Effects of ammonioalkyl sulfonate internal salts on electrokinetic micropump performance and reversed-phase high-performance liquid chromatographic separations

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Abstract

Ammonioalkyl sulfonate internal salts are explored owing to their potential for improving electrokinetic pumps used to perform miniaturized HPLC separations. The internal salts investigated can be added at high molarity since they are net-neutral, and furthermore show potential for increasing electroosmotic pumping owing to their large positive dielectric increment. Streaming potential measurements of buffered aqueous systems with varying concentrations of ammonioalkyl sulfonate internal salts have been used to measure these dielectric increments, which increase with the length of the alkyl linker. Due to their positive dielectric increments and their decremental effect on solution conductivity, all of the measured species are predicted to improve the pressure generation (up to 85%) and efficiency performance (up to 140%) of electrokinetic pumps when added at 1 M concentration. RP-HPLC separations with an ammonioalkyl sulfonate (TMAPS) have been performed and indicate that separation performance is essentially unaffected by these species. These results indicate the potential for a variety of ammonioalkyl sulfonates to be used to improve electrokinetic pump performance for miniaturized HPLC.
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1. Introduction

Internal salts play important roles as buffers, detergents, and ion-pairing reagents in a variety of separation systems. Here, we investigate specific fluid properties of solutions of several internal salts and observe the effects of these species on reversed-phase HPLC separations. The purpose of this work is to identify species with potential for enhancing the performance of electrokinetic micropumps used for miniaturized HPLC.

Micropumps have received a great deal of recent attention owing to their ability to improve the performance of chemical analysis systems by reducing footprint, reagent volumes, and electrical power needs. Micropumps have been investigated as a means to move fluids and actuate microscale mechanical components [1,2]. In particular, electrokinetic micropumps (EK pumps) have been shown to generate pressures above 57 MPa (8000 p.s.i.) [3] or flow-rates above 1 μl/min [2], making them attractive for miniaturization of HPLC systems [4] and actuation of microscale mechanical components [5].

EK pumps use electroosmosis in charged porous media to mobilize fluid [2,3,6]. EK pumps are ideally suited for micro total-analysis systems, since they can straightforwardly generate controlled flow-

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rates (nl/min or μl/min) and high pressures (>10 MPa) required for chromatographic separations.

Ammonioalkyl sulfonate internal salts are investigated here due to their potential for improving electrokinetic pump performance. Within the pH range in which these species are zwitterionic, the positively-charged ammonium and negatively-charged sulfonate groups each hold localized charges which are separated by an alkyl chain; the separation of these charges leads to a large dipole moment and an increase in the dielectric constant when these species are added to aqueous mixtures. Because these molecules are uncharged in the zwitterionic configuration, they can be used at high molarity without leading to high conductivities or changes in the structure and thickness of the electrical double layer.
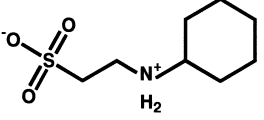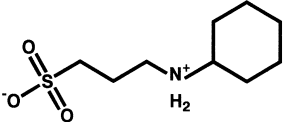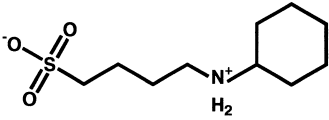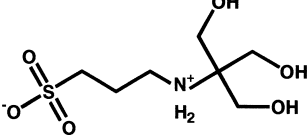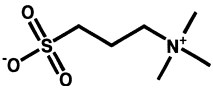
Several ammonioalkyl sulfonates were considered (Table 1), including cyclohexylamino alkyl sulfonates (CHES, CAPS, CABS), tris(hydroxymethyl)methylamino alkyl sulfonates (TAPS) and trialkylammonio alkyl sulfonates (TMAPS). These compounds are all commercially available (all are marketed as biological buffers except TMAPS, which is marketed as an ion-pairing reagent for CE), and form a convenient set of materials for comparison. CHES, CAPS and CABS form a homologous set, allowing effects of linker length to be directly investigated. TMAPS, TAPS, and CAPS all possess a propane linker between ammonium and sulfonate, allowing for elucidation of the relative importance of molecular structure independent of alkyl linker length.

