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Review

Retention behaviours and separation of carboxylic acids by ion-exchange chromatography

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Abstract

The use of high-performance suppressed ion chromatography for the separation of aliphatic carboxylic acids has become an attractive and viable method during the past years. This paper summarises and critically concludes that some new results have been achieved in separation and detection of low-molecular-mass organic anions. Theoretical and practical considerations of ion-exchange selectivity to control retention behaviour are presented. The major factors that determine the separation ability of ion-exchange chromatography (pK_a values, the aliphatic nature and valency of solutes, eluent pH and the chemical composition of stationary phases) are discussed. The question of isocratic vs. gradient elution and different separation modes are examined briefly. The potentials and limitations of the developed methods and their specific application areas are outlined.

Keywords: Reviews; Carboxylic acids

Contents

1. Introduction

Ion chromatography has gained broad acceptance as a tool for the analysis of aliphatic carboxylic acids and their carboxylate salts in a wide variety of complex matrixes and had been widely used to determine the answers to several theoretical and practical questions or to serve the environmental chemistry, food chemistry, biomedical research and pharmaceutical industries. Therefore, a rapid and

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simple method for determining these compounds is required.

Carboxylic acids are separated usually by three different liquid chromatographic methods: anion-exchange [1,2], ion-exclusion and reversed-phase chromatography [3–5]. Traditional high-performance liquid chromatography methods use refractive index (RI), ultraviolet (UV) absorption and derivatization fluorescence techniques for the detection of the acids. The most common application is the ion-exclusion technique. It is especially attractive as an adjunct to ion-exchange chromatography since selectivities obtained by these methods are quite different. Strong inorganic acid anions are excluded from the resin phase in a single peak according to the Donnan principle and elute at the void volume. Weaker and protonated species, existing largely in the molecular form, are retained on the stationary phase by a combination of ion-exclusion and hydrophobic interactions. For low-molecular-mass components, ion-exclusion is the preferable retention mechanism and all fully ionised solutes will elute at the same retention time, without resolution. Simultaneous determination of short-chain carboxylic acids in mixtures with mono- and divalent inorganic anions still represents a rather difficult separation problem.

Recently, ion chromatography has developed rapidly as a sensitive and simple separation method for inorganic and weak aliphatic organic acids. In general, the ion-exclusion modes of ion chromatography by isocratic elution cannot provide the complete chromatogram of the homologous series of weak acids and inorganic anions. The combination of anion-exchange separation and eluent-suppressed electrical conductivity detection using a latex-based pellicular stationary phase offers several advantages over existing techniques. Recent developments indicate that gradient elution ion chromatography is about to replace ion-exclusion chromatography for the separation of a wide variety of organic and inorganic substances.

This review will discuss factors controlling retention behaviours of aliphatic carboxylic acids in anion chromatography using different separation and detection methods. In this paper, we also consider the influence of ion-exchange equilibria on the retention of some mono- and dicarboxylate anions. We shall limit our discussion mainly to the application of high-performance suppressed ion chromatography (HPIC).

2. Retention behaviours

An understanding of the factors that affect the retention behaviour for carboxylate anions is useful in designing optimal elution conditions. The most effective utilization of HPIC requires accurate characterisation of the solute retention. Some of the factors that affect anion retention have also been studied in the author's laboratory [6–8].

2.1. Ion-exchange selectivity

The ability to control ion-exchange selectivity is the most important means of manipulating separation in ion-chromatography. The separation of organic ions is affected by the relative sample and eluent charges, eluent concentration and the pH of the mobile phase. In light of their acid–base chemistry, it is instructive to examine their retention behaviour in ion-exchange chromatography. The more hydrophilic carboxylic acids are those with pK_a values below 4, with aliphatic character, and the hydroxy-substituted acids (e.g. malic, lactic, tartaric, etc.; Table 1). Selectivity in an ion-exchange system is quantified through the use of the selectivity coefficient ($K_{A/E}$). The ion-exchange equilibria for the eluent (E^{x-}) and analyte (A^{y-}) species:

Table 1
Dissociation constants of organic acids in aqueous solutions (T= 25°C) [36]

Carboxylic acid	pK_{a1}	pK_{a2}	
Formic	3.75		
Acetic	4.75		
Propionic	4.87		
Lactic	3.86		
Pyruvic	2.49		
Oxalic	1.23	4.19	
Malonic	2.83	5.69	
Succinic	4.16	5.61	
Tartaric	2.98	4.34	
Fumaric	3.02	4.39	
Maleic	1.83	6.07	
Citric	3.13	4.76	pK_{a3}=6.40

$$yR_x\text{-}E + xA^{y-} \overset{K_{A/E}}{\Leftrightarrow} xR_y\text{-}A + yE^{x-} \quad (1)$$

where R refers to the stationary phase, and x and y are the charges of a given anion. The selectivity coefficient, $K_{A/E}$, is given by:

$$K_{A/E} = \frac{(A^{y-})^x [E^{x-}]^y}{[A^{y-}]^x (E^{x-})^y} \quad (2)$$

where () is the concentration in the stationary phase and [] is the concentration in the mobile phase.

