Capillary zone electrophoretic studies of ion association between inorganic anions and tetraalkylammonium ions in aqueous–dioxane media

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

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

Abstract

Ion association between inorganic anions and symmetrical tetraalkylammonium ions, R_4N^+ (R = Me, Et, Pr, *n*-Bu, *n*-Am, and 2-methyl butyl {isoamyl = iAm}) was investigated using ordinary silica capillary by capillary zone electrophoresis. An improved version of the Williams–Vigh method was used for the first time to measure the mobilities of the inorganic anions. Plots of log K_{ass} against log dielectric constant in various media, revealed a smaller change in K_{ass} compared to dielectric constant. These plots suggest that the Bjerrum's equation is inadequate in accounting for the associations of ions in a CZE setup.
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1. Introduction

Aqueous–organic mixed solutions such as aqueous–dioxane binary solutions find a broad application in industries ranging from the manufacture of paints to detergents and deodorants. Equally broad is their use in many areas of fundamental and advanced scientific research, e.g. studies of analytes interactions in capillary electrophoresis and ion chromatography, synthesis, and mechanistic studies in organic chemistry and the prediction of drug solubility and chemical stability in pharmaceutical sciences. The physicochemical properties of binary aqueous–organic solvent systems, e.g. dielectric constants or viscosity, can be varied continuously by merely changing their solvent composition. 1,4-Dioxane has been widely researched [1–5] as an organic component in binary solutions, partly because of its uniquely low dielectric constant ($\varepsilon = 2.2$) compared to water's ($\varepsilon = 78.8$) and high miscibility with water. Therefore, aqueous–dioxane

solvent system is particularly suitable for studies in which analysis over a wide range of dielectric constants is required.

Conductometric techniques have been extensively used for a long time in the study of ion association in various solvent systems, e.g. isomeric dichloroethene [6] and aqueous–dioxane [1]. Through such measurements, Fuoss and Kraus [1] were able to confirm Bjerrum's theory [7] by examining iAm$_4$N$^+$–nitrate association in aqueous–dioxane mixture at various compositions. Apart from conductometry [8,9], capillary zone electrophoresis and HPLC have emerged as alternative techniques for studying ion interaction in solutions.

For nearly a decade capillary electrophoresis has been applied to the investigation of ion association with large association constants ($K_{ass} \geq 20$) in aqueous [10–17], non-aqueous [18–20], and mixed aqueous–organic media [21,22]. In these investigations, interactions between the associating species have been followed by measuring the mobilities of either a positively charged species or bulky, low-mobility negatively charged organic species, which can be easily measured using ordinary silica or coated capillary [10–17]. On the other hand, studies on ion associations, in which association is monitored

* Corresponding author.
E-mail address: motomizu@cc.okayama-u.ac.jp (S. Motomizu).

by measuring the mobilities of the highly mobile anions, especially those accompanied by small association constants ($K_{ass} < 10$) are few. Findings from weak interactions can be used in the design of the CE separation methods, e.g. in the separation of synthetic peptides using weak interactions between small organic molecules like trifluoroacetic acid and the peptides [23].

Weak ion association between inorganic anions and tetraalkylammonium ions in aqueous solutions using neutral coated capillary has already been reported [24]. Similar interactions were later examined in aqueous–ethanol mixed solutions [25] using an ordinary silica capillary, in which the association constants showed a modest increase with an increase in the proportion of ethanol in the migrating buffer. It was, therefore, of interest to investigate similar associations in mixed solvents, which differ markedly in their dielectric constants. Such solvent systems are expected to provide a wide range of dielectric constants on which to investigate ion association. As a result apolar dioxane with very low dielectric constant and water with relatively high dielectric constant were selected as solvents.

In this study the association constants, K_{ass} between the small inorganic anions (Br^-, SCN^-, NO_3^-, $S_2O_3^{2-}$, $S_4O_6^{2-}$, $[Co(NO_2)_6]^{3-}$, $[Fe(CN)_6]^{3-}$, and $[Fe(CN)_6]^{4-}$) and symmetrical tetraalkylammonium ions (Me_4N^+; Et_4N^+; Pr_4N^+; Bu_4N^+; Am_4N^+, iAm_4N^+ = tetra-(2-methyl butyl)-ammonium {tetraisoamylammonium} ion) were determined in aqueous–dioxane solvent of variable composition by using an ordinary silica capillary. Consequently, the Williams–Vigh method [26] had to be employed for the measurement of the effective mobility of anions most of which are highly mobile. The Williams–Vigh method was improved to reduce the analysis time, since the original version would have required a longer capillary and hence taken longer analysis time for accurate measurement of the individual anion's mobilities.

In addition, this study presents a qualitative examination of the suitability of Bjerrum's theory to account for observations in inorganic anions–tetraalkylammonium ions associations. Since Bjerrum's theory was confirmed by conductometric measurements, which uses electrolytic solutions essentially different from CZE's, re-examination of the theory from CZE point of view was thought necessary.

2. Experimental

2.1. Instrumentation

A Hewlett Packard ^{3D}CE system (Waldbronn, Germany) equipped with a diode array detector was used for electrophoretic mobility measurements. A water circulation thermostatic equipment was connected to the system to maintain constant temperature in the vial compartment. An ordinary fused silica capillary (Agilent Technologies, Germany), 50 μm i.d., 375 μm o.d., 40 cm effective length, and 48.5 cm total length was employed for all measurements of the an-

ion's effective mobility. The capillary cartridge was maintained at $25.0 \pm 0.1\,°C$ and the detection of anions was done at 220 nm. HP Chemstation software in a Windows environment controlled the system functions, processed the signals and collected the generated data.

2.2. Reagents and solutions

Milli-Q Labo water (18.0 MΩ cm, Nihon Millipore, Tokyo, Japan) was used in the preparation of all the reagent solutions which were filtered through 0.45 μm syringe driven filters (Advantec, Tokyo, Japan) before use.

Stock migrating buffer solutions containing 0%, 16.5%, 33%, 49.6%, and 66.2% (m/m) dioxane (Sigma-Aldrich, St. Louis, USA) corresponding to 0%, 16%, 32%, 48%, and 64% (v/v), respectively, were prepared by adding an appropriate amount of dioxane to the formate buffer, made from formic acid and sodium formate (Wako Pure Chemicals, Osaka, Japan). The pH of the buffer solution in pure aqueous solution was 3.80. Despite not making pH measurements on adding dioxane, the same concentration of formic acid and sodium formate was maintained in aqueous–dioxane buffers. In this analysis, all the electrolytic solutions contained a formate buffer at a concentration of 10 mM total formate.

