Review

Factors affecting selectivity in ion chromatography

James S. Fritz*

Department of Chemistry, Iowa State University and Ames Laboratory - US Department of Energy, Ames, IA 50011-3111, USA

Available online 30 January 2005

Abstract

Methods for separation of ions by ion-exchange, ion-pair, and zwitterion ion chromatography share at least one common thread – the induced formation of a cation–anion pair in the stationary phase. Selectivity can be defined as the relative ability of sample ions to form such a pair. Examples are given in anion-exchange chromatography to show the effect of variations in the geometry, bulkiness and polarity of the resin cation on selectivity. The type of resin matrix, the hydrophobic nature of the resin surface and the degree of solvation also affect chromatographic behavior. The selectivity series observed in ion chromatography seems to be best explained by the interplay of two components: electrostatic attraction (ES) and the enforced-pairing (EP) that is brought about by hydrophobic attraction and by water-enforced ion pairing. Selectivity in ion-pair chromatography (IPC) and in zwitterion ion chromatography (ZIC) is affected by both the mobile phase cation and anion. This leads to elution orders for anions that are different from conventional ion-exchange chromatography (IC) of anions where cations are excluded from the stationary phase and have little effect on a separation. The elution order of anions in ZIC is similar to that in IC except for small anions of 2− charge, which are retained more weakly in ZIC. A unique advantage of ZIC is that sample ions can be eluted as ion pairs with pure water as the eluent and a conductivity detector. The mechanism for separation of anions on a zwitterionic stationary phase has been a subject for considerable debate. The available facts point strongly to a partitioning mechanism or a mixed mechanism in which partitioning is dominant with a weaker ion-exchange component.
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Keywords: Selectivity; Ion chromatography

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* Tel.: +1 515 294 5987; fax: +1 515 294 3578.
  E-mail address: kniss@ameslab.gov.

1. Introduction

Here, work is reviewed concerning how chromatographic ion separations are affected by variations in the physical and chemical constitution of the stationary phase and by changes in the chemical nature of the mobile phase. The effects observed in ion-exchange chromatography (IC), ion-pair chromatography (IPC), and zwitterion chromatography (ZIC) and to a limited extent, liquid–liquid extraction, are compared. Pertinent theories concerning the mechanism of the separation processes are also discussed briefly. A major goal is to arrive at a simple and unified theory that explains the similarities and differences between conventional IC and ZIC.

2. Ion-exchange chromatography

2.1. Polymeric matrix effects

Selectivity in anion IC is affected by the polymeric matrix of the ion exchanger. In one study [1], columns were prepared by applying a permanent coating such as cetylpyridinium chloride (CPC) to a porous cross-linked polystyrene resin (XAD-1) or to a polyacrylate resin (XAD-8). Polarizable anions (nitrate and especially iodide) gave significantly larger retention factors (relative to chloride) on the coated polyacrylate resin. Conversely, the relative retention of sulfate was 2.4 times higher and that of thiosulfate was 3 times higher on the coated polystyrene material.

Polarizable anions are often found to give relative long retention times and tailed peaks when chromatographed on anion exchangers with a polydivinylbenzene polymeric matrix [2,3]. This is most likely the result of hydrophobic interaction of the sample anions with the resin. Incorporation of ca. 10% methanol into the mobile phase has been used to alleviate this difficulty.

In preparing anion-exchange columns by permanently coating a cationic surfactant onto a reversed-phase silica column, the underlying surface can be made less hydrophobic by applying an initial coating of a nonionic surfactant. Peak shape and chromatographic efficiency were markedly better when a C_{18} silica column was coated first with Triton X-100 and then with the cationic surfactant CPC [4]. This provides further confirmation of this hypothesis. Ion-exchange retention behavior is determined by both the lower neutral surfactant layer and the upper charged surfactant layer acting in concert, and not by the upper layer alone.

The physical nature of the polymeric matrix can certainly affect selectivity. Two basic types of polymeric ion exchangers have been used. Early gel-type resins were basically polystyrene materials with a limited degree of cross-linking. These were often designated as X-4, X-8, etc., to indicate the percentage of styrene-divinylbenzene (DVB) added to the polymerization mixture as the cross-linking tended to restrict the access of larger ions to the interior of the resin where the bulk of the actual ion exchange took place. The polymeric

ion exchangers used in modern ion chromatography tend to be macroporous materials. The structure of each spherical bead consists of a network of micro particles with numerous pores and channels. The bulk of the ion exchange takes place at exchange sites on the solid micro particles within the resin bead. The pore size and chemical nature of the polymer will affect the ease with which competing ions can enter the resin and undergo ion exchange.

Many ion-exchange materials currently used for ion chromatography are "pellicular," meaning that ion exchange only occurs at a thin layer on the outer part of the spheres. Cation exchangers are "surface sulfonated" and anion exchangers have positively-charged latex particles attached to the sulfonated layer. However, the selectivity of these pellicular materials is still affected by the porosity and degree of cross-linking of the interior polymer as well as the latex particles.

2.2. Resin functional groups

Okada [5] compared the effects $-NH_3^+$ and $-NEt_3^+$ groups in anion exchangers of the same polymeric matrix and almost the same exchange capacity (0.4 mmol/g). The $-NH_3^+$ resin had a more concentrated charge and a stronger electrostatic field than the resin with $-NEt_3^+$, where the + charge was more dispersed. Going from $-NH_3^+$ to $-NEt_3^+$ resulted in decreased electrostatic and hydrogen bonding interactions with sample anions and increased ion-induced dipole and London dispersion interactions. The latter two effects resulted in preferable binding of larger ions by $-NEt_3^+$. As an example, the ratio of retention factors of ClO_4^-:Cl^- was 17.4 for the resins with $-NEt_3^+$ but only 1.89 for those with $-NH_3^+$ groups.