The volumetric distribution coefficient for solute A is designated as D_A and is given by:

$$D_A = \frac{(A^{y-})}{[A^{y-}]} = K_{A/E}^{1/x} \left[\frac{(E^{x-})}{[E^{x-}]} \right]^{y/x} \quad (3)$$

Analytical trace conditions are achieved when the number of moles of A^{y-} ions is much less than the number of moles of E^{x-} ions. Supposing that $c_A \ll c_E$ in the mobile and in the stationary phase, respectively; $K_{A/E}$ = constant and E^{x-} occupies x ion-exchange sites on the stationary phase, the ion-exchange capacity of the column, Q, is given by: $(E^{x-}) = Q/x$; then Eq. (3) becomes:

$$D_A = K_{A/E}^{1/x} \left(\frac{Q}{x} \right)^{y/x} [E^{x-}]^{-y/x} \quad (4)$$

The logarithm of Eq. (4) provides the relationship:

$$\log D_A = \text{constant} - \frac{y}{x} \log[E^{x-}] \quad (5)$$

where

$$D_A = k' \frac{V_0}{V_{st}}$$

and

$$\log k' = \text{constant}' - \frac{y}{x} \log[E^{x-}] \quad (6)$$

Eqs. (4)–(6) show that the retention of solutes is determined by the selectivity coefficient, the ion-exchange capacity and the concentration of the eluent in the mobile phase. This well known equation indicates that log D_M is linearly dependent on both log $[E^{x-}]$ and log Q. The absolute value of the slope in both cases is y/x. Thus, elution times can be brought into a practical range by increasing the eluent concentration and/or decreasing the column's capacity. These relationships, especially the one involving the eluent concentration, proved to be valid for mono- and divalent carboxylate eluted with monovalent ions on high capacity ion-exchange resins.

2.2. Mobile phase-related factors

Separation modes for carboxylic acids can be based on their ionic character as the main interaction mechanism. When choosing separation schemes for underivatized carboxylic acids, there are some basic characteristics of the analytes and the eluent that should be considered. These are pK_a values, the aliphatic nature and valency of solutes, and the eluent pH. For example, the divalent solute as the malonic acid, (pK_{a1} = 2.83 and pK_{a2} = 5.69) contains two carboxylic groups, is doubly negative at a high pH interval. From the ionisation equilibria (see Fig. 1), it is evident that this occurs at higher than pH 7.5 and the monovalent species exist profoundly at pH 4. Thus, the composition of such an analyte can be easily governed by the eluent pH. Consequently, the pH and eluent concentration are powerful parameters for adjusting the retention by an ion-exchange mechanism.

The versatility of ion chromatography has been significantly developed by the application of multiple species eluents. However, ion-exchange equilibria are strongly dependent on interactions between the species in the mobile phase. The strength of an eluent can be manipulated simply by changing the pH. Phthalate, phosphate, citrate and carbonate buffer eluents were most frequently utilised for this purpose in anion separation.

When carbonate and bicarbonate anions are used,

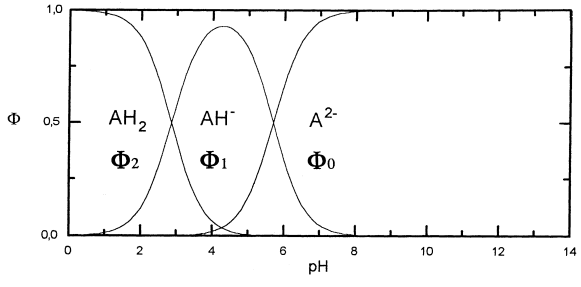

Fig. 1. Proton ionisation equilibria of malonic acid. Φ is the partial molar fraction of malonic species.

the resulting eluent is buffered and has an elution strength that can be varied easily by varying the ratio of the two anions. As the pH of the mobile phase is changed, simultaneous ion-exchange equilibria and protonation will take place in the chromatographic system. Carbonate eluent as a multispecies eluent contains three competing anions (CO_3^{2-}, HCO_3^- and OH^-). In order to have reliable retention behaviours, all forms of the eluent and solute components must be considered at the same time. Current research in this field is appropriately directed towards a fundamental understanding of how changes in eluent and sample composition affect the separation process. The '*multiple eluent species*' and '*multiple eluent/analyte species*' models were developed and derived in different forms by some authors [9–11] as a means of considering all competing eluent ions by taking into account their differing selectivities. Modelling of the observed retention behaviour by these models requires an evaluation of solute/eluent and intereluent selectivity coefficients. These constants may be obtained from observed retention volumes. These data and the effective column capacity are substituted into a retention equation derived from ion-exchange equilibrium and provide a sequence of equations with unknown selectivity coefficients.

