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Short communication

Analysis of active chemical species generated by electrolysis using non-aqueous capillary electrophoresis
Detection of the anion radical and the divalent anion of tetracyanoquinodimethane

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Abstract

We have investigated detection of the anion radical and the divalent anion of tetracyanoquinodimethane (TCNQ) by acetonitrile–CE under anaerobic conditions. With electrolysis at a potential of 0.0 V (vs. Ag/AgCl), an acetonitrile solution of TCNQ turned green, characteristic of the TCNQ anion radical ($TCNQ^{-}$). Only one peak of the anionic compound was observed in CE of the electrolysis solution and it should be that of $TCNQ^{-}$. Then, the electrolysis potential was shifted to −0.8 V expected to be sufficient potential for the further reduction of $TCNQ^{-}$, and the solution turned almost colourless. In CE analysis of the latter solution, another anionic component possessing a larger electrophoretic mobility than that of $TCNQ^{-}$ was detected, and it was decomposed immediately under aerobic conditions. This product was strongly suggested to be the divalent anion of TCNQ, and the present method would contribute notably to detection of the unstable species.
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1. Introduction

Active chemical species like radicals have been always investigated as interesting targets in chemistry, namely as intermediates of valuable reactions, useful reagents for synthesis, functional molecules and so on. In practical studies, serious efforts have been made to keep these usually unstable species from decomposing during their identification, structural analysis and study of their physical features. Thus, investigators have used special techniques to stabilize the active species and to minimize the intervals between generation and detection. An example of the former is the use of matrices at ultra-low temperatures, and those of the latter are several flow analysis methods [1–5]. Detection methods are always required to be highly selective, such as that of electron-spin-resonance (ESR) spectroscopy in radical analysis, to identify such unstable species in situ, because isolation of an active species for detailed analysis will be difficult or practically impossible in many cases.

On the other hand, if we can remove active species from coexisting compounds, we will be able to analyze the targets more certainly and easily. Makino et al. reported high-performance liquid chromatography (HPLC) separation of several spin-adducts (radicals) followed by on-line ESR spectro-

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scopic analysis of the separated components and they successfully characterized each structure of the radicals [6,7]. It would have been difficult to identify each of these radicals by ESR only without the HPLC separation, although the detected radicals of the spin-adducts were exceptionally stable enough to survive in a column during the separation. Furthermore, separation of an active species means transfer of the species from the matrix where it generated to a separation medium. There would be quenchers coexisting in the generation matrix, and the separation medium can be designed as a more comfortable living medium compared with the generation matrix. Therefore, separation may contribute to extend lifetime of the species practically and thus, to achieve the detection of the species.

In our previous work, a *para*-benzoquinone anion radical generated by electrolysis was detected using capillary electrophoresis (CE) with an acetonitrile system, and hydrogen-bonding activity of the species was studied [8]. This would be the first separation of a relatively unstable radical, indicating the usefulness of this method in analysis of an active species. We have employed CE as the tool in our previous and the present work for the following reasons. (1) Various solvents including pure non-aqueous solvents are available for the separation media. Similar media to those for generation of targets can be used for their separation analysis. On the other hand, targets can be more stable in non-aqueous systems than in aqueous systems or vice versa. We can select the suitable media according to the situation. (2) Short analysis time can be achieved if needed. (3) There are no stationary phases, which can be undesirable for stabilization of the active species. (4) Capillaries are closed systems which are useful for preventing external scavengers such as oxygen molecules. (5) The migration behaviour of targets will give us information about the physical and chemical features of analytes, namely hydrodynamic sizes, charges, and abilities of interactions.

In this work, we have tried to detect other species generated by electrolysis: the radical anion and the divalent anion of tetracyanoquinodimethane. Especially, the divalent anion would be a troublesome target as a highly reduced form which is subject to decomposition.

2. Experimental

2.1. Materials

Tetracyanoquinodimethane (TCNQ) was purchased from Nacalai Tesque and purified by recrystallization from acetone. Tetrabutylammonium perchlorate (TBAP) as an electrolyte for both electrolysis and electrophoresis was prepared as reported in a previous paper [9]. TCNQ and TBAP were dried in a high vacuum for 1 h before use. Acetonitrile of spectra-grade was obtained from Nacalai Tesque and dried for more than 2 days with molecular sieves 3A and then distilled. All other chemicals were of analytical grade.

2.2. Apparatus

All electrophoretic measurements were performed on a laboratory-made system. A Matsusada HCZE-30 PNO high-voltage power supply (Siga, Japan), a Jasco CE-970 detector (Tokyo, Japan), and a Jasco 807-IT integrator were employed. A DB-1 coated capillary, was used in all CE measurements and it was obtained from J&W Science (CA, USA). The capillary has an internal diameter of 50 μm and an outer diameter of 365 μm. Its total length was 50 cm where the effective length was 25 cm.

