Ion-exclusion Chromatography of Weak Acids Using a Carbonic Acid Solution as Eluent

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Ion-exclusion chromatographic separation of organic acids using a carbonic acid solution as eluent is presented. This permits conductivity detection of ionic species without a suppressor as a post-column reactor. It was found that the proposed method was applicable to the separation of organic acids having pK_a over *ca* 3.5. This technique was applied to the determination of acetic acid in pharmaceuticals without tedious sample preparation.

In recent years, ion-exclusion technique, first reported by Wheaton and Bauman[1] for the separation of nonionic from ionic materials, has become of wider interest because of its ability to separate strong acids as a class from weak acids and also from each other.

Tanaka *et al.*[2] have indicated that ion-exclusion chromatography (IEC) using a hydrogen-type cation-exchange resin and flow-coulometric detection could be used for the separation of many weak acids from each other and for their determination. They[3] also have recently described the ion-exclusion behavior of a large number of strong and weak acids using water as eluent. Furthermore, Turkelson and Richards[4] have reported the use of IEC to separate most of the Krebs cycle acids, demonstrating that ion-exclusion can also separate weak acids from each other by the use of dilute hydrochloric acid solution as eluent.

Ion chromatography exclusion (ICE) technique, one of the modes of ion chromatography,[5] has also been successfully applied to the separation and analysis of many organic acids in various samples having complex matrices, *i.e.* brine,[6] foods,[7] and biologicals.[8] In this technique, using a dilute hydrochloric acid solution as eluent and conductivity detection, the separator column is connected with a so-called halide suppressor, which permits a sensitive detection of the ionic species in a sample solution with the reduction of the background conductivity. Although it has been proven that the ICE technique was a useful one for the analysis of organic acids, unfortunately, it has some disadvantages because of the use of the halide suppressor. In the suppression reaction procedure, the formation of silver chloride precipitation in the suppressor column causes an increase of back pressure in the system. Consequently, the retention time of the analyte will change and the detector response of the analyte will be diminished. Thus, the identification of unknown species will be somewhat difficult and the analytical precision in the quantification of the species will be poor, even though these problems may be improved by replacement with a different suppressor and/or by cutting off the consumed part of the suppressor.

In the previous communication,[9] we have briefly reported a new ion-exclusion technique using a carbonic acid solution as eluent and conductivity detection without a suppressor as a post column reactor. The present paper describes the elution behavior of several organic acids and the application to the acetate analysis to some pharmaceuticals.

Experimental

Materials. All reagents used were of reagent grade. All standard solutions, except for the solution of lithium lactate, valeric and hexanoic acid, were prepared by dissolving the acid and/or the corresponding sodium salt in distilled and deionized water (DI water). Valeric and hexanoic acid standard solutions were prepared by dissolving the acid in aliquots of 1 mol dm^{-3} NaOH solution and diluting with DI water. In addition, acetic acid standard solution for calibration was prepared by dissolving the potassium salt in DI water. The salt was dried in an electric drier oven at 110 °C for 24 h. Klinisalz® B Ringer's solution (Eisai) and Celtol® Intramuscular lGm (Takeda) were obtained from commercial sources. The aqueous solution of Celtol® injections was prepared by dissolving 0.5325 g of it (500 mg as Potency) in 5.0 cm^3 of the distilled water for injection and held in water bath at 25 °C.

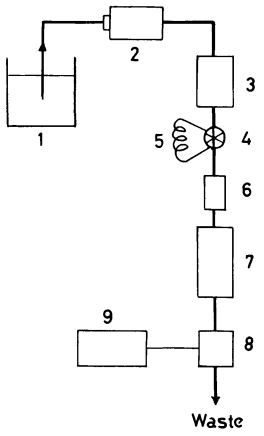

Fig. 1. Flow diagram of IEC system.
1: Eluent reservoir, 2: Pump, 3: Dionex Anion Suppressor (P/N 30828), 4: Injection valve, 5: Sample loop, 6: Dionex Anion Suppressor (P/N 38029), 7: Dionex ICE Separator (9 mm i.d. × 250 mm, P/N 30888), 8: Conductivity detector, 9: Recorder.

