System Peaks of Cyclodextrins in Capillary Electrophoresis

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Summary

Using a running buffer containing cyclodextrins (CDs) and 2-[*N*-cyclohexylamino]-ethanesulfonic acid (CHES), positive system peaks were observed in the analysis of a ganglioside mixture by CE-UV. These system peaks were related to CDs in the running buffer because these peaks were also detected when a plug of solution devoid of CDs but having the same CHES concentration and pH as the running buffer was injected. Neutral CDs were separated owing to the formation of inclusion complexes with the anionic CHES ion. One possible explanation for the positive system peaks is that the anionic CD-CHES inclusion complex is displaced by co-ions with higher UV absorptivity.

1 Introduction

In capillary electrophoresis (CE) with UV absorbance detection, it is well known that, in many cases, the electropherogram shows not only the peaks corresponding to injected analytes but also some additional peaks that do not belong to any injected analytes. The term "system peaks" was introduced to denote these peaks. Over the last several years, a number of papers have addressed this topic [1–9]. Among the approaches, the migration vacancy provides a simple model to explain the origin of the system peaks [6]. It has been shown that, on using a background electrolyte containing more co-ionic species, the injection of a sample without these species can produce the corresponding number of migration vacancies, *i.e.*, system peaks.

In the analysis of a ganglioside mixture by CE, several electrolyte systems were evaluated for the separation of underivatized gangliosides. It was found that the separation efficiency could be enhanced if cyclodextrins were added into the running buffer. However, in addition to peaks corresponding to the gangliosides, several system peaks were also observed (**Figure 1**). The number of the system peaks equals the number of cyclodextrins in the running buffer. The intensities of the system peaks were proportional to the concentration of cyclodextrins (CDs) in the running buffer.

Unlike other reports, system peaks observed in this system originate from neutral compounds (CDs) not ionic compound. The system peaks are demonstrated and one possible cause of these peaks is discussed in this report.

2 Experimental

2.1 Chemicals and Reagents

2-[*N*-Cyclohexylamino]-ethanesulfonic acid (CHES), α-cyclodextrin, β-cyclodextrin, and γ-cyclodextrin were purchased from Sigma (St. Louis, MO). Sodium hydroxide was purchased from J.T. Baker (Phillipsburg, NJ). Deionized (18 MΩ) water (Milli-Q water system, Millipore, Bedford, MA) was used in the preparation of all the solutions. A sample of the ganglioside mixture (0.5 mg/mL) was prepared in the deionized water. The buffer used for characterization the system peaks is 8.25 mM α-CD, 8.25 mM β-CD, 8.25 mM γ-CD, and 30 mM CHES at pH = 9.3.

2.2 Instrumentation

CE system was made in-house using a CZE 1000R (Spellman, Plainview, NY, USA) power supply. CE columns were fused-silica capillary (Polymicro Technologies, Phoenix, AZ) 50-mm i.d., 375-mm o.d. and 100 cm length (90 cm to detector). A small area of the polyimide coating was burned off to form a window for UV detection. On column detection was perform on an UV detector (UV-C Rainin, Emeryville, CA, USA) operated at wavelength of 200 nm. Before each run, the capillary column was washed with 1 M NaOH following with 0.1 M NaOH, water, and running buffer. The capillary was then equilibrated with running buffer for 20 min. The UV spectra of the system peaks were obtained by on line coupling of CE with a scanning UV spectrophotometer (UV-3000, Thermo Separation Products, San Jose, CA, USA).

