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# Micellar electrokinetic chromatography with acid labile surfactant

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ABSTRACT

We present a study of a degradable surfactant, sodium 4-[(2-methyl-2-undecyl-1,3-dioxolan-4-yl)methoxy]-1-propane sulfonate that is also known as an acid-labile surfactant (ALS). The performance of ALS as a pseudostationary phase is assessed and compared with established pseudostationary phases such as sodium dodecyl sulphate (SDS), volatile surfactants and polymeric micelles. ALS achieves separation efficiency of 100,000–145,000 theoretical plates and relative standard deviation (RSD) of electrophoretic mobility ($n = 5$) of less than 3%. Retention factors with ALS are strongly correlated with those with SDS. This is shown by the $R^2 = 0.79$ for all eleven analytes and an $R^2 = 0.992$ for specifically the non-hydrogen bonding (NHB) analytes. However, ALS displays different selectivity than SDS for hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) solutes ($R^2$ of 0.74 and 0.88, respectively). ALS is degraded to less surface active compounds in acidic solution. These less surface-active compounds are more compatible with the electrospray ionization mass spectrometry (ESI-MS). ALS has a half-life of 48 min at pH 4. ALS has the potential to couple micellar electrokinetic chromatography (MEKC) with the ESI-MS. ALS can be used as a pseudostationary phase for a high efficiency separation and later acid hydrolyzed to enable an ESI-MS analysis.



## 1. Introduction

Micellar electrokinetic chromatography (MEKC) is a high efficiency separation method that conveniently gives efficiencies greater than 100,000 theoretical plates [1–3]. In MEKC surfactant is present above its critical micelle concentration (CMC). The resultant micelles act as a pseudostationary phase. Analytes which partition into the micelle migrate with an electrophoretic mobility that is a function of both the electrophoretic mobility of the micelle and the partition coefficient [2]. MEKC has been used to separate neutral and charged analytes ranging from small molecules to macromolecules such as peptides, proteins and saccharides [4–12]. MEKC has become a popular separation technique because it gives higher theoretical plate count and consumes less toxic and expensive organic solvent than high performance liquid chromatography (HPLC) [13,14].

Electrospray ionization mass spectrometry (ESI-MS) is a powerful detection technique, capable of giving rich structural information from aqueous and aqueous/organic solutions [15–17]. ESI-MS′ soft ionization enables the transformation of large molecules into gas-phase ions without decomposition [18]. This has made ESI-MS an immensely popular technique to study large biomolecules. The combination of high efficiency separation and selectivity of MEKC with the versatility of ESI-MS is extremely tempting [19]. However, coupling the two techniques is difficult at best. Low molecular mass surfactants such as sodium dodecyl sulphate (SDS) that are commonly used in MEKC have low volatility, are very surface-active and suppress the analyte signal in the ESI-MS [20]. Some solutions have been developed to circumvent the problem of hyphenating MEKC with ESI-MS [21,22]. One method is partial-filling MEKC (PF-MEKC) [3,23,24]. Briefly, in PF-MEKC there are three plugs inside the capillary. The plugs consist of background electrolyte (BGE), followed by a micellar solution and lastly a sample solution. When voltage is applied, the analytes migrate into the micellar region and are separated. The separated analytes continue to move towards the BGE region that is free of surfactant. The analytes elute out of the capillary and are introduced into an ESI-MS system. The micellar plug is left behind and does not interfere with the ESI-MS analysis. Another method is the use of volatile surfactants such as perfluorooctanoic acid (PFOA). PFOA has low boiling point compared to SDS (190 °C vs. ≫320 °C). PFOA is volatile enough that it does not concentrate on the surface of the droplets and thereby suppress the analyte ESI-MS signal [25,26]. Similarly, Goetzinger and Cai employed organic micellar system of lauric acid and monoamines to couple MEKC with the ESI-MS [27]. Thirdly, high molecular mass surfactants that form polymeric micelles have also been used to unify MEKC with ESI-MS [28–31].