Approximate low-concentration pK_as for the ammonioalkyl sulfonates considered are shown in Table 1. In all cases, the sulfonate pK_a is near 1, while the

Table 1
Molecules considered. Chemical structures are shown in zwitterionic form, the dominant form present in the specified optimal pH range

Internal salt	Structure	Sulfonate pK_a	Ammonium pK_a	Optimal pH range
CHES		1	9.3	4–6.3
CAPS		1	10.4	4–7.4
CABS		1	10.7	4–7.7
TAPS		1	8.4	4–5.4
TMAPS		1	13	4–10

ammonium pK_a ranges from 8.4 to 10.7. We define the optimal pH range as the range in which both moieties are three pH units from their pK_a, such that at least 99.8% of the ammonioalkyl sulfonates are net-neutral, a necessity for practical implementation at high concentration.

In the following sections, we explore the effects of several uncharged ammonioalkyl sulfonate internal salts (often referred to as ammonioalkyl sulfonates here for brevity), with a view toward optimizing HPLC using electrokinetic pumps. Firstly, we report the changes in material properties, specifically conductivity, permittivity, and viscosity, and predict the mechanical effects of these changes on electrokinetic micropump performance. Secondly, we infer chemical and surface interactions by observing the effects of these solutes on reversed-phase separation.

2. Theory

In general, addition of solutes to aqueous solutions changes the solution conductivity, permittivity, and viscosity; and changes the character of electrical double layers at the interface between the solution and solid walls. In addition, through ion-pairing and interaction with walls, solutes may change separation performance and affect analyte–analyte interactions. In this section, analytical relations are presented for electrokinetic pump performance as a function of bulk fluid properties. These relations highlight the potential for ammonioalkyl sulfonate internal salts to lead to improved pump performance.

EK pump systems can be represented by simple equations if a number of key assumptions are made. While these simplified equations exhibit some errors when used to quantitatively predict EK pump performance, they are easily-interpreted and do successfully predict how performance scales with fluidic and geometric properties [7]. Here, we consider a simplified system that assumes that the characteristic pores in the media are large as compared to the thickness of the electrical double layer [8], and that the porous media can be approximated as a system of capillaries with diameters equal to the characteristic pore size. We assume further that surface conductance and convective contributions to charge transport may be ignored. Finally, we assume that bulk fluid

properties are applicable to the double layer. For this case, the performance of electrokinetic pumps may be characterized with three equations [2,7,9]. The maximum pressure per volt generated in an EK pump is given by:

$$\frac{\Delta P_{max}}{V} = \frac{8\varepsilon\varepsilon_0\zeta}{d^2} \tag{1}$$

where ΔP_{max} is the maximum pressure (observed at zero flow-rate), V is the applied voltage, ε_0 is the permittivity of free space, ε is the dielectric constant, ζ is the wall zeta potential, and d is the characteristic pore size of the porous medium. The maximum flow-rate generated per applied volt is given by:

$$\frac{Q_{max}}{V} = \frac{\varepsilon\varepsilon_0\zeta A}{\mu l} \tag{2}$$

where Q_{max} is the maximum flow-rate (observed at zero downstream fluidic resistance), A is the pump open cross-sectional area, μ is the dynamic viscosity, and l is the length of the pump. Finally, we can define the efficiency as:

$$\eta = \frac{Q\Delta P}{VI} = \frac{\Delta P}{V}\frac{Q}{V}\frac{l}{A\sigma} \tag{3}$$

where VI is the applied electrical power and $Q\Delta P$ is the generated mechanical power. σ is the bulk conductivity of the pumped solution. Of these three performance values, efficiency is the most useful metric, since it is independent of geometric design and therefore the most general.

The parameters in Eqs. (1)–(3) can be classified as geometric (A, l), interfacial (ζ), or fluidic (ε, μ, σ). Here we consider modification of fluid properties (ε, μ, σ) through the addition of ammonioalkyl sulfonates in their uncharged, zwitterionic state to aqueous buffers. Since these molecules are stable and uncharged, they are unreactive and do not significantly affect interfacial phenomena. However, they can have a large impact on the viscosity, conductivity, and permittivity of the solution. In addition to the typical viscosity increase observed with high molarity solutes, ammonioalkyl sulfonates generally decrease conductivity by decreasing ion mobility, and increase permittivity due to their large dipole moments. Since the change to the dielectric constant is typically linear with concentration, it is

typically described using a linear dielectric increment $d\varepsilon/dC$:

$$\varepsilon(C) = \varepsilon(0) + \frac{d\varepsilon}{dC}\,C \tag{4}$$

where C is the concentration of solute and the linear dielectric increment is a property of the specific solute–solvent system.