The pairing cations in the migrating solutions consisted of symmetrical tetraalkylammonium ions. Except for the tetraisoamylammonium ion, iAm_4N^+, all the tetraalkylammonium salts were dissolved in water as chloride salts (Tokyo Kasei, Tokyo, Japan) to prepare 0.20 M stock solutions. The tetraisoamylammonium ion was obtained from its iodide salt (Tokyo Kasei, Tokyo, Japan) by dissolving the salt in 60% (v/v) ethanol–aqueous solution, vigorously shaking with silver oxide for 36 h, centrifuging and finally filtering the supernatant solution through 0.45 μm syringe-driven filter. The final stock solution contained 0.1 M tetraisoamylammonium hydroxide.

Single anion stock solutions (0.01 M) were prepared from their respective sodium salts (Wako Pure Chemicals, Osaka, Japan). The sample solution consisted of one or more anions, each diluted to 3×10^{-4} M in a 10 mM total formate buffer solution. Mesityl oxide (0.02%, v/v) was added to each sample solution as a neutral marker. As previously reported [25], associations which involved $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$ were carried out under constant ionic strength ($I = 31$ mM) [16] and the tetraalkylammonium ion concentration was examined in the range 0–21 mM. On the other hand, associations with Br^-, NO_3^-, SCN^-, ReO_4^-, $HCrO_4^-$, $S_2O_3^{2-}$, $S_4O_6^{2-}$, and $[Co(NO_2)_6]^{3-}$, were carried out without adjusting the ionic strength of the migrating solution [25]. These anions were analyzed by diluting tetraalkylammonium ions stock solutions to a series of migrating solutions containing 0–35 mM cation concentration. With an exception of the iAm_4N^+ ion electrolyte in which Cr(VI) existed as CrO_4^{2-}, Cr(VI) existed as $HCrO_4^-$ in all other migrating solutions.
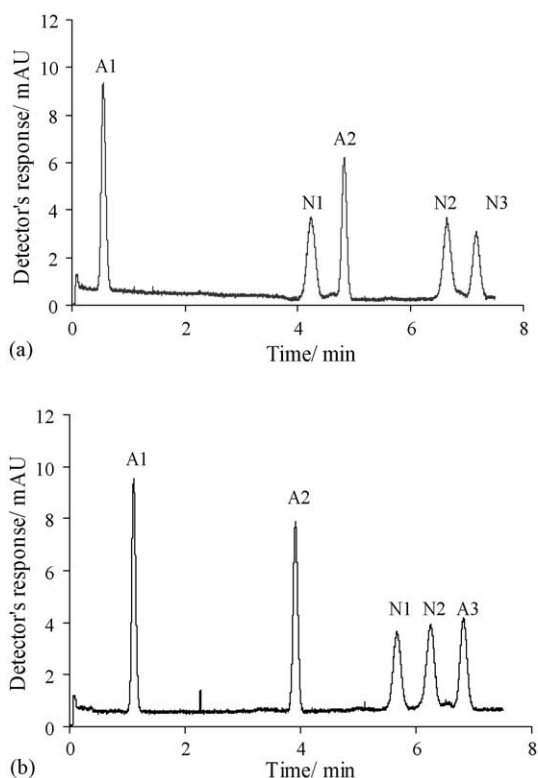
Fig. 1. Electropherograms obtained by the Williams–Vigh method: (a) original and (b) improved version. A1, first injection analyte's peak; N1, first injection neutral marker's peak; A2, second injection analyte's peak; N2, second injection neutral marker's peak; N3, third injection neutral marker's peak.

2.3. Methods: anion's effective mobilities measurements

Both single separation (double injection) and the improved double separation (triple injection) modes of the Williams–Vigh method [26] were used to measure the mobilities of the inorganic anions. The mobilities of hexacyanoferrate (II) and hexacyanoferrate (III) were carried out exclusively using the double separation mode, while all other mobility measurements were made using both modes. The procedure used for single separation has been outlined in the previous report [25].

The original double separation mode [26] carried out as outlined previously [25] yielded an e-gram shown in Fig. 1a. In the improved version, the same series of steps as in the original version were carried out, except that the voltage was applied for a longer period of time. As a result all the analyte peaks appeared before neutral marker peak from the first separation (Fig. 1b). For example, in the original version, voltage could be applied for about 60 s, while in the improved version voltage could be applied for 100 s. Since the application of voltage for longer time intervals leads to improved reproducibility of results, this approach facilitated reliable measurement of mobility for the highly mobile anions without a need of a longer capillary and hence saved the analysis time. To calculate the effective mobilities of the

anions the same formula as in the original version was used [26].

The silica capillary was conditioned at the beginning and the middle of each association analysis, by flushing with 0.1 M NaOH solution (5 min) followed by Milli-Q water (5 min). Between successive runs, the capillary was flushed with the corresponding migrating solutions for 3 min. Initially, the buffer solution constituted the migrating solution and the sample underwent an appropriate mode of the Williams–Vigh method (single/double separation). After a triple run, a fresh migrating solution was prepared by increasing the concentration of the tetraalkylammonium ions while keeping the buffer concentration constant and the triplicate mobilities measurements done. The concentrations of cations in the migrating solutions were further increased at regular intervals and the corresponding mobilities determined until the preset maximum tetraalkylammonium ion concentrations were reached. Electrophoretic mobilities of the individual anions at each cation concentration were averaged to give the anion's effective electrophoretic mobility, μ_{eff}.

3. Results and discussion

3.1. Effect of dioxane on mobility of anions and association constants

A cation, C^{n+} and an anion, A^{m-} can form an ion associate according to the equilibrium:

$$C^{n+} + A^{m-} \rightleftharpoons CA^{(n-m)+}$$
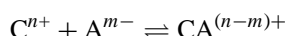
Since the cationic pairing ions are present in excess over the analyte ions, A^{m-}, and the ion associates are not so stable, only 1:1 ion associate formed are considered.

