April, 1987] © *1987 The Chemical Society of Japan* *Bull. Chem. Soc. Jpn.*, ***60***, *1327—1332 (1987)* 1327

# Simultaneous Determinations of Inorganic and Organic Acids by Ion Chromatography

Hisaaki Itoh* and Yoshio Shinbori
Faculty of Pharmaceutical Sciences, Josai University, Sakado, Saitama 350-02

(Received September 13, 1986)

The elution behavior of seven common inorganic anions ($F^-$, $Cl^-$, $NO_2^-$, $Br^-$, $NO_3^-$, $SO_4^{2-}$, and phosphate) and a large number of organic acids in an AS-5 separator column using a weak carbonate eluent was examined and the possibility of simultaneous determinations of the inorganic and organic acids in a single injection was investigated. For pharmaceutical samples, such as a Ringer's solution which contains some inorganic anions and a single organic anion as anion contents, each anion could be simultaneously analyzed with a relative standard deviation of below 0.6%. Furthermore, it was found that even in a sample such as the liquor of canned goods having a complex matrix, both inorganic and dicarboxylic acid contents could be simultaneously analyzed without any tedious pretreatment, except for a simple dilution with distilled and deionized water. For the liquor samples, the relative standard deviation for five measurments was less than 5.0%.

Ion chromatography, first described in 1975,[1] has become a widely used analytical technique for the determination of ionic species in aqueous samples.[2–14] Today, based on the developments of column technology and a detection system, many separator columns with different selectivities and detection systems have been made available commercially. It should also be noted that with the spread of the application area and an increased need to analyze samples having complex matrices, the key point of success of an analysis primarily depends upon the best selection of a combination of the separator column and the detection mode.

An AS-5 separator column, which was packed with poly(styrene-divinylbenzene) copolymer base pellicular ion exchange resins (particle size: 15 μm), is one type of anion separator column that is commercially available from Dionex (California, USA). It makes it possible to rapidly and sensitively analyze such anions as $I^-$, $SCN^-$, and $CrO_4^{2-}$,[15] which have relatively high affinities on the resins, using a mixed solution of *p*-hydroxybenzonitrile, sodium carbonate, and sodium hydrogencarbonate (0.8, 4.4, and 5.4 in mmol dm$^{-3}$, respectively) as the eluent and conductivity detection. Furthermore, it should be noted that this separator can also be used for the separation of common inorganic anions (except for nitrate and bromide ions) by using a weak eluent such as 2.5 mmol dm$^{-3}$ sodium carbonate/0.25 mmol dm$^{-3}$ sodium hydrogencarbonate.

In previous papers we described both the elution behavior of seven common inorganic anions in AS-1[16] and AS-4[17] separator columns by using carbonate-hydrogencarbonate as the eluent as well as the separation characteristics of many organic acids in an ICE separator column[18,19] using carbonic acid as the eluent and its application to the determination of valproic acid in drugs and patient plasma.[20] The present paper describes the elution behavior of seven common inorganic anions and a large number of organic acids in an AS-5 separator column using a weak carbonate-hydrogencarbonate eluent and its application to simultaneous determinations of inorganic and organic acids in various pharmaceuticals and canned goods.

## Experimental

**Materials.** All reagents used were of reagent grade. All inorganic anion standard solutions were prepared by dissolving the corresponding sodium salts in distilled and deionized water. Organic acid standard solutions were also prepared by dissolving the acid or the corresponding alkali salts in distilled and deionized water, and/or dissolving the acid in aliquots of 1 mol dm$^{-3}$ NaOH solution and then diluting with distilled and deionized water. The active carbon for the chromatograph was from commercial sources and was used after a pretreatment: wash with 1-propanol by means of a Soxhlet extractor for 3 h, filter using suction rinsing with distilled and deionized water, then dry in an electric drier oven at 150 °C for 24 h.

**Chromatographic System.** A Dionex Model 2000i ion chromatograph equipped with an AG-5 guard column, an AS-5 anion separator column, an AFS-1 anion fiber suppressor, and a conductivity detector was used. In applications to canned-good samples, a UVIDEC-100-VI variable UV-VIS detector (Japan Spectroscopic Co., Ltd.) was also used in series between the separator column and the suppressor. A sample loop with a volume of 50 mm$^3$ was used. For conductivity suppression, a 0.0125 mol dm$^{-3}$ $H_2SO_4$ solution was used with a flow rate of c.a. 3 cm$^3$ min$^{-1}$ for all the eluent solutions used. The column temperature was not regulated but the eluent solution temperature was regulated by immersing the eluent reservoir into a water bath controlled at 25 °C. Chromatograms were recorded by the use of a Pantos U-228 dual-pen recorder.

