Bull. Chem. Soc. Jpn., **77**, 1465–1473 (2004) 1465

An Investigation of Weak Ion Association Equilibria between Inorganic Anions and Tetraalkylammonium Ions in Ethanol–Aqueous Media Using Capillary Zone Electrophoresis

Julius Mbuna, Toshio Takayanagi, Mitsuko Oshima, and Shoji Motomizu*

Department of Chemistry, Faculty of Science, Okayama University, 3-1-1 Tsushimanaka, Okayama 700-8530

Received February 4, 2004; E-mail: motomizu@cc.okayama-u.ac.jp

Weak ion association equilibria between inorganic anions, ranging in charge from −1 to −4, and symmetrical tetraalkylammonium ions, R_4N^+ (R = Me, Et, Pr, Bu, *n*-Am), were investigated in formate buffer solutions containing a variable composition of ethanol (0–62.9% (m/m)). The individual effective mobilities of the inorganic anions in a series of tetraalkylammonium chloride solutions were measured using an ordinary silica capillary maintained at 25.0 ± 0.1 °C under a negative potential (−10 kV) and direct UV detection ($\lambda = 220$ nm). Under the stated experimental conditions, the association constants, K_{ass}, showed a modest increase as the amount of ethanol in the buffer was increased. The concepts of medium effect and Gibbs free energy of transfer were applied to weak ion equilibria in order to investigate changes in association constant as the ethanol content in the buffer solutions was increased. The above treatment on selected associations revealed that the increased instability of the inorganic anion in mixed ethanolic solutions is responsible for the observed increase in K_{ass}.

Capillary electrophoresis has traditionally been carried out in aqueous solutions, a medium particularly suitable for the analysis of hydrophilic analytes. The last two decades have seen an increase in research done in nonaqueous solvents as a strategy for enhancing some separation parameters like the analytes' selectivity, especially for analytes which prove difficult to resolve in aqueous media.[1–4] Nonaqueous capillary electrophoresis is credited with a number of merits including better separation of hydrophobic analytes,[5] a wider range of physicochemical parameters to manipulate,[6,7] the use of expanded chemical equilibria,[8,9] the generation of the low Joule heating effect,[10] and the suitability for MS detection.[11]

Mixed organic–aqueous solvents can offer greater potential in analysis and separation, since such solvent systems tend to utilize the superior properties of the constituent solvents. Moreover, organic–aqueous solutions do not require the purification that is often necessary in analysis done in neat solvents. It is therefore expected that investigations in such solvents systems will facilitate the analysis of a wider range of analytes. Apart from the possibility of enhancing analytes' selectivity and other separation parameters, findings from such studies can be extended to evaluate some deductions of various association theories (e.g., Bjerrums) in a multi-ion electrolyte environment. Mixed organic–aqueous solvents in CZE have been used to separate a wide range of analytes.[12–16] In a series of published works, Sarmini and Kenndler investigated various aspects of organic analytes separation in methanol–aqueous,[17] ethanol–aqueous,[18] 1-propanol–aqueous,[19] and acetonitrile–aqueous[20] with the intention of examining the effect of the organic components on mobility, acidity constants, and selectivity of substituted aromatic acids. More recently, Porras et al.[21] analyzed the complexation between β-cyclodextrin and benzoate ion in separate binary methanol–aqueous and acetonitrile–aqueous mixed solvents. The authors used the medium effect concept to determine the species and/or its properties responsible for the observed trend in the complexation constant as the proportion of organic component (methanol, acetonitrile) in the buffer was varied. However, most of the previous research mainly investigated strong interactions, which involved measuring the mobility of relatively large anions. Despite being thought to operate in some biological fluids, weak ion association involving small inorganic anions has received relatively less attention compared to strong ion association. In this work, an attempt was therefore made to determine small K_{ass} values by monitoring the mobility of highly mobile inorganic anions in a variable composition of ethanol–aqueous media using ordinary silica capillary electrophoresis.

Ethanol, being a low molecular weight alcohol, satisfies key criteria for use in organic–water media[17] such as miscibility in water and the ability to dissolve buffer components. Tetraalkylammonium ions were selected as pairing cations because they are symmetrical and hence facilitate a comprehensive comparison and interpretation of the analytical results. Instead of a neutrally-coated capillary, the association between the tetraalkylammonium ions and inorganic anions was investigated in mixed water–ethanol using an ordinary silica capillary, known for their relatively lower cost, durability, and usability over a wider pH range. In order to determine the association constants between the tetraalkylammonium ions and the inorganic anions in this investigation, it was necessary to accurately measure the mobility of anions at fixed cation concentrations. Despite its ability to measure the mobility of any neutral or charged species using a coated or uncoated capillary in aqueous,[22] organic–aqueous,[23] and nonaqueous media,[24] the William–Vigh method[25] has rarely been employed in the measurement of electrophoretic mobility of small inorganic anions using an ordina-

Published on the web August 6, 2004; DOI 10.1246/bcsj.77.1465

ry silica capillary.

The aims of this work were twofold. First, to determine the association constants K_{ass} between the tetraalkylammonium ion and the inorganic anions in aqueous–ethanol mixed solvents using an ordinary silica capillary. Second, to account for the observed trend in association constants K_{ass} by applying the concept of the medium effect and the Gibbs free energy of transfer.

Experimental

Reagents. All the reagent solutions were prepared using 18.0 MΩ cm Milli-Q Labo water (Nihon Millipore, Tokyo, Japan) and filtered through 0.45 µm syringe driven filters (Advantec, Tokyo, Japan).

All the stock buffer solutions contained 0.15 M (M: mol dm^{-3}) formic acid and 0.25 M sodium phosphate (Wako Pure Chemicals, Osaka, Japan) as buffer components and an appropriate amount of ethanol (HPLC grade, Wako Pure Chemicals, Osaka, Japan) to make 0%, 15.7%, 31.4%, 47.2%, and 62.9% (m/m) ethanolic buffer solutions. After dilution of the stock buffer solutions to 10 mM total formate concentration, the apparent pHs of the background electrolyte solutions in the absence of a pairing cation were 3.90, 3.95, 3.96, 3.97, and 3.99 for 0%, 15.7%, 31.4%, 47.2%, and 62.9% (m/m) solutions, respectively.

