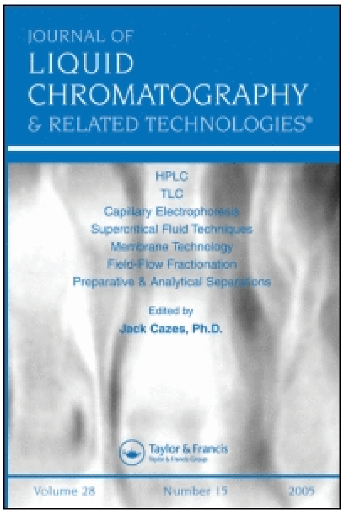

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DONNAN EXCLUSION - ION EXCLUSION CHROMATOGRAPHY

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ABSTRACT

A review of Donnan exclusion and ion exclusion chromatography is presented by extracting the phenomena of "pure" ionic exclusion by ion-exchangers from literature and by giving its theoretical background in terms of Donnan equilibrium distribution, not intending a complete collection of the literature on conventional ion-exclusion chromatography.

OUTLINE OF IONIC EXCLUSION STUDIES FROM LITERATURE

The phenomenon that co-ions of an ion-exchanger are excluded from the ion-exchanger by electrostatic repulsion with their fixed charged groups (anions from cation-exchangers or cations from anion-exchangers) was first utilized by Wheaton and Bauman (1,2) as "ion exclusion" in the chromatographic separation of electrolytes and nonelectrolytes. The separation of hydrochloric acid and acetic acid, hydrochloric acid and dichloroacetic acid, sodium chloride and ethyl alcohol, and other pairs of strong electrolyte and non- or weak electrolyte components were quantitatively separated with strong acid type cation-exchange resin Dowex 50W column and water eluent. The features of this ion exclusion chromatography are that water is usually the best eluent and that ionic components are eluted at the column interstitial volume, while nonionic components are eluted at the total column liquid volume in a typical case. These features may lead to a remarkable simplification of operation and, therefore, sometimes to an economical industrial utility. A number of separations on this principle were tried by several investigators in the early years. For example, the separation of sodium chloride and boric acid (4), thiocyanate and sulphide (5), and hydrochloric and acetic acid (6) were performed using strong acid type cation-exchanger of sodium form or hydrogen form.

The same techniques of column operation were applied to the mutual separation among organic weak acids or nonionic components (1,2,6,8,9,10). Acetic acid and acetone, or acetone and formaldehyde were separated with cation-exchanger Dowex 50W or anion-exchanger Dowex 1 (2). Harlow and Morman (6) developed a method for the separation and automatic determination of acids by ion exclusion chromatography with a column of hydrogen form Dowex 50W-X12 and water eluent. The elution of individual acids was automatically recorded by base titration for the effluent. By this technique, they could analyze a sample containing several acids such as hydrochloric, formic, acetic, propionic and other aliphatic acids. The same method was employed in the determination of dicarboxylic acids such as oxalic, malonic, succinic, glutaric acids, etc. (7), and also applied to the determination of trace amounts of organic acids in silage (8). Harlow and Morman presented retention data for over 50 acids of differing structure (6).

Although these data gave us very useful information for practical separability, an essential explanation for ion exclusion mechanism could not be shown, because most organic acids have more or less tendency of adsorption onto the ion-exchange resin called ion exclusion-partition chromatography. A rough tendency that weaker acids are eluted later than stronger acid could be seen, but there are also some exceptions; for instance, formic acid (pK 3.75) is eluted before chloroacetic acid (pK 2.75) and propionic acid (pK 4.87) before acryric acid (pK 4.26) by their data. The dependence of distribution coefficient for aliphatic monocarboxylic acid (C_1-C_6) and dicarboxylic acid (C_2-C_7) on their moleculal weight was studied in detail by Lehotay and Traiter (9) at different crosslinkages of the strongly acidic cation-exchanger. A remarkable increase in the distribution coefficient value with increasing molecular weight was observed in both mono- and dicarboxylic series, but this tendency was clearly beyond that expected from the less dissociation for the larger acid, which indicates that other nonionic factors are superimposed on ion exclusion mechanism in the distribution of the sample acid between the resin phase and the outer solution. In case of aromatic carboxylic acids, the elution behavior can be explained with more difficulty by pure ion exclusion, especially in the weak acids (10). For these solutes, adsorption by π-bonding effect or higher solubility to more "organic" resin phase than outer aqueous solution causes retarded elution from an ion-exchanger column; therefore it seems not proper to use a term "ion exclusion chromatography" for this case.

