Analysis of Ion Association Reactions between Monovalent Polycyclic Aromatic Cations and Anions in Aqueous Solution as Studied by Capillary Zone Electrophoresis

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Ion association reactions between monovalent polycyclic aromatic cations and anions have been studied through the mobility change in capillary zone electrophoresis. When the cationic reagent possessing aromatic moieties was used, isomers of aromatic anions were well resolved. Apparent electrophoretic mobility of the anions decreased with an increase in the concentration of the cationic reagent in the migrating solution; the magnitude of the decrease was large when bulkier cations were used. The ion association constants obtained by analyzing the mobility change increased with an increase in the number of aromatic rings of either the pairing cations or the anions. The ion associates formed consist of monovalent cations and anions, and the ion association constant for an ion associate formed between 1-propylbenzo[*f*]quinolinium ion and pyrene-1-carboxylate ion was in the magnitude of $10^{2.74}$ dm^3 mol^{-1}. Such ion associability was interpreted from the contribution of the stacking of aromatic moieties in an aqueous solution, in addition to the increase in the hydrophobicity.

Capillary zone electrophoresis (CZE) is widely used for the separation and determination of ionic substances. The use of ion association reagents has been proved to be effective for resolution improvement of similar compounds, such as positional isomers.[1—3] As for the mechanism of electrophoretic migration in CZE, the electrophoretic mobility of an analyte reflects its dissolved state. By utilizing the electrophoretic mobility, one can perform some reaction analysis by measuring the apparent electrophoretic mobility at various concentrations of the interacting reagent in the migrating solution. Ligand–protein interaction,[4] protein–sugar interaction,[5] binding of a fluorescently labeled molecule to antibody,[6] binding of a neutral compound to dodecyl sulfate micelles,[7] and binding of 2-phenoxypropionic acid enantiomer to β-cyclodextrin[7] have been analyzed so far by the CZE method.

The authors aimed at clarifying the ion–ion interaction of organic compounds in an aqueous solution by the CZE method. Many types of interactions have already been clarified: for example, hydrophobicity of the reagents,[8—10] multipoint interaction,[11] association probability,[12] hydration,[13] stacking of aromatic moieties,[14] and hydrophilicity–electrostatic interactions.[15] Each of such interactions has been proved to be useful for the resolution improvement of positional isomers of the aromatic anions. On the study of the stacking of aromatic moieties, however, only pyridinium type ions have been investigated.[14] In this study, polycyclic cations are investigated to enhance the stacking of aromatic moieties, that is, aromatic–aromatic interaction, between monovalent cations and anions. Contribution of aromatic carbon to the ion associability is also deduced from the ion association constants.

Experimental

Apparatus. An Applied Biosystems 270A-HT Capillary Electrophoresis System equipped with a UV detector was used. A fused silica capillary purchased from GL Sciences was attached to the system; the capillary was 72 cm in total length, 50 cm in effective length from the sample injection point to the UV detector, and had a 50 μm inner diameter. A Hitachi D-2500 Chromato-Integrator was used for recording electropherograms.

Reagents. Migrating buffer components, NaH_2PO_4 and Na_2HPO_4 were purchased from Wako and used without further purification; the pH of the buffer solution was adjusted to 7.0 by mixing these two components. Ion association reagents of 1-ethylpyridinium bromide ($1\text{-EP}^+\cdot Br^-$), 1-ethylquinolinium iodide ($1\text{-EQ}^+\cdot I^-$), tetrabutylammonium bromide ($TBA^+\cdot Br^-$), trimethyloctylammonium chloride ($OTMA^+\cdot Cl^-$), and benzyltrimethylammonium chloride ($BTMA^+\cdot Cl^-$) were purchased from Tokyo Kasei Kogyo. 1-Propylpyridinium bromide ($1\text{-PP}^+\cdot Br^-$) and benzyltriethylammonium chloride ($BTEA^+\cdot Cl^-$) were purchased from Aldrich. 1-Propylquinolinium bromide ($1\text{-PQ}^+\cdot Br^-$), 2-propylisoquinolinium bromide ($2\text{-PiQ}^+\cdot Br^-$), and 1-propylbenzo[*f*]quinolinium bromide ($1\text{-PBfQ}^+\cdot Br^-$) were synthesized from corresponding heterocyclic compounds, such as quinoline, isoquinoline, and benzo[*f*]quinoline, and 1-bromopropane in a refluxed ethanol solution. The products were recrystallized in ether–ethanol solution. Purity of the synthesized reagents was investigated by ion-pair extraction with lauryl sulfate ion (Wako) or dodecylbenzenesulfonate ion (Wako) into chloroform; the purity of each reagent was more than 95% and the reagents are suitable for the aim in the present study. The aromatic cations examined in this study are abbreviated as C^+ and are summarized in Fig. 1.

