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Retention behavior of nitrite on a reversed-phase column with an acidic aqueous eluent containing alcohol as a modifier

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Abstract

The retention behavior of nitrite on two octadecyl modified reversed-phase columns, a poly(vinylalcohol) gel-based Asahipak ODP-50 and a silica gel-based LiChrosorb C-18 columns, was studied. Nitrite was retained only on the ODP column when dilute sulfuric acid was used as an eluent. With the addition of alcohol as an organic modifier, nitrite was retained on both columns. The capacity ratio and the peak shape of nitrite on the ODP column were controlled by the concentrations of sulfuric acid and alcohol in the eluent. The capacity ratio, k' , of nitrite can be described as the sum of the contribution of the ion-exchange process between nitrite anion and a fixed quaternary ammonium group and those of the esterification processes between nitric acid and hydroxyl groups. The latter process consists of the distribution of the hydrophobic nitrite ester yielded by the reaction with alcohol in the mobile phase and in the solvated layer on the ligand. The reaction of nitrous acid with a hydroxyl group remaining on the gel surface also participates in the retention of nitrite. The retention of nitrite on the LiChrosorb C-18 column was controlled by the esterification process with alcohol in the eluent.

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1. Introduction

A poly(vinylalcohol) gel (PVA gel) and its alkyl-modified gels are the materials prepared for reversed-phase liquid chromatography. These materials have a hydrophilic property due to the alcoholic hydroxyl group and the quaternary ammonium group included in the framework of the gel matrix [1,2]. In an acidic media, the ammonium group is protonated to work as a weak anion-exchange site [3]. A PVA and its C₄-, C₈- and C₁₈-modified gel columns were reported as separators of some common inorganic

anions such as bromate, bromide, nitrite, nitrate and carbonate [4,5]. The retention of bromate, bromide and nitrate were controlled by anion-exchange equilibrium. On the other hand, unique behavior of nitrite was observed. The retention of nitrite increased significantly with the increasing sulfuric acid concentration in the eluent [4]. Retention of nitrite on an octadecyl-modified PVA column with an acidic aqueous eluent was applied to the nitrite determination in a solution with a chloride matrix such as brine samples [6].

In the present study, the retention behavior of nitrite is reported using a dilute acidic eluent containing an organic modifier on PVA-based and silica

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gel-based octadecyl-modified reversed-phase columns. The proposed retention mechanism of nitrite on a reversed-phase column with acidic alcohol eluent is similar to that of ion pair chromatography [7–10].

2. Materials and method

The chromatographic system used was a Model LC 6AD pump (Shimadzu, Kyoto, Japan), a Model 860 CO column oven (Jasco, Tokyo, Japan) and a Shimadzu variable wavelength UV monitor Model LC10A. The wavelength of the UV monitor was set at 210 nm. The column oven temperature was controlled at $40 \pm 0.1^\circ\text{C}$. The chromatograms were processed with a Shimadzu Chromatopac Model CR-5A.

The analytical columns were an octadecyl-modified PVA gel column, Asahipak ODP-50 (particle diameter 5 μm , Asahi Chemical Industry, Tokyo, Japan) and an octadecyl-modified silica gel column, LiChrosorb RP-18 (5 μm , E. Merck, Germany). A Polygosil 60-5 (5 μm , Macherey-Nagel, Düren, Germany) silica gel column was used as a reference column. The Asahipak ODP-50 column was obtained from Asahi Chemical Industry. The other columns were packed in stainless-steel tubes in our laboratory. After packing, the columns were washed sequentially by isopropanol, hexane, isopropanol and acetonitrile, and then equilibrated with the eluent.