Chromatographic System. The Dionex Model 10 Ion Chromatograph equipped with conductivity detector was modified. The flow diagram of the modified system is shown in Fig. 1. Two Dionex anion suppressors[10] were used: One (9 mm i.d.×100 mm) between the pump and the injection valve and another (6 mm i.d.×60 mm) between the injection valve and the ICE Separator.[11] There are two reasons for introducing a suppressor before the ICE Separator. First, this column plays a role of a so-called guard column for the ICE Separator. Second, it neutralizes the sample solution and converts the sample ions into the corresponding acid forms. Thus, the system can be continually operated for 20 to 30 h depending upon the sample matrix and the flow rate without column regeneration. In addition, with a view to faster analysis, both the separator valve and the suppressor valve were bypassed by connecting the top of the suppressor column directly to the injection valve and the bottom of the ICE Separator directly to the conductivity cell inlet using a short length of tubings (0.5 mm i.d). The eluent reservoir contains a sodium carbonate solution and/or a standard sodium hydrogencarbonate/sodium carbonate solution which is commonly used for the anion analysis in ion chromatography. This solution is converted into a definite concentration of carbonic acid solution by passing it through the suppressor column. The injection volume was 50 mm^3. The strip chart recorder was operated at 1 mV full scale range and a chart speed of 30 cm h^{-1}. A 0.5 mol dm^{-3} sulfuric acid solution was used for column regeneration at flow rate of 1.0 cm^3 min^{-1}.

Results and Discussion

Elution Behavior of Organic Acids. In the previous paper,[9] it was shown that the presence of carbonic acid in the eluent dramatically improves the separation of organic acids such as formic, acetic, propionic, and butyric. Figure 2 typically shows the variation of the chromatographic capacity factor (k') of four organic acids with the concentration of carbonic acid in the eluent. The increase of each k' value was observed with the increase of the concentration of carbonic acid, that is, the decrease of pH of the eluent (pH 5.0 to 4.3). This is a characteristic effect of the pH of the eluent in ion-exclusion chromatographic separation.[4,12]

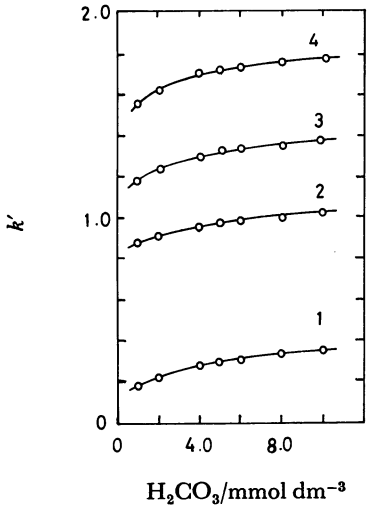

Fig. 2. Variation of the chromatographic capacity factor (k') with the concentration of carbonic acid solution.
Notations: 1 Formic acid, 2 acetic acid, 3 propionic acid, 4 butyric acid.

The factors which affect the separation characteristics in IEC are pK_a, structure and concentration of solute, temperature, eluent pH, nature of the resin, such as degree of cross linkage, and eluent flow rate *etc.*[1,6] Among these factors, generally, the pK_a of the solute can be used to predict its elution behavior. Because of a Donnan membrane effect, at a given eluent pH, the distribution of the solute between the occluded liquid inside the resin pores and the interstitial liquid of the resin particles depends upon its pK_a. The lower the pK_a, the smaller the distribution, that is, the lower the pK_a, the faster the elution. Indeed, Tanaka *et al.* have shown that this general rule holds for a large number of inorganic acids and also for some organic acids such as formic and acetic.[3]

