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Note

Use of non-ionic and zwitterionic surfactants to enhance selectivity in high-performance capillary electrophoresis

An apparent micellar electrokinetic capillary chromatography mechanism

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High-performance capillary electrophoresis (HPCE) has initially attracted much attention as a high-efficiency separation technique. Within separations science, the impact of efficiency and selectivity in achieving resolution is often discussed. As a newly emerging technique, one question surrouding open tubular HPCE is whether or not it is an inherently high-efficiency, low-selectivity process.

Terabe *et al.*[1] have demonstrated that selectivity can be added to open tubular HPCE by using ionic surfactants in the micellar electrokinetic capillary chromatography (MECC) format. Here, at or above the critical micelle concentration (CMC) of the surfactant, micelles act as a pseudophase. Based on the difference in partitioning of various solute molecules between the pseudophase and bulk solvent and the difference in mobility of the electroosmotic flow (EOF) of the bulk solvent *vs.* the mobility of the ionic pseudophase, separation is achieved.

What we have observed in our laboratory is the enhancement of separation of closely related species by the use of non-ionic and zwitterionic surfactants. These surfactants are only effective in achieving separations at or above the CMC. Examples are given and an explanation for the observation is proposed.

EXPERIMENTAL

The apparatus used has previously been described[2]. The capillaries used were aryl pentafluoro (APF) deactivated capillaries[3] with inner diameters ranging from 20 to 50 μm.

The octyl glucoside and CHAPS ({3-[3-(chloroamidopropyl) dimethylammonio]-1-propanesulfonate}) used were obtained from Calbiochem (San Diego, CA, U.S.A.). For the octyl glucoside, both the Ultrol and regular grade were evaluated. In this particular application, the regular grade was found to be suitable. Desipramine and nortriptyline were purchased from Sigma (St. Louis, MO, U.S.A.). The peptides were obtained from Peninsula Labs. (Belmont, CA, U.S.A.).

Specific conditions for each experiment are noted in the figure legends.

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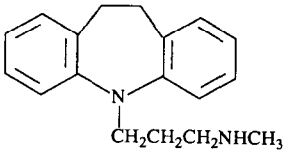
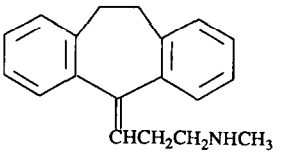
RESULTS AND DISCUSSION

Two specific examples of selectivity enhancement by using non- or zwitterionic surfactants will be used:

(1) The separation of the tricyclic antidepressant desipramine from a closely related substance, nortriptyline.



Desipramine



Nortriptyline

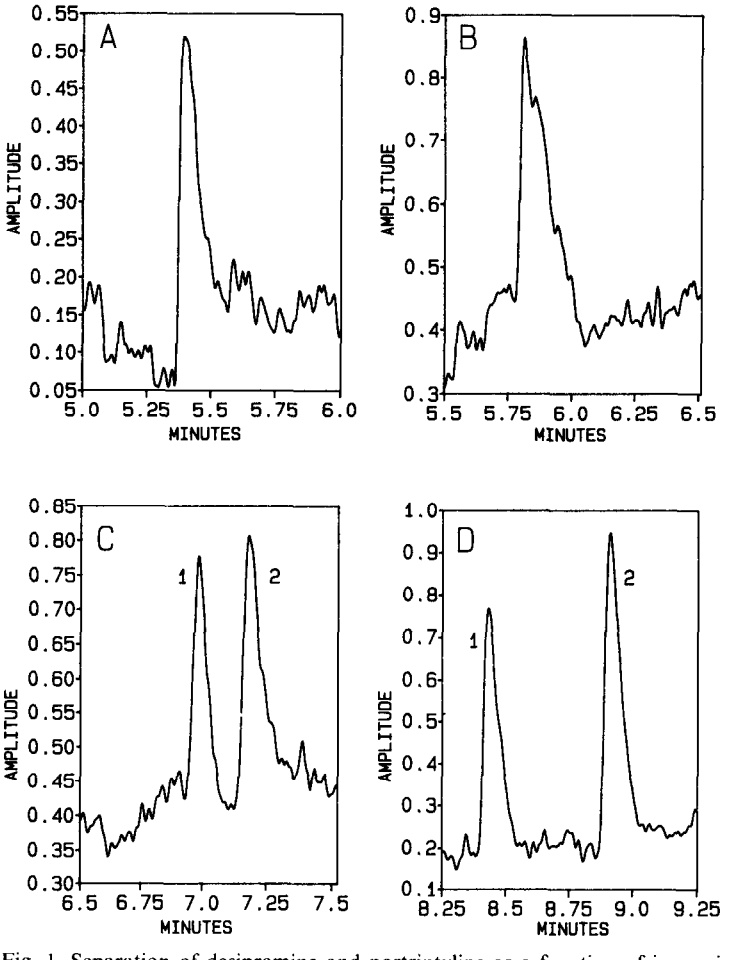


Fig. 1. Separation of desipramine and nortriptyline as a function of increasing amounts of octyl β-D-glucoside in 67 mM phosphate (100 mM Na^+) pH 7.0 buffer. (A) No octyl glucoside; (B) 10 mM octyl glucoside; (C) 20 mM octyl glucoside (CMC); (D) 30 mM octyl glucoside (above AN). Conditions: 50 μm I.D. × 375 μm O.D. fused-silica APF capillary, 60 cm to detection; detection: 213 nm on-column; field strength: 300 V/cm. Peaks: 1 = desipramine; 2 = nortriptyline.

