ELSEVIER

Journal of Chromatography A, 956 (2002) 139–145

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of total acidity and of divalent cations by ion chromatography with *n*-hexadecylphosphocholine as the stationary phase

Wenzhi Hu[a], Kyoshi Hasebe[b], Kazuhiko Tanaka[c], James S. Fritz[d,*]

[a]*Division of Chemistry, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan*

[b]*Division of Material Science, Graduate School of Environmental and Earth Science, Hokkaido University, Sapporo 060-0810, Japan*

[c]*National Institute of Advanced Industrial Science and Technology at Seto, Seto 489-0884, Japan*

[d]*Ames Laboratory, US Department of Energy and Department of Chemistry, Iowa State University, 332 Wilhelm Hall, Ames, IA, USA*

Received 11 September 2001; received in revised form 15 December 2001; accepted 2 January 2002

Abstract

An ion chromatographic (IC) method is reported for simultaneous determination of total acidity (H^+), Ba^{2+}, Ca^{2+}, and Mg^{2+} in aqueous samples. A standard ODS silica column modified by coating with *n*-hexadecylphosphocholine was used as the separation column. Water alone was used as the eluent, with conductivity detection of the sample ions. An excess of sodium iodide was added to each sample so that both H^+ and divalent cations were always eluted with iodide as the counterion. The elution order was Ba^{2+}, Mg^{2+}, Ca^{2+}, and H^+ with H^+ being eluted much later than the divalent cations. Acetic acid and several other weak acids could also be separated because all the protons were transposed from acetic acid (HAc) to HI by the sodium iodide. Detection limits for 100 μl injection, $S/N=3$ were in the low micromolar range for the divalent cations and approximately 0.3 m*M* for H^+/I^-. This method was used successfully for simultaneous determination of total acidity, magnesium and calcium in HCl-type of hot-spring water.

Keywords: Acidity; Stationary phases, LC; Zwitterion chromatography; Ion chromatography; Organic acids; Hydrochloric acid; Sodium iodide; Metal cations; Hexadecylphosphocholine

1. Introduction

The acidity or basicity of solutions plays a critical role in biological, physiological, environmental and chemical industrial processes. While the pH of a sample is typically sensed by electrochemical or other means, the total acidity must be determined by titration [1,2]. The essential principle of acid–base titration has remained largely unchanged since its inception more than 150 years ago.

DeBorba et al. recently reported a method for determining acidity of analytical samples by using cation-exchange chromatography with conductivity detection [3]. The chromatographic column was packed with a cation-exchanger containing strongly acidic sulfonic acid groups. Such a resin should be ideal for separating H^+ from other cations but carboxylate or phenolic exchange sites formed dur-

*Corresponding author. Tel.: +1-515-294-5987; fax: +1-515-294-3578.

E-mail addresses: hu@ees.hokudai.ac.jp (W. Hu), kniss@ameslab.gov (J.S. Fritz).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00040-7

ing the sulfonation process made it necessary to protonate these weakly acidic groups by operating at a lowered eluent pH. As with any separation by cation-exchange chromatography, the eluting ions gave the eluent a relatively high background conductance. Nevertheless, a linear range between 0.05 and 1.00 m*M* was reported for the hydronium ion concentration.

Another form of ion chromatography (IC) uses an ordinary reversed-phase silica column that has been coated with a zwitterionic surfactant that contains both a fixed positive group (usually a quaternary N^+) and a negative group (such as SO_3) in close proximity. A column of this type has the unique property of taking up ions in pairs; a sample anion is attracted to the N^+ atom and a sample cation is attracted simultaneously to the negative sulfonate group. Retention of sample ions is usually much weaker than with a conventional cation- or anion-exchange column. This means that water alone can often be used as the mobile phase, a situation that is ideal for detection of sample ions by conductivity. Methods in which either anions or cations are separated on a column containing a zwitterionic stationary phase have been given the name electrostatic ion chromatography (EIC) [4] or zwitterionic ion chromatography (ZIC) [5].