Table 1 lists k' values of mono- and dicarboxylic acids and some substituted derivatives of these acids obtained by using carbonic acid solution (5.0 mmol dm^{-3}) as eluent together with their pK_a values. These k' data show that the general rule mentioned above holds only for acids of low molecular weight such as formic, acetic, succinic and glutaric *etc.*

Table 1. Chromatographic k' data

Acids	pK_a[a]	k'
Oxalic	1.19 (4.28)[b]	0
Pyruvic	2.49	0
Malonic	2.85 (5.69)	0
(*S*,*S*)-Tartaric	3.03 (4.36)	0
L-Malic	3.4 (5.1)	0.08
Formic	3.752	0.28
Lactic	3.862	0.28
Succinic	4.20 (5.64)	0.40
Glutaric	4.343 (5.42)	0.64
Adipic	4.430 (5.41)	0.97
Acetic	4.756	1.02
4-Oxovaleric	4.59	1.15
Propionic	4.874	1.33
Pimelic	4.509 (5.4)	1.64
Butyric	4.820	1.75
Valeric	4.86	2.85
Suberic	4.5 (4.7)	3.1
Hexanoic	4.88	5.0

a) Landolt-Börnstein, "Zahlenwerte und Funktionen," II B., 7 Teil, Springer (1960). b) Parenthesis: pK_2 value.

However, as the solutes become more aliphatic in nature, that is, with increase of the hydrophobic moiety, Van der Waals forces and/or the hydrophobic interactions between the hydrophobic moiety of the solute and the hydrocarbon portion of the resin become more dominant. Thus, the elution sequences differ from that predicted from the pK_a of solutes only. The k' values obtained show a tendency to increase with that of the hydrophobic moiety, that is, the molecular weight of solute regardless of its pK_a. In addition, hydroxy and keto carboxylic acids show smaller k' values than those of the corresponding normal acids because the introduction of such a hydrophilic group makes the acid stronger and increases the hydrophilicity of the solute.

In summary, although the definite correlation between k' value and pK_a of the solute was not observed from the results shown in Table 1, it can be seen that this technique is applicable for the separation of organic acids having pK_a over *ca* 3.5.

Application to the Determination of Acetic Acid in Pharmaceuticals. In order to evaluate the applicability of the present system, the determination of acetic acid in pharmaceutical samples, Klinisalz® B Ringer's solution and Celtol® injections, was undertaken. Klinisalz® B Ringer's solution contains NaCl, KCl, CH_3COONa, KH_2PO_4, $MgCl_2$, and xylitol as components. Celtol® injections contains cephacetrile sodium (CEC), which is one of the 3-(acetoxymethyl)-cephalosporins, and it is known that it partially decomposes to liberate acetic acid in aqueous solution and that the resulting desacetyl derivative has only a low potency. Thus, from the standpoint of quality control for both pharmaceuticals, it is required to analyze the acetic acid content.

The calibration curves constructed by using the peak height of standard acetate solution were linear over the range of 0.1—10 μg cm^{-3} and 0.5—30 μg cm^{-3} (as CH_3COO^-) at 3 μS and 10 μS, respectively. A relative standard deviation of 1.3% was obtained for five replicate injections of 1.0 μg cm^{-3} level at 10 μS. In addition, the detection limit established using 50 mm^3 standard injection was 40 ng cm^{-3}.

Both samples were directly injected without sample preparation except for diluting with DI water. The CEC solution was held in a cooling bath at 10 °C after dilution since CEC was stable in aqueous solution at low temperature.[13]

The analytical results based on peak height measurements and chromatograms are shown in Table 2 and Fig. 3, respectively. For Klinisalz® B Ringer's solution, 11.81 μg cm^{-3} and 11.85±0.08 μg cm^{-3} are values calculated from the appended component table as taken and obtained from five replicate determinations, respectively. These data show that the method we describe is simple, precise and sensitive, which is applicable for routine analysis of the acetate of these pharmaceutical samples.