A Shimadzu Model GC-8A gas chromatograph with a capillary column, ULBON HR54 (0.25 mm \times 25 m, Shinwa Kako, Kyoto, Japan) was employed to certify the production of the nitrite esters in acidic solutions containing nitrous acid and alcohol. An Ionchromatographic Analyzer Model IC 100 (Yokogawa Electric, Tokyo, Japan) was used for the study of nitrous acid stability in acidic solutions. Sodium nitrite, sodium nitrate, uracil and other chemicals of analytical-reagent grade were purchased from Nacalai Tesque (Kyoto, Japan). Methanol, ethanol, isopropanol and acetonitrile of HPLC-reagent grade were purchased from Wako Chemicals (Osaka, Japan). Deionized pure water for eluent was prepared with a Nanopure water purification system (Barnstead, Newton, MA, USA).

3. Results and discussion

3.1. Retention of nitrite on a reversed-phase column

Using a dilute sulfuric acid eluent, nitrite was retained on an ODP column and the addition of alcohol to the eluent resulted in a remarkable increase of the retention of nitrite.

The retention behavior of nitrite on the ODP column with an acidic alcohol eluent may be interpreted as the association of the three different processes. First, the nitrite anions are retained by anion-exchange equilibrium with the quaternary ammonium group in the gel matrix. Second, nitrous acid distributes onto the gel by reaction with a residual hydroxyl group on the PVA gel surface. Third, the nitrite ester formation with alcohol in the eluent participates in the retention. The yielded ester distributes on to the bonded alkyl chain. Alternatively, the alcohol molecule bound in the solvated layer on the ligand catches up with the nitrite through the esterification reaction. The last process involves the hydrophobic interaction of the nitrite ester with the stationary phase.

The capacity ratio, k' , of the solute on the ODP column can be described as the sum of the contribution of the ion-exchange reaction in the nitrite anion form, k'_{ieX} , and the esterification reactions in the nitrous acid form, k'_{est} , which consists of the second and the third processes;

$$k' = k'_{\text{ieX}} + k'_{\text{est}} \quad (1)$$

In the mobile phase, nitrous acid dissociates to the nitrite anion;

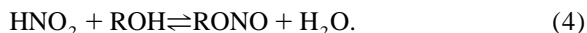


The contribution of nitrite retention through ion exchange, k'_{ieX} , is expressed by the dissociation constant of the solute, K_1 , the counter ion, I , and the equilibrium constant of the ion-exchange reaction, K ;

$$k'_{\text{ieX}} = \frac{K_1}{[\text{H}^+] + K_1} \left(\frac{[\text{I}^e]_s}{[\text{I}^e]_m} K \right)^{1/e} \quad (3)$$

where the subscripts s and m refer to the stationary and the mobile phases, and the superscript e the charge of the counter ion.

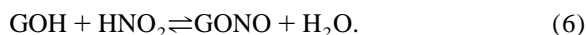
The contribution of the nitrite retention through esterification, k'_{est} , can be described in terms of the following equilibria. Esterification of nitrous acid with alcohol, ROH, in the mobile phase,



Distribution of alcohol on to the octadecyl ligand, L,



Distribution of nitrous acid on to the gel through the ester formation with a bound hydroxyl group on the PVA gel matrix, GOH,



Distribution of nitrous acid on to the ligand by esterification with the solvated alcohol molecule on the ligand,



Distribution of nitrite ester on to the ligand,



Coincident distribution of nitrous acid and alcohol on to the ligand,



The total ligand concentration, $[\text{L}]_{\text{tot}}$, is expressed by the sum of the concentrations of the free ligand, $[\text{L}]$, the ligand on which the alcohol molecule is adsorbed, $[\text{L-ROH}]$, and the ligand occupied by the nitrite ester, $[\text{L-RONO}]$,

$$[\text{L}]_{\text{tot}} = [\text{L}] + [\text{L-ROH}] + [\text{L-RONO}]. \quad (10)$$