A novel anion-exchange resin was prepared by reacting chloromethylated polystyrene particles with diethylenetriamine to produce a functional group with three nitrogen atoms [6]. By adjusting the pH of the mobile phase, this group would have a net charge ranging from 1+ to 3+. With an eluent (sodium perchlorate) that was completely ionized, the retention times of sample anions decreased steadily going from pH 2.2 to 8.2. An interesting feature of this resin was the unusually long retention time of sulfate compared to chloride and other monovalent anions. This may have been the result of very strong electrostatic attraction between the 2− sulfate and the high net charge on the resin cation.

Barron and Fritz investigated the effect of functional group structure on the selectivity of low-capacity anion exchangers for monovalent [7] and divalent [8] anions. A number of macroreticular anion-exchange resins of low capacity were prepared by chloromethylation of XAD-1 under mild conditions, followed by reaction with the appropriate tertiary amine. These resins were then evaluated to determine their relative selectivities for 17 monovalent anions. While the relative affinity of the resins for some anions changes very little as the resin structure is altered, other anions exhibit pronounced changes. Increasing the size of the R group in the $-NR_3^+$ exchange sites in a series from R = methyl to octyl substantially

increased the retention (relative to chloride) of larger ions like nitrate, chlorate, iodide and thiocyanate. However, the relative retention of divalent anions (sulfate, thiosulfate, oxalate) decreased as R became larger.

Virtually all anion-exchange resins contain a quaternary ammonium group as the cation. By using a resin with the bulkier tetraalkylphosphonium group, the retention of nitrate is increased substantially. An ion exchanger of this type selectively removes nitrate from contaminated water supplies [9].

Replacement of one or more of the alkyl groups in an anion exchanger with a more polar substituent has a major effect on selectivity. The classical Type II anion exchangers which have $-N(CH_3)_2CH_2CH_2OH$ have a much lower affinity for common anions compared to OH^- than the Type I exchangers with $-N(CH_3)_3$ functional groups [10]. Dionex Corporation used this principle to design anion exchangers with which sodium or potassium hydroxide could be used as an effective eluent.

2.3. Solvation effects

Incorporation of some organic solvent into the aqueous mobile phase is an intriguing way to modify selectivity in IC. Rubin and Stillian [11] pointed out that the earlier polymers used in Dionex ion exchangers would swell so much that even a small amount of organic solvent could not be tolerated in the eluent. By increasing the polymer cross-linking to 50% their modern ion exchangers are compatible with all solvents commonly used in HPLC.

In discussing solvation effects, it was stated that solvent molecules are oriented around ions in an ordered manner. Use of a mixed organic–aqueous mobile phase allows intrusion of organic molecules into the aqueous solvation sphere. For an ion to adsorb onto an ion-exchange site, it must first rearrange and eventually partially shed its solvation sphere to allow close approach to the ion-exchange site; the closer it can approach the more tightly bound it becomes. Likewise, an ion-exchange site must reorient its solvation sphere to permit the ion to approach. Combining these concepts leads to the notion of selective mediation.

The use of a mixed aqueous–organic mobile phase often has the practical advantage of providing faster separations. As one example, a mixture of nine anions was separated on a Dionex AS-11 column with 45 mM NaOH in 40% methanol as the stationary phase [11].

The first work in non-suppressed cation chromatography illustrates the vital role of solvation within the ion-exchange phase [12]. Attempts to separate mixtures of alkali metal and alkaline earth cations with a column containing a lightly sulfonated macroporous polystyrene-DVB resin all failed. However, a sulfonated microporous polystyrene–4% DVB resin that was normally used for gel permeation chromatography gave excellent separations of 1+ and 2+ cations.

Later research has reaffirmed these early results [13]. A separation of alkali metal ions was first attempted in wa-

ter alone using the lightly sulfonated macroporous cation exchanger with aqueous 3 mM methanesulfonic acid as the eluent. Under these conditions the sample cations exhibited very similar retention times. The selectivity of the macroporous resin for alkali metal ions was improved considerably by chemically introducing hydroxymethyl groups [14] prior to sulfonation.

These results seem to indicate that solvation of the resin plays a role in imparting selectivity for the various sample ions. Microporous cation-exchange resins form a gel and are highly hydrated within. With sulfonated macroporous resins the hydrated alkali metal ions may be repelled somewhat by the hydrophobic resin matrix. The presence of hydroxymethyl groups on the macroporous resin makes it less hydrophobic and improves selectivity for the hydrated alkali metal cations. When the sulfonated macroporous resin was used with the same acidic eluent in 100% methanol instead of water, a very good chromatographic separation was obtained (Fig. 1). Now the alkali metal ions are solvated more with methanol than with water and the resin matrix is probably coated with a thin layer of methanol, which make the ions and resin surface more compatible with one another.

Okada and Harada [15] studied the local structures of chloride and bromide within an anion-exchange resin by means of X-ray adsorption fine structure (XAFS). The resins containing a quaternary ammonium cation and Cl^- or Br^- as the counter ion were equilibrated under various partial vapor pressures to allow the anions to have various hydration numbers. At an average hydration number of 3, all water molecules are coordinated with the anion. As more water molecules are added, the more heavily hydrated anion dissociates from the cationic groups on the ion exchanger. It was also shown an average of 2.1 water molecules are stripped off in transfer of Cl^- from the bulk solution to the resin. An average of 2.6 water molecules are stripped off Br^- in the same transfer.
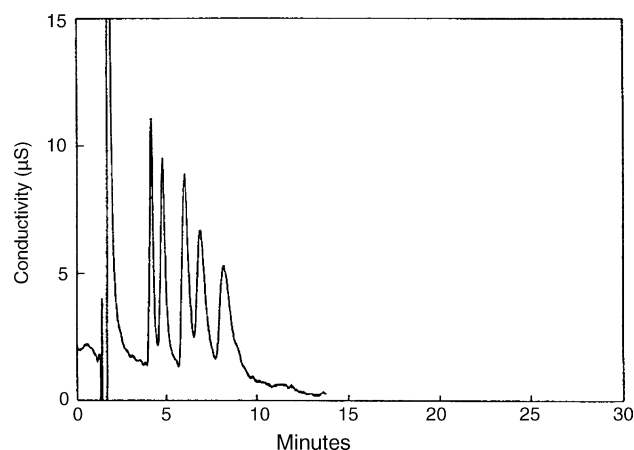