The corresponding association constant, K_{ass}, is obtained by:

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

The effective electrophoretic mobility, μ_{eff}, of an anion A^{m-} can be calculated by Eq. (2):

$$\mu_{eff} = \frac{1}{1 + K_{ass}[C^{n+}]}\mu_A + \frac{K_{ass}[C^{n+}]}{1 + K_{ass}[C^{n+}]}\mu_{CA} \tag{2}$$

When the charge of the cation and the anion is the same, Eq. (2) reduces to:

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

where μ_A is the mobility of an anion and μ_{CA} is the mobility of an ion associate.

The experimentally obtained effective mobility at each cation concentration were corrected for viscosity [21] and then treated by a non-linear least-squares method in order to compute K_{ass} (Table 1) and the corresponding effective

Table 1
log K_{ass} between tetraalkylammonium ions and inorganic anions determined at 25 °C and variable aqueous–dioxane composition in the migrating buffer

Pairing cation	Pairing anion	Dioxane, % (m/m)				
		0 (1.90)	16.5 (1.81)	33.0 (1.69)	49.6 (1.54)	66.2 (1.32)
Me$_4$N$^+$	Fe(CN)$_6^{4-}$	0.99 ± 0.14	1.29 ± 0.08	1.20 ± 0.17	1.30 ± 0.10	1.29 ± 0.18
	Fe(CN)$_6^{3-}$	1.14 ± 0.17	1.30 ± 0.05	1.40 ± 0.12	1.39 ± 0.09	1.35 ± 0.12
	Co(NO$_2$)$_6^{3-}$	0.19 ± 0.09	0.64 ± 0.12	0.77 ± 0.18	0.80 ± 0.14	1.07 ± 0.09
	S$_4$O$_6^{2-}$	0.99 ± 0.11	1.10 ± 0.10	1.09 ± 0.09	1.00 ± 0.12	1.21 ± 0.11
	S$_2$O$_3^{2-}$	0.48 ± 0.10	0.75 ± 0.18	1.01 ± 0.14	0.72 ± 0.11	0.99 ± 0.15
	ReO$_4^-$	0.62 ± 0.15	0.71 ± 0.10	0.69 ± 0.18	0.58 ± 0.16	0.90 ± 0.13
	HCrO$_4^-$	0.66 ± 0.08	0.67 ± 0.15	0.72 ± 0.12	0.62 ± 0.10	0.85 ± 0.17
	Br$^-$	0.1 ± 0.09, 0.1[a]	0.20 ± 0.10	0.45 ± 0.18	0.54 ± 0.18	0.59 ± 0.15
	NO$_3^-$	0.30 ± 0.11	0.45 ± 0.11	0.48 ± 0.09	0.23 ± 0.08	0.76 ± 0.14
	SCN$^-$	0.24 ± 0.10	0.60 ± 0.09	0.56 ± 0.14	0.46 ± 0.15	0.61 ± 0.11
Et$_4$N$^+$	Fe(CN)$_6^{4-}$	0.95 ± 0.14	1.30 ± 0.18	1.48 ± 0.11	1.30 ± 0.12	1.31 ± 0.13
	Fe(CN)$_6^{3-}$	1.23 ± 0.07	1.48 ± 0.13	1.50 ± 0.08	1.57 ± 0.18	1.53 ± 0.11
	Co(NO$_2$)$_6^{3-}$	0.44 ± 0.06	0.84 ± 0.08	0.80 ± 0.18	0.81 ± 0.10	0.71 ± 0.11
	S$_4$O$_6^{2-}$	1.26 ± 0.14	0.99 ± 0.07	0.96 ± 0.05	1.0 ± 0.11	1.16 ± 0.14
	S$_2$O$_3^{2-}$	0.47 ± 0.10	0.59 ± 0.07	0.88 ± 0.18	0.66 ± 0.11	0.86 ± 0.18
	ReO$_4^-$	0.51 ± 0.13	0.66 ± 0.16	0.73 ± 0.18	0.72 ± 0.18	0.76 ± 0.19
	HCrO$_4^-$	0.63 ± 0.11	0.60 ± 0.18	0.71 ± 0.16	0.76 ± 0.19	0.74 ± 0.18
	Br$^-$	0.20 ± 0.03, 0.24[a]	0.43 ± 0.12	0.53 ± 0.14	0.57 ± 0.10	0.64 ± 0.13
	NO$_3^-$	0.36 ± 0.08	0.34 ± 0.16	0.42 ± 0.18	0.35 ± 0.11	0.54 ± 0.19
	SCN$^-$	0.30 ± 0.06	0.36 ± 0.11	0.52 ± 0.18	0.54 ± 0.08	0.65 ± 0.18
Pr$_4$N$^+$	Fe(CN)$_6^{4-}$	1.25 ± 0.10	1.38 ± 0.11	1.26 ± 0.09	1.47 ± 0.03	1.50 ± 0.11
	Fe(CN)$_6^{3-}$	1.30 ± 0.16	1.50 ± 0.03	1.68 ± 0.07	1.57 ± 0.09	1.69 ± 0.12
	Co(NO$_2$)$_6^{3-}$	0.35 ± 0.09	0.84 ± 0.14	0.85 ± 0.16	0.85 ± 0.07	1.02 ± 0.19
	S$_4$O$_6^{2-}$	1.14 ± 0.11	1.21 ± 0.12	1.15 ± 0.09	1.35 ± 0.11	1.38 ± 0.16
	S$_2$O$_3^{2-}$	0.47 ± 0.09	0.65 ± 0.08	1.01 ± 0.11	1.02 ± 0.19	1.21 ± 0.17
	ReO$_4^-$	0.71 ± 0.16	0.71 ± 0.14	0.81 ± 0.17	0.79 ± 0.19	0.81 ± 0.19
	HCrO$_4^-$	0.59 ± 0.10	0.72 ± 0.15	0.83 ± 0.12	0.83 ± 0.18	0.88 ± 0.19
	Br$^-$	0.32 ± 0.13, 0.39[a]	0.40 ± 0.09	0.67 ± 0.11	0.61 ± 0.17	0.70 ± 0.07
	NO$_3^-$	0.13 ± 0.09	0.31 ± 0.10	0.37 ± 0.14	0.54 ± 0.19	0.58 ± 0.15
	SCN$^-$	0.58 ± 0.13	0.48 ± 0.08	0.52 ± 0.10	0.67 ± 0.09	0.74 ± 0.09
Bu$_4$N$^+$	Fe(CN)$_6^{4-}$	1.09 ± 0.02	1.46 ± 0.07	1.54 ± 0.10	1.58 ± 0.15	1.70 ± 0.14
	Fe(CN)$_6^{3-}$	1.29 ± 0.10	1.45 ± 0.13	1.60 ± 0.15	1.74 ± 0.18	2.13 ± 0.19
	Co(NO$_2$)$_6^{3-}$	0.30 ± 0.07	0.87 ± 0.13	0.96 ± 0.17	0.90 ± 0.13	0.99 ± 0.13
	S$_4$O$_6^{2-}$	1.10 ± 0.13	1.23 ± 0.11	1.29 ± 0.14	1.26 ± 0.07	1.34 ± 0.13
	S$_2$O$_3^{2-}$	0.47 ± 0.02	0.85 ± 0.12	0.71 ± 0.15	1.07 ± 0.18	1.13 ± 0.14
	ReO$_4^-$	0.80 ± 0.14	0.84 ± 0.18	0.99 ± 0.16	0.96 ± 0.11	1.06 ± 0.18
	HCrO$_4^-$	0.69 ± 0.06	0.89 ± 0.13	0.92 ± 0.16	1.00 ± 0.11	1.10 ± 0.19
	Br$^-$	0.33 ± 0.12, 0.44[a]	0.49 ± 0.09	0.46 ± 0.14	0.57 ± 0.13	0.72 ± 0.18
	NO$_3^-$	0.29 ± 0.09	0.60 ± 0.09	0.70 ± 0.11	0.62 ± 0.14	0.75 ± 0.17
	SCN$^-$	0.84 ± 0.10	0.85 ± 0.07	0.92 ± 0.08	0.90 ± 0.14	0.94 ± 0.09
Am$_4$N$^+$	Fe(CN)$_6^{4-}$	1.21 ± 0.13	1.39 ± 0.04	1.43 ± 0.08	1.46 ± 0.08	1.47 ± 0.03
	Fe(CN)$_6^{3-}$	1.18 ± 0.18	1.48 ± 0.06	1.53 ± 0.08	1.50 ± 0.13	1.58 ± 0.11
	Co(NO$_2$)$_6^{3-}$	0.26 ± 0.10	0.99 ± 0.19	0.98 ± 0.11	1.01 ± 0.09	1.21 ± 0.12
	S$_4$O$_6^{2-}$	1.04 ± 0.07	1.25 ± 0.04	1.23 ± 0.06	1.35 ± 0.10	1.38 ± 0.06
	S$_2$O$_3^{2-}$	0.33 ± 0.12	0.62 ± 0.06	0.50 ± 0.06	1.03 ± 0.12	1.05 ± 0.19
	ReO$_4^-$	0.92 ± 0.09	1.21 ± 0.09	1.15 ± 0.17	1.16 ± 0.06	1.21 ± 0.13
	HCrO$_4^-$	0.75 ± 0.08	0.96 ± 0.05	0.91 ± 0.09	1.03 ± 0.14	1.04 ± 0.18
	Br$^-$	0.33 ± 0.14	0.64 ± 0.07	0.57 ± 0.18	0.77 ± 0.11	0.78 ± 0.17
	NO$_3^-$	0.49 ± 0.11	0.73 ± 0.07	0.77 ± 0.09	0.80 ± 0.09	0.82 ± 0.12
	SCN$^-$	0.78 ± 0.13	0.96 ± 0.06	1.02 ± 0.07	1.04 ± 0.09	1.07 ± 0.09

