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Short communication

Specific analyte–electrolyte additive interaction in transient isotachophoresis–capillary electrophoresis

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Abstract

While cationic surfactants are usually included in the separation electrolyte to reverse the electroosmotic flow, the presence of the surfactant may also offer a means of capillary electrophoresis (CE) separation selectivity control over the anionic analytes, especially those that are prone to ion-pairing interaction. For one such analyte anion, iodide, the formation of several ion-association/partition products with cetyltrimethylammonium chloride (CTAC) was first discovered when optimizing (decelerating) iodide mobility (in order to achieve effective transient isotachophoretic stacking). At comparatively high concentrations of iodide (≥0.01 m*M*) and the cationic surfactant well above the critical micelle concentration (25 m*M*), an additional peak due to interactions with the CTAC micelle was recorded, with a UV absorption spectrum fairly different from those of both interacting partners and also the iodide–monomeric surfactant ion pair. Never observed before in normal CE mode, this phenomenon is believed to have occurred due to the enrichment effect of the initial isotachophoresis state.

Keywords: Isotachophoresis–electrophoresis; Iodide; Surfactants; Inorganic anions; Cetyltrimethylammonium chloride

1. Introduction

The mainstream methodology for capillary electrophoresis (CE) separation of inorganic anions implies a co-electroosmotic migration of the analytes under the action of an anodic electroosmotic flow (EOF). Suitable EOF modifiers, providing an EOF in the desired direction, include long-chain alkyltrimethylammonium salts, e.g. cetyltrimethylammonium bromide or chloride (CTAC). As evidenced for the first time by Jones and Jandik [1], another objective for the use of an EOF modifier in the separation electrolyte (SE) may be the variations in selectivity that can be achieved by changing the modifier concentration. The authors ascertained the observed selectivity changes as being due to ion pairing between certain analyte anions and the EOF modifier. Since then, cationic surfactants have often been effectively used to enhance the CE resolution of anions by virtue of ion-pairing effects [2–5] (see also Ref. [6] for an overview and Ref. [7] for multiple effects). Likewise, manipulation of the separation selectivity for inorganic ions can be attained by

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applying micellar electrolytes involving the same cationic surfactants but at concentrations above the critical micelle concentration [8]. Micellar electrokinetic chromatography using alkyltrimethylammonium micelles has been utilized to produce separations that offer additional selectivity for anions differing in ion-pair formation ability and ionic/partition interaction with the micelles, including the highly polarizable/hydrophobic iodide ion [8–10].

In our parallel research [11], CTAC surfactant was employed to selectively alter (decelerate) the effective mobility of iodide. There are two main reasons why the iodide migration velocity should be delayed. Firstly, when the separation of iodide from highly saline matrices is a challenge, its signal response can be deteriorated by a system signal of the matrix chloride [12] and/or an overlapping effect from the adjacent bromide that may occur at a high excess in such samples [12–14] (e.g., in seawater the concentration of bromide exceeds that of iodide by a factor of 1500). Secondly, the moderate detection limits attained using conventional UV detectors rule out the possibility of determining the trace levels at which iodide exists in the majority of practical samples. This makes it highly desirable for the application of a suitable sample-enrichment procedure to be combined on-line with the following CE step. Transient isotachophoresis (ITP) provides such an option, even for loaded samples [11], but with the condition that iodide can be retarded so as to migrate at a greater distance behind the matrix chloride (performing the role of a leading anion). When modifying the iodide mobility to meet this requirement, we observed the appearance of a second peak for iodide, which might be attributed to a specific electrostatic interaction with, or partition into, the CTAC micelle. In the present contribution, the origin of this finding and the underlying analyte–electrolyte chemistry were investigated in relation to the accompanying sample stacking by transient ITP.

