Capillary Electrophoretic Characterization of Water-soluble Carbon Nanodots Formed from Glutamic Acid and Boric Acid under Microwave Irradiation

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Water-soluble carbon nanodots (CND) were synthesized under microwave irradiation from glutamic acid or glutamic acid–boric acid mixture. The CNDs were collected in an aqueous solution through size fractionation by centrifugal filtration. The CNDs thus prepared were subjected to characterization by capillary electrophoresis (CE). A peak signal of anionic substance was detected in the electropherogram, and it was found to be a major component of the CNDs. The effective electrophoretic mobility of the major component was almost identical over the pH range between 6.7 and 11.6, suggesting that the functional group of amine or boric acid moiety was not included in the CNDs. The effective electrophoretic mobility decreased at an acidic pH of less than 5, and it was suggested that carboxylate moiety was included in the CNDs. A signal of less-charged CNDs was also detected in the electropherogram, and the CNDs were characterized by a CE format of micellar electrokinetic chromatography. Two or four peaks were detected just after the electroosmotic flow; the less-charged CNDs were thus hydrophilic. The affinity interaction was also examined between the major anionic CNDs and a hydrophobic pairing cation. The peak signal of the major anionic CNDs broadened, and its theoretical number of plates decreased in the presence of tetrabutylammonium ion in the separation buffer. A small portion of the anionic CNDs were a little hydrophobic at different degrees, and their effective electrophoretic mobility decreased by the hydrophobic interaction, resulting in peak broadening of the anionic CNDs.

Keywords Carbon nanodot, characterization, capillary electrophoresis, micellar electrokinetic chromatography, affinity capillary electrophoresis

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Introduction

Recently, various types of carbon clusters have been developed, including fullerene, carbon nanotube, graphene, and carbon nanodot (CND). Among the carbon clusters, CNDs possess efficient and stable photoluminescent characteristics, and CNDs are now of great interest in analytical and biomedical research.[1-3] Unlike metal-based quantum dots, CNDs are less toxic, and biomedical and clinical applications are expected on the basis of their luminescent properties.[4,5] Concerned with analytical applications, some of the present authors (Morita *et al.*) have prepared CNDs from glucose and polyethylene glycol by microwave irradiation, and the CNDs were utilized for the detection of nitrite by quenching of the photoluminescence.[6] Nitrogen-contained and sulfur-contained CNDs were also prepared from amino acids.[7] The doping of N or S to CNDs developed the quantum yield.[7] Graphene quantum dots have

been used as an additive in capillary electrophoresis, and the resolutions of cinnamic acids and its derivatives were developed through aromatic-aromatic interactions.[8]

The CNDs have been characterized by physical and spectroscopic analyses, including SEM, TEM, XRD, IR, Raman, XPS, MALDI-TOF-MS, TGA, *etc*.[9] Chemical characterization and analytical separation were also examined by HPLC and CE. Carbon nanoparticles (CNPs), prepared by the flame of a paraffin oil lamp, were fractionated by anion-exchange HPLC,[10] and the maximum emission wavelength of the CNPs was shorter with such CNPs as with a shorter elution time. Carbon dots derived from graphite nanofiber were also fractionated by anion-exchange HPLC, and the functional groups on their surface were characterized by FT-IR and XPS.[11] Capillary electrophoresis was also used for the characterization of CNDs.[12-16] Nano diamond prepared by detonation was characterized by CE.[12] While a broad peak attributed to the nanodiamond was detected with a tris buffer, a borate buffer accelerated condensation of the nanodiamond.[12] A core-shell fluorescent hybrid was prepared from the core material of iron oxide or citrate-stabilized silver nanoparticles and carbon materials.[13] A CE characterization was examined with magnetite

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carbon dots. While a tight zone was detected at pH 7, a sharp peak followed by a much broader band was detected at pH 12. The broad band suggested dissolution/degradation of the carbon dots into the fragments.[13] Amine/carboxylic acid-functionalized CNDs were prepared from citric acid and 1,2-ethylenediamine, and the CNDs were characterized by CE.[14] The CNDs were found to be composed of positively-charged, negatively-charged, and electrically neutral CNDs.[14] Carbon dots were synthesized from citric acid and 1,2-ethylenediamine by a hydrothermal reaction; the carbon dots were used for the labeling of antibodies.[15]

