ELSEVIER

Journal of Chromatography A, 755 (1996) 37–42

JOURNAL OF CHROMATOGRAPHY A

Indirect photometric detection of inorganic anions in microcolumn ion chromatography using octadecylsilica immobilized with bovine serum albumin as stationary phase

Toyohide Takeuchi*, Rahmiana Zein[1], Edison Munaf[1], Tomoo Miwa

Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-11, Japan

Received 29 April 1996; revised 7 June 1996; accepted 11 June 1996

Abstract

Octadecylsilica immobilized with bovine serum albumin was capable of separating inorganic anions. The stationary phase retained anions under acidic conditions, and the use of aqueous solution containing sodium iodide and tartaric acid as the eluent allowed the indirect photometric determination of chloride and nitrate in water and serum samples. The mobile phase conditions were optimized.

Keywords: Stationary phases, LC; Mobile phase composition; Chloride; Nitrate; Inorganic anions

1. Introduction

Various stationary phases have been utilized in ion chromatography since its development [1], involving polymer-based or silica-based ion-exchangers, silica gel [2], alumina [3] and other materials [4]. Alumina is amphoteric and it can retain both anions and cations depending on the eluent conditions [3]. Alumina retains anions at pH values below the isoelectric point (p*I*), whereas it retains cations at pH values higher than the p*I*.

Since proteins are also amphoteric, it is expected that they can retain anions or cations depending on the pH of the surrounding solution. As there are a large number of proteins commercially available, with different ionic and hydrophobic properties, it is expected that protein-immobilized stationary phases will possess various selectivities and work as both anion and cation-exchangers by changing the pH of the eluent.

Protein-bonded stationary phases have been developed in liquid chromatography (LC) for the resolution of enantiomers [4–8], including bovine serum albumin (BSA) [4], human serum albumin [5], mucoids [6–8], etc. Several protein-bonded stationary phases are now commercially available.

This paper describes ion chromatography of inorganic anions using octadecylsilica immobilized with BSA (BSA-ODS) as the stationary phase. Eluent conditions are examined for the indirect photometric determination of anions. BSA-ODS employed in this

*Corresponding author.

[1] R. Zein and E. Munaf are on leave from the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Andalas University Padang, West Sumatra, Indonesia.

0021-9673/96/$15.00
PII S0021-9673(96)00570-5

work was originally developed for the determination of small molecules coexisting with proteins such as in the case of serum samples.

2. Experimental

2.1. Apparatus

A microcolumn liquid chromatograph was assembled from an MF-2 microfeeder (Azumadenki Kogyo, Tokyo, Japan) equipped with a 0.5-ml MS-GAN 050 gas-tight syringe (Ito, Fuji, Japan) as a pump, an ML-522 microvalve injector with an injection volume of 0.11 μl (Jasco, Tokyo, Japan), a 0.35 mm I.D. microcolumn, a UV-970 or UVIDEC-100 UV detector (Jasco) with a laboratory-made flow cell, and a Chromatopac C-R4A data processor (Shimadzu, Kyoto, Japan).

The microcolumn was prepared from fused-silica tubing of 0.35 mm I.D. (GL Science, Tokyo, Japan) as reported previously [9], and 5 μm BSA-80Ts (TOSOH, Tokyo, Japan) was employed as the packing. The BSA-80Ts columns were prepared by using methanol as the slurry and packing solvent. Experiments were carried out at room temperature (ca. 20°C).

2.2. Reagent

Guaranteed reagent-grade solvents and reagents were obtained from Nacalai Tesque (Kyoto, Japan), unless otherwise noted. These reagents were employed as received. Sodium or potassium salts of anions were used as the standard analytes. Purified water was prepared by using a Milli-Q Plus system (Millipore S.A., Molsheim, France). Eluents and samples were prepared from the purified water.

Bovine serum (Nacalai Tesque) was diluted 100-fold with purified water, and the solution was passed through a 20×0.5 mm I.D. microcolumn packed with Develosil LOP ODS (30 μm; Nomura Chemical, Seto, Japan) to remove hydrophobic components, followed by loading the solution into the microvalve injector (0.11 μl).

3. Results and discussion

3.1. Selection of eluent

In ion chromatography aromatic acids such as phthalate, benzoate and salicylate are usually utilized as the visualization agent for indirect photometric detection of inorganic anions [10]. However, these visualization agents were not successfully applied to the present system using BSA-ODS as the stationary phase.

Aqueous solutions of various acids in the presence of sodium iodide were examined as the eluent in this work. It is expected that acids maintain the pH value lower than the p*I* whereas sodium iodide works as the visualization agent. Among various acids examined, citric acid and tartaric acid achieved better selectivity. Therefore, tartaric acid was employed in the following experiments. Unfortunately, the p*I* of the immobilized BSA is not certain.