The ion exclusion phenomenon was observed also for crosslinked dextran gels such as Sephadex G-10 or G-25 used in gel chromatography, based on steric exclusion (11).

Since these gels usually contain fixed carboxylate groups as much as about 1 % of ordinary ion-exchange resin capacity, anionic species are excluded from the gel phase when water is employed as eluent. The elution order $Na_5P_3O_{10}$-Na_2HPO_4-NaCl was observed and glucose, having larger size than NaCl, was eluted the latest. With an increased concentration of eluent background electrolyte or sample salt itself, each elution volume increased except for glucose and the behavior came to show pure steric exclusion effect.

Ion exclusion effects in the chromatography of various compounds of biological interest such as polyphosphoric esters (12), amino acids and proteins (13,14, 15) were investigated using Sephadex (12), Sepharose (13), controlled pore glass (14), and DEAE-Sephadex and CM-Sephadex (15). Crone found two interesting empirical relationships on the retention of these biological components; one is the linear plot between $1/K_D$ and $1/I$ for agarose gels where K_D is the distribution coefficient and I the ionic strength of the eluent solution (13), and the other is the linearity between K_D of amino acids and their net positive or negative charge with anion or cation exchange Sephadex (15). The latter is very important, because this result may approach a fundamental clearance for ionic exclusion phenomena.

To give a correct explanation for ionic exclusion, a system, in which only ionic exclusion is involved and any other effects such as adsorption or steric exclusion are absent, should be investigated. Tanaka et al. (16, 17,18) studied such a system using many inorganic and organic acids of small size, and strong acid type cation-exchange resin for liquid chromatographic use. They obtained a linear relationship between pK_1 (the first-step dissociation constant) of the sample acid and capacity factor (retention volume, distribution coefficient) under a condition with water eluent (16,18). It may be difficult to give a theoretical basis for their results, because the pH may changes with the kind and the amount of sample component when water is employed as eluent. The quantity directly related to the retention should be the anionic charge and not the pK_1 itself. They also studied the effect of organic solvent content and temperature of the eluent on the retention volume (16,17). They concluded that the increased retention with organic solvent and at elevated temperature was due to the increased pK_1 value caused by the lowered dielectric constant of the eluent. The chromatographic determinations of phosphate, phosphite and hypophosphite ions with the 70 % acetone-water eluent (17), and of phosphate, pyrophosphate, tripolyphosphate and trimetaphosphate ions with the 80 % dioxane to water gradient eluent, were performed by purely ionic exclusion.

Waki and Tokunaga (20,21,22,23) extended the ionic exclusion system from the conventional very dilute media to much more concentrated eluents and gave a theoretical basis for the ionic exclusion behavior by studying an appropriate system consists of small size anions of widely different charges, a sulphonated crosslinked dextran cation-exchanger SP-Sephadex C-25 and a concentrated tetramethylammonium chloride eluent (when necessary, pH-buffered). They named "Donnan exclusion chromatography" (DEC) for such a purely ionic exclusion chromatography by the reason that the ionic exclusion behavior can be quantitatively expressed by a simple Donnan equilibrium relation. The conventional "ion exclusion chromatography" with water or very dilute eluents can, of course, be included in DEC in principle. In the application of DEC, a number of phosphorus oxoanions of different charges were separated to each other at their dissociated states using SP-Sephadex C-25, some metal ions using QAE-Sephadex A-25, and also mono-, di-, and trivalent organic acids were separated to each other (24).

As a special application of Donnan exclusion chromatography, the determination of stability constants for unstable ion-pairs in concentrated media such as trimetaphosphate complexes of calcium and magnesium were studied (23). A further description of the theory and the applications of Donnan exclusion chromatography is given in the following section.

