bleeding and contamina-

tion, regeneration, homogeneous packing, stationary phase immobilization, cross sample interferences, etc., can be eliminated or minimized. The cross-flow field is established by physical arrangement of ions in the buffered mobile phase and semipermeable membranes are not needed. Accordingly, the length and shape of the column are more flexible than the open bed in flow field flow fractionation. In the present communication we will report a theory which underlies the mechanism of retention for this separation technique.

THEORETICAL CONSIDERATIONS

Assuming that the flow of the mobile phase in a capillary column is laminar with velocity (16),

$$v(r) = \frac{(P_i - P_o)\, R^2}{4\eta L} [1 - (r/R)^2]$$

$$= 2 \langle v \rangle [1 - r'^2] \qquad (1)$$

where P_i and P_o are pressures at the capillary inlet and outlet, and R and L are the radius and length of the capillary, respectively. The viscosity of the mobile phase is denoted as η and r' is the relative radial distance.

The flux density of a solute, J, can be conveniently broken into lateral and axial components, i.e., $J = J_r + J_x$. We have

$$J_r = -D_r \nabla_r C + UC = 0 \qquad (2)$$

$$J_x = -D_x \bar{k} (\partial C/\partial x) + vC \qquad (3)$$

The gradient operator ∇_r acts upon the radial coordinates only and $\bar{k}$ is the unit vector along the column axis, x. D_r and D_x are the diffusion coefficients of the solute molecules in the r and x directions, respectively. For simplicity, we will assume they are identical, i.e., $D_r = D_x = D$.

In the presence of a cross-flow electrical field, the radial velocity of the solute, U, can be expressed as,

$$U = \frac{zeE}{f} \tag{4}$$

where z is the number of charges on the molecule and e is the elementary charge of an electron. The field strength E in the column is a parameter dependent of r and can be written as the gradient of the electrical potential, Ψ,

$$E = -\frac{\partial \Psi}{\partial r} \tag{5}$$

Since the external potential is applied across the diffuse double layer along the capillary wall, and since it is unlikely for the solute molecules to get into the Helmholtz layer, Ψ may be conveniently expressed as

$$\Psi = \Phi \exp[-KR(1 - r')] \tag{6}$$

where Φ is the potential at the outer Helmholtz plane (OHP) and is assumed to be proportional to the applied voltage, V, i.e., $\Phi = \alpha V$. K is the so called Debye reciprocal length (17) which depends on the electrolyte concentration in the mobile phase.

The "frictional" force, f, of a spherical macromolecule with a radius, ρ, can be approximated by

$$f = 6\pi\eta\rho \tag{7}$$

On substituting these values into (2) and solving for C, one obtains,

$$C(r,x,t) = C_o(x,t)\exp\left\{-\frac{\lambda}{KR}[1 - \exp(-KR(1 - r'))]\right\} \tag{8}$$

where

$$-\frac{\lambda}{KR} = \frac{zeV\alpha}{6D\pi\eta\rho}$$

is always negative because the charges, ze, on the molecule must be opposite in sign to the applied potential, V. Accordingly, the quantity λ is always positive.

Equation (9) can be simplified further for $r' \approx 1$,

$$C = C_o \exp [-\lambda(1 - r')] \tag{10}$$

and for $r' << 1$,

$$C = C_o \exp (-\lambda/KR) \tag{11}$$

Equation (10) gives the solute concentration distribution close to the capillary wall where the solute molecules are under the influence of the double layer potential. $C_o(x,t)$ is the transient solute concentration on the capillary wall, or more exactly, on the OHP surface. Equation (11), on the other hand, is the concentration distribution in the bulk solution. In the case when λ/KR is large, the bulk concentration is seen to be negligibly small. The solute molecules will be predominently retained within the double layer, resulting in a long retention time. When λ/KR is small, most of the solute molecules will be in the bulk solution and moving with the mobile phase, resulting in a short retention. Since the quantity λ/KR depends on the number of charges on the molecule, the magnitude of the applied potential, the diffusion and size of the solute molecules, and the viscosity of the mobile phase, one can selectively retain different solute components in a mixture simply by controlling these parameters.

