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ULTRAVIOLET VISUALIZATION OF INORGANIC IONS BY REVERSED-PHASE ION-INTERACTION CHROMATOGRAPHY

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SUMMARY

A method that uses standard reversed-phase high-performance liquid chromatographic columns and UV detectors to separate and quantitate low levels of poorly absorbing or UV-transparent inorganic anions is described. The anions are separated on a reverse phase column in an ion-interaction mode with UV-absorbing quaternary ammonium compounds as the ion-interaction reagents (IIR) and detection is completely non-specific. Present detection limits are of the order of 0.5 nmoles of anion directly injected. Some important parameters in terms of retention of the samples and the system peak such as buffer and IIR concentration and nature are also discussed.

INTRODUCTION

Low levels of inorganic ions can presently be determined by a number of modern chromatographic techniques. The use of ion chromatography both with and without a suppressor column has been reviewed recently[1]. Inorganic ions have been separated on a reversed-phase support using a hydrophobic quaternary ammonium compound as an "ion-pairing"(PIC) reagent and conductivity detection[2]. Both inorganic ions and some substituted carboxylic acids have been separated on a reversed-phase column using octylamine as the PIC reagent[3]. UV absorbance at 215 nm was used to detect these ions as they have an inherent absorbance at that low wavelength. This same mode of detection was used to separate inorganic anions on a cyano-bonded silica column with a cetyltrimethylammonium compound used as the PIC reagent[4]. An indirect photometric technique was used to detect UV-transparent ions after separation on specially prepared ion-exchange resins[5].

Recently there have appeared a number of papers on the determination of non-absorbing ionic species using reversed-phase high-performance liquid chromatographic (HPLC) systems[6–9]. This has been accomplished by the addition of a UV-absorbing ion-interaction reagent (IIR)[10], to the eluent. A mechanism for this visualization technique has been suggested[11] and is based upon the ion-interaction model of retention for ionic samples in reversed-phase chromatography[10]. The effect of ionic strength on retention and detector response with this technique has been in-

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vestigated[12]. Separation and quantitation of a series of alkyl sulfonates and alkylamines[7,8,12] by this technique has been accomplished.

This work demonstrates that separation and quantitation of poorly absorbing inorganic anions, using hydrophobic quaternary ammonium compounds that absorb in the UV as the IIR, can also be accomplished with this visualization technique. Some important parameters in terms of retention of samples and the system peak will also be discussed.

This approach compares favorably to other methods of ion analysis by HPLC in terms of speed and detection limit. It has the added advantage that any ion can be detected by this technique and therefore is especially useful for UV-transparent ions such as Cl^- or SO_4^{2-}. The detection method is therefore completely general in nature with little difference in sensitivity between different ions. It is an especially attractive method since it employs conventional HPLC technology that is already in wide use (reversed-phase columns and a UV detector).

EXPERIMENTAL

The chromatographic system consisted of an IBM Model 9533 pump module, a 25-cm silica and a 5-cm Supelco LC-18DB precolumn, a Rheodyne Model 7125 injector with a 50-μl sample loop, a 5-cm Supelco LC18DB analytical column, a Perkin-Elmer LC-55 variable-wavelength detector and a Perkin-Elmer Model 56 recorder. The column was thermostated at 25 ± 0.1°C with a column jacket and a Haake Type FE temperature controller. The constant detector output of approximately 0.5 V at a nominal background absorbance of 1 a.u. was subtracted by a simple d.c.-offset circuit[13]. Peak area was recorded on a Hewlett-Packard Model 3390A reporting integrator. The mobile phase was prepared from HPLC grade water which was filtered through a 0.45-μm Millipore filter membrane. Ion-interaction reagents not available commercially were prepared in this laboratory as either the chloride or bromide salts[13] via the Menschutkin reaction[14]. Benzyltributylammonium chloride was obtained from Aldrich (Milwaukee, WI, U.S.A.) and used as received. All other chemicals were of analytical reagent grade.

The IIRs in the chloride or bromide form were converted to the hydroxide form by passing a water solution of the IIR over Bio-Rad AG 1-X8 anion-exchange resin. Since capacity factors are very strongly dependent on ionic strength extreme care is required in preparation of the eluent. Exactly a two-fold molar excess of acetic acid was added to the IIR (hydroxide form). The solution was then adjusted to a total ionic strength of 10 m*M* by addition of 1 *M* acetic acid–sodium acetate buffer. At 25°C and 1 ml/min the back pressure of the system was approximately 40 bar. Sample mixtures were prepared in the eluent.