Tetraalkylammonium ions (tetramethylammonium ion, Me_4N^+; tetraethylammonium ion, Et_4N^+; tetrapropylammonium ion, Pr_4N^+; tetrabutylammonium ion, Bu_4N^+; tetra-*n*-pentylammonium ion, Am_4N^+), which acted as pairing cations, were dissolved in water as chloride salts (Tokyo Kasei, Tokyo, Japan) to prepare 0.20 M stock solutions for each salt. The source of anionic analytes was their respective sodium salts (Wako Pure Chemicals, Osaka, Japan) prepared as single anion 0.01 M stock solutions. The sample solution consisted of one or more anions, each diluted to 3×10^{-4} M in a buffer solution containing 10 mM total formate ion concentration. Mesityl oxide (0.02% (v/v)), used as a neutral marker, was added to the sample solution. For association with Br^-, NO_3^-, SCN^-, ReO_4^-, $HCrO_4^-$, $S_2O_3^{2-}$, $S_4O_6^{2-}$, and $[Co(NO_2)_6]^{3-}$, the tetraalkylammonium ions stock solutions were diluted to a series of migrating solutions containing 0–35 mM cation concentration. It is worth noting that Cr(VI) in CrO_4^{2-} exists as $HCrO_4^-$ under the experimental pH conditions. Dilution to another series of migrating solutions containing 0–21 mM tetraalkylammonium ions was made for the associations which involved $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$ ions, since the two anions were analyzed at a constant ionic strength ($I = 31$ mM).[26]

Apparatus. Electrophoretic mobility of the anions was measured by a Hewlett Packard ^{3D}CE system (Waldbronn, Germany) equipped with a diode array detector in which the vials compartment is fitted with a water-cooled temperature-controlling device. Electrophoresis was carried out in a 50 µm i.d., 375 µm o.d., 40 cm effective length and 48.5 cm total length ordinary fused silica capillary (Agilent Technologies, Germany). System control, signal processing, and data collection were done by HP Chemstation software.

Measurement of Effective Mobility. Both single separation (double injection) and double separation (triple injection) modes of the William–Vigh method[25] were used to measure the mobility of the anions. Except for the analysis of hexacyanoferrate(II) and hexacyanoferrate(III), which were carried out exclusively using the double separation mode, all other mobility measurements were made using both modes. The single separation mode was used for solutions in which the cation concentration was not more than 10 mM, while the double separation mode was adopted above 10 mM cation concentration. Briefly, the single separation involved injecting (by pressure) a sample containing a neutral marker (mesityl oxide) into the capillary, applying pressure (50 mbar) to transfer the sample plug for some time (3 min) followed by the application of a voltage (−10 kV) for a fixed time interval (3 min). Finally, a neutral marker was injected, after which pressure was applied to move the bands past the detector. The effective electrophoretic mobility, μ_{eff} of the anion, was calculated from the migration times recorded on the electropherogram.[25]

In double separation mode, the first step involved injection (by pressure) of a sample containing a neutral marker into the capillary, transfer of the sample plug by pressure (50 mbar) for some time (2 min) followed by the application of a voltage (−10 kV) for a fixed time interval (1.3 min), to effect the first separation. The second step was a repetition of the first, except that the transfer of the sample plug by pressure was done such that the sample plugs remained in thermostated part of the capillary during the application of the voltage to effect the second separation. The third step involved the injection of the neutral marker and the application of pressure to move the bands past the detector. As in single separation mode, the effective electrophoretic mobility, μ_{eff}, of the anion was calculated from the peak times in the electropherogram.[25]

Experimental Procedure. The silica capillary was conditioned twice, at the beginning and middle of each association analysis, by flushing with 0.1 M NaOH solution for 5 min followed by purified water for 5 min. In between runs, the capillary was flushed with the corresponding migrating solutions for 2 min. The capillary cartridge was maintained at a constant temperature (25 ± 0.1 °C) and anions were detected directly at 220 nm. Starting each analysis with a buffer as a migrating solution, the sample solution underwent an appropriate mode of the William–Vigh method. Subsequent runs were made by preparing a fresh migrating solution containing a buffer (10 mM) and a raised concentration of the pairing cation (in 5 mM tetraalkylammonium salt increments), until the operating current was about 20 µA corresponding to a point beyond which the Joule heating effect becomes serious. In order to control the Joule heating effect and ensure complete dissolution of the ionic solutes, the highest cation concentration examined for hexacyanoferrate(II) and hexacyanoferrate(III) associations was 21 mM. For the remaining anions' associations the maximum concentration of the cations analysed was 35 mM. Triplicate determinations of effective electropheretic mobility were made at each cation concentration and averaged to give the anion's effective electrophoretic mobility, μ_{eff}.

Results and Discussion

Anions' Mobility Profile. The double separation (triple injection) mode was employed in all mobility measurements in which a significant difference in ionic strength ($\Delta I \geq 10$ mM) existed between the sample and the running electrolyte. Consistent with the reported observation,[25] some differences in the mobility of anions were noted between single separation (double injection) and double separation modes (triple injection) for solutions with notable difference in ionic strength between the migrating and the sample solutions. The initial isotachophoretic effect has been suggested as a possible reason for such mobility differences. Consequently, double separation was used in mobility measurements for all hexacyanoferrate(II) and hexacyanoferrate(III) analysis as well as in analysis of other anions above 10 mM cation concentration.

Assuming 1–1 ion associate formation between a cation, C^{n+} and an anion, A^{m-}

$$C^{n+} + A^{m-} \rightleftharpoons CA^{(n-m)+}.$$

The association constant, K_{ass} can be expressed as:

$$K_{ass} = \frac{[CA^{(n-m)+}]}{[C^{n+}][A^{m-}]}. \tag{1}$$

The effective electrophoretic mobility, μ_{eff}, of an analyte ion A^{m-} can be obtained by:

$$\mu_{eff} = \frac{[A^{m-}]}{[A^{m-}] + [CA^{(n-m)+}]}\mu_A + \frac{[CA^{(n-m)+}]}{[A^{m-}] + [CA^{(n-m)+}]}\mu_{CA}, \tag{2}$$

where μ_A is the mobility of an anion and μ_{CA} is the mobility of an ion associate. Taking mass balance into account and simplifying Eq. 2, μ_{eff} becomes:

$$\mu_{eff} = \frac{1}{1 + K_{ass}[C^{n+}]}\mu_A + \frac{K_{ass}[C^{n+}]}{1 + K_{ass}[C^{n+}]}\mu_{CA}, \tag{3}$$

for associating ions with different charges, and:

$$\mu_{eff} = \frac{1}{1 + K_{ass}[C^{n+}]}\mu_A, \tag{4}$$

for associating ions possessing the same charges.

After correcting the experimental effective mobility at each cation concentration for viscosity, a Non-Linear Least-Squares Method was used to compute K_{ass} (Table 1) and the corresponding effective electrophoretic mobility of the anion in absence of the pairing ion according to either Eq. 3 or 4. By using K_{ass} and the electrophoretic mobility (from Eq. 3 or 4), theoretical mobility of a particular anion can be computed to yield the simulated curves shown in Fig. 1. As the curve fitting suggests, a fair agreement exists between the simulated and the experimental data.