Controlled-potential electrolysis was performed in a bulk electrolysis cell of 100 ml with a three-electrode system consisting of a carbon electrode, an Ag/AgCl reference electrode and a Pt-wire counter electrode [3]. A Hokuto Denko HA-501 potentiostat (Tokyo, Japan) was used for electrolysis. A Shimadzu SPD-M10A photodiode array detector with an optical path length of 1.0 cm equipped with a personal computer and a Shimadzu LC-1-AD pump was used to measure the absorption spectra [3].

2.3. Procedure

2.3.1. Electrolysis

The initial concentrations of TCNQ in sample solutions were $4.2 \cdot 10^{-4}$ and $3.9 \cdot 10^{-5}$ mol/l for electrophoretic analysis and spectral observation, respectively, in 100 ml of acetonitrile containing TBAP as a supporting electrolyte. The concentra-

tions of TBAP were 0.01 and 0.1 mol/l in the former and the latter solutions, respectively. First, electrolysis potential was set at 0.0 V (vs. Ag/AgCl) for generation of the TCNQ anion radical ($TCNQ^-$). After the analysis of $TCNQ^-$, potential was shifted to −0.8 V for further reduction of $TCNQ^-$. These potentials are sufficiently negative to reduce TCNQ and $TCNQ^-$ (see below) [10]. N_2 gas was sufficiently bubbled into the sample solution before electrolysis to remove dissolved oxygen molecules. During the electrophoresis and the spectral observation, electrolysis of the solution being stirred and bubbled with N_2 gas was continued to prevent reduced forms of TCNQ from reoxidation.

2.3.2. Electrophoresis

At the beginning of daily measurements, capillaries were rinsed with pure acetonitrile for 10 min with suction. The capillaries were further rinsed with distilled acetonitrile for 1 min and then filled with a running solution before each run. As the running solution, acetonitrile containing 0.025 mol/l TBAP was prepared in an atmosphere of N_2 and was bubbled with N_2 gas for 30 s before each run. Sample solutions were injected by a hydrodynamic method on the cathodic side at a height of 10 cm for 15 s. Applied potential was fixed at −10 kV where running current was 11 μA. Detection wavelengths were set at 410 nm for the TCNQ anion radical or 230 nm for all anionic products. We also measured absorption spectra of peak components using a wavelength-scanning function of the CE-970 detector. Peak assignment was made based on migration times, consideration of absorption spectral information, and co-injection of an electrolysis solution containing "relatively stable" $TCNQ^-$.

3. Results and discussion

Fig. 1 indicates spectral change of a TCNQ sample solution during electrolysis. First, the potential was set at 0.0 V (vs. Ag/AgCl) and a component having an absorption maximum around 410 nm was generated from TCNQ as shown in (A). This should be the TCNQ anion radical ($TCNQ^-$), judging from reported reduction potentials and exist-

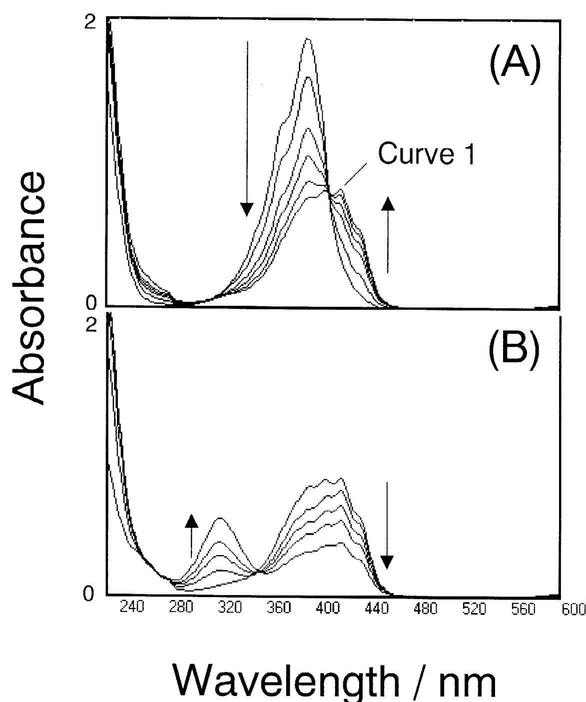

Fig. 1. Spectral change of TCNQ in acetonitrile containing 0.1 mol/l TBAP with electrolysis at (A) 0.0 V (vs. Ag/AgCl) and (B) −0.8 V. Initial concentration of TCNQ is $3.9 \cdot 10^{-5}$ mol/l.