   In this study, the present authors prepared CNDs from boric acid and glutamic acid by microwave irradiation, as well as from glutamic acid, since the introduction of hetero atoms can develop the quantum yield of CNDs.[7] The prepared CNDs are abbreviated as BG-CND and G-CND, respectively. Although boron-doped CND was synthesized from boric acid and sucrose by Sadhanala and Nanda, only the photoluminescence and CND size were examined.[16] Chemical characterization of the CNDs in aqueous solution is also important from the viewpoints of the surface functional group, the charged status in solution, the reactivity and specificity, and the luminescence property, in addition to the physical and structural information. Along with the photometric investigation of both CNDs prepared in this study, the CNDs were subjected to CE characterization. Three types of the CE format were examined: capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), and affinity capillary electrophoresis (ACE). Anionic and less-charged CNDs were found in BG-CND and G-CND by CE investigations. The CE results suggested that both CNDs are highly hydrophilic on anionic CNDs and less-charged CNDs.

Experimental

Reagents and chemicals

   Boric acid and glutamic acid were purchased from Koso Chemical (Saitama, Japan) and Sigma-Aldrich (St. Louis, MO, USA), respectively. The buffer components used in CE were sodium tetraborate decahydrate (borax, from FUJIFILM Wako Pure Chemical, Osaka, Japan), 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES, Dojindo Laboratories, Kumamoto, Japan), *N*-tris(hydroxymethyl)methyl-3-amino-propanesulfonic acid (TAPS, Dojindo), or *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS, Dojindo). The anionic surfactant of sodium dodecyl sulfate (SDS, from FUJIFILM Wako Pure Chemical) was also used in MEKC. Tetrabutylammonium chloride (TBA⁺Cl⁻) as a hydrophobic ion-pair reagent was from Tokyo Chemical Industry (Tokyo, Japan). Other reagents used were of analytical grade. Water used was purified by Milli-Q Gradient A10 (Merck Millipore Japan, Tokyo, Japan), throughout.

Apparatus

   An Agilent Technologies ³DCE (Agilent Technologies, Waldbronn, Germany) was used as a CE system equipped with a photodiode array detector. A fused-silica capillary was purchased from GL Sciences (Tokyo, Japan); it was attached to the CE system after being cut to a required length. The dimensions of the capillary were 375 μm o.d., 75 μm i.d., 64.5 cm in the total length, and 56 cm in the effective length from the injection end to the detection point. A photometric detection window was made to the capillary by burning a small portion of the polyimide coating. The capillary was held in a cassette cartridge, and the cartridge was set in the CE system. The capillary was flushed daily with 0.1 mol L⁻¹ NaOH for 2 min and with the purified water for 2 min to refresh the inner wall of the capillary.

   A TOA DKK (Tokyo, Japan) pH meter, MH-25G, with a combined glass electrode was used to measure the pH of the separation buffer after being calibrated with standard pH solutions. A commercial microwave oven (600 W, Hitachi, Japan) was used for the synthesis of CNDs, and a TOMY Seiko SRX-201 was used for centrifugation.

Microwave-assisted synthesis of CNDs

   Two types of CNDs were synthesized by microwave irradiation;[6] they were from glutamic acid (G-CND) and from a mixture of boric acid and glutamic acid (BG-CND). The BG-CND was synthesized as follows. Aliquots of 0.3 g boric acid (5 mmol) and 0.7 g glutamic acid (5 mmol) were dissolved in 20 mL of water. The solution was transferred to a PFA pressure vessel (60 mL, Flon Industry, Tokyo, Japan) and irradiated in a microwave oven for 10 min. After heating, the crude product was cooled to room temperature and dispersed in water, followed by centrifugation at 4000 × *g* for 20 min.[7] The supernatant was treated with a molecular-weight cutoff membrane (Amicon Ultra-4, Millipore), as equivalent to <10 kDa, and thus BG-CND dispersed solution was prepared;[7] the cloudy solution of raw CND got clear by the treatments. G-CND was also synthesized in a similar manner to BG-CND, employing a single precursor, 1.0 g of glutamic acid.

