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Review

Principles and applications of ion-exclusion chromatography

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ABSTRACT

Ion-exclusion chromatography is a relatively old technique that has been attracting increased attention. It is particularly useful for separating hydrophilic molecular species from one another and from large amounts of ionic materials. The basic principles and methodology of ion-exclusion chromatography are presented and illustrated with typical examples. Several recent developments in ion-exclusion chromatography are discussed. These include methods for the enhancement of conductivity detection of weakly ionized substances and a new method for the chromatographic separation and detection of water.

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1. INTRODUCTION

Ion-exclusion chromatography actually involves the chromatographic separation of molecular species rather than ions. Of course, ions can often be readily converted into molecular species as is the case when anions of weak acids are acidified. Another possible reason for including ion-exclusion chromatography in a symposium devoted to ion chromatography is that an ion-exchange column is generally used for separations performed by ion-exclusion chromatography.

The most widely accepted explanation of ion-exclusion chromatography is that separation occurs because of differences in partition of molecular solutes between the

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eluent and eluent that is immobilized within the resin phase. Sample anions are excluded from the resin phase by the fixed charges of the sulfonate groups of the cation-exchange resin. If an anion-exchange column is used, sample cations are excluded by the fixed positive charges on the resin. Thus sample ions pass rapidly through the column and elute as a group. Molecular substances are free to partition into the occluded solvent within the resin and are therefore separated from the anions and from one another. The ability to separate molecular substances from much larger amounts of ions is one of the appealing advantages of ion-exclusion chromatography.

The mechanism of the separation process is undoubtedly more complex than the explanation that has just been given. Partly for this reason ion-exclusion chromatography has often been given other names such as Donnan exclusion ion-exclusion chromatography, ion-exclusion partition chromatography and ion-moderated partition chromatography.

Excellent and comprehensive reviews of ion-exclusion chromatography have been given by Gjerde and Mehra [1] and by Haddad and Jackson [2]. The goal of the present paper is to review the fundamental theory and typical applications in a concise manner and to focus on some selected recent developments.

2. METHODOLOGY AND THEORY

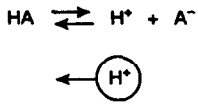
Separations by ion exclusion are carried out on a cation-exchange column, or occasionally on an anion-exchange column. The cation resin contains sulfonate groups ($-SO_3^-$) or mixed sulfonate and carboxylate groups [1]. The resin is most commonly used in the H^+ form, although another cation can serve as the counter ion. In fact, effective separation of sugars necessitates the use of a resin in the Ca^{2+} form. The introduction of ionic groups causes a microporous resin to take up water and become a gel. However, macroporous ionic resins have a large surface due to numerous pores and channels that can hold occluded water. In either case molecular solutes can partition between the mobile phase solvent and the occluded solvent within the resin phase. Assuming this is the only operative mechanism, the solute retention volume (V_R) is given by:

$$V_R = V_0 + DV_i$$

where V_0 is the interstitial volume of eluent (the eluent outside the resin beads), V_i is the internal volume of eluent (the occluded eluent within the resin beads), and D is the distribution ratio of a given solute. If a solute cannot enter the resin phase because of very large size (size exclusion) or because it is an ion (ion exclusion), the value of D is zero. If a solute is entirely molecular and entirely free to enter the resin phase, the value of D is 1. However, solutes may vary in their ability to enter the resin occluded phase and D can therefore be some value between zero and 1. This short range of D values provides only a narrow window for separations. For this reason, columns used in ion-exclusion chromatography frequently are fairly long and of large diameter in order to provide a good separation of samples containing several molecular compounds.

Acids are found to elute in the decreasing order of their acid dissociation constants, the stronger acids eluting first. Even fairly weak acids may be partially ionized and thereby exist as a mixture of the molecular acid and the acid anion. The

presence of more than one species results in broad peaks. It is common practice to avoid this complication by incorporating a low concentration (1 to 10 m*M*) of a strong acid (sulfuric acid for example) to the eluent. The H^+ from this acid represses the ionization of a weak acid solute (HA) and sharpens the chromatographic peak.



