

# Ion chromatography on reversed-phase materials coated with mixed cationic and nonionic surfactants

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## Abstract

Columns suitable for use in anion chromatography can be prepared by coating a packed reversed-phase HPLC column (C<sub>18</sub> silica or polystyrene particles) with a cationic surfactant. The efficiency is improved dramatically by first coating the column with a nonionic surfactant and then subsequently with the cationic surfactant. The thickness of the first coated layer as well as the chemical structure of the surfactant have a major effect on the column performance. Actual separations are included to demonstrate the convenience and practical use of the coated columns. Using this approach, columns with 12 900 theoretical plates for the 15-cm column (or 86 000 plates/m) were produced, giving well shaped peaks with an average asymmetry factor of 1.09. The coated layers were found to be stable, giving retention times with an average relative standard deviation of 1.6% for 12 consecutive runs.

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**Keywords:** Stationary phase, LC; Dynamically coated column; Surfactants; Nonionic surfactant; Cationic surfactant

## 1. Introduction

Since its introduction in 1975, ion chromatography (IC) has become the dominant analytical method for determining inorganic anions and some organic anions [1]. The original dual-column system, which used a separation column in conjunction with a second column to suppress the background conductance, has undergone many modifications and improvements [2]. However, the separation power of IC columns, measured in theoretical plates, continues to be lower than that of HPLC columns for separation of neutral organic analytes. Thus, improvement in separation efficiency would appear to be both desir-

able and feasible both in suppressed and nonsuppressed IC systems [3,4].

Coating a porous, granular material with a liquid ion-exchanger offers a convenient way to prepare exchangers for use in IC. Static coating of porous poly(styrene-divinylbenzene) or polyacrylate resin with a quaternary ammonium salt [5,6] or latex [7], as well as dynamic and permanent coating of commercial HPLC columns with a surfactant [5,8–10] or dye [11] have been investigated. Although dynamic coating is a convenient and simple process, the coated columns have tended to be only moderately efficient—about 6400–10 000 theoretical plates/m [12,13]. Control of the exchange capacity and separation selectivity has also been difficult to achieve [14].

We now describe rapid, simple methods for

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permanently coating an HPLC column with a cationic surfactant to produce an efficient column for separation of anions by IC. Importantly, coating with both a nonionic surfactant as well as a cationic surfactant was found to improve separation efficiency and to reduce the time required for separation. Performance data are presented and the likely mechanism is discussed.

## 2. Experimental

### 2.1. Reagents

Acetonitrile was of HPLC grade from Merck (Darmstadt, Germany). The eluent additives and analyte chemicals were of analytical reagent grade and were used as obtained from Aldrich (Milwaukee, WI, USA) or Ajax (Auburn, Australia). All eluents were prepared daily. Stock solutions were used to prepare all sample solutions by dilution with the mobile phase. A Millipore Milli-Q water system (Millipore, Milford, MA, USA) was used to further deionise distilled water for all eluents and sample mixtures.

### 2.2. Instrumentation

The ion chromatograph used in these experiments was a Dionex (Sunnyvale, CA, USA) Model DX600 column instrument equipped with a GS50 gradient pump, an AS50 Thermal compartment, an AS50 autosampler, a UI 20 universal interface, and a Waters 484 tunable absorbance detector. Data were acquired by using PEAKNET 6.2 software installed on a P-III 550 computer. The separation columns used were a Dionex IonPac NS1 (150×4 mm I.D.) polymeric column, Dionex Acclaim 120 C<sub>18</sub> (100×4.6 mm I.D.), Waters (Milford, MA, USA) Nova-Pak C<sub>18</sub> (150×3.9 mm I.D.), Phenomenex (Torrance, CA, USA) Synergi 4 μm Max-PR 80 Å C12 (150×4.6 mm I.D.) and CERI (Chemicals Evaluation and Research Institute, Japan) L-column ODS (150×4.6 mm I.D.)