Procedure for the CE measurements

   Stock solutions of BG-CND and G-CND were obtained as dark brown. The CND solutions were diluted with water and used for spectral and the CE measurements.   In the CE measurements, the diluted CND solution was injected into the capillary from the anodic end by applying a pressure of 50 mbar for 5 s, after the capillary had been equilibrated with a separation buffer. Each end of the capillary was then dipped in cathodic and anodic buffer vials, and a DC voltage of 15 or 25 kV was applied to the capillary for CE separation. An analyte of CND was photometrically detected at 200 nm. The capillary cassette was thermostated at 25°C by circulating temperature-controlled air during the CE measurements. Electropherograms were recorded and analyzed by a ChemStation software (Agilent Technologies, Ver. B04.02).

Results and Discussion

Spectrum properties of the CNDs

   Prior to CE characterization, the spectrum properties of the CNDs were investigated. The UV-vis absorption maximum was at 313 nm, and the absorption was very intense at shorter wavelengths (Fig. S1, Supporting Information). Since the CE analysis of the CNDs was made by photometric detection, a detection wavelength at 200 nm was chosen.   The photoluminescence (PL) of BG-CND was observed with an emission wavelength at 378 nm when excited at 328 nm (Fig. S1 and Table S1, SI). The quantum yields were measured at low concentrations of the CNDs with the absorbance range below 0.05, where a linear relationship between the absorbance and the PL intensity was held (Fig. S2, SI). The quantum yields of BG-CND and G-CND, thus-determined, were 0.42 and 0.10, respectively (Table S1). Therefore, the introduction of boron to CND was found to be effective for the development of the PL. In comparison, Sadhanala and Nanda synthesized boron-doped
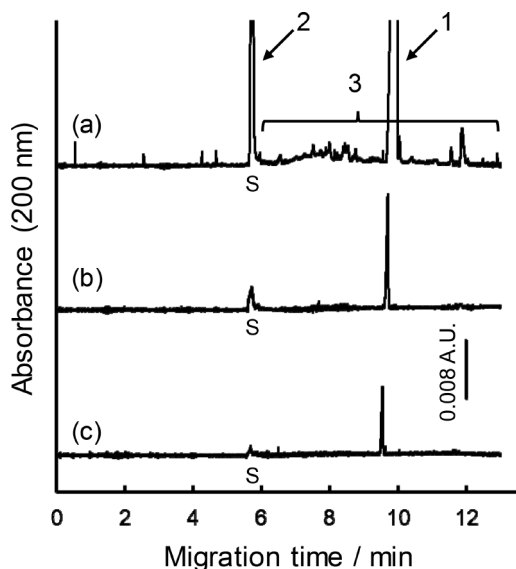
Fig. 1 Electropherograms of the BG-CND at different dilution factors. Separation buffer: 10 mmol L⁻¹ borax (pH 9.3). Dilution factor of the BG-CND solution: (a), 10 fold; (b), 100 fold; (c), 200 fold. CE conditions: applied voltage, 15 kV; detection wavelength, 200 nm; capillary temperature, 25°C; injection period, 50 mbar × 5 s. Signals assigned are: 1, a major component of anionic CNDs; 2, less-charged component(s) of CNDs; 3, various minor components of anionic CNDs. S: solvent (EOF).

CNDs from boric acid and sucrose in a muffle furnace for a different baking time, and the quantum yields of the CNDs were a few percent.[16]

The emission intensity was almost identical in the pH range between 4.5 and 9.5, but decreased under the acidic and alkaline pH conditions (Fig. S3, SI). The effect of the salt concentration was not significant; the emission intensity of BG-CND was almost identical up to 2 mol L⁻¹ NaCl (Fig. S4, SI). These results suggest that the CNDs were well dispersed in the aqueous solutions.