Factors other than partition between the mobile and occluded solvent phases are likely to affect the chromatographic behavior of molecular solutes. There may be a size effect in which molecules of larger size have greater difficulty in entering the resin. Older work [3] has indicated that the extent of resin cross-linking also plays a role. Finally, as the size of the molecule increase there is likely to be an increasing hydrophobic interaction between the molecule and the polymeric matrix. This interaction is especially strong when the molecule contains a benzene ring. Addition of an organic solvent modifier to the aqueous eluent will reduce hydrophobic interactions of organic solutes, sharpen the peaks, and reduce the retention time. However, an organic solvent can be added to the eluent to *increase* the retention of a polar solute such as ammonia [4].

2.1. Detectors

It is best to select a detector that responds well to the solutes to be determined but not to the eluent itself. A variable-wavelength UV detector, a refractive index detector, or some type of electro chemical detector will often meet these criteria. However, conductivity continues to be the most popular and widely used detector for ion-exclusion chromatography. As already discussed, it is often necessary to add a low concentration of a strong acid to the eluent to repress the ionization of weakly acidic sample constituents. This means that a relatively small increase in an already high background conductivity needs to be measured.

Two strategies have been used to remedy this situation. The first is to reduce the background conductance of the eluent.

(1) The oldest method is to place a Ag^+-form cation-exchange column between the separation column and the detector cell and to use hydrochloric acid as the strong acid added to the eluent. The hydrochloric acid is effectively removed by the ion-exchange suppressor:

$$H^+Cl^- + \text{resin-}Ag^+ \rightarrow \text{resin-}H^+ + AgCl(s)$$

The silver chloride stays in the suppressor column and gradually plugs it. One answer to this problem was to use a plastic tube as the suppressor and periodically cut off the plugged portions.

(2) A cation-exchange membrane can be used as the suppressor. This is kept in the tetrabutylammonium cationic form (TBA^+) by continuously circulating TBA^+Cl^- solution over the outside of the membrane. The H^+Cl^- of the eluent is thereby converted to TBA^+Cl^- which has a significantly lower conductance (Table I).

TABLE I

RELATIVE CONDUCTANCE OF 1 m*M* SOLUTIONS USED IN ION-EXCLUSION CHROMATOGRAPHY

TBA = Tributylamine; OSA = octanesulfonic acid; TDFHA = tridecafluoroheptanoic acid.

Ion pair	Conductance (μS)
H^+Cl^-	425
TBA^+Cl^-	100
TBA^+OSA^-	40
TBA^+TDFHA^-	30

A still lower conductance is obtained by using a strong acid in the eluent that has an anion that is larger and less conducting than chloride [5].

(3) Tanaka and Fritz [6] showed that addition of a somewhat weaker and less conducting acid to be the eluent will still repress the ionization of carboxylic acid solutes and given sharp chromatographic peaks. An eluent containing 5 m*M* benzoic acid has a background conductivity of only 5.6 μS cm^{-1} compared to 81.6 μS cm^{-1} for 5 m*M* sulfuric acid. The detector response for alkane carboxylic acids is higher for the benzoic acid eluent than for the eluent containing sulfuric acid. Later it was found that succinic acid worked as well as benzoic acid and required a much shorter time for initial equilibration of the column.

A second strategy for improving the sensitivity of conductivity detection in ion-exclusion chromatography is to enhance the conductivity of the sample solute(s).

(1) Carbon dioxide and carbonates can be determined by ion-exclusion chromatography on a H^+-cation-exchange column using pure water as the eluent. The conductivity detection signal is weak owing to the small degree of ionization of carbonic acid. Tanaka and Fritz [7] showed that the detection signal could be increased several fold by inserting ion-exchange "enhancement" columns between the separation column and the detector cell. The first enhancement column is a cation exchanger that converts carbonic acid into potassium bicarbonate, which is more completely ionized and therefore more conducting.

$$H_2CO_3 + \text{resin-}K^+ \rightarrow \text{resin-}H^+ + K^+HCO_3^-$$

This reaction appears to be quantitative, which might seem surprising because of the weakly acidic nature of carbonic acid. However, the concentration of K^+ in the resin phase is very high (*ca.* 4 *M*) and is therefore able to shift the equilibrium strongly to the right.

