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Approaches to gradient elution in ion-exclusion chromatography of carboxylic acids

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ABSTRACT

A range of eluents has been examined with a view to determining which can be used to manipulate the selectivity of retention in ion-exclusion chromatography when applied to the separation of common water-soluble carboxylic acids. The column used was a Bio-Rad HPX-87H Organic Acids column and the eluents examined included water and dilute solutions of sulfuric acid, phosphoric acid, *p*-toluenesulfonic acid, methanesulfonic acid and benzoic acid. Both conductivity and ultraviolet detection were utilized. The use of water alone as an eluent gave poorly shaped peaks, whilst the remaining eluents gave satisfactory peak shape. The best performance was obtained using methanesulfonic acid as eluent. Studies on gradient elution in ion-exclusion chromatography were also undertaken. Three approaches to gradient separation were investigated. The first approach was to utilize a concentration gradient in which the eluent concentration was decreased over the run, thereby increasing the degree of solute ionization and thus solute retention times. This method proved to be of limited utility. The second approach involved increasing the amount of an organic modifier (acetonitrile) in the eluent, and satisfactory gradients were produced by this method. In the third approach, a varying concentration of β-cyclodextrin was introduced into the eluent as a means of reducing the retention times of aromatic solutes through the formation of inclusion compounds with β-cyclodextrin. Once again, satisfactory gradient separations were produced with this approach.

INTRODUCTION

Carboxylic acids can be analyzed chromatographically by employing reversed-phase [1], ion-exchange [2,3] or ion-exclusion [4,5] techniques. The reversed-phase and ion-exchange methods are somewhat limited in that it is difficult to separate low-molecular weight acids using these approaches. On the other hand, ion-exclusion chromatography using a high-capacity cation-exchange resin in the H^+ form with an acidic eluent offers separation of a wider range of acids than is possible with either of the alternative methods. Typical ion-exclusion columns designed for the separation of carboxylic acids contain sulfonated polystyrene–divinylbenzene copolymers. Large column dimensions are necessary to provide sufficient occluded mobile phase to permit a reasonable degree of retention of solute acids.

The theory of ion-exclusion chromatography has been discussed by a number of authors [6–8] and it is evident that solutes are retained by a number of mechanisms, including electrostatic effects, adsorption, and perhaps size exclusion. Paramount amongst these is the electrostatic interaction of the solute with the charged functional groups on the resin surface. These functional groups can be considered to comprise a charged membrane separating the flowing mobile phase from occluded, static mobile phase trapped in the pores of the resin. Ionic solutes are rejected, or excluded, from the resin because of their inability to penetrate the charged

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"membrane" and so are eluted at the void volume of the column. In contrast, non-ionic (or weakly ionic) substances may partition between the occluded liquid phase and the flowing mobile phase. The degree of partition determines the extent of retention on the column. Although ion-exchange resins are used, true ion-exchange reactions are not involved.

The degree of retardation increases with decreasing the degree of ionisation (or increasing p*K*a) [7]. Strong acids which are fully ionised are totally excluded and are eluted at the void volume. Very weak acids (pK_a greater than 6.4) tend not to be excluded at all and permeate totally into the resin, giving retention times which are independent of pK_a. Many acids with pK_a values intermediate between these extremes show an elution order which can be predicted from pK_a values. However, other acids (particularly aromatic carboxylic acids and long-chain aliphatic carboxylic acids) show retention times which are longer than expected from consideration of their pK_a values alone. In these cases, hydrophobic adsorption effects are considered to contribute to the retention process. Finally, some acids (especially difunctional aliphatic carboxylic acids) show less retention than expected from their pK_a values. For these species, it has been postulated [9–11] that size-exclusion effects may restrict access to the occluded liquid.