Another potential solution to couple MEKC with ESI-MS is cleavable surfactants. An example is sodium 4-[(2-methyl-2-undecyl-1,3-dioxolan-4-yl) methoxy]-1-propane sulfonate, also known as acid-labile surfactant (ALS, Fig. 1). ALS was introduced

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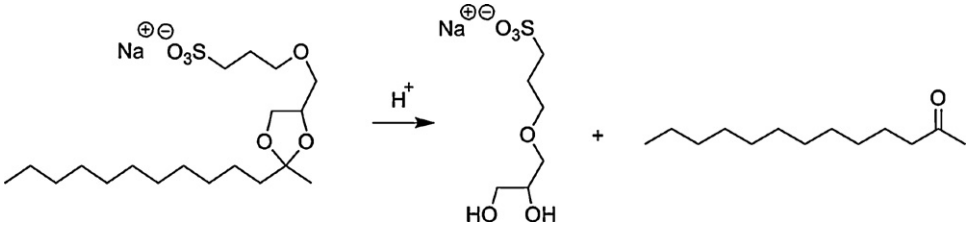


**Fig. 1.** Acid hydrolysis of ALS into its less surface-active successor compounds.

in 1999 to replace SDS in PAGE because it has similar denaturing and electrophoretic properties as SDS [32]. ALS can be degraded under acidic condition to give less surface active products (Fig. 1). ALS is available commercially under the name of RapiGest, but the ALS used in this study was synthesized in our lab. ALS has been shown to separate proteins comparably to SDS in a microfluidic electrophoresis device [33]. However, no study of ALS in MEKC of small molecules has been reported.

In this work, two aspects of ALS relevant to MEKC-MS are examined. First, ALS is assessed as a pseudostationary phase for MEKC. The micelle mobility, separation efficiency and selectivity of ALS are compared with SDS. Since ALS is degraded under acidic conditions, the stability of the ALS under MEKC conditions is also investigated to ensure that it will be stable during the duration of MEKC separation and analysis. Second, we assess the compatibility of ALS with the ESI-MS. The kinetics of ALS cleavage and the surface activity of the degradation products are determined.

## 2. Experimental

### 2.1. Materials and reagents

Solutions were prepared with ultrapure (18 MΩ) water (Barnstead, Chicago, IL, USA). The chemicals were of reagent grade or better. 2-Tridecanone, glycerol, *p*-toluenesulfonic acid monohydrate, 1,3-propane sultone, naphthalene, 2-naphthol, anthracene, alkylphenone homologous series (acetophenone–hexanophenone), resorcinol, phenol, 4-nitroaniline, benzyl alcohol, atenolol, di-sodium tetraborate, sodium phosphate monobasic monohydrate, SDS and formic acid (FA) were used as received from Sigma–Aldrich (Milwaukee, WI, USA). Reagent grade benzene, toluene, ethyl acetate, hexane, methanol, acetonitrile (ACN) and anhydrous ethyl alcohol were from Fisher Scientific (Fairlawn, NJ, USA). The pH of the background electrolyte (BGE) was measured using a Model 445 digital pH meter (Corning, Acton, USA) and were adjusted using 1 M HCl and/or 1 M NaOH. Methanol was used as the EOF marker. All solutions were filtered through 0.2 μm nylon filters (Barnstead) prior to analyses.