Figure 1 also shows the change in mobility of the anions investigated with an increase in Bu_4N^+ concentration at different ethanol contents in the running buffer. The curves show a variable decrease in mobility of the anions, which reflects their varying extents of association with the pairing cation. Hexacyanoferrate(III) appears to undergo the most extensive association at any given ethanol composition by virtue of its charge and size, followed by hexacyanoferrate(II). Dielectric friction[27] may be responsible for the lower initial mobility in hexacyanoferrate(II) as compared to hexacyanoferrate(III) due to its relatively larger charge. Interestingly, hexanitrocobaltate(III) ion showed a significantly lower initial mobility than hexacyanoferrate(III) despite possessing same overall charge, suggesting that the two anions experience different levels of hydrodynamic friction.[23] However, an examination of the crystal radii of the two anionic species[28] does not show a significant difference in size. Subsequently, the anions would be expected to be hydrated to a more or less same extent. Thus, such a difference in mobility may be due to the variable extents of hydration undergone by the ligands NO_2^- and CN^-, in which NO_2^- is believed to be more hydrated based on its higher hydrophilic character.[29]

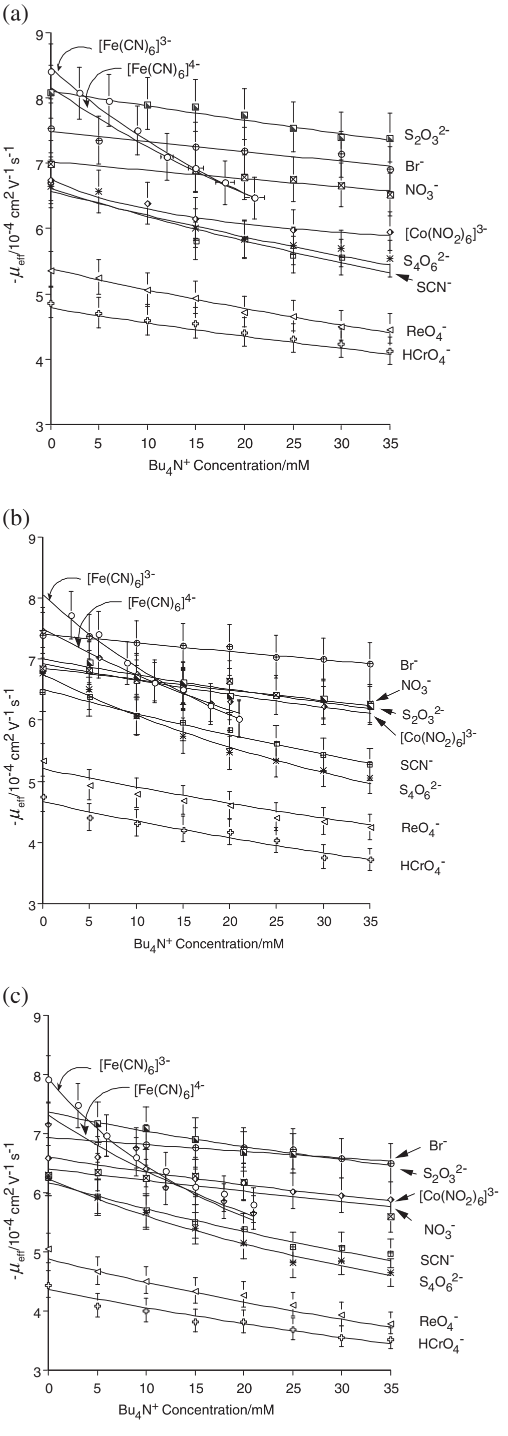

Fig. 1. Variation of anions' effective electrophoretic mobility with increase in tetrabutylammonium ion (Bu_4N^+) concentration at different ethanol contents in the buffer. Solid curves: theoretical curve fitting of experimental data by Eq. 3 or 4. μ_{eff}: effective electrophoretic mobility. CE conditions: sample, one or more anions (3×10^{-4} M each) + 10 mM formate buffer; migrating solution, formate buffer (10 mM) + (0–21 or 0–35 mM) $Bu_4N^+Cl^-$; injection, 3 s at 50 mbar; applied voltage, −10 kV; capillary, fused silica capillary; detection wavelength, 220 nm at anode. μ_{eff}: determined in (a) 0%, (b) 31.4%, (c) 62.9% (m/m) ethanolic buffer solution. Error bars: ±95% confidence interval.

Table 1. Logarithmic Values of K_{ass} between Tetraalkylammonium Ions and Inorganic Anions Determined at 25 °C and Variable Ethanol–Water Composition

Anion	$\mathrm{Me_4N^+}$					$\mathrm{Et_4N^+}$				
% (m/m) EtOH	0	15.7	31.4	47.2	62.9	0	15.7	31.4	47.2	62.9
$\mathrm{Fe(CN)_6{}^{4-}}$	0.99 ± 0.14	0.96 ± 0.18	1.22 ± 0.03	1.21 ± 0.12	1.27 ± 0.08	0.95 ± 0.14	1.15 ± 0.10	1.17 ± 0.11	1.21 ± 0.14	1.36 ± 0.14
$\mathrm{Fe(CN)_6{}^{3-}}$	1.14 ± 0.17	1.26 ± 0.19	1.30 ± 0.03	1.44 ± 0.09	1.46 ± 0.09	1.23 ± 0.07	1.26 ± 0.09	1.38 ± 0.03	1.48 ± 0.17	1.63 ± 0.18
$\mathrm{Co(NO_2)_6{}^{3-}}$	0.19 ± 0.09	0.39 ± 0.16	0.60 ± 0.18	0.80 ± 0.12	0.98 ± 0.16	0.44 ± 0.06	0.43 ± 0.14	0.30 ± 0.09	0.47 ± 0.10	0.86 ± 0.12
$\mathrm{S_4O_6{}^{2-}}$	0.99 ± 0.11	1.08 ± 0.17	1.10 ± 0.15	1.33 ± 0.13	1.25 ± 0.11	1.26 ± 0.14	1.00 ± 0.06	1.07 ± 0.10	0.98 ± 0.11	1.29 ± 0.08
$\mathrm{S_2O_3{}^{2-}}$	0.48 ± 0.10	0.65 ± 0.18	0.91 ± 0.10	0.98 ± 0.18	1.08 ± 0.17	0.47 ± 0.10	0.48 ± 0.14	0.71 ± 0.16	0.95 ± 0.16	0.88 ± 0.13
$\mathrm{ReO_4{}^-}$	0.62 ± 0.15	0.65 ± 0.18	0.36 ± 0.16	0.58 ± 0.05	0.84 ± 0.10	0.51 ± 0.13	0.40 ± 0.09	0.47 ± 0.16	0.47 ± 0.15	0.82 ± 0.14
$\mathrm{HCrO_4{}^-}$	0.66 ± 0.08	0.53 ± 0.19	0.70 ± 0.20	0.70 ± 0.19	1.01 ± 0.10	0.63 ± 0.11	0.53 ± 0.11	0.40 ± 0.11	0.45 ± 0.14	0.68 ± 0.16
$\mathrm{Br^-}$	0.01 ± 0.09	0.09 ± 0.09	0.11 ± 0.10	0.36 ± 0.15	0.66 ± 0.17	0.20 ± 0.03	0.09 ± 0.09	0.27 ± 0.10	0.19 ± 0.03	0.32 ± 0.10
$\mathrm{NO_3{}^-}$	0.30 ± 0.11	0.13 ± 0.10	0.26 ± 0.13	0.38 ± 0.16	0.28 ± 0.03	0.36 ± 0.08	0.09 ± 0.10	0.33 ± 0.14	0.15 ± 0.13	0.32 ± 0.13
$\mathrm{SCN^-}$	0.24 ± 0.10	0.34 ± 0.12	0.62 ± 0.15	0.56 ± 0.16	0.54 ± 0.14	0.30 ± 0.06	0.43 ± 0.13	0.54 ± 0.13	0.41 ± 0.17	0.46 ± 0.12