RESULTS AND DISCUSSION

Retention of samples and system peak

Data for the retention of sample anions and the system peak, as defined by Denkert *et al.*[7], *versus* the concentrations of various IIRs are shown in Fig. 1. At a given concentration of IIR the more hydrophobic the IIR, specifically as the aromatic group is changed from benzyl to naphthyl and the alkyl group from propyl to butyl,

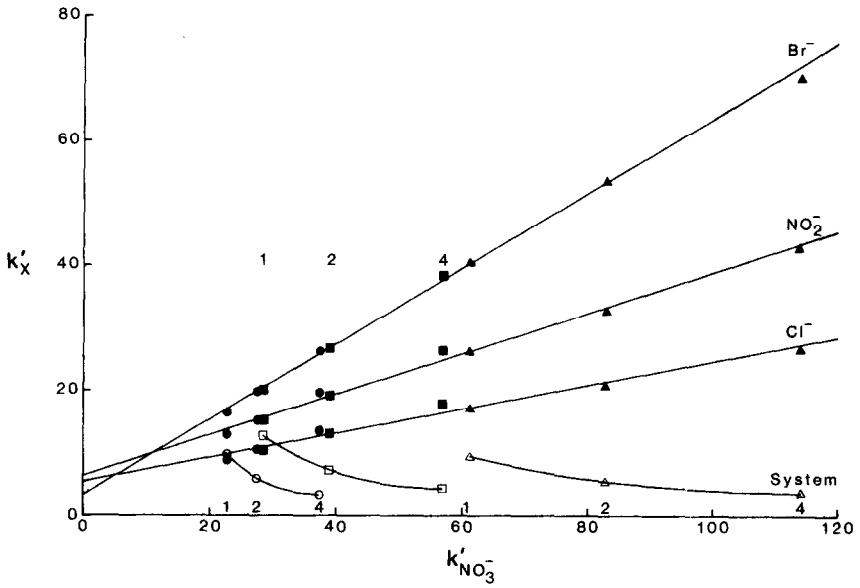

Fig. 1. k' of each anion and the system peak *vs.* the k' of an arbitrary reference anion which in this case is NO_3^-. ●, Benzyltributylammonium as the IIR; ■, α-naphthylmethyltripropylammonium and ▲, α-naphthylmethyltributylammonium. Open symbols denote the system peak. Numvbers above or below columns of data are the eluent concentrations (mM) of the particular IIR. Eluent is 10 mM acetic acid/sodium acetate buffer in water at pH = 4.75. Flow-rate was 2 ml/min. Wavelength of detection was set to give a nominal background absorbance of 1.0 for each IIR and concentration. Wavelength was set to λ_{max} (262 nm) for benzyltributylammonium.

the greater is the capacity factor (k') for each sample anion. The k' for the system peak, however, remains approximately constant, *i.e.*, it is independent of the nature of the IIR. As the concentration of the IIR is increased the k' values for sample anions also increase but the system peak k' decreases. This decrease would be due to a concave adsorption isotherm for the IIR where the slope, k', of the isotherm gradually decreases with increasing concentration of IIR in the eluent.

The motivation for studying the effect of concentration and nature of the IIR was our observation that peaks which eluted prior to the system peak invariably exhibited less sensitivity than peaks which elute after the system peak. This is most surprising since one would consider that species with large k' values should be effectively more dilute according to the well known equation

$$C_{max} = \frac{W}{V_0 (1 + k')} \cdot \sqrt{\frac{N}{2\pi}} \tag{1}$$

where C_{max} corresponds to the peak height maximum concentration of the eluted peak, W is the amount of injected sample, V_0 is the void volume of the column and N is the number of plates for the column. This phenomenon has been observed by others[12].

Thus we sought conditions which would cause elution of the system peak prior

to all anions of potential interest at k' values less than 10. As can be seen in Fig. 1 we were not able to establish such conditions by manipulation of the mobile phase concentration of IIR or the nature of the IIR alone. The nature of the buffer, pH and ionic strength also have little effect on the k' of the system peak but have a large effect on the k' of the sample anions. These parameters as well as the type of column used and the effect of an organic modifier such as methanol or acetonitrile will be discussed in detail in a subsequent report.