Carboxylate anions of benzoic acid (BC), 1-naththoic acid (1-NC), 2-naththoic acid (2-NC), anthracene-1-carboxylic acid (1-AC), anthracene-2-carboxylic acid (2-AC), and anthracene-9-carboxylic

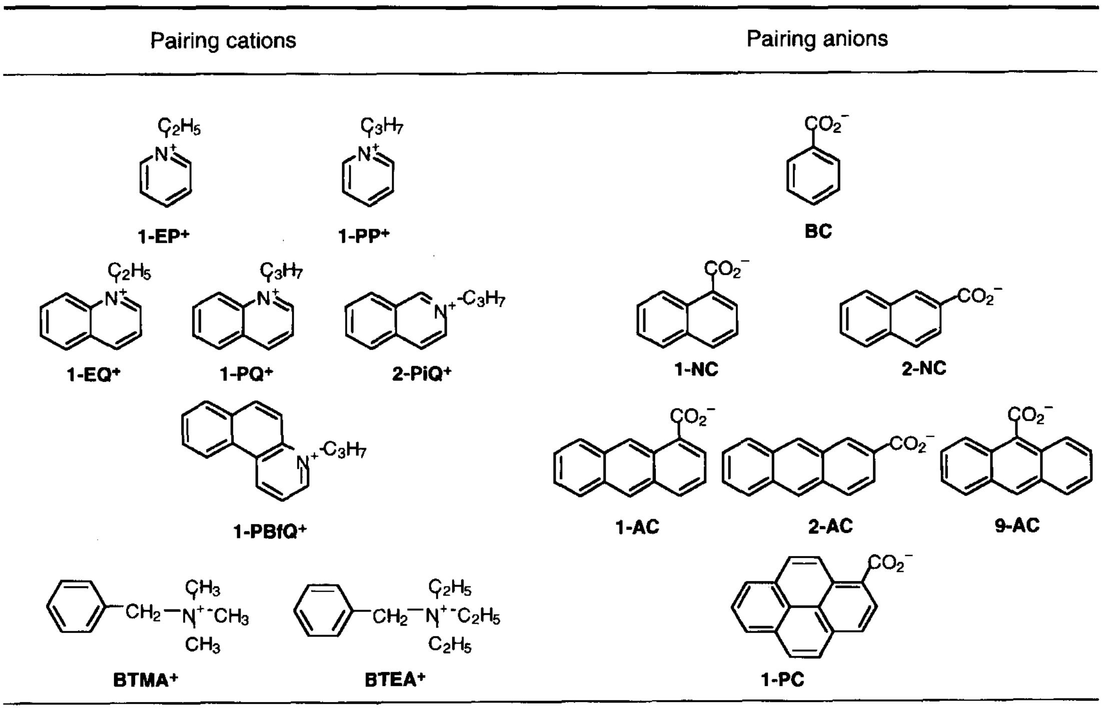

Fig. 1. Aromatic cations and anions examined as ion association reagents.

acid (9-AC) were purchased from Tokyo Kasei Kogyo. Pyrene-1-carboxylic acid was purchased from Aldrich. The carboxylic acids were neutralized with an equivalent amount of NaOH and used as analytes; they are abbreviated as A^- and are also summarized in Fig. 1.

The water used was de-ionized and distilled.

Procedure for the CZE Measurement. A migrating solution containing the pH buffer and an ion association reagent was poured into both a cathodic and an anodic reservoirs, as well as into a capillary. A sample solution containing the analyte anions was injected into the capillary from the anodic end for 3 s hydrodynamically by the vacuum system (injection volume: about 9 nl). Then a voltage of 20 kV was applied and electrophoresis was started. The analyte anions were photometrically detected at 230, 240, or 250 nm. Throughout the experiments, the capillary was held in a thermostated compartment controlled at 35 °C. To evaluate the velocity of the elctroosmotic flow, 3%(v/v) ethanol was added in the sample solutions to detect it. The electrophoretic mobility of the analytes was calculated in an ordinary manner.

