Table 1
Detection limits (*DL*) of gold at different concentrations of carrier solution in the sample (*C*) and injection times (*t*)

C (%)	*t* (s)	*DL* (μg mol^{-1})
10	50	0.07
20	50	0.08
30	50	0.11
40	20	0.19
50	20	0.20
70	20	0.29

Experimental conditions: −7 kV, 220 nm; fused-silica capillary (70 cm × 0.05 mm I.D.).

observed between the slopes of the regression equations (found to be related to peak height) of the calibration graphs and the composition of the carrier solution.

Table 2 summarizes some results for the reproducibilities obtained for different concentrations of gold and carrier solution in the sample. The relative standard deviations of peak heights and peak areas for successive injections were found to be less than 4.2%, and for the migration time it was below 0.87% ($n = 10$). Good reproducibilities were observed under all conditions used, but the sensitivity appeared to increase when the carrier solution content in the sample was decreased. This is because stacking conditions were met to a greater extent, so the sampling injection time could consequently be increased to obtain sharp peaks.

3.4. Application

The method was applied to the monitoring of gold(III) concentration during a study of $AuCl_4^-$ transport through a solid-supported liquid membrane. In these experiments, gold was selectively transported and concentrated using two-compartment cells containing two aqueous phases separated by a hydrophobic membrane, impregnated with an organic phase permeable to gold. One of the compartments (feed solution) contained gold(III) and 0.5–1 *M* sodium chloride (pH 2) and the other compartment contained a stripping solution.

Fig. 4 shows the electropherogram of a sample after appropriate dilution. For comparison, the same sample was analysed by CZE according to the described procedure and by flame AAS. The values obtained were 2.50 and 2.46 μg ml^{-1} using the CZE and AAS techniques, respectively, i.e., not significantly different. The first peak in Fig. 4 is NO_3^- (a contaminant), which was confirmed by using a standard addition method.

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Table 2
Relative standard deviations of peak area (R.S.D.-A), peak height (R.S.D.-h) and migration time (R.S.D.-t) of $AuCl_4^-$ at different concentrations of carrier solution in the sample (*C*) and injection times (*t*)

C (%)	Au (μg ml^{-1})	*t* (s)	R.S.D.-A (%)	R.S.D.-h (%)	R.S.D.-t (%)
40	5	10	3.36	1.90	0.78
40	5	20	1.56	0.95	0.80
40	2	20	1.90	3.40	0.67
30	5	20	3.37	2.20	0.75
30	3	50	1.10	2.70	0.42
30	3	20	2.30	3.20	0.87
20	5	20	3.25	0.56	0.54
20	3	50	4.20	4.10	0.30

Other conditions as in Table 1.

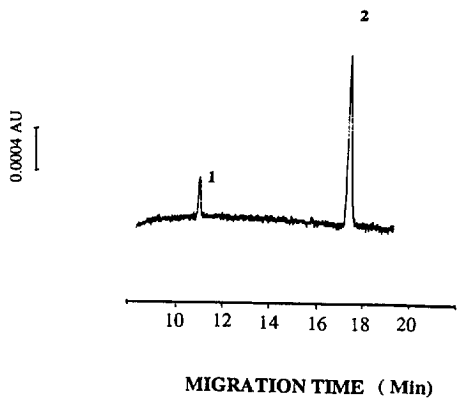

Fig. 4. Electropherogram of 2.5 μg ml^{-1} Au(III) sample. Sample preparation: dilution with 0.025 *M* HCl, injection; other conditions as in Fig. 3. Peaks: 1 = NO_3^- (contaminant), 2 = $AuCl_4^-$.

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Short communication

Migration behavior of niacin derivatives in capillary electrophoresis

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Abstract

The migration behaviour of niacin derivatives was investigated by capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). When the pH of the buffer solution is lower than the pK_a of the pyridine ring in the niacin derivatives, they are positively charged by protonation on the pyridine ring and migrate electrophoretically. The mobilities of niacin derivatives in CZE were controlled by the pH of the migrating buffer. Good separation of thirteen niacin derivatives was achieved at pH 2.8. Further, to shorten the analytical time and to achieve a more complete separation, an investigation by MEKC using sodium dodecyl sulfate micelles was performed. All thirteen niacin derivatives were eluted within 30 min and a satisfactory separation was achieved.