An anion-exchange column in the hydroxyl form serves as a second enhancement column. This converts potassium bicarbonate to potassium hydroxide and further increases the conductivity.

$$K^+HCO_3^- + 2\,\text{resin-}OH^- \rightarrow \text{resin}_2\text{-}CO_3^{2-} + K^+OH^- + H_2O$$

(2) A Japanese group [8] used this enhancement principle to improve the conductivity detection of carboxylic acids, dicarboxylic acids, and fluoride. A hollow-fiber, cation-exchange membrane was used as the enhancement unit, and an appropriate electrolyte flowed over the outside to provide continuous regeneration. They found sodium sulfate regenerant to give better chromatographic peaks than sodium hydroxide. The carboxylic acid solutes were converted to the highly ionized sodium salts

$$RCO_2H + \text{cation exchanger-}Na^+ \rightarrow \text{cation exchanger-}H^+ + Na^+RCO_2^-$$

Similarly, the sulfuric acid in the eluent was converted to the lower conducting sodium sulfate. A very large peak enhancement of approximately 34-fold was obtained using this system.

(3) Okada and Dasgupta [9] used a similar membrane system to convert nitric acid in the eluent to sodium nitrate and weakly acidic solutes (HA) to the sodium salt (Na^+A^-); sodium salts of weak acids are of course bases. The increase in pH is measured using 4-nitrophenol as indicator.

3. SELECTED APPLICATIONS

3.1. Inorganic acids and anions

Fluoride [10,11], phosphite and hypophosphite [12], arsenate [12] and nitrite [13–15] are readily determined by ion-exclusion chromatography. Bicarbonate [6,16] and sulfide [16] are anions of very weak acids and can be determined using only water as the eluent. Determination of sulfite in food [17–19] is now of considerable interest owing to a U.S. Federal regulation limiting the permissible amounts. A high-pH buffer is used to extract both "free" sulfite and sulfite that is bound to carbonyl compounds in food. Free sulfite only is determined after extraction with a pH 2.0 buffer.

Borate is separated by ion-exclusion chromatography as boric acid [20,21] but the ionization is so slight that sensitivity of conductivity detection is poor. Use of an aqueous eluent containing mannitol or fructose forms a complex that is more highly ionized and better detected by conductivity [22].

3.2. Organic acids

Determination of low-molecular-weight organic acids is probably the most common use of ion-exclusion chromatography. Acids such as formic, acetic, hydroxyacetic, citric, tartaric, oxalic, malonic and ascorbic acid have been determined in a large number of aqueous samples. Good compilations are available in recent books [1,2,4]. The eluent is always predominately aqueous and usually contains a low concentration of a strong acid to maintain the sample acids in the molecular form. A number of acids can often be separated with good resolution in a single run.

3.3. Sugars

Sugars can be separated by ion-exclusion chromatography on a cation-exchange column in the H^+ form [23]. However, better separations are obtained on a Ca^{2+}-form column where complexation probably plays a major role. Sucrose, a disaccharide, elutes before dextrose, fructose and other monosaccharides. Oligomers elute still earlier in order of decreasing molecular weight.

Detection of sugars presents a problem. A differential refractometer or a UV spectrophotometer at 195 nm can be used but the sensitivity is somewhat limited. Cowie *et al.* [24] were able to detect reducing carbohydrates potentiometrically using a metallic copper electrode.

3.4. Polar molecular compounds

Low-molecular-weight alcohols and glycols have been determined by ion-exclusion chromatography using conductivity [23,25] or refractive index [26] as the detector. Dimethylsulfoxide has been determined in sea water using a UV detector [27]. McClure [28] determined parts-per-billion (10^9) concentrations of formaldehyde in aqueous solution using post-column detection with acetylacetone and direct UV detection at 420 nm.

3.5. Determination of water

If an alcohol such as methanol can be separated by ion-exclusion chromatography using water as the eluent, why not do the reverse and separate water using a methanol eluent? Stevens *et al.* [29] did just this. They added a small amount of sulfuric acid to the eluent and detected the chromatographic water peak by a decrease in conductivity. The main drawback with this method was a non-linear calibration curve with very poor detection sensitivity in some concentration regions.