Me$_4$N$^+$, tetramethylammonium ion; Et$_4$N$^+$, tetraethylammonium ion; Pr$_4$N$^+$, tetrapropylammonium ion; Bu$_4$N$^+$, tetrabutylammonium ion; Am$_4$N$^+$, tetraamylammonium ion; K_{ass} determined by Eq. (2) or (3), error: 3σ; log D values in bracket; D, dielectric constant.

[a] Conductometric values from Ref. [29].

electrophoretic mobilities of the anion in the absence of the pairing ion. By substituting K_{ass} and the corresponding electrophoretic mobilities of the anion in either Eq. (2) or (3), electrophoretic mobilities of a particular an-

ion were computed to yield the simulated curves shown in Fig. 2. A good agreement exists between the simulated and the experimental data as illustrated by the curve fitting (Fig. 2).
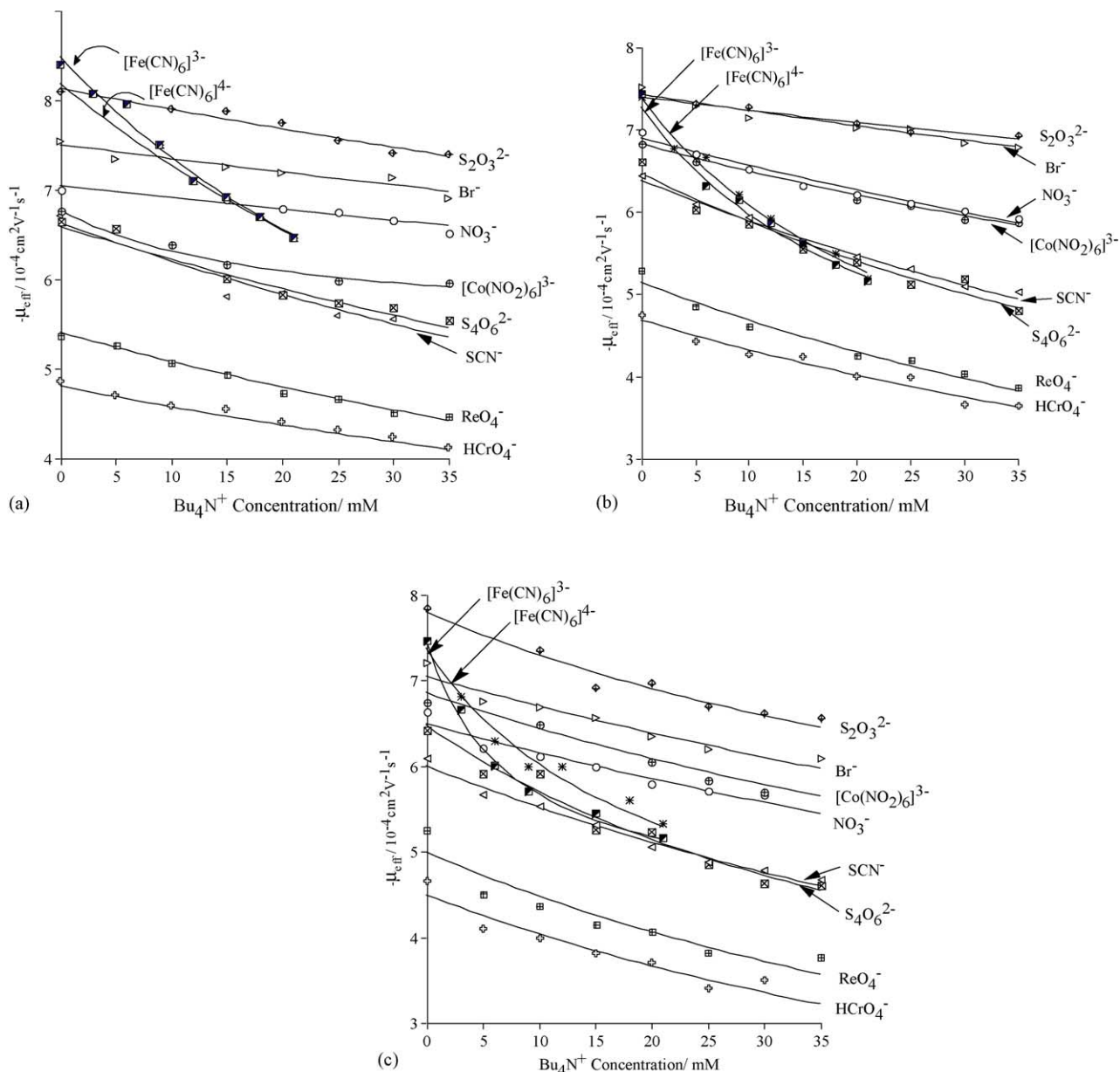
Fig. 2. Effect of tetrabutylammonium ion (Bu$_4$N$^+$) concentration and dioxane content in the migrating buffer on the effective electrophoretic mobilities of anions. Solid curves: theoretical curve fitting of experimental data by Eq. (2) or (3). μ_{eff}: effective electrophoretic mobility. CE conditions: 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$_4$N$^+$Cl$^-$; sample 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) 33.0%, and (c) 66.2% (m/m) dioxane–aqueous in the migrating buffers.