### 2.2. Synthesis of ALS

The synthesis of the ALS precursor 4-hydroxymethyl-2-methyl-2-undecyl-1,3-dioxolane (HMUD) followed that of Jaeger et al. with some modifications [34]. 10 g of 2-tridecanone (0.05 mol), 5.6 g of glycerol (0.06 mol) and 50 mg of *p*-toluenesulfonic acid monohydrate catalyst were dissolved in 250 mL of benzene. The reaction mixture was refluxed with stirring for 45 h in a 500-mL round-bottom flask (RBF) fitted with a Dean-Stark apparatus. The reaction mixture was cooled to room temperature and washed with 500 mL of 5% (m/v) sodium bicarbonate aqueous solution, dried using $Na_2SO_4$ and rotary evaporated under vacuum. HMUD is a viscous light yellow liquid. HMUD was purified by flash chromatography (silica, 200–400 mesh, 60 Å; 1:6, v/v, ethyl acetate:hexane as eluent) before being used in the next step of the synthesis. HMUD was analyzed by a direct injection high resolution ESI-MS, $m/z$ = 272.42 (predicted 272.42). The IR spectra of the reaction mixture at the start and the end of the reaction showed the disappearance of the carbonyl peak at 1720 $cm^{-1}$ of 2-tridecanone and the appearance of hydroxyl peak of HMUD at 3430 $cm^{-1}$. The yield was 67–72%.

The synthesis of ALS from HMUD was performed according to the procedure of Yamamura et al. [35]. Briefly, equimoles of powdered NaOH and HMUD were placed in an RBF with 200 mL of toluene. The mixture was stirred at 50 °C while equimolar 1,3-propane sultone was added over 30 min. The suspension was stirred further at 70–75 °C for 6 h. Upon adding the reaction mixture into boiling ethanol, a white precipitate (ALS) formed. The ALS was collected after the removal of the solvent and recrystallized from ethanol. The identity of ALS was confirmed by direct injection high resolution ESI-MS, $m/z$ = 393.23 (predicted 393.23). The Melting point was 258–262 °C. The yield was 70–80%.

### 2.3. Instrumentation

MEKC experiments were performed on a Beckman-Coulter P/ACE MDQ system (Fullerton, CA, USA) equipped with a UV absorbance detector monitoring 214 nm. 50 μm ID (363 μm OD) bare fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with a total length of 51.5 cm and effective length of 41.5 cm were used. The capillary temperature was maintained at 25 °C. New capillaries were flushed with 1.0 M NaOH for 60 min at 20 psi (138 kPa), followed by 0.1 M NaOH for 30 min at 20 psi (138 kPa). Samples were hydrodynamically injected using 0.3 psi (2.1 kPa) for 3 s. The capillary was rinsed with 0.1 M NaOH for 30 min at the start of each day prior to any analysis. The capillary was flushed with ACN, 0.1 M NaOH and BGE respectively for 5 min each at 20 psi (138 kPa) prior to each run.

High resolution ESI-MS analyses were performed on an Agilent Technologies 6220 TOF-ESI-MS (Santa Clara, CA, USA) by direct injection. The cleavage rate study was performed on an Agilent Technologies HP MSD1100 ESI-MS system (Santa Clara, CA, USA) by direct injection. 475 μL of atenolol (25 mM) dissolved in 0.5% formic acid (FA, pH 2.5) was spiked with 25 μL of ALS (25 mM) dissolved in deionized water. The solution mixture was homogenized and injected in to the ESI-MS every 30 min for 16 h.

Surface tension measurements were taken using a Fisher surface tensiometer model 20 (Fisher Scientific, Pittsburgh, PA). Before each measurement the platinum–iridium ring and 50-mL beaker used were cleaned in benzene.

## 3. Results and discussion

### 3.1. Comparison of MEKC with ALS and SDS

SDS is the most widely used surfactant for MEKC separations of both small molecules such as metabolites and macromolecules such as proteins, peptides and saccharides [2,10,36–38]. SDS is well characterized, easily available, inexpensive, and highly soluble in aqueous media. It also has low UV absorbance and high solubilization power.