The average zone velocity of a component can be expressed as

$$\bar{v} = \frac{\langle Cv \rangle}{\langle C \rangle} \tag{12}$$

Under near-equilibrium condition, the time dependent axial flux of the component takes the form,

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} + \bar{v} \frac{\partial C}{\partial x} \tag{13}$$

The solution of this equation is known to give a gaussian distribution. The column efficiency in terms of height equivalent to a theoretical plate (HETP), H, for the solute can be shown to be

$$H = \frac{2D}{\bar{v}} \tag{14}$$

The retention, Re, is evaluated by,

$$Re = \frac{\bar{v}}{\langle v \rangle}$$

$$= 2\left[1 - \frac{\lambda - 3}{\lambda - [1 - \exp(-\lambda)]} - \frac{6}{\lambda^2}\right]$$

$$= 4\left[\frac{1}{\lambda - 1} - \frac{6}{\lambda^2}\right] \qquad (15)$$

The last expression of (15) is obtained when λ is large, say, greater than 10. For really large λ values, equation (15) can be further simplified to give

$$Re = \frac{4}{\lambda} = \frac{24D\pi\eta\rho}{zeV\alpha KR} \qquad (16)$$

It is seen from (16) that better retention will be obtained by chosing a mobile phase of smaller viscosity and by applying a greater potential to the column. The radius of the capillary does not need to be very small.

DISCUSSION

The use of electrical double layers as the virtual "stationary phase" on an open capillary column for separations and determinations of charged macromolecules has many scientific merits. First, an open capillary column is theoretically expected to have greater resolving power than a conventional packed column, particularly for high molecular weight compounds. The upper limit in molecular sizes will be the diameter of the capillary which is way beyond the pore size of any gel materials. Accordingly, it would be the method of choice for characterization of very large molecules, biological cells or even viruses.

Secondly, since it requires no chemical stationary phase, problems often encountered in conventional HPLC, such as stationary phase immobilization, column bleeding, column regeneration, non-homogeneous packing, sample contamination, memory effects, etc., are either non-existant or greatly diminished.

Thirdly, retention depends not only on the magnitude but also on the polarity of the applied potential. A negative potential will only retard the migration of positively charged particles, and vice versa. Accordingly, positively and negatively charged molecules can be resolved separately. Furthermore, since the number of charges on a molecule depends on the pH value in the solution, pH programming together with potential programming will greatly enhance resolution and efficiency.

Besides, the minimal requirement in sample preparation, the possibility of miniturization, and the easiness in interfacing with other instrument make this technique very promising in becoming a universal separation tool for proteins, enzymes and other biochemical substances.

The real mechanism of this separation technique is still not clearly understood, but the above theory does provide a conceivable way of treatment. It relates solute retention to many experimental parameters in workable function forms. This relationship will be very useful in future development of the technology. At the present state of art, the major difficulties we experienced are the lack of a sensitive detector with essentially zero void volume and the elimination of electrode interferences at high potentials. However, the preliminary results clearly demonstrate that the approach is along the correct theoretical/experimental lines.

In our preliminary separation of protein mixtures consisting of ribonuclease A (MW 13,700), chymotrypsinogen A (MW 25,000), ovalbumin (MW 45,000), aldolase (MW 158,000) and blue dextran 2000 (MW 2,000,000), degassed phosphate buffer solutions were used as the mobile phase. The applied potential for these systems was limited by the electrolysis of water. A maximum of 5 volts has been safely used without observing serious bubble formation on the capillary column during separation, normally completed within 10 minutes. The potential limit may be extended if solvent of higher decomposition potential is employed.

Other electrode interferences, such as redox of the solute molecules or buffer ions, can be significant. Some of these reactions

may be beneficial and some adversary to separation. To make this technique practical, these interferences will have to be studied more carefully.

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