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Micellar electrokinetic chromatography of charged and neutral drugs in acidic running buffers containing a zwitterionic surfactant, sulfonic acids or sodium dodecyl sulphate Separation of heroin, basic by-products and adulterants

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Abstract

A procedure for the separation of charged and neutral solutes in acidic micellar running buffers has been developed. The procedure is based on a zwitterionic surfactant 3-N,N-dimethylmyristylammoniopropanesulfonate (MAPS) and alkylsulfonates added to the running buffer at pH 4.0. The alkylsulfonates increase the migration-time window for elution of neutral substances by increasing electro-osmotic mobility and by creating a negative micellar electrophoretic mobility. The test solution contained opiates and adulterants found in heroin seizures. Both the test solution and actual heroin seizures were successfully separated using a running buffer containing 50 m*M* 6-aminocaproic acid adjusted to pH 4.0 with 50 m*M* MAPS, 5 m*M* 1-heptanesulfonic acid and 10% acetonitrile. The procedure offers an alternative to micellar electrokinetic chromatographic separations based on charged surfactants in alkaline buffers.

Keywords: Buffer composition; Heroin; Opiates; Surfactants

1. Introduction

Micellar electrokinetic chromatography (MEKC) is accomplished by the use of surfactants in the running buffer. Surfactants are classified as anionic, cationic, zwitterionic and non-ionic and at concentrations above the critical micelle concentration they aggregate to form micelles. MEKC with charged micelles is able to separate neutral as well as charged solutes. The majority of investigations on MEKC is based on alkaline running buffers and anionic surfactants such as sodium dodecyl sulphate (SDS). Anionic surfactants have a migration toward the anode, which is in the opposite direction of the electro-osmotic flow (EOF). The interaction of neutral solutes with micelles is based predominantly on hydrophobic interaction with the alkyl chain moiety. Hydrophilic solutes which interact weakly with the micelle will therefore elute at or near the EOF, while hydrophobic solutes which interact strongly with the micelle will elute at or near the micelle. In alkaline running buffers with charged surfactants, separation of charged and neutral solutes can be carried out in the same run.

Analysis of seized drugs represents an analytical challenge because of the complexity of the material. For example, seized heroin is never 100% pure; it contains numerous manufacturing by-products and

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adulterants. Several capillary electrophoresis methods have been developed for the analysis of illicit drugs [1–10]. Excellent separations of heroin, its basic by-products and adulterants are obtained via MEKC with charged surfactants in alkaline running buffers [11–16]. In order to establish the identity of any controlled drug, the criteria should be at least two independent analytical parameters [17]. In an attempt to develop a separation system which was independent of the alkaline MEKC systems used, an acidic MEKC system was developed for the separation of charged and neutral solutes. Substances found in seized heroin was used as model substances for the investigation.

In acidic running buffers the EOF is low and negative surfactants such as SDS, which migrate in the opposite direction of the EOF, will result in exceptionally long migration-times for solutes which interact strongly with the micelle. One solution to this problem is to utilize non-ionic or zwitterionic surfactants in combination with additives, which could increase the migration-time window for neutral substances. Swedberg demonstrated the use of non-ionic and zwitterionic surfactants for the separation of tricyclic antidepressants and peptides at pH 7.0 [18]. Hansen and co-workers demonstrated the use of zwitterionic and non-ionic surfactants for the separation of basic drugs at pH 4.0 [19]. However, neutral surfactants with the lack of electrophoretic mobility migrate with the same velocity as the EOF and would not be able to separate uncharged solutes. In this investigation, MEKC at pH 4.0 with non-ionic and zwitterionic surfactants were combined with anionic additives such as sulfonic acids and SDS. The anionic additives were found to extend the migration-time window by increasing the electroosmotic mobility and by creating a negative micellar electrophoretic mobility. Both the test solution containing the main basic opiates and other neutral and acidic adulterants found in heroin seizures were successfully separated by MEKC at pH 4.0.

