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Separation of inorganic analyte anions on dye-coated stationary phases

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ABSTRACT

The separation of inorganic analyte anions on a dye-coated stationary phase was studied. The dye employed in this study is typically used for pH titrations and is composed of hydrophobic groups and a fixed charge site. The mobile phase variables that affect analyte anion retention were studied and include: concentration of dye, type and concentration of organic modifier, mobile phase pH, type and concentration of counter anion, and ionic strength. Different stationary phases were studied including a polymer-based packing and a silica-based ODS packing. Linear regression studies were done on the polymer column using conductivity detection. Correlation coefficients were found to be greater than 0.999 over a range of 1 to 1000 ppm, with detection limits between 0.5 and 1.0 ppm.

INTRODUCTION

In ion-interaction chromatography, the hydrophobic counter ion is sorbed onto the stationary phase and forms a charged double layer. The analyte ions of interest are then separated in the diffuse secondary layer. The ion-interaction retention model has been studied and theoretical interpretations have been developed [1–11].

An area of ion-interaction chromatography that has not received as much attention is the addition of dyes to the mobile phase. DiNunzio and Freiser [12] reported on the separation of aliphatic acids on a chemically bonded ODS column that was coated with brilliant green. The separation was described as true ion-pair chromatography since the mobile phase was composed of only organic solvents. One study described the separation of organic and inorganic anions using methylene blue as the counter ion [13], while Golombek and Schwedt [14] used a methyl green coated column for the separation of inorganic anions. The separation of metal ions was described by Jones and Schwedt [15] where several different dyes were studied.

In the studies where a dye was used as an ion-interaction reagent (IIR), detection was usually done using a conductivity detector or by indirect UV detection where the counter anion was UV-active. In this study, however, the dye is used as both the IIR and for indirect visible detection. This paper describes the mobile and stationary phase variables that affect the separation of inorganic and organic analyte anions on dye-coated stationary phases. The analyte anions were detected either by conductivity or indirect visible detection. The results that were obtained will be discussed.

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EXPERIMENTAL

Chemicals

HPLC-grace acetonitrile was obtained from Baxter Scientific Products (McGaw Park, IL, U.S.A.). HPLC-grade water was obtained by passing deionized water through a Nanopure water purification unit. Ethyl violet, citric acid, potassium hydrogen phthalate (KHP), salicyclic acid, sodium benzoate, succinic acid, *p*-hydroxybenzoic acid, inorganic salts and metal salts were obtained from Aldrich (Milwaukee, WI, U.S.A.). All chemicals were reagent grade.

Apparatus

The liquid chromatographic apparatus used in this study consisted of a Spectra-Physics Model 8800 high-performance liquid chromatography (HPLC) pump, Spectra-Physics Model 8875 full loop autosampler, Spectra-Physics Model SC101-100 variable-wavelength UV–visible detector, and Spectra-Physics ChromJet integrator. The columns used in this study were: a 150 × 4.1. mm Hamilton PRP-1 column (Hamilton, Reno, NV, U.S.A.), a 150 × 4.6 mm PLRP-S column (Polymer Labs., Amherst, MA, U.S.A.) and a 5-μm, 150 mm × 4.6 B&J ODS column (Baxter Healthcare Corp., McGaw Park, IL, U.S.A.). The PRP-1 column is a spherical, 10-μm poly(styrene–divinylbenzene) packing. The PLRP-5 column is composed of a spherical 5-μm poly(styrenedivinylbenzene) packing. Flow-rates of 1.0 ml/min were used, unless noted. Aqueous analyte samples of approximately 500 μg/ml were used. Sample aliquots of 10 μl were used, except for the calibration curves where 20-μl injections were used. Inlet pressures of 500–1000 p.s.i. were observed. A wavelength of 620 nm was used for the indirect visible detection.

Ethyl violet was purchased as a chloride salt and was converted to a fluoride salt by passing the dye through an Amberlite IRA-400 anion-exchange column (Aldrich) that was charged in the F^- form.

Mobile phase preparation

The ethyl violet dye was quantitatively transferred (appriopriate volume of a 0.01 *M* ethyl violet solution) to a beaker that contained the aqueous buffer solution. The desired pH was achieved by adding acid or base. The aqueous solution was diluted to the appropriate volume and the organic modifier was then added. The solution was mixed and then filtered through a 0.45-μm PTFE membrane.

Column loading

Column loading was determined by running the mobile phase through the column and UV–visible detector until the breakthrough occurred. The number of μmoles of ethyl violet adsorbed onto the stationary phase was calculated from the breakthrough volume [3]. The column was then allowed to equilibrate for an additional 30–60 min.