2. Experimental

2.1. Instrumentation and procedure

A CAPI-3100 (Otsuka Electronics, Osaka, Japan) CE system equipped with a diode-array detector was used. Fused-silica capillaries of 100 cm (87.7 cm to the detector)×75 mm I.D. were obtained from Otsuka Electronics. A new capillary was flushed with 0.1 *M* NaOH for 10 min and then with water and SE for 30 min. Before each run, the capillary was purged with the SE for 3 min. A negative voltage of −4 kV was applied for separations. The detection wavelength was set as specified below. Samples and the terminating electrolyte solution were introduced by negative pressure (0.5 kg/cm^2). All experiments were conducted at 25 °C (capillary chamber).

2.2. Reagents and solutions

All standards and electrolytes were prepared with analytical-reagent-grade chemicals in deionized water obtained by a Millipore Labo system (Tokyo, Japan). Stock solutions of iodide and nitrate (final concentration 1 m*M* each) were prepared from their sodium salts. Mixed anion solutions of low concentrations (0.1, 0.01 and 0.001 m*M*) in 0.5 *M* NaCl were prepared by serial dilutions of these standards. CTAC and 2-(*N*-morpholino)ethanesulfonic acid (MES) were purchased from Nacalai Tesque (Kyoto, Japan).

The SE consisted of 500 m*M* sodium chloride and 25 m*M* CTAC. The pH was adjusted to 2.4 with 1 *M* HCl. The terminating electrolyte was 500 m*M* MES at pH 6.0 (NaOH). Electrolytes were filtered through a 0.5-mm membrane filter prior to use.

3. Results and discussion

As shown in our recent study [11], a combination of the sample chloride ($79.1\cdot10^{-5}$ cm^2 V^{-1} s^{-1}) and a rather slow migrating MES ($28.0\cdot10^{-5}$ cm^2 V^{-1} s^{-1}), introduced as a separate, terminating electrolyte zone (both ITP supporting ions were at 500 m*M* concentration), works well to accommodate I^- into the ITP migration range. When the effective mobility of iodide was decelerated down to $32.9\cdot10^{-5}$ cm^2 V^{-1} s^{-1} by applying the CTAC-containing SE, this analyte anion produced a nicely stacked peak (even when injected from a seawater sample). In contrast, a rather weakly interacting with CTAC nitrate, the mobility of which remained at the margin of the

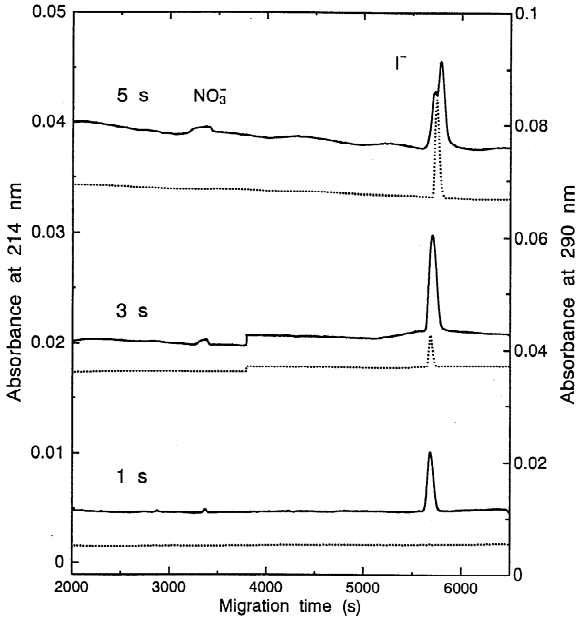

Fig. 1. The 10^{-5} *M* iodide signal at various sample loading times. Detection, 214 nm (solid-line electropherograms), 290 nm (dotted-line electropherograms). Sample, 0.01 m*M* I^- and 0.01 m*M* NO_3^- in 0.5 *M* NaCl. Other conditions, see Experimental.