A chromatogram obtained by a 15 cm column is shown in Fig. 1, where aqueous solution containing 0.3 m*M* tartaric acid and 1 m*M* sodium iodide is used as the eluent. In Fig. 1, 0.5 m*M* each of chloride, bromide and nitrate are separated, but sulfate could not be identified under the conditions used. As observed in common indirect photometric detection, analyte peaks in Fig. 1 were negative.

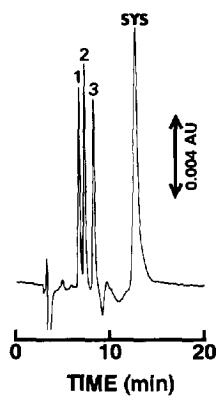

Fig. 1. Separation of inorganic anions on a BSA-ODS column. Column, 15 cm×0.35 cm I.D., packed with BSA-80Ts; eluent, aqueous solution containing 1 m*M* sodium iodide and 0.3 m*M* tartaric acid (pH 3.3); flow-rate, 2.8 μl min^{-1}; wavelength of detection, 225 nm; sample, 0.5 m*M* each of chloride (1), bromide (2), and nitrate (3); SYS=system peak; injection volume, 0.11 μl.

Since sodium iodide gave the maximum absorptivity at 225 nm [molar extinction coefficient, 1.2×10^4 $(\text{mol } l^{-1})^{-1}$ cm^{-1}], the chromatogram was traced at 225 nm. It should be noted that the system peak denoted by SYS in Fig. 1 also appeared when purified water was injected.

Under the conditions in Fig. 1, iodate eluted prior to chloride, whereas nitrite eluted between chloride and bromide. Fluoride could not be identified. When 0.15 m*M* tartaric acid without sodium iodide was used as the eluent, iodide and thiocyanate eluted after nitrate in this order. Under the latter conditions, phosphate, sulfate and thiosulfate were not eluted from the column. Eluent conditions are now being investigated so that wide range of anions can be eluted from the column.

3.2. Effect of tartaric acid concentration

The concentration of tartaric acid affected the retention and the signal intensity of analyte anions. Fig. 2 shows the effect of tartaric acid concentration on the retention time of chloride, bromide, nitrate and the system peak. It is seen that the retention time of the analyte anions is not significantly affected by the acid concentration, whereas that of the system peak decreases with increasing acid concentration.

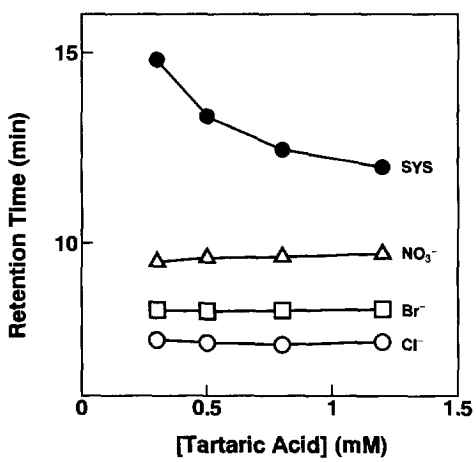

Fig. 2. Retention time against tartaric acid concentration. Operating conditions as in Fig. 1 except for the eluent; eluent, aqueous solution containing 1 m*M* sodium iodide and tartaric acid as indicated (pH 2.9–3.3).

When 1 m*M* sodium iodide without tartaric acid was employed as the eluent the retention of the analyte anions was too small to be resolved. This indicates that the acidic condition is essential for the retention of anions.

The effects of the tartaric acid concentration on the signal intensity are illustrated in Fig. 3. The peak areas of the analyte anions increased with decreasing acid concentration in the region of 0.3–1.2 m*M*. The results show that the acid concentration should be reduced to get better sensitivity. The pH values of the eluents under the conditions in Fig. 3 are 2.9–3.3. This means that the p*I* value of the immobilized BSA employed is higher than 3.3.

3.3. Effect of sodium iodide concentration

The retention times of the analyte anions decreased with increasing sodium iodide concentration, as shown in Fig. 4. This is because iodide competes for the ion-exchange sites with the analyte anions.