### 2.3. Coating procedure

Dynamically coated columns were prepared using

commercial reversed-phase columns from Dionex and Waters. The columns were coated by passing solutions of 5 mM didodecyltrimethylammonium bromide (DD), 5 mM cetylpyridinium chloride (CPC), or 5 mM solutions of a nonionic surfactant (Triton X100, Tween 20 or Brij 35) at 1 ml/min for 1–2 h. In some cases, coating with a mixed solution of ionic and nonionic surfactant was employed. If desired, the progress of coating could be monitored continuously by a UV–Vis detector at 200 nm.

Separations on columns coated with DD were performed with 5.0 mM sodium perchlorate at a flow-rate of 2.0 ml/min with direct UV detection of sample anions. Separations on the other columns were performed with 10 mM sodium carbonate as the eluent, a flow-rate 1.0 ml/min, and a suppressed conductivity detection system.


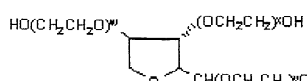
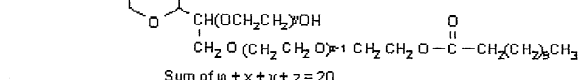


The separation column was equilibrated with eluent until the baseline was stabilised. Sample injection was made at this point. The suppressor was operated at 50 mA using the recycle mode.

Retention factors,  $k'$ , were calculated according to the expression:  $k' = (t_R - t_0)/t_0$ . The system dead time,  $t_0$ , used to calculate the retention factor  $k'$ , was measured by injecting water onto the system. Peak asymmetry ( $A$ ) was calculated using  $A = (RW5\% + LW5\%)/(2 \times LW5\%)$ , where RW5% and LW5% are widths of the right and left portions of the peak at 5% of the peak height. Theoretical plates were calculated using the half-height method.

## 3. Results and discussion

The chemical structures of the surfactants used in this research are given in Table 1. Based on previous reported success [10], didodecyltrimethylammonium bromide (DD) was used first to coat commercial HPLC columns. Sodium perchlorate (5.0 mM) was found to be the most efficient eluent with analyte detection at 200 nm. Fig. 1 shows good separation of acetate, nitrite, bromide, nitrate and oxalate but the peaks for the organic anions and thiocyanate were broad and had long retention times. Coating the same column with a mixture of 5 mM DD and 2 mM Tween 20 resulted in much shorter retention times for the organic anions while maintaining reasonable peak resolution (Fig. 2).

Table 1  
Nonionic and cationic surfactants utilized for coating columns

Abbreviation	Name	Structure	Average $M_n$
Triton X-100	Polyoxyethylene (10) isoocetylphenyl ether	$C_8H_{17}$ —  — $(OCH_2CH_2)_nOH$ $n \sim 10$ 	
Tween 20	Polyoxyethylene (20) sorbitan monolaurate	 $CH_2O(CH_2CH_2O)_xCH_2CH_2O-C(=O)-CH_2(CH_2)_8CH_3$ Sum of $w + x + y + z = 20$	1228
Brij 35	Polyoxyethylene (23) lauryl ether	$C_{12}H_{25}(OCH_2CH_2)_nOH$ $n \sim 23$	ca. 1198
CPC	Cetylpyridinium chloride	 $Cl^-$ $\cdot H_2O$	340
DD	Didodecyltrimethylammonium bromide	 $CH_2(CH_2)_{14}CH_3$	462.6

Retention times for anions are given as a function of the concentration of Tween 20 in the coating solution in Table 2, using the ODS L-column and the Dionex NS1 column. There was a sharp drop in

retention times between 0 and 1 mM Tween 20 and then a slower decrease up to 5 mM Tween 20. The percentage reduction in retention times was particularly large for polarizable anions such as bromide, nitrate, molybdate and chromate. Some hydrophobic species, such as benzoate, and phthalate showed an initial decrease in retention, followed by a small increase at higher concentrations of Tween 20.