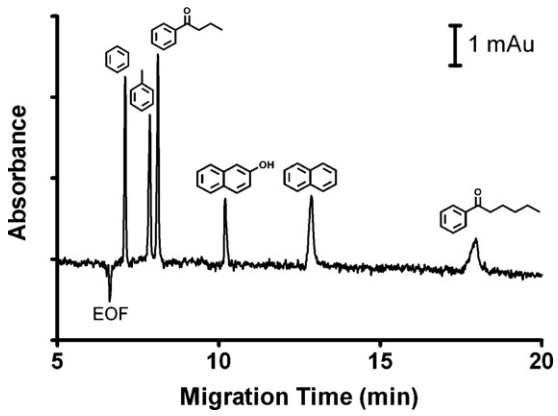


**Fig. 2.** Separation of six model analytes using ALS as a pseudostationary phase: benzene, toluene, butyrophenone, 2-naphthol, naphthalene, hexanophenone. Conditions: EOF marker, methanol; detection, 214 nm; applied voltage, 15 kV; BGE, 50 mM $NaH_2PO_4$, 100 mM borate, 30 mM ALS, pH 7.04; capillary (50 μm ID) length, 51.5 cm; effective length, 41.5 cm.

ALS has a critical micelle concentration (CMC) of 0.5 mM in pure water [35]. This is lower than the 8 mM CMC of SDS [1]. This potentially would allow ALS to be used for MEKC at a lower surfactant concentration, hence avoiding the higher viscosity and conductivity associated with SDS [39]. ALS also has comparable background absorbance as SDS. These properties make ALS an attractive surfactant to perform MEKC separation.

Fig. 2 shows an MEKC separation of six model analytes using ALS. Table 1 compares MEKC separations using ALS and SDS in terms of separation efficiency ($N$) and repeatability. ALS displayed separation efficiencies of 95,000–145,000 theoretical plates for more polar analytes ($k'$ = 0.088–0.24). SDS achieved 110,000–180,000 theoretical plates for the same analytes. Separation efficiency decreases for less polar analytes. It ranges from 60,000–110,000 for analytes with $k'$ = 0.7–3.1 in ALS. Theoretical plates of the same analytes using SDS were 55,000–150,000.

Hexanophenone gives surprisingly low separation efficiency (15,000 theoretical plates) for ALS due to peak fronting. This peak shape and decrease in separation efficiency are consistent with the nonlinear, anti-Langmuir type isotherm observed for hexanophenone in MEKC with latex nanoparticles [40].

Considering other systems to bridge MEKC with the ESI-MS, polymeric micelles [36,41–44] yield efficiencies up to 600,000 theoretical plates [45] and the volatile surfactant PFOA yields 84,000–89,250 theoretical plates [25]. Thus while ALS provides slightly lower separation efficiency than SDS, ALS can still be considered a good MEKC agent as it gives $N$ > 100,000 [1]. ALS also outperforms some other MS compatible systems.

Another important factor in MEKC agent is the repeatability of the migration time ($t_m$). SDS is very reliable when it comes to repeatability. The relative standard deviation (RSD) of the electrophoretic mobility of the 11 model analytes are less than 2% ($n$ = 5) for SDS in Table 1. ALS yields comparable electrophoretic mobility repeatability, except for benzyl alcohol and benzene which are slightly higher. Polymeric micelle used with MEKC-ESI-MS analysis showed RSD of 0.8–0.9% ($n$ = 3) [46]. The repeatability of ALS is comparable with SDS and polymeric micelle [36]. No repeatability RSD has been reported for volatile surfactants.

Further, ALS is stable under standard (neutral) MEKC conditions. After 8 weeks at room temperature, the net electrophoretic mobility of 4-nitroaniline in 30 mM ALS (pH 7) was equivalent to that of a fresh solution ($-2.22 \times 10^{-5}$ $cm^2/V\,s$ vs. $-2.07 \times 10^{-5}$ $cm^2/V\,s$).