Anion	$\mathrm{Pr_4N^+}$					$\mathrm{Bu_4N^+}$				
% (m/m) EtOH	0	15.7	31.4	47.2	62.9	0	15.7	31.4	47.2	62.9
$\mathrm{Fe(CN)_6{}^{4-}}$	1.25 ± 0.10	1.31 ± 0.10	1.27 ± 0.09	1.27 ± 0.03	1.50 ± 0.02	1.09 ± 0.02	1.10 ± 0.18	1.16 ± 0.07	1.25 ± 0.15	1.22 ± 0.18
$\mathrm{Fe(CN)_6{}^{3-}}$	1.30 ± 0.16	1.41 ± 0.07	1.30 ± 0.06	1.45 ± 0.07	1.78 ± 0.02	1.29 ± 0.10	1.31 ± 0.16	1.40 ± 0.08	1.70 ± 0.05	1.56 ± 0.10
$\mathrm{Co(NO_2)_6{}^{3-}}$	0.35 ± 0.09	0.60 ± 0.07	0.71 ± 0.08	0.51 ± 0.12	0.64 ± 0.14	0.30 ± 0.07	0.61 ± 0.18	0.82 ± 0.17	0.97 ± 0.18	0.92 ± 0.09
$\mathrm{S_4O_6{}^{2-}}$	1.14 ± 0.11	1.11 ± 0.11	1.26 ± 0.13	1.20 ± 0.04	1.12 ± 0.05	1.10 ± 0.13	1.10 ± 0.11	1.15 ± 0.09	1.19 ± 0.09	1.19 ± 0.09
$\mathrm{S_2O_3{}^{2-}}$	0.47 ± 0.09	0.61 ± 0.10	0.75 ± 0.17	0.92 ± 0.15	0.72 ± 0.16	0.47 ± 0.02	0.81 ± 0.09	0.71 ± 0.15	0.74 ± 0.19	1.09 ± 0.08
$\mathrm{ReO_4{}^-}$	0.71 ± 0.16	0.69 ± 0.18	0.75 ± 0.15	0.62 ± 0.17	0.63 ± 0.16	0.80 ± 0.14	0.85 ± 0.12	0.84 ± 0.14	0.95 ± 0.18	0.94 ± 0.10
$\mathrm{HCrO_4{}^-}$	0.59 ± 0.10	0.70 ± 0.17	0.71 ± 0.10	0.66 ± 0.16	0.72 ± 0.13	0.69 ± 0.06	0.88 ± 0.14	0.86 ± 0.13	0.93 ± 0.17	0.88 ± 0.18
$\mathrm{Br^-}$	0.32 ± 0.13	0.23 ± 0.07	0.30 ± 0.12	0.38 ± 0.12	0.28 ± 0.10	0.33 ± 0.12	0.42 ± 0.04	0.30 ± 0.13	0.36 ± 0.15	0.24 ± 0.13
$\mathrm{NO_3{}^-}$	0.13 ± 0.09	0.41 ± 0.13	0.43 ± 0.11	0.66 ± 0.13	0.37 ± 0.13	0.29 ± 0.09	0.59 ± 0.04	0.44 ± 0.06	0.55 ± 0.17	0.50 ± 0.17
$\mathrm{SCN^-}$	0.58 ± 0.13	0.48 ± 0.15	0.75 ± 0.15	0.76 ± 0.08	0.65 ± 0.14	0.84 ± 0.10	0.83 ± 0.03	0.82 ± 0.09	0.81 ± 0.16	0.89 ± 0.12

Anion	$\mathrm{Am_4N^+}$				
% (m/m) EtOH	0	15.7	31.4	47.2	62.9
$\mathrm{Fe(CN)_6{}^{4-}}$	1.21 ± 0.13	1.23 ± 0.16	1.23 ± 0.16	1.29 ± 0.16	1.30 ± 0.15
$\mathrm{Fe(CN)_6{}^{3-}}$	1.18 ± 0.18	1.13 ± 0.17	1.28 ± 0.11	1.60 ± 0.09	1.56 ± 0.14
$\mathrm{Co(NO_2)_6{}^{3-}}$	0.26 ± 0.10	0.48 ± 0.03	0.68 ± 0.16	0.92 ± 0.15	0.69 ± 0.19
$\mathrm{S_4O_6{}^{2-}}$	1.04 ± 0.07	1.01 ± 0.05	1.05 ± 0.13	1.05 ± 0.17	1.32 ± 0.10
$\mathrm{S_2O_3{}^{2-}}$	0.33 ± 0.12	0.47 ± 0.14	0.66 ± 0.16	0.34 ± 0.13	1.05 ± 0.12
$\mathrm{ReO_4{}^-}$	0.92 ± 0.09	0.97 ± 0.15	0.96 ± 0.12	1.08 ± 0.14	1.13 ± 0.13
$\mathrm{HCrO_4{}^-}$	0.75 ± 0.08	0.63 ± 0.05	0.71 ± 0.15	0.98 ± 0.17	0.84 ± 0.15
$\mathrm{Br^-}$	0.33 ± 0.14	0.18 ± 0.10	0.50 ± 0.13	0.62 ± 0.12	0.43 ± 0.12
$\mathrm{NO_3{}^-}$	0.49 ± 0.11	0.40 ± 0.14	0.57 ± 0.08	0.65 ± 0.13	0.61 ± 0.16
$\mathrm{SCN^-}$	0.78 ± 0.13	0.72 ± 0.16	0.91 ± 0.11	0.97 ± 0.10	0.88 ± 0.09

K_{ass} determined by Eq. 3 or 4, error: 3σ. $\mathrm{Me_4N^+}$ = Tetramethylammonium ion. $\mathrm{Et_4N^+}$ = Tetraethylammonium ion. $\mathrm{Pr_4N^+}$ = Tetrapropylammonium ion. $\mathrm{Bu_4N^+}$ = Tetrabutylammonium ion. $\mathrm{Am_4N^+}$ = Tetra-*n*-pentylammonium ion.

An increase in the proportion of ethanol in the buffer is accompanied by a small increase in the steepness of the curves (Fig. 1(c) compared to (a)), implying a likely increase in the extent of association for most anions. In addition, Fig. 1 confirms the earlier observation[23] that an increase in ethanol content in the buffer solution not only alters the mobility of the individual anions to different extents but may also alter the migration order, e.g., a reversal of NO_3^- and $Co(NO_2)_6^{3-}$, $S_4O_6^{2-}$ and SCN^- at 62.9% (m/m) ethanol.

Trends in Association Constants (K_{ass}). Table 1 contains association constants between tetraalkylammonium ions and the inorganic anions at various ethanol contents in the buffer. A fair agreement exists between the association constants determined at 0% ethanol (Table 1) and those previously determined using a neutrally-coated capillary in aqueous solution.[30] Despite concerns over the mounting error inherent in computing increasingly small K_{ass} by the Non-Linear Least-Squares method,[21] a good agreement exists between CZE[30] and the conductometric data.[31] Thus, uncertainities associated with such small K_{ass} will not be expected to affect the trends shown by K_{ass}.

For all associations, the increase in ethanol content in the buffer led to an increase in K_{ass} as originally predicted by Bjerrum[32] due to a decrease in the dielectric constant. At each ethanol composition, anions with a higher charge (-3, -4) generally exhibited a greater interaction (Table 1) than those with a lower charge (-1). Compared to a strong association,[21] there is a correspondingly smaller change in K_{ass} as the proportion of ethanol in the buffer is increased.