RESULTS AND DISCUSSION

Retention of inorganic and organic analyte ions on reversed stationary phases using a mobile phase containing a hydrophobic ion of opposite charge is determined

by two major equilibria [3,4,8–11]. The first equilibria describes the interaction that takes place between the stationary phase and the hydrophobic ion (eqn. 1), while the second equilibria describes the ionic interaction that takes places between the analyte ion and the counterion associated with the retained hydrophobic ion (eqn. 2). The second equilibria takes place in what has been termed the diffuse secondary layer. These equilibria are shown by eqns. 1 and 2, respectively:

$$A + EV^+ + C^- + M^+ \rightleftarrows A \cdots EV^{+-}C + M^+ \quad (1)$$

$$A \cdots EV^{+-}C + X^- + M^+ \rightleftarrows A \cdots EV^{+-}X + C^- + M^+ \quad (2)$$

where A represents the stationary phase, EV^+ represents an ion-interaction reagent (UV-active counter cation) in the mobile phase, C^- is the counter anion associated with the ion-interaction reagent, the buffer and/or added inert electrolyte, M^+ is a cation associated with the counter anion and/or the analyte anion, and X^- is the analyte anion. The variables that will affect the separation are; the reversed stationary phase, the type and mobile phase concentration of the IIR, the mobile phase concentration of organic modifier, the type and mobile phase concentration of counter anion in the mobile phase, and mobile phase pH. It should be noted that the inorganic analyte anions studied in this paper had no retention on the stationary phases in the absence of the IIR whereas organic analyte anions have some retention depending on the hydrophobicity of the organic analyte anion.

The IIR used in this study has the added feature of being chromophoric which allows for the indirect visible detection of the analyte anions. Both conductivity and indirect detection were used in this study. When using indirect visible detection, the absorbance of the UV–visible detector should not exceed its linear working range (usually an absorbance range of 0.8 or less). As an analyte anion travels down the column (eqns. 1 and 2) the concentration of the UV-active IIR band changes relative to the background absorbance. The IIR in the band either increases do to removal from the column and provides a positive chromatographic peak, or decreases due to its uptake onto the column and produces a negative chromatographic peak. The IIR in the mobile phase is responsible for the retention and resolution of the analyte anions and in their indirect visible detection.

Effect of ethyl violet concentration

The first mobile phase parameter that was studied was the concentration of ethyl violet. The retention of the ethyl violet onto the stationary phase was high and required either a long breakthrough time (> 12 h) or a high mobile phase concentration of organic modifier (> 20% acetonitrile). The amount of ethyl violet retained on the column was calculated from the breakthrough volumes [3]. It was found that as the mobile phase concentration of ethyl violet was increasd, the amount of ethyl violet adsorbed onto the stationary phase increased. Since ethyl violet is quite hydrophobic, it produces a significant amount of surface anion-exchange sites on the column. At a concentration of $2.0 \cdot 10^{-4}$ *M* of ethyl violet, there are 145 μequiv./column of anion-exchanges sites. This concentration compares with that found for mobile phases using tetraalkylammonium salts [4] and low-capacity anion exchangers [16].