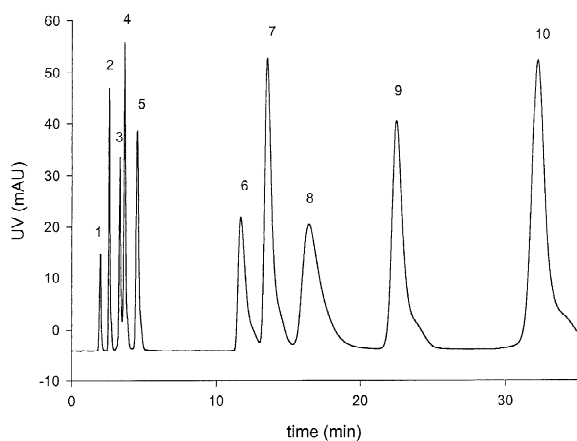


Fig. 1. Separation of a standard solution containing different inorganic and organic anions. Conditions: column: L-column ODS coated with 5 mM didodecyltrimethylammonium bromide; eluent: 5 mM  $NaClO_4$ ; flow-rate, 2.0 ml/min; detection, UV at 200 nm; sample: 100  $\mu$ l containing 30  $\mu$ g/ml acetate, 2  $\mu$ g/ml nitrite, 2  $\mu$ g/ml bromide, 2  $\mu$ g/ml nitrate, 2  $\mu$ g/ml 4-hydroxybenzoate, 30  $\mu$ g/ml oxalate, 30  $\mu$ g/ml molybdate, 10  $\mu$ g/ml benzoate, 10  $\mu$ g/ml phthalate, 30  $\mu$ g/ml thiocyanate. Peaks: 1=acetate; 2= nitrite; 3=bromide; 4=nitrate; 5=oxalate; 6=4-hydrobenzoate; 7=molybdate; 8=benzoate; 9=phthalate and 10=thiocyanate.

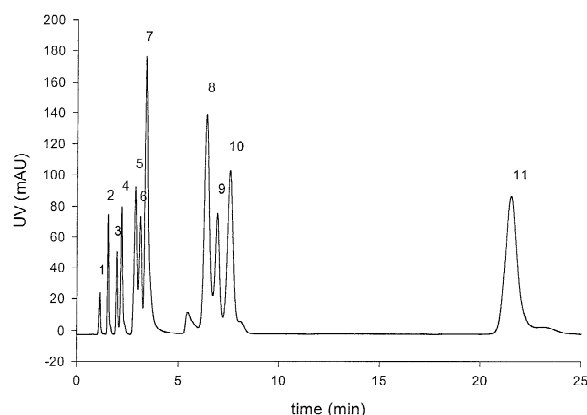


Fig. 2. Separation of a standard solution containing different inorganic and organic anions. Same conditions as Fig. 1 except the column was coated with a mixture of 5 mM didodecyltrimethylammonium bromide and 2 mM Tween 20. Peaks: 1=acetate; 2= nitrite; 3=bromide; 4=nitrate; 5=oxalate; 6=4-hydrobenzoate; 7=molybdate; 8=iodide; 9=phthalate; 10=benzoate and 11= thiocyanate.

Table 2

Retention times ( $t_R$ ) of anions on coated columns using 2 ml/min 5 mM NaClO<sub>4</sub> (L-column ODS) and 1.5 ml/min 2 mM NaClO<sub>4</sub> (Dionex NS1 column) as eluent. Each column was coated with the indicated concentration of Tween 20 mixed with 5 mM DD

Anion [Tween 20] used in coating solution (mM)	$t_R$ (min)									
	L-column ODS					Dionex NS1 column				
	0	1.0	2.0	3.0	5.0	0	3.0	5.0	10.0	
Acetate	1.92	1.18	1.26	1.03	1.082	2.72	1.37	0.97	1.06	
Nitrite	2.62	1.74	1.63	1.82	1.45	3.84	1.95	1.39	0.95	
Bromide	3.35	2.15	2.19	2.03	1.68	4.87	2.48	1.78	0.95	
Nitrate	3.71	2.36	2.45	2.193	1.88	5.6	2.95	2.12	1.05	
4-Hydroxybenzoate	4.57	4.34	3.44	3.6	3.96	7.15	3.98	3.24	3.28	
Oxalate	12.0	7.00	3.05	2.45	2.54	38.7	5.67	2.29	3.97	
Tungstate	13.3	3.22	3.25	3.5	5.52	32.0	6.28	2.61	3.89	
Chromate	13.2	5.13	4.5	5.17	3.64	17.0	7.08	6.80	3.88	
Molybdate	13.8	5.97	3.58	4.32	3.20	47.3	6.41	2.39	1.50	
Iodide	15.6	7.49	7.07	6.17	4.58	19.9	11.9	9.06	2.72	
Benzoate	16.4	14.3	7.8	7.9	9.98	22.3	9.86	6.71	6.59	
Phthalate	23.2	16.6	7.26	8.69	9.76	48.9	12.2	5.15	3.93	
Thiocyanate	32.2	23.7	21.4	18.5	13.8	58.5	38.1	33.4	8.90	