THEORY AND APPLICATION OF DONNAN EXCLUSION CHROMATOGRAPHY

When a cation-exchanger is immersed in the solution of electrolyte B^+A^-, the distribution of the electrolyte and a sample anion X^{x-} are given by Donnan equilibrium,

$$[A^-]_r\,[B^+]_r\,\bar{y}_A\,\bar{y}_B = [A^-]\,[B^+]\,y_A\,y_B \qquad (1)$$

$$[X^{x-}]_r\,[B^+]_r^x\,\bar{y}_X\,\bar{y}_B^x = [X^{x-}]\,[B^+]^x\,y_X\,y_B^x \qquad (2)$$

where $[\ \ ]_r$ and $[\ \ ]$ are molar concentrations, y's activity coefficients and r or bar refers to exchanger phase. The distribution coefficient of the sample anion can be expressed as

$$K_D = R^x \qquad (3)$$

where

$$R = \frac{y_X^{1/x}\ y_B}{\bar{y}_X^{1/x}\ \bar{y}_B} \cdot \frac{[B^+]}{[B^+]_r} \qquad (4)$$

Since $[\,B^+\,]_r \gg [\,B^+\,]$ for all cation-exchangers at the dissociated state of the ion exchange group, R is smaller than unity as far as a remarkable $\bar{y}_X$ lowering due to adsorption does not take place. Therefore K_D is less than unity in pure ionic exclusion system (K_D for neutral species is unity) and in the order of

neutral species > mono- > di- > trivalent anion > ····.

This is also true for gels containing impurity ion-exchange groups when the outer solution is dilute.

The R increases with increasing concentration of the bulk electrolyte, since the increase in $[\,B^+\,]$ is more rapid than that in $[\,B^+\,]_r$ in spite of the electrolyte invasion. As a result, the K_D value of an anion increases with the B^+A^- concentration, though K_D of neutral component is unvaried at unity. The approximate manner of the K_D change for differently charged anions can be drawn using the relation of eqns (1) to (4) under proper assumptions. Assuming for simplification that the presence of other components than the bulk solute B^+A^- in both phases is negligible in quantity and therefore $[\,B^+\,]_r = C_r + [\,A^-\,]_r$ where C_r is the volume base ion-exchange capacity, and that the activity coefficient of any ion has no difference between the exchanger phase and the outer solution, the distribution coefficient of the sample anion can be written as

$$K_D = \left(\frac{2[\,B^+\,]}{C_r + \sqrt{C_r^2 + 4[\,B^+\,]^2}} \right)^x \qquad (5)$$

The typical model of K_D change with outer electrolyte concentration is shown in Fig. 1 where it is assumed that C_r does not vary with the bulk electrolyte concentration for simplified calculations. The calculations are made for an ion-exchanger of assumed capacity C_r=1 which corresponds to a considerably swollen type, and