Capillary zone electrophoresis of the CNDs

Capillary zone electrophoresis is a powerful tool to characterize CNDs based on its charge. Hu *et al.* utilized CZE separation to characterize the CND prepared from 1,2-ethylenediamine and citric acid.[14] In this study, we used CZE characterization for the CNDs of BG-CND and G-CND prepared by microwave radiation. Typical electropherograms of BG-CND are shown in Fig. 1 with different dilution factors. A major peak 1 was detected in the electropherograms. The migration time of peak 1 took a longer time than the electroosmotic flow (EOF), and the major component of BG-CND was found to be anionic. A CZE separation was examined with the major peak 1 to verify the signal to be the CNDs (Fig. S5, SI). The major peak 1 was clearly resolved from glutamic acid; it was not glutamic acid, but anionic CNDs. As for the peak shape of peak 1, it is a sharp signal, as is commonly detected with popular ionic molecules. The sharpness of the peak is distinct from the peak shape of CZE or MEKC signals of graphene.[17-19] This result suggests that the major component of BG-CND is rather uniform; the mass/charge ratio is not so much dispersed. Markova *et al.* have detected a similar sharp peak for core-shell CNDs by fluorimetric detection.[13] Hu *et al.* have detected main sharp peaks for amine/carboxylic acid-functionalized CNDs.[14] Thus, a major peak 1 detected in this study would be a major CND

component.

Another distinct peak 2 was also detected at an identical migration time with the EOF; the CNDs were less-charged or electrically neutral. A considerable number of small peaks 3 were also detected in the electropherogram (Fig. 1a) with a raised baseline. A wide variety of anionic CNDs as minor components were also contained in the BG-CND sample.

A similar variation of the CZE signals in the electropherogram was also obtained with G-CND: a major peak of anionic CNDs, a peak of a certain amount of neutral CND, and a considerable number of various anionic CNDs as minor components (Fig. S6, SI).

The major anionic CNDs, a peak 1 in Fig. 1, was characterized through the effective electrophoretic mobility (μ_{eff}). The effective electrophoretic mobility was obtained from the migration times of the anionic CNDs (t_{CND}) and EOF (t_{EOF}), using Eq. (1) in a usual manner,

$$\mu_{eff} = \frac{L_D L_T}{V} \left(\frac{1}{t_{CND}} - \frac{1}{t_{EOF}} \right), \tag{1}$$

where L_T, L_D, and V are the total length of the separation capillary, the effective length of the capillary from the injection end to the detection point, and the applied voltage. The electrophoretic mobility of the anionic CND was examined under neutral to weakly alkaline pH conditions. The result is shown in Fig. 2a. The negative value of μ_{eff} means that the CND was anionic. The μ_{eff} values of the anionic major component of BG-CND and G-CND were almost identical in the pH range examined. When any boric acid group or amine moiety is included in the CNDs, the functional group may dissociate H⁺ at this pH region and the anionic charge of the CNDs would increase. However, the μ_{eff} values are almost identical in the pH range, and the BG-CND does not contain either a boric acid group or the amine moiety. The elemental composition of the precursor of BG-CND (*i.e.* the equimolar mixture of glutamic acid-boric acid) has a nitrogen content of 5.7% and a boron content of 6.2%; therefore, it is expected that nitrogen and boron are contained in the BG-CND as other forms, such as B–C and B–N composites. Figure S7 (SI) depicts the FT-IR spectra of (a) glutamic acid, (b) boric acid and (c) BG-CND. The results revealed that the obtained BG-CND showed vibration bands, indicating the incorporation of B and N in the structure of CNDs, which arose from B–C stretching at 1100 cm⁻¹, C–N/C=N stretching at 1250 cm⁻¹, B–O stretching at 1360 cm⁻¹ and B–N stretching at 1430 cm⁻¹.