## Results and Discussion

**Elution Behavior of Inorganic Anions.** Figure 1 exemplifies a chromatogram of seven common anions obtained by using a 2.5 mmol dm$^{-3}$ $Na_2CO_3$/0.25 mmol dm$^{-3}$ $NaHCO_3$ solution as the eluent. Figure 2 shows the variation in the retention time of seven common anions with a change of the pH of the eluent solution; this was obtained by changing the composition of sodium carbonate/sodium hydrogencarbonate. However, the total concentration of the eluent was held constant at 2.75 mmol dm$^{-3}$. It can be seen that

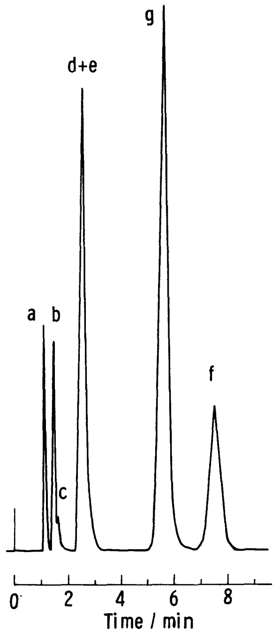

Fig. 1. A typical chromatogram of common seven anions. Column: AS-5 separator column with AG-5 guard column; eluent: 2.5 mmol dm$^{-3}$Na$_2$CO$_3$/0.25 mmol dm$^{-3}$NaHCO$_3$; flow rate: 2.0 cm$^3$ min$^{-1}$; anion fiber suppressor regenerant: 12.5 mmol dm$^{-3}$ H$_2$SO$_4$ solution at c.a. 3 cm$^3$ min$^{-1}$; detection: conductivity at 30 $\mu$S FS; Peak notation: a=fluoride, b=chloride, c=nitrite, d=bromide, e=nitrate, f=phosphate, g=sulfate.

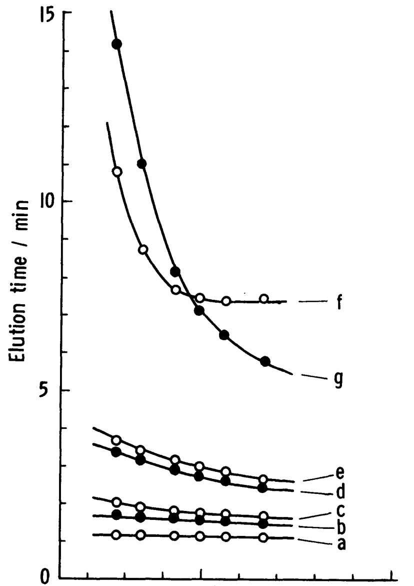

Fig. 2. Variations in the retention time of common seven anions with pH of the eluent solution. All chromatographic conditions except for eluent composition are the same as shown in Fig. 1. The total concentration of the eluent was held constant at 2.75 mmol dm$^{-3}$.

Table 1. Carboxylic Acids

| Acids | Notation | Acids | Notation |
|---|---|---|---|
| Formic | 1 | Oxalic | 11 |
| Acetic | 2 | Malonic | 12 |
| Propionic | 3 | Succinic | 13 |
| Butyric | 4 | Glutaric | 14 |
| Pivalic | 5 | Adipic | 15 |
| Pyruvic | 6 | Pimelic | 16 |
| Levulinic | 7 | DL-Malic | 17 |
| Lactic | 8 | (*R*,*R*)-Tartaric | 18 |
| Glycolic | 9 | Maleic | 19 |
| Crotonic | 10 | Fumaric | 20 |
|  |  | Itaconic | 21 |

the effect of the pH of the eluent solution on the retention time of polyvalent ions (SO$_4^{2-}$ and phosphate) is much greater than that of monovalent ions (Cl$^-$, Br$^-$, and NO$_3^-$) and that the difference of the retention time between monovalent and polyvalent ions. Thus, the separation of these ions was very good. It should also be noted that the elution behavior of these ions in an AS-5 separator column is fairly different from that in another previously reported separator columns.[16,17]

**Elution Behavior of Organic Acids.** Having noticed the observations described above and directing simultaneous determinations of inorganic and organic acids, the elution characteristics of a large number of organic acids in an AS-5 separator column were investigated.