From Eqs. (1)–(4), it is clear that solutes with large positive dielectric increments can have a positive effect on electrokinetic pump performance by increasing the dielectric constant of the fluid. Solutes with high dipole moments (well above that of water) lead to positive dielectric increments because of the additive effects of their dipole moments to the system. Because many ammonioalkyl sulfonates can be added at high molarity ($>1\ M$), they can have a significant effect on the solution permittivity. The charges at the ammonium and the sulfonate groups are localized, and the dipole moments observed correspond roughly to that predicted by isolated unit charges separated by a length defined by the alkyl linker [10]. In the following sections, we will present measurements of the key parameters in Eqs. (1)–(4), and use these measurements to predict the effect of ammonioalkyl sulfonates on EK pump performance. We then show RP-HPLC separations using these additives to show that these additives do not degrade separation performance.

3. Experimental techniques

3.1. Chemicals

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA), except for TMAPS, which was purchased under the trade name Z1-Methyl (Waters, Milford, MA, USA).

3.2. Streaming potential

To enable prediction of EK pump performance using Eqs. (1)–(4), measurements of zeta potential, conductivity, permittivity, and viscosity of buffered aqueous solutions of ammonioalkyl sulfonates in silica capillaries were performed. Solutions of ammonioalkyl sulfonates of varying concentrations

were prepared and buffered at pH within the optimal range specified in Table 1. Phosphate buffers with pH near 7.0 were used for all species except for TAPS, for which citrate buffers with pH near 4.7 were used. Bulk conductivity was measured using a conductivity meter (Corning 441, Corning, NY, USA). Solution viscosities were inferred by observing the upstream pressure (strain-gauge flow-through transducer, Senso-Metrics SP70D, Simi Valley, CA, USA) when a syringe pump (Cole-Parmer 74900, Vernon Hills, IL, USA) was used to induce a controlled 150 μl/min flow-rate through a 0.30-m length of 150 μm I.D. capillary. All measurements were made at room temperature ($20\pm2\ °C$).

The streaming potential induced by fully-developed laminar Poiseuille flow in a silica capillary with thin electrical double layers [8] was used to measure the effect of ammonioalkyl sulfonates on ε of aqueous solutions (Fig. 1). Buffers at controlled cation concentration and pH were prepared, and bulk fluid conductivity and viscosity were measured as described above. The buffer was then used to fill 25–35 cm long, 150 μm I.D. capillaries. Pressure (0–200 p.s.i.) was applied to one end of the test capillary using a syringe pump. Platinized platinum electrodes were connected to an electrometer (Keithley 614, Cleveland, OH, USA), and signals indicating pressure and voltage were acquired by a PC running LabView 5.0 (National Instruments, Austin, TX, USA). Experimental parameters (forcing pressure, capillary diameter, capillary length, electrode capacitance, buffer conductivity) were chosen such that the flow was laminar, surface conductivity could be ignored, hydrodynamic starting length was a
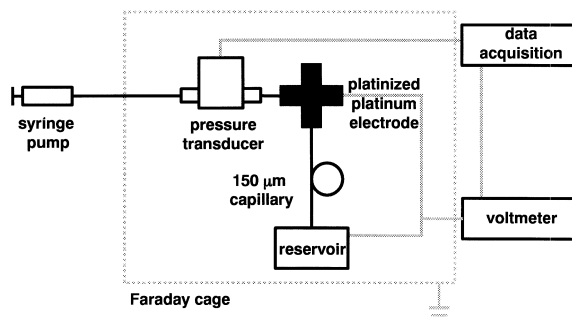

Fig. 1. Streaming potential measurement apparatus.