This behaviour can be attributed to hydrophobic absorption onto the layer of Tween 20, which would not be expected to occur for hydrophilic ions such as acetate.

A potential disadvantage in coating prepacked columns was that the coating expands the volume of the solid particles in the column and can result in a higher back-pressure. DD coating solutions are cloudy and appear to give a relatively thick coated layer. In one case repeated coating, removal of coating and re-coating resulted in the creation of a void in the column packing at the inlet end owing to expansion and contraction of the packing. Attempts to obtain a thinner coating layer by adding 20% (v/v) of acetonitrile to the coating solution resulted in an IC column of significantly lower efficiency.

In the next series of experiments, CPC was selected for use because it is readily soluble in water and gives a clear solution for coating. CPC has previously been used successfully to create columns for anion chromatography [5]. Several nonionic surfactants were also tested to modify the IC performance of coated columns: Triton X100, Tween 20 and Brij 35. Of these, Triton X100 was the most promising.

When a packed column was treated with a mixture of a cationic surfactant and a nonionic surfactant, the coating presumably consisted of a mixture of the two

surfactants in a single adsorbed layer. However, we found that coating first with a layer of a nonionic surfactant and then subsequently with the cationic surfactant CPC created a more efficient column. It is noteworthy that a column coated in this way gave shorter retention times and sharper peaks than one coated with CPC alone. However, coating first with CPC and then with a nonionic surfactant resulted in a column with almost no retention of anionic analytes. The ion-exchange capacity of the CPC layer was evidently rendered inactive by the outer layer of nonionic surfactant.

Performance data for several coated columns are summarised in Table 3. Columns 1 and 2 were coated in layers using aqueous solutions of Triton X-100 and CPC. Columns were coated with a solution of the nonionic surfactant in 30% acetonitrile in water, and then with an aqueous solution of CPC to form the second layer. This procedure resulted in a thinner first layer and gave a column with better performance. Column 1 with an average plate number  $N=7300$  and column 3 with  $N=6200$  were by far the best columns. For comparison, average  $N$  values for the other columns were: Column 2,  $N=920$ ; column 4,  $N=2600$ ; column 5,  $N=3030$ ; column 6,  $N=2800$ . The average retention time was lowest for column 3 (5.6 min). This compared to an average of 7.1 min on the same

Table 3  
Retention times ( $t_R$ ) of anions on coated columns using 10 mM sodium carbonate as eluent

Anion Column <sup>a</sup>	$t_R$ (min)					
	1	2	3	4	5	6
Fluoride	1.97	2.12	1.63	1.53	1.62	1.90
Acetate	2.74	3.32	2.13	1.74	1.88	2.45
Formate	3.18	4.00	2.30	1.93	1.98	2.73
Fluoroacetate	4.44	6.46	2.78	1.88	2.20	3.25
Chloride	5.93	8.63	7.17	2.41	2.61	3.95
Nitrite	9.45	15.8	4.66	3.08	3.49	5.84
Phosphate	13.3	17.0	5.51	3.08	3.68	6.53
Difluoroacetate	15.2	28.9	7.57	3.38	4.60	8.60
Bromide	17.8	32.5	7.44	5.06	5.34	9.58
Sulfate	18.1	25.8	8.87	4.37	5.34	11.7
Nitrate	25.2	54.5	10.6	6.68	7.27	13.7

<sup>a</sup> Columns: 1=Dionex C<sub>18</sub> (10 cm), Triton X-100–CPC coating; 2=Dionex NS1 (15 cm), Triton X–CPC; 3=Waters NovaPak C<sub>18</sub> (15 cm), Triton (30% ACN)–CPC; 4=Waters NovaPak C<sub>18</sub>, Tween 20 (30% ACN)–CPC; 5=Waters NovaPak C<sub>18</sub>, Brij 35 (35% ACN)–CPC; 6: Waters NovaPak C<sub>18</sub>, CPC coating.

column coated only with CPC and an average of 11.6 min on column 1. The asymmetry factors were also lower on column 3.