Eluents employed in ion-exclusion chromatography can be water [12] or a dilute solution of a strong mineral acid such as sulfuric acid [13] or an aliphatic sulfonic acid [14,15]. Weak acids such as phosphoric acid [16] and benzoic acid [13] are also commonly used. The use of an acidic eluent gives improved peak shape and ensures that the retention time is independent of solute concentration. Organic modifiers have also been employed as eluent components in ion-exclusion chromatography [12]. Solvents such as methanol have little effect on the retention times of low-molecular-weight aliphatic acids, but cause a decrease in the retention time of larger aliphatic acids [12]. Gradient elution in ion-exclusion chromatography has been reported using eluents comprising 6–64% methanol in 0.5 m*M* sulfuric aicd [13]. A further limitation on the type of eluent used is that UV detection (typically in the wavelength range 210–215 nm) is generally employed, and the cluent must be transparent at the detection wavelength. With all of the eluents currently used, the separation selectivity is rather limited, with the pH of the eluent exerting the greatest influence on retention.

The potential of gradient elution has been exploited widely in most forms of liquid chromatography, but has not been used to any significant extent in ion-exclusion chromatography. In this study, selectivity effects in ion-exclusion chromatography arising from variation of the composition of the eluent are investigated. These selectivity effects are then applied to gradient elution of mixtures of aliphatic and aromatic carboxylic acids by ion-exlusion.

EXPERIMENTAL

Instrumentation

The liquid chromatograph comprised a Millipore-Waters (Milford, MA, USA) M-600 E multisolvent gradient pump and a Waters Model U6K injector. Two detectors were used, namely a Waters Model 480 variable-wavelength UV–VIS detector and a Waters Model Model 430 conductivity detector. The column used was a Bio-Rad (Richmond, CA, USA) Aminex HPX-87H Organic Acids ion-exclusion column (300 × 7.8 mm I.D.) packed with 9-μm sulfonated styrene–divinylbenzene resin with 8% cross-linking. Chromatograms were recorded on a BBC Goerz Metrawatt (Vienna, Austria) SE 120 chart recorder.

Reagents and procedures

The carboxylic acids used as solutes are listed in Table I, together with the pK_{a1} value for each acid.

TABLE I

pK_{a1} VALUES OF THE CARBOXYLIC ACIDS INVESTIGATED

Acid	pK_{a1}	Acid	pK_{a1}
Oxalic	1.23	Acetic	4.75
Maleic	1.83	Propionic	4.87
Malonic	2.83	Isobutyric	4.84
Tartaric	2.98	Mandelic	3.66
Citric	3.13	Phthalic	2.89
Malic	3.40	Terephthalic	3.82
Succinic	4.20	*p*-Hydroxybenzoic	4.48
Formic	3.75	Benzoic	4.19
		Salicylic	3.00

TABLE II

RETENTION TIMES OF CARBOXYLIC ACIDS FOR DIFFERENT ELUENTS (1 m*M*)

Solute acid	Retention time (min)					
	Water	H_3PO_4 (pH 3.02)	H_2SO_4 (pH 3.63)	Benzoic acid (pH 3.62)	*p*-Toluenesulfonic acid (pH 3.00)	Methanesulfonic acid (pH 2.74)
Citric	4.0	5.7	6.3	4.5	5.7	5.3
Tartaric	4.0	5.7	6.8	4.5	5.7	5.5
Malonic	4.0	6.0	N.A.	4.5	6.3	5.5
Malic	4.0	6.6	7.6	5.3	6.7	6.3
Succinic	N.A.[a]	9.2	N.A.	7.0	9.2	8.6
Formic	6.3	9.7	10.9	7.0	10.0	9.5
Acetic	8.7	11.7	12.1	9.5	11.3	10.9
Isobutyric	N.A.	15.6	N.A.	12.8	15.8	14.7

[a] N.A. = Data not available.

These acids were obtained as analytical-grade reagents and were used without further purification. Eluents were prepared by diluting the required amounts of the eluent acid in a 1-l volumetric flask, followed by degassing using a Bransonic 220 (Branson, CO, USA) ultrasonic bath prior to use. The water used was purified on a Millipore (Bedford, MA, USA) Milli Q water purification system and filtered through a Millipore solvent clarification apparatus using a Millipore type 0.45-μm HA filter. The eluent flow-rate was fixed at 1.0 ml/min. Working standard solutions were prepared daily from stock solutions of 10 000 ppm (aliphatic) or 1 000 ppm (aromatic) carboxylic acids.