The signal intensity is strongly affected by the sodium iodide concentration. Fig. 5 shows the peak areas against the sodium iodide concentration. It is seen that 0.5 m*M* gave the largest peak area. On the other hand, 1 m*M* sodium iodide gave the largest peak heights. This is because the retention time is also affected by the iodide concentration, as demon-

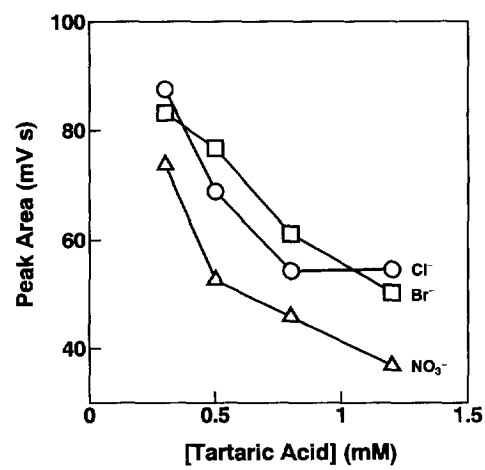

Fig. 3. Peak area against tartaric acid concentration. Operating conditions as in Fig. 2.

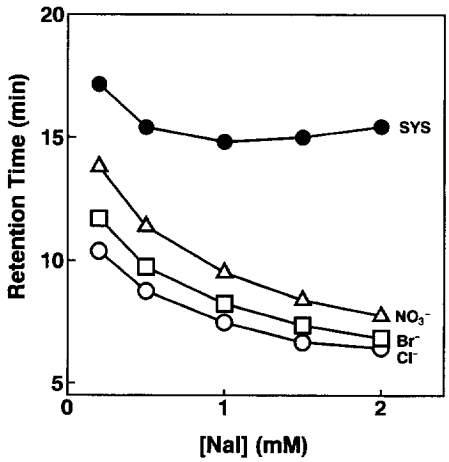

Fig. 4. Retention time against sodium iodide concentration. Operating conditions as in Fig. 1 except for the eluent; eluent, aqueous solution containing 0.3 m*M* tartaric acid and sodium iodide as indicated (pH 3.3–3.4).

strated in Fig. 4. The background absorbance of the 1 m*M* solution was 0.15.

In indirect photometric detection, the analyte signal can be expressed in terms of the absorptivity of the analyte and eluent ions and the equivalent molar concentration of the analyte [10,11]. The peak area of the analyte is therefore independent of the eluent concentration if we can assume one-to-one interaction between the analyte and the visualization agent and that the degree of dissociation of the analytes is not influenced by the eluent conditions. In the present system, the analyte ions could be replaced by iodide and/or tartrate, resulting in complex dependence of the peak area on the eluent concentration.

3.4. Analytical figures of merit

Under the conditions in Fig. 1 the linearity of the signal intensity of analyte anions was examined. Fig. 6 illustrates the peak area of chloride, bromide, nitrate and the system peak against the analyte concentration. It is found that the calibration curves for chloride and bromide were overlaid because they are almost transparent at the detection wavelength (225 nm). On the other hand, nitrate gave smaller peak areas than chloride and nitrate. This can be explained by the fact that nitrate slightly absorbs UV light at 225 nm, leading to a compensation of the depressed background caused by analyte–iodide replacement.

Relative standard deviations of the retention time, peak height and peak area for five successive measurements were 0.3–0.4, 0.6–1.5 and 1.6–3.0%, respectively.

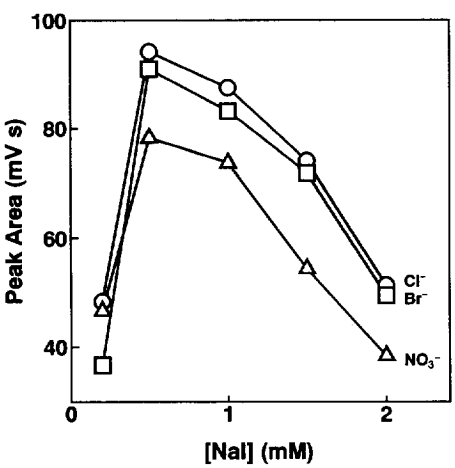

Fig. 5. Peak area against sodium iodide concentration. Operating conditions as in Fig. 4.

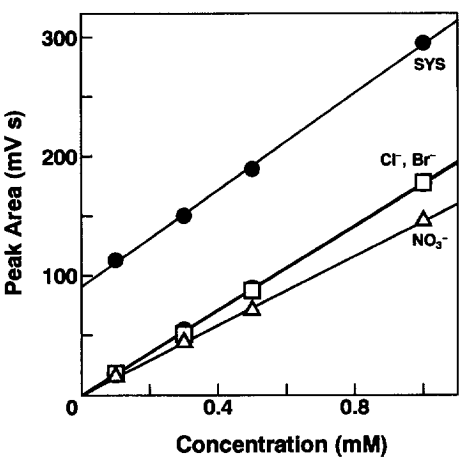

Fig. 6. Calibration curves for chloride, bromide, nitrate and system peak. Operating conditions as in Fig. 1.