Fig. 1. compares the separation of several inorganic anions at two different

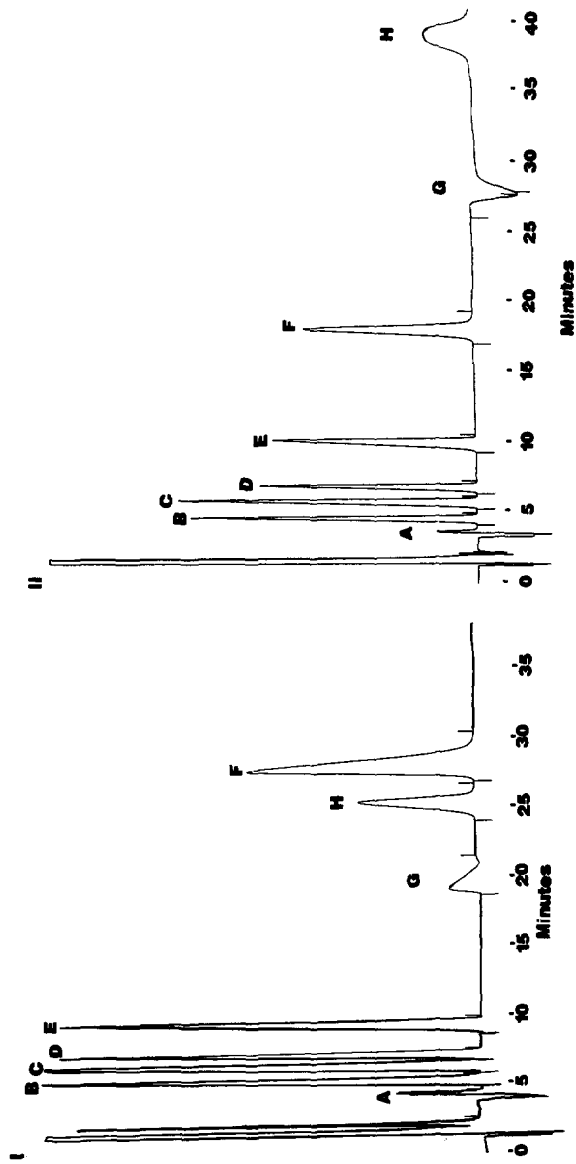

Fig. 1. The separation of several inorganic anions at different mobile phase concentrations of ethyl violet. Mobile phase: (I) 0.1 m*M* ethyl violet, 2.0 m*M* KHP, p*H* 4.0, acetonitrile–water (20:80), (II) same as I except 0.3 m*M* ethyl violet. Peaks: A = F^-; B = Cl^-; C = NO_2^-; D = Br^-; E = NO_3^-, F = SO_4^{2-}; G = system peak; H = I^-.

mobile phase concentrations of ethyl violet. Chromatogram I shows the separation where 0.1 m*M* ethyl violet was used while chromatogram II shows the separation where the ethyl violet concentration was increased to 0.3 m*M*. The analyte anions were baseline resolved using 0.3 m*M* ethyl violet, however the separation took longer than when the 0.1 m*M* ethyl violet mobile phase was used. It is interesting to note that almost all of the analyte anions had lower retention times when the mobile phase concentration of ethyl violet was increased, except for I^- which increased in retention.

Mobile phase variables —effect on ethyl violet adsorption

The concentration of organic modifier also had an effect on the amount of ethyl violet adsorbed onto the stationary phase. As the mobile phase concentration of organic modifier was increased, the amount of ethyl violet adsorbed onto the stationary phase decreased. This decrease in the amount of adsorbed ethyl violet produced a corresponding decrease in the number of anion-exchange sites present on the stationary phase.

The mobile phase ionic strength was found to affect the amount of ethyl violet adsorbed onto the stationary phase. The amount of ethyl violet adsorbed onto the stationary phase was found to increase with increasing ionic strength. The increase in the amount of ethyl violet adsorbed led to an increase in the apparent number of anion-exchange sites present. Although more anion-exchange sites were present at a higher mobile phase ionic strength, retention of the analyte anions decreased due to increased competition for the anion-exchange sites from the higher concentration of counter anions (see eqn. 2).

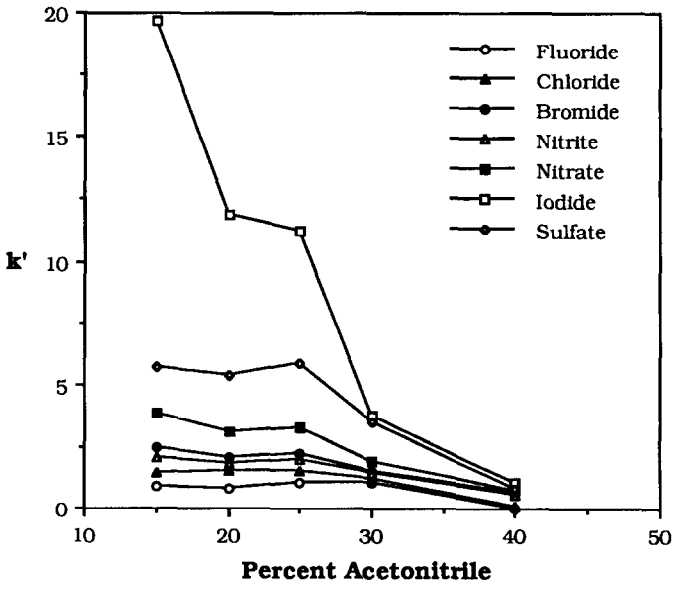

Fig. 2. The effect of acetonitrile concentration on analyte anion retention. Mobile phase conditions: 0.1 m*M* ethyl violet, 1.0 m*M* KHP, pH 4.5, acetonitrile–water. k' = Capacitiy factor.

Effect of organic modifier on anion retention

Fig. 2 shows how retention of the analyte anions were affected by the concentration of acetonitrile that covered a range of 15% to 40%. Retention of the anions is dependent on the number of anion-exchange sites provided by the adsorbed ethyl violet. As the concentration of acetonitrile was increased, the amount of adsorbed ethyl violet decreased which led to a lower number of anion-exchange sites present. Resolution of the anions was found to be better at lower concentrations of acetonitrile due to the higher number of anion-exchange sites present on the stationary phase.