Most of the mobility trends of the inorganic anions in aqueous–dioxane media are similar to those exhibited in aqueous–ethanol media [25]. As shown in Fig. 2, the mobilities of anions decreased to different extents as the concentration of the pairing cation increased. As the composition of the dioxane was increased, a noted increase in the slope of the mobility curves is observed (Fig. 2a and c), which may indicate a possible increase in the extent of association. It is worth noting that most of the anions maintain their migration order as the content of dioxane in the buffer increased

although the mobilities of the some anions are continuously altered to the extent that there is a reversal in the migration order at higher dioxane content and TBA$^+$ concentration. For example, at 66.2% (m/m) dioxane (Fig. 2c) the migration order of hexacyanoferrate (II) and hexacyanoferrate (III) is reversed. The observed reversal is an indication of a considerable difference in the extent of ion association undergone by the two anions. In comparison to mobility profiles shown in aqueous–ethanol media, mobilities of most anions appear to change at a slightly higher rate in aqueous–dioxane media.

In addition, Fig. 2 can be used to examine the effect of dioxane on the mobility of the differently charged anions at a specific ionic strength in the absence of pairing ion (i.e. at $[Bu_4N^+] = 0$). Roy and Lucy [27,28] have reported that the combination of ionic strength and increasing proportion of organic solvent results in dramatic change in mobilities of the highly charged ions. In this work, however, the mobilities of hexacyanoferrate (II) and hexacyanoferrate (III) (Fig. 2) appeared to be only slightly affected by an increase on diox-ane content at ionic strength ($I = 10$ mM). Despite the small effect of dioxane on the mobilities of highly charged anions (hexacyanoferrate (II)/hexacyanoferrate (III)), an interesting reversal of the μ_{eff} was noted between hexacyanoferrate (II) and thiosulfate at 66.2% (m/m) dioxane. According to Hubbarb–Onsager equation, hexacyanoferrate (II) is ex-pected to experience more dielectric friction than hexacyano-ferrate (III) in the absence of the pairing cation by virtue of its higher charge. However, this analysis found that hexacyano-ferrate (III) is more affected by dielectric friction than hexa-cyanoferrate (II). As expected, in the absence of the pairing cation, the monovalent ions examined, e.g. nitrate, showed no significant change in mobility upon increasing the con-tent of the dioxane in the migrating buffer since they are less affected by the dielectric friction.

Among the methods used for K_{ass} determination, con-ductometric technique is one of the most widely used. A fair agreement exists between the data determined by CZE and those obtained by conductivity measurement in pure aqueous solution (Table 1). Similarly, most of the trends shown by the association constants between inorganic anions and tetraalkylammonium ions in aqueous–ethanol [25] are also observed in this analysis (Table 1). In general, K_{ass} between the inorganic anions and the tetraalkylammonium ions showed an increase as the dioxane content in the migrating buffer was increased. Table 1 shows that anions with higher charge/size ratio experienced stronger interaction than those with lower charge/size ratio. It can also be seen from Table 1 that the bulkier cations show increased interaction than the less bulky cations. In agreement with earlier reports [24,25], hexacyanoferrate (III) shows the strongest interaction, while the bromide anion shows the weakest interaction at nearly all dioxane compositions. The difference in their levels of inter-actions can be ascribed mainly to a relatively high charge/size and to a smaller extent, increased hydrophobic interactions of the hexacyanoferrate (III) compared to the bromide ion. Generally, K_{ass} values in aqueous–dioxane solvent system are higher than those determined in aqueous–ethanol solvent system [25] and aqueous media [24], for similar associ-ations. A comparatively lower dielectric constant of the aqueous–dioxane media may be responsible for the observed increase in the magnitude of K_{ass}. Interestingly, K_{ass} values (Table 1) consistently show that hexacyanoferrate (III) interacted with cations more extensively than the hexacyano-ferrate (II), despite the latter possessing a higher charge. A probable explanation for this observation may lie in their con-tact distances as they form ion associates. Hexacyanoferrate

(II) is expected to be more hydrated than the hexacyanofer-rate (III) by the virtue of its higher charge and hence possess larger contact distance. Since Stern et al. [6] showed that an inverse relationship exists between contact distance and K_{ass}, it may be suggested that the smaller K_{ass} with most anions is due to increased contact distance in hexacyanoferrate (II).