An excellent separation of several inorganic anions on column 1 with 10 mM sodium carbonate eluent is shown in Fig. 3. However, bromide and sulphate were only partially resolved and 4-hydroxybenzoate required >70 min for elution. Fig. 4 shows a separation of acetate and three fluoroacetates on the same column. Column 3, which was coated with Triton X-100 from a solution containing 30% acetonitrile gave separations that were significantly faster (Fig. 5). Nine of the ten anions were eluted within 11 min; the peaks were sharp and well resolved except for oxalate and nitrate.

The most efficient column for anion chromatography was prepared by coating a Phenomenex Synergi column with 5 mM Triton X100 from 30% acetonitrile and then aqueous 5 mM CPC. The eluent was 2 mM sodium perchlorate and direct UV detection at 210 nm was used. The performance data in Table 4 show an actual average plate number of 12 900 for the 15-cm column or 86 000 plates/m. The peaks were well shaped with an average asymmetry factor of 1.09. A chromatographic separation is shown in Fig. 6, and the performance of this column in comparison to a column coated only with a cationic surfactant can be seen by comparing Figs. 1 and 6. Similar chromatographic separations were obtained using a carbonate eluent and suppressed

conductivity detection, with only a slight reduction in efficiency attributable to peak broadening in the suppressor.

The stability and reproducibility of retention times for these columns was excellent. For 13 consecutive

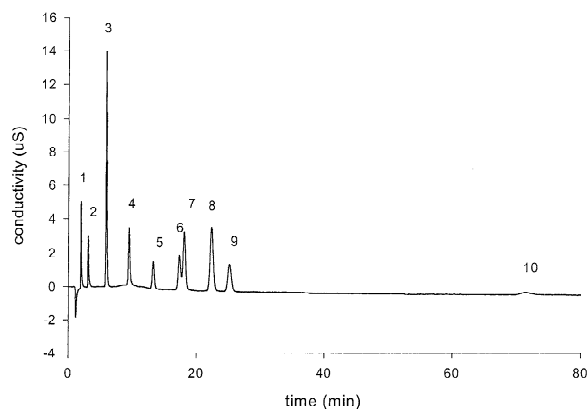


Fig. 3. Separation of a standard solution containing different inorganic and organic anions. Conditions: column: Dionex Acclaim 120 C<sub>18</sub> 5  $\mu$ m 120  $\text{\AA}$  (4.6 $\times$ 100 mm I.D.) coated first with 5 mM Triton X-100 and then 5 mM CPC; eluent: 10 mM Na<sub>2</sub>CO<sub>3</sub>; flow-rate, 1.0 ml/min; detection, suppressed conductivity with suppressor operated at 50 mA in the recycle mode; sample: 50  $\mu$ l containing 2  $\mu$ g/ml fluoride, 10  $\mu$ g/ml formate, 5  $\mu$ g chloride, 5  $\mu$ g/ml nitrite, 5  $\mu$ g/ml bromide, 5  $\mu$ g/ml nitrate, 10  $\mu$ g/ml phosphate, 10  $\mu$ g/ml sulphate, 20  $\mu$ g/ml 4-hydroxybenzoate, 20  $\mu$ g/ml oxalate. Peaks: 1=fluoride; 2=formate; 3=chloride; 4=nitrite; 5=phosphate; 6=bromide; 7=sulphate; 8=oxalate; 9=nitrate and 10=4-hydrobenzoate.