3 Results and Discussion

3.1 System Peaks in CE-UV

Gangliosides were analyzed by CE-UV using a running buffer containing CHES and α-, β-, γ-CDs. In addition to the gangliosides peaks, three system peaks and an electroosmotic vacancy were observed (Figure 1). In order to characterize these system peaks, a plug of solution devoid of CDs but having the same CHES concentration and pH as the running buffer was injected. Three system peaks and one electroosmotic vacancy were detected as shown in **Figure 2**. Because the

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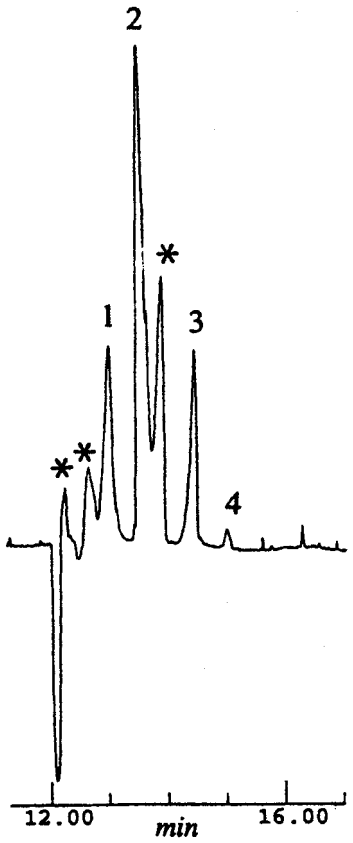

Figure 1. Electropherogram of gangliosides (GD1a GD1b GT1b GQ1b GM1) mixture with 20mM CHES, 8.25 mM α-CD, 8.25 mM β-CD and 8.25 mM γ-CD pH = 9.3 as the running buffer. (*: system peaks,1:GM1, 2:GD1a/b, 3:GT1b, 4:GQ1b).

only difference between the sample plug and the running buffer is the CDs, it is believed that these peaks are more likely due to the CDs in the running buffer. To confirm this speculation, a spiking procedure based on the enrichment of the sample with CDs was used. The electropherograms obtained by injection of a sample plug containing different combinations of CDs are shown in **Figure 3**. The results showed that the three system peaks a, b, c were related to the of γ-, α-, and β-CDs respectively. Injection of a plug containing CDs of different concentrations indicated that the absorbance was inversely proportional to the concentration of CDs (data not shown). A negative peak was observed when the concentration of the CD in the sample plug was greater than that in the running buffer. The absorbance of EOF peak I was proportional to the total concentration of injected CDs. A positive EOF peak was observed when the total concentration of injected CDs was lower than the CDs in the BGE. A negative EOF peak was observed when the total concentration of injected CDs was higher than the CDs in the BGE.

3.2 Separation of CDs in CHES Buffer

CDs are toroidally shaped polysaccharides formed from 6, 7, and 8 glucopyranose units [10] and uncharged except at very high pH. Therefore, electrophoretic separation would not normally be expected at the pH used in this study (pH = 9.3). Separation of CDs has been reported by the formation of inclusion complexes with the anionic 2-anilinonaphthalene-6-sulfonic acid in the running buffer [11]. This mechanism is also capable of explaining the separation of CDs in this system. CD molecules form inclusion complexes with CHES in