Results and Discussion

CZE Separation of Aromatic Anions with Aromatic Cations. Electropherograms separating seven kinds of monovalent aromatic anions with 2-PiQ$^+$ as a pairing cation are shown in Fig. 2. In the absence of the pairing cation, the positional isomers were not resolved {Fig. 2(a)}, whereas they were well resolved in the presence of 7 mM (M = mol dm^{-3}) 2-PiQ$^+$ in the migrating solution {Fig. 2(b)}. The resolution was also improved when other pairing cations were used. Such resolution improvement of the positional isomers was similar to our previous study using some quaternary ammonium ions as ion association reagents.[8,11]

Along with the increase in the number of the aromatic ring of the pairing cation or with the increase in the concentration of the pairing cation, the baseline became noisy, which is attributed to the increased UV-absorptivity of the migrating solution. Therefore, pairing cations possessing quinoline ring or isoquinoline ring are adequate for practical use of the improvements of the resolution and of the detection limit of analytes.

Changes in Apparent Electrophoretic Mobility. The apparent electrophoretic mobility of the aromatic anions, $-\mu'_{\mathrm{ep}}$, was plotted against the concentrations of the pairing cations; three examples using 1-PP$^+$, 2-PiQ$^+$, and 1-PBfQ$^+$ are shown in Fig. 3. Most of the $-\mu'_{\mathrm{ep}}$ values decreased with increasing concentrations of the pairing cations. Such decreases can be attributed to the formation of the ion associates with the pairing cations, which results both in an increase in apparent molecular mass and in a decrease in apparent charge. It is also noticed from Fig. 3 that even lower concentrations of the modifying reagent, ion association reagent in this case, can contribute to the resolution improvement when bulkier cations are used; the concentrations required for sufficient mobility decrease are 1-PP$^+$ > 2-PiQ$^+$ > 1-PBfQ$^+$.

Determination of Ion Association Constants. Ion association constants can be determined by analyzing the change in the electrophoretic mobility in the same manner as our previous studies.[8,12] The ion association reaction in an aqueous solution between a pairing cation, C^+, and a pairing anion, A^-, is written as in. Eq. 1 with its equilibrium constant given by Eq. 2:

$$C^+ + A^- \overset{K_{\mathrm{ass}}}{\rightleftharpoons} C^+\cdot A^- \tag{1}$$

$$K_{\mathrm{ass}} = \frac{[C^+\cdot A^-]}{[C^+][A^-]}, \tag{2}$$

where K_{ass} (dm^3 mol^{-1}; M^{-1}) is an ion association constant. This ion association constant, K_{ass}, can be related to

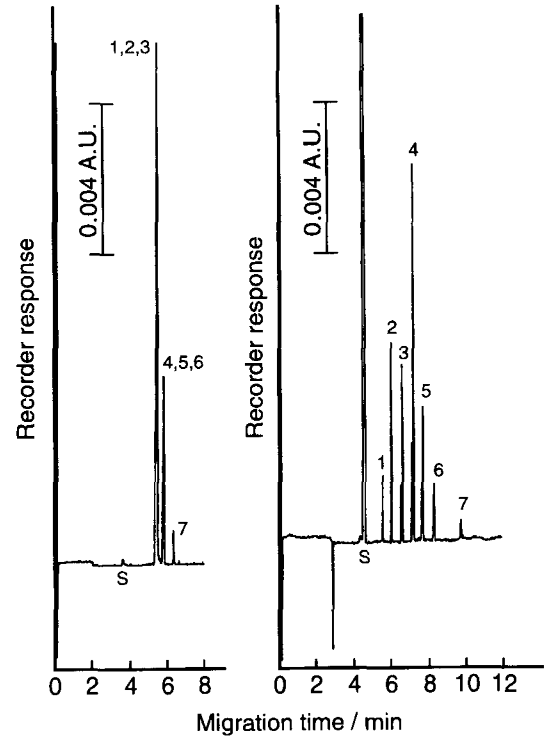

Fig. 2. Typical electropherograms of aromatic anions with 2-PiQ$^+$ as an ion association reagent. CE conditions: applied voltage, 20 kV; detection wavelength, 240 nm; capillary room temperature, 35 °C; injection period, 3 s (injection volume, about 9 nl). Migrating solution: a) 10 mM phosphate buffer (pH 7.0); b) 10 mM phosphate buffer + 7 mM 2-PiQ$^+$·Br$^-$. Sample solution: 1×10^{-5} M anions. Signal identifications: 1, 1-PC; 2, 2-AC; 3, 1-AC; 4, 9-AC; 5, 2-NC; 6, 1-NC; 7, BC. S: EOF (dip peak).