2. Experimental

2.1. Chemicals

6-Aminocaproic acid (6-ACA) and 3-N,N-dimethylmyristylammoniopropanesulfonate (MAPS) was from Fluka (Steinheim, Germany). N-Nonyl-β-D-glucopyranoside (NGP), SDS, 1-pentanesulfonic acid, 1-hexanesulfonic acid, 1-heptanesulfonic acid and phosphorus acid were supplied by Sigma (St. Louis, MO, USA). HPLC grade acetonitrile and methanol were supplied by Rathburn (Walkerburn, UK). Dodecanophenone was supplied by Aldrich (Buchs, Switzerland). Deionized water was obtained from a Milli-Q system (Millipore, MA, USA). The drug standards were supplied either by the Norwegian Medicinal Depot (Oslo, Norway) or the National Institute of Forensic Toxicology (Oslo, Norway). Drug seizures were supplied by the Bureau of Crime Investigation (Oslo, Norway).

2.2. CE system

MEKC was performed with a Dionex capillary electrophoresis system I, equipped with automatic sampling and execution of the electrophoretic run. An uncoated fused silica capillary, 65 cm×50 μm, with the detector window 5 cm from the outlet, was used. Hydrodynamic injection was performed by either gravity or pressure. The system was run at ambient temperature with an applied voltage of 27 kV, the current not exceeding 25 μA. The compounds were detected on-column at 214 nm, the UV source being a deuterium lamp. Electropherograms were recorded and processed with a Macintosh LC computer using Dynamax HPLC Method Manager program (Rainin, Woburn, MA, USA).

Water and methanol were used as EOF markers and dodecanophenone was used as a micelle marker. The running buffer consisted of 50 m*M* 6-ACA, 50 m*M* MAPS, 5 m*M* 1-heptanesulfonic acid and 10% acetonitrile added as organic modifier. The buffer was adjusted to pH 4.0 with 1.0 *M* phosphorus acid. Prior to analyses the capillary was filled with 0.1 *M* NaOH. After 20 min the capillary and the buffer cups were rinsed with deionized water. Finally, the running buffer was flushed into the capillary and the buffer cups. The capillary was automatically filled with new running buffer between each run to ensure reproducibility.

2.3. Sample preparation

The test solution consisted of procaine, morphine, codeine, heroin, noscapine, papaverine, nicotin-

amide, caffeine, barbitone, paracetamol, allobarbitone, phenobarbitone and diphenhydramine (internal standard). All drugs was prepared in running buffer in a concentration of 0.1 mg/ml.

Drug seizures were homogenized to a fine powder and dissolved in running buffer at a concentration of 1 mg/ml. All samples were then vortexed for 2 min at 12 000 rpm prior to dilution to 0.5 mg/ml when internal standard was added at a concentration of 0.05 mg/ml.

2.4. Determination of electrophoretic mobility

Electro-osmotic mobility and electrophoretic mobility was calculated from the equations below [20]:

$$\mu_{eo} = v_{eo} L_T / V \text{ and } v_{eo} = L_D / t_{eo}$$

$$\mu_i = v_i L_T / V \text{ and } v_i = (L_D / t_i) - (L_D / t_{eo})$$

where μ_{eo} = electro-osmotic mobility ($cm^2\ V^{-1}\ s^{-1}$), μ_i = electrophoretic mobility of component i ($cm^2\ V^{-1}\ s^{-1}$), v_{eo} = electro-osmotic velocity ($cm\ s^{-1}$), v_i = electrophoretic velocity of component i ($cm\ s^{-1}$), L_T = total capillary length (cm), L_D = effective capillary length (cm), t_i = migration time of component i (s), t_{eo} = migration time of EOF marker (s), V = applied voltage (V).

3. Results and discussion

All separations were carried out at pH 4.0. At pH 4.0 the test solution is a mixture of charged and neutral substances. The opium alkaloids and nicotinamide are positively charged and have a positive electrophoretic mobility, while the barbiturates, caffeine and paracetamol are neutral with zero electrophoretic mobility. The surfactants investigated were the non-ionic surfactant NGP and the zwitterionic surfactant MAPS.