Most applications of this type have involved the separation of anions using a column material coated with a sulfobetaine surfactant. Recently Hu et al. [6,7] reported that cations can be separated with water alone as the mobile phase by using a column coated with zwitterionic micelles of a phosphocholine type as the stationary phase. However, the possibility of separating and determining hydrogen ions was not investigated.

We have now found that a zwitterionic column of the phosphocholine type retains hydrogen ions much more strongly than most mono- and divalent cations. This is the basis of a completely new, simple and highly useful IC system for simultaneous determination of total acidity and several divalent cations. The separations are achieved without the need of competing ions in the eluent, as is required in conventional IC. Although a mobile phase of water can produce multiple peaks by different combinations of anions and cations, this phenomenon can be avoided simply by adding an excess of a salt such as sodium iodide to the sample.

2. Experimental

2.1. Apparatus

The high-performance liquid chromatography (HPLC) system used throughout this study was a Shimadzu (Kyoto, Japan) LC-10A IC system. It consisted of a LC-10AT pump, a SIL 10A auto-injector (injectable range, 5–400 μl), a CTO-10A column oven (room temperature), a CDD-6A conductivity detector, and a CR-6A Chromatopac data system. An auto-titrator (ATU-501) obtained from TOA-DKK (Tokyo, Japan) was used to perform the titrations.

2.2. Reagents

n-Hexadecylphosphocholine (HDPC, $C_{21}H_{46}NO_4$-P) used to establish the zwitterionic micellar stationary phase was obtained from Anatrace (Maumee, OH, USA). Its purity was better than 99% (recommended by the manufacturer). Other chemicals used to prepare the samples and the eluent were analytical grade and were obtained from Wako (Osaka, Japan). Water was obtained from a WG261 Autostill water purification system (Yamato, Tokyo, Japan).

2.3. Column preparation

The column used was L-column (250×4.6 mm I.D.; 5 μm particle size, 120 Å pore size, 17% C/Si, 340 $m^2\ g^{-1}$) obtained from Chemicals Evaluation and Research Institute, Tokyo, Japan). This column was modified by passing 50 ml of 30 m*M* HDPC solution through the column at a flow-rate of 1.0 ml/min. After a water rinse the column was found to contain 0.716 mmol of HDPC [8]. To maintain a constant amount of the zwitterionic surfactant on the stationary phase, the eluents used throughout this study always contained approximately 10 μ*M* HDPC.

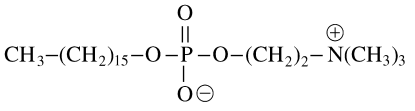
3. Results and discussion

3.1. Principles

Virtually all methods employing a zwitterionic

surfactant have used a reversed-phase silica column coated with a surfactant of the sulfobetaine type. Anions are generally retained more strongly than cations on this type of column. As stated by Okada and Patil [9], EIC is essentially a method for separation of anions. The rules for this type of anion separation are distinctly different from conventional ion chromatography. In EIC sample ions are taken up in pairs and are also eluted in pairs. The sample anion is attracted to the positively charged nitrogen of the surfactant and the counter cation is simultaneously attracted to the negative sulfonate group. A plot of log k' (k' is the retention factor) vs. the ionic concentration of the mobile phase tends to be very flat [5]. This means that strongly retained anions such as thiocyanate and perchlorate take a long time to elute. Mori et al. [10] addressed this difficulty by using a column coated with a mixture of zwitterionic and cationic surfactants.

In the present research a reversed-phase silica column coated with a phosphocholine zwitterionic surfactant (HDPC) is used to separate H^+ and several divalent metal cations. Again, sample cations and anions are taken up and eluted in pairs. The cation (C^+) is attracted to the negative phosphate group and the anion (A^-) pairs up with the positive nitrogen, as shown below.



With a column coated with a phosphocholine surfactant sample ions are retained more weakly than with sulfobetaine columns. Now, water alone can be used as the mobile phase and sensitive detection is possible with a conductivity detector. We also found that H^+ is retained much more strongly than 1+ and 2+ metal ions, although trivalent metal ions such as Al^{3+} and La^{3+} are retained more strongly than H^+. With this new system it is feasible to separate and determine both H^+ and several 2+ metal ions in sample mixtures.