### 3.2. Stability of ALS under acidic conditions

ALS is hydrolyzable under acidic conditions [47,48]. This property is what makes ALS attractive in proteomics because its less surface-active products (Fig. 1) are compatible with ESI-MS [32,49]. While being acid-hydrolyzable is advantageous for ESI-MS detection, ALS needs to be stable over the range of BGE pH used in MEKC. Techniques such as sample stacking with reverse migrating micelle are performed under acidic conditions (pH <4) to suppress the EOF [50]. ALS must be stable for long enough to be used in such experiment.

To test the stability of ALS, repetitive MEKC separations were performed using 30 mM ALS in pH 4.0 buffer. The net migration time (i.e., observed migration time − migration time of the EOF) of benzyl alcohol is consistent over 60 h (see Supplementary Fig. 1), with an RSD of 4.8% and a slope that is statistically equivalent to zero ($(-5.6 \pm 20) \times 10^{-5}$). These results coupled with those at pH 7 (Section 3.1) indicate that ALS is hydrolytically stable enough to be used conveniently for MEKC separations.

### 3.3. Mobility and selectivity of ALS

The migration time window for a neutral compound in MEKC is between the migration time of the EOF ($t_{eof}$) and the micelle ($t_{mc}$). Common ways to calculate the mobility of the micelle are to use a homologous series or a single marker [51]. The homologous series method was introduced by Bushey and Jorgenson in 1989 [52], and has been used extensively over the years [51]. In this procedure a homologous series (commonly alkylphenones) are separated under MEKC conditions. Initially, the $t_m$ of the compound with the highest carbon number is assigned as the $t_{mc}$. The retention factors for the other members of the homologous series are calculated using:

$$k'_{EKC} = \frac{t_m - t_{eof}}{t_{eof}(1 - (t_m/t_{mc}))} \quad (1)$$

**Table 1**
Comparison of efficiency, repeatability and retention factor ($k'$) between SDS and ALS as a pseudostationary phase.

| Analytes | Efficiency SDS | Efficiency ALS | RSD (%) of electrophoretic mobility, $n$ = 5, SDS | RSD (%) of electrophoretic mobility, $n$ = 5, ALS | $k'$ SDS | $k'$ ALS |
|---|---|---|---|---|---|---|
| Resorcinol | 170,000 | 140,000 | 1.9 | 1.3 | 0.13 | 0.15 |
| Phenol | 175,000 | 125,000 | 1.2 | 1.3 | 0.26 | 0.12 |
| Benzyl alcohol | 175,000 | 145,000 | 0.4 | 2.0 | 0.30 | 0.074 |
| 4-Nitroaniline | 180,000 | 115,000 | 1.1 | 1.2 | 0.64 | 0.13 |
| Benzene | 145,000 | 95,000 | 0.6 | 2.2 | 0.84 | 0.088 |
| Toluene | 110,000 | 105,000 | 0.2 | 1.1 | 1.5 | 0.24 |
| 2-Naphthol | 95,000 | 95,000 | 0.4 | 0.3 | 2.8 | 0.74 |
| Naphthalene | 55,000 | 60,000 | 0.4 | 0.5 | 7.1 | 1.4 |
| Butyrophenone | 150,000 | 110,000 | 1.1 | 1.3 | 8.6 | 0.29 |
| Valerophenone | 120,000 | 105,000 | 0.2 | 0.9 | 25.9 | 0.7 |
| Hexanophenone | 90,000 | 15,000 | 0.7 | 0.3 | 75.5 | 3.1 |

*Experimental conditions*: 50 mM $NaH_2PO_4$, 100 mM borate, 30 mM surfactant (SDS or ALS), pH 7.04. Detection: 214 nm; total capillary length: 51.5 cm; effective length: 41.5 cm; applied voltage: 15 kV.