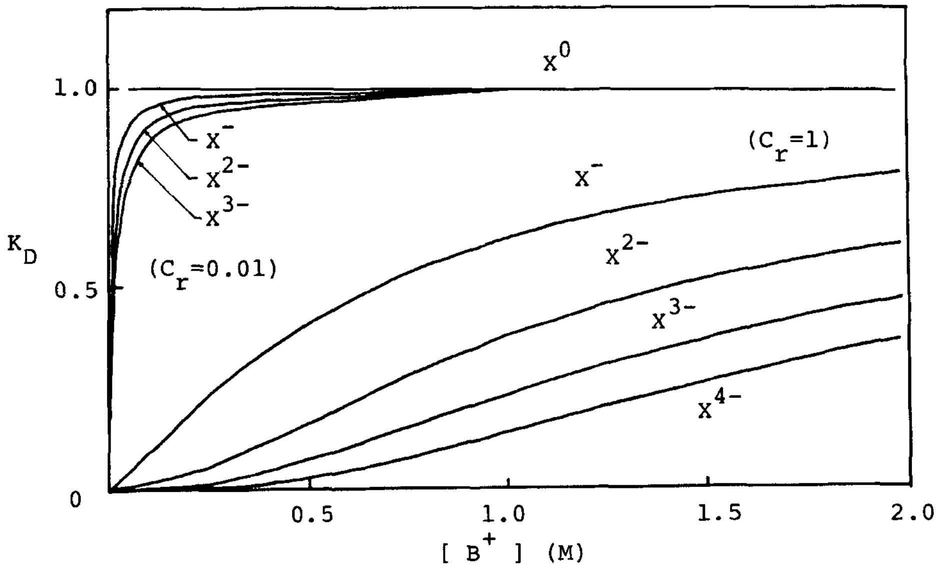

FIGURE 1

The K_D variation calculated for different charges with outer electrolyte concentration.

for a gel of impurity ion-exchange capacity $C_r = 0.01$. At very low outer concentrations, all K_D values for anions become zero, so the conventional separations of nonionic solutes and ionic solutes have been carried out with water eluents. However, this condition is powerless for the mutual separations between differently charged anions, and eluent of high electrolyte concentration should be used in such a case. For example, the condition of 0.5 to 1.0 M concentration is considered to be best for the separation of monovalent and divalent anions, and the more concentrations are recommended for higher charged ones. This behavior should somewhat change depending on the ion-exchange capacity, but a similar trend in the K_D change was confirmed by the measurements with phosphorus oxoanions for SP-Sephadex C-25 and tetramethylammonium chloride eluents of various concentrations (22). The separation between charged ions with a non-ion-exchanging gel which contains only impurity ion-exchange groups may be very difficult, because the concentrated electrolyte eluents bring about undistinguishable K_D values approaching unity. Even if there could be found a good condition in theory for the separation, such a system may be unstable and a slight variation in conditions may give a tremendous disturbance to the eluting position. On the other hand, the use of ion-exchangers of extremely high capacity like Dowex 50W-X8 or -X12 may also be unrecommendable because of inconvenience in employing extremely concentrated electrolyte eluents and by kinetic reasons.

Another important empirical finding is that R in eqn (3) is constant irrespective to sample species under a given eluent condition and therefore there is a linear relationship between log K_D and ionic charge x. Fig. 2 shows such a relationship in the system of SP-Sephadex C-25 and 1.00 M tetramethylammonium chloride eluent. The K_D here was chromatographically determined by the equation

$$V_e = V_0 + K_D \cdot V_D \qquad (6)$$

where V_e is the elution volume of a sample ion, V_0 the void volume and V_D the net internal volume of ion-exchanger phase. V_0 was assumed from the elution volume of ions of very high charge. V_D was calculated from the elution volume for neutral species such as ammonia or crotonaldehyde assuming $K_D = 1$. It can also be seen from Fig. 2 that K_D values are decided only by the ionic charge of the sample ion but molecular structure has no effect on the elution position as far as the

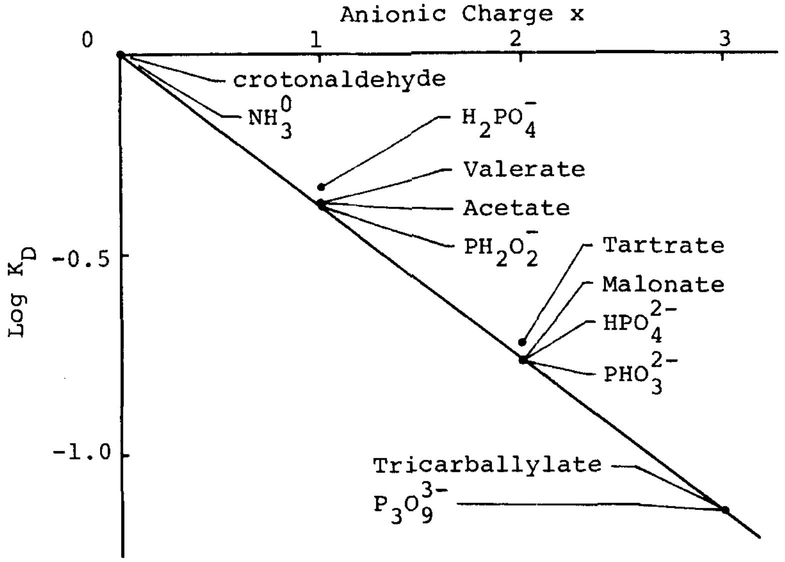

FIGURE 2

The relationship between charge and distribution coefficient K_D.

molecular size is not very large, as indicated by the identical K_D values for acetate and valerate anions in the same aliphatic monocarboxylate series. Fig. 3 is an example of DEC separation for various oxopolyanions of phosphorus based on the charge difference (20). For large ions whose molecular sizes are beyond about 10 Å in length, however, a steric exclusion effect may be superimposed on the ionic exclusion in the chromatography (24).

