

CHROM. 22 621

Alkanesulphonates as eluents for the determination of nitrate and nitrite by ion chromatography with direct UV detection

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(First received February 27th, 1990; revised manuscript received May 13th, 1990)

ABSTRACT

A homologous series of alkanesulphonates were investigated for use as eluents in the ion chromatography of nitrate and nitrite with direct UV detection. The alkyl chain length has a large positive effect on elution strength when a poly(styrene–divinylbenzene)-based ion-exchange column is used. This effect is much less apparent with silica-based columns. Sulphonates from methane- to 1-hexanesulphonate gave excellent separations of nitrate and nitrite, allowing the determination of either without interference in the presence of a 1000-fold molar excess of the other, and making a 1-min assay for nitrate and nitrite possible. The alkanesulphonates can also be used with conductivity detection, allowing the specificity of UV detection to be combined with the versatility of conductivity measurements.

INTRODUCTION

High-performance liquid chromatography (HPLC) is a widely used technique for the determination of inorganic anions [1,2]. As only a few anions show appreciable UV absorbance in the available region of the spectrum, conductivity detection has most commonly been used. A variety of anion-exchange columns are available which give excellent resolution with aromatic eluents such as phthalate, which are commonly used in single-column ion chromatography with conductivity detection. However, the use of optical detection for direct measurements of UV-absorbing ions such as nitrate and nitrite is prevented by the high background absorbance of aromatic eluents. Direct measurements have two advantages in the determination of nitrate and nitrite: they allow the use of standard HPLC apparatus with UV detectors, and they avoid potential interferences from other ions with low UV absorbance. There is therefore a need for information about alternative eluents that can be used with optical detection. Ideally these would be effective at low concentrations so that they could also be used with conductivity detection.

The alkanesulphonates provide a series of potential eluents with low UV absorbance and a number of other desirable properties. They are anionic over a wide pH range and form a homologous series with varying degrees of hydrophobic character. They are non-volatile and readily available in pure form.

Methanesulphonate was used together with a silica-based anion-exchange column as an eluent for the HPLC of anions by Ivey [3], who reported the separation of

several anions, including nitrite and nitrate, using low concentrations (2–10 mM), and was able to use both conductivity and absorbance measurements. However, higher concentrations were necessary in subsequent investigations with different columns, varying from 11 mM chloromethanesulphonate [4] and 12–20 mM methanesulphonate [5] to 200 mM methanesulphonate [6] with silica-based columns of different capacities. The highest concentrations were reported to cause column damage [6]. High concentrations of methanesulphonate (30 mM) were also used with a polymethacrylate-based column [7]; in contrast, Jackson [8] obtained a good separation of nitrate and nitrite with low concentrations (*ca.* 3 mM) of 1-heptane- and 1-octanesulphonate using the same type of column. This suggests that the longer chain alkanesulphonates might be valuable eluents for ion chromatography.

We report here a comparison of the effects of a series of alkanesulphonates with different alkyl chain lengths on the separation of nitrate and nitrite on commercially available low-capacity resin- and silica-based anion-exchange columns.

EXPERIMENTAL

HPLC system

An HPLC system with a Kontron 420 pump, a Kontron 460 autosampler and a Kontron 432 UV–visible detector set at 210 nm was used. In experiments with conductivity detection the pump and autosampler were connected in series with a Kontron 430 UV–visible detector and a Tecator 6200 ion analyzer. In both instances a Kontron 450 data system was used for control of the apparatus and collection and treatment of the results. The autosampler was operated with the injection volume set at 5, 10 or 50 μl .

Columns

A Wescan Anion/R column (250 mm \times 4.6 mm I.D.), the material being a poly(styrene–divinylbenzene)-based anion exchanger with a capacity of 0.2 mequiv./g, was used together with an Alltech Anion/R precolumn (10 mm \times 4.6 mm I.D.). For comparison, a Tecator Anion/Si silica-based anion-exchange column (250 mm \times 4.6 mm I.D.) with an Alltec Anion/S precolumn (10 mm \times 4.6 mm I.D.) and a Vydac 300IC polymer-protected silica-based anion-exchange column (50 mm \times 4.6 mm I.D.) were used with some eluents. A Wescan Anion/R precolumn (30 mm \times 4.6 mm I.D.) was used as an analytical column for the development of a rapid assay for nitrate and nitrite. The column temperature was maintained at 30°C in all instances.