The equilibrium constants for Eqs. (4)–(9) are given as K_2 , K_3 , K_4 , K_5 , K_6 and K_7 , respectively. From Eqs. (4)–(6) and Eq. (9), K_7 is shown as a product term of K_2 and K_6 , or K_3 and K_5 , namely, $K_7 = K_2K_6 = K_3K_5$. The relationship between k'_{est} and the concentration of alcohol in the mobile phase can be expressed as follows,

$$\begin{aligned} k'_{\text{est}} &= \phi \frac{[\text{L-RONO}] + [\text{GONO}]}{[\text{HNO}_2] + [\text{NO}_2^-] + [\text{RONO}]} \\ &= \phi \frac{(K_7[\text{L}]_{\text{tot}} + K_3K_4[\text{GOH}])[\text{ROH}] + K_4[\text{GOH}]}{\left(1 + \frac{K_1}{[\text{H}^+]} + K_2[\text{ROH}]\right)(1 + K_3[\text{ROH}])}. \end{aligned} \quad (11)$$

where ϕ is the phase ratio of the column. When the eluent is a dilute sulfuric acid solution, substitution of Eq. (2) and Eq. (11) to Eq. (1) yields

$$\begin{aligned} k' &= \frac{K_1}{[\text{H}^+] + K_1} \left(\frac{[\text{SO}_4^{2-}]_s}{[\text{SO}_4^{2-}]_m} K \right)^{1/2} \\ &+ \phi \frac{(K_7[\text{L}]_{\text{tot}} + K_3K_4[\text{GOH}])[\text{ROH}] + K_4[\text{GOH}]}{\left(1 + \frac{K_1}{[\text{H}^+]} + K_2[\text{ROH}]\right)(1 + K_3[\text{ROH}])}. \end{aligned} \quad (12)$$

Eq. (12) represents the dependence of the k' value on the alcohol and the hydrogen ion concentrations.

3.2. Effects of organic modifiers

Fig. 1 shows chromatograms on the ODP column obtained with an eluent containing 0.5 mM sulfuric acid and 5.0% (v/v) of various organic modifiers. The retention of nitrite increased significantly with the addition of alcohols, such as methanol, ethanol or isopropanol, to the eluent. The effects on nitrite retention and peak width varied with the alcohol used as an organic modifier. When di- or trialcohol of ethyleneglycol or glycerol was applied, however, the retention of nitrite was slightly reduced probably due to the hydrophilic property of the yielded mononitrite ester. Addition of acetonitrile repressed the increase in nitrite k' under acidic eluent. Nitrate eluted before nitrite with serious peak broadening and tailing. Both nitrite and nitrate eluted after the water shock observed at about 3 min from the start at a flow-rate of 0.5 ml/min. Uracil, added as a marker, eluted at the same retention time as water from the ODP column throughout the study. The peak position of uracil is shown in Fig. 1f.

3.3. Effects of acid concentration in an aqueous ethanol eluent

The peak retention and shape of nitrite and nitrate were susceptible to the hydrogen ion concentration in the mobile phase. The effects of sulfuric acid concentration in 30% (v/v) ethanol on the nitrite peak are shown in Fig. 2. Without sulfuric acid, nitrite eluted at the water shock in a narrow band, and nitrate coeluted but tailed as shown in Fig. 2a. With