The set of equations is solved by iterative minimisation of the differences between the measured and calculated retention data by varying the solute/eluent coefficients and the intereluent coefficient. In general, these constants may be used to predict retention data for a variety of eluent compositions. The calculated ion-exchange selectivity constants can be used in the preparation of retention surface diagrams for anions studied. An example of such a retention surface diagram is shown for some carboxylate ions in Fig. 2 [12]. This typical diagram gives a clear picture of the relationship between the value of log k', the eluent concentration, and pH. This figure indicates that the retention is influenced strongly by the pH of the eluent. A decrease in eluent pH leads to an increase in the retention because the predominant form of driving species is the monovalent HCO_3^- at lower than pH 10.

In alkaline solutions, the NaOH can also operate as a single eluent, however OH^- is the weakest eluting anion. Consequently, it is usually used at

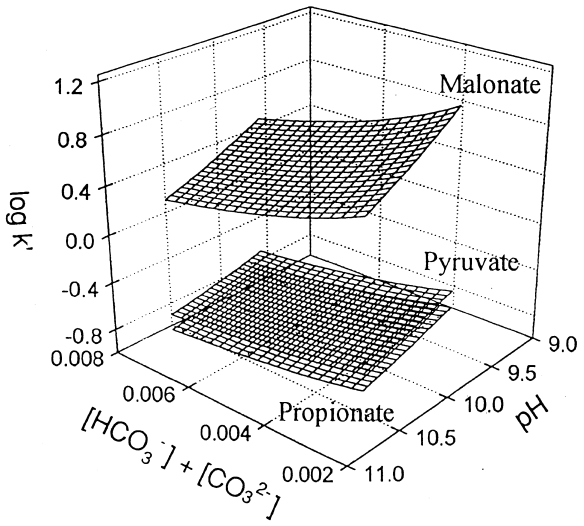

Fig. 2. Calculated retention surfaces for carboxylate anions eluted with carbonate buffer. Reproduced with permission from Ref. [12].

higher concentrations than the carbonate buffer. The observed capacity factors of the carboxylate analytes are summarised in Fig. 3 [6]. Close examination of these figures highlights several interesting aspects of the retention characteristics of the analytes studied. Plotting the log k' values, where $k' = (V_R - V_0)/V_0$, as the function of log c_{eluent}, the following conclusions are established:

- The changes in the eluent concentration in turn had a significant effect on the analyte retention characteristics. Increasing the eluent concentration leads to decreased capacity factors. Inorganic anions, such as chloride, nitrate and sulphate, have k' values that are different to those of mono- and dicarboxylate anions and they can be separated simultaneously in one run.
- The fact that several of the plots cross one another indicates that the elution order of the anions can be reversed by increasing the eluent concentration. At NaOH concentrations below 35 m*M*, nitrate elutes before diprotic carboxylate. When the eluent concentration is increased to 75 m*M* NaOH, the elution order is reversed and nitrate is retained longer than all anions, except fumarate.