1. Introduction

Niacin (nicotinic acid) is a type of vitamin that is widely distributed in animals and vegetables. It is converted into NAD (nicotinamide adenine dinucleotide) and NADH (nicotinamide adenine dinucleotidic acid), which behave as coenzymes for many redox enzymes in the cells. Niacin leads to many derivatives by an in vivo metabolic pathway. Consequently, to establish in detail the dynamic behaviour of niacin in vivo, an analytical technique with high resolution and sensitivity for these derivatives is required [1]. Previously, we have reported that some niacin derivatives could be separated by capillary tube isotachophoresis (cITP) [2]. However, cITP could not offer a complete separation. Niacin derivatives have a pyridine ring in their structure, and most of them behave similarly as weak bases. The small difference in the electrophoretic mobilities makes it difficult to separate them by cITP.

Capillary electrophoresis (CE) has attracted interest in many areas recently because of the high resolution. Capillary zone electrophoresis (CZE), one of the separation modes of CE, is a powerful separation technique for many ionic substances. CZE has a high resolution for ionic species but not for non-ionic species. For the separation of non-ionic species, micellar electrokinetic chromatography (MEKC) can be applied [3,4]. Therefore, the separations of many substances have been attempted by using CE. The separation of water- or fat-soluble vitamins has been performed by using CZE and MEKC [5–9]. However, investigations of the migration behaviour of niacin derivatives in detail have not yet been reported.

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In this study, the migration behaviour of niacin derivatives was investigated by CZE and MEKC. First we attempted to separate niacin derivatives on the basis of proton dissociation equilibrium at the pyridine ring in the CZE mode. The electrophoretic mobilities were calculated from the migration times. Further, the migration behaviour of the niacin derivatives in buffer containing sodium dodecyl sulfate (SDS) was investigated by MEKC, and their distribution coefficients into SDS micelles were evaluated.

2. Experimental

2.1. Apparatus

A polyimide-coated fused-silica capillary (70 cm × 50 μm I.D.) was purchased from GL Science (Tokyo, Japan). A Model HCZE-30PNO.25 high-voltage power supply (Matsusada Precision Devices, Shiga, Japan) was used for applying high voltages. An ISCO (Lincoln, NE, USA) CV^4 variable-wavelength absorbance detector was used to measure the absorbance at 185 or 210 nm on the column at a position 20 cm from the negative end of the capillary. The measurements of migration times and recording of electropherograms were carried out with a Model D-2500 Chromato-Integrator (Hitachi, Tokyo, Japan). The sample solution was introduced into the capillary by raising the positive end of the capillary about 4 cm higher than the other end. The electroosmotic velocities of the bulk solution and the electrophoretic velocities of SDS micelles were calculated from the signals of methanol and Sudan III, respectively.

2.2. Reagents

All reagents were of analytical-reagent grade. β-Picoline, 3-pyridinemethanol, isonicotinic acid hydrazide, pyridine-3-aldehyde, 3-acetylpyridine, N-methylnicotinamide, thionicotinamide, nicotinic acid ethyl ester, nicotinic acid and pyridine-3-sulfonic acid were purchased from Tokyo Kasei (Tokyo, Japan), pyridine, nicotinamide, Sudan III and SDS from Wako (Osaka, Japan) and 6-aminonicotinamide from Sigma (St. Louis, MO, USA).

Sample solutions were prepared by dissolving the above reagents in water at 50–100 m*M*, except for thionicotinic acid and 6-aminonicotinic acid, which were dissolved in hydrochloric acid, and nicotinic acid, which was dissolved in potassium hydroxide solution. Mixed samples were prepared by mixing equal volumes of each solution.

Buffer solutions were prepared by mixing 0.02 *M* chloroacetic acid solution (pH 2.8) or 0.02 *M* potassium phosphate solution (pH 7.0) or 0.02 *M* boric acid solution (pH 9.1) with 0.01 *M* potassium hydroxide solution in order to adjust the pH to the required value. A buffer solution of pH 5.0 was prepared by mixing 0.02 *M* acetic acid with 0.02 *M* sodium acetate solution. For MEKC, SDS was dissolved in 0.02 *M* boric acid–0.01 *M* potassium hydroxide buffer solution. The buffer solution passed through a 0.2-μm cellulose acetate filter (Toyo Roshi Kaisha) prior to use. The solutions of niacin derivatives were stored in a refrigerator.