Electrically neutral surfactants have no electrophoretic mobility and migrate with the EOF ($t_{eo} = t_{mc}$). When t_{eo} equals t_{mc} no separation of neutral substances is possible. The resolution (R) of two neutral solutes migrating closely together can be described by the equation [21]:

$$R = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k}{1 + k} \cdot \frac{1 - (t_{eo}/t_{mc})}{1 + (t_{eo}/t_{mc})k}$$

where N is the plate number, α the separation factor, k is the retention factor and t_{eo} and t_{mc} are the electro-osmotic migration time and the micelle migration time, respectively. The uncharged compounds elute in the migration-time window between t_{eo} and t_{mc}.

In this investigation alkylsulfonic acids of different chain lengths and SDS were added to the acidic micellar running buffers in order to improve the separation of neutral substances. The ability of the additives to alter t_{eo} and t_{mc} was studied. To achieve resolution of all substances in the test solution, the addition of organic modifiers were also investigated.

3.1. Neutral surfactants added to the running buffer

Fig. 1a shows a capillary zone electrophoretic separation of the test mixture at pH 4.0. The first eluting positively charged bases were only partly separated. The uncharged compounds caffeine, paracetamol and the three barbiturates co-eluted at t_{eo}. The effect of adding the neutral surfactant NGP to the running buffer is shown in Fig. 1b. Except for the partly separated morphine and codeine, the positively charged bases were now satisfactorily separated. The separation of the bases is primarily a result of their different electrophoretic mobility and their different hydrophobic interaction with the micelles. The uncharged compounds (peaks 8–12) co-eluted as one peak and migrated with the same velocity as the EOF and the micelles ($t_{eo} = t_{mc}$).

As shown in Fig. 1c, the zwitterionic surfactant MAPS had the same effect on the separation of the basic compounds as the non-ionic surfactant NGP. However, the zwitterionic surfactant also increased the resolution of the uncharged compounds caffeine, paracetamol and the barbiturates. As reported by Kristensen and Hansen [22], this could indicate that the MAPS micelles have a negative electrophoretic mobility. The negative electrophoretic mobility is caused by a small overall negative charge. The negative electrophoretic mobility, which is in the opposite direction of the EOF, results in a narrow migration-time window for elution of uncharged

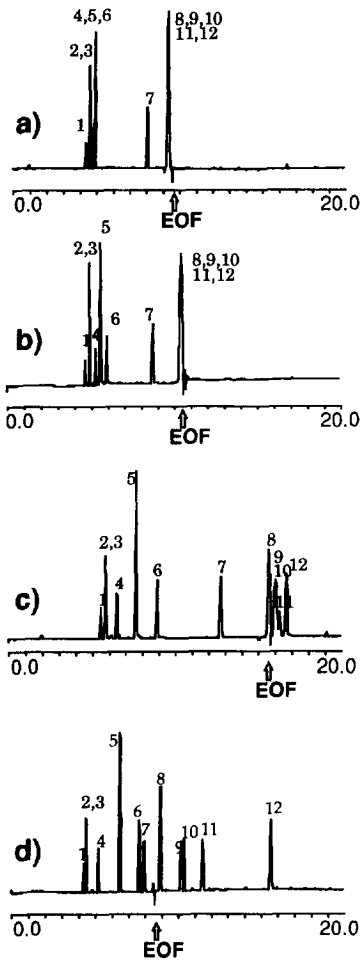

Fig. 1. Electropherograms of the test solution separated at pH 4.0 with running buffers consisting of 50 m*M* 6-ACA (a), 50 m*M* 6-ACA, 50 m*M* NGP (b), 50 m*M* 6-ACA, 50 m*M* MAPS (c), 50 m*M* 6-ACA, 50 m*M* MAPS, 5 m*M* heptanesulfonic acid (d). Peaks: 1 = procaine, 2 = morphine, 3 = codeine, 4 = heroin, 5 = noscapine, 6 = papaverine, 7 = nicotinamide, 8 = caffeine, 9 = barbitone, 10 = paracetamol, 11 = allobarbitone, 12 = phenobarbitone. For experimental details see text.

solutes. Partial separation of the neutral solutes in the test solution was therefore obtained.