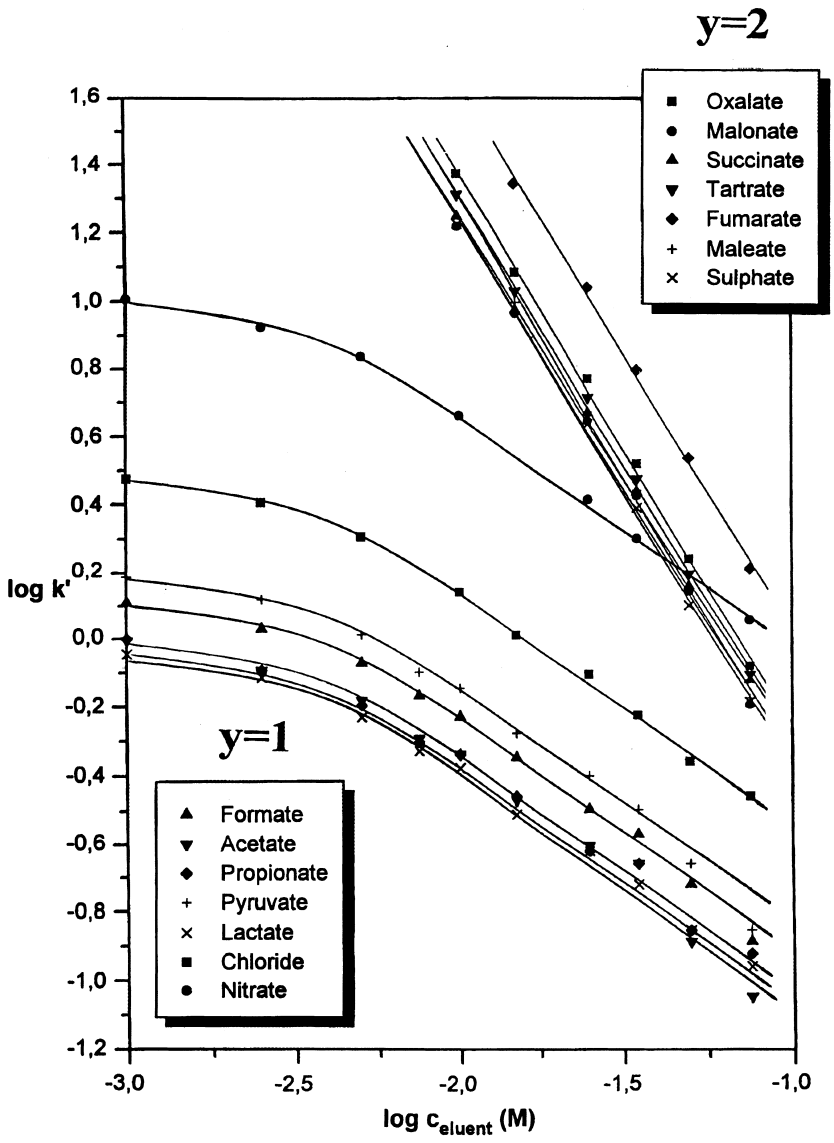

Fig. 3. Effect of eluent concentration on the retention behaviour of monovalent ($y=1$) and divalent ($y=2$) anions.

- Monocarboxylate anions eluted before dicarboxylate anions. Increased solute charges lead to increased capacity factors.
- Retention is longer when there is another functional group in the molecule. In general, keto- or -OH groups on the hydrocarbon chain predict higher retention volumes (pyruvate vs. propionate and tartarate vs. succinate).
- The retention behaviour of dicarboxylic acids depends on the relative position of the carboxyl groups on the hydrocarbon chain. Geometric isomers (maleate and fumarate) can be separated.
- Retention is higher as there is π bound on the hydrocarbon chain (fumarate, maleate vs. succinate).

2.3. Stationary phase-related factors

Retention behaviours of analytes are also determined by some major factors of the stationary phases

used in anion chromatography: (1) the morphological structure of the stationary phases (pellicular, macroporous, gel-types), (2) the chemical composition of the matrix (polystyrene, polymethacrylate, silica-based), (3) the chemical structure of the functional groups (dimethyl-ethylamine, dimethyl-allylamine, trimethylamine) and (4) the ion-exchange capacities. The majority of the stationary phases used in ion chromatography are pellicular polystyrene-based, latex materials [13]. Latex-based pellicular covering is a term used to describe the attachment of colloidal ion-exchange resin of one change to a much larger substrate particle of the opposite charge. These packings offer high speed, high efficiency and moderate loading capacity while maintaining very short diffusion paths. These features combine to give better resolution and shorter separation times. The chemically and mechanically stabile substrate core is ethylvinylbenzene crosslinked with 55% divinylbenzene, which permits the use of these packings with HPLC solvents. A pellicular resin has active ion-exchange capacity only at or near the surface of the bead. Fig. 4 shows an expanded view of an aminated pellicular anion-exchange bead. The anion-exchange latex is held on the surface primarily by the electrostatic attraction of the positively charged latex particles to the negatively charged surface active sites.

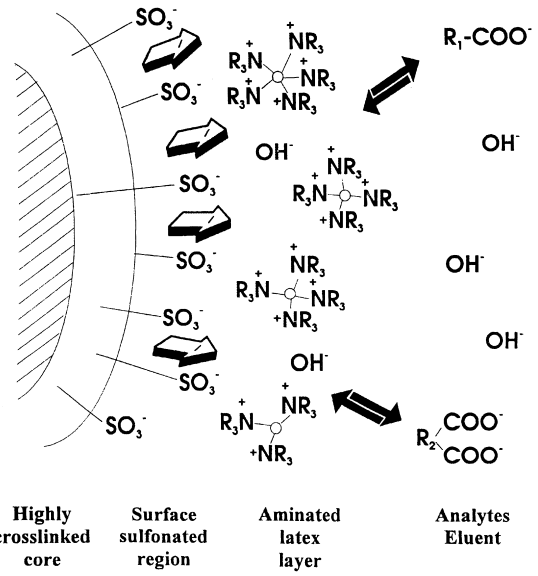

Fig. 4. Schematic view of the mixed mode retention effects on a latex-based pellicular phase.

The k' data from Fig. 3 can be used to show the retention mechanism of carboxylates on the pellicular phase with sodium hydroxide eluents. The slopes of these linear plots should be equal to (-1) times the charge of the sample anions divided by the charge of the eluent, as with $-y/x$ in Eq. (6). Since the charge of the OH^- eluent is (-1), the slopes should be equal to the charge of the analytes. Studies of retention in ion chromatography showed results that were in general agreement with the theoretical ion-exchange model [14,15], but, in this case, some significant deviations from the predicted dependencies indicated that the actual mechanism was more complex. All slopes in Fig. 3 are definitely less for the mono- and divalent carboxylates than those predicted on the basis of the stoichiometric pure ion-exchange model.

A logical hypothesis for this unusual retention behaviour of anions may be made based on the chemical structure of the highly crosslinked latex-based pellicular phase. This polymer configuration concentrates a vast number of anion-exchange sites into a very narrow layer on the surface-sulfonated highly crosslinked (55%) core. It can be seen in Fig. 4 that there are two sites of interaction on the stationary phase, namely the anion-exchange site in the latex-bonded layer and the electrostatic repulsion or ion-exclusion site in an oppositely charged underlayer, which excludes anions through Donnan potential. In general, highly crosslinked polymers show stronger Donnan exclusion effects than resins of lower crosslinking [16].