negligible fraction of the capillary length, and the electrical equilibration time of the system was fast as compared to temporal pressure gradients. Forcing pressure was scanned, and the pressure–voltage relation was fit to a line. With known fluid parameters (μ, σ), linear fits to the pressure–voltage curve give $\varepsilon\zeta$ using the Smoluchowski equation [11]:

$$\varepsilon\zeta = \frac{\mu\sigma}{\varepsilon_0}\frac{\Delta V}{\Delta P} \tag{5}$$

For each buffer (i.e. cation concentration and pH), streaming potential was used to measure $\varepsilon\zeta$ over a range of concentrations of ammonioalkyl sulfonates, and a line was fit to the result. Since the dielectric constant of additive-free buffer is known (80.2 at 20 °C), the intercept of the linear fit (corresponding to zero ammonioalkyl sulfonate concentration) specifies the zeta potential at that pH and cation concentration. The double layer structure and the magnitude of the zeta potential are unaffected by small uncharged species such as the ammonioalkyl sulfonates considered here, therefore ζ may be divided from the slope of the resulting linear fit, resulting in the dielectric increment $d\varepsilon/dC$ of the ammonioalkyl sulfonate in question.

3.3. Reversed-phase HPLC

Reversed-phase HPLC of a five-peptide standard (Sigma H2016) was used to compare separation performance with and without ammonioalkyl sulfonate additives. Separations were performed on a HP 1100 HPLC system (Palo Alto, CA, USA). Compounds were separated on a 16 mm×15 cm Zorbax 3.5μ 300SB-C$_{18}$ column (Agilent, Palo Alto, CA, USA). The column flow-rate was 1.0 ml/min. In all cases, the mobile phase consisted of 10 m*M* acetate pH 4 in a gradient from 0% to 28% acetonitrile in water. Ammonioalkyl sulfonates of varying concentrations were added equally to both elution buffers so as to enable comparison of separation performance with and without additives. Data acquisition and processing were performed using Chemstation (Agilent) and baseline correction was performed using ABSPDF (Sandia National Laboratories, Livermore, CA, USA) [12].
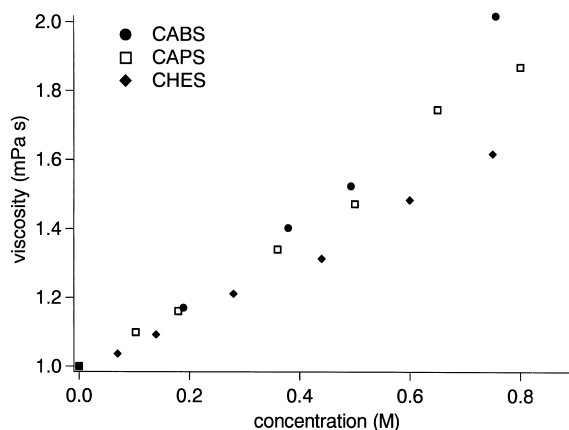

Fig. 2. Observed solution viscosity in aqueous solutions, 10 m*M* phosphate pH 7, of cyclohexylamino alkyl sulfonates.

4. Results

Measured values of viscosity and conductivity are shown in Figs. 2 and 3 for various buffered solutions of cyclohexylaminoalkane sulfonates. From these and measurements of streaming potential, the zeta potential (Table 2) and dielectric constants (Fig. 4) may be inferred. Since the measurements of zeta potential come from linear fits to the observed streaming potential with varying concentrations of ammonioalkyl sulfonates, these data should evince any effects of the additive on the zeta potential.
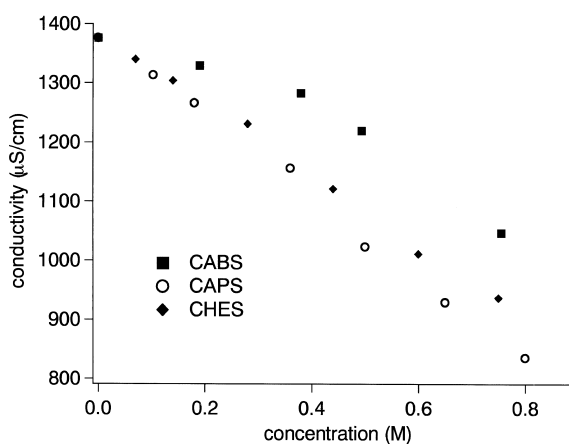

Fig. 3. Observed solution conductivity in aqueous solutions, 10 m*M* phosphate pH 7, with varying concentration of cyclohexylamino alkyl sulfonates.