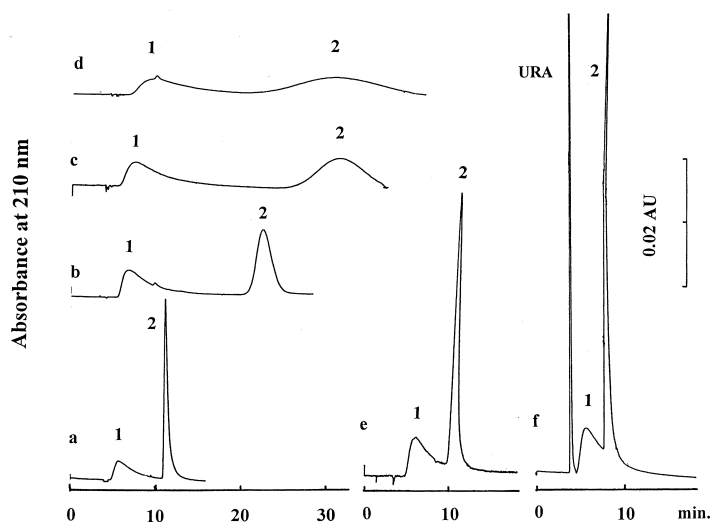


Fig. 1. Effects of organic modifiers in 0.5 mM sulfuric acid eluent. Column, Asahipak ODP-50 (150 mm×4.6 mm I.D.); flow-rate, 0.5 ml/min; detection, UV 210 nm; organic modifier (5.0%, v/v). (a) None; (b) methanol; (c) ethanol; (d) isopropanol; (e) glycerine; (f) acetonitrile. Peaks: (1) nitrate; (2) nitrite. Sample: volume, 5 μ l, (a); nitrate, 25 ng; nitrite, 100 ng; other traces, nitrate, 50 ng; nitrite, 200 ng; uracil, 100 ng.

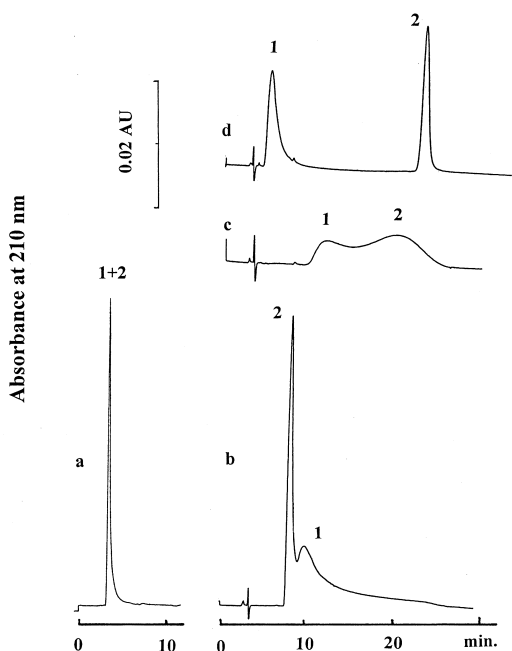


Fig. 2. Effect of sulfuric acid concentration in a 30% ethanol eluent. Column, Asahipak ODP-50; flow-rate, 0.5 ml/min; detection, UV 210 nm. Sulfuric acid concentration: (a) 0; (b) 0.02; (c) 0.05; (d) 5.0 mM. Sample volume, 5 μ l. Peaks: (1) nitrate (50 ng); (2) nitrite (200 ng).

the addition of sulfuric acid to the eluent, the peaks became wider and nitrate eluted after nitrite. Above 0.02 mM in the sulfuric acid concentration, the elution order of two components reversed and the width of both peaks extended extraordinarily.

In acidic eluent, the quaternary ammonium group on the gel matrix, the pK value of which was estimated between 4 and 5 [3], protonates according to the eluent pH. The dissociation of nitrous acid also depends on the pH. With the decrease of eluent pH, the ratio of the protonated quaternary ammonium group increases, while the concentration of the nitrite anion decreases. On the other hand, in acidic eluent, nitrite ester formation progresses, with a free molecule and a molecule bound to the ligand alcohol molecules. The distribution of nitrite on to the hydroxyl group on the gel matrix through the ester formation also takes place according to Eq. (7). As a result, the retention of nitrite increased with sulfuric acid concentrations. Above 1.0 mM, the k' of nitrite was relatively constant, while the peak width decreased with increasing acid concentration. The retention of nitrate reduced with the sulfuric acid concentration due to the increase of the counter ion concentration.