The effective electrophoretic mobility of CNDs was also examined under acidic pH conditions. The electroosmotic flow was not fast enough to detect the anionic CNDs of peak 1 under the acidic pH condition, and a pressure-assisted CE was examined; a pressure of 50 mbar was continuously applied to the injection vial of the anodic end during electrophoresis. The results are shown in Fig. 2b. The electrophoretic mobility of CNDs is almost identical between Figs. 2a and 2b, and thus the pressure-assisted CE fairly worked. The effective electrophoretic mobility of CNDs tended to decrease under pH conditions below 5. The decrease in μ_{eff} would be attributed to the protonation of CNDs. The pH range of the protonation suggested that the anionic charge is introduced with the carboxylate moiety. Although the CND is not either uniform or monoprotic acid, as well as it might be positively charged under strongly acidic conditions, the decrease in μ_{eff} has been treated as an acid dissociation constant of a monoprotic acid as a rough approximation. The apparent acid dissociation constant, K_a', could be determined with the monoprotic acid through the change in the μ_{eff} values using[20]
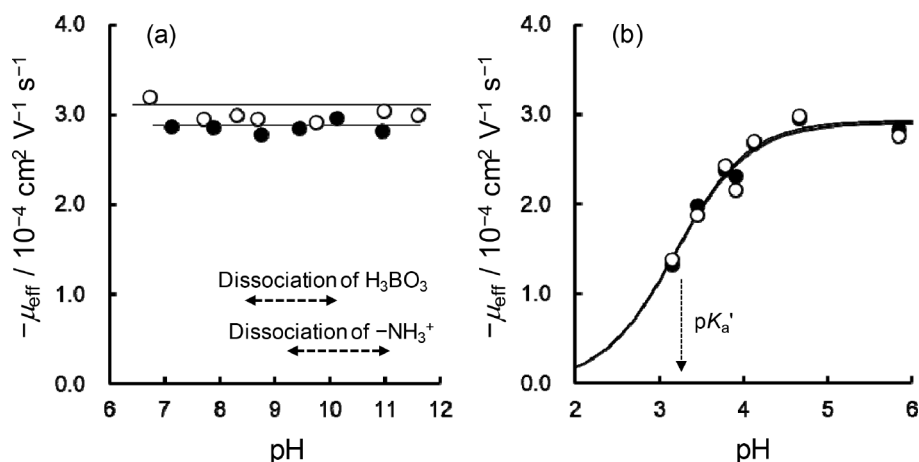
Fig. 2   Effective electrophoretic mobility of the major anionic CNDs at (a) neutral to weakly alkaline pH conditions, and (b) acidic pH conditions under pressure-assisted CE.   Separation buffer: (a) 10 mmol L^{-1} buffer component. Ionic strength was controlled at 10 mmol L^{-1} with NaCl. Buffer component: pH 6.7 – 7.7, HEPES–NaOH; pH 8.3 – 8.7, TAPS–NaOH; pH 9.7 – 11.6, CAPS–NaOH. (b) 20 mmol L^{-1} buffer component. Ionic strength was controlled at 20 mmol L^{-1} with NaCl. Buffer component: pH 3.1 – 3.8, HCOOH–NaOH; pH 3.9 – 4.7, CH$_3$COOH–NaOH; pH 5.8, MES–NaOH. Sample solution: 100 fold diluted BG-CND or 200-fold diluted G-CND solution. CE conditions: applied voltage, 25 kV; detection wavelength, 200 nm; capillary temperature, 25°C; injection period, 50 mbar × 5 s. ○: BG-CND; ●: G-CND. Assisting pressure during CZE (b): 50 mbar.

$$\mu_{eff} = \frac{10^{-pK_a'} \times \mu_{ep}}{10^{-pH} + 10^{-pK_a'}}, \tag{2}$$

where μ_{ep} is the electrophoretic mobility of the anionic CND as one species. Software of R program (Ver. 3.4.1) was used for the analysis.[21]   The pK_a' values with their standard error were determined to be 3.19 ± 0.06 and 3.19 ± 0.09 for BG-CND and G-CND, respectively. The pK_a' values suggest the presence of the carboxylate moiety.