Fortier and Fritz [30] devised a unique equilibrium system for spectrophotometric detection of water after separation by ion-exclusion chromatography. This method has been studied and refined by continuing research of Chen and Fritz [31,32]. Water is separated chromatographically from the sample matrix on a short column packed with cation-exchange resin in the H^+ form using dry methanol as the eluent. Detection of the water peak is made possible by addition of a low concentration of cinnamaldehyde to the methanol eluent. In the presence of an acid catalyst, such as a H^+-cation exchanger, cinnamaldehyde reacts with methanol to form the dimethylacetal.

$$C_6H_5CH{=}CHCHO + 2\,CH_3OH \overset{H^+}{\rightleftarrows} C_6H_5CH{=}CHCH(OCH_3)_2 + H_2O$$

The UV spectra of cinnamaldehyde and the acetal are quite different; only the cinnamaldehyde absorbs strongly around 300 nm. Since most of the cinnamaldehyde has been converted to the acetal, the background absorbance at 300 nm is low. However, a water zone passing through the column will shift the equilibrium towards the formation of more cinnamaldehyde and the absorbance at 300 nm will increase.

$$H_2O + \text{acetal} \overset{H^+}{\rightleftarrows} \text{aldehyde} + 2CH_3OH$$

In methanol the equilibrium constant, K, has been measured [31].

$$K = \frac{[\text{aldehyde}]}{[\text{acetal}]\,[H_2O]} = 5.3 \cdot 10^{-4}$$

The detector signal (A_{det}), which is the change in absorbance when water passes through the detector cell is given by the following equation

$$A_{det} = kC_{ca}(C_{samp} - C_{blank})$$

where k is a proportionality constant related to the equilibrium constant K, C_{ca} is the total concentration of cinnamaldehyde added to the eluent, C_{samp} is the water concentration of sample, and C_{blank} is the water concentration in the eluent itself. As predicted by this equation, the detector signal has been shown experimentally to be a linear function of the total aldehyde and of the concentration of water present.

Typically, a sharp water peak is obtained in approximately 2 min. The water peak is always well separated from an earlier injection peak that is due to the sample matrix. Under favorable conditions a very short column (length 2.5 cm) can be used and a water peak obtained in as little as 20 s [32].

Samples that can react with methanol pose a special problem for water determination. For example, how can small amounts of water in acetone be determined when acetone can react with methanol to produce a much larger quantity of water?

$$CH_3COCH_3 + 2CH_3OH \overset{H^+}{\rightleftarrows} CH_3C(OCH_3)_2CH_3 + H_2O$$

The key to this problem is that the above reaction will not take place unless H^+ is present to catalyze the reaction. By using a cation-exchange column in the Li^+ form, water in the acetone can be separated chromatographically from the acetone. A H^+-form column placed in series then catalyzes the cinnamaldehyde–acetal equilibrium shift that is necessary for detection of the water. Reaction of acetone with methanol to form water is also catalyzed in this second column, but separation of the acetone and initial water has already taken place in the first column.

The detection limit of water is limited in part by the amount of water in the

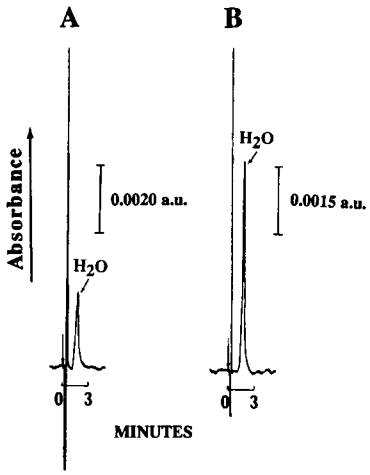


Fig. 1. Determination of water by ion-exclusion chromatography. For conditions, see text. (A) 26 ppm H_2O in anhydrous decahydronaphthalene; (B) 46 ppm H_2O in anhydrous acetonitrile.

methanol eluent. It is impossible to remove all of the water from methanol by conventional methods such as addition of molecular sieves or distillation after adding a reagent such as calcium hydride. We have recently found that addition of an *ortho* ester (plus an acid catalyst) to methanol will reduce the water content to an extremely low level [33]. Fig. 1 shows chromatograms for determination of only 26 ppm and 46 ppm of water, respectively in "anhydrous" samples of two organic liquids. These were run with a methanol eluent that had been dried by addition of trimethylorthoformate.

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