The same type of separation was made for the organic acid anions of different charges such as monovalent acetate, divalent tartrate and trivalent tricarballylate anions (24).

The DEC principle can be applied also to the separation of cations with an anion-exchanger, consider-

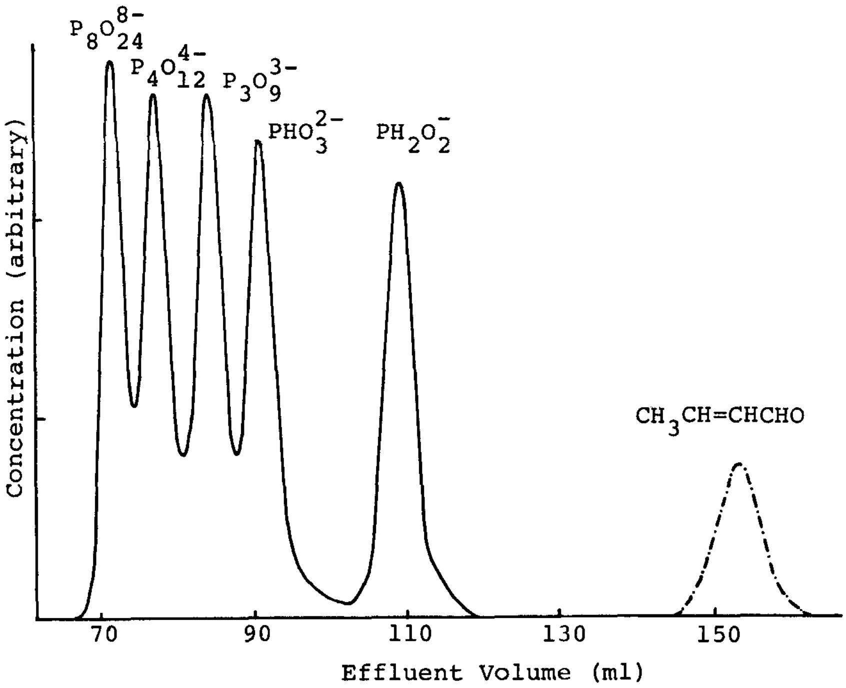

FIGURE 3

Separation of phosphorus oxoanions by DEC (20).
Colmn; SP-Sephadex C-25 $(CH_3)_4N^+$-form, 95.8 X 1.5 cm.
Eluent; 1.00 M $(CH_3)_4NCl$ (pH 9.8). Flow rate; 0.75 ml/min.
Sample; each 5 X 10^{-3} mmol(as P).
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ing the reversed sign of electrical charge for everything. Fig. 4 represents the separation of cobalt, potassium and silver with nitrate form QAE-Sephadex A-25 and 0.3 M sodium nitrate eluent. Here silver has about -0.5 mean charge by partial ion-pair formation with nitrate ion, therefore the separation from monovalent cation K^+ is successful (20). In the practical separation by DEC, the charges of ions to be separated are