Fig. 1. Separation of Li^+ (2 ppm), Na^+ (5 ppm) K^+ (16 ppm), Rb^+ (24 ppm), and Cs^+ (48 ppm) on a 15-cm polymeric cation-exchange column with 3 mM methanesulfonic acid in methanol as the eluent. From ref. [43] with permission.

In the IC separation of organic cations, it has long been known, or at least suspected, that the mechanism involved more than electrostatic attraction between the fixed negative sites in the ion exchanger and the sample cations. Hoffman and co-workers [16,17] suggested that two mechanisms occur in such cases: ion exchange and hydrophobic interaction between the sample cations and the resin matrix. Dumont et al. [18] also concluded that much of the selectivity for protonated amine cations comes from hydrophobic interaction between the carbon chain of the analyte ions and the polymer matrix of the ion exchanger. By operating in a nonaqueous mobile phase (methanol, ethanol, 2-propanol) the hydrophobic interactions were greatly reduced and aliphatic amine cations from one to ten carbon atoms had almost the same retention factor.

2.4. Mechanism of ion-exchange chromatography

Selectivity in ion-exchange chromatography may be defined as the relativity of sample ions to form an ion pair with the sites of opposite charge within the stationary phase. It is helpful to divide the factors that affect selectivity into two general classes.

(1) Electrostatic attraction (ES): This attraction is stronger when the sample ion has a higher charge and when the paired ions are very close to one another.
(2) Enforced pairing effects (EP): Stronger ion pairing occurs within the stationary phase than in the aqueous solution due to a combination of effects that may include hydrophobic attraction, hydrogen bonding, lower dielectric constant and what has been termed "water-structure induced ion pairing."

The latter theory was developed by Diamond and co-workers to explain elution orders observed in anion-exchange chromatography [19,20]. Large, poorly hydrated univalent ions in aqueous solution intrude into the surrounding water structure without being able to strongly orient the water molecules around themselves into coordinate hydration shells. This contributes to a tightening of the water structure around the ions. The chemical results are such that large ions tend to be rejected by the water phase and they are more easily extracted by nonstructured organic solvents than are smaller ions.

The strong retention of polarizable inorganic and organic anions in ion-exchange chromatography is likely the result of EP effects. Variations in selectivity due to changes in the hydrophobic/hydrophilic properties of the R groups can be attributed more to EP effects than to ES.

The exchange reaction in IC can be written:

$$RN^+E^- + A^- \rightleftharpoons RN^+A^- + E^-$$
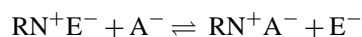
where E^- is the eluent anion and A^- a sample anion. The fixed RN^+ sites on the ion exchanger result in a strong electrostatic "wall" that excludes cations from the mobile phase from entering the stationary phase. This leads to the conclusion that cations will have little if any effect on the exchange equilibrium. However, the nature of the fixed counter ions within the ion exchanger has a major effect.

The situation in conventional ion chromatography is very favorable for the exchange of one ion for another. For example, an anion-exchange column prepared by coating a reversed-phase HPLC column with a cationic surfactant has a positive zeta potential. This facilitates the approach of anions from the mobile phase to the mobile-stationary phase interface and to close proximity of the exchange sites. Since the coated stationary phase contains only positive sites fixed to a hydrophobic matrix, cations from the mobile phase would be excluded.

The physical nature of the ion exchangers used in modern IC is also conducive to exchange of sample and eluent ions. For example, the most widely used anion exchangers have a thin coating of charged latex particles coated onto the outer surface of a spherical substrate. The line charge density of the latex polymer structure as well as the favorable geometry favors the electrostatic component of the ion exchanger mechanism. A small ion with a 2− charge, such as sulfate, ranks high in the selectivity series in IC due to this strong electrostatic component.

3. Ion-pair chromatography

In this type of anion chromatography, which is also called ion-interaction chromatography (IIC) or mobile-phase ion chromatography (MPIC), anions or cations are separated on an ordinary HPLC column with an aqueous–organic mobile phase containing an organic pairing ion with an inorganic co-ion. A tetraalkylammonium salt with an inorganic co-ion ($R_4N^+Co^-$) is often used as a pairing reagent for analyte anions and an alkane sulfonate salt with an alkali metal co-ion ($Co^+RSO_3^-$) for cationic analytes [21]. During the conditioning step the pairing reagent is in a dynamic equilibrium between the mobile phase and the hydrophobic stationary phase in the column.

$$R_4N^+Co^- \text{ (solution)} \rightleftharpoons R_4N^+Co^- \text{ (stationary phase)}$$

or

$$Co^+RSO_3^- \text{ (solution)} \rightleftharpoons Co^+RSO_3^- \text{ (stationary phase)}$$
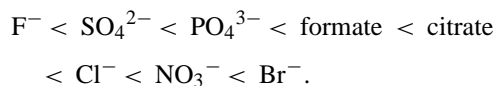
This equilibrium is controlled by varying the type and concentration of the pairing reagent, and the proportion of organic solvent in the mobile phase.