RESULTS AND DISCUSSION

Nature of the eluent acid

Ion-exclusion chromatography is most commonly performed using sulfuric acid as eluent, with UV detection. This eluent was compared with water, phosphoric acid, methanesulfonic acid, *p*-toluenesulfonic acid and benzoic acid eluents, using both UV and conductivity detection. Table II lists retention times obtained at an eluent strength of 1 m*M* and shows that the elution order was almost identical for each eluent. That is, no selectivity effects arose when the nature of the eluent acid was varied. Detection limits were calculated for each eluent using both UV and conductivity detection. In some cases, a particular detection mode was inappropriate (*e.g.* UV detection with UV-absorbing eluents, such as benzoic acid). Again, no large variations in detection limits were observed for the different eluents tested but methanesulfonic acid offered the best overall performance in terms of separation and chromatographic efficiency and was therefore employed in all further work.

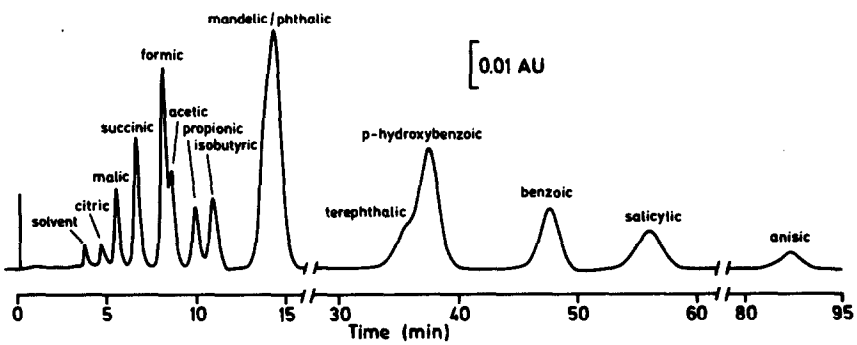

Fig. 1. Isocratic separation of aliphatic (20–500 ppm) and aromatic (5–50 ppm) carboxylic acids. The eluent was 10 m*M* methanesulfonic acid containing 1% (v/v) acetonitrile. Spectrophotometric detection at 210 nm was used.

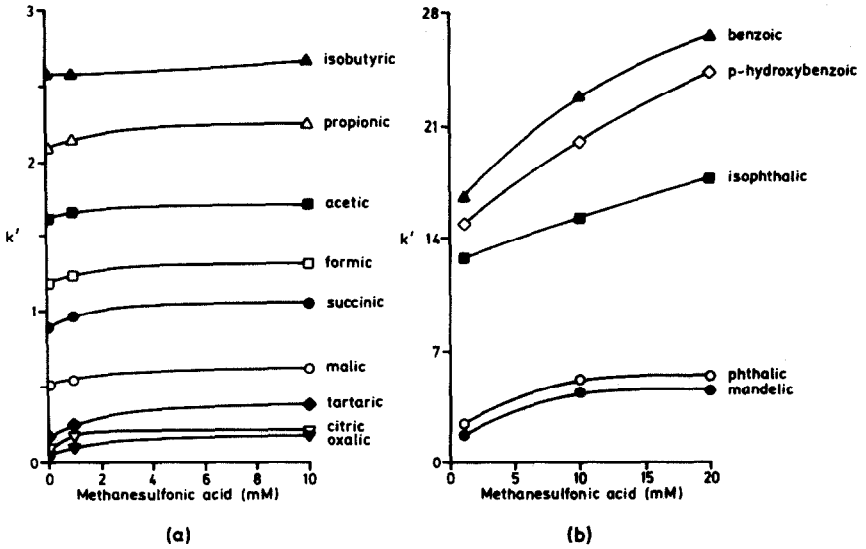

Fig. 2. Effect of the eluent concentration of methanesulfonic acid on the capacity factors of (a) aliphatic and (b) aromatic carboxylic acids. Other conditions as in Fig. 1.