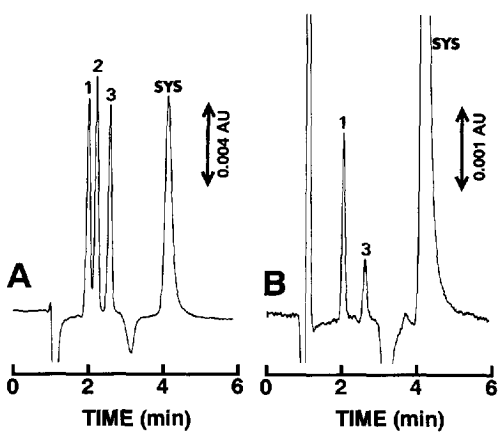

Fig. 7. Separations of (A) standard mixture and (B) components in tap water on a 7.5 cm column. Column, 7.5 cm×0.35 mm I.D., packed with BSA-80Ts; flow-rate, 5.6 μl min^{-1}; standard sample, 0.5 m*M* each; other operating conditions as in Fig. 1.

3.5. Application

Chloride and nitrate contained in various water samples were determined by using a 7.5 cm column.

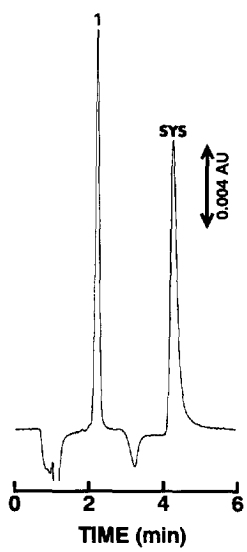

Fig. 8. Separations of components in bovine serum on a 7.5 cm column. Operating conditions as in Fig. 7 except for the sample; sample, 100-fold diluted bovine serum; other operating conditions as in Fig. 1.

Fig. 7 demonstrates the separations of a standard mixture (A) and components in tap water (B). The concentration of chloride and nitrate in the tap water were 90 and 39 μ*M*, respectively, corresponding to 3.2 and 2.4 mg l^{-1}. The concentration detection limits at $S/N=3$ for chloride, bromide and nitrate under the conditions in Fig. 7 were 3.3, 3.2 and 3.9 μ*M*, corresponding to mass detection limits of 0.36, 0.35 and 0.43 pmol, respectively.

The present system was suitable for the determination of anions contained in serum because adsorption of proteins onto the BSA-80Ts stationary phase is minimized. Fig. 8 demonstrates the chromatogram of the components in 100-fold diluted bovine serum sample. The serum was diluted with purified water and passed through a microcolumn (20×0.5 mm I.D.) packed with Develosil LOP ODS to remove hydrophobic components from the sample. The concentration of chloride in the bovine serum was determined to be 104 m*M*.

4. Conclusion

BSA-ODS columns were successfully applied to the separation of inorganic anions. The analytes could be visualized by using sodium iodide as the eluent under acidic conditions. By careful selection of the eluent conditions, separation of other anions as well as cations may also be achieved on the BSA-ODS column. It is also expected that other protein columns will generate different selectivities.

Acknowledgments

The authors wish to thank TOSOH for the kind offer of the BSA-80Ts packings employed in this work.

References

[1] H. Small, T.S. Stevens and W.C. Bauman, Anal. Chem., 47 (1975) 1801.
[2] R.L. Smith and D.J. Pietrzyk, Anal. Chem., 56 (1984) 610.
[3] G.L. Schmitt and D.J. Pietrzyk, Anal. Chem., 57 (1985) 2247.

[4] S. Allenmark, J. Liq. Chromatogr., 9 (1986) 425.
[5] E. Domenici, C. Bertucci, P. Salvadori, G. Felix, I. Cahagne, S. Montellier and I.W. Wainer, Chromatographia, 29 (1990) 170.
[6] J. Hermansson, J. Chromatogr., 269 (1983) 71.
[7] T. Miwa, M. Ichikawa, M. Tsuno, T. Hattori, T. Miyakawa, M. Kayano and Y. Miyake, Chem. Pharm. Bull., 35 (1987) 682.
[8] J. Haginaka, C. Seyama and N. Kanasugi, Anal. Chem., 67 (1995) 2539.
[9] T. Takeuchi and D. Ishii, J. Chromatogr., 213 (1981) 25.
[10] H. Small and T.E. Miller, Jr., Anal. Chem., 54 (1982) 462.
[11] D.R. Jenke, Anal. Chem., 56 (1984) 2468.