Table 1 lists the various kinds of carboxylic acids examined. Figure 3 shows the variation in the retention time of carboxylic acids with a change of the pH of the eluent solution. The change of the pH of the eluent solution hardly affected the elution time of the monovalent anions. All of the monocarboxylic acids, regardless of the aliphatic and unsaturated acids (including keto acids), were first eluted for approximately the same elution time as fluoride anion. These results show that since any of the acids examined are fully dissociated in the pH of the eluent solution used, the retention of these ions are based on the eluting power of carbonate ions. Furthermore, these results also imply the possibility of a simultaneous determination of inorganic and carboxylic acids in a single injection.

**Applications to the Analysis of Pharmaceuticals.** For analytical chemists and analysts who work, especially, in laboratories of pharmaceutical companies and/or in hospitals, measurements of both organic acids and inorganic anions in a single sample solution are among the most frequently encountered determinations from the standpoint quality control. In such case, if possible, it would be the most desirable way for analysts to simultaneously determine both ions in a single measurement without any tedious treatment. The ion chromatography system described in this article offers a simple, rapid, and precise means for such an analysis. The sample preparation required is only a simple dilution with distilled and deionized water.

Figure 4 shows a typical chromatogram of a Rin-

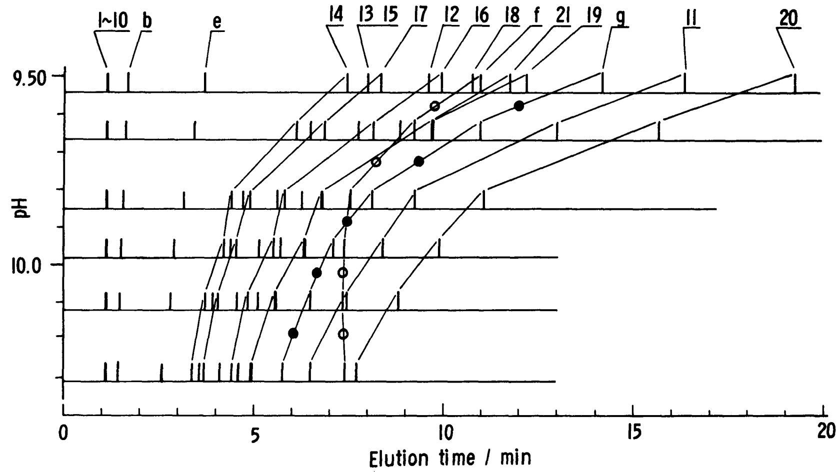

Fig. 3. Variations in the retention time of carboxylic acids with pH of the eluent solution. All chromatographic conditions are the same as shown in Fig. 2. Notations for inorganic and organic anions are the same as shown in Fig. 1 and Table 1, respectively.

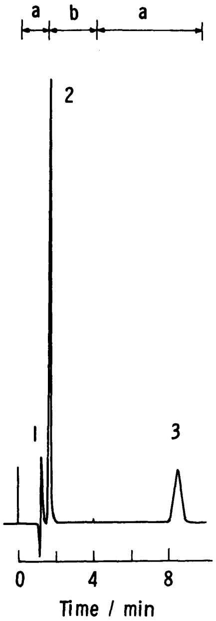

Fig. 4. A chromatogram of a Ringer's solution. All chromatographic conditions except for the eluent composition and the detector range are the same as shown in Fig. 1. Eluent: 1.25 mmol dm$^{-3}$ $Na_2CO_3$/ 1.50 mmol dm$^{-3}$ $NaHCO_3$ (pH=9.72); detector range: a=10 $\mu$S, b=30 $\mu$S; peak notation: 1=acetate, 2=chloride, 3=phosphate.