Since sample ions are both taken up and eluted in pairs with water as the eluent, the chromatographic peak for a sample cation (C^+) will actually be a cation–anion pair (C^+A^-). The retention time for such a peak will be a function of how tightly A^-, as well as C^+, is retained by the phosphocholine. It is expected that the retention time for C^+ can be either increased or decreased by the choice of the accompanying anion, A^-.

When a sample contains more than one anion, multiple cation–anion peaks can result when water alone is the eluent. For example, cations C_1 and C_2 can combine with anions A_1 and A_2 to form four peaks: C_1 A_1, C_1 A_2, C_2 A_1 and C_2 A_2. In order to obtain only one peak for each sample cation, the sample must be made to contain only a single anion or an excess of the sodium salt of a strongly-retained anion must be added to the sample. Since all sodium salts are eluted very rapidly by water, this extra peak will not interfere with the later sample peaks.

3.2. Separation of H^+ and metal cations

A significant advantage of the new system is its ability to separate ions using water as the mobile phase. This leads to excellent sensitivity using conductivity detection. Another very striking property of the HDPC-coated column is that hydrogen ions are eluted *after* mono- and divalent metals cations.

Cations such as Na^+, Ba^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+} and Zn^{2+} are eluted with chloride as the counter ion within ~4 min, whereas a sharp peak for H^+/Cl was obtained much later at ca. 14 min. It is also possible to separate H^+ from a much higher concentration of some inorganic salts. This is shown in Fig. 1 where 10 m*M* HCl is separated from 1.0 *M* NaCl.

The strong retention of H^+ appears to be a unique property of the alkylphosphocholine. Zwitterionic surfactants of the sulfobetaine type, such as 3-(*n*-dimethylmyristylammonio)propanesulfonate, gave a retention time for H^+ that was much too short for a practical separation. In the new IC system with only water as the mobile phase, the sample cations and anions always elute as cation–anion pairs. The retention time of each ion combination is determined both by the cation and the anion. The retention time for H^+ has a very strong dependence on the counter anion. Thus it is feasible to separate different species of acids. This is demonstrated in Fig. 2 where five acids are eluted in the order: H_3PO_4, HCl, HNO_3, H_2SO_4 and $HClO_4$.

3.3. Control of analyte ion partitioning

With water as the mobile phase, partitioning of the

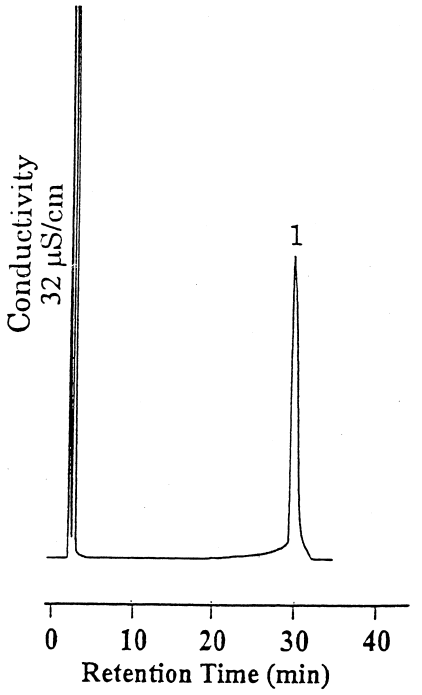


Fig. 1. Separation of 10 m*M* hydrochloric acid and 1.0 *M* sodium chloride on a silica C_{18} column (250×4.6 mm) coated with HDPC. Mobile phase was water alone at a flow-rate of 1.0 ml/min; 100 μl sample, conductivity detection.