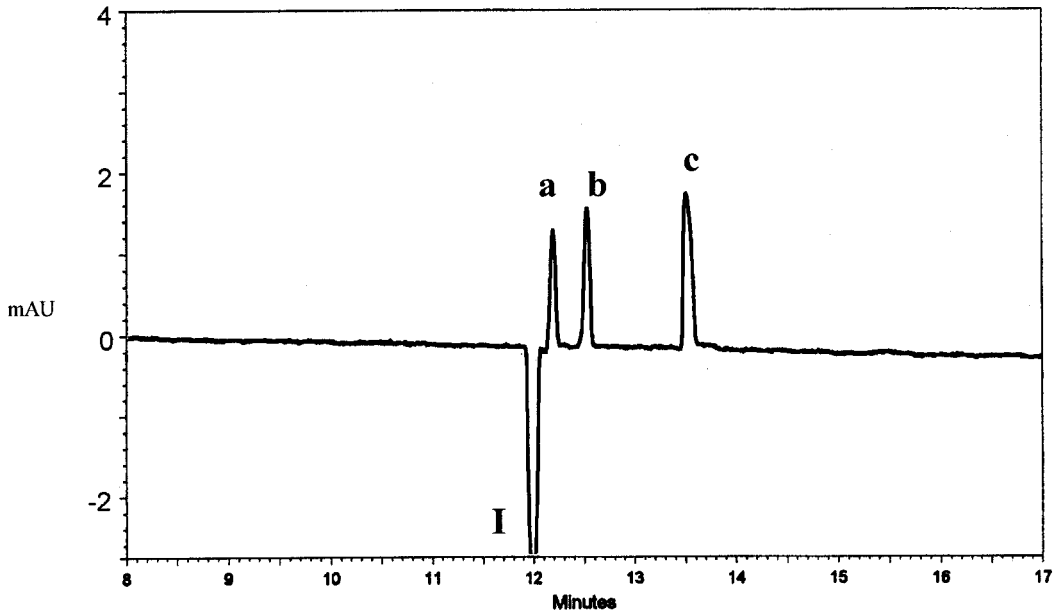

Figure 2. Electropherogram for injection of 30 mM CHES at pH = 9.3 with running buffer containing 30 mM CHES, 8.25 mM α-CD, 8.25 mM β-CD and 8.25 mM γ-CD at pH = 9.3.