Mobile phases

Methanesulphonic acid and the sodium salts of methane-, 1-butane-, 1-hexane- and 1-octanesulphonic acid were obtained from Aldrich; the last three were of the quality recommended for use as ion-pairing reagents. Sodium ethanesulphonate was of the highest quality available from TCI Tokyo Kasei. 2-(N-Morpholino)ethanesulphonic acid (MES) and tris(hydroxymethyl)aminomethane (Tris) were obtained from Sigma. All eluents used with the resin column were buffered with 0.5 mM Tris–0.25 mM methanesulphonic acid (pH \approx 8.2). Eluents for use with the silica-based columns were buffered with 0.5 mM MES–0.25 mM NaOH (pH \approx 6.2). Distilled water for eluent preparation was filtered through a Millipore Milli-Q system with

a 0.22- μm exit filter before the addition of sodium sulphonates, as vacuum filtration of the eluents after the addition of the long-chain sulphonates results in excessive foam formation. All eluents were degassed under vacuum on an ultrasonic bath before use.

Analysis of results

For singly charged eluents and analytes the expected relationship between eluent concentration, C , and capacity factor, k' , is given by $Ck' = \text{constant}$ [9]. From this it is possible to derive a relationship between retention time, t_R , and C :

$$t_R = (a t_M)/C + t_M \quad (1)$$

with two unknowns, a , which is equal to Ck' , and t_M , which is the time for transit of the mobile phase. Values of Ck' for different eluents were calculated by fitting the experimental values for retention time at different eluent concentrations to eqn. 1 using non-linear regression.

RESULTS

The chain length of alkanesulphonates has a large effect on the efficiency with which anions can be eluted from the poly(styrene-divinylbenzene) anion-exchange column. Fig. 1 shows the effects of the concentration of methane-, ethane-, 1-butane- and 1-hexanesulphonate on the retention times of nitrate and nitrite at a fixed flow-rate. Octanesulphonate was even more effective than hexanesulphonate, but the concentrations required were so low that deformation of the peak shape was observed as a result of column overloading when 5 nmol nitrate and 5 nmol nitrite were injected (results not shown). Perchlorate was a slightly weaker eluent than butanesulphonate but more effective than ethanesulphonate.

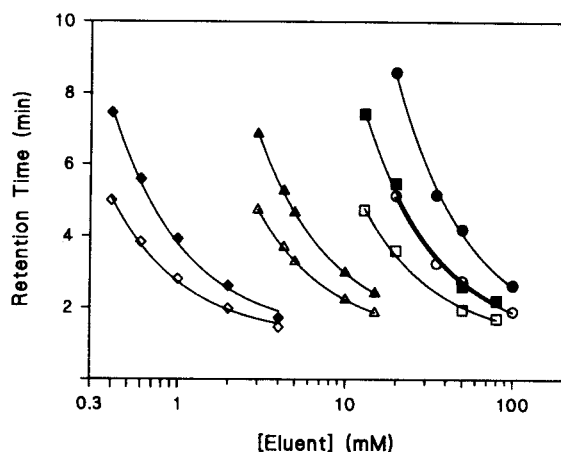


Fig. 1. Retention times for nitrate (solid symbols) and nitrite (open symbols) at different sodium alkanesulphonate concentrations; the flow-rate was 2 ml/min. The column used was a 25-cm Wescan Anion/R with an Alltec Anion/R precolumn. In addition to sodium alkanesulphonate, the eluent contained 0.5 mM Tris-0.25 mM methanesulphonic acid. \circ, \bullet = Methanesulphonate; \square, \blacksquare = ethanesulphonate, $\triangle, \blacktriangle$ = 1-butanesulphonate; \diamond, \blacklozenge = 1-hexanesulphonate. The lines through the experimental points were obtained by fitting the results to the theoretical relationship (eqn. 1) between retention time and eluent concentration.