We did, however, observe that the presence of an alkyl sulfonate in the eluent did significantly and systematically alter the system peak k'. The effect of concentration of hexanesulfonate in the eluent on the system peak and sample anion retention is shown in Fig. 2. As expected, all retentions decrease. Fig. 2 shows that by adding approximately 0.5 mM hexanesulfonate to the eluent containing 4 mM IIR the k' of the system peak can be reduced by a factor of two. This effect is most probably due to an increase in the stationary phase concentration of IIR in equilibrium with a given mobile phase. Indeed, upon equilibrating a column with IIR, larger breakthrough volumes are observed indicating increased partitioning of the IIR in the presence of the alkyl sulfonate. This increased partitioning causes a shift

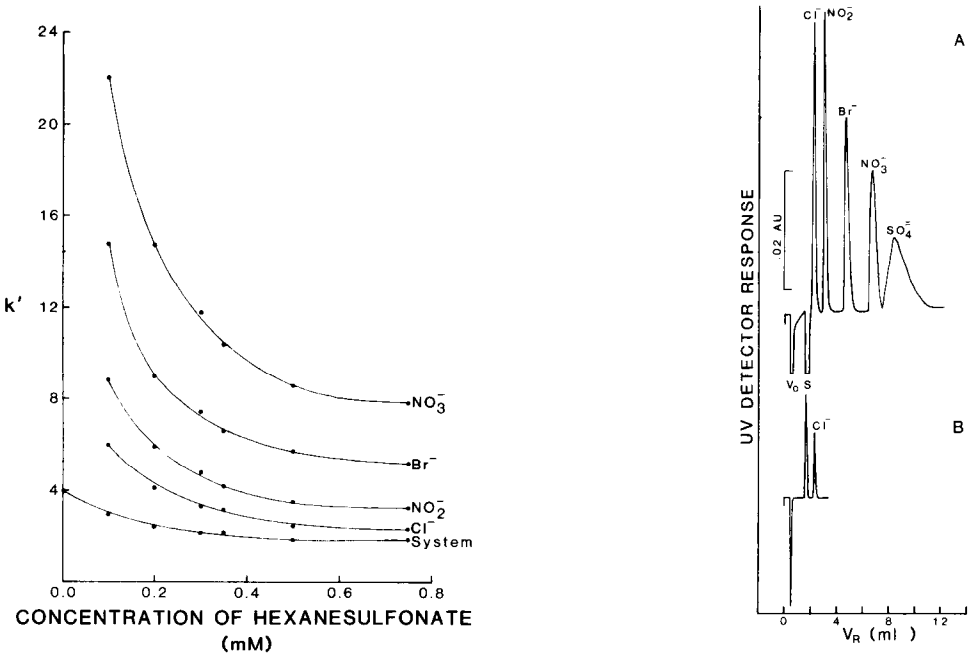

Fig. 2. k' of each anion and the system peak *vs.* the eluent concentration of hexanesulfonate. The mobile phase is 4 mM in naphthylmethyltributylammonium and 10 mM acetic acid sodium acetate buffer in water at pH = 4.75. Flow-rate was 1 ml/min.

Fig. 3. A, Separation of five inorganic anions. Sample concentration is 1 mM in each anion. Wavelength of detection was 316 nm with a background absorbance of approximately 1.1 a.u. Flow-rate was 1 ml/min. Mobile phase was 0.25 mM hexanesulfonate, 4 mM α-naphthylmethyltributylammonium and 10 mM acetic acid sodium acetate in water at pH = 4.75. Samples were made in the eluent. B, Injection of α-naphthylmethyltributylammonium chloride dissolved in the eluent. Eluent conditions as in Fig. 3A.

in the adsorption isotherm so that the slope of the isotherm is smaller. Thus the k' of a pulse of IIR must decrease.

The effect of hexanesulfonate on the sample ions is much more dramatic. Although not shown in Fig. 2, the k's for NO_3^- and Cl^- with no hexanesulfonate in the eluent are approximately 114 and 27 respectively. It is obvious that a very small amount of hexanesulfonate in the eluent competes very effectively with the sample anions for retention on the stationary phase. A more detailed study of the nature and concentration of this competing ion will be discussed in a subsequent report.

A mobile phase consisting of 4 m*M* α-naphthylmethyltributylammonium ion, 10 m*M* acetic acid–sodium acetate (pH = 4.75) and 0.25 m*M* sodium hexanesulfonate in water was used to obtain the chromatograms in Fig. 3A and B. Fig. 3A is a representative sample chromatogram of a separation of Cl^-, NO_2^-, Br^-, NO_3^- and SO_4^{2-}. The first negative or "vacancy" peak corresponds to the void volume of the column and is induced by the initial equilibration of the sample with the eluent and the column[8]. The second vacancy peak is the "system" peak and occurs at the k' of the IIR as shown in Fig. 3B. These vacancy peaks are followed by each of the sample peaks. A thorough explanation of the origin and nature of these induced peaks has appeared in a recent paper[11].