Ideally a plot of $\log k'_{EKC}$ vs. the carbon number should be linear. If it is not, new $\log k'_{EKC}$ are estimated by extrapolating the regression line to the member of the homologous series with the highest carbon number. The new $t_{mc}$ is calculated by rearranging equation (1). The new $t_{mc}$ is used to recalculate the $\log k_{EKC}$ of each homologous member and again plotted against the carbon number. The process is reiterated until the difference between consecutive values is less than 0.001 min or the correlation coefficient reaches $R^2 = 0.9999$. For MEKC with SDS, the homologous series acetophenone–hexanophenone yielded a linear plot of $\log k'_{EKC}$ vs. carbon number ($R^2 = 0.9999$) and a mobility of SDS micelles of $-3.74 \times 10^{-4}$ cm$^2$/V s. This value is consistent with values reported in the literature for SDS which range from $-3.9 \times 10^{-4}$ to $-4.4 \times 10^{-4}$ cm$^2$/V s [53,54].

Unfortunately, attempts to use the homologous series method with ALS resulted in positive deviation of the $\log k'_{EKC}$ vs. carbon number plot and the iterative calculations did not converge. Although the homologous series has been successfully utilized to determine $t_{mc}$ over the years, there are some cases where it has failed [51,55,56]. The failure in Ref. [55] displayed a different pattern (i.e. negative deviation) than observed herein, and had been attributed to restricted hydrophobic domains. The other instance of failure was for a siloxane polymeric micelle [55]. This siloxane polymer contains a similar hydrophilic cyclic ketal linkage to that in ALS (Fig. 1). None of the pseudostationary phases for which the homologous series method has been successful contain such a polar linker [51]. However, more investigations need to be done before any concrete conclusion can be reached.

In the single-marker method, a hydrophobic compound that is fully partitioned into the pseudostationary phase is used to calculate the electrophoretic mobility of the pseudostationary phase. Compounds that have been used as micelle markers include Sudan III, dodecanophenone, anthracene and decanophenone [4,51,56,57]. Using anthracene with SDS yielded a micelle mobility of $-3.70 \times 10^{-4}$ cm$^2$/V s, in excellent agreement with the value determined using the homologous series method. The mobility of ALS micelles based on anthracene is $-2.33 \times 10^{-4}$ cm$^2$/V s. The elution window in MEKC is determined by the EOF and the micelle mobility. Faster micelle mobility results in greater elution window and hence larger peak capacity. The migration window time ratios ($t_{mc}/t_{eof}$) of ALS and SDS are 6.6 and 9.5 respectively. This shows that ALS has a smaller migration window time ratio than SDS. ALS has slower mobility compared to polymeric micelles such as sodium 10-undecenyl sulphate ($-4.3 \times 10^{-4}$ cm$^2$/V s) and siloxane polymer ($-5.3 \times 10^{-4}$ cm$^2$/V s) [44,58]. However, the ALS mobility does fall within the range of common pseudostationary phases ($-2.35$ to $-5.3 \times 10^{-4}$ cm$^2$/V s) [58].

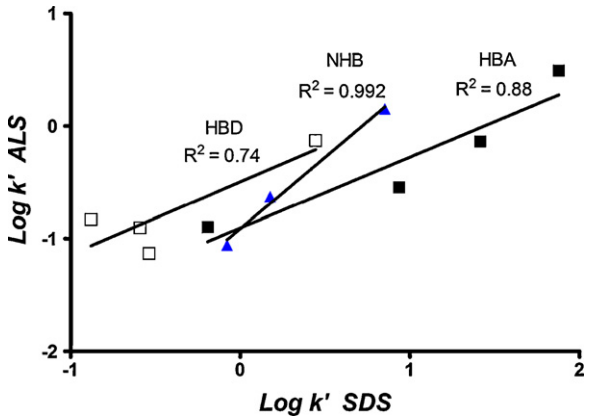


**Fig. 3.** Log $k'$ of eleven analytes using ALS and SDS as pseudostationary phase. (□) Hydrogen bond donating (resorcinol, phenol, benzyl alcohol, 2-naphthol), $R^2 = 0.74$; (■) hydrogen bond acceptor (4-nitroaniline, butyrophenone, valerophenone, hexanophenone), $R^2 = 0.88$; (▲) non-hydrogen bonding (benzene, toluene, naphthalene), $R^2 = 0.992$. Conditions: EOF marker, methanol; detection, 214 nm; applied voltage, 15 kV; BGE, 50 mM $NaH_2PO_4$, 100 mM borate, 30 mM ALS or SDS, pH 7.04.