The greater elution strength of the higher homologues can be explained by interactions between the hydrophobic alkyl chain and the poly(styrene-divinylbenzene) matrix, and might not be observed with other kinds of column material. The behaviour of methane- and 1-hexanesulphonate was therefore investigated with two different types of silica-based column. Table I shows the elution strength of the alkanesulphonates as the product of eluent concentration and the capacity factor for nitrate. This product was found by fitting experimental results to the theoretical relationship between retention time and eluent concentration (eqn. 1), and is a measure of the eluent concentration needed to obtain a capacity factor of 1 for the anion considered. While 1-hexanesulphonate as eluent was 68 times stronger than methanesulphonate when the poly(styrene-divinylbenzene)-based column was used, the elution strength was increased only 1.2 times when the silica-based column was used and 2.0 times with the polymer-protected silica column.

The resolution of nitrate and nitrite obtained with the poly(styrene-divinylbenzene) column also depends on the type and concentration of the eluent. Fig. 2 shows the relationship between the capacity factor obtained with different eluent concentrations and the resolution obtained. The best resolution was obtained with either methanesulphonate or with ethanesulphonate (results not shown). The results for 1-butanedisulphonate (not shown) were between those for methane- and 1-hexanesulphonate. In addition to the effect of chain length on the resolution, there was also the expected increase in resolution when the capacity factor was increased by lowering the concentration of the eluent. However, even in the worst cases, the resolution observed was always more than adequate, and other criteria such as the analysis time and the eluent concentration can be considered when selecting analytical conditions.

The use of high concentrations of eluent produces the risk of greater contamination by impurities present in the reagents; this was a problem with the quality of the ethanesulphonate used, which gave rise to a higher background absorbance than with the other eluents investigated. It is also possible that high eluent concentrations could lead to column damage and microbial growth, although

TABLE I

ELUTION STRENGTH OF ALKANESULPHONATES WITH DIFFERENT TYPES OF COLUMN

Results were obtained by fitting the data from a series of experiments with different eluent concentrations to the theoretical relationship between retention time and concentration as described under Experimental.

Column	Type	Product of eluent concentration and capacity factor for nitrate (mM)	
		Methanesulphonate ^a	1-Hexanesulphonate
Wescan Anion/R	Resin	130	1.9 ^b
Vydac 300IC	Silica	113	56 ^c
Tecator Anion/Si	Silica	14	12 ^d

^a Eluent concentrations from 15 to 100 mM were used.

^b Eluent concentrations from 0.4 to 4 mM were used.

^c Ck' was calculated using the observed capacity factor from a single experiment where the eluent concentration was 10 mM.

^d Ck' was calculated from a single experiment where the eluent concentration was 5 mM.

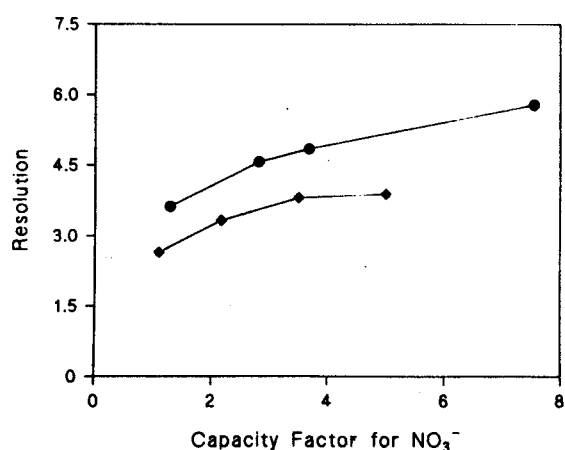


Fig. 2. Relationship between capacity factor for nitrate and resolution of the nitrate and nitrite peaks obtained with different concentrations of (●) methanesulphonate and (◆) 1-hexanesulphonate. The capacity factor (k') was calculated from the retention time (t_R) and the transit time for the mobile phase (t_M) according to the expression $k' = (t_R - t_M)/t_M$; t_M was taken as the time when the negative peak from the water injection was observed in the chromatogram; in the case of 1-hexanesulphonate the measured t_M with 4 mM eluent was used in all instances.

we did not experience such problems. In contrast, low eluent concentrations can give rise to peak deformation as a result of overloading, as we observed with 1-octanesulphonate and concentrations of 1-hexanesulphonate under 1.0 mM. As a reasonable compromise we selected 5 mM 1-butanedisulphonate for further investigations. Using this eluent, we obtained the chromatograms shown in Fig. 3 for two nitrate and nitrite standard mixtures differing a 1000-fold in concentration. The retention times and resolution obtained under these conditions are given in Table II. Some analogous results for the performance of the polymer-protected silica column are also given in Table II. The resolution obtained is excellent with both methane- and 1-hexanesulphonate.