3.2. Bjerrum's theory and inorganic anions–tetraalkylammonium ions association: a qualitative assessment

Bjerrum's theory [7] was put forward as an attempt to ex-plain deviations of the experimental results from the predic-tions made by the Debye–Hückel theory [30], on the behavior of ions in solutions. Bjerrum introduced the concept of ion association to explain the observed deviations with the use of the equation:

$$K = \frac{4\pi N}{1000} \left(\frac{|z_1 z_2| e^2}{aDkT} \right)^3 Q(b) \tag{4}$$

$Q(b)$ is defined by the integral: $Q \equiv \int_2^b e^Y \, dY$ and $Y = |z_1 z_2| e^2 / rDkT$; where K is the association constant, $b = |z_1 z_2| e^2 / aDkT$, N the Avogadro's number, z_1 and z_2 are ionic charges, k the Boltzmann constant, D the dielectric constant, e the electronic charge, r the distance between the ions, a the distance of closest approach between ions, and T the absolute temperature.

Other theories on ion association were later proposed by Denison and Ramsey [31], Fuoss [32], Ebeling [33] and, more recently Yokoyama and Yamatera [34]. However, it is the Bjerrum's theory that has been widely used despite its short-comings such as counting as associated ions, which are not in actual contact. This theory has been successful in explain-ing certain aspects of ion association and was confirmed by conductometric measurements [1] of iAm_4N^+– NO_3^- asso-ciation in aqueous–dioxane media.

It can be deduced from Eq. (4) that the association con-stant, K, will increase if the associating species bear oppo-site charges and the dielectric constant of the media is as low as possible. Fig. 3a shows the conductometrically de-termined variation of association constants with dielectric constant for the association between iAm_4N^+ and NO_3^- in aqueous–dioxane media [1]. Consistent with the Bjerrum's theory, Fig. 3a exhibits a nearly exponential increase in as-sociation constant as the dielectric constant decreases. By conductometric measurements, the Bjerrum's theory appears to be obeyed because such measurements were possibly made in fairly dilute solutions, which contained a few ions in so-lutions. Interestingly, the curve fitting of the experimental results from the conductometric measurement indicates that association will cease at definite aqueous–dioxane composi-tion (corresponding to x-intercept). However, Bjerrum's the-ory should be merited for correctly predicting an increase in association constant, K_{ass} for associations carried out in aqueous–organic media.
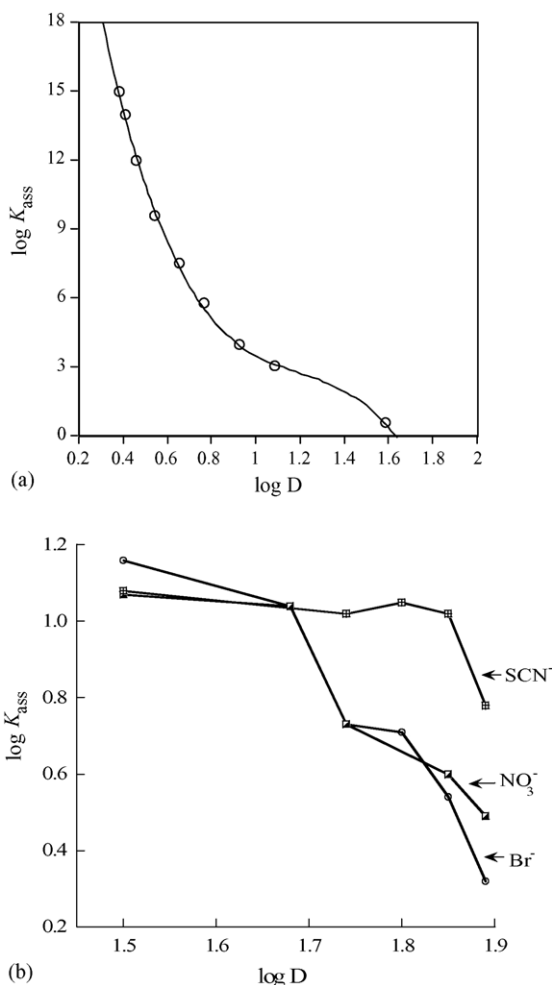
Fig. 3. Dependence of association constants on dielectric constants in the association between inorganic anions and tetraalkylammonium ions (a) conductometric determination between $i\mathrm{Am_4N^+}$ and nitrate in aqueous–dioxane media; solid line is a curve fitting (b) CE determination of association between tetra-*n*-pentylammonium ($\mathrm{Am_4N^+}$) ion and the monovalent anions in aqueous–methanol migrating electrolyte. Dielectric constants from Ref. [35].

Methanol and acetonitrile are popular CE reagents, which can give a reasonably wide range of dielectric constants when added to the migrating electrolyte. However, methanol was chosen because it is relatively less toxic. Association in methanolic migrating electrolyte was one of the analyses carried out to re-examine the Bjerrum's theory by CZE method. Dioxane was not added to the migrating solution to alter dielectric constants because measurement of anion's mobilities by the procedure used in this analysis would be difficult due to high viscosity of the aqueous–dioxane solvent. The dielectric constant was therefore varied by adding an appropriate amount of methanol in the migrating electrolyte in presence of the formate buffer (10 mM). Contrary to the Bjerrum's equation's predictions, a plot of $\log K_{\mathrm{ass}}$ against log dielectric constant determined by CZE measurement (Fig. 3b) shows a smaller increase in K_{ass} as the dielectric constant [35] decreases. This plot was ob-

tained by the association between the inorganic monovalent anions, $\mathrm{Br^-}$, $\mathrm{NO_3^-}$, $\mathrm{SCN^-}$, and tetra-*n*-pentylammonium ions ($\mathrm{Am_4N^+}$) in methanol–aqueous media (0–100%, m/m). Compared against association done in aqueous–dioxane media (Fig. 3a), the association in aqueous–methanol media (Fig. 3b) shows a negligibly small increase in K_{ass} on varying dielectric constant, and if plotted on the same axis (plot not shown), CE results will appear as near-horizontal, straight lines for $\log K_{\mathrm{ass}}$–$\log D$ relationship. The increase in K_{ass} shown by Fig. 3b is smaller compared to a substantial decrease in dielectric constant. This suggests that dielectric constant is not the only factor influencing ion association in solutions. Had dielectric constant been the only factor affecting association, a major change in K_{ass} would have been observed in tetraalkylammonium ions–anions association on increasing the proportion of methanol in the migrating solution.