Fig. 1 shows the isocratic separation of fourteen aliphatic and aromatic carboxylic acids using 10 m*M* methanesulfonic acid [containing 1% (v/v) acetonitrile] as eluent. The aliphatic acids are eluted in the early part of the chromatogram, followed by the aromatic acids which show very strong retention on the column. The retention times of the aliphatic carboxylic acids increase with increasing pK_{a1} and with the exception of the difunctional succinic acid, the elution order can be predicted from the pK_{a1} values. This indicates that the electrostatic ion-exclusion effect predominates for these solutes. The strong retention of aromatic acids is attributable to hydrophobic adsorption effects.

Effect of eluent concentration

The effect of concentration of methanesulfonic acid in the eluent on the capacity factors of aliphatic and aromatic carboxylic acids is shown in Fig. 2. Although most of the organic acids are too weak for their ionisation to be affected significantly by pH changes in the range 1.80–3.52, an increase of eluent concentration caused a moderate increase in capacity factors. This result agrees with a previous study [4]. This is a characteristic effect of the pH of the eluent in ion-exclusion chromatographic separation [8]. Stronger acids, such as oxalic and citric acids, show a more pronounced dependence on the eluent concentration. As this parameter is increased (and the eluent pH is lowered) these acids became less ionized, leading to decreased electrostatic (ion-exclusion) repulsion and hence increased retention times. The aromatic acids (Fig. 2b) show quite strong dependence on the concentration of methanesulfonic acid in the eluent.

The effect of the eluent concentration on column efficiency is listed in Table III, which shows that there is a general increase in column efficiency as the

TABLE III

COLUMN EFFICIENCIES AT DIFFERENT METHANESULFONIC ACID CONCENTRATIONS

Acid	Theoretical plates per column (*N*)		
	0.5 m*M*	1.0 m*M*	10.0 m*M*
Citric	4 225	4 556	6 148
Malic	5 378	6 601	8 100
Malonic	4 726	5 814	7 836
Formic	13 514	14 400	16 256
Acetic	8 251	11 556	13 275
Propionic	8 755	9 025	10 796
Isobutyric	4 160	5 025	6 944

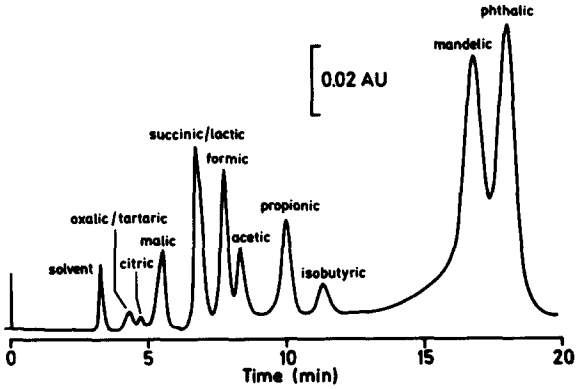

Fig. 3. Gradient elution from 50 to 1 m*M* methanesulfonic acid. Other conditions as for Fig. 1.

eluent concentration is increased. This result is in accordance with a previous study [17]. The plate number (N) is strongly influenced by the degree of ionization of the acids, with the relatively strong acids (*e.g.* citric) showing the lowest N values.

The above results suggest that the eluent concentration can exert a limited degree of retention selectivity in ion-exclusion chromatography, especially between the stronger and weaker carboxylic acid solutes. Gradient elution by changing the eluent concentration of methanesulfonic acid should therefore be possible for mono- and dibasic carboxylic acids and the less strongly retained aromatic carboxylic acids. Fig. 3 shows a chromatogram obtained by changing the eluent concentration of methanesulfonic acid over the range 50.0–1.0 m*M*. The gradient employed was a negative gradient, *i.e.* from high to lower concentration of eluent, since this process increases the eluotropic strength. The baseline change is minimal, but the separation time and resolution are improved only marginally in comparison to isocratic separation.