the apparent electrophoretic mobility of the analytes; the relationship between $-\mu'_{ep}$ and K_{ass} can be represented as in Eq. 3:

$$-\mu'_{ep} = \frac{1}{1+K_{ass}[C^+]}(-\mu_{ep}) + \frac{K_{ass}[C^+]}{1+K_{ass}[C^+]}(-\mu_{ep,IA}), \qquad (3)$$

where $-\mu_{ep}$ and $-\mu_{ep,IA}$ are the electrophoretic mobility of a certain analyte anion and its ion associate, respectively. The ion associates investigated in this study are formed between monovalent cations and anions; therefore, the mobility of the ion associate, $-\mu_{ep,IA}$, can be assumed to be zero from the view point of its apparent charge. Ion association constants were determined by a non-linear least-squared method[8,11] based on Eq. 3; the K_{ass} values obtained are summarized in Table 1.

Ion Associability of the Aromatic Anions with Aliphatic Quaternary Ammonium Ions. Ion associability of the aromatic anions with aliphatic quaternary ammonium ions, such as BTMA$^+$, BTEA$^+$, OTMA$^+$, and TBA$^+$, were also investigated from the mobility change; the K_{ass} values obtained are also summarized in Table 1.

Ion associability of aromatic anions with aliphatic quaternary ammonium ions, such as OTMA$^+$ and TBA$^+$, increased with an increase in the bulkiness of the analyte anions. However, the increases in K_{ass} values with such cations are rather small compared to the sizes of increases with the aromatic cations. Increases in K_{ass} values with ion association reagents of BTMA$^+$ and BTEA$^+$ are similar to those of OTMA$^+$ or TBA$^+$. Therefore, such cations as BTMA$^+$ and BTEA$^+$ are considered to be classified among aliphatic quaternary ammonium ions, although they have a benzene ring.

Contribution of Stacking of Aromatic Moieties to Ion Associability. In the previous studies, some factors contributing to ion associability were clarified: hydrophobicity of the reagents,[8—10] multipoint interaction,[11] association probability,[12] hydration,[13] stacking of aromatic moieties,[14] and hydrophilicity–electrostatic interactions.[15] The aim of this study is to enhance the ion associability through stacking of aromatic moieties; some polycyclic ions were also investigated. Ion association constants obtained with monovalent aromatic cations and anions are in the wide range from $10^{0.20}$ M^{-1} for 1-EP$^+$–BC ion associate to $10^{2.74}$ M^{-1} for 1-PBfQ$^+$–1-PC ion associate.

To evaluate the wide range of the K_{ass} values, the ion association constants are plotted against the total carbon number except the functional group (–COO$^-$) of ion associate; the to-