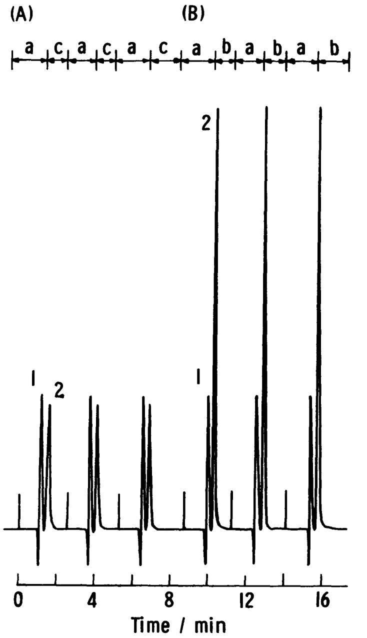

Fig. 5. Chromatograms of a Ringer's solution obtained by the repeated injections. All chromatographic conditions except for the detecor range are the same as shown in Fig. 4. Detector range: a=10 $\mu$S; b=30 $\mu$S; c=100 $\mu$S; Peak notation: 1=lactate, 2=chloride.

ger's solution which contains acetate, chloride, and phosphate as anion components. The detector range was switched from 10 to 30 μS immediately after the elution of an acetate peak and then again to 10 μS at 4 min after injection. Figure 5 also shows chromatograms obtained by repeated injections of a Ringer's solution, which contains lactate and chloride as anion components. In this case, the detector range was also switched from 10 to 100 μS (A) and to 30 μS (B) immediately after the elution of an lactate peak. The analytical results are summarized in Table 2. The determination of each ion was performed by comparing its peak height with that of a standard. The relative standard deviation of all ions in five measurements was less than 0.6%. These results probably indicate that the present system is effectively applicable for a simultaneous determination of organic and inorganic anions in such a routine analysis of pharmaceutical samples as a Ringer's solution.

**Applications to the Analysis of the Liquor of Can-**

Table 2. Analytical Results for Ringer's Solutions

| Sample | Content (mmol dm$^{-3}$)$^{a)}$ Cl$^-$ (45) 30 µS$^{b)}$ | Cl$^-$ (45) 100 µS$^{b)}$ | Acetate (20) 10 µS$^{b)}$ | H$_2$PO$_4^-$(10) 10 µS$^{b)}$ | Sample | Content (mmol dm$^{-3}$)$^{a)}$ Cl$^-$ (108) 30 µS$^{b)}$ | Cl$^-$ (108) 100 µS$^{b)}$ | Lactate (28) 10 µS$^{b)}$ |
|---|---|---|---|---|---|---|---|---|
| | 45.0 | 45.1 | 19.7 | 10.0 | | 108.7 | 108.5 | 28.1 |
| | 45.0 | 44.9 | 19.6 | 10.0 | | 109.6 | 108.7 | 28.0 |
| A$^{c)}$ | 45.3 | 44.7 | 19.6 | 10.1 | B$^{g)}$ | 109.3 | 107.9 | 28.2 |
| | 45.5 | 44.9 | 19.8 | 10.1 | | 109.0 | 108.5 | 28.2 |
| | 45.1 | 44.7 | 19.7 | 10.0 | | 109.6 | 108.7 | 28.4 |
| Mean$^{d)}$ | $45.1_8$ | $44.8_6$ | $19.6_8$ | $10.0_4$ | Mean$^{d)}$ | $109.2_4$ | $108.4_6$ | $28.1_4$ |
| S.D.$^{e)}$ | $0.2_2$ | $0.1_7$ | $0.0_8$ | $0.0_5$ | S.D.$^{e)}$ | $0.3_9$ | $0.2_8$ | $0.1_7$ |
| R.S.D.$^{f)}$ (%) | 0.5 | 0.4 | 0.4 | 0.5 | R.S.D.$^{f)}$ (%) | 0.4 | 0.3 | 0.6 |

a) Values indicated in the content table. b) Detector range used. c) ×200 dilution. d) Mean value. e) Standard deviation. f) Relative standard deviation. g) ×500 dilution.