Table 2
Observed zeta potentials measured using streaming potential at silica surfaces

Internal salt	pH	Zeta potential (mV)	Source
CHES	6.88±0.18	45.9	This work
CAPS	7.04±0.02	45.4	This work
CABS	7.04±0.03	49.1	This work
TMAPS	6.90±0.10	50.0	This work
None	7.0	51.2	[11]

Cation concentration for all solutions is 15 mM K$^+$. Literature value for zeta potential without internal salts is obtained by interpolating between data points from Ref. [11] (10 mM, 100 mM) using the model proposed by Ref. [13].

Table 3
Observed dielectric increments for a variety of ammonioalkyl sulfonates

Molecule	dε/dC (M^{-1})	Source	Technique
TAPS	23±4	This work	Streaming potential
CHES	20±5	This work	Streaming potential
CAPS	38±7	This work	Streaming potential
CABS	68±4	This work	Streaming potential
TMAPS	42±5	This work	Streaming potential
TMAPS	47.6	[7]	EK pump performance
TEAES	42.2	[10]	Dielectric spectroscopy
TEAPS	58.6	[10]	Dielectric spectroscopy
TEABS	72.9	[10]	Dielectric spectroscopy

TEAES and TEABS are ethyl and butyl homologs, respectively, of TEAPS (triethylammoniopropane sulfonate). Intervals listed are for 99% confidence and incorporate statistical uncertainty of linear fit as well as experimental uncertainty in conductivity, viscosity, and voltage measurements.

Since the inferred zeta potential results for several additives match well with each other (5% standard deviation around mean) and with previous results on buffered salt systems [11], these data support our assumption that ammonioalkyl sulfonates do not affect double layer structure.[1] The slope of the linear fits in Fig. 4 give the dielectric increment. Dielectric increments for various ammonioalkyl sulfonate internal salts, both from this and previous works, are tabulated in Table 3. The data for CHES, CAPS, and CABS shows, as has previously been observed [10] with TMAPS homologs, that dielectric increments for homologous ammonioalkyl sulfonate series with
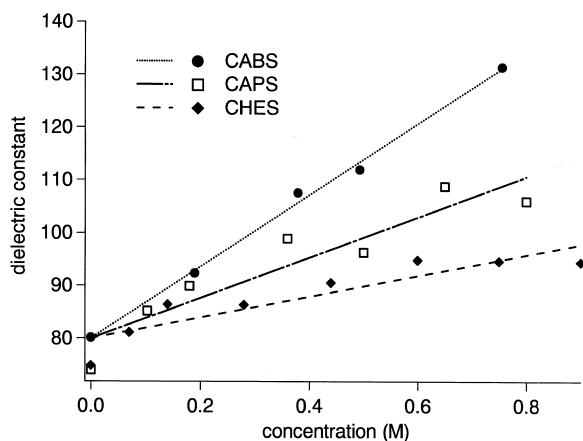

Fig. 4. Observed dielectric constant for aqueous solutions, 10 mM phosphate pH 7, of cyclohexylamino alkyl sulfonates.

[1]Changes in permittivity do lead to a change in the thickness of the electrical double layer; however, in the thin-double-layer limit this effect leads to very small changes in the observed zeta potential.

C$_2$–C$_4$ linkers increase with length of alkyl chain. Results for TMAPS agree to within 11% with previous results [7] using EK pumps.