With the pellicular resins used in suppressed IC, the outer latex particles are fully functionalized and the ion-exchange capacity of the stationary phase is manipulated easily by changing the latex particle diameter. Consequently, it is also possible to manipulate separation parameters, such as the retention time and resolution, by varying the column capacity (see Eq. (4)). On a low capacity column (45 μekv/column), a gradient procedure was developed [17] with borate eluent for the determination of most of the carboxylate components. The different retention characteristics of a high capacity (170 μekv/column) column were used to identify interfering components (see Table 2).

Table 2
Retention times (t_R) of low-molecular-mass carboxylate components on two different columns

Component Name	Composition	AS11 gradient	AS10 borate isocratic
		45 μekv/column; t_R (min)	7 m*M*, 170 μekv/column; t_R (min)
3-Hydroxybutyrate	$H_3CHOHCH_2CCOO^-$	5.0	7.2
Lactate	$H_3CHOHCCOO^-$	5.6	7.9
Acetate	H_3CCOO^-	5.8	7.9
Glycolate	HOH_2CCOO^-	6.2	8.6
Propionate	$H_3CH_2CCOO^-$	7.0	8.6
2-Hydroxybutyrate	$H_3CH_2CHOHCCOO^-$	7.0	8.8
Formate	$HCCOO^-$	8.8	12.0
Butyrate	$H_3CH_2CH_2CCOO^-$	8.6	9.6
Pyruvate	$H_3COCCOO^-$	10.0	16.0
Valerate	$H_3CH_2CH_2CH_2CCOO^-$	10.8	12.6
Oxalate	$(COO)_2^{2-}$	21.1	

Adapted from Ref. [17] with permission.

Dionex [18] demonstrated that two different retention modes, ion-exchange and reversed-phase, operate independently on the multi-phase column packing (PAX-500). Using this separator, the gradient elution system can control the reversed-phase selectivity for neutral organic molecules and ion-exchange selectivity for acidic anions. The hydrophobic porous core is the region where reversed-phase retention occurs. The outer layer of the bead is the site of ion-exchange retention.

3. Separation and detection methods

Over the past several years, improvements in separation and detection methods, especially for weak acid anions, have remained an important area of research in ion chromatography.

Because ionisation of organic acids depends on the pH, their elution behaviour would be affected by changing the pH of the mobile phase in the pH range determined by their pK_a values. Using an eluent pH higher than 8, all species, i.e. basic eluents and sample analytes, are in an anionic form. Conductivity detection using NaOH eluent is not very sensitive in a single column system because of the high background conductivity. A suppressed system however yields a sensitive method as the background conductivity is reduced, NaOH is transformed into water and sample anions remain mostly in an anionic form. The pH of the eluent after passing the suppressor device is between seven and eight. The separation system can be seen in Fig. 5 [6]. Fig. 6 shows a separation of eight mono-, di- and trivalent carboxylate anions and sulphate ions using 50 m*M* NaOH as the eluent and an isocratic method. Mostly baseline resolution was achieved between every peak. Under these conditions, citrate is strongly retained. Chemically suppressed conductivity detection was accomplished using a micromembrane

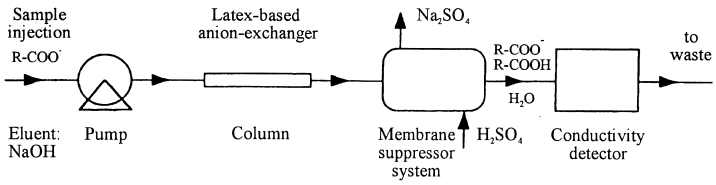

Fig. 5. Block diagram showing the instrumental components used for suppressed IC of carboxylic acids.

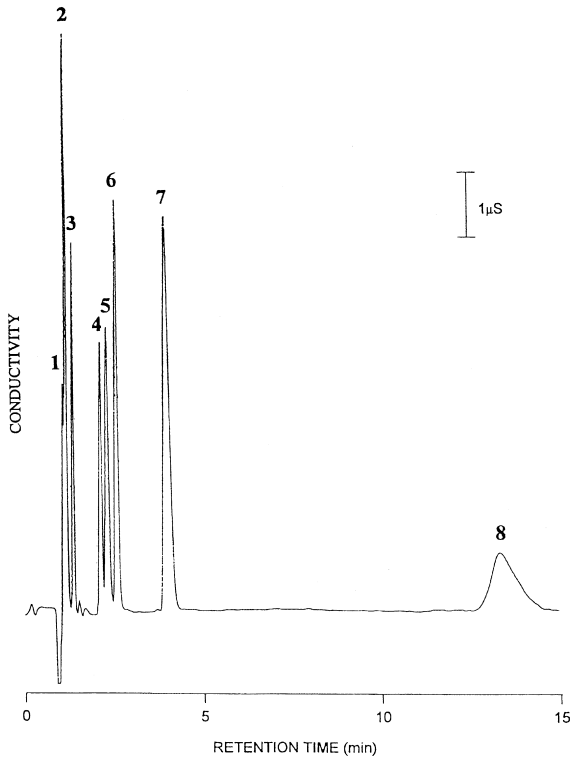

Fig. 6. Chromatogram of carboxylate- and inorganic anions. The concentrations of the anions in the samples varied in the range of 0.05-0.1 m*M*. Detection was by suppressed conductivity. C_{eluent} = 50 m*M* NaOH, Peaks: 1 = acetate, 2 = pyruvate, 3 = chloride, 4 = sulphate, 5 = succinate, 6 = oxalate, 7 = fumarate and 8 = citrate. Figs. 3–6 are reproduced with permission from Ref. [6].

suppressor that was continuously regenerated with sulphuric acid.