3.4. Effects of ethanol concentration in an acidic eluent

The effects of ethanol concentration in 5.0 mM sulfuric acid eluent are shown in Fig. 3. The retention of nitrite increased with ethanol concentration, and after arriving at the maximum value the retention decreased. The relationship between k' and the ethanol concentration in the eluent on the two reversed-phase columns is plotted in Fig. 4 (curves a–c). The respective retention volume of nitrite with a pure water eluent on each column was used as void volumes.

The maximum k' values of the nitrite on the ODP and the RP-18 columns were between 10 and 15% (v/v) ethanol concentration with 0.5 mM sulfuric acid eluent. With 5.0 mM sulfuric acid eluent, k' was at a maximum with the lower ethanol concentration.

The evident difference between two reversed-phase columns in the retention of nitrite was observed in the absence of ethanol. The k' value of nitrite on the ODP column depended on the acid

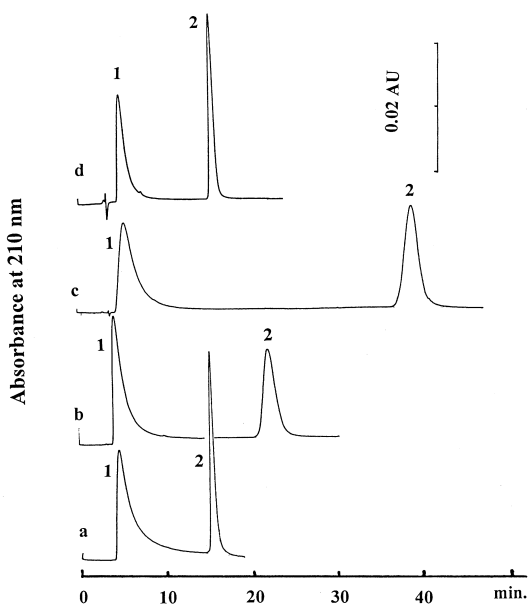


Fig. 3. Effect of ethanol concentration in 5.0 mM sulfuric acid eluent. Column, Asahipak ODP-50; flow-rate, 0.5 ml/min; detection, UV 210 nm. Ethanol concentration: (a) 0; (b) 0.5; (c) 10.0; (d) 40.0 (% v/v).

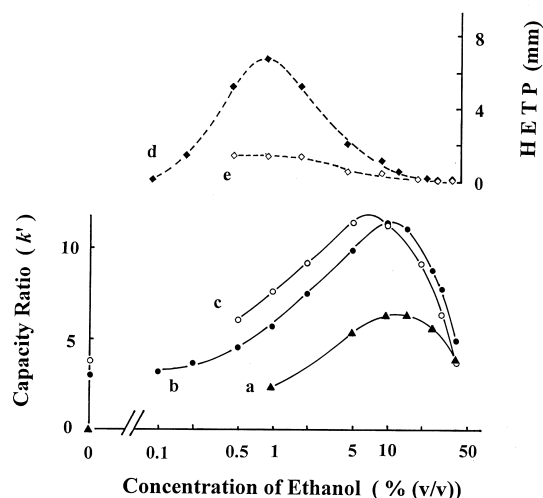


Fig. 4. The capacity ratio and HETP of nitrite as a function of ethanol concentration. Column: (a) LiChrosorb RP 18 (150 mm × 4.6 mm I.D.); (b–e) Asahipak ODP-50 (150 mm × 4.6 mm I.D.). Sulfuric acid concentration: (a,b,d) 0.5 mM; (c,e) 5.0 mM.

concentration, while nitrite was not retained on the silica-based RP-18 column.

Without the organic modifier, Eq. (12) is rewritten as

$$k' = \frac{K_1}{[H^+] + K_1} \left(\frac{[SO_4^{2-}]_s}{[SO_4^{2-}]_m} K \right)^{1/2} + \phi \frac{K_4 [H^+] [GOH]}{[H^+] + K_1} \quad (13)$$

At 5.0 mM sulfuric acid concentration, considering the dissociation constant of nitrite, $K_1 = 4.6 \times 10^{-4}$, as the contribution of the first term to the right of k' in Eq. (13) being much smaller than that of the second term, the k' value equals the constant value of $\phi K_4 [GOH]$.