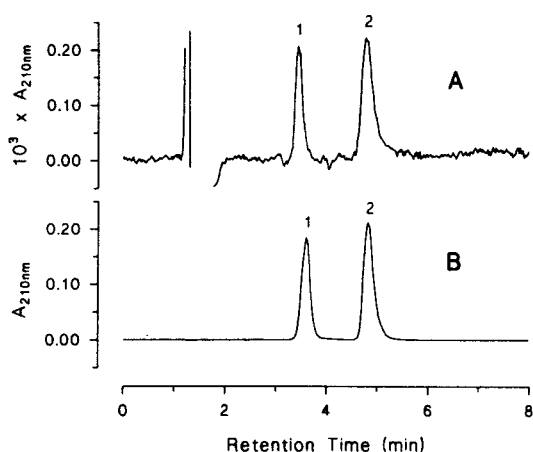


Fig. 3. Chromatograms of two standard mixtures of nitrate and nitrite. The eluent was 5 mM sodium 1-butanedisulphonate containing 0.5 mM Tris-0.25 mM methanesulphonic acid and the column was a 25-cm Wescan Anion/R analytical column with an Alltec Anion/R precolumn. The flow-rate was 2 ml/min. (A) 50 μ l of 1 μ M NaNO₃-1 μ M NaNO₂; (B) 50 μ l of 1 mM NaNO₃-1 mM NaNO₂. Peaks: 1 = nitrite; 2 = nitrate.

TABLE II

EXAMPLES OF ANALYTICAL RESULTS OBTAINED WITH ALKANESULPHONATES USING DIFFERENT COLUMNS

The eluent flow-rate was 2 ml/min with the Wescan column and 3 ml/min with the Vydac column.

Eluent	Concentration (mM)	25-cm Wescan Anion R column			5-cm Vydac 300IC column		
		t_R (min)		Resolution	t_R (min)		Resolution
		NO_2^-	NO_3^-		NO_2^-	NO_3^-	
Methanesulphonate	20	5.1	8.6	5.8	2.1	3.2	3.3
1-Butanesulphonate	5	3.3	4.7	3.7	—	—	—
1-Hexanesulphonate	10	—	—	—	1.6	2.3	3.0
1-Hexanesulphonate	1	2.8	3.9	3.3	—	—	—

One practical effect of a high resolution of two components is the ability to determine either without interference in the presence of a large excess of the other. This is illustrated in Fig. 4, which shows the effect of increasing concentrations of the other ion on the apparent peak height and peak area of nitrate or nitrite. The results show that with the eluent used, either anion can be determined without interference in the presence of a 1000-fold molar excess of the other. These measurements were performed in the absence of interfering components. High concentrations of ions such as chloride in the sample are likely to influence the resolution obtained. This was investigated in a series of experiments (not shown) analogous to those in Fig. 4. Samples with 1 nmol of nitrate and nitrite containing up to 1 mg (17 μmol) of sodium chloride were injected. The area of the nitrate peak was constant at chloride-to-nitrate molar ratios up to about 5000, whereas baseline distortion affected the area of the nitrite peak at chloride-to-nitrite ratios over about 500. High chloride concentrations also caused the