ence of equal absorption points meaning only one product was generated in this reduction $\{E_1^0=0.17$ and $E_2^0=-0.37$ V vs. SCE for redox couples ($TCNQ/TCNQ^-$) and ($TCNQ^-/TCNQ^{-2}$), respectively [10]}. Electrolysis was performed at 0.0 V sufficiently until the electrolysis current became nearly zero meaning electrolysis was completed, and the last spectrum in (A) (curve 1) will be the spectrum of $TCNQ^-$ only. Next, the potential for electrolysis was shifted to −0.8 V, and the obtained spectra are shown in (B). The spectra indicate formation of another component possessing an absorption maximum at 320 nm. This product will be $TCNQ^{2-}$ according to the reduction potentials mentioned above and the equal absorption points shown in (B) also.

Fig. 2 shows electropherograms of the TCNQ sample solution before electrolysis (A), electrolyzed at 0.0 V (B) and at –0.8 V (C and D). Detection wavelengths were fixed at 410 nm (A and B) for

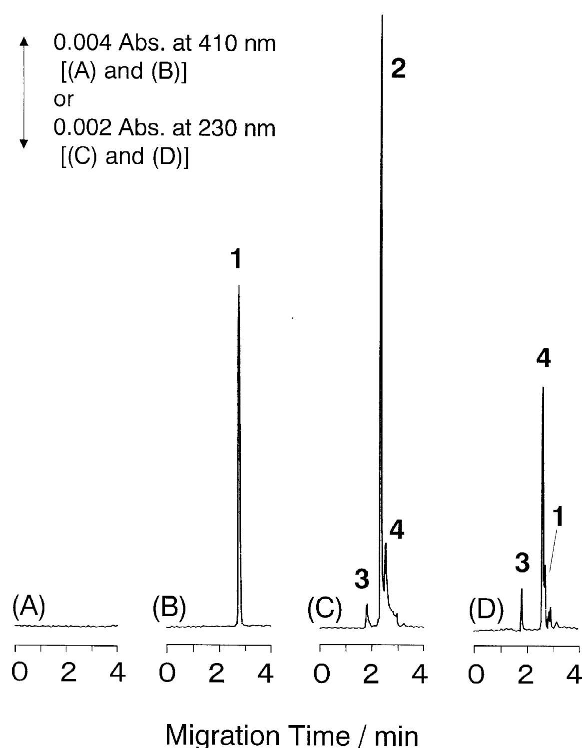

Fig. 2. Electropherograms of the TCNQ sample solution before electrolysis (A), electrolyzed at 0.0 V (B) and at −0.8 V (C and D). Conditions: running solutions, acetonitrile containing 0.01 mol/l TBAP; capillary tube: 500 mm (effective length 250 mm)× 0.05 mm I.D.; applied voltage, −10 kV (current, 11 μA); injection, a hydrodynamic method on the cathodic side at a height of 10 cm for 15 s; detection wavelength, 410 nm (A and B) or 230 nm (C and D). Peak assignment: 1, $TCNQ^{-}$; 2, $TCNQ^{2-}$; 3, unknown; 4, a main decomposition product from $TCNQ^{2-}$.

detection of $TCNQ^{-}$ or 230 nm (C and D) for detection of all anionic products originating from TCNQ. In this separation system, anionic components are detected on the anodic side, because the electroosmotic flow was minimal when DB-1 coated capillaries were used [8,9,11,14]. No peaks were seen in the electropherogram of the sample solution before electrolysis Fig. 2A. With electrolysis at 0.0 V, the sample solution turned green, and this colour will be characteristic of $TCNQ^{-}$ judging from Fig. 1A. Fig. 2B shows the electropherogram of this solution and only peak 1 was observed. Fig. 3 illustrates absorption spectra of the peak components in Fig. 2, and the component of peak 1 had an absorption maximum at nearly 410 nm. This is consistent with

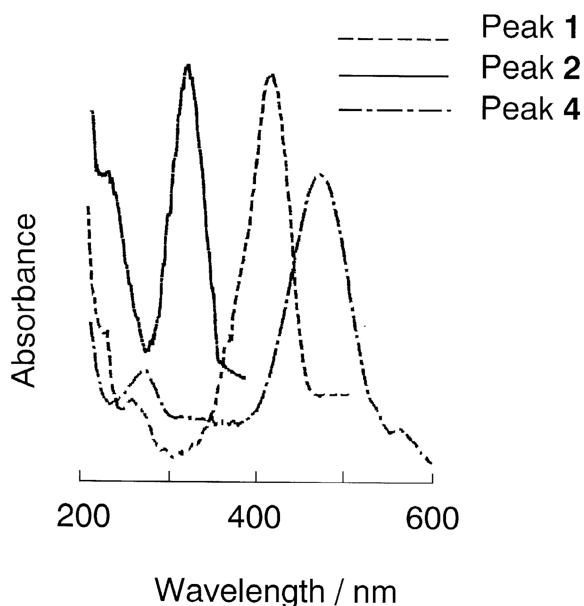

Fig. 3. Absorption spectra of the peak components in Fig. 2.

the spectrum of $TCNQ^{-}$ in Fig. 1A. Thus, the component of peak 1 should be $TCNQ^{-}$.