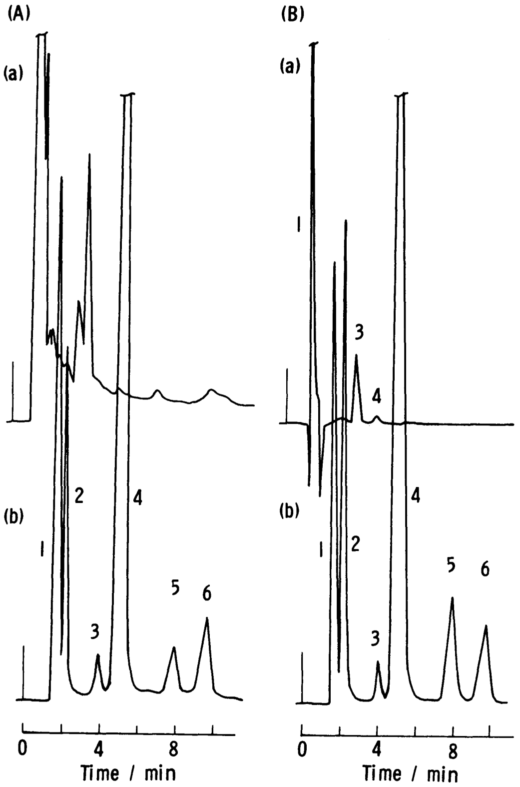

Fig. 6. Chromatograms of the liquor of a canned watermelon. All chromatographic conditions except for the detector range of the conductivity monitor, flow rate, and the use of UV detector are the same as shown in Fig. 1. Detector range of conductivity detector: 10 µS; flow rate: 1.5 cm$^3$ min$^{-1}$; UV detector: 235 nm, 0.01 AU. Peak notation: 1=monocarboxylate, 2=chloride, 3=nitrate, 4=malate, 5=sulfate, 6=phosphate.

**ned Goods.** A Ringer's solution is a typical example having a clean matrix. However, it is more general for one to have to analyze ionic species in a sample having a complex matrix. In order to estimate the applicability of the present system to an analysis of a sample having a complex matrix, the analysis of anions in the liquor of canned goods was attempted.

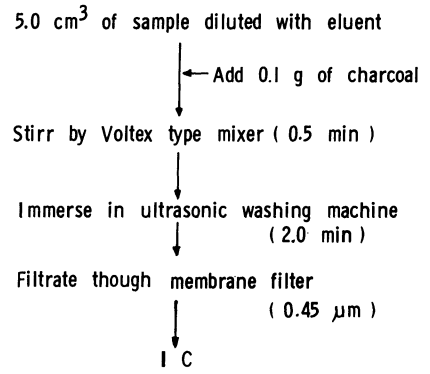

Fig. 7. A sample pretreatment procedure.

Figures 6(A) and 9(A) show typical chromatograms of the liquor of canned watermelons and peachs, respectively, that were diluted with distilled and deionized water and then injected into the sample loop of the chromatograph through a membrane filter (Millipore, HA type, 0.45 µm). In these figures, chromatograms (a) and (b) were obtained by monitoring with a UV detector at 235 nm and a conductivity detector at 10 µS, respectively. In both chromatograms (b) of Figs. 6(A) and 9(A), an identification of each peak is possible by comparing their retention times with those of standard anions (as shown in each figure). However, since both chromatograms (a) in Figs. 6(A) and 9(A) show relatively complex profiles (notably, in the former, the nitrate peak is eluted as an adjacent one to a relatively large unknown one which does not show any clear peak at the corresponding retention time in the chromatogram (b)), a sample pretreatment was attempted in order to confirm the response of each ion in the conductivity detector and the assignment of each peak in chromatogram (b).

After considerable exploratory experiments using Amberlite XAD-2 and XAD-4 resins (Rohm & Haas Co.), Dowex 50W-X8 resin (200—400 mesh, Dow Chemical Co.), SEP-PAK® C$_{18}$ cartridges (Waters Associates), Darco G-60 (active carbon, Darco Dept., Atlas Powder Co.), and active carbon for a chromatograph (Wako Pure Chemical Industries Ltd.), the sample pretreatment procedure using active carbon for a chroma-

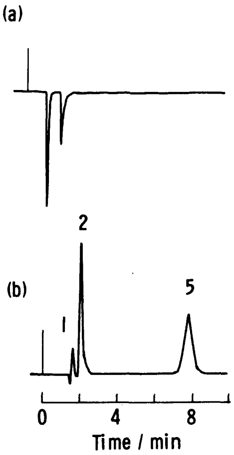

Fig. 8. A typical chromatogram of a blank solution prepared by following the pretreatment procedure using the eluent solution only. All chromatographic conditions and peak notations are the same as shown in Fig. 6.

tograph was determined (Fig. 7).