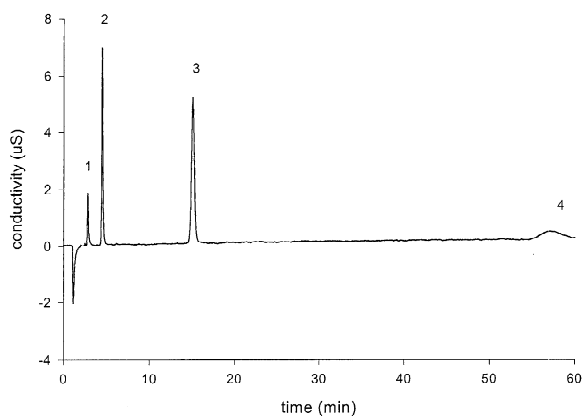


Fig. 4. Separation of a standard solution containing acetate and fluoroacetates. Conditions: column: Dionex Acclaim 120  $C_{18}$  5  $\mu\text{m}$  120  $\text{\AA}$  ( $4.6 \times 100$  mm I.D. coated first with 5 mM Triton X-100 and then 5 mM CPC; eluent: 10 mM  $\text{Na}_2\text{CO}_3$ ; flow-rate, 1.0 ml/min; detection by suppressed conductivity with suppressor operated at 50 mA in the recycle mode; sample: 50  $\mu\text{l}$  containing 10  $\mu\text{g/ml}$  acetate, 10  $\mu\text{g/ml}$  fluoroacetate, 10  $\mu\text{g/ml}$  difluoroacetate, 50  $\mu\text{g/ml}$  trifluoroacetate. Peaks: 1=acetate; 2=fluoroacetate; 3=difluoroacetate; 4=trifluoroacetate.

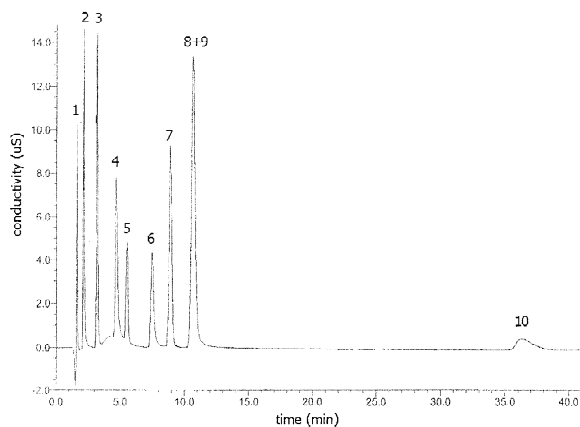


Fig. 5. Separation of a standard solution containing different inorganic and organic anions with carbonate eluent using a multicoated column. Conditions: column: Waters Nova-Pak  $C_{18}$  ( $150 \times 3.9$  mm I.D.) coated first with 5 mM Triton X-100 with 30% ACN and then 5 mM CPC; eluent: 10 mM  $\text{Na}_2\text{CO}_3$ ; flow-rate, 1.0 ml/min; detection, suppressed conductivity operated at 50 mA in the recycle mode; sample: 50  $\mu\text{l}$  containing 2  $\mu\text{g/ml}$  fluoride, 10  $\mu\text{g/ml}$  formate, 5  $\mu\text{g/ml}$  chloride, 5  $\mu\text{g/ml}$  nitrite, 5  $\mu\text{g/ml}$  bromide, 5  $\mu\text{g/ml}$  nitrate, 10  $\mu\text{g/ml}$  phosphate, 10  $\mu\text{g/ml}$  sulphate, 20  $\mu\text{g/ml}$  4-hydroxybenzoate, 20  $\mu\text{g/ml}$  oxalate. Peaks: 1=fluoride; 2=formate; 3=chloride; 4=nitrite; 5=phosphate; 6=bromide; 7=sulphate; 8=oxalate; 9=nitrate; 10=4-hydroxybenzoate.