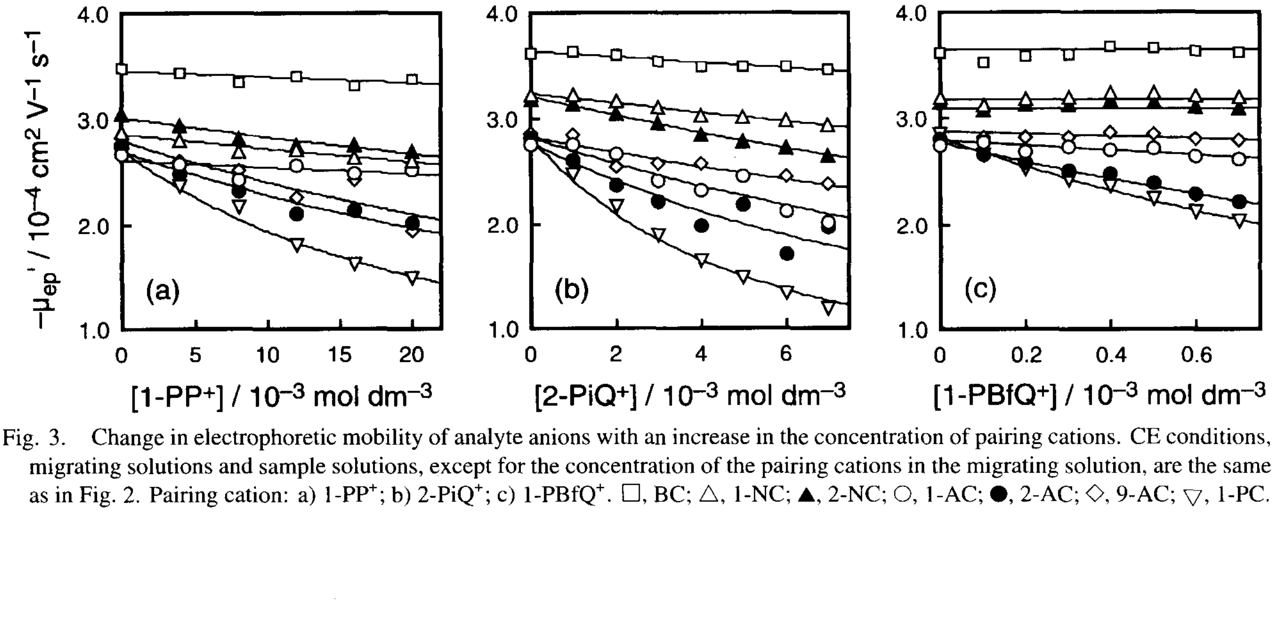

Fig. 3. Change in electrophoretic mobility of analyte anions with an increase in the concentration of pairing cations. CE conditions, migrating solutions and sample solutions, except for the concentration of the pairing cations in the migrating solution, are the same as in Fig. 2. Pairing cation: a) 1-PP$^+$; b) 2-PiQ$^+$; c) 1-PBfQ$^+$. □, BC; △, 1-NC; ▲, 2-NC; ○, 1-AC; ●, 2-AC; ◇, 9-AC; ▽, 1-PC.

Table 1. Ion Association Constants Obtained by the Electrophoretic Method

Pairing	$\log (K_{ass}/\,M^{-1})^{a)}$						
cations	BC	1-NC	2-NC	1-AC	2-AC	9-AC	1-PC
$1\text{-}EP^+$	0.20	0.41±0.42	0.71±0.11	0.37±0.52	0.49±0.30	1.24±0.75	1.54±0.18
$1\text{-}PP^+$	0.24	0.71±0.25	0.80±0.29	0.43	1.27±0.20	1.22±0.60	1.60±0.13
$1\text{-}EQ^+$	0.94±0.07	1.05±0.30	1.48±0.12	1.82±0.12	2.18±0.18	1.43±0.10	2.50±0.38
$1\text{-}PQ^+$	1.09	1.08±0.31	1.46±0.12	1.79±0.09	2.10±0.05	1.44±0.12	2.34±0.10
$2\text{-}PiQ^+$	0.87±0.34	1.17±0.15	1.47±0.08	1.70±0.41	1.89±0.42	1.45±0.52	2.25±0.12
$1\text{-}PBfQ^+$	—$^{b)}$	—$^{b)}$	—$^{b)}$	—$^{b)}$	2.56±0.10	—$^{b)}$	2.74±0.07
$BTMA^+$	0.38±0.20	0.68±0.46	0.86±0.20	0.87±0.23	1.14±0.11	0.91±0.16	1.23±0.07
$BTEA^+$	0.43±0.74	0.86±0.04	1.04±0.25	1.14±0.12	1.41±0.04	1.04±0.12	1.50±0.03
$OTMA^+$	0.60±0.53	0.97±0.21	0.97±0.13	0.81±0.20	1.13±0.10	1.28±0.13	—$^{b)}$
TBA^+	0.46 (0.5±0.3)$^{c)}$	1.06±0.29 (0.87±0.12)$^{c)}$	1.10±0.41 (1.17±0.06)$^{c)}$	0.84±0.09	1.38±0.16	1.30±0.29	1.76±0.15

a) Error: 3σ. If the error is not cited, the deviation of the values is large. b) Not obtained because of the noisy baseline of the electropherograms at sufficient concentrations of $1\text{-}PBfQ^+$. c) Reported values cited from Ref. 12.