Both the hydrophilic and hydrophobic groups determine the selectivity of a surfactant in MEKC. ALS has a similar hydrophobic tail as SDS, but a different palisade region. Thus we expect ALS to offer different selectivity. Fig. 3 shows the log of retention factor ($k'$) of eleven different analytes using ALS and SDS as the pseudostationary phase. Retention factors with ALS are strongly correlated with those with SDS, as evidenced by the $R^2$ of 0.79 for the eleven analytes in Fig. 3, and an $R^2 = 0.992$ for specifically the non-hydrogen bonding (NHB) analytes. However, ALS displays different selectivity than SDS for hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) ($R^2$ of 0.74 and 0.88, respectively). The HBD solutes show higher affinity for ALS (top line) whereas the HBA solutes have higher affinity for SDS (bottom line). This behavior is similar to what was observed in the selectivity comparison between lithium perfluorooctanesulfonate (LiPFOS) and SDS, where the hydrophobic interaction is the primary driving force between micelle–solute interactions [59].

The $k'$ of hydrophobic analytes with ALS are much lower than with SDS (Table 1). For example, the $k'$ of naphthalene is 1.4 and 7.1 using ALS and SDS respectively. ALS resolves hydrophobic analytes well (Fig. 2). However, ALS is at a disadvantage to separate

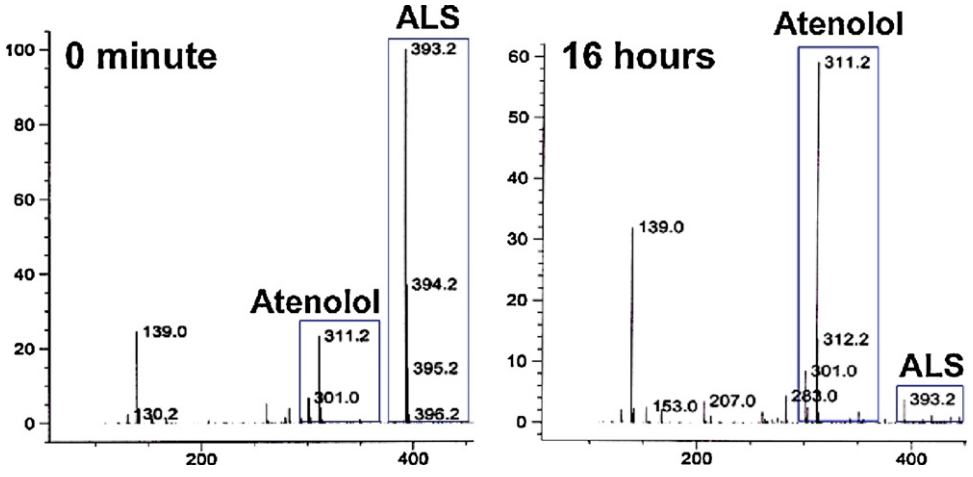


**Fig. 4.** Mass spectra of atenolol and ALS at 0 minute and 16 h. Conditions: 475 μL of atenolol (25 mM in 0.5% formic acid) spiked with 25 μL of ALS (25 mM in deionized water). Conditions: negative mode; fragmentation energy, 80 V; solvent system, methanol. Atenolol peak is $[M+FA-H]^-$. ALS peak is $[M]^-$.