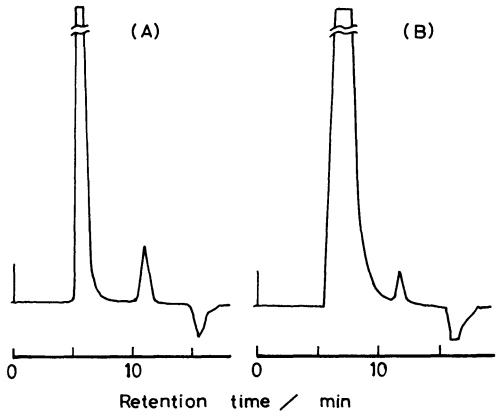

Fig. 3. Chromatograms of acetic acid in (A) Klinisalz® B Ringer's solution (×50 dilution) and (B) CEC injections solution (×200 dilution).
Chromatographic conditions. (A) Eluent: 5.0 mmol dm^{-3} carbonic acid solution, flow rate: 0.85 cm^3 min^{-1}, detector setting: 10 μS. (B) Eluent: 8.0 mmol dm^{-3} carbonic acid solution, flow rate: 0.75 cm^3 min^{-1}, detector setting: 3 μS.

Table 2. Replicate determination of acetic acid in pharmaceuticals. Peak heights[a]

Klinisalz® B Ringer's solution (×50 dilution)	CEC injection solution (×200 dilution) A[b]	B[c]
25.5	9.8	33.7
26.0	10.0	33.4
25.8	10.4	33.5
25.7	9.6	33.0
26.0	10.2	33.2
Average 25.8	10.0	33.4
σ[d] 0.2_0	0.2_9	0.2_4
R.S.D./%[e] 0.8	2.9	0.7

a) Unit: mm. b) Sampling at 20 min after dissolution. c) Sampling at 6.0 hours after dissolution. d) Standard deviation. e) Relative standard deviation.

References

1) R. M. Wheaton and W. C. Bauman, *Ind. Eng. Chem.*, **45**, 228 (1953).
2) K. Tanaka, T. Ishihara, and H. Sunahara, *Bunseki Kagaku*, **24**, 235 (1975).
3) K. Tanaka, T. Ishizuka, and H. Sunahara, *J. Chromatogr.*, **174**, 153 (1979).
4) V. T. Turkelson and M. Richards, *Anal. Chem.*, **50**, 1420 (1978).
5) H. Small, T. Stevens, and W. C. Bauman, *Anal. Chem.*, **47**, 1801 (1975).
6) W. Rich, F. Smith, Jr., L. McNeil, and T. Sidebottom, "Ion Chromatographic Analysis of Environmental Pollutants," ed by J. D. Mulik, E. Sawicki, Ann Arbor Science,

Michigan (1979), Vol. 2, Chap. 2, pp. 17—29.

7) Dionex Application Note 19, "Analysis of Strong and Weak Acids in Coffee Extract," April (1979).

8) W. R. Rich, E. Johnson, L. Lois, B. E. Stafford, P. M. Kabara, and L. J. Marton, "Liquid Chromatography in Clinical Analysis," ed by L. J. Marton, P. M. Kabara, The Humana Press, New Jersey (1981), Chap. 17, pp. 393—407.

9) H. Itoh and Y. Shinbori, *Chem. Lett.*, **1982**, 2001.

10) Column packed with 20—40 μ Dionex DC-X8 resin, a microporous strong acid cation exchanger, which consists of a stylene/divinyl benzene copolymer containing sulphonic acid groups.

11) 9 mm i.d.×200 mm column packed with 9±0.5 μ DC-X8 resin.

12) R. M. Wheaton and W. C. Bauman, *Ann. N.Y. Acad. Sci.*, **57**, 159 (1953).

13) H. Itoh, Y. Shinbori, and N. Tamura, *Bunseki Kagaku*, **32**, 571 (1983).