A popular but complicated method for the simultaneous separation of inorganic and organic anions is to couple ion-exclusion and ion-exchange separation modes by the multidimensional method [19]. The weakly ionised carboxylic acids pass rapidly through an anion-exchange column and suppressor membrane, where they are not retained. This early part of the chromatogram was separated on an ion-exclusion column. At the same time, the fully ionised inorganic anions are separated on the anion-exchange column.

Singly charged carboxylic acids ($C_{n<4}$) exhibit a very low affinity to the anion separator, so that, a weak eluent strength can provide sufficient resolution to separate these components. However, weak eluents do not clearly elute double and triple charged acids. For a wide variety of organic acids, high-performance anion-exchange chromatography on a latex-based pellicular phase with gradient elution became a powerful tool for analytical separation. Gradient elution times are short relative to other techniques and detection limits of suppressed conductivity are as good as, or better than, those obtained with other types of detection. Gradient elution, however, is only practical when steps are taken to minimise the charge in background conductivity of higher eluent concentration.

In suppressed ion chromatography, gradients are performed by choosing eluents that can be suppressed to produce little or no background conductivity. For anion-exchange gradients, salts of weak bases with $pK_a > 7$ can be used. These include the sodium salts of boric acid ($pK_a = 9.1$), and *p*-cyanophenol ($pK_a = 8.0$). Of course, the best weak acid to use in water itself; the salt being sodium hydroxide. Carbonate-containing eluents are generally not acceptable for gradient elution because of the relatively low p*K*a of 6.3 for carbonic acid. An example of the ability of gradient elution to separate and elute a large number of both organic and inorganic ions in a single run is shown in Fig. 7a. Here, the initial eluent is 0.75 m*M* NaOH, which is dilute enough to separate the weakly retained monoprotic acids. The eluent at the end of the gradient is 85 m*M* NaOH, which is concentrated enough to elute the much more strongly retained triprotic isomers of citric acid. The system can separate up to 36 anions with charges from -1 to -3 in one gradient run. Another example of gradient elution using cyanophenate is shown in Fig. 7b. The separator column is used to obtain better selectivity of the diprotic organic acids. This illustrates the separation of 22 anions in 22 min. This would require three isocratic elution steps if separated individually.

In some suppressed systems, there is a non-linear dependence of peak height on sample amount. This is common when analytes produce very weak organic acids ($pK_a > 7$) in the suppressor and is caused by the decrease in analyte dissociation with increasing concentration. The detector signal is thus strongly dependent on the pK_a of the analyte. If, however, the detector influent background was alkaline instead of slightly acidic, all weak acids would be negatively