Figure 2 shows how the extent of the interaction ($\log K_{ass}$) between tetraalkylammonium ions and the representative inorganic anions (charge -1 to -4) is affected by the bulkiness of the tetraalkylammonium ions as the amount of ethanol in the buffer is varied. For anions bearing charges from -2 to -4, a fluctuating trend is observed as the number of the carbon atoms in the cation increases. A similar K_{ass} trend is observed for the multi-charged anions as the amount of ethanol in the buffer is increased. On the other hand, single charged anions showed a steady increase in K_{ass} as the alkyl group became bulkier or as the amount of ethanol was raised. Nitrate ion (Fig. 2) showed a relatively smaller increase in K_{ass} compared to perrhenate ion as the ethanol content in the buffer was increased. Based on their crystal radii, nitrate ion (179 pm) is expected to be more hydrated than the perrhenate (260 pm).[28] Consequently, nitrate ion possesses more hydrophilic character than the perrhenate ion[33] and thus experiences comparatively weaker interactions with increasingly hydrophobic cations. Furthermore, a more hydrated nitrate implies an increase in contact distance in R_4N^+–NO_3^- ion pairs resulting in weaker interactions than in R_4N^+–ReO_4^- ion pairs.

Figure 3 compares the association constants between two cations, Me_4N^+ and Bu_4N^+, which differ markedly in size, with representative anions (charge -1 to -4) as the amount of ethanol is varied between 0–62.9% (m/m). An apparent feature of Fig. 3 is that $\log K_{ass}$ appears to increase at a higher rate for Me_4N^+–anion than that of Bu_4N^+–anion association. In their investigation of contact distances between ion pairs in isomeric dichloroethane solvents, Stern et al.[34] showed that there is an inverse relationship between contact distance and association constants. In terms of charge density, Me_4N^+ undergoes more extensive hydration than Bu_4N^+ in aqueous solution. The addition of ethanol is expected to cause a greater change in the extent of hydration of Me_4N^+ than it would in Bu_4N^+ hydration. Assuming that an anion is equally hydrated in both Me_4N^+ and Bu_4N^+ electrolytic solutions, the contact distance between ion pairs in Me_4N^+–anion is expected to be less than

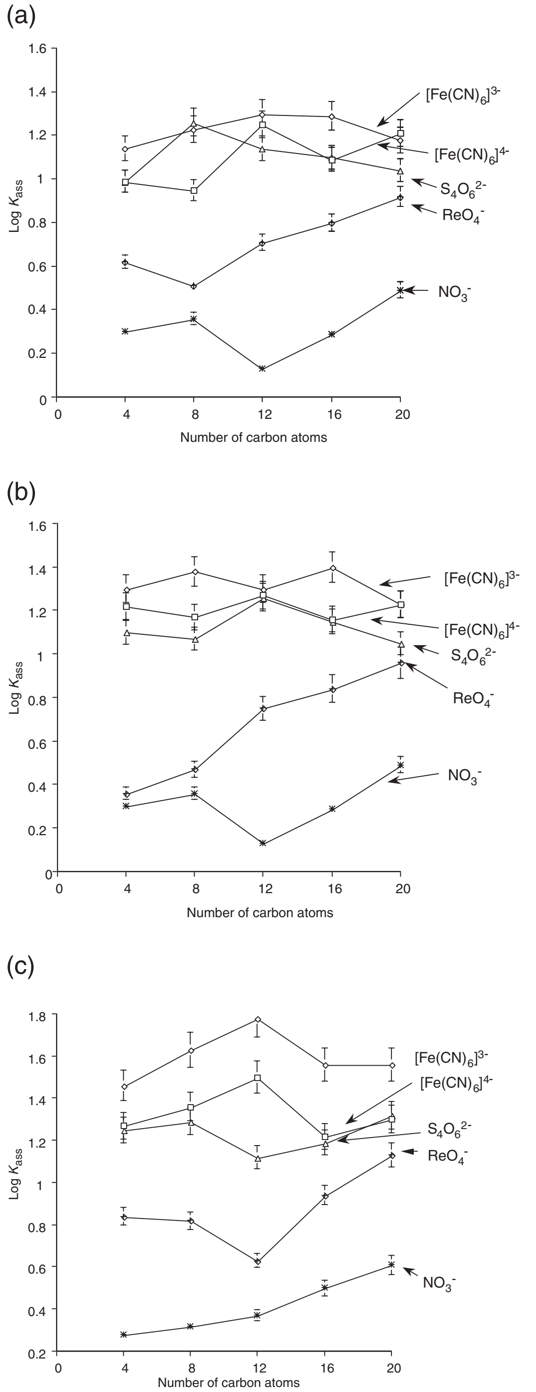

Fig. 2. Variation of $\log K_{ass}$ with an increase in bulkiness of the alkyl group in tetraalkylammonium ion upon association with inorganic anions. (a) 0%, (b) 31.4%, (c) 62.9% (m/m) ethanolic buffer. Error bars: ±95% confidence interval.

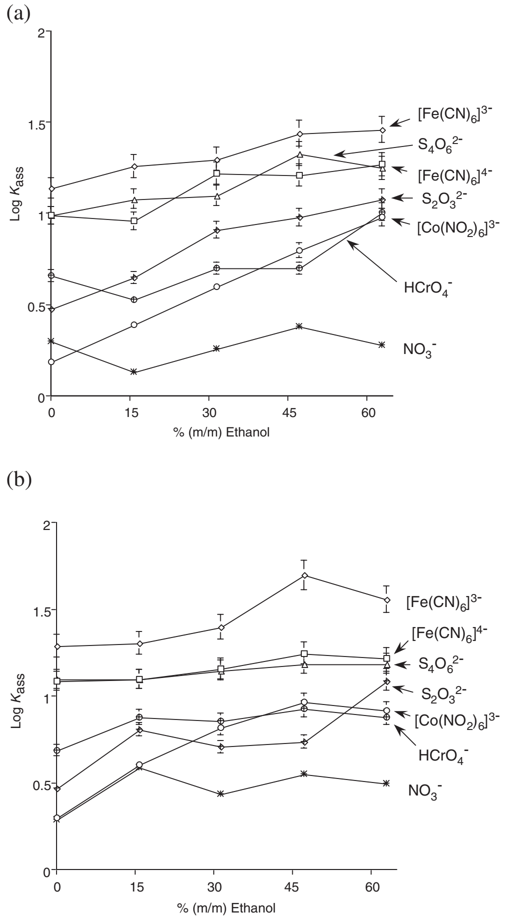

Fig. 3. Changes in $\log K_{ass}$ at different ethanol contents upon association between inorganic anions and (a) tetramethylammonium ion and (b) tetrabutylammonium ion. Error bars: ±95% confidence interval.

in Bu_4N^+–anion. Thus, Me_4N^+–anion association will increase at a more substantial rate than Bu_4N^+–anion and hence the greater rate of change of $\log K_{ass}$ for Me_4N^+. Interestingly, there appears to be more fluctuations with the hexacyanoferrate(III) and the tetrathionate ion in Me_4N^+ than in Bu_4N^+ association. The rest of the anions, on the other hand, show a steady increase in K_{ass} as the amount of ethanol in the buffer was increased.