Having measured the dielectric increments, conductivities, and viscosities of these solutions, it is straightforward to predict (using Eqs. (1)–(4)) the effect that addition of ammonioalkyl sulfonates will have on electrokinetic pump pressure, flow and efficiency. Results are displayed in Fig. 5. In general, all species show improvement in predicted pressure performance due to the $P_{max} \sim \varepsilon$ dependence in Eq. (1). This improvement can be as high as 85% for CABS at 1 M. Here, performance is roughly proportional to the length of the alkyl linker. Flow performance, which scales with ε/μ (Eq. (2)), is dependent on the viscosity effects of the additives. Here, the lower viscosity of TMAPS makes it the best performer (20% increase in predicted flow at 1 M); in fact, all of the cyclohexylaminoalkane sulfonates show decreases in predicted flow performance. Efficiency, which scales with $\varepsilon^2/\mu\sigma$, is universally increased. The prominent effect of conductivity change can be seen here, as TMAPS, CAPS, and CABS all show large predicted efficiency increases, with CAPS showing the largest (140%) increase at 1 M due to its dramatic reduction of conductivity. While the magnitude of various effects changes from species to species, the forms of the predicted performance changes reported here match well with the
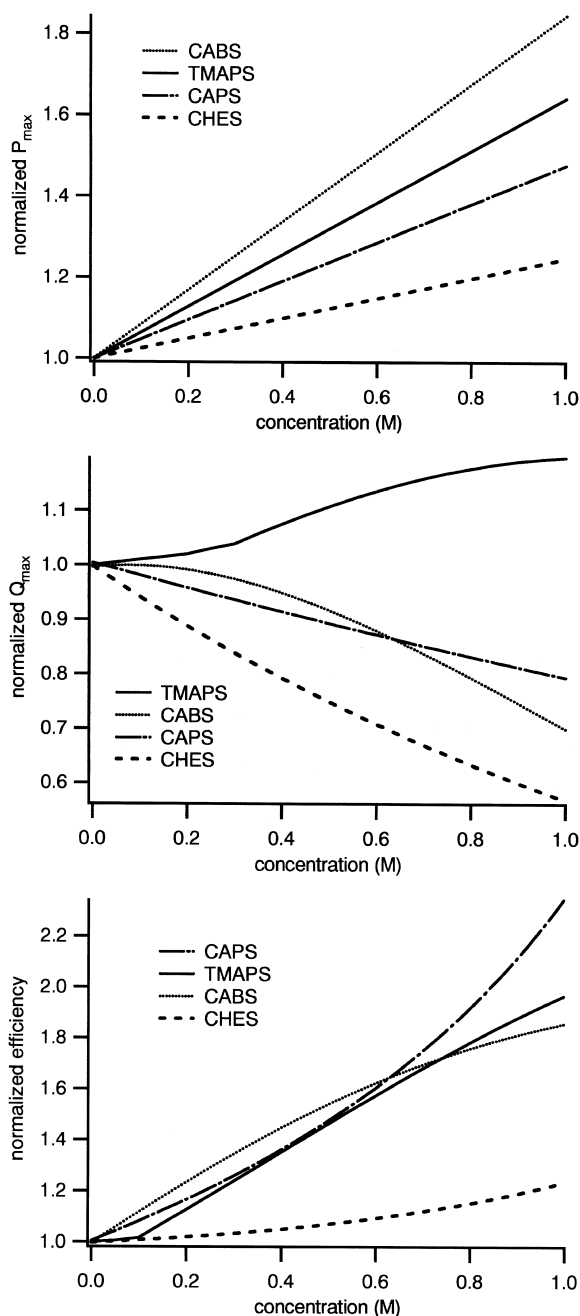
Fig. 5. Predicted EK pump performance as a function of ammonioalkyl sulfonate concentration.
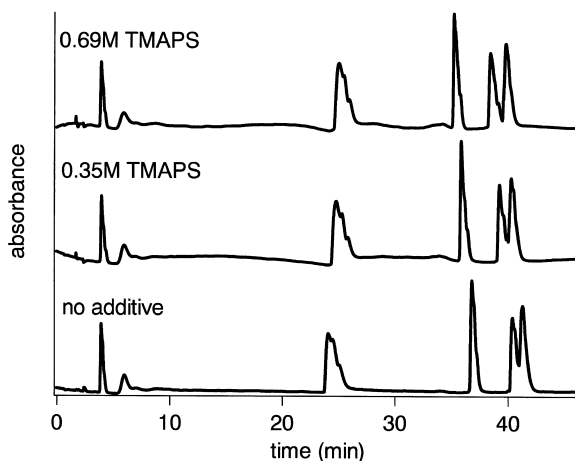