After conditioning, the sample is introduced as the mobile phase continues to be pumped through the column. Analyte retention has been suggested to follow a double-layer model in which the organic pairing ion occupies a primary layer on the stationary phase and the other ions in the system compete for the secondary layer [22]. Sample anion or cations are separated by differences in their affinity for the pairing ion sites on the stationary phase.

Ion-pair chromatography is actually quite broad in scope. Chromatographic behavior can be distinctly different when organic ions, rather than inorganic ions, are to be separated. This diversity is illustrated by considering four specific examples.

(1) Separation of inorganic anions on a poly(styrene-divinylbenzene) adsorbent in the presence of tetraalkyl-ammonium salts [23]. The first of the two major equilibria encountered involves retention of the R_4N^+ salt as a double layer on the stationary phase surface. The second equilibrium is between analyte anions and those occupying the secondary layer of the double layer. The mobile phase variables are the structure and concentration of the R_4N^+ salt, solvent composition, pH, and the concentration of the co-ion accompanying the R_4N^+ salt. The retention factor, k', for inorganic anions was adjusted to a desired range by varying the composition of the aqueous–organic mobile phase from 17.5 to 35% acetonitrile. The retention order of anions was similar to that in conventional anion-exchange chromatography: $ClO_4^- > I^- > NO_3^- > Br^- > NO_2^- > Cl^- >$ citrate > formate > $F^- > OH^-$, and strong retention of divalent anions.

(2) Further insight into IP chromatography was provided by a study on the separation of aromatic carboxylate and sulfonate anions with a tetrapentylammonium salt as the pairing ion [22]. Here, it was shown that the co-ion has a strong influence on the partitioning equilibrium of the pairing reagent between the mobile and stationary phases. Table 1 shows that the retention factor, k', of tetrapentylammonium salts increased in the following order with various co-ions: $OH^- < F^- <$ formate $< Cl^- < Br^- < NO_3^-$. Sodium salts of various inorganic anions added to the mobile phase (which also contained a lower concentration of $R_4N^+Br^-$) reduced the retention times of the organic sample anions in the order:

$$F^- < SO_4^{2-} < PO_4^{3-} < \text{formate} < \text{citrate}$$
$$< Cl^- < NO_3^- < Br^-.$$

Sulfate now has a much weaker affinity for the stationary phase than it did in the previous example where inorganic anions were separated. This implies that the separation mechanism is different when organic anions are separated.

(3) The chromatographic behavior of C_2–C_{12} alkanesulfonates on a polymeric column (PRP-1) or a C_{18} silica column with a metal ion salt as the only counter ion in the mobile phase [24]. Retention of the sulfonates was believed to be accompanied by double layer formation at the stationary phase surface. The sulfonate analyte constitutes the primary layer due to hydrophobic interaction between the sulfonate chain and the stationary phase surface, and the diffuse secondary layer is made up of counter cations.

The retention factors, k', of the analytes *increased* substantially when the concentration of sodium acetate in the mobile phase was increased in steps from 10^{-4} to 10^{-2} M. When the mobile phase contained metal ion chlorides at constant ionic strength, the retention factors of C_6–C_{10} alkanesulfonates increased in the order: $Al^{3+} > Ba^{2+} > Mg^{2+} > Na^+ > Li^+$. These trends suggest that an appreciable association is taking place between the cation and the sulfonate analytes. The association reduces the charge at the anionic center and hence increases the hydrophobic interaction between the analyte and the stationary phase.

(4) Roberts [25] found that the chromatographic behavior of protonated amines on a bonded-phase silica column is influenced markedly by the identity of the mobile phase anion. For example, the retention factor values of protonated nordoxepin, nortriptyline and amitriptyline all increased approximately 6-fold across the following series of anions employed as mobile-phase modifiers:

$$H_2PO_4^- < HCO_2^- < CH_3SO_3^- < Cl^- > NO_3^-$$
$$< CF_3CO_2^- < BF_4^- < ClO_4^- < PF_6^-.$$
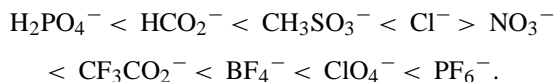
In contrast, a neutral hydrophobic analyte, acenaphthene, showed no significant changes with respect to the mobile phase anion.

These effects were felt to be consistent with nonstoichiometric, double-layer ion-pairing models [26] and the rank of the anion in the Hofmeister series. Although the Hofmeister effect is not completely understood, it is well accepted that the rank of an ion in the series is a measure of its propensity to accumulate at or near interfacial regions and that the physical quantity responsible for the effect is intimately tied to the solvation properties of the ion.

3.1. Mechanism of ion-pair chromatography

It is apparent from these examples that IPC covers a broad range of chromatographic conditions. In the first example, the pairing ion is attached to the stationary phase so strongly that it may be considered almost as a permanent coating. Sulfate is strongly retained and the elution order of monovalent anions is virtually the same as encountered in ion-exchange

Table 1
Retention of tetrapentylammonium salts on PRP-1 conditions: 3:7 CH_3CN:H_2O mobile phase, conductivity or refractive index detector

Analyte	k'
$R_4N^+OH^-$	0.27
$R_4N^+F^-$	2.81
$R_4N^+Form^-$	3.05
$R_4N^+Cl^-$	3.27
$R_4N^+Br^-$	3.75
$R_4N^+NO_3^-$	4.44

Adapted from [22].

chromatography. The retention factors of analyte anions decrease with a higher ion concentration in the mobile phase, but the slope of a plot of $\log k'$ versus log eluent concentration is lower than the theoretical slope for an ion-exchange mechanism.