Surfactant assisted capillary zone electrophoresis of CND

Since less-charged CND is detected with BG-CND and G-CND, they are also characterized by capillary electrophoresis. A CE format of MEKC with anionic surfactant is useful for the electrophoretic separation of less-charged substances.[22,23] However, the general MEKC, partition of an analyte to the anionic micelle, would not be applied in characterization of the CNDs. Because CNDs are relatively large particles against small surfactant molecules, the anionic surfactants would be adsorbed on the surface of the CNDs and the less-charged CND would apparently be anionic. The CE separations of the less-charged CNDs in the MEKC format are shown in Fig. 3, where SDS has been used as an anionic surfactant. It can be seen in the electropherograms that a CZE peak that appeared at the migration time of the EOF split into two or four peaks for BG-CND and G-CND, respectively. Since the CNDs are composed of various sizes and shapes, the number of the detected peaks do not correspond to the number of CND groups directly. It is reported that a cluster of Au nanoparticles in different average diameters has been resolved by CZE.[24] Therefore, the resolution of the CND clusters by the MEKC format suggests that the adsorption property of dodecyl sulfate ion (DS$^-$) to CNDs would similarly be categorized into two or four groups. The migration time of the apparently anionic CNDs, however, slightly shifted from the migration time of the EOF. Therefore, the surfactant of hydrophobic DS$^-$ is not likely to be adsorbed on the less-charged CNDs, and the CNDs are highly hydrophilic. In MEKC, the hydrophobicity of the analyte is popularly evaluated through
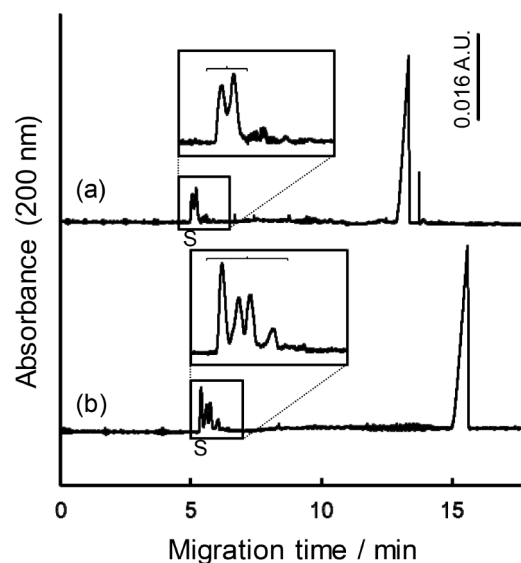

Fig. 3   Electropherograms of the CNDs in the presence of SDS in the separation buffer. Separation buffer: 5 mmol L^{-1} NaH$_2$PO$_4$ + 5 mmol L^{-1} Na$_2$HPO$_4$ (pH 7.0) + 50 mmol L^{-1} SDS. Sample solution: (a), 50-fold diluted BG-CND solution + 50 mmol L^{-1} SDS; (b) 50-fold diluted G-CND solution + 50 mmol L^{-1} SDS. CE conditions: applied voltage, 25 kV; detection wavelength, 200 nm; capillary temperature, 25°C; injection period, 50 mbar × 5 s. S: solvent (EOF).

the capacity factor (k') in the micelle partition.[22,23] Although the generation of the apparently anionic charge is different between the micelle partition and the adsorption of surfactant, as well as the CND clusters are not uniform, the hydrophobicity/hydrophilicity of the CNDs are evaluated through the capacity factor of the less-charged CNDs for a reference. The migration time of the micelle is essential for calculating the capacity factor, and it was measured with Sudan II as a micelle marker. The apparent k' values obtained with the less-charged CNDs are
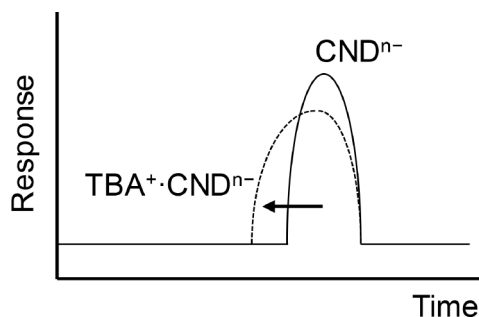
Scheme 1   Schematic illustration of peak broadening of an anionic CND peak. Some portion of the anionic CND interacts with a pairing cation, TBA+, in the separation buffer, resulting the decrease in the number of theoretical plates.

0 and 0.04 for the two peaks of BG-CND and in the range between 0 and 0.19 for the four peaks of G-CND. The small apparent k' values suggest that the less-charged CNDs are highly hydrophilic.

In the presence of SDS in the separation buffer, the effective electrophoretic mobility of anionic CNDs slightly changed (2.91×10^{-4} and 2.95×10^{-4} cm^2 V^{-1} s^{-1} for G-CND and BG-CND, respectively), and the interaction with the surfactant is also weak with the anionic CNDs.