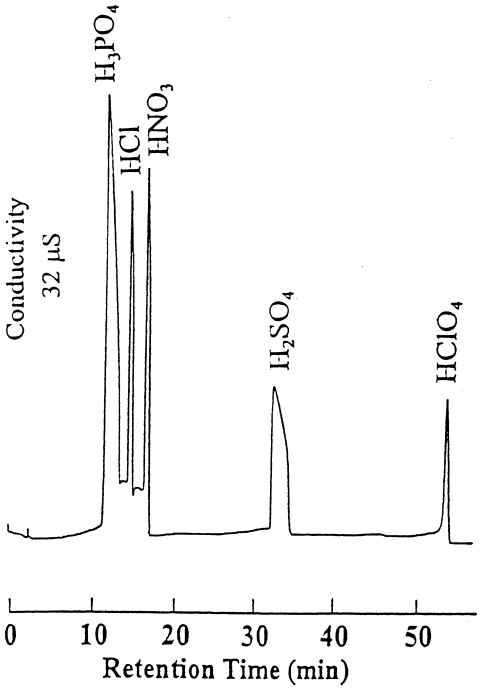


Fig. 2. Separation of a 20 μl sample containing 10 m*M* concentrations of each of five acids. Conditions were the same as Fig. 1.

anions and cations needs to be controlled in order to avoid cross distribution of the various species. Without control of the partitioning, cations of analytical interest may combine with several different anions and therefore appear on the chromatogram as a multiplicity of cation–anion peaks. In previous studies this undesirable phenomenon was prevented by the addition of a controlling salt to the sample [11,12] or by use of a short ion-exchange column inserted between the sample injector and the separation column [13].

If H^+ is to be determined in a solution containing three different anions using only water as the mobile phase, the H^+ will be divided among three different peaks: H^+/A_1, H^+/A_2 and H^+/A_3. The fraction of the total H^+ in each peak will depend on the relative affinity of each ion pair for the stationary phase, with the greatest fraction of the H^+ occurring in the latest-eluting peak. By adding at least a 5-fold excess of the sodium salt of a more strongly retained anion to the sample, the sample acidity can be forced to elute in a single chromatographic peak. This principle is illustrated in Fig. 3 which shows the chromatographic peak for HCl alone (left), after addition of sodium iodide (right).

Addition of an excess of sodium salt such as NaI also permits quantitative measurement of total acidity when the sample contains weaker acids. Suppose we have a weak acid HA which is incompletely ionized and add NaI which is completely ionized:

$$HA \rightleftharpoons H^+ + A^-$$

$$NaI \rightarrow Na^+ + I^-$$

Of the four possible ion pairs, H^+/I^- is by far the most tightly held by the HDPC stationary phase. This fact, together with the addition of a higher concentration of NaI, shifts the various equilibria so that *all* of the H^+ is contained in a single peak as H^+I^-. The other ion pairs Na^+/A^- and Na^+/I^- are more weakly held by the HDPC and elute well before the H^+/I^- peak.

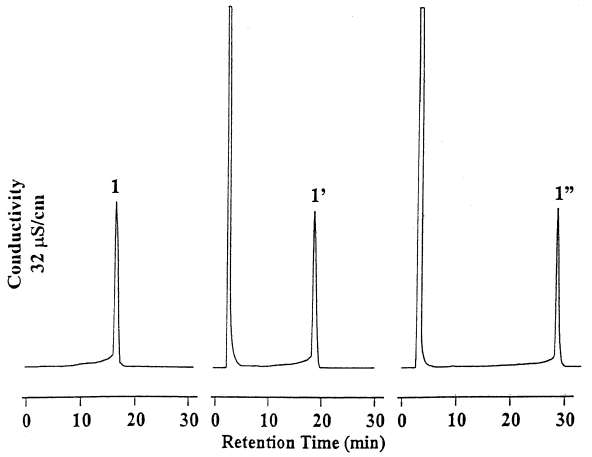


Fig. 3. Chromatograms of 2.0 m*M* HCl (left trace), 2.0 m*M* HCl with a 5-fold excess of sodium nitrate added to the sample (center trace), and 2.0 m*M* HCl with a 5-fold excess of sodium iodide. Eluent was water containing 10 μ*M* HDPC; conductivity detection.