Application of the Medium Effect Concept and the Gibbs Free Energy of Transfer to Weak Ion Association. Medium Effect: The medium effect is defined as the logarithm of the transfer activity coefficients, ${}_{m}\gamma_i$, which is a ratio of the activity coefficient for a given chemical species, *i*, as it is undergoes transfer between two solvents, i.e.:

$$ {}_{m}\gamma_i = \frac{{}_{S1}\gamma_i}{{}_{S2}\gamma_i}, \tag{5} $$

where ${}_{S1}\gamma_i$ = the activity coefficient in solvent 1 (usually water) and ${}_{S2}\gamma_i$ = the activity coefficient in solvent 2.

The medium effect gives a measure of the chemical potential of a species and an indication of its stability upon transfer from one solvent to another under standard conditions. In aqueous–organic solvent systems, the medium effect shows the relative stability of chemical species *i* in water relative to an organic solvent. It therefore assumes a positive value if the species is more stable in water and negative if the species is more stable in an organic solvent.

Sarmini and Kenndler had earlier investigated changes in the ionic mobilities, acidity constants, and separation selectivities of substituted aromatic acids in aqueous–organic media consisting of methanol,[17] ethanol,[18] 1-propanol,[19] and acetonitrile[20] as organic solvents. In these water–organic solutions, the medium effect was used to explain the changes in stability of a proton, which is responsible for changes in the pK_a of analytes, as the proportion of the organic component was varied.

The medium effect is related to the standard Gibbs free energy of transfer, ΔG_t° by:

$$ \ln {}_{m}\gamma_i = \frac{\Delta G_t^\circ}{RT} = \frac{({}_{S1}G_i^\circ - {}_{S2}G_i^\circ)}{RT}, \tag{6} $$

where ΔG_t° = standard Gibbs free energy of transfer, ${}_{S1}G_i^\circ$ = Gibbs free energy in the standard state in solvent S1, ${}_{S2}G_i^\circ$ = Gibbs free energy in the standard state in solvent S2, R = molar gas constant, T = absolute temperature.

The standard Gibbs free energy of transfer, ΔG_t° was previously used by Penn et al.[35] to explain the stability of tioconazole cation in the presence of β-cyclodextrin. Porras et al.[21] further extended the concept of the medium effect to gain an insight into the roles played by individual species involved in a chemical equilibrium in dictating a shift in equilibrium position. Consequently, it is possible to identify the cause of the observed trend in equilibrium constants. Such a finding could be important in analytes' separations, which involve an ion association mechanism since separation selectivity can be readily optimized to effect a particular separation.

The association constant K_{ass} between a cation, C^{n+}, and an anion, A^{m-}, in the equilibrium expression:

$$ C^{n+} + A^{m-} \rightleftharpoons CA^{(n-m)+}, $$

can be written as (in activity terms):

$$ K_{ass} = \frac{a_{CA^{(n-m)+}}}{a_{C^{n+}}a_{A^{m-}}}. \tag{7} $$

A transfer from water to organic solvent is accompanied by a change in the ion association constant expressed as:

$$ \log {}_{W}K_{ass} - \log {}_{S}K_{ass} = \Delta pK_{ass}^{S-W} = \log \frac{{}_{m}\gamma_{CA}}{{}_{m}\gamma_{C}\,{}_{m}\gamma_{A}} $$
$$ = \log {}_{m}\gamma_{CA} - \log {}_{m}\gamma_{C} - \log {}_{m}\gamma_{A}, \tag{8} $$

where $\log {}_{W}K_{ass}$ = association constant in water, $\log {}_{S}K_{ass}$ = association constant in organic solvent.

Due to the non-availability of consistent thermodynamic data, the discussion on the medium effect and Gibbs free energy of transfer will be limited to four tetraalkylammonium ions (Me_4N^+, Et_4N^+, Pr_4N^+, and Bu_4N^+) and one anion (SCN^-). Figure 4 shows variations in the individual medium effects of Me_4N^+, Et_4N^+, Pr_4N^+, Bu_4N^+ (cations), SCN^- (anion), and their corresponding ion associates. To obtain the medium effect of each ionic species involved in the ion association, it was necessary to convert the percentage v/v to percentage m/m in terms of ethanol composition. The Gibbs free energy of transfer for tetraalkylammonium ions corresponding to the experimental ethanol compositions (m/m) were then obtained by interpo-

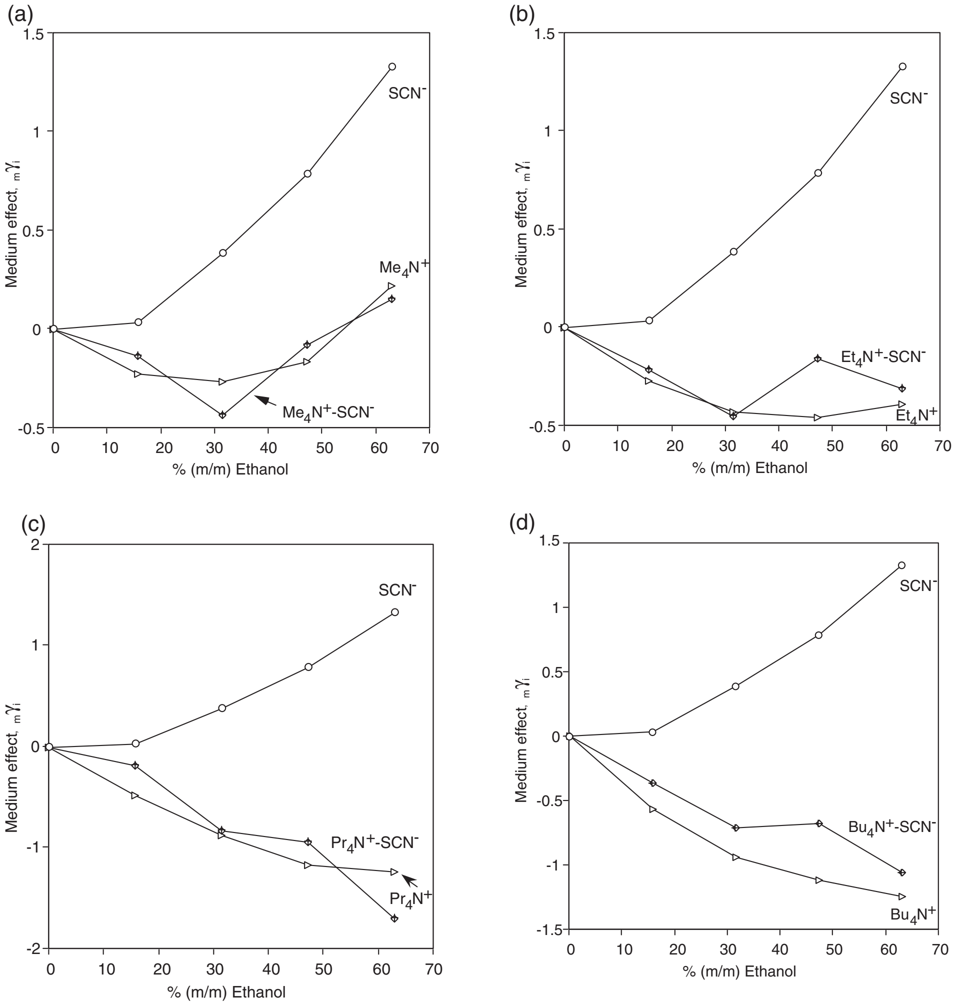

Fig. 4. Medium effect of SCN^-, individual tetraalkylammonium ions and their corresponding ion associates in ethanolic solutions at 25 °C.

lation of the data contained in a comprehensive review by Kalidas et al.[36] Similarly, the Gibbs free energy of transfer for thiocyanate ion were determined by the interpolation of data published by Marcus.[37] By Eq. 6, the medium effect of tetraalkylammonium ion and thiocyanate ion were computed, after which the medium effect of the corresponding ion associates were calculated using Eq. 8. Using the medium effect of the three chemical species, plots depicting individual variations in the medium effect as a function of ethanol content in the buffer solutions were made (Fig. 4).