3.2. Addition of heptanesulfonic acid to the zwitterionic micellar buffer

In order to improve the resolution of the uncharged compounds, heptanesulfonic acid was added to the zwitterionic micellar buffer. The aim was to decrease the t_{eo}/t_{mc} ratio, either by increasing t_{mc} or by decreasing t_{eo}. To our knowledge there are no reports of adding sulfonic acids to a zwitterionic micelle system at acidic pH to increase the migration-time window for uncharged compounds.

As shown in Fig. 1d, a significantly improved resolution of the uncharged compounds (caffeine, paracetamol and the barbiturates) and a significant increase in EOF was obtained after addition of heptanesulfonic acid, compared with Fig. 1c. No significant effects on the separation of the basic compounds were found, however, papaverine (peak 6) and nicotinamide (peak 7) eluted more closely. The effect is dependent on the concentration of heptanesulfonic acid. The EOF increased when 1, 5 and 10 m*M* heptanesulfonic acid was added to the running buffer. Selectivity differences were also seen as the heptanesulfonic acid concentration increased. The migration order of papaverine and nicotinamide was switched when 10 m*M* heptanesulfonic acid was added. In addition, the resolution between the positively charged procaine and the unresolved peak of morphine and codeine decreased because of the increased EOF. Optimal resolution of the charged and neutral solutes with 5 m*M* heptanesulfonic acid was achieved and 5 m*M* of the additive was used in further studies.

3.3. Addition of alkylsulfonates and SDS.

The migration-time window is related to electroosmotic mobility and electrophoretic mobility of the micelles. Different additives used in MEKC can alter the electrokinetic mobilities. The effects encountered when adding heptanesulfonic acid to the micellar solution led to a more detailed study on the use of negatively charged additives. Alkylsulfonates of different chain lengths and SDS were investigated. Fig. 2 shows the t_{eo}/t_{mc} ratio plotted vs. composition of the micellar buffer. The migration-time window is large when t_{eo}/t_{mc} ratios are small. Buffer 1 contains the non-ionic surfactant NGP and with no separation of uncharged solutes a t_{eo}/t_{mc} ratio of 1 was obtained. Buffer 2 contains the zwitterionic surfactant MAPS and the small decrease in the t_{eo}/t_{mc} ratio confirms the possibility of a partial separation of neutral solutes. The running buffers 3–6 contain MAPS to which was added 5 m*M* of sulfonic acids

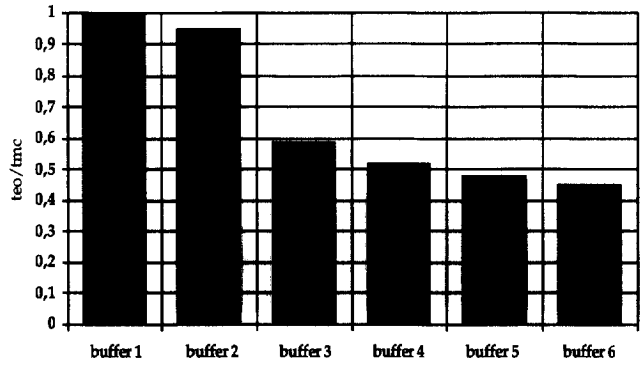

Fig. 2. The t_{eo}/t_{mc} ratio plotted vs. composition of the running buffer at pH 4.0. 50 m*M* 6-ACA, 50 m*M* NGP (buffer 1), 50 m*M* 6-ACA, 50 m*M* MAPS (buffer 2), 50 m*M* 6-ACA, 50 m*M* MAPS, 5 m*M* pentanesulfonic acid (buffer 3), 50 m*M* 6-ACA, 50 m*M* MAPS, 5 m*M* hexanesulfonic acid (buffer 4), 50 m*M* 6-ACA, 50 m*M* MAPS, 5 m*M* heptanesulfonic acid (buffer 5), 50 m*M* 6-ACA, 50 m*M* MAPS, 5 m*M* SDS (buffer 6).