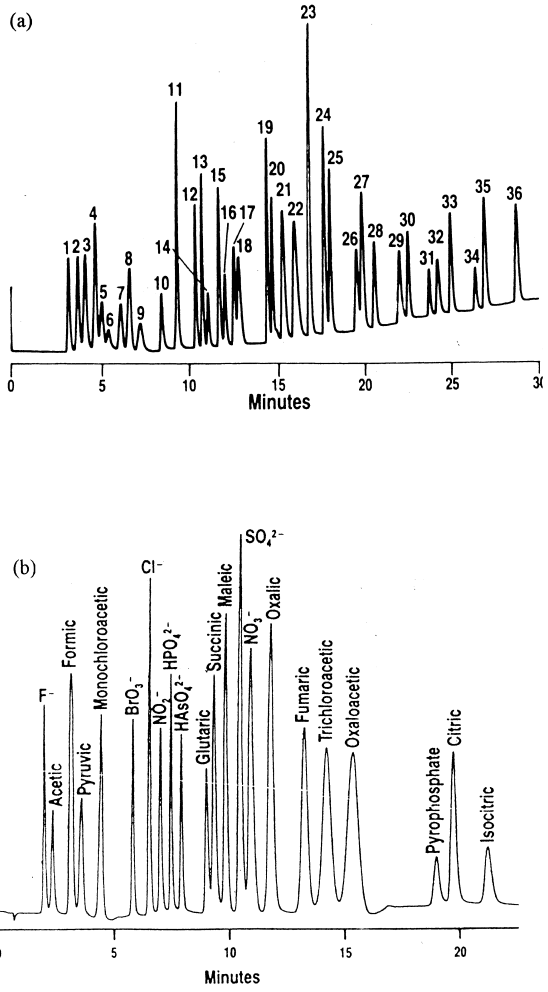

Fig. 7. Gradient elution of carboxylate- and inorganic anions. (a) (1) Fluoride (1.5 ppm); (2) α-hydroxybutyrate; (3) acetate; (4) glycolate; (5) butyrate; (6) gluconate; (7) α-hydroxyvalerate; (8) formate (5 ppm); (9) valerate; (10) pyruvate; (11) monochloroacetate; (12) bromate; (13) chloride (3 ppm); (14) galacturonate; (15) nitrite (5 ppm); (16) glucoronate; (17) dichloroacetate; (18) trifluoroacetate; (19) phosphite; (20) selenite; (21) bromide; (22) nitrate; (23) sulphate; (24) oxalate; (25) selenate; (26) α-ketoglutarate; (27) fumarate; (28) phthalate; (29) oxalacetate; (30) phosphate; (31) arsenate; (32) chromate; (33) citrate; (34) isocitrate; (35) *cis*-aconitate and (36) *trans*-aconitate. Column, Dionex AS5A 150×4 mm, 5 μm latex-coated resin. Eluent, NaOH gradient. (b) Column, Dionex AS6; eluent, *p*-cyanophenate gradient. Reproduced with permission from Ref. [2].

ionised and would thus be sensed by conductance. In order to solve the problem of detection, a sequential suppressed and single column separation system has

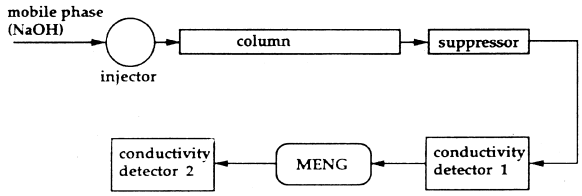

Fig. 8. Block diagram showing the instrumental components used for sequential suppressed IC. Reproduced with permission from Ref. [20,21].

been developed [20,21] (see Fig. 8). This technique combines the advantages of both suppressed and non-suppressed conductivity detection. Following the detector (1) of a conventional NaOH eluent suppressed IC system, a constant concentration of NaOH is introduced by the microscale electrolytic NaOH generator (MENG), and this is followed by a second conductivity detector, which records the decrease in the eluent background signal. Thus the detector (1) signal is related to $(\lambda_{H^+} + \lambda_{A^-})$ and the analyte HA concentration (HA is not fully ionised). Similarly, the detector (2) signal is directly related to $(\lambda_{OH^-} - \lambda_{A^-})$ and the analyte concentration. Together, the two detector outputs provide detectabilities at the μg/l level for very weak acids. A specific application of chemiluminescence detection is the analysis of oxalate in biological samples with tris(2,2′-bipyridyl)ruthenium(II) as the postcolumn reagent [22]. Ion-exchange chromatography has successfully been coupled to mass spectrometric detection by an electrospray interface for the analysis of organic acids [23]. The MS also provides the analyst with a tool to deconvolute the coelution of acids. Disadvantages at monocarboxyl acids are the poor sensitivity because of their low molecular mass and volatility.

Among optical detectors, UV detectors are the most commonly used type of detector in the detection of carboxylic acids. A wavelength of 210 nm is most frequently selected because this is the region where absorbing acetic acid has its maximum absorptivity. However, because many carboxylic acids absorb weakly, the detection sensitivity is limited in the range of 0.01–10 m*M* [24]. Refractive index detection is useful for detecting substances having little or no measurable UV absorption.

Table 3
Routine quality control for the chromatographic determination of carboxylic acids

Solutes	Sample	Ion-exclusion column	Ion-exchange column	E^a	E^b	D^c	D^d	Reference
Carboxylic acids, inorganic anions	Water	Waters Fast Fruit juice	Waters IC Pac A	1.0 m*M* Octane–sulfonic acid	3.0 m*M* Sodium octanesulfonate	P	C	[19]
Carboxylic acids, inorganic ions	Coffee	Dionex HPICE	Dionex HPIC-AS1	10 m*M* HCl	3.0 m*M* $NaHCO_3$ 2.4 m*M* Na_2CO_3	C	C	[18]
F^-, formate, acetate, Cl^-, Br^-, NO_3, I^-, SO_4^{2-}	Solder fluxes	Waters Ion-exclusion	Waters IC-Pak A	1.0 m*M* Octane–sulfonic acid	4.0 m*M* Sodium octane–sulfonate	C	C	[37]
Lactate, pyruvate	Plasma	Dionex HPICE	Dionex HPIC-AS1	10 m*M* HCl	0.66 m*M* $NaHCO_3$	C	C	[31]
Organic acids	Biological materials	Dionex HPICE	Dionex HPIC-AS1	10 m*M* HCl	0.66 m*M* $NaHCO_3$	C	C	[31]
SO_4^{2-}, acetate, formate	Brine	Dionex JE-B-1	Dionex HPIC-AS1	H_2O	3.0 m*M* $NaHCO_3$ 2.4 m*M* Na_2CO_3	C	C	[38]
Vanillylmandelic	Urine	Dionex HPICE	Dionex HPIC-AS1	10 m*M* HCl 14% ACN[g]	6 m*M* Na_2CO_3	C	C	[31]
Carboxylic acids	Sweet wine		Shimpack IC-AI		0.975 m*M* Phthalic acid, pH 4.15		C	[39]
Organic acids	Fruit juice		Dionex OMNI Pac Pax-500 (chem. suppr.)		0.6–20 m*M* NaOH in ethanol–methanol gradient elution		C	[26]
Oxalate	Urine and plasma				2 m*M* Tris(2,2′-bipyridyl)–ruthenium(II)		PCR	[22]
Organic acids	Pharmaceuticals				NaOH gradient elution		MS	[23]
Carboxylic acids inorganic anions	Biological material		Shimpack IC-AI		0.975 m*M* Phthalic acid, pH 4.15		C	[40]
Short chain mono- and dicarboxylic acids	Drinking water		Dionex AS 10		8–125 m*M* NaOH gradient elution		C	[34]