Effect of organic modifier

Addition of an organic modifier, such as methanol, acetonitrile or acetone, to the eluent may influence the participation of solute adsorption effects in the retention process. This influence should be greatest for aromatic acids. Fig. 4 shows the retention changes for both aliphatic and aromatic acids caused by the addition of acetonitrile to the methanesulfonic acid eluent. For aliphatic carboxylic acids (Fig. 4a), the capacity factors were reduced slightly by the addition of acetonitrile, whereas very significant changes were observed for the aromatic acids (Fig. 4b). Some eluent selectivity therefore exists, especially between the aromatic and aliphatic carboxylic acids.

Gradient elution was therefore performed by varying the percentage of acetonitrile over the range

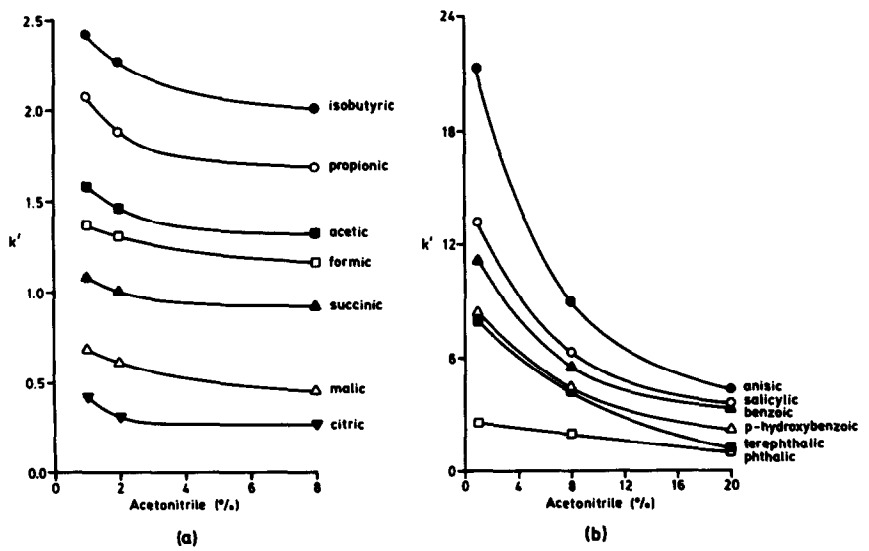

Fig. 4. Effect of the eluent concentration of acetonitrile on the capacity factors of (a) aliphatic and (b) aromatic carboxylic acids. Other conditions as in Fig. 1.

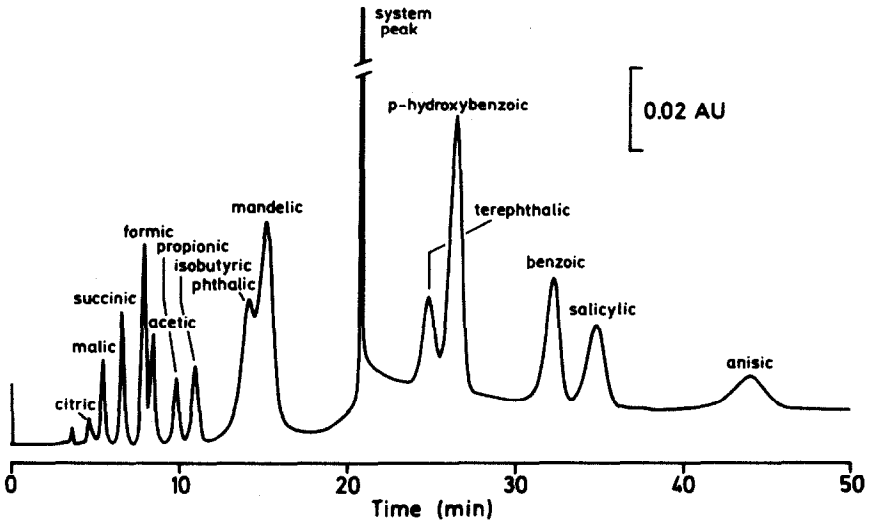

Fig. 5. Gradient elution performed by varying the percentage (1 to 15%) of acetonitrile in 10 m*M* methanesulfonic acid eluent. Other conditions as in Fig. 1.