Fig. 6. Baseline-corrected RP-HPLC separation with varying concentrations of TMAPS. See Experimental section for details.

dicted their effect on EK pump performance, the effect of ammonioalkyl sulfonates on reversed-phase HPLC separations were investigated. TMAPS was chosen, as it can be used to improve pressure, flow, and efficiency in the 0–1 M region. RP-HPLC of a five-peptide standard with mobile phases containing varying concentrations of TMAPS are shown in Fig. 6. Here, it can be seen that the effect of the TMAPS additive on separation performance is negligible (Table 4), indicating that TMAPS can be used without interfering with RP-HPLC performance.

5. Discussion

While the simplified theory presented here can successfully predict pump performance [7], it is applicable only if a number of limiting assumptions are made. Eqs. (1)–(3) assume a thin debye layer model and treat the fluidic boundary conditions as a pure slip at the interface with a slip velocity defined by the wall zeta potential. Thin debye layers are also required for the assumed charge transport, which ignores excess surface conductance. Convective contributions to the charge transport are ignored in Eq. (3). These assumptions are all well met if the counterion concentration is large—for example, 1 mM of univalent counterion concentration leads to electrical double layers which are ~3 nm and much

performance changes measured for EK pumps using TMAPS additives [7].

Having observed bulk fluid properties and pre-

Table 4
Summary of separation results for the RP-HPLC separation in Fig. 6

TMAPS conc (M)	$R_{3,4}$	$R_{4,5}$	Normalized t_3	Normalized t_4	Normalized t_5
0	4.58	1.04	1	1	1
0.35	4.73	1.31	0.98	0.97	0.98
0.69	3.51	1.38	0.96	0.95	0.96

$R_{x,y}$: resolution of peaks x and y. Normalized t_i: elution time of peak i, normalized to value at zero TMAPS concentration.

smaller than the typical pore sizes (100–1000 nm) of EK pump media. Eqs. (1)–(3) furthermore assume that bulk properties are applicable in the double layer, and that the low-permittivity monolayer at the surface may be ignored. Since the additives considered here have higher dipole moments than water, the spatial region in which orientational restriction will modify the local permittivity can be expected to be larger upon addition of internal salts than it is for additive-free buffer. Thus, we might expect that this theory would break down at high (>1 M) buffer concentrations as the thickness of the diffuse double layer becomes small as compared to the region of orientational restriction. However, at the sub-molar concentrations considered, this assumption is sound.

The results from the previous section show how permittivity increases resulting from addition of ammonioalkyl sulfonate internal salts can significantly improve miniaturized pump performance, which, when applied to miniaturized separations, improves separation performance. For a given pressure, improved pump performance allows smaller voltages to be used, reducing substrate voltage hold-off requirements, electrolysis, and bubble generation. For a given voltage, increased pressure improves chromatographic performance by allowing separation media with smaller pore size. While the pressure source can be fluidically separated from the separations buffer, practical implementation is simplest if the separations buffers themselves can be pumped. While it is clear that the effect of each ammonioalkyl sulfonate must be confirmed independently before a general conclusion is drawn, results with RP-HPLC separations using TMAPS suggest that high concentrations of some ammonioalkyl sulfonates can be added to RP-HPLC buffers without affecting separations performance.

6. Conclusions

Fluidic and chromatographic effects of ammonioalkyl sulfonate additives on electrokinetic pumping and RP-HPLC separation performance have been evaluated. Streaming potential measurements of buffered aqueous systems with varying concentrations of ammonioalkyl sulfonate internal salts have been used to measure the dielectric increment of these species and predict their effect on electrokinetic pump performance. All measured species show significant positive dielectric increments that increase with the length of the alkyl linker. Due to their positive dielectric increments and decremental effect on solution conductivity, all of the measured species are predicted to improve the pressure generation (up to 85%) and efficiency performance (up to 140%) of electrokinetic pumps when added at 1 M concentration. RP-HPLC separations with one ammonioalkyl sulfonate (TMAPS) have been performed and indicate that separation performance is affected negligibly. These results indicate the potential for a variety of ammonioalkyl sulfonates to be used to improve electrokinetic pump performance for miniaturized HPLC.

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