In examples 3 and 4, organic analyte ions are separated with inorganic pairing ions in the mobile phase. Retention factors of the analyte ions vary according to the chemical nature of both the analyte and pairing ions. Retention factors increase with a higher ionic concentration in the mobile phase. This is the opposite to that observed in the first example and in ion-exchange chromatography. The most likely mechanism is one in which the analyte and pairing ions partition between the mobile phase and the stationary phase where they are retained as ion pairs.

$$C^+ \text{ (mobile)} + A^- \text{ (mobile)} \rightleftharpoons C^+A^- \text{ (stationary)}$$

We may conclude that the mechanism of IPC can vary between two extremes, ion-exchange and partitioning similar to that in HPLC. A mixed mode mechanism seems likely for many types of IP separations.

4. Liquid–liquid extraction

Liquid–liquid extraction is another instance in which a cation–anion combination can be transferred from an aqueous to an organic liquid phase. For example, chloro ion association complexes of iron(III) and gold(III) are extracted as the ion pairs, $H_3O^+FeCl_4^-$ and $H_3O^+AuCl_4^-$, respectively [27]. These extractions must involve specific interactions between the extracted ion pair and an ether or carbonyl oxygen of the organic liquid. In the iron(III) complex, the interaction is between the organic oxygen and iron.

The analogy of ion exchange to liquid solvent extraction is a compelling one that was recognized quite some time ago [28]. Navtanovich et al. [29,30] cite the work of Ivanov et al. [31,32] who established the selectivity of extraction of various anions into a toluene solution containing the tetraoctylammonium cation. The order of increasing selectivity for monovalent anions was found to be:

$$OH^- < F^- < \text{acetate} < HCO_3^- < HSO_4^- < Cl^- < Br^-$$
$$< \text{benzoate} < NO_3^- < I^- < ClO_4^-.$$
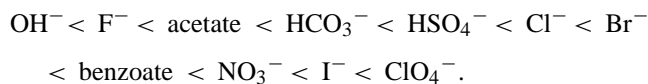
Another study in which a xylene solution of a benzyltrialkylammonium salt with alkyl chains of 7–9 carbons was used places SCN^- between I^- and ClO_4^- in the selectivity series.

Ion-pair chromatographic separations have been carried out on a C_8 reversed-phase silica column coated with an organic liquid, 1-pentanol [33]. Phenylethylamine derivatives were separated as ion pairs with octylsulfate. The aqueous mobile phase contained a phosphate pH buffer and was saturated with 1-pentanol in order to preserve the liquid coating on the column. Liquid–liquid distribution studies have demonstrated that the solvating properties of the organic phase have a very large influence on the selectivity of separations. Hydrogen bonding the solute and solvents is of particular significance, and it is important to have systems where the hydrogen-bonding ability of the organic phase can be varied [34].

5. Zwitterion ion chromatography

The concept of using zwitterionic substances in ion separations dates back to at least 1981 when Knox and Jurand described a new form of ion-pair chromatography in which a zwitterion pairing agent, 11-aminoundecanoic acid, was used in the separation of nucleotides [35]. Yu and coworkers [36,37] employed a column containing a zwitterionic stationary phase bonded to silica particles in the chromatographic separation of ions. They were able to separate organic cations and organic anions simultaneously on the same column by what they believed to be an ion-exchange process. The column had sufficient hydrophobic character to also permit the simultaneous chromatographic separation of neutral solutes. A typical eluent consisted of an ammonium phosphate pH buffer in an aqueous solution containing 15% methanol.

In 1993 Hu et al. introduced a new form of ion chromatography in which a silica C_{18} column was permanently coated with a zwitterionic reagent and pure water served as the mobile phase [38]. This method was called electrostatic ion chromatography (EIC).

A C_{18} reversed-phase silica column (15 cm long) was permanently coated with 3-[(cholamidopropyl)dimethylamino]-1-propanesulfonate (CHAPS) or CHAPSO as the zwitterion reagent. The mobile phase was pure water. A conductivity detector provided sensitive detection of the eluted ion pairs because the background conductivity of the pure water was so low. Excellent separations of NaCl, NaNO$_2$, KBr and NaNO$_3$ were obtained in 5–7.5 min. A good separation of Na$_2$SO$_4$, NaCl, KNO$_2$, NaNO$_3$, KI and NaSCN was obtained with an aqueous solution of a 3 mM phosphate buffer as the mobile phase.

In 1994, Hu et al. [39] showed that multiple peaks were obtained when the sample contains different cations as well as different anions. The separations in Fig. 2 on a CHAPS-coated C_{18} column with pure water as the eluent show well-resolved peaks for different cation–anion pairs. The elements in each peak were confirmed by ICP-AES detection on a separate run. The proportion of an element in multiple peaks was shown to be determined by the relative affinities of the various cation–anion combinations for the zwitterion stationary phase.

In more recent work the column used for the separation of anions is usually prepared by applying a permanent coating of a sulfobetaine, such as 3-(*N,N*-dimethylmyristylammonio) propanesulfonate, to a C_{18} column of the type commonly used in HPLC. This is done simply by passing a solution of the sulfobetaine through a packed C_{18} column. The
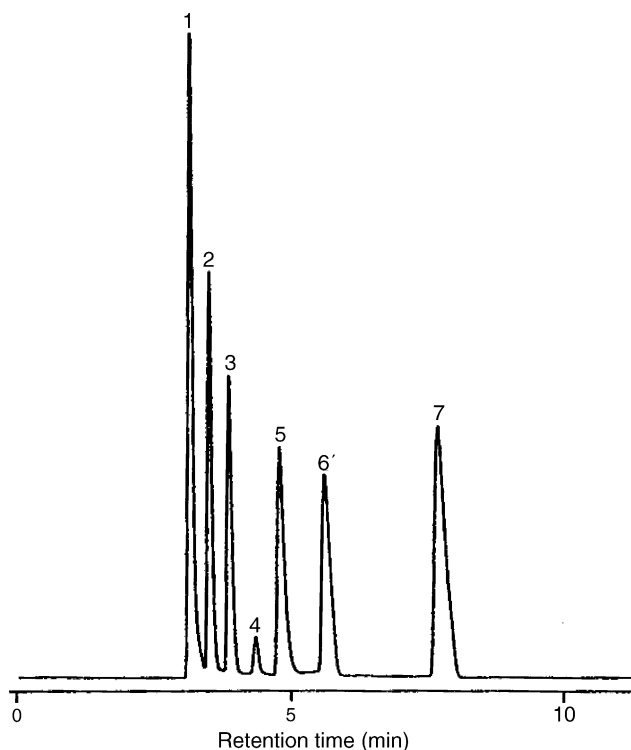
Fig. 2. Chromatographic separation on an ODS column coated with CHAPS using pure water as the eluent with conductivity detection. Key: (1) $2Na^+–SO_4{}^{2-}$; (2) Na^+Cl^-; (3) Na^+Br^-; (4) $Ca^{2+}–2Br^-$; (5) $Na^+–I^-$; (6) $Na^+–SCN^-$; (7) $Ca^{2+}–2SCN^-$. From ref. [39] with permission.