of increasing chain lengths and SDS. A significant decrease in the t_{eo}/t_{mc} ratio was observed when a sulfonic acid or SDS was added and the ratio decreased as a function of the chain length of the alkyl group. The t_{eo}/t_{mc} ratio decreased because the additives have changed the electro-osmotic mobility (μ_{eo}) and the micellar electrophoretic mobility (μ_{mc}). Fig. 3 shows μ_{eo} and μ_{mc} plotted vs. the composition of the running buffer. The small negative electrophoretic mobility of the MAPS micelles is confirmed with buffer 2. Both μ_{eo} and μ_{mc} increased as a function of the chain length of the additive. The increase in μ_{eo} is larger than the negative increase in μ_{mc} which results in decreased migration times for all compounds. The increased μ_{eo} is probably caused by hydrophobic adsorption of the alkylsulfonates and SDS to the capillary wall, resulting in an increased negative surface charge. This interaction increased with the chain length of the additive. The interactions with the zwitterionic micelle and the negatively charged additives leads to an increased surface charge of the micelle. The interactions could be either electrostatic or hydrophobic. Both interactions will increase μ_{mc}.

The electrophoretic mobility of all compounds in the test solution, as a function of the added sulfonic acid and SDS, is shown graphically in Fig. 4. Positive values on the *y*-axes correspond to compounds with positive electrophoretic mobility, negative values on the *y*-axes correspond to compounds with negative electrophoretic mobility and zero on the *y*-axes corresponds to the mobility of the EOF.

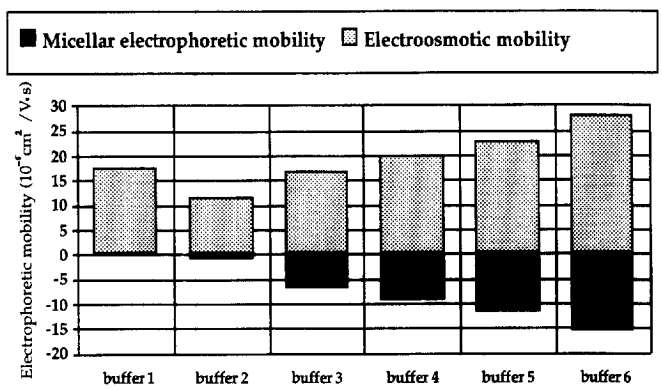

Fig. 3. Effective electro-osmotic mobility and effective micellar electrophoretic mobility (10^{-5} cm^2 V^{-1} s^{-1}) plotted vs. composition of the running buffer. Running buffers as in Fig. 2.