3. Results and discussion

3.1. CZE separation

First, the migration behaviour of niacin derivatives was investigated by CZE. The relationship between the pH of the migrating buffer and the electrophoretic mobility of niacin derivatives obtained in the CZE mode is shown in Fig. 1. When 0.02 *M* borate buffer (pH 9.1) was used as the migrating solution, most of the niacin derivatives (**1–11**) were eluted at the same velocity as the electroosmotic flow (EOF). Because these niacin derivatives are electrically neutral in this buffer solution, the electrophoretic mobility is zero. Therefore, these substances move at the same velocity and no separation could be achieved. On the other hand, nicotinic acid (**12**) and pyridine-3-sulfonic acid (**13**) were eluted after methanol. They have a strong acid dissocia-

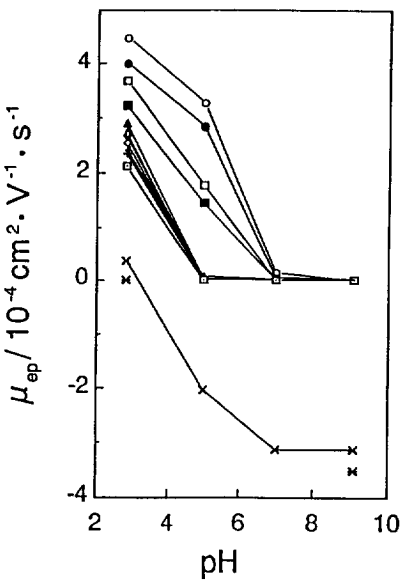

Fig. 1. Effect of the pH on electrophoretic mobility. Buffers: pH 2.8, chloroacetic acid; pH 5.0, acetic acid; pH 7.0, phosphoric acid; pH 9.1, boric acid. Voltage, 15 kV; capillary length, 70 cm. Compounds: ○ = pyridine (**1**); ● = β-picoline (**2**); □ = 3-pyridinemethanol (**3**); ■ = 6-aminonicotinamide (**4**); ▲ = pyridine-3-aldehyde (**5**); △ = isonicotinic acid hydrazide (**6**); ◆ = 3-acetylpyridine (**7**); ◇ = nicotinamide (**8**); + = thionicotinamide (**9**); ┼ = N-methylnicotinamide (**10**); ⊡ = nicotinic acid ethyl ester (**11**); × = nicotinic acid (**12**); ✳ = pyridine-3-sulfonic acid (**13**).

tion group which is negatively charged at this pH. Therefore, they migrated electrophoretically in the opposite direction to the EOF. The electrophoretic mobilities of the niacin derivatives, except nicotinic acid and pyridine-3-sulfonic acid, increased with decreasing pH. When the pH of the buffer solution is lower than the pK_a of the pyridine ring in the niacin derivatives, they are positively charged by protonation on the pyridine ring. The degree of ionization depends on the pK_a of these species. The electrophoretic mobilities of the niacin derivatives can be controlled on the basis of the acid dissociation equilibrium. The pK_a values of the niacin derivatives used in this study are about 3–5 [10].

A good separation of the thirteen niacin derivatives was achieved at low pH (2.8). At pH 2.8, the electrophoretic mobilities of nicotinic acid (**12**) and pyridine-3-sulfonic acid (**13**) are nearly zero. The positive charge due to protonation of the pyridine ring is neutralized by the negative charge of the strong acid dissociation groups, and therefore the net charge of these substances is close to zero. Pyridine-3-sulfonic acid migrated with the EOF and was detected at this pH. However, pyridine-3-sulfonic acid was not eluted at pH 7 and 5. The migration behaviour of this compound at various pH values can be considered as follows. The EOF at pH 9.1 is so fast that pyridine-3-sulfonic acid can be eluted even if it has a negative charge to migrate electrophoretically in the opposite direction to the EOF. At pH 2.8, although the EOF is slow, pyridine-3-sulfonic acid can be eluted because the electrophoretic mobility in the opposite direction to the EOF is zero, as mentioned previously. However, the electrophoretic mobility of negatively charged pyridine-3-sulfonic acid in the opposite direction to the EOF is larger than the EOF at pH 5 and 7. Therefore, pyridine-3-sulfonic acid was not eluted to the detector side and could not be detected at these pH values.

Fig. 2 shows the electropherograms at pH 9.1 and 2.8. At pH 9.1, most of the niacin derivatives except nicotinic acid (**12**) and pyridine-3-sulfonic acid (**13**) could not be separated, whereas at pH 2.8, the thirteen niacin derivatives were separated completely. The elution order seems to follow approximately the pK_a order. However, about 50 min were required for the elution of all the niacin derivatives including pyridine-3-sulfonic acid (**13**). At low pH, the EOF becomes small and it therefore becomes difficult to detect neutral and negatively charged species at low pH. Similar investigations were carried out at lower pH. More peaks than the number of sample species were observed at pH 2.0. These peaks were assumed to originate from the reaction products between niacin derivatives in the acidic medium.

It was found that the separation of niacin derivatives by CZE was improved by controlling the pH. However, in the CZE mode, a longer migration time was required for the detection of all the niacin derivatives and broadening of the peak of pyridine-3-aldehyde also occurred.