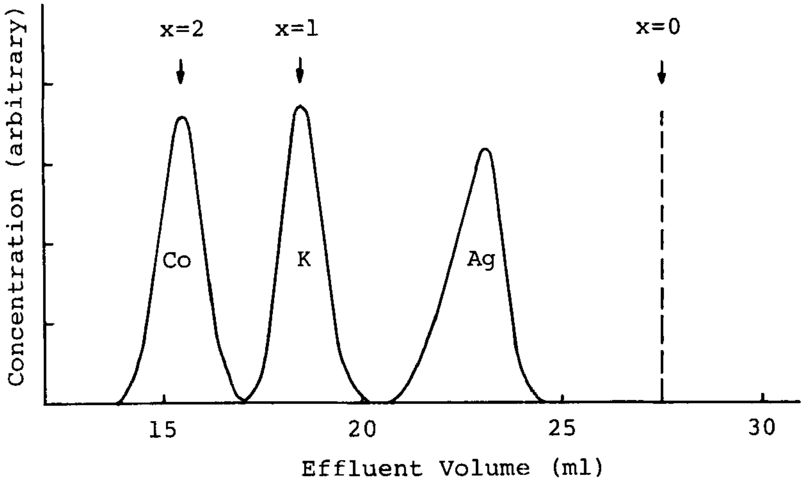

FIGURE 4

Donnan exclusion chromatography for cations (20). Column; QAE-Sephadex A-25 NO_3^--form, 39.0 X 1.0 cm. Eluent; 0.3 M $NaNO_3$. Flow rate; 0.4 ml/min. Sample; each 0.025 mmol.

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not necessarily integers. Between the eluting positions for monovalent ion and neutral component, there is usually a large elution space to be utilized. The Donnan exclusion for nonintegral charges can be treated as the case of an equilibrium mixture sample, as described below, briefly.

In an equilibrium mixture M-L system (M:cation, L:sample anion), the apparent distribution coefficient of L at excess M can be expressed by

$$K_D = K_{D,L}\,\alpha_L + K_{D,ML}\,\alpha_{ML} + K_{D,M_2L}\,\alpha_{M_2L} + \cdots \quad (7)$$

assuming that any cationic M-L complexes are not formed where the K_{D,M_jL} is the distribution coefficient characteristic to the charge of M_jL and α_{M_jL} its mole fraction for all L species. When all α's are calculable from known stability constants, K_D (therefore the eluting position of L) can be predicted.

Let's consider the simplest binary system of a partly dissociated monobasic acid HL. The distribution coefficient of L is

$$K_D = \frac{[\,HL^0\,]_r + [\,L^-\,]_r}{[\,HL^0\,] + [\,L^-\,]} = \frac{K_{D,HL^0} + K_{D,L}\cdot K_1/[\,H^+\,]}{1 + K_1/[\,H^+\,]}$$

$$= 1 - \bar{i}(\,1 - K_{D,-1}) \qquad (8)$$

introducing the first dissociation constant K_1 of the acid, where $\bar{i}$ is the average anionic charge and $K_{D,-1}$ is the K_D of monovalent anion which is a constant irrespective of species for a given eluent. For water or extremely dilute eluents

$$K_D = 1 - \bar{i} \qquad (9)$$

since all anions are completely excluded and R in eqn (4) becomes zero. Anycase, the plot of K_D vs. $\bar{i}$ should give a straight line with a negative slope, such a behavior being obtained with amino acids (15). Similarly, K_D for polybasic acids in the water eluent system is written as

$$K_D = \frac{1}{1 + K_1/[\,H^+\,] + K_1K_2/[\,H^+\,]^2 + \cdots} \qquad (10)$$

Any simple relationship between K_D and pK_1 may not be present. Only when K_2 is very small compared with K_1, and the eluent pH is kept constant, the approximate linearity may be found for the acids whose pK_1 values range around the eluent pH value (16,18).

A complex formation may sometimes effectively be utilized in the DEC separation. For instance, the separation of ortho-, pyro- and tripolyphosphate anions is difficult at their completely dissociated forms, since the charges are -3, -4 and -5, respectively. In general, the quantitative separation between adjacent charges for ions higher than trivalent is not easy even if a large column is used. In such a case, the separation may be improved by lowering their charges through protonation or ion-pair formation by the use of the eluent of lower pH and different composition (20).

Finally, it must by said that the Donnan exclusion chromatography having a unique nature, that the eluting position depends only on the ionic charge at a given eluent condition, may be applicable to the evaluation of acid dissociation constants or stability constans of labile unstable complexes. The stability constants of trimetaphosphate complexes with magnesium and calcium were determined by this technique (23).

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