column coating is an inner salt, or zwitterion, of the type formula:

$$R–N^+(CH_3)_2CH_2\ CH_2CH_2SO_3{}^-.$$

This sulfobetaine type of column will be referred to as a SB column.

A zwitterion column that may be used for the chromatographic separation of cations is prepared in a similar manner by coating a C_{18} column with *n*-hexadecyl phosphocholine. In case the negative charge is adjacent to the long hydrocarbon chain and the positively charged nitrogen is at the other end of the molecule.

$$CH_3—(CH_2)_{15}—O—\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}—O—(CH_2)_2—\overset{+}{N}(CH_3)_3$$
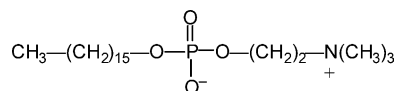
A column of this type will be referred to as PC.

The chromatographic properties of both SB and PC columns differ in several ways from the anion- and cation-exchange columns used in conventional ion chromatography.

(1) Sample cations and anions are taken up simultaneously. In conventional IC a sample anion (or cation) is simply exchanged for an eluent anion (or cation) that is already on the ion-exchange column packing.

(2) In ZIC, it is sometimes feasible to elute sample ions with only pure water as the mobile phase. In such cases each chromatographic peak contains both a sample cation and

a sample anion. Multiple peaks may be obtained when the sample contains more than one anion and more than one cation. For example, cations C_1 and C_2 can combine with anions A_1 and A_2 to form four peaks: C_1A_1, C_1A_2, C_2A_1 and C_2A_2. The relative amounts of an ion (such as C_1) is a function of the relative affinity of each ion pair (such as C_1A_1, C_1A_2) for the stationary phase. Multiple peaks for cations can be avoided by passing the sample through an anion exchanger to give only one kind of anion, or more simply by adding an excess of salt such as NaI to the sample [40]. Na^+I^- is so weakly retained that it elutes before any sample ions, and I^- combines more strongly than other anions with the sample cations so that only one peak is obtained for each cation.

(3) An eluent containing the sodium salt of another anion facilitates the separation of anions by ZIC. A plot of retention factor, k', against the eluent concentration shows a rather sharp increase or decrease in k' between zero and about 1 mM eluent concentration, followed by a plot that is almost flat with increasing concentration [41]. This is in marked contrast to the situation in conventional IC where a similar plot shows a linear decrease in $\log k'$ with increasing log of eluent concentration.

(4) Anions can be determined directly in saline matrixes by ZIC. Hu et al. [42,43] determined low concentrations of bromide, nitrate and iodide in actual seawater using a 20-fold diluted artificial seawater as the eluent. Okada and Patil [44] found that a plot of the adjusted retention time of iodide versus the concentration of salt in the eluent attained a maximum somewhere between 0.01 and 0.10 M salt. These results suggest that the mechanism of ZIC must be quite different from that in conventional ion chromatography.

(5) The elution order for anions has been reported to be: $F^- < HPO_4{}^{2-} < SO_4{}^{2-} < Cl^- < NO_2{}^- < Br^- < NO_3{}^- < ClO_3{}^- < I^- < ClO_4{}^-$ [41,45]. The early elution of hydrogen phosphate and sulfate is quite different from the elution order observed in ion-exchange chromatography.

In cation chromatography on a zwitterion column of the PC type with pure water as the eluent, the following elution order was observed: Na^+, $K^+ \ll Ba^{2+} < Mg^{2+} < Ca^{2+} < H^+$ [40]. The retention times of these cations, and H^+ in particular, were affected very strongly by the anion that was present. The elution order for acids was: $H_3PO_4 < HCl < HNO_3 \ll H_2SO_4 \ll HClO_4$. These results emphasize the fact that elution behavior in ZIC is affected by both the sample cation and anion rather than only by the nature of the sample cation or anion as is the case in ion-exchange chromatography.

Since peaks are eluted as ion pairs and the retention times depend on the nature of both the cation and the anion, the retention time of a peak can be altered by addition of an ion of opposite charge to the desired sample ion. This ion is added to the sample only; pure water continues to be used as the mobile phase.

This sample addition technique is best illustrated by an example [40]. Divalent anions such as Ba^{2+}, Mg^{2+} and Ca^{2+} were eluted very quickly from a PC column when chloride was the counter anion and the peaks were poorly resolved. However, addition of an excess of Na^+I^- to the sample enabled the sample cations to be eluted as the $M^{2+}I_2^-$ ion pairs. The retention times were longer and the peaks were well resolved. An even better separation with longer retention times was obtained when Na^+SCN^- was added to the sample. The excess Na^+I^- or Na^+SCN^- eluted very quickly and did not interfere with the desired separation.