Although the active carbon was pretreated (described in the experimental section), a blank solution prepared by following a pretreatment procedure using only an eluent solution showed three peaks; this corresponds to the retention time of monocarboxylate, chloride, and sulfate ions (Fig. 8). However, in this experiment, no further pretreatment of the active carbon was performed since the reproducibility of the peak heights of these peaks were extremely good and no other peaks were observed. Furthermore, in this procedure, a liquor of canned goods was diluted with an eluent solution in the place of distilled and deionized water since a decrease of about 30—50% in the peak height of each peak obtained by the conductivity detector before and after the pretreatment procedure was observed when a liquor of canned goods was diluted with distilled and deionized water. However, diluting with an eluent solution resulted in no decrease in the peak height of each peak (as shown below).

Figure 6(B) shows typical chromatograms of liquor of a canned watermelon prepared by following the pretreatment procedure. Chromatogram (a) clearly shows three peaks which can be assigned to monocarboxylate (unidentified), nitrate, and malate ions, respectively. On the other hand, chromatogram (b) shows no changes in the elution profile of nitrate, malate, and phosphate peaks; however, by excluding the first eluted peak, only an increase of the peak heights of the chloride and sulfate peaks (which correspond to the peak height of each ion observed in Fig. 8) can be observed. This means that by the presence of the eluent, any loss of malate and inorganic anions in the sample solution could not be observed before and after the pretreatment procedure. Moreover, it was confirmed that peak 3 of chromatogram (b) in Fig. 6(B) as well as in Fig. 6(A) was based only on a nitrate ion, by comparing the response of the sample with that of a standard nitrate ion obtained from both UV and conductivity monitors. Finally, these results indicate that by using a conductivity detector chloride, nitrate, malate, sulfate, and phosphate ions in the liquor of a canned watermelon can be simultaneously analyzed without any tedious pretreatment, except for simple dilution with distilled and deionized water and/or an eluent solution.

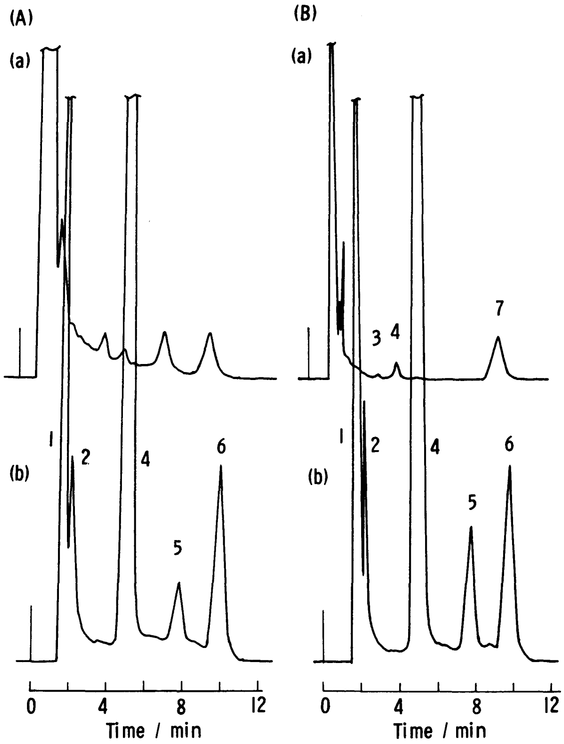

Fig. 9. Chromatograms of the liquor of a canned peach. All chromatographic conditions and peak notations except for fumarate ion are the same as shown in Fig. 6. Peak notation: 7=fumarate.

Figure 9(B) shows chromatograms of a liquor of canned peachs, prepared by following the pretreatment procedure. In chromatogram (a) obtained from a UV monitor, three peaks can be assigned to nitrate, malate, and fumarate ions, respectively. On the other hand, chromatogram (b), obtained from the conductivity monitor, did not show any changes of the elution profile, except for an increase in the peak heights of chloride and sulfate peaks. In addition, although fumarate and phosphate ions are eluted at the extremely near position, it can be concluded that peak 6 of chromatogram (b) in Fig. 9(B) as well as in Fig. 9(A) is based on only phosphate ions since fumarate ion shows no response for the conductivity detector at 10 μS in the concentration level observed.