Bjerrum's theory was further investigated by examining the association between the anions and the tetraalkylammonium ions at variable dioxane composition in the migrating buffer. Fig. 4 shows plots of $\log K_{\mathrm{ass}}$ against log dielectric constants [35] for the association between four tetraalkylammonium ions and anions bearing different charges. In most associations, the association constants show variable increase with decrease in dielectric constant. However, a closer examination reveals that increases in K_{ass} are small compared to large decrease in dielectric constant, e.g. in the $\mathrm{NO_3^-}$ – $\mathrm{Am_4N^+}$ association, $\log K_{\mathrm{ass}}$ increases from 0.49 to 0.82 for a corresponding dielectric constant change ($\log D$) of 1.9–1.3. As with associations carried out by varying methanol composition in the migrating electrolyte (Fig. 3b), the increase in K_{ass} is small. In addition, a comparison of $\log K_{\mathrm{ass}}$–log dielectric constant for tetraalkylammonium ion–nitrate associations done in aqueous–dioxane (Fig. 5a) with those determined in aqueous–ethanol (Fig. 5b) media, shows that the association constants in aqueous–dioxane are only slightly higher. Such small differences in K_{ass} are unexpected from Bjerrum's point of view, for media, which differ so substantially in their dielectric constants.

Since Bjerrum's theory was confirmed by conductometric determination of the association between nitrate and $i\mathrm{Am_4N^+}$, it was necessary to carryout a CZE determination for the association between $i\mathrm{Am_4N^+}$ and some inorganic anions. Table 2 summarizes $\log K_{\mathrm{ass}}$ between the inorganic anions and $i\mathrm{Am_4N^+}$ at variable dioxane composition in the buffer. As explained earlier (Section 2.3) $i\mathrm{Am_4N^+}$ was obtained differently because of its insolubility in water. While the results (Table 2) show a general, sharp increase in $\log K_{\mathrm{ass}}$ compared to similar associations in Table 1, the increase is noticeably sharper for the traditionally, weakly associated anions (e.g. $\mathrm{NO_3^-}$, $\mathrm{Br^-}$). The magnitude of K_{ass} values indicate that fairly strong associations occur at low dioxane compositions in the migrating buffer. However, only a marginal increase in K_{ass} is observed on increasing the proportion of dioxane in the migrating buffer. Thus, K_{ass} remained virtu-
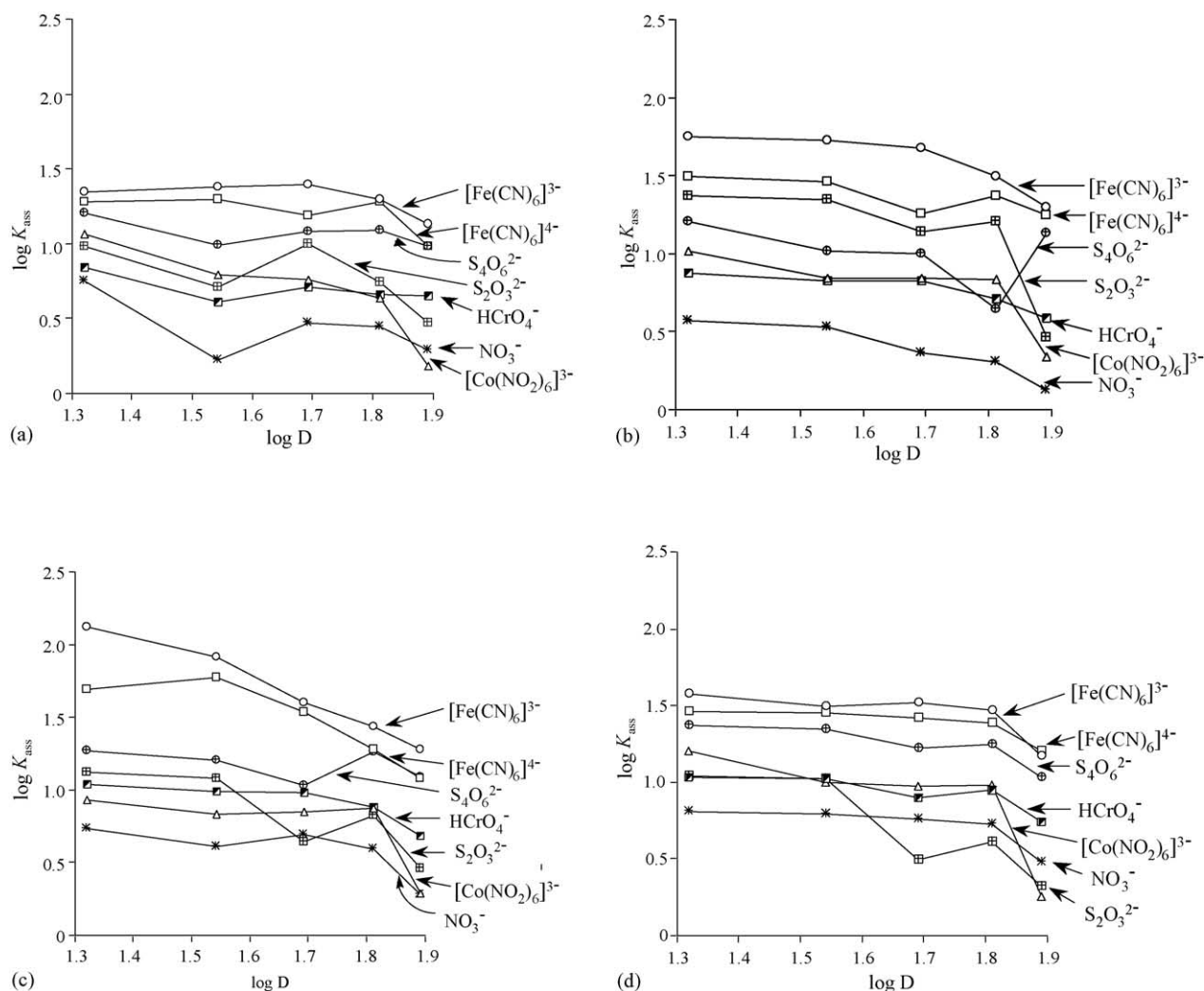
Fig. 4. Effect of dielectric constant on the association constants between inorganic anions and tetraalkylammonium ions (a) Me_4N^+, (b) Pr_4N^+, (c) Bu_4N^+, and (d) Am_4N^+ in buffers containing dioxane as an additive.

ally constant as the composition of dioxane was raised. These results suggest that the addition of dioxane to the migrating buffer has a slight or no effect in K_{ass} for iAm_4N^+–inorganic anions association.