TABLE I

SOME TYPICAL CAPACITY FACTORS OF COMMON INORGANIC AND ORGANIC ANIONS

Mobile phase was 4 m*M* naphthylmethyltributylammonium hydroxide, 10 m*M* acetic acid sodium acetate buffer, pH 4.75, and 0.25 m*M* sodium hexanesulfonate in water. All samples were made up in and diluted with the eluent. System peak capacity factor is 2.36.

Anion	*Capacity factor*	*Anion*	*Capacity factor*
Fluoride	0.60	Tartrate	15.3
Iodate	1.82	Sulfate	16.4
Chloride	3.48	Sulfite	16.9
Bromate	3.83	Citrate	18.9
Nitrite	5.02	Iodide	55.9
Bromide	7.84	Perbromate	>60
Nitrate	11.5	Perchlorate	>60
Chlorate	14.7	Periodate	>60

Table I contains a list of capacity factors for those anions shown in Fig. 3 and also a number of other common inorganic anions as well as several low-molecular-weight organic anions. These capacity factors were obtained under the same conditions as in Fig. 3. Under these conditions the only two peaks with any significant overlap are chloride and bromate (k' 3.48 and 3.83 respectively) and under these conditions a total of seven different anions can be separated with capacity factors less than 12. It should be stressed that those ions with capacity factors larger than 12 can be eluted at more reasonable capacity factors by simple changing the eluent condition (for example, by increasing the buffer concentration or by changing the nature of the buffer). Under these revised conditions the less retained ions would elute in or near the void volume.

Quantitation

Fig. 4 is a plot of peak height (a.u.) *versus* nmoles of sample injected. Linearity is excellent up to 15 nmoles injected (Fig. 4B). At higher amounts injected a noticeable rolloff occurs where the observed peak height is lower than expected (Fig. 4A). This can be explained by a combination of factors. The chromatograms of Fig. 5 are the results of constant volume (50 μl) injections of serial dilutions of 1 mM each of Cl^-, NO_2^-, Br^- and NO_3^-. It is apparent for well retained peaks that as the sample concentration becomes large the time of the peak maximum shifts to shorter time and the peak becomes more asymmetric. The shift in k' would explain peak heights deviating from those expected except that a shift to smaller k' should cause a positive deviation from the expected peak height (eqn. 1) but a negative deviation is observed. The fact that the peak becomes much more asymmetric explains this apparent anomaly. As a peak becomes more asymmetric the peak height decreases significantly[15].