The potential was changed to −0.8 V and the sample solution turned almost colourless. This observation also is consistent with the spectral change in Fig. 1B. After the electrolysis current became almost zero, the sample solution was injected for CE analysis. Fig. 2C is an electropherogram of the sample solution injected immediately after the sampling. The component of peak 2 had a larger electrophoretic mobility than that of $TCNQ^{-}$ (see below) and had an absorption maximum at nearly 320 nm as shown in Fig. 3. This is consistent with the spectral observation for $TCNQ^{2-}$ in Fig. 1B and thus, the component of peak 2 should be $TCNQ^{2-}$. Fig. 2D is an electropherogram of the sample solution injected after standing for 5 min from sampling under an aerobic condition. The difference between the two electropherograms (C and D) will be interpreted as follows. $TCNQ^{2-}$ would exist as the main component in the sample solution on electrolysis under the anaerobic condition and then change immediately into the component of peak 4 predominantly during the standing under the aerobic condition without being electrolyzed. The sample solution turned dark brown upon standing for 5 min.

This colour change would be attributable to formation of the component of peak 4 having an absorption maximum at nearly 470 nm as shown in Fig. 3. Product analysis of the component of peak 4 and another unknown product of peak 3 is in progress.

The electrophoretic mobilities of $TCNQ^{-}$ and $TCNQ^{2-}$ were estimated as 45.9 and 52.0 cm^{2} min^{-1} kV^{-1}, respectively, assuming electroosmotic mobility was negligible in this separation system [8,9,11]. This difference in electrophoretic mobilities of the two anions may seem to be slight, taking into consideration that $TCNQ^{2-}$ is a divalent anion while $TCNQ^{-}$ is a mono anion. This may be interpreted by strength of interaction of the two anions with tetrabutylammmonium ion (TBA^{+}) of the counter cation [12,13] and/or strength of solvation toward the two anions. The interaction with TBA^{+}, if any, should have serious effects on effective charges and hydrodynamic radii of the anions. It will be reasonable that divalent anions interacted with counter cations much more strongly than mono anions do. Additionally, non-aqueous media are greatly advantageous to such electrostatic interactions, compared with aqueous media [9,11–23]. On the other hand, it will be also reasonable that divalent anions are solvated by acetonitrile more strongly than mono anions are [24], and it would cause larger increase in the hydrodynamic radius for $TCNQ^{2-}$ compared with $TCNQ^{-}$ in this case. Anyway, the difference in strength of the interaction with TBA^{+} and strength of solvation could reduce the difference in electrophoretic mobilities between $TCNQ^{2-}$ and $TCNQ^{-}$.

It should be mentioned that the closed separation system in a capillary would contribute greatly towards preventing decomposition of $TCNQ^{2-}$ during separation. Solubility of O_2 in acetonitrile is considerably high compared with that in water. If there had been a certain opening toward air in the separation system, O_2 would penetrate into the acetonitrile solution and oxidative decomposition of $TCNQ^{2-}$ will occur immediately during separation. As shown in Fig. 2, $TCNQ^{2-}$ in the sample solution after standing for 5 min under an aerobic condition was decomposed completely, while most of $TCNQ^{2-}$ in the capillary existed during its separation time of 3 min. The closed system will be a great advantage of CE for separation and detection of active species.

In conclusion, we have performed separation analysis of $TCNQ^{-}$ and $TCNQ^{2-}$ generated with electrolysis in acetonitrile using acetonitrile–CE. Especially, $TCNQ^{2-}$ was successfully detected by the present method. On the other hand, the present method needs transfer of sample solutions from the generation system to the separation system and this can be a serious drawback for detection of more unstable species. In our preliminary experiments, the reduced species generated by electrolysis could not live during the transfer from the electrolysis cell to the capillary (unpublished data). This would be attributable to very slight exposure to O_2 and such transfer must facilitate exposure to external compounds causing decomposition of the active species. The time for transfer also should be greatly decreased. Improvement of our system from these points of view is in progress.

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