Anions have been separated on a SB-coated column using sodium salt of tetraborate [45], sulfate or carbonate [46] as the mobile phase. These anions rank low in the anion selectivity series. There is very little change in the retention times of sample anions in the concentration of salt employed in the mobile phase. Cook et al. [41] reported that the retention factor of nitrite and iodide actually increases substantially going from pure water to about 2 mM sodium sulfate in the mobile phase. This was attributed to breaking up the interaction between paired positive and negative charges on adjacent zwitterion molecules by the ions in mobile phase and thereby making the zwitterion ionic sites more available to sample ions.

A problem with anion ZIC is that ions that are higher in the selectivity series tend to have overly long retention times with the mobile phase ions that have been commonly used.

The rather meager data available indicate that use of a salt of an anion that is higher in the selectivity series, such as chlorate or perchlorate, would be effective in reducing the retention times of strongly retained sample anions.

5.1. Mechanism of zwitterion ion chromatography

To explain the experimental results for a process they called electrostatic ion chromatography (ESI), Hu et al. [38] envisioned a mechanism in which the analyte anion is electrostatically attracted to the positive charge in the zwitterionic stationary phase. But the negative charge in the stationary phase is very close to the positive charge, and as a result, repulsion of the analyte anion occurs simultaneously. The analyte cation will encounter similar electrostatic attraction and repulsion. The authors reasoned that neither the positive nor the negative charge of the zwitterionic stationary phase can work as an ion-exchange site, so the analyte anions and their counter cations are forced into an "ion-pairing-like form" at some distance from the zwitterion charges (see Fig. 3).

As additional experimental results have accumulated it has become apparent that a satisfactory mechanism for ZIC must include EP as well as ES effects. Actually, there are a number of similarities between ion-pair and zwitterion ion chromatography. In both of these the uptake of anions is affected by the particular cation that is present, retention of anions is enhanced by increased ionic strength of the aqueous phase, and most importantly the elution order of anions is almost identical. Actually, a very similar elution order is ob-
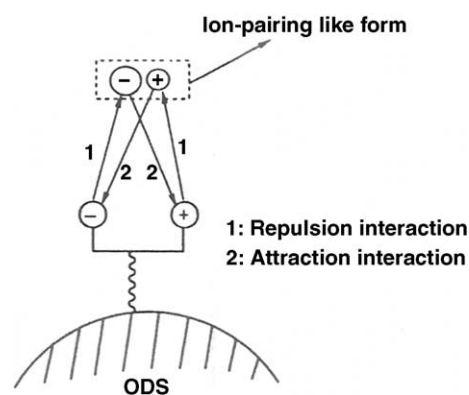

Fig. 3. Simultaneous electrostatic attraction and repulsion interactions between analyte ions and the zwitterionic charged stationary phase. From ref. [38] with permission.

served for anions in conventional ion-exchange chromatography except for small, highly charged ions such as sulfate and hydrogenphosphate.

A column coated with a betainesulfonate (BS) reagent is used for the separation of anions. This coating is a zwitterion with positively charged quaternary ammonium (N^+) group located next to a hydrophobic alkyl chain. A negatively charged sulfonate group (O^-) is separated from the N^+ by three methylene groups. When pure water is used as the mobile phase, sample anions (A^-) most likely migrate to the vicinity of the N^+ and the adjacent alkyl chain by an electrostatic and enforced-pairing mechanism. Concurrently, the sample cations (C^+) are attracted to the O^- sites.

When C^+ is monovalent, such as Na^+, it is retained somewhat loosely by O^-, while A^- is retained more tenaciously. This imparts a negative zeta potential to the interface between mobile and stationary phases. Cook et al. [41] liken this situation to a Donnan membrane. Whatever the detailed mechanism may be, the net result is that a pair of ions, C^+A^-, is taken up by the stationary phase. As elution with pure water continues, an equilibrium is established in which the ion pairs of the sample move along the column and hopefully are separated.

The mechanism of ZIC continues to be a subject of some debate and perhaps a certain degree of confusion. Jiang and Irgum [47] concluded that the mechanism is different from that in conventional ion-exchange chromatography. Noting that anions elute according to increasing chaotropic properties in the Hofmeister series, they suggested it is reasonable to assume that an exclusion can be in effect, but partitioning with the polymeric substrate cannot be ruled out since the hydrophobic character of an ion is also connected to the level of hydration.

Cook et al. [41] reviewed previously published retention mechanism in electrostatic ion chromatography (EIC) and proposed a new mechanism based on electroosmotic flow measurements on fused-silica capillaries coated with zwitterion surfactants. The first part of their mechanism proposes that equilibration of the bound zwitterions with a mobile

phase containing a suitable electrolyte causes the establishment of a charged layer created by the terminal sulfonate groups of the zwitterion, which acts as a Donnan membrane. The magnitude and polarity of the charge on this membrane depends on the nature of the mobile-phase ions. The Donnan membrane exerts weak electrostatic repulsion or attraction effects on analyte anions. A second component of the retention mechanism is chaotropic interaction of the analyte anion with the quaternary ammonium functional group of the zwitterion. This interaction exerts the major effect on the separation selectivity, such that analyte anions are eluted in order of increasing chaotropic interactions in accordance with the Hofmeister series. A subsequent paper proposed a mechanism for the chromatography of cations on a column coated with a phosphocholine zwitterion [48].

An additional clue to the mechanism of ZIC is provided by a study in which the zwitterionic surfactant used as the stationary phase, ammonium sulfobetaine-1, had a greater distance between the positively and negatively charged groups than the surfactants used in previous studies, zwittergent-3-14 and C12N35 (see Fig. 1 from ref. [43].