1–15%, with the methanesulfonic acid concentration being maintained at 10.0 m*M*. The chromatogram obtained is shown in Fig. 5. There is little baseline change and both the separation time and resolution are improved considerably when compared to the isocratic run, especially for the aromatic carboxylic acids. As expected, the separation of the aliphatic acids does not differ greatly from that shown in Fig. 1. A disadvantage of this approach is the presence of an extraneous peak in the chromatogram, the retention time of which was constant regardless of the concentration limits of the gradient or the slope of the gradient ramp. The height of the peak was proportional to the percentage of acetonitrile in the eluent, suggesting that this peak originated from the acetonitrile itself. Such a system peak has been noted previously [17]. It is possible that other organic modifiers could be used to eliminate this peak; however, this was not investigated.

Effect of β-cyclodextrin in the eluent

Cyclodextrins (CDs) are cyclic oligosacharides, constructed from α-(1,4)-linked glucose units arranged in a torus, with the most common CDs being α, β- and γ-CD, containing six, seven and eight glucose units, respectively. The structures of these compounds are typified by a central cavity which gives rise to their remarkable ability to form inclusion complexes with various guest molecules. In addition, CDs are stable within a wide range of pH and do not absorb in the full UV region commonly used in chromatographic detection.

For the above reasons β-CD has been investigated as a mobile phase modifier in reversed-phase liquid chromatography (RPLC) [18] and, more recently, in ion-exclusion chromatography [19]. In the latter study, we have shown that the value of the inclusion constant for a solute (*i.e.* the equilibrium constant for the formation of the inclusion complex) can be determined using the ion-exclusion chromatography retention volume of that solute in an eluent containing β-CD. In the present study β-CD is utilized as an eluent modifier in order to reduce the retention times of those solutes forming inclusion complexes. Fig. 6 shows the effect of varying eluent concentrations of β-CD on the retention times of carboxylic acids. It can be seen that retention times of the aliphatic carboxylic acids showed slight decreases (Fig. 6a), whereas those for most of the aromatic acids were decreased significantly (Fig. 6b). This result is in accordance with our previous study [19] wherein aromatic carboxylic acids were found to have much greater inclusion constants than aliphatic acids.