The low retention of nitrite on the RP-18 column with a dilute acid eluent suggests that neither anionic site nor reactive hydroxyl group exist on the gel surface under the present conditions.

The dependence of the height equivalent of a theoretical plate (HETP) of the column on the concentration of ethanol is illustrated in Fig. 4 (curves d and e). With 0.5 mM sulfuric acid eluent, HETP increased with the increasing ethanol con-

centration to arrive at an exceedingly large maximum value of 6.8 mm at 1.0% (v/v), and then reduced with the concentration of ethanol.

The peak broadening and tailing originate due to the existence of multiple sorption sites which hold the solute by various means. The peak broadening is also due to the fact that the esterification and the reverse hydrolysis reactions are very slow kinetic processes compared to equilibration in usual reversed-phase or ion-exchange chromatography.

At higher acid concentrations, the peak shape of nitrite becomes narrower because the esterification process is predominant compared to that of ion exchange. With higher ethanol concentrations, the k' of nitrite on the ODP column and that on the RP-18 column were very close, as shown in Fig. 4 (lower panel). The higher concentrations of alcohol proceed with the reaction of nitrous acid with ethanol, moreover, the ester formation with the hydroxyl group on the gel matrix (according to Eq. (6)) is a minor process compared to that with the ethanol molecule (according to Eq. (7), Eq. (8) or Eq. (9)). Consequently, on both columns, the retention of nitrite is controlled mainly by the competition between alcohol and the nitrite ester for the ligand, as usually observed in reversed-phase chromatography.

The reactivity of a residual hydroxyl group, a silanol group, on the surface of silica gel with nitrous acid was examined on a Polygosil column. With 0.5 mM sulfuric acid eluent, nitrite had no retention, confirming that the silanol group is inactive with nitrite under the experimental conditions.

The formation of the nitrite ester from a mixture of nitrite and alcohol solution was verified by gas chromatography and UV spectrophotometry. Sodium nitrite solution was added to a mobile-phase solution in a sealed Reacti-vial (Pierce, IL, USA) controlled at 40°C, and after mixing the head space gas was applied to a capillary column gas chromatograph. Immediately after mixing the solution, the corresponding nitrite ester peak was obtained. However, it was difficult to find direct evidence of the nitrite reaction with the fixed hydroxyl group on the ODP gel matrix.

The amount of the nitrite peak area varied with the eluent components because of the change in absorptivity. For the well-separated peak, however, the peak area obtained by duplicate or triplicate in-

jections with the individual eluent compositions were within $\pm 3\%$ agreement throughout the present study.

To study the stability of nitrous acid during chromatography, the sodium nitrite solution was incubated at 40°C under the various conditions as in the analytical column. After incubation, the solution was analyzed by ion chromatography using a conventional carbonate eluent. Oxidation of nitrous acid to nitric acid took place in the aqueous acidic solution. When 50 ppm of nitrous acid in 0.5 and 5.0 mM sulfuric acid solutions were incubated for 30 min, there was an approximate decrease of 4 and 10% of nitrous acid, respectively. On the other hand, a corresponding amount of nitric acid was produced. The oxidation of nitrous acid was suppressed remarkably in the presence of alcohol. With the addition of 5 and 20% of ethanol to the respective 0.5 and 5.0 mM sulfuric acid solutions, the yield of nitric acid with incubation was suppressed to below 1% of the original nitrous acid in both solutions.

The proposed retention model appears to account for the experimentally observed results. It is possible to predict the dependence of k' on pH and alcohol concentration in the mobile phase by Eq. (12), if all constants and column properties are known.

The polymer-based ODP-50 column was stable and the reproducibility of the retention data was sufficient with the acidic eluent, while the silica-based RP-18 column deteriorated during the study

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