Experimental evidence is shown in Fig. 4 where NaI was added in excess (approximately 1 *M*) to a sample of 2.0 m*M* oxalic acid (left), 2.0 m*M* tartaric acid (center) and 2.0 m*M* ascorbic acid (right). In each case the peak was H^+I^-, which was confirmed using a pH meter, an anion-exchange IC, and the EIC retention time for a known HI solution. By comparing the peak areas of the H^+/I^- peak with a standard 4.0 m*M* HI sample, it was calculated that the protons of oxalic acid and tartaric acid were completely converted to H^+/I^-, but the protons of ascorbic acid were only about 39% converted. Experiments with other weak acids (formic, acetic, pyruvic, malonic, propionic and phthalic acid) led to the conclusion that many weak acids can be converted quantitatively to H^+I^- by this technique.

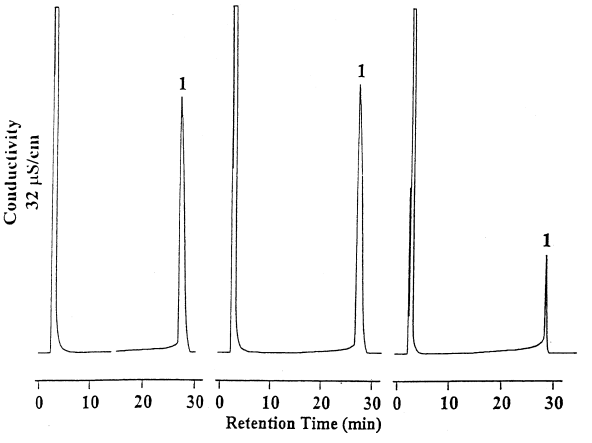


Fig. 4. Chromatograms of oxalic acid (left trace), tartaric acid (center trace) and ascorbic acid (right trace) with a 5-fold excess of sodium iodide added to the sample. Other conditions as in Fig. 1.

3.4. Simultaneous determination of total acidity (H^+), Ba^{2+}, Mg^{2+} and Ca^{2+}

The elution behavior of other cations in this IC system were studied. Fig. 5 shows two typical chromatograms for separation of divalent cations together with H^+ using iodide (left trace) and thiocyanate (right trace) as the counterions. These model divalent cations (Ba^{2+}, Mg^{2+} and Ca^{2+}) were base-line separated and were eluted much faster than H^+. The effluents for the divalent cations were identified by connecting this IC system to an inductively coupled plasma atomic emission spectrometry (ICP-AES) detector (chromatograms are not shown). Ba^{2+} was eluted first, followed by Mg^{2+}, Ca^{2+} and then H^+. This order of cation elution was quite different from that observed in conventional cation-exchange IC. No separation was observed for the

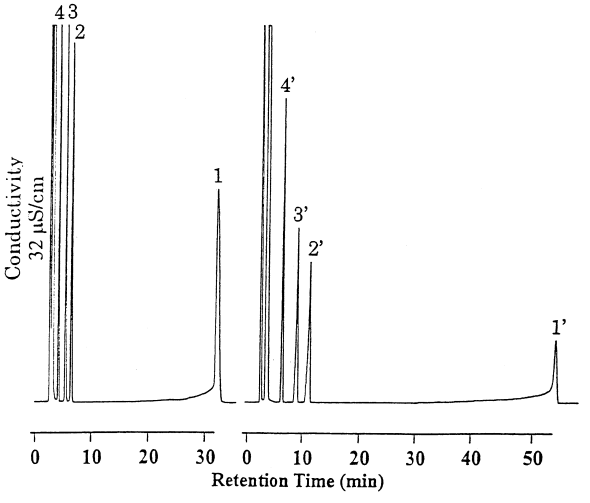


Fig. 5. Simultaneous determination of total acidity, Ba^{2+}, Mg^{2+} and Ca^{2+} using iodide (left trace) and thiocyanate (right trace) as the counterions. Sample: 1.0 m*M* each of Ba^{2+}, Mg^{2+} and Ca^{2+}; 3.0 m*M* H^+ (HCl). Other HPLC conditions as Fig. 1. Peaks: 1, $1=H^+$; 2, $2=Ca^{2+}$; 3, $3=Mg^{2+}$; 4, $4=Ba^{2+}$.