All the plots in Fig. 4 show that the thiocyanate ion becomes increasingly unstable (in ethanolic solution) as the amount of ethanol in the buffer is increased. While both tetraalkylammonium ion and its ion associate generally showed increasing stability as the ethanol proportion was raised, tetraalkylammonium ions generally displayed a slightly higher stability. The increased stability of the tetraalkylammonium ions in ethanolic solutions may be attributed to an increase in the hydrophobic interactions between the organic solvent (ethanol) and the cation, coupled by an increase in the breakup of water structures. It is noteworthy that the Me_4N^+–SCN^- association (Fig. 4(a)) shows a distinctly different medium effect trend from the other tetraalkylammonium ion–thiocyanate associations. At about 30% (m/m) ethanol content, the respective medium effects of the cation and ion associate reach their minima, denoting the maximum stability ever attained in ethanolic solution. Beyond 30% (m/m), all three species displayed increasing instability in ethanolic solution.

Gibbs Free Energy of Transfer (ΔG_t°). The standard Gibbs free energy of transfer may be used to explain the observed trends in K_{ass} as the organic solvent (ethanol) content in the buffer is increased, as well as identify a likely cause for the shift in the equilibrium position.

For an equilibrium:

$$C^{n+} + A^{m-} \rightleftharpoons CA^{(n-m)+},$$

an increase in stability (in water) of the reactants or a decrease in stability of the product (in mixed solvents) as the amount of ethanol is increased shifts the equilibrium to the left, resulting into smaller K_{ass} values. On the other hand, an increase in stability (in mixed solvents) of the product or a decrease in stability of the reactants displaces the equilibrium to the right, yielding a higher equilibrium constant.

ΔG_t° for the tetraalkylammonium ions and SCN^- were obtained by interpolation of literature data[36,37] as previously explained, while those of the corresponding ion associates were

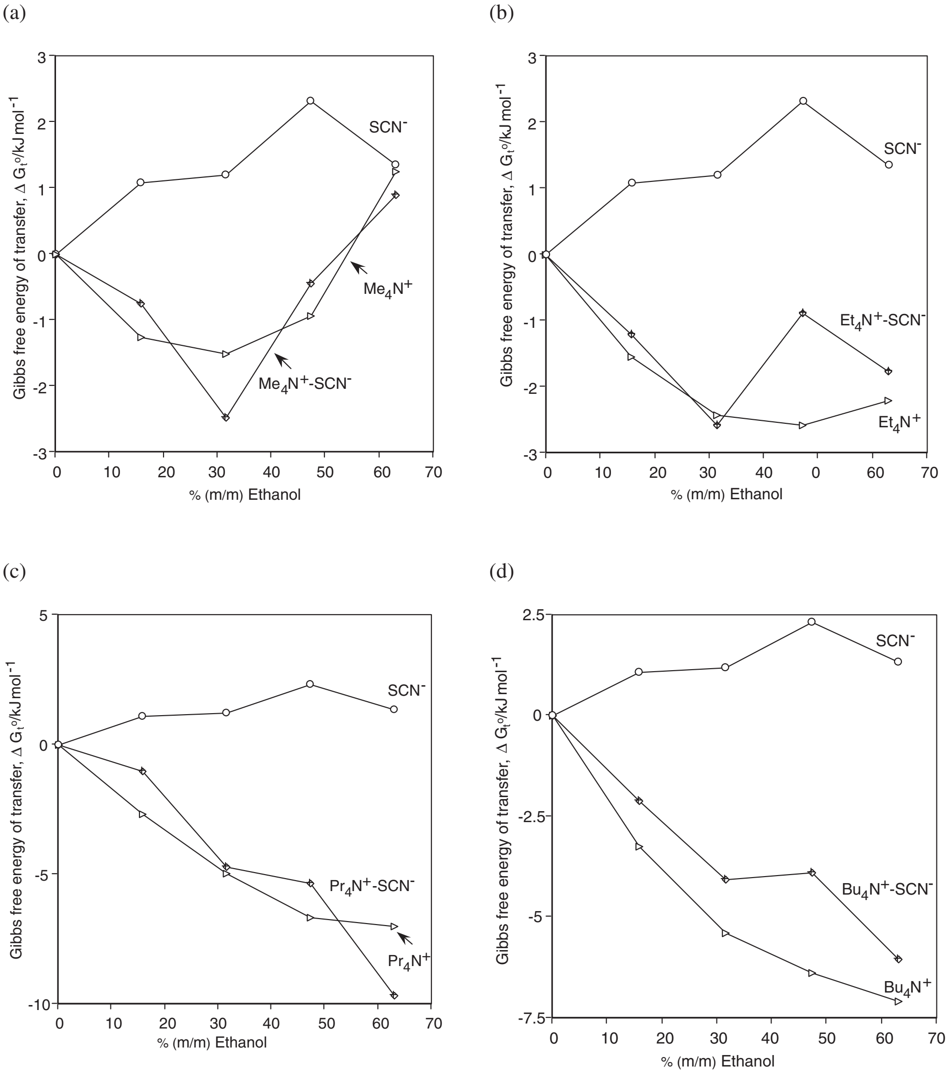

Fig. 5. Standard Gibbs free energy of transfer, ΔG_t°, for SCN^-, the individual tetraalkylammonium ions and their corresponding ion associates at 25 °C.

determined by Eq. 6 from the ion associate's medium effect value (Eq. 8).

Except for the Me_4N^+–SCN^- association, tetraalkylammonium ions–thiocyanate associations (Fig. 5(b–d)) showed a similar trend, i.e., ΔG_t° for SCN^- increased while ΔG_t° for the tetraalkylammonium ions and its ion associate decreased (became more stable in ethanolic solution). Despite the cation displaying a higher stability than the ion associate (Fig. 5(b–d)), this stabilizing effect is offset by the rising instability of the thiocyanate anion. Consequently, the overall effect is a shift of equilibrium to the right in favour of formation of the ion associate. This may account for the observed increase in K_{ass} as the ethanol content in the buffer is increased for the tetraalkylammonium ion–thiocyanate association. It could be suggested that the observed increase in equilibrium constants is largely due to the increasing instability of thiocyanate (in ethanolic solution).