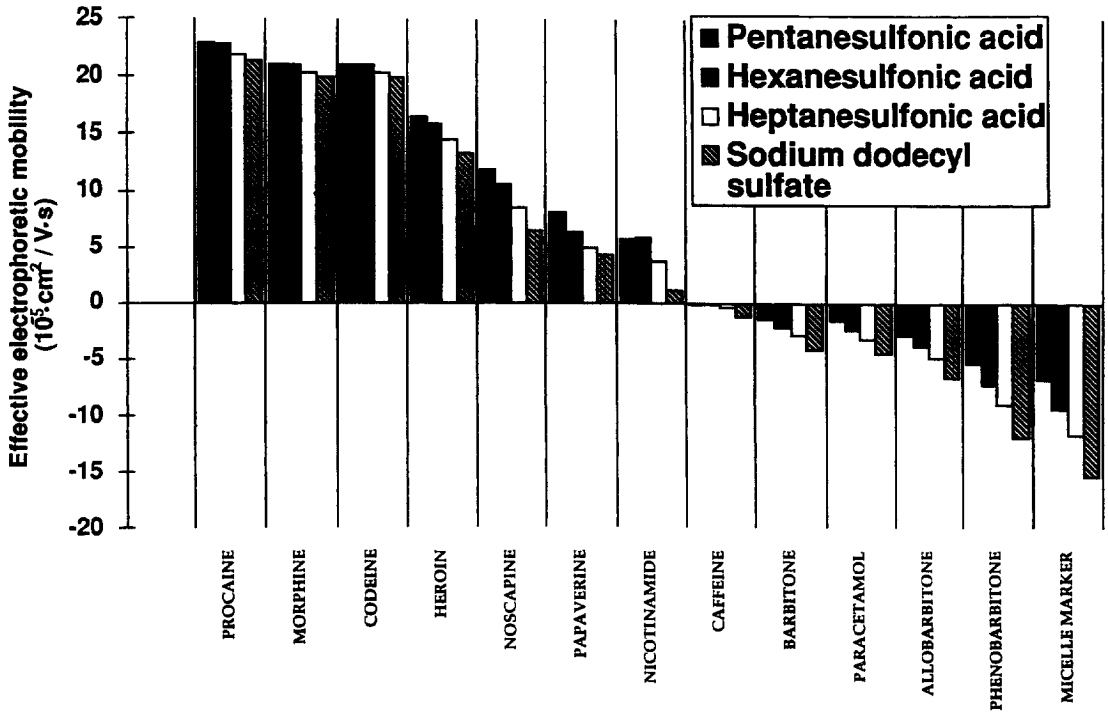

Fig. 4. The effective electrophoretic mobility of all solutes in the test solution as a function of the sulfonic acids and SDS added to the running buffer.

The positively charged solutes have a positive electrophoretic mobility which decreases with the chain length of the additive. All positively charged solutes elute before the EOF. The solutes which are non-ionic at pH 4.0 have a negative electrophoretic mobility because of partitioning into the negatively charged micelles. Their mobility increases with the chain length of the additive.

3.4. Applications

The separation system developed was used for the separation of heroin and related substances in heroin seizures. However, the buffer system was not able to separate morphine and codeine. In order to achieve the desired separation, organic solvents were investigated as modifiers. A complete separation of morphine and codeine was achieved after the addition of 10% acetonitrile to the running buffer.

The acidic MEKC system offers a different selectivity than the commonly used alkaline MEKC systems based on SDS as surfactant. The positively charged alkaloids are all eluted before t_{eo}. Neutral solutes elute in the migration-time window between t_{eo} and t_{mc}. The migration of negatively charged solutes is dependent on their charge/mass ratio. Charged molecules such as carboxylic acids migrate slowly and will usually be eluted after t_{mc}. In MEKC systems based on SDS in alkaline buffers, all substances commonly found in heroin seizures are eluted between t_{eo} and t_{mc}. The acidic MEKC system can therefore offer additional information to confirm the identity of unknown substances. Fig. 5 shows two electropherograms of typical heroin seizures. Ten different heroin seizures were analyzed and the main compounds identified using relative migration-times. The relative standard deviation on the relative migration-times were in the range 0.32–1.94% (within-days) and 0.63–2.59% (between-days).

4. Conclusions

Neutral and positively charged solutes can be

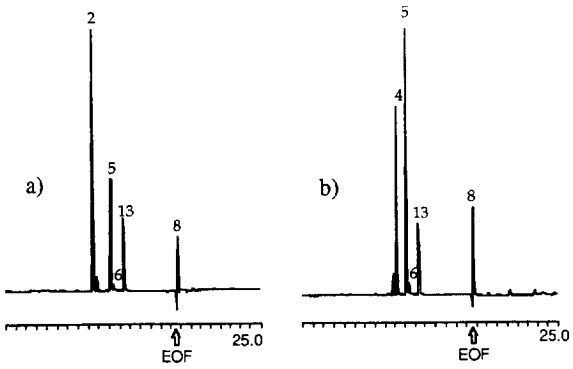

Fig. 5. Electropherograms of two heroin seizures (a) and (b). Running buffer: 50 m*M* 6-ACA (pH 4.0), 50 m*M* MAPS, 5 m*M* heptanesulfonic acid, 10% acetonitrile. Peak identification as in Fig. 1. Peak 13: diphenhydramine (internal standard).

separated by MEKC in acidic running buffers based on a zwitterionic surfactant. The migration-time window for separation of neutral solutes is increased by adding sulfonic acids or SDS to the running buffer. These additives increases the migration-time window by increasing the EOF and by increasing the micellar electrophoretic mobility in the opposite direction to the EOF. The effect is dependent on the alkyl chain of the additives, a stronger effect is seen with increasing chain lengths of the additives. The method developed offers an alternative to the commonly used alkaline MEKC systems for the separation of charged and neutral solutes.

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