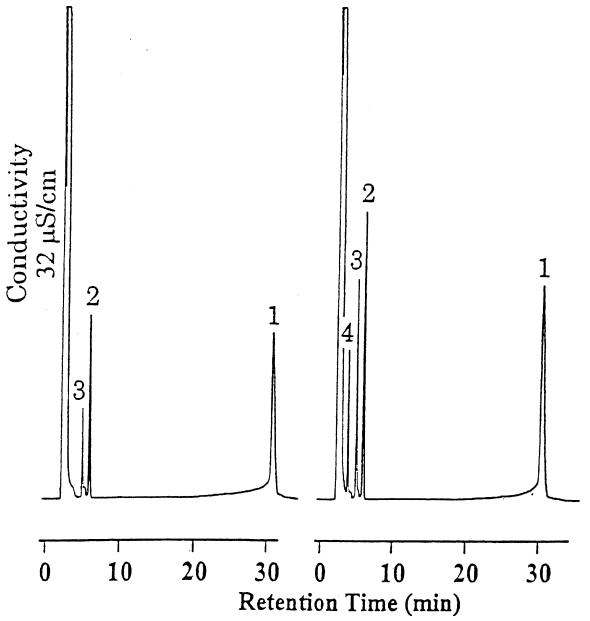


Fig. 6. Simultaneous determination of total acidity, Ca^{2+} and Mg^{2+} in hydrochloric acidic hot-spring water. The actual sample (left trace) and the sample after addition of 0.2 m*M* each of HCl, $CaCl_2$, $MgCl_2$ and $BaCl_2$ (right trace). Peaks: $1=H^+$; $2=Ca^{2+}$; $3=Mg^{2+}$; $4=Ba^{2+}$. Other HPLC conditions as Fig. 3.

monovalent cations which were eluted near the void volume of this IC system.

As a practical demonstration of the practical applicability of this method, an acidic (HCl-type) hot spring water was analyzed five times and the results were compared to those from a conventional cation-exchange IC and an auto-titration system. A typical chromatogram obtained from this sample is shown in Fig. 6. Comparative analytical data (Table 1) show the new method to be very simple and reliable for the simultaneous determination of total acidity and the divalent cations.

3.5. Analytical data

Aqueous solutions of several inorganic and organic acids were prepared and analyzed by the new zwitterion system to study the reproducibility, detection limits and analytical performance of the system. Plots of the H^+/I^-, H^+/ClO_4^- or H^+/SCN^- peak areas vs. concentration of the sample acid gave linear calibration curves with an average correlation factor $r^2=0.9992$. The slopes and intercepts for HCl, HNO_3 and acetic acids were almost identical, whereas the slopes for diprotic acids (oxalic and tartaric) were essentially twice those of the monoprotic acids.

The detection limit (100 μl) injection, $S/N=3$) for the H^+I^- system was 0.32 m*M* and the calibration curve was linear up to 6.8 m*M* H^+. Somewhat higher detection limits were obtained for $H^+ClO_4^-$ and H^+SCN^-. The detection limit for H^+ was not as low as might be expected. A low concentration (10 μ*M*) of HDPC must be added to the aqueous mobile phase to maintain the HDPC coating on the column. It seems likely that a small portion of the sample H^+ may combine with the micro-molar concentrations of HDPC in the mobile phase. A higher concentration of HDPC in the mobile phase was found to decrease the H^+ peak area.

Identical standard samples (0.50, 0.70 and 1.0 m*M* oxalic acid aqueous solutions) were analyzed 30 times under the same HPLC conditions as described in Fig. 4 (left trace). The relative standard deviations (RSDs) of retention time, peak areas and peak heights were smaller than 0.78% for these model samples. Small amounts of HCl (0.10, 0.50, and 1.0 m*M*, respectively) were added to a standard sample of 1.0 m*M* oxalic acid solution. Recoveries of added HCl between 99.2 and 100.8% were obtained from these spiked samples.