tal carbon number used can be considered as one measure of hydrophobicity, as in Fig. 4. It is noticed from Fig. 4 that such ion associates possessing many carbon atoms are more associable than the ones possessing fewer carbon atoms, and that there is favorable relationship ($r = 0.942$) between $\log K_{ass}$ and the total carbon number although other structural effects, such as the position of the charged site, are involved. The slope of the relationship, 0.14, should be the contribution of one carbon atom of the ion associates. The authors previously reported, using similar plots, that the contribution of methylene group of aliphatic ion to $\log K_{ass}$ in an aqueous solution is about 0.06.[8—10] The present result, 0.14, is about twice as large as the previous value. The slope, 0.14, is considered to be the total contribution of carbon on ion associability. The contribution of hydrophobicity related to carbon number, 0.06, is involved in the value of 0.14; therefore, the difference, 0.08, can be attributed to stacking of aromatic moieties, that is aromatic–aromatic interaction, in an aqueous solution.

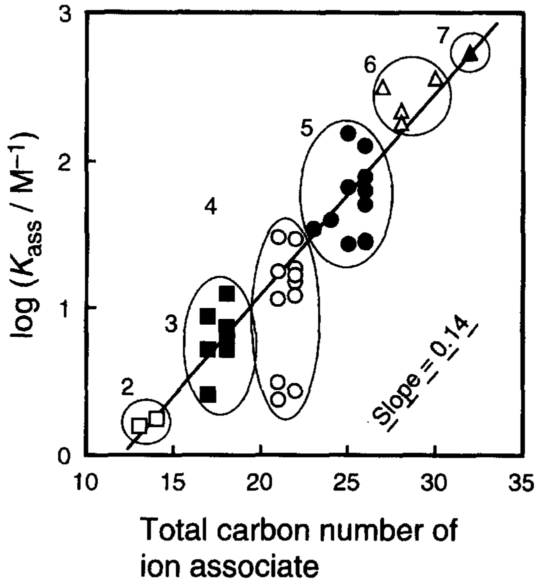

Fig. 4. Plots of $\log K_{ass}$ values against the total carbon number of ion associate. Numbers in the figure show the total aromatic ring of ion associates.

The K_{ass} values are grouped according to the total number of aromatic rings of ion associates, as is shown with the total number of the ring in Fig. 4. There are differences in the composition of ion associates in the total number of aromatic ring; a total number of four for pyridine–anthracene (1+3) and quinoline or isoquinoline–naphthalene (2+2), that of five for pyridine–pyrene (1+4) and quinoline or isoquinoline–anthracene (2+3), and that of six for quinoline or isoquinoline–pyrene (2+4) and benzo[*f*]quinoline–anthracene (3+3). However, the ion association constants are not so much different among such groups. The classification based on the total number of aromatic ring should also support the contribution of aromatic carbon to ion associability.

Comparison of Ion Associability among the Anionic Isomers. The authors have explained that naphthalene anions possessing an anionic group on β-position are more associable with quaternary ammonium ions than those possessing a group on α-position, owing to the basicity.[12] In the case of polycyclic aromatic cations, similar results are obtained; the degree of ion associability is in the order of 2-NC > 1-NC for each aromatic cation. Also in anthracene anions, the degree of ion associability is in the order of 2-AC > 1-AC, and higher associability of an anion possessing an anionic group on 2-position was proved. In the comparison of the three anthracene anions, 9-AC seems least associable among the isomers, although there are some exceptions. Since there are such differences in ion associability, resolution improvement by CZE can be realized using such ion association reagents as we investigated.

Conclusion. In the present study, improvement of the ion associability between monovalent cations and anions was achieved by utilizing stacking of aromatic moieties. The ion associate of interest was formed between organic ions, and its ion association constant was unexpectedly large: $10^{2.74}$

M^{-1} for 1-PBfQ$^+$–1-PC ion associate in an aqueous solution. Resolution improvement of positional isomers of organic anions was also attained in the presence of a cationic reagent in the migrating solutions. The ion association reaction in an aqueous solution thus examined would be promising for designing the separation and determination of ionic organic analytes by capillary zone electrophoresis.

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