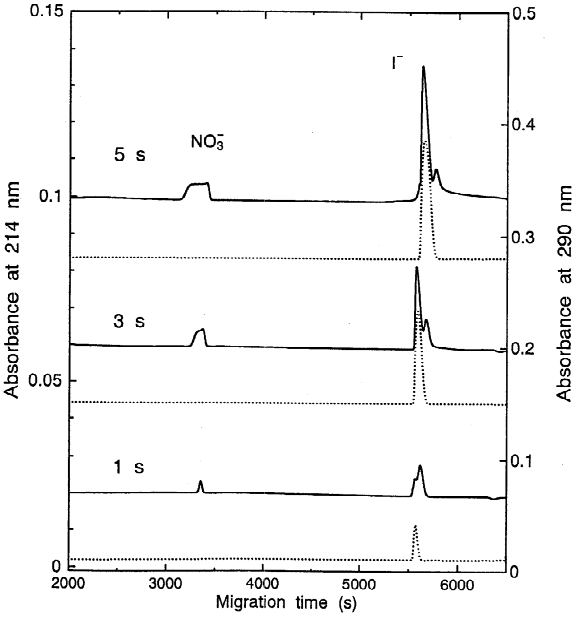

Fig. 2. The 10^{-4} *M* iodide response at various sample loading times. Sample, 0.1 m*M* I^- and 0.1 m*M* NO_3^- in 0.5 *M* NaCl. Other conditions as for Fig. 1.

ionic range used for ITP focussing, exhibited a noticeable peak-destacking effect (Figs. 1 and 2). Such transient ITP–CE conditions yielded an improvement in detectability (in other words, an increase in the actual iodide concentration in the migrating zone) of more than two orders of magnitude compared to the common CE mode and thereby provided the possibility of quantifying iodide at the low-μg/l level [11].

At the higher sample concentrations examined in the present study, iodide exhibited quite different migration behavior. Following an increase in loading of the 0.01 m*M* I^- sample, the peak due to iodide exhibited broadening and even became split at the longest sampling time tested (see the corresponding solid-line trace in Fig. 1; the data for intermediate 2 and 4 s injections are not shown here and hereafter for simplicity). Electropherograms recorded at 290 nm, on the other hand, displayed only a single sharp peak, the position of which coincided with the peak shoulder observed at a sampling time of 5 s and 214 nm. The double-peak observation turned out to be yet more evident at increased iodide concentrations in the sample. The response from I^- introduced into the capillary at 0.1 m*M* was greatly affected by the amount loaded, as indicated by the detection of two partly co-migrating peaks at the 1 s sampling time (Fig. 2). It is noteworthy that, while the first peak rose gradually with increasing sampling time, variations in the sample load exerted almost no influence on the height of the second peak. Again, only one peak, belonging to a faster-migrating iodide species, was detected at 290 nm.

Fig. 3 and Table 1 summarize, respectively, the UV absorption spectra and the wavelengths for the absorption maxima of separated iodide species recorded on-line using the diode-array detector. For the sake of clarity, note that CTAC does not absorb at $\lambda > 210$ nm and the iodide ion has maximum absorbance at 226 nm. As can be concluded from analysis of the spectral data, at lower sample loads iodide migrated as the only species with λ_{max} at 230 nm. Loading of a greater amount of iodide (as a product of concentration and sampling time) favored the transformation of this species into two forms, presumably differently associated with the micelle, with the larger the amount, the more pronounced the