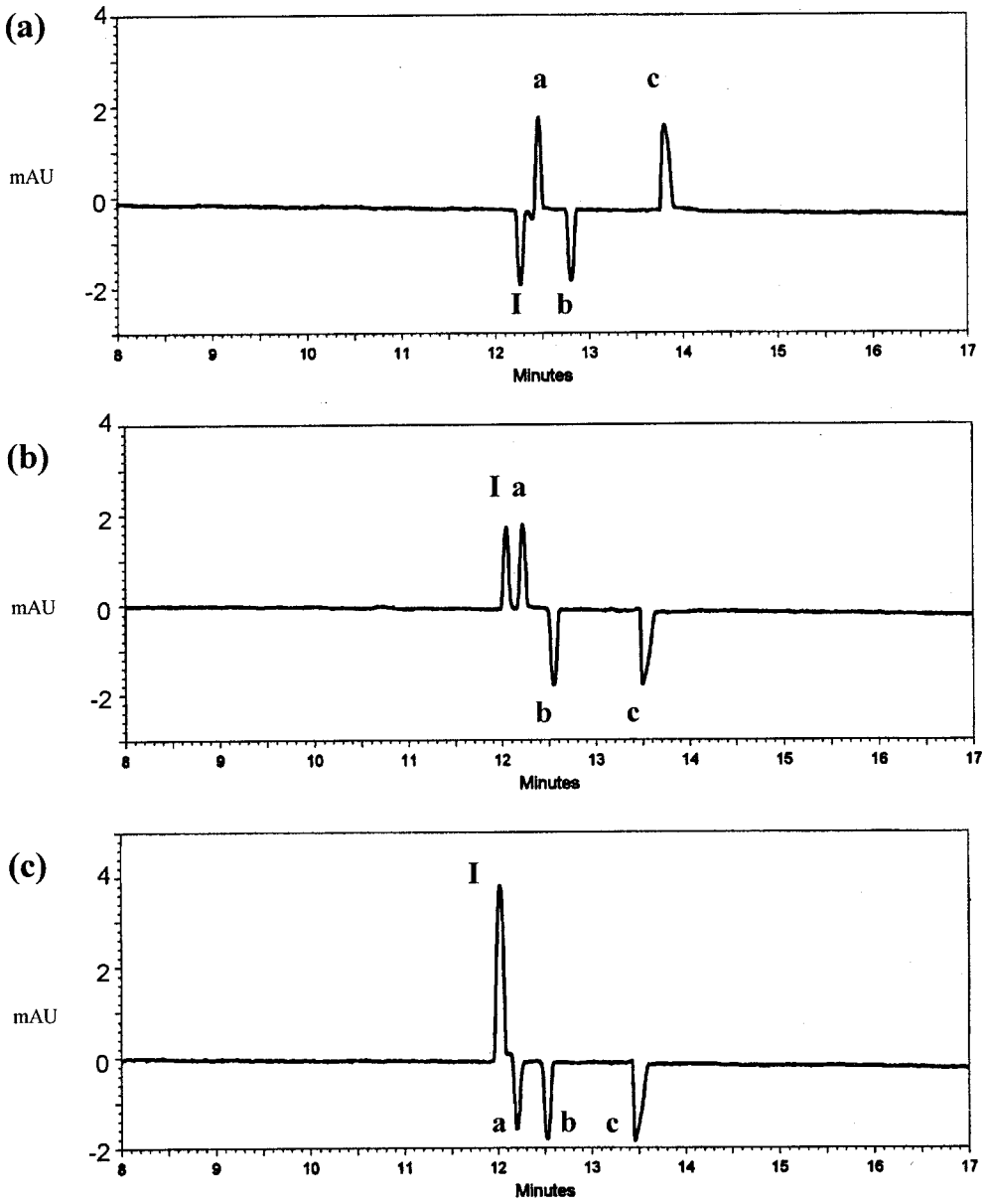

Figure 3. Electropherograms for injection of (a) 16.5 mM α-CD (b) 16.5 mM α-CD and 16.5 mM β-CD (c) 16.5 mM α-CD, 16.5 mM β-CD and 16.5 mM γ-CD dissolved in 30 mM CHES at pH = 9.3, other conditions are the same as in Figure 2.

the running buffer. Since CHES is an anionic species in the running buffer, the CD-CHES complexes migrate under the electrical field. The elution order of CDs depends on the stability of the CD-CHES complex. It is very likely that CHES preferentially forms an inclusion complex with β-CD and therefore has the longest retention time.

3.3 Occurrence of the CD System Peaks

In vacancy CE, before the injection of a plug of background electrolyte (BGE), the electrode vessels and the separation column are filled with a solution in which the sample (analyte) is dissolved in the BGE. The electropherogram is almost identical to that of a conventional CE experiment except that the analyte is detected as a negative peak. This is because the UV-absorbing analyte in the BGE is displaced by a low UV-absorbing coion. Our system is very similar to vacancy CE [12], if CDs (more correctly, CD-CHES complexes) are considered as the analytes. However, unlike the conventional zone vacancy CE, positive peaks instead of negative peaks were detected when a plug of BGE was injected. One possible explanation is that, unlike the conventional vacancy CE,

the CDs (more correctly, CD-CHES complexes) in the background electrolyte are displaced by a co-ion with higher UV absorptivity.

Since CD-CHES complexes are anionic in the running buffer, the ion which displaces the CD-CHES complex, should be an anion too. There are three anions that can displace the CD-CHES; these ions are CHES, carbonate (from atmospheric CO_2) and hydroxide. Because the UV-absorptivity of CHES and CD-CHES are similar (data not shown), a significant change in UV absorbance is not expected if the CD-CHES complexes are displaced by the CHES ions. Besides CHES ion, both carbonate and hydroxide can displace CD-CHES complexes. To produce positive peaks, the co-ion must have higher UV-absorptivity than the CD-CHES complex. A UV-absorbance study indicated that the UV-absorptivity of hydroxide was much higher than that of CHES (data not shown). Due to the high absorptivity of hydroxide, a positive peak will be observed if the CD-CHES is displaced with hydroxide ion. Beside hydroxide, carbonate (from atmospheric CO_2) may also displace the CD-CHES complex. There are two items of evidence indicating that hydroxide is the more likely candidate. First, at the wavelength used in this study, the molar absorptivity of carbonate is about ten times less than that of hydroxide [13]. Secondly, the UV spectra of the system peaks (obtained by on-line coupling of CE with a scanning UV spectrophotometer) were similar to the spectrum of hydroxide.

4 Conclusion

Unlike other reports, system peaks observed in this system originate from neutral compounds (CDs) not ionic compound. Positive system peaks were observed if a plug of BGE devoid of CDs was injected into the separation column. The exact mechanism for the formation of the system peaks is not clear. One possible explanation is that the CD-CHES anion is displaced by the high UV absorptivity hydroxide ion. Finally, this approach also provides a method for the separation of CDs of different size.

Acknowledgments

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