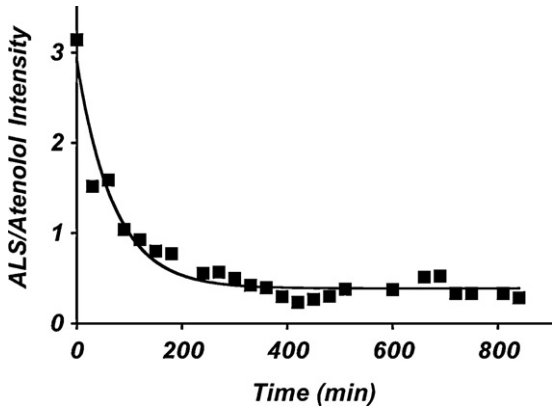


**Fig. 5.** Ratio of ALS/atenolol ESI-MS signal intensity over a period of 16 h. Conditions: 475 μL of atenolol (25 mM in 0.5% formic acid), pH 2.5, spiked with 25 μL of ALS (25 mM in deionized water). Conditions: fragmentation energy, 80 V; solvent system, methanol; half-life of ALS, 48 min.

hydrophilic analytes. For instance, SDS resolves resorcinol and 4-nitroaniline ($k'$ = 0.13 and 0.64 respectively) whereas ALS does not ($k'$ = 0.15 and 0.13 respectively).

### 3.4. Cleavage rate of ALS and compatibility with ESI-MS

There are two requirements that ALS needs to fulfill before it can link MEKC with online ESI-MS. First, ALS must decompose into less surface active compounds. Second, the hydrolysis of ALS should be as rapid as possible. Preliminary studies monitored the hydrolysis via surface tension. The surface tension of a 25 mM ALS (pH 7) solution was measured as 46 dynes/cm, which is in good agreement with literature (41 dynes/cm) [35]. After 19 h hydrolysis with 0.5% formic acid, the surface tension increased to 58 dynes/cm. The surface tension right after the addition of 0.5% formic acid was 30.5 dynes/cm ($n$ = 3). This shows that the increase in surface tension is due to hydrolysis of ALS and not to the presence of formic acid.

To more directly study the effect of ALS on ESI-MS, the ESI-MS signal of atenolol as a model analyte was monitored. The $m/z$ of atenolol (311.2) is easily resolved from that of ALS (393.2). Prior to hydrolysis (time = 0), the ALS dominates the ESI signal (Fig. 4) in an equimolar solution of ALS and atenolol. With degradation of the ALS (e.g., 16 h in Fig. 4), the atenolol signal increases. As a control, the same experiment using SDS shows that SDS signal still dominates over the atenolol signal after 16 h (see Supplementary Fig. 2). To monitor the hydrolysis kinetics, the ESI signal intensity ratio of ALS over atenolol was plotted vs. time (Fig. 5). The half-life of ALS hydrolysis is 48 min at pH 2.5 in water. Alternately the half-life of ALS in a 50/50 ACN/0.5% FA solution is 170 min (see Supplementary Fig. 3). Fig. 4 and 5 confirm that the degradation products of ALS are less surface-active than the original ALS, consistent with literature claims [32,33]. Unfortunately, the long half-life of ALS makes online coupling of MEKC-ESI-MS unrealistic. However, the high separation efficiency and selectivity of ALS make it attractive for MEKC-fraction collection mass spectrometry [60,61].

## 4. Concluding remarks

ALS was used as pseudostationary phase for high efficiency separation of model neutral analytes. ALS achieved separation efficiency slightly lower than that achieved with SDS, but greater than that of alternate ESI friendly pseudostationary phase. ALS is stable for a reasonable period of time under acidic condition allowing its usage as a pseudostationary phase. ALS also offers a different selectivity than SDS. ALS is acid hydrolyzed into less surface-active compounds that are more compatible with the ESI-MS. Unfortunately, the cleavage rate of ALS is too slow to be used in an online MEKC-ESI-MS system.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.09.065.

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