Detection limits for Ba^{2+}, Mg^{2+} and Ca^{2+} using

Table 1
Results of determination of total acidity, magnesium ions and calcium ions obtained using the present method (EIC), auto-volumetric titration, and conventional cation-exchange IC

	Total acidity (m*M*)	Ca^{2+} (m*M*)	Mg^{2+} (m*M*)
EIC	1.86±0.04	0.33±0.01	0.13±0.01
Volumetric titration	1.87±0.05	–	–
Cation-exchange IC	–	0.32±0.01	0.14±0.01

iodide as the counterion were also examined. The detection limit for Ba^{2+} was 1.5, 1.8 μ*M* for Mg^{2+}, and 2.1 μ*M* for Ca^{2+} (100 μl injection volume, $S/N=3$).

4. Conclusions

The phosphocholine stationary phase (HDPC) used here, in contrast to the more commonly used zwitterion phases of the sulfobetaine type, has a much greater affinity for H^+ than for most other inorganic cations. Unlike conventional ion chromatography where the mobile phase must contain a sufficiently high ionic concentration to move the sample ions along the column, the proposed method uses water alone as the as the eluent. This means that sample ions can be detected with excellent sensitivity with a conductivity detector. The new method provides a simple and reliable way to simultaneously determine total acidity and several divalent cations in aqueous samples.

With HDPC as the stationary phase and water as the mobile phase, sample cations elute as ion pairs with a counter anion. However, multiple peaks may be obtained for each sample cation when the sample contains more than one type of anion. This situation is avoided and only a single peak obtained for H^+ and for each divalent cation by adding a 5-fold excess of sodium iodide to the sample while continuing to use only water as the eluent. Most weak acids, as well as strong acids, elute quantitatively as $H^+ I^-$ under these conditions. In this way the total acidity, and not merely the equilibrium concentration of H^+, can be measured.

The retention time of H^+ is influenced by the counter anion. This phenomenon makes it possible to separate the acids in a mixture.

References

[1] W.E. Harris, B. Kratochevil, An Introduction to Chemical Analysis, Saunders College Publishing/CBS, New York, 1981.
[2] A.G. Dickson, C. Goyet (Eds.), Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide System in Seawater, ORNL/CDIAC-74, US Department of Energy, 1994.
[3] B.M. DeBorba, C.M. Kinchin, D. Sherman, T.K. Cook, P.K. Dasgupta, K. Srinivasan, C.A. Pohl, Anal. Chem. 72 (2000) 96.
[4] W. Hu, T. Takeuchi, H. Haraguchi, Anal. Chem. 65 (1993) 2204.
[5] H.A. Cook, G.W. Dickinoski, W. Hu, J.S. Fritz, P.R. Haddad, Anal. Chem. 73 (2000) 3022.
[6] W. Hu, P.R. Haddad, K. Hasebe, K. Tanaka, Anal. Commun. 36 (1999) 97.
[7] W. Hu, Langmuir 15 (1999) 7168.
[8] W. Hu, P.R. Haddad, Anal. Commun. 35 (1998) 191; W. Hu, P.R. Haddad, Anal. Commun. 370 (2001) 48.
[9] T. Okada, J.M. Patil, Anal. Commun. 36 (1999) 9.
[10] M. Mori, W. Hu, J.S. Fritz, H. Tsue, T. Kaneta, S. Tanaka, Fresenius J. Anal. Chem. 370 (2001) 429.
[11] W. Hu, H. Tao, H. Haraguchi, Anal. Chem. 66 (1994) 2514.
[12] W. Hu, H. Haraguchi, J. Chromatogr. A 723 (1996) 251.
[13] K. Hasebe, T. Sakuraba, W. Hu, J. Liq. Chromatogr. Rel. Technol. 2 (4) (1999) 561.