Despite showing an increase in K_{ass}, as with other tetraalkylammonium associations, the ΔG_t° profile for TMA^+–SCN^- is markedly different from those shown by other tetraalkylammonium ions-thiocyanate associations (Fig. 5(b–d)). Figure 5(a) shows that SCN^- undergoes a steady increase in ΔG_t°, reflecting an increase in the instability with rising levels of ethanol in the buffer solution. On the other hand, Me_4N^+ and its ion associate showed an increase in stability (in ethanolic solution) up to about 30% (m/m), beyond which both species experienced a decrease in stability. Up to 30% (m/m) ethanol, a similar explanation as that used for other tetraalkylammonium ions–thiocyanate pairs holds for the increase in K_{ass}. However, beyond 30% (m/m) ethanol content, the increase in K_{ass} can be ascribed to the combined instability of both SCN^- and Me_4N^+ (favour ion formation), which offsets the increased instability of the ion associate (favours ion dissociation). Overall, ion formation is favoured leading to an increase in K_{ass}. A significant increase in K_{ass} for Me_4N^+–SCN^- association above 30% (m/m) (Table 1) may be ascribed to this combined instability of SCN^- and Me_4N^+ over the Me_4N^+–SCN^- ion associate.

Conclusion

The potential for the use of ordinary silica capillaries, preferable for their low cost and robustness, to investigate weak ion associations between tetraalkylammonium ions and inorganic anions has been demonstrated. The main advantage of this method among CE techniques is its use of an ordinary silica capillary to measure the mobility of highly-mobile anions and subsequently determine their association constants. Compared with conductometric methods of association constant determination, the CZE method offers several advantages including precise control of experimental conditions and the ability to determine association constants in a multi-ion environment. While ion chromatography can be used to carry out similar determinations, the CZE method is cost effective (lower equipment acquisition/maintenance costs) and uses smaller volumes of reagents. In addition, this work provides ion association constants data for the interaction between inorganic anions and tetraalkylammonium ions in buffers with variable ethanol–aqueous compositions which augments the existing data. The data may also be used for further thermodynamic evaluations and comparison purposes with data derived from other analysis techniques.

The medium effect and Gibbs free energy of transfer have revealed an increasing instability in anions as the proportion of ethanol in the buffer is raised as a cause for the observed increase in K_{ass} for the tetraalkylammonium ion–inorganic anion association. It is suggested that to obtain an increase in K_{ass} in such associations, an anionic species which becomes increasingly unstable in ethanolic solution can be used. For this purpose, more hydrophilic anions could be employed. Conversely, if a decrease in K_{ass} is desired, a hydrophobic anion, which is likely to be more stable in ethanolic solutions, can be employed.

This study was supported by a Grant-in-Aid for Scientific Research (B) (No. 13440220) from the Japan Society for Promotion of Science (JSPS).

References

1 Y. Walbroehl and J. W. Jorgenson, *J. Chromatogr.*, **315**, 135 (1984).

2 R. S. Sahota and M. G. Khaledi, *Anal. Chem.*, **66**, 1141 (1994).

3 I. Bjørnsdottir, J. Tjornelund, and S. H. Hansen, *Electrophoresis*, **19**, 2179 (1998).

4 S. P. Porras, M.-L. Riekkola, and E. Kenndler, *Chromatographia*, **53**, 290 (2001).

5 J. L. Miller, M. G. Khaledi, and D. Shea, *Anal. Chem.*, **69**, 1223 (1997).

6 M. Chiari and E. Kenndler, *J. Chromatogr., A*, **716**, 303 (1985).

7 I. Bjørnsdottir and S. H. Hansen, *J. Chromatogr., A*, **771**, 313 (1995).

8 T. Okada, *J. Chromatogr., A*, **695**, 309 (1995).

9 A. M. Stalcup and K. H. Gahm, *J. Microcol. Sep.*, **8**, 145 (1996).

10 K. D. Altria and S. M. Bryant, *Chromatographia*, **46**, 122 (1997).

11 A. J. Tomlinson, L. M Benson, J. W. Gorrod, and S. Naylor, *J. Chromatogr., B*, **657**, 373 (1994).

12 S. Fujiwara and S. Honda, *Anal. Chem.*, **59**, 487 (1987).

13 C. Schwer and E. Kenndler, *Anal. Chem.*, **63**, 1801 (1991).

14 G. M. Janini, K. C. Chan, J. A. Barnes, G. M. Mushik, and H. J. Issaq, *Chromatographia*, **35**, 497 (1993).

15 B. B. VanOrman, G. G. Liversidge, and G. L. MacIntire, *J. Microcol. Sep.*, **2**, 176 (1990).

16 W. Schützner and E. Kenndler, *Anal. Chem.*, **64**, 1991 (1992).

17 K. Sarmini and E. Kenndler, *J. Chromatogr., A*, **806**, 325 (1998).

18 K. Sarmini and E. Kenndler, *J. Chromatogr., A*, **811**, 201 (1998).

19 K. Sarmini and E. Kenndler, *J. Chromatogr., A*, **818**, 209 (1998).

20 K. Sarmini and E. Kenndler, *J. Chromatogr., A*, **833**, 245 (1998).

21 S. P. Porras, K. Sarmini, S. Fanali, and E. Kenndler, *Anal. Chem.*, **75**, 1647 (2003).

22 J. B. Kim, K. Otsuka, and S. Terabe, *J. Chromatogr., A*, **912**, 343 (2001).

23 I. R. Kimberly and C. A. Lucy, *J. Chromatogr., A*, **964**, 213 (2002).

24 S. P. Porras, M.-L. Riekkola, and E. Kenndler, *Electrophoresis*, **23**, 367 (2002).

25 B. A. William and G. Vigh, *Anal. Chem.*, **68**, 1174 (1996).

26 S. Motomizu and T. Takayanagi, *J. Chromatogr., A*, **853**, 63 (1999).

27 S. Fu and C. A. Lucy, *Anal. Chem.*, **70**, 173 (1998).

28 Y. Marcus, "Ion Properties," Marcel Dekker, New York (1997), Table 3.

29 W. R. Jones and P. Jandik, *J. Chromatogr., A*, **546**, 445 (1991).

30 J. Mbuna, T. Takayanagi, M. Oshima, and S. Motomizu, *J. Chromatogr., A*, **806**, 191 (1998).

31 "Stability Constants of Metal-Ion Complexes (Part A)," ed by E. Hogfeldt, Pergamon Press, Oxford (1982), pp. 84–129.

32 H. S. Harned and B. B. Owen, "Physical Chemistry of Electrolyte Solutions," Reinhold, New York (1964), pp. 283–294.

33 M. Boström, D. R. M. William, and B. W. Ninham, *J. Biophys.*, **85**, 686 (2003).

34 K. H. Stern, F. Healey, and A. E. Martel, *J. Chem. Phys.*, **19**, 1114 (1950).

35 S. G. Penn, E. T. Bergström, D. M. Goodall, and J. S. Loran, *Anal. Chem.*, **66**, 2866 (1994).

36 C. Kalidas, G. Hefter, and Y. Marcus, *Chem. Rev.*, **100**, 819 (2000).

37 Y. Marcus, *Pure Appl. Chem.*, **62**, 899 (1990).