(2) The separation of heptapeptides angiotensin III and [Val^4]-angiotensin III. These two peptides differ in the substitution of isoleucine for valine at the fourth residue from the N-terminus.

Micelle formation is affected by numerous variables including pH, ionic strength and temperature. Initial studies were done under defined conditions of sodium ion concentration and pH where CMC and aggregation number (AN) are known for the surfactants used[4].

In Fig. 1, at 100 m*M* sodium ion and pH 7.0, the electropherogram panel shown, establishes that the separation of desipramine from nortriptyline occurs at CMC and improves at the AN for octyl glucoside. The experiment was repeated with CHAPS with similar results.

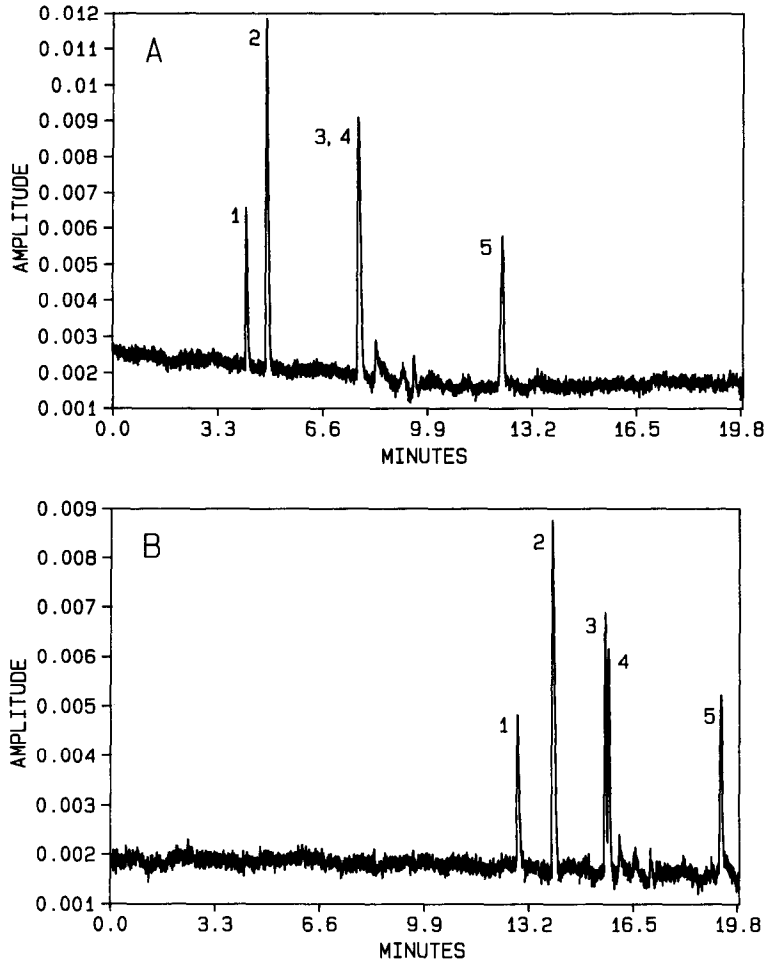


Fig. 2. Enhancement of selectivity is demonstrated in (B) by the addition of 80 m*M* octyl glucoside to 250 m*M* phosphate (pH 7.0) electrophoresis buffer. Peaks: 1 = bradykinin; 2 = releasing luteinizing hormone; 3 = [Val^4]-angiotensin III; 4 = angiotensin III; 5 = angiotensin II. Conditions: 17 μm I.D. × 375 μm O.D. fused-silica APF capillary, 70 cm to detection; detection: 210 nm on-column; field strength: 250 V/cm.

Fig. 2 shows electropherograms comparing separations of six peptides before and after addition of octyl glucoside. The addition of 80 m*M* octyl glucoside to the electrophoresis buffer notably affected selectivity; particularly of the angiotensin III pair.

Separation by MECC, as was previously mentioned, depends on both the partitioning of solute between the bulk solvent and the micelle pseudophase, and also the difference in electrophoretic mobility of the bulk solvent *vs.* the micelle. For non-ionic or zwitterionic surfactants, even though these surfactants bear no net charge, the explanation for the mobility of micelles formed from such surfactants fits perfectly within what is known about double ion layer formation on unionized surfaces. It is known from colloid and surface science that the three major charging mechanisms for a surface in contact with a polar medium are ionization, ion adsorption and ion dissolution[5]. For the suspended non-ionic or neutral ionic micellar structures, ion adsorption giving rise to a mobility in an applied field is as predictable as the formation of an electric double layer in Teflon capillary giving rise to an electroosmotic flow in that material.

The potential advantages of non- or zwitter-ionic surfactants for use in HPCE include:

(1) These materials have much less impact on the magnitude of the EOF mobility than the charged surfactants were observed to have had. Unlike CTAB, they cannot reverse the EOF direction.

(2) These materials are known to have little impact on protein structure as witnessed by excellent recovery of biological activity when those surfactants are used[4].

Uses of these surfactants for macromolecular separations are currently being explored by this laboratory.

REFERENCES

1 S. Terabe, S. K. Otsuka, K. Ichikawa, K. A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1985) 111–113.
2 H. Lauer and D. McManigill, *Anal. Chem.*, 58 (1986) 166.
3 S. A. Swedberg, *Anal. Biochem.*, in press.
4 J. Neugebauer, *A Guide to the Properties and Use of Detergents in Biology and Biochemistry, Document No. 8183-687*, Calbiochem Corporation, San Diego, CA.
5 D. J. Shaw, *Introduction to Colloid and Surface Chemistry*, Butterworths, London, 1975, pp. 133–135