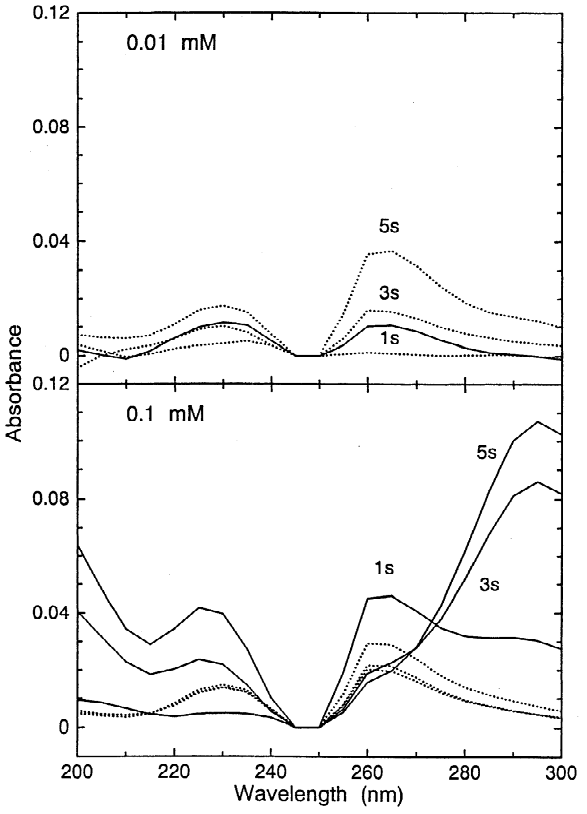

Fig. 3. UV absorption spectra of iodide species. Solid line for peak 1 (for the 0.01 m*M* sample this peak was observed only at the 5 s injection) and dotted line for peak 2.

bathochromic shift effects for both association products (see Fig. 3). Furthermore, as the sample load increased, the relative content of these species changed in favor of the fast-migrating species, with λ_{max} around 290 nm at higher loads. A comparison of the migration and absorption patterns brings one to the conclusion of the co-existence of three iodide-related species, the number of which thus corresponds to the number of maxima in the UV spectra (see Table 1).

Table 1
Maximum absorbance wavelengths for iodide peaks at different concentrations and sample introduction times

Concentration (m*M*)	Sampling time (s)	λ_{max} (nm)	
		Peak 1	Peak 2
0.01	1	–[a]	230
	5	230, 260	230, 260[b]
0.1	1	260	230, 260[b]
	5	290	230, 260[b]

[a] Not detected.
[b] The largest absorbance maximum.

For a more explicit interpretation of the above results, one should take into account that, in a given cationic micellar system, iodide inclines both to a strong interaction with the CTAC surfactant and to be retained in its micelle due to partitioning effects [8]. However, unlike in common micellar electrokinetic chromatography, under the acidic SE conditions employed, virtually no dynamic modification of the capillary wall occurred. Consequently, the electroosmotic flow was not reversed, but rather halted. As a result, the observed migration of the micelle, as well as of that part of the surfactant remaining in the monomeric form, was towards the cathode at the injection end of the capillary. This explains why iodide, predominately existing in an ion-association equilibrium with the monomeric CTAC at lower concentrations (the maximum absorption of the ion associate at 230 nm; peak 2 in the table), has a longer migration time. Micellar interactions, though stronger in magnitude (as witnessed by larger bathochromic shifts), resulted in a weaker retardation of iodide. Differences in the binding behavior of iodide may be ascribed to two types of interaction with the micelle: (i) the formation of an ion-associate species, with I^- distributed on the surface of the positively charged micelle ($\lambda_{max} = 260$ nm) and (ii) partition of iodide (in the form of the hydrophobic I^-–CTAC ion pair) into the core of the micelle (the resultant species has λ_{max} at 290 nm). Obviously, these two iodide species exist in an equilibrium which is too fast for their resolution. On the other hand, the occurrence of separate peaks for the iodide forms, associated with monomeric CTA^+ and bound to micelles, can be explained by a rather slow kinetics of iodide exchange between the different species (as was judiciously pointed out by one of the referees). However, on the basis of the present experiments we can only speculate which form delays the species distribution.

As a concluding remark, the high sample preconcentration factors achieved by transient ITP should be emphasized as a prerequisite for the formation of strongly associated iodide species. For this reason, such interactions have not been observed

in similar migrating systems without a transient ITP stage [8–10] or in our experiments when the concentration of iodide was reduced to 0.001 m*M*. Presumably, similar phenomena could take place in the case of other anionic species with a strong affinity for cationic surfactants (or possibly analogous electrolyte additives). Turning to the analytical output of this study, the transient ITP–CE determination of iodide, occurring in a sample at moderate concentrations, is recommended to be carried out at lower energy wavelengths (see dotted traces in Figs. 1 and 2).

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