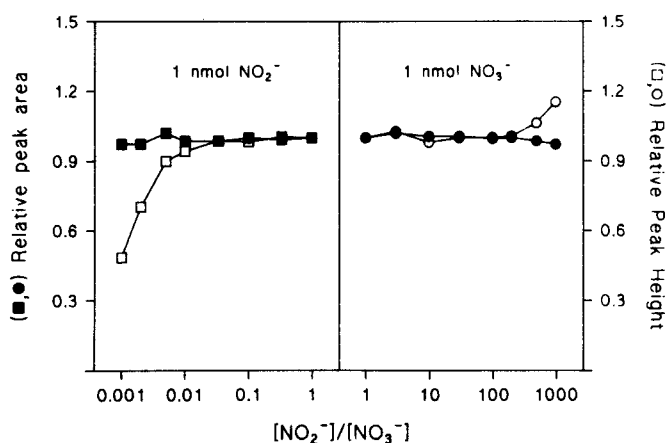


Fig. 4. Interference between nitrite and nitrate when measuring one in the presence of an excess of the other. Left, peak height and area obtained for 1 nmol of nitrite in the presence of various amounts of nitrate between 1 μmol and 1 nmol; right, corresponding heights and areas obtained with 1 nmol nitrate and various amounts of nitrite between 1 nmol and 1 μmol .

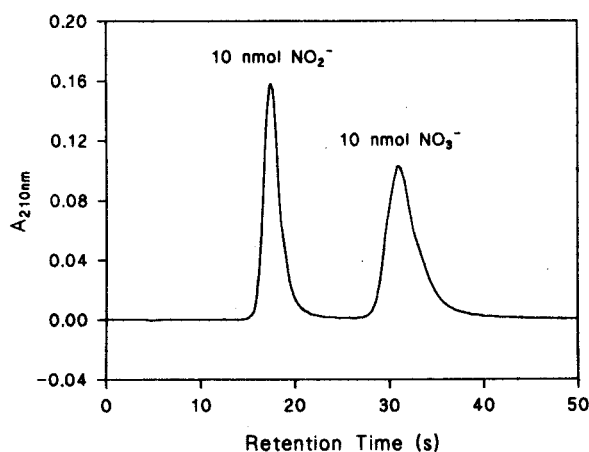


Fig. 5. Rapid assay of nitrate and nitrite using a 3-cm Wescan Anion/R precolumn as an analytical column. The eluent was 40 mM sodium methanesulphonate containing 0.5 mM Tris–0.25 mM methanesulphonic acid at a flow-rate of 4 ml/min. A volume of 10 μ l of 1 mM nitrate and 1 mM nitrite was injected.

nitrite peak to be displaced in the direction of the nitrate peak, and the two peaks were only partially resolved when the injected sample contained 1 mg of sodium chloride.

Another advantage of high resolution is the possibility of using shorter columns. Veuthey *et al.* [10] used a 3-cm resin-based ion-exchange precolumn as the analytical column with sodium hydrogenphthalate as eluent to reduce the analysis time for several inorganic anions. As resolution is proportional to the square root of the column length, whereas transit time is proportional to the length, 50% of the resolution can be maintained when the column length and thus the analysis time at a given flow-rate are reduced by 75%. As the back-pressure depends on the length of the column, it is possible to decrease the analysis time further by increasing the flow-rate when shorter columns are used. Thus the choice of methanesulphonate, which gives the optimum peak separation, as the eluent at a concentration of 40 mM and increasing the flow-rate from 2 to 4 ml/min with a 3-cm column results in a 1-min assay with a resolution of 2.5, as shown in Fig. 5.

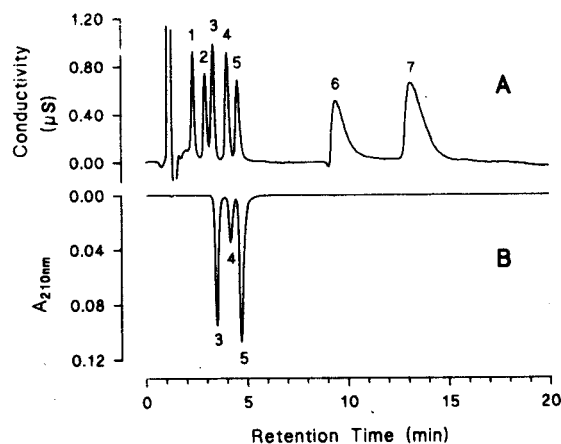


Fig. 6. Chromatograms of a standard mixture of seven anions using both (A) conductivity detection and (B) UV detection. Conditions as in Fig. 3. Injection volume, 50 μ l. The sample contained (1) 1 mM F^- , (2) 0.5 mM Cl^- , (3) 0.5 mM NO_2^- , (4) 0.5 mM Br^- , (5) 0.5 mM NO_3^- , (6) 2 mM HPO_4^{2-} and (7) 1 mM SO_4^{2-} .