Peak broadening of the anionic CNDs with ion association reaction with TBA+

The CNDs are not a molecule, but a carbon cluster with much variation in their size and form, and a detected CZE peak of the major anionic species includes various types of CNDs. A variety of anionic CND clusters were consecutively detected, and the sole CZE peak of anionic CNDs consequently appeared. Since the major peak is composed of various types of CNDs, the degree of the interaction with any affinity reagent would be different among the anionic CND clusters. When the degree of the interaction is different stepwise, the one signal may split into several peaks, as is seen in less-charged CNDs with an anionic surfactant. Or, any peak fronting/tailing would occur to the CND peak, when a considerable portion of the consecutive CND clusters interacts with an affinity reagent with a different, but consecutive, degree.

One approach concerning the affinity interaction is utilization of the hydrophobic ion-association reaction.[25] The hydrophobic CND of anionic forms would interact with a hydrophobic pairing cation, such as tetrabutylammonium ion (TBA+), during electrophoretic migration in the separation capillary, as is given by an equilibrium reaction:

$$\text{TBA}^+ + \text{CND}^{n-} \rightleftharpoons \text{TBA}^+ \cdot \text{CND}^{n-}. \qquad (3)$$

Net charge of the anionic CND would decrease from $-n$ to $-(n-1)$ due to the equilibrium reaction, and the effective electrophoretic mobility would also decrease by the equilibrium reaction. The decrease in the effective electrophoretic mobility is accompanied in some portion of the consecutive CNDs in the major anionic CZE peak, resulting in the peak broadening of the major peak, as is illustrated in Scheme 1.

The affinity interaction was thus examined with TBA+ by adding it to the separation buffer; the results are shown in Fig. 4. The migration time of an anionic CND peak took longer with increasing concentrations of TBA+Cl−. The longer detection time of the peak is attributed to the slower EOF with higher salt
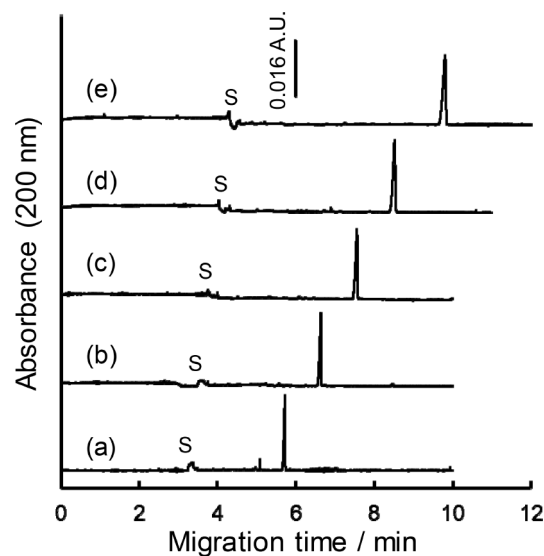

Fig. 4   Electropherograms of the BG-CND in the presence of TBA+Cl− in the separation buffer. Separation buffer: 10 mmol L^{-1} borax (pH 9.3) + TBA+Cl−. Concentration of TBA+Cl−/mmol L^{-1}: (a), 0; (b), 5; (c), 10; (d), 15; (e), 20. Sample solution: 200-fold diluted BG-CND solution. CE conditions: applied voltage, 25 kV; detection wavelength, 200 nm; capillary temperature, 25°C; injection period, 50 mbar × 5 s. S: solvent (EOF).

concentrations of the separation buffer. However, the effective electrophoretic mobility of the anionic BG-CND was almost identical over the TBA+Cl− concentration range examined (Fig. S8, SI). Therefore, the major components in peak 1 are less associable with TBA+, and they are hydrophilic. A sole peak of the anionic CNDs in the presence of TBA+ also suggests that the difference in the little hydrophobicity of the anionic CNDs is consecutive, in contrast to the adsorption property of DS− to the less-charged CNDs.

The peak of the anionic BG-CND, however, was broadened at higher TBA+Cl− concentrations. The peak broadening was quantitatively evaluated through the number of theoretical plates (N). The number of theoretical plates was calculated from the migration time of the peak and the peak width in an ordinary manner. The results are shown in Fig. 5. The number of theoretical plates decreased with both BG-CND and G-CND. Since the effective electrophoretic mobility of the anionic CNDs was not affected by the addition of TBA+Cl− or NaCl, diffusion improvement of the CNDs by the temperature increase would not be applied. The peak broadening would thus be attributed to the affinity interaction of the part of BG-CND with TBA+ in consecutive difference, as is illustrated in Scheme 1.