The third approach to gradient elution in ion-

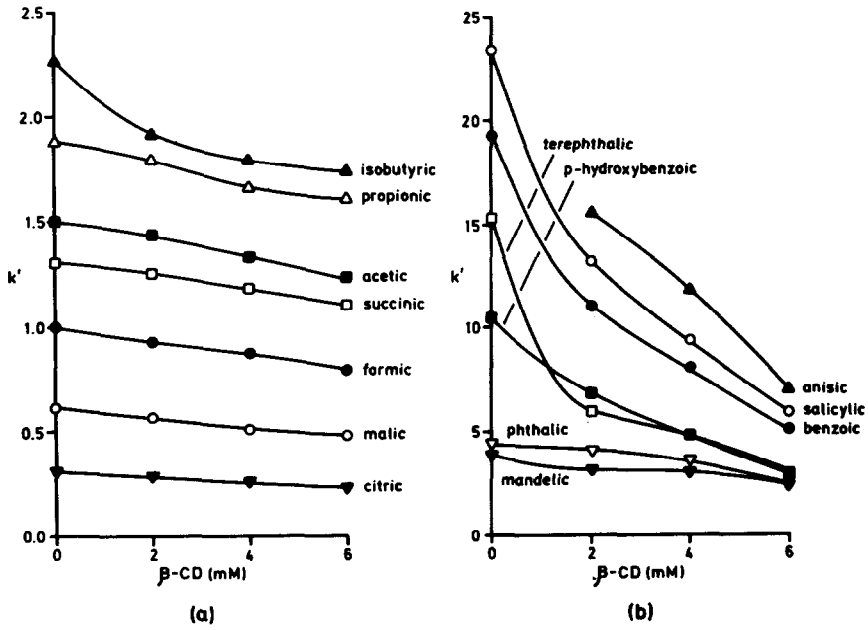

Fig. 6. Effect of the addition of β-cyclodextrin on the capacity factors of (a) aliphatic and (b) aromatic carboxylic acids. Other conditions as in Fig. 1.

exclusion chromatography involved increasing the concentration of β-CD in the eluent. A chromatogram obtained with a gradient from 0 to 6.0 m*M* β-CD in an eluent containing 10.0 m*M* methanesulfonic acid and 2% acetonitrile is shown in Fig. 7. Some baseline drift is apparent (due to the slight absorbance of β-CD at the detection wavelength used), however, the analysis time and resolution are

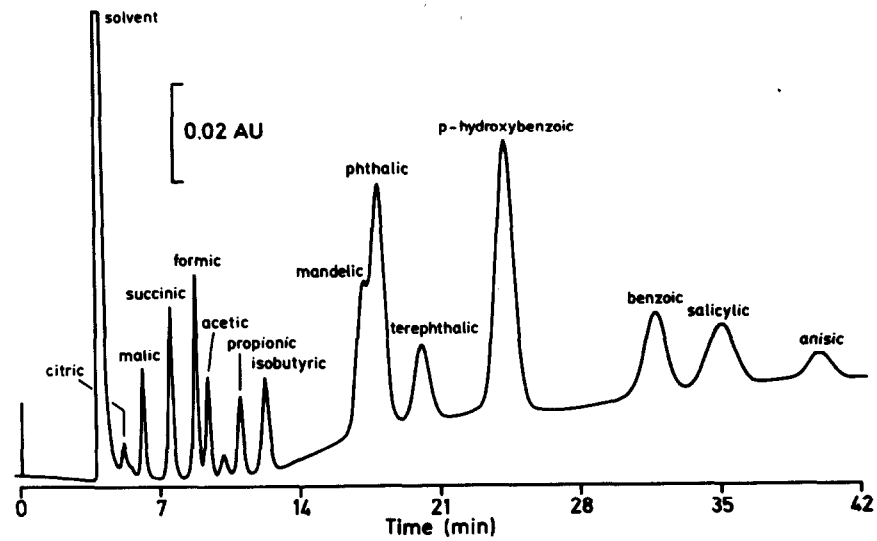

Fig. 7. Gradient elution performed by varying the concentration (1 to 6 m*M*) of β-cyclodextrin in 10 m*M* methanesulfonic acid eluent. Other conditions as in Fig. 1.

superior to those for the other gradients shown in Figs. 3 and 5. Calibration plots were constructed by injecting mixtures of carboxylic acids at various concentration and using the gradient described above. Linear calibration was obtained up to at least 1000 ppm for the aliphatic carboxylic acids and 100 ppm for the aromatic carboxylic acids.

CONCLUSIONS

This study has shown that methanesulfonic acid is a useful eluent for ion-exclusion chromatography of carboxylic acids. Some eluent selectivity can be attained through variation of the concentration of methanesulfonic acid in the eluent, but greater selectivity effects occur through the addition of acetonitrile or β-CD to the eluents. Gradient elution in ion-exclusion chromatography can be achieved by varying the concentration of methanesulfonic acid, acetonitrile or β-CD. The latter two approaches are more effective than the first, but acetonitrile produces a system peak in the chromatogram which may cause interference. The β-CD gradient causes a significant reduction in the retention times of aromatic acids as a result of the formation of inclusion complexes. Since such complexes are formed only with solutes of appropriate size, the β-CD gradient is more selective than the alternative approaches investigated in this work.

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