The comparative results showed that ammonium sulfobetaine-1: (1) gave shorter retention times for thiocyanate, iodide and nitrate, (2) shorter retention times for anions with 10 mM sodium sulfate than 10 mM sodium chloride, (3) a linear plot of $\log k'$ against log sodium sulfate eluent concentration where $\log k'$ decreased as the eluent concentration was increased (see Fig. 4 from ref. [43]). All of this points to a greater degree of ion-exchange character for the sulfobetaine-1 than for the two other surfactants in which the positive and negative charges were separated by only three carbon atoms.

We may conclude from this that the degree of charge separation in the stationary phase has an important bearing on the retention mechanism. A small, divalent anion such as sulfate is retained in conventional ion exchange primarily by electrostatic attraction for N^+ sites in the ion exchanger. But in SB phases where the charges are separated by only three carbon atoms, sulfate must encounter considerable electrostatic repulsion from nearby O^- sites. This would explain its low position in the elution series.

Chromatographic separation of anions on columns coated with a zwitterionic reagent is analogous to conventional ion-exchange chromatography in that both contain fixed cationic sites within the stationary phase. However, in zwitterionic stationary phases the anionic sites are in close proximity to the cationic sites. The normally strong ES attraction for anions like sulfate for the positively charged sites on the zwitterion is largely neutralized by the ES repulsion of the nearby negatively-charged sites. The EP component of sulfate is very weak owing to its small size and high degree of hydration. The net attraction of sulfate for the stationary phase is thus very weak. In a similar manner ES attraction of Na^+, a commonly used cation in anion chromatography, for negative sulfonate sites is weakened by ES repulsion from the positive sites.

The opposing attraction and repulsion ES effects in a zwitterionic stationary phase are of much less consequence for anions like bromide, iodide and perchlorate. These anions are drawn to the stationary phase more by HP attraction than by ES forces.

A recent paper brings the differences in chromatographic behavior between ion-exchange and zwitterion chromatography into sharp focus [49].

(1) The elution of anions on a column coated with zwittergent-3-14 with an aqueous mobile phase containing 7.0 mM $Na_2B_2O_7$ and 2.0 mM H_3BO_4 follows the order: sulfate and fluoride < chloride < nitrite < bromide < nitrate < chlorate. With a conventional anion-exchange column and the same mobile phase, the elution order is the same except for sulfate, which now elutes much later (between bromide and nitrate).

(2) A plot of log retention factor (k') against $\log Na_2B_2O_7$ concentration in the mobile phase shows a linear increase in k' (slope = 0.79) with a higher eluent concentration. This behavior is the opposite to that which occurs in conventional anion exchange, where plots of $\log k'$ versus log [eluent] are linear but have negative slopes.

(3) Very high concentrations of chloride or sulfate (up to 1.2 M or higher) enhance the retention of bromide, iodide and chlorate on a zwittergent-3-14 column. Separations under similar conditions in conventional anion chromatography are virtually impossible.

The available facts on the separation of anions on a zwitterion stationary phase point strongly to a partitioning mechanism or a mixed mode in which partitioning is dominant with only a weak ion-exchange component. As in IP, the driving force in ZIC is the tendency of analyte ions and co ions to pass from the mobile phase and pair up as C^+A^- in the hydrophobic stationary phase. The retention factors of sample ions are determined by the relative ability of pairs of ions to partition between the mobile and stationary phases. This is determined primarily by the hydrophobic properties of the ion pairs rather than by the relative concentrations of eluent and sample ions in the mobile phase. This is different from ion-exchange chromatography in which analyte and eluent ions compete for specific charged sites and a higher concentration of eluent ions reduces the k' of analyte ions. The areas of ES charge are much more diffuse in ZIC than in conventional IC.

6. Conclusions

Examination of selected journal publications in IC, IPC, solvent extraction and ZIC reveals that these separation techniques have several things in common, as pertains to the separation of anions. In each case retention of anions involves transfer of sample anions to a stationary phase (that is primarily organic in nature) where a cation–anion pair is formed. The strength of this ion pair, and hence, the relative retention,

is affected by: (1) the properties of the sample anion, and (2) the environment within the stationary phase.

There are two major components in the pairing of sample anions. One component is electrostatic attraction. This component is stronger when the positive ion (usually a group containing N^+) is a point charge and is weaker when the positive charge is more diffuse, such as when the $-NR_3^+$ contains larger, more hydrophobic R groups. ES is also stronger when the sample anion is a small, poorly hydrated ion with a higher charge (SO_4^{2-}, HPO_4^{2-}, etc.). The ES component is weaker when the sample ion is a larger, polarizable anion such as I^-, SCN^-, ClO_4^- or RCO_2^-.

The second major component includes hydrophobic attraction and water-structure induced pairing. We refer to this as enforced pairing. Pairing is stronger when the sample anion is larger and more polarizable, and also when the cation R groups are larger and when the environment within the stationary phase is more hydrophobic. A tendency for larger ions to migrate from aqueous solution to an aqueous/organic stationary phase interface has been noted even in the absence of oppositely charged sites in the organic phase. This has been observed for ions in IP and solvent extraction, as well as for the retention of neutral species in IC and ZIC. The presence of positively charged sites in the organic phase will of course increase the EP attraction for anions.

In IPC and ZIC a larger, polarizable cation in solution will increase the tenacity with which sample anions are retained by the stationary phase. In anion chromatography the cation in solution is excluded from the stationary phase and thus has almost no effect on the exchange of one anion for another. However, the size and hydrophobicity of the fixed cationic sites in the stationary phase can have a major effect on the retention of sample anions.

In ZIC the distance between + and − charged sites seems to affect the retention mechanism. In most systems + and − sites are separated by three carbon atoms. Ions such as SO_4^{2-} that are retained primarily by ES attraction are subjected to conflicting forces: attraction by the positive sites and repulsion by the negative.

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