From CZE investigations (Table 2 and Figs. 3–5), it can be suggested that Bjerrum's theory does not fully account for associations in CZE electrolytic solutions. If association of ions was solely dependent on dielectric constant, ion association

Table 2
log K_{ass} between tetraisoamylammonium ions (iAm_4N^+) and inorganic anions at 25 °C at variable aqueous–dioxane compositions in the migrating buffer

Anion	Dioxane, % (m/m)				
	0 (1.90)	16.5 (1.81)	33.0 (1.69)	49.6 (1.54)	66.2 (1.32)
$Fe(CN)_6^{4-}$	1.50 ± 0.12	1.59 ± 0.09	1.55 ± 0.07	1.51 ± 0.15	1.70 ± 0.10
$Fe(CN)_6^{3-}$	1.66 ± 0.18	1.69 ± 0.19	1.58 ± 0.18	1.61 ± 0.09	1.71 ± 0.11
$Co(NO_2)_6^{3-}$	1.32 ± 0.12	1.37 ± 0.15	1.34 ± 0.17	1.39 ± 0.07	1.41 ± 0.15
$S_4O_6^{2-}$	1.57 ± 0.18	1.47 ± 0.15	1.57 ± 0.11	1.49 ± 0.07	1.50 ± 0.16
$S_2O_3^{2-}$	1.50 ± 0.19	1.20 ± 0.18	1.26 ± 0.16	1.29 ± 0.13	1.28 ± 0.18
ReO_4^-	1.55 ± 0.18	1.57 ± 0.18	1.56 ± 0.15	1.57 ± 0.18	1.57 ± 0.16
$HCrO_4^-$	1.15 ± 0.06	1.27 ± 0.05	1.25 ± 0.18	1.28 ± 0.11	1.31 ± 0.04
Br^-	1.22 ± 0.10	1.21 ± 0.14	1.09 ± 0.16	1.24 ± 0.07	1.21 ± 0.15
NO_3^-	1.21 ± 0.09	1.25 ± 0.11	1.27 ± 0.14	1.28 ± 0.09	1.26 ± 0.18
SCN^-	1.44 ± 0.14	1.43 ± 0.14	1.44 ± 0.16	1.45 ± 0.13	1.43 ± 0.15

K_{ass} determined by Eq. (2) or (3), error: 3σ, log D values in bracket; D, dielectric constant.
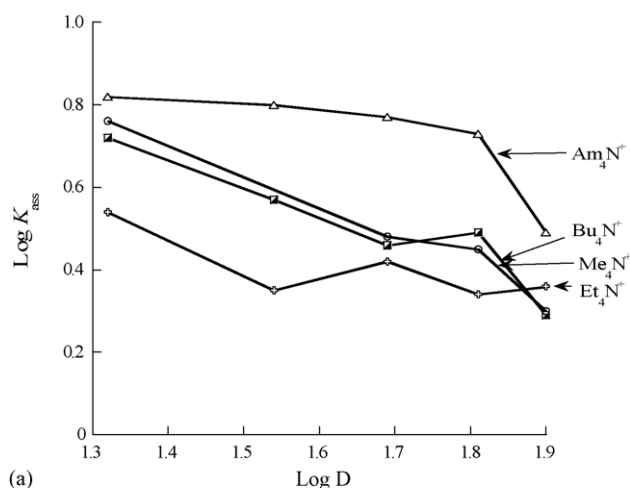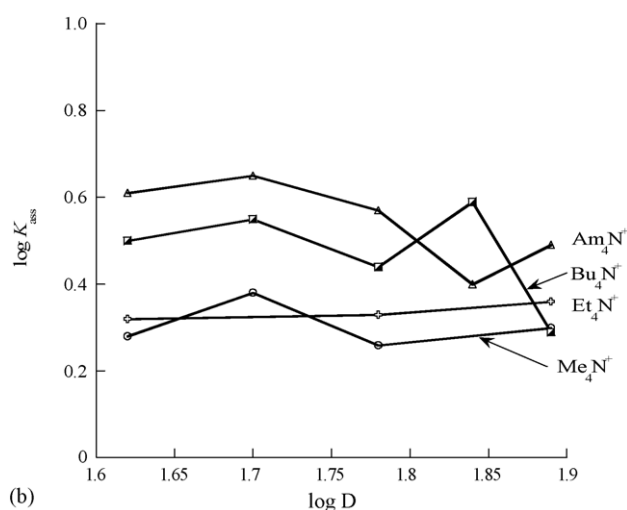
Fig. 5. Effect of dielectric constant on the association constants between nitrate and tetraalkylammonium ions in (a) dioxane–water migrating buffers and (b) ethanol–water migrating buffers.

lary in the determination of the mobilities of the highly mobile anions and subsequently their association constants upon interaction with cations. The addition of dioxane to the migrating buffer in inorganic anion–tetraalkylammonium ions associations produced a higher increase in association constants than a similar addition of ethanol. Contrary to the expectations that use of dioxane as a buffer additive would lead to dramatic increase in K_{ass} by virtue of its ability to greatly alter dielectric constant, the results demonstrated that only moderate increase in K_{ass} could be attained. CZE measurements have shown that the Bjerrum's theory based primarily on electrostatic attraction and dielectric constant is limited in accounting for the association between the inorganic anions and the tetraalkylammonium ions. It is possible that besides the dielectric constants, a combination of other forces such as solvent–ion, inter-particles non-ionic interactions, etc., may be playing a contributory role in the association process.

carried out in aqueous–methanol migrating electrolyte and aqueous–dioxane media would have been accompanied by substantial differences in K_{ass}. Earlier works [17,24,25] have shown that K_{ass} is generally affected by the size of the alkyl group as well as type of media. It can be suggested that association is a complicated process, which is affected by factors other than dielectric friction and electrostatic interactions. It is worth emphasizing that the Bjerrum's theory does not consider other factors like size of associating species, ion–solvent interactions, etc., which seem to influence association significantly.

Acknowledgement

4. Conclusions

Improvements in anion mobility measurements were achieved by employing modified version of the original Williams–Vigh's-triple injection mode-method. The results obtained further show the potential of ordinary silica capil-

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