Long-chain sulphonates can also be used with coupled conductivity and UV detection as a powerful method for determining both UV-absorbing and UV-transparent anions. Fig. 6 shows the separation of seven anions using 1-butanedisulphonate as eluent. It is noteworthy that with conductivity detection the water peak from injection is the only system peak observed, and that the injection peak using UV detection is negligible.

The position of the phosphate peak in the seven anions standard depends on the pH of the eluent. In the chromatogram in Fig. 6 this was buffered at about pH 8.2, so that phosphate is present in the HPO_4^{2-} form. In general, buffering the eluent at different pH values was found to have little effect on nitrate and nitrite [as long as nitrite ($\text{p}K_a = 3.4$) is not appreciably protonated] but changes the position of elution of weak acids and the zwitterionic buffers commonly used in biochemistry. This can be useful in separating nitrate or nitrite from interfering components of this type.

DISCUSSION

The choice of a method for the determination of nitrate and nitrite by HPLC depends on a number of factors. The detector and column types available will usually be a major consideration, together with the concentrations expected in the sample and the nature of interfering compounds. Other considerations include the number of samples to be analysed and hence the significance of the cost and time of the assays, and the possible interest of simultaneous measurements of other components.

Although ion-pair chromatography on reversed-phase columns [11,12] may have advantages in particular situations, ion-exchange chromatography on specialized columns for inorganic anions is the most straightforward approach. Direct UV detection for nitrate and nitrite is probably the method of choice unless it is desired to measure other non-absorbing anions at the same time. This approach is sensitive, straightforward and uses the most commonly available detector, and interference is essentially limited to other UV-absorbing anions. However, when non-absorbing anions are to be measured in the same sample, it is necessary to use conductivity or indirect UV detection with absorbing eluents [13].

Of the potential eluents available for use with direct UV detection, the results presented here show that the alkanedisulphonates have several advantages. The existence of a homologous series means that for a wide range of types of resin-based column, conditions can be found allowing elution within reasonable times without the use of excessively high eluent concentrations. The high elution strength of the longer chain homologues means that eluent concentrations giving a low background in conductivity measurements can be used. This opens up the possibility of combining the selectivity of UV detection with the universality of conductivity detection. For maximum detector response in conductivity measurements, the use of alkanedisulphonates will be inferior to weakly acidic eluents because of the signal enhancement resulting from the use of a mixture of charged and uncharged forms of the eluent [14]. However, this enhancement is associated with the presence of system peaks which are not observed with alkanedisulphonates.

With silica-based columns the hydrophobicity of the eluent is of minor importance, and the retention of nitrate and nitrite is mostly affected by the concentration of the eluent and the capacity of the column. Rapid elution with low

eluent concentrations depends on a low column capacity, leading to the danger of column overloading if the sample contains high concentrations of interfering ions. The advantages of alkanesulphonates as eluents allowing the use of low concentrations are thus most apparent when columns with hydrophobic carrier material are used.

Although this investigation was concerned with nitrate and nitrite, UV detection can also be used for the measurement of other inorganic anions, including bromide and chloride [5]. We have found alkanesulphonates to be useful in simultaneous determinations of chloride by measurement of absorbance at 190 nm and sulphate by measurement of conductivity in sea-water samples, where the chloride peak in conductivity detection was obscured by the injection peak.

The separations obtained with alkanesulphonates appear to be at least as good as those obtained with the aromatic compounds commonly used for ion chromatography, and they are therefore of considerable potential interest as eluents for the determination of inorganic anions using HPLC.

ACKNOWLEDGEMENTS

The HPLC apparatus used was partly funded by a grant from the Carlsberg Foundation. We thank Dr. F. Østergaard Andersen for the loan of columns and access to equipment for conductivity detection.

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