Table 4

Performance data for Phenomenex Synergi column ( $4.6 \times 150$  mm) coated with Triton X-100 in 30% acetonitrile and then with aqueous cetylpyridinium chloride; eluent 2.0 mM sodium perchlorate; UV detection at 210 nm

Ion	$t_R$ (min)	Asymmetry factor (A)	Theoretical plates per column (N)
Acetate	3.48	0.94	4900
Nitrite	4.55	1.03	12 700
Bromide	5.39	0.99	15 600
Nitrate	6.21	1.03	14 500
Oxalate	12.6	1.30	12 300
Iodide	13.7	1.13	16 700
4-Hydroxybenzoate	15.0	1.08	12 500
Chromate	16.3	1.26	13 300
Thiocyanate	38.8	1.00	14 900
Phthalate	40.9	1.09	11 700

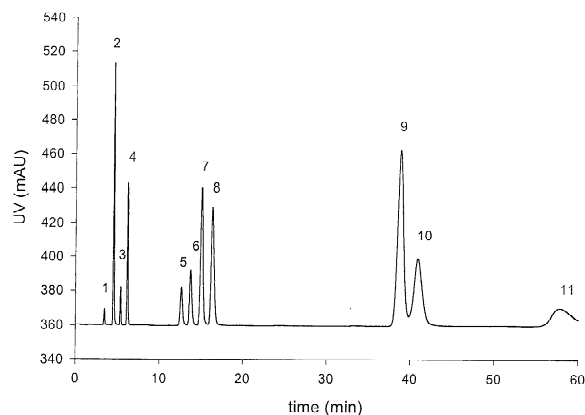


Fig. 6. Separation of a standard solution containing different inorganic and organic anions. Conditions: column: Phenomenex Synergi 4  $\mu\text{m}$  Max-PR 80  $\text{\AA}$  ( $4.6 \times 150$  mm I.D.) coated first with 5 mM Triton X-100 with 30% ACN and then 5 mM CPC; eluent: 2 mM  $\text{NaClO}_4$ , flow-rate, 1.0 ml/min; detection, UV detection at 210 nm; sample: 50  $\mu\text{l}$  containing 30  $\mu\text{g/ml}$  acetate, 2  $\mu\text{g/ml}$  nitrite, 2  $\mu\text{g/ml}$  bromide, 2  $\mu\text{g/ml}$  nitrate, 2  $\mu\text{g/ml}$  4-hydroxybenzoate, 30  $\mu\text{g/ml}$  oxalate, 30  $\mu\text{g/ml}$  chromate, 10  $\mu\text{g/ml}$  benzoate, 10  $\mu\text{g/ml}$  phthalate, 30  $\mu\text{g/ml}$  thiocyanate. Peaks: 1=acetate; 2=nitrite; 3=bromide; 4=nitrate; 5=oxalate; 6=iodide; 7=4-hydroxybenzoate; 8=chromate; 9=thiocyanate; 10=phthalate and 11=benzoate.

in-day runs on column 1, the relative standard deviation of retention times for fluoride, formate, chloride, nitrite, phosphate, bromide, sulphate, oxalate and nitrate were 0.6, 0.95, 1.15, 1.3, 2.0, 1.4, 2.8, 3.0 and 1.2%, respectively, for an average of 1.6%.

#### 4. Conclusions

The performance of an ion-exchange column relies on rapid equilibration between the eluent anion and the various analyte anions at each exchange site on the resin. These equilibria are affected strongly by electrostatic effects, such as attraction of the various anions for the ion-exchanger matrix as well as by the various electrostatic attractions of anions for the positive functional groups on the resin. Addition of an organic solvent has been used in the past as a means to reduce interactions with the resin matrix that could cause peak tailing and/or peak broadening. We believe that the nonionic surfactant acts in a similar manner, perhaps by providing an intermediate phase existing between the functional group (residing in the adsorbed layer of cationic surfactant) and the resin substrate.

In a study using anion-exchange columns prepared by coating neutral resin particles with CPC, the retention times of analyte anions were found to be influenced by the chemical nature of the particles [5]. Thus, the retention times of bromide, nitrate and iodide, relative to that of chloride, increased substantially going from Rohm and Haas XAD-1 (polystyrene) to XAD-8 (polyacrylate), while the relative retention times of sulphate and thiosulphate decreased. These findings demonstrate that the underlying material in coated columns also affects chromatographic behaviour in ion chromatography. Our present study provides further confirmation of this hypothesis. Ion-exchange retention behaviour is determined by both the lower neutral surfactant layer and the upper charged surfactant layer acting in concert, and not by the upper layer alone.

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