The analytical results are summarized in Table 3. The concentration of the fumarate ion in the liquor of canned peach was determined by comparing its peak height with that of standard fumarate ion obtained from the UV monitor. The relative standard deviation

Table 3. Analytical Results for Liquors of Canned Goods[a]

| Ions | Peach[b] | | Watermelon[b] | |
|---|---|---|---|---|
| | Mean[c]±S.D.[d] | R.S.D.[e] | Mean[c]±S.D.[d] | R.S.D.[e] |
| $Cl^-$ | <10[f] | | 44.3± 2.2 | 5.0 |
| $NO_3^-$ | Trace | | 18.0± 0.2 | 1.1 |
| Malate | 1940 ±51 | 2.6 | 1570 ±45 | 2.8 |
| $SO_4^{2-}$ | 38.0± 0.4 | 1.0 | 22.4± 0.5 | 2.2 |
| Phosphate[g] | 244 ± 9 | 3.7 | 105 ± 4 | 3.8 |
| Fumarate[h] | 7.4± 0.3 | 4.0 | — | — |

a) In mg dm$^{-3}$, the concentration of the original liquor. b) ×20 dilution. c) Mean value. d) Standard deviation. e) Relative standard deviation (%). f) Estimated by assuming the response based on chloride ion only. g) As $HPO_4^{2-}$. h) Estimated from UV detector response.

of all ions in five measurements was less than 5.0%.

These results indicate that the present system, using a conductivity detector and/or, if necessary, combined with a UV detector, an AS-5 separator column, and a dilute sodium carbonate sodium hydrogencarbonate solution as eluent, is also applicable to a simultaneous determination of dicarboxylic acids and inorganic anions in such samples having complex matrices as the liquor of canned goods. Furthermore, it should be noted that the pretreatment procedure developed in this study is a useful technique for further application studies.

The authors wish to express their thanks to Professor Shigero Ikeda of Osaka University for his helpful discussion.

## References

1) H. Small, T. S. Stevens, and W. C. Bauman, *Anal. Chem.*, **47**, 1801 (1975).

2) J. D. Mulik and E. Sawicki, "Ion Chromatographic Analysis of Environmental Pollutants," Ann Arbor Science, Michigan (1979), Vol. 2.

3) G. Muto and K. Oikawa, "Ion Chromatography," Kodansha Scientific, Tokyo (1983), Chap. 6.

4) R. D. Rocklin and E. L. Johnson, *Anal. Chem.*, **55**, 4 (1983).

5) W. F. Koch, *J. Res. of N.B.S.*, **88**, 157 (1983).

6) R. D. Rocklin, *Anal. Chem.*, **56**, 1959 (1984).

7) K. Hasegawa, H. Hiraki, and M. Miyazaki, *Bunseki Kagaku*, **34**, T71 (1985).

8) H. Nagashima, K. Kuboyama, and K. Ono, *Bunseki Kagaku*, **34**, 381 (1985).

9) H. Honma, K. Suzuki, M. Yoshida, and H. Yanashima, *Bunseki Kagaku*, **35**, 536 (1986).

10) K. Fujimura and M. Tsuchiya, *Bunseki Kagaku*, **35**, T50 (1986).

11) M. Yamamoto, H. Yamamoto, Y. Yamamoto, S. Matzushita, N. Baba, and T. Ikushige, *Anal. Chem.*, **56**, 832 (1984).

12) L. K. Tan and J. E. Dutrizac, *Anal. Chem.*, **58**, 1383 (1986).

13) K. Ohsawa, Y. Yoshimura, S. Watanabe, H, Tanaka, A. Yokota, K. Tamura, and K. Imaeda, *Analytical Science*, **2**, 105 (1986).

14) C. D. Chrswell, D. R. Mroch, and R. Markuszewski, *Anal. Chem.*, **58**, 319 (1986).

15) Product information from Dionex, "High Performance Ion Chromatography Anion Selectivity 5 Column," Aug. (1983).

16) H. Itoh and Y. Shinbori, *Bunseki Kagaku*, **29**, 239 (1980).

17) H. Itoh and Y. Shinbori, *Bunseki Kagaku*, **31**, T39 (1982).

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

19) H. Itoh and Y. Shinbori, *Bull Chem. Soc. Jpn.*, **58**, 3244 (1985).

20) H. Itoh, Y. Shinbori, and N. Tamura, *Bull. Chem. Soc. Jpn.*, **59**, 997 (1986).