[a]Eluent for ion-exclusion column. [b]Eluent for ion exchange column. [c]Detector for ion-exclusion system. [d]Detector for ion-exchange system.
C = conductivity. P = potentiometric. MS = mass spectrometric detection. PCR = postcolumn reaction detection. ACN[g] = acetonitrile.

4. Selected applications

Ion chromatography has been successfully applied to the analysis of carboxylic acids in many extremely diverse types of sample (see Table 3). In some cases, improvements in methods may exist that have not yet been brought into common practice. Also, the applications of ion chromatography are expanding and changing rapidly in this area.

There are several practical uses for the routine analysis of beverages like juice, wine and milk, as commercial products [25,26]. A complete profile of the carboxylic and inorganic acids, including the minor ones, are used to characterise the juice. Knowledge of the carboxylic and inorganic acids profile is very important in the control of the quality and genuineness of a juice. Small carboxyl acids are extremely important in wines and juice drinks because they contribute to taste and product stability [27]. It is essential for the wine maker to know the content of the carboxylic acid of the grape juice as this provides data for deciding the best time to harvest the grapes and for controlling the fermentation and also provides for the overall quality of the wine produced [28].

Various diseases have been correlated with increased concentrations of organic acids in biological fluids. Therefore, ion chromatography is very welcome in many areas, e.g. in serum, plasma, urine and cerebrospinal fluid monitoring, in diet and drug therapy, in bacterial drug therapy [29] and in other biomedical applications in clinical diagnosis, providing fast analysis and involving a minimum of sample preparation for routine analysis. One popular application is the determination of urinary oxalate [30]. Oxalate can cause painful kidney stones, but it has also been suggested that it has properties as an anticarcinogen. A feasibility study using ion-exchange–ion-exclusion coupled chromatography to analyse pyruvate and lactate in serum has also been done [31].

Air, surface and ground waters, and soil can be contaminated by unwanted carboxylic acids originating from different sources. Aliphatic monocarboxylic acids are industrial chemicals with a wide variety of applications. They are used in the preparation of polymers, resins and dyes in the manufacture of various esters for solvent purposes, in the food industry, in cosmetics and as chemical intermediates. The physiological consequences of exposure to carboxylic acid functions are primarily irritation of eyes, skin or mucous membranes, with short-chain acids (formic, acetic and propionic acids) also being responsible for burns [32]. The discharge of unconditioned silage liquors into soils, sewers and rivers can initiate environmental responses by containing short chain aliphatic acids [33]. The problem of determining carboxylic acids produced by bacterial anaerobic degradation of tetrachloroethane in ground water also can be a part of environment impairment. Low-molecular-mass carboxylic acids can be found in environmental water samples depending on the emitting sources and the ongoing biological and chemical activity. For example, glycolate is formed by photosynthesis and biopolymers are degraded in water by fermentative processes to formate, acetate, lactate and propionate [17]. In atmospheric waters, these acids can also be present.

During ozonation of drinking water, organic matter in the source water is oxidised to form a variety of carboxylic acids. A fast HPIC method was developed to identify and quantify selected ozonation by-products (hydroxybutyric, acetic, glycolic, butyric and formic acid) [34].

Fumaric, maleic, tartaric and succinic acids are used as counterions to isolate and stabilise basic pharmaceutical compounds and intermediates. A silica-based amine HPLC column was used with a conductivity detector and methanol, with sodium dihydrogenphosphate as the mobile phase, to separate these acids [35].

5. Conclusion

The most powerful separation and detection technique for aliphatic carboxylic acids in complex matrices seems to be high-performance suppressed ion chromatography with gradient elution using a latex-based pellicular stationary phase. HPIC offers a reliable methodology for the simultaneous analysis of mono-, di- and trivalent carboxylate anions and inorganic ions. Separation parameters are possible to manipulate by mobile phase- and stationary phase-related factors. Current research in this field is appropriately directed towards a fundamental under-

standing of how changes in the eluent and sample composition affect the separation process. Improvement in detection and separation modes have remained an important area of research in ion chromatography. However, recent developments indicate that HPIC is about to replace ion-exclusion chromatography for the analysis of organic ions.

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