When a small portion of the slightly hydrophobic CNDs interact with TBA+, the effective electrophoretic mobility of the TBA+–CND^{n-} associates would be reduced according to the degree of the interaction, and they migrate relatively faster than CND^{n-} in the presence of EOF. Thus, the consecutive TBA+–CND^{n-} associates would be detected as fronting signal to the CND peak, as illustrated in Scheme 1. The fronting of the peak was evaluated through the peak symmetry factor.[26] When the peak is highly symmetric, the value is close to 1, while fronting peaks lead to a peak symmetry factor >1, and tailing peaks lead to a peak symmetry factor <1. The results of the peak symmetry factor are also shown in Fig. 5. The peak symmetry factor tended to increase with increasing concentrations of TBA+, suggesting that the peak fronting is more intense.
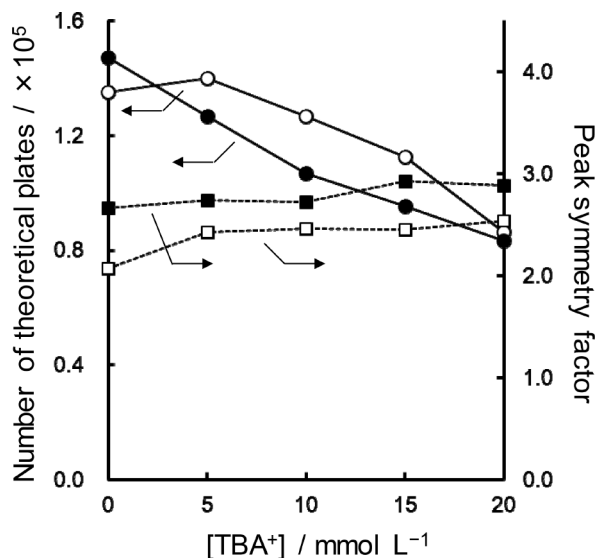
Fig. 5   Changes in the number of theoretical plates of the anionic CNDs, as well as the peak symmetry factor of the CNDs, with increasing concentrations of TBA$^+$. Symbols: ○ and □, BG-CND; ● and ■, G-CND. Separation buffers, sample solution, and CE conditions are the same as in Fig. 4.

Therefore, some portion of the anionic CNDs are hydrophobic with a different degree, but consecutive. When NaCl was used as an affinity reagent instead of TBA$^+$Cl$^-$, the decrease in N and the peak fronting was slight, and the affinity interaction of Na$^+$ with any CND was less than TBA$^+$.

Conclusions

In this study, two types of water-soluble CNDs, BG-CND and G-CND, were prepared by microwave irradiation in a wet process from boric acid and glutamic acid, as well as from glutamic acid. The thus-prepared CNDs were characterized by CE. A major anionic CND peak and a less-charged CND peak were detected by CZE with a considerable number of small peaks of anionic CNDs. The effective electrophoretic mobility of the major anionic CND was almost identical over a neutral to alkaline pH range, and either the amine or boric acid moiety was not included in the anionic CND. Meanwhile, the effective electrophoretic mobility decreased at an acidic pH range, suggesting the functional groups of the carboxylate moiety. The anionic surfactant of SDS was examined to give a negative charge to the less-charged CNDs, but the surfactant was not likely adsorbed on the CNDs and the less-charged CNDs were hydrophilic. When affinity interactions were examined with hydrophobic TBA$^+$, the effective electrophoretic mobility of the anionic CND slightly changed. However, the CZE peak of the anionic CND was broadened and peak fronting was observed, suggesting that a small portion of the anionic CND were somewhat hydrophobic with consecutive degree. In this way, capillary electrophoresis is useful for the characterization of water-soluble CNDs in an aqueous solution in the formats of CZE, MEKC, and affinity CE.

Acknowledgements

Supporting Information

Conditions of the spectrophotometric measurements and the results are shown in Figs. S1 to S4, as well as in Fig. S7. Spectral data are given in Table S1. CZE separations of BG-CND and G-CND are shown in Figs. S5, S6, and S8. These materials are available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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