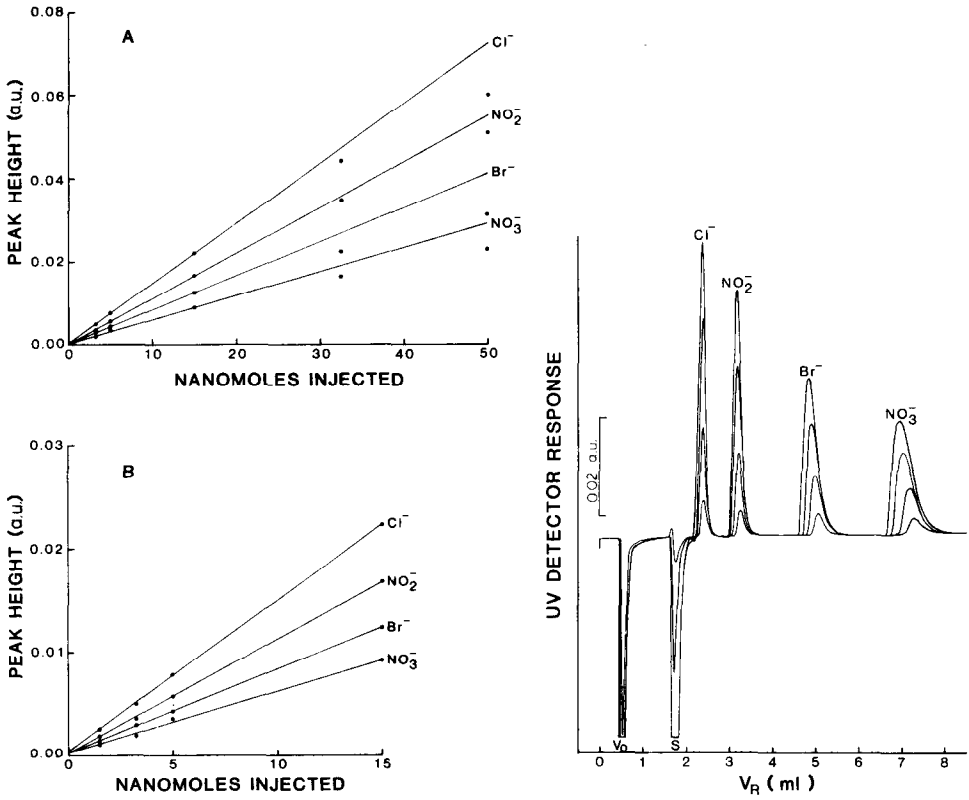

Fig. 4. A, Detector response in a.u. (peak height) *vs.* nanomoles of sample injected. 50-μl injections of 1, 0.65, 0.3, 0.1 and 0.065 mM each of Cl^-, NO_2^-, Br^- and NO_3^-. All other conditions as in Fig. 3. B, Expanded scale of Fig. 4A showing linearity of response at low amounts of sample injected. 50-μl injections of 0.3, 0.1, 0.065 and 0.03 mM each of Cl^-, NO_2^-, Br^- and NO_3^-.

Fig. 5. Detector response and retention volume *vs.* the concentration of injected sample. 50-μl injections of 1, 0.65, 0.3 and 0.1 mM each of Cl^-, NO_2^-, Br^- and NO_3^-. All other conditions as in Fig. 3.

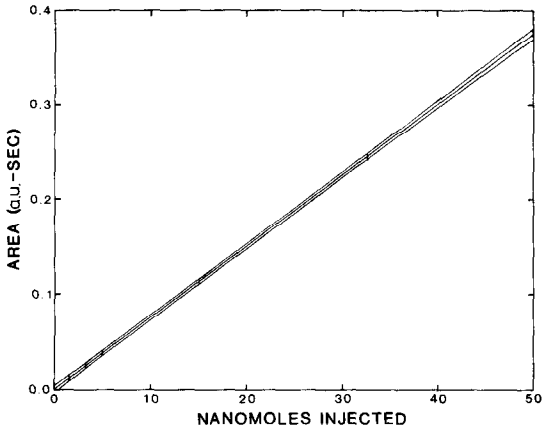

Fig. 6. Regression plot of peak area *vs.* nanomoles injected for Br^-. 50-μl injections of 1, 0.65, 0.3, 0.1, 0.065 and 0.03 m*M* Br^-. All other conditions as in Fig. 3.

The peak height decreases faster than it should be increasing due to a decrease in k'. Sachok *et al.*[8] show similar results for pentanesulfonate and hexanesulfonate. Peak height measurement is therefore not a suitable method for accurate quantitation at high sample concentration.

Fig. 6 shows a regression plot for area determination *versus* nanomoles injected. Regression parameters are shown in Table II showing sensitivity, linearity and precision for each anion. As can be seen from this data linearity is very good for area determination even at high amounts of injected sample. Conservative detection limits are 0.8 nmole for Cl^-, 0.9 nmole for NO_2^-, 0.5 nmole for Br^- and 1.0 nmole for NO_3^- and were determined by the method of Hubaux and Vos[16] at the 90% confidence level. These detection limits compare favorably with those stated for ion chromatography with or without a suppressor column[1].

This method of UV visualization of ionic samples show great promise in terms of speed of analysis and ease of operation. Another advantage is that the analysis can be performed on any conventional HPLC unit equipped with a reversed-phase

TABLE II

REGRESSION PARAMETERS OF PEAK AREAS *vs.* NANOMOLES INJECTED

See Table I for conditions. All anions were determined in the presence of the other three. 50-μl injections of 1, 0.65, 0.3, 0.1, 0.065 and 0.03 m*M* each of Cl^-, NO_2^-, Br^- and NO_3^-.

Anion	*Slope (a.u. sec/nmole)**	*Intercept (a.u. · sec)*	*Detection limit** (nmoles)*
Cl^- (n = 23)	0.642 ± 0.005	0.012 ± 0.001	0.8
NO_2^- (n = 23)	0.640 ± 0.008	0.004 ± 0.002	0.9
Br^- (n = 24)	0.752 ± 0.005	0.0004 ± 0.001	0.5
NO_3^- (n = 21)	0.819 ± 0.011	−0.002 ± 0.003	1.0

* Slope values and uncertainties are multiplied by 100; all certainties are expressed as one standard deviation.

** By the method of Hubaux and Vos[16] at the 90% confidence level.

column and a variable wavelength detector. Detection limits could be lowered even further if the method can be made to work at even lower concentrations of IIR in the eluent. Work is currently in progress towards this goal.

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