Effect of pH

The effect that the mobile phase pH had on analyte retention is shown in Fig. 3. Retention times did not change for most of the anions over the pH range of 4 to 10 (ionic strength held constant). Three anions, however, were affected by pH; sulfate which decreased in retention, and phosphate and iodide which increased in retention. The increase in retention for phosphate is attributed to the change in ionization over the pH range studied. The mobile phase pH appears to have had an effect on the polarizability of sulfate and iodide which in turn affected their retention [17]. Fig. 4 shows the separation of Cl^-, NO_2^-, Br^-, NO_3^-, I^-, SO_4^{2-} at pH 6.0. Several differences are readily apparent when comparing Fig. 1 (I) with a mobile phase pH of 4.0, to Fig. 4 with a pH of 6.0. At pH 4.0, all of the anions were baseline resolved and the system peak did not interfere with the separation. At pH 6.0, the retention times for I^- and SO_4^{2-} were significantly different when compared to pH 4.0. SO_4^{2-} had a much lower retention time whereas I^- had an increase in retention. Also at pH 6.0, the system peak interfered with the Cl^- peak and NO_3^- and SO_4^{2-} were not baseline

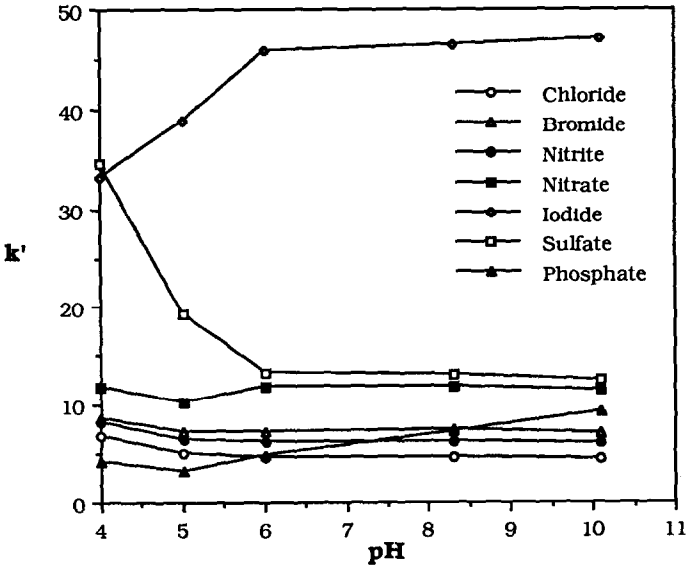

Fig. 3. The effect of mobile phase pH on analyte anion retention. Mobile phase conditions: 0.1 m*M* ethyl violet, 1.0 m*M* KHP, acetonitrile–water (20:80).

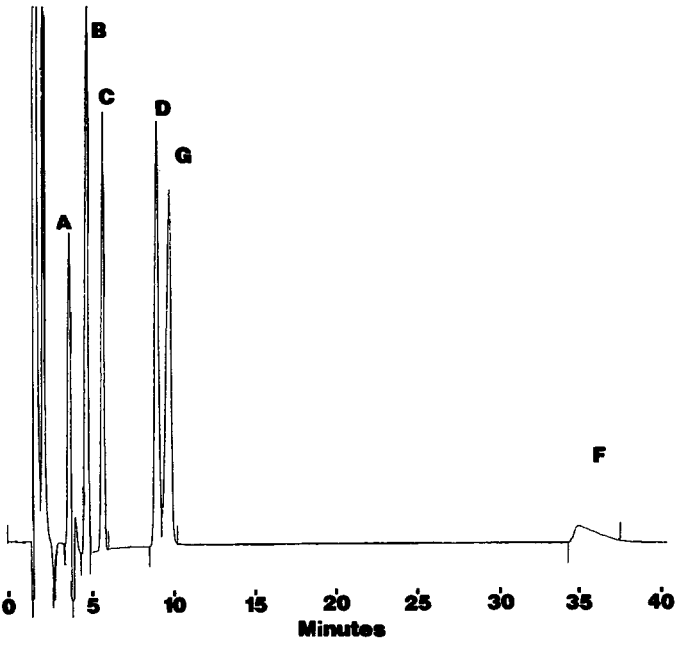

Fig. 4. The separation of several inorganic analyte anions at pH 6.0. Mobile phase conditions: 0.1 m*M* ethyl violet, 2.0 m*M* KHP, pH 6.0, acetonitrile–water (20:80). Peaks: A = Cl^-; B = NO_2^-; C = Br^-; D = NO_3^-; E = system peak; F = I^-; G = SO_4^{2-}.

resolved. This study indicated that a lower mobile phase pH would provide a better separation without any interference from the system peak.

Effect of ionic strength

The concentration of counter-anion (KHP) in the mobile phase was increased in order to determine what effect ionic strength would have on anion retention. All of the anions studied decreased in retention as the concentration of KHP was increased. This is due to increased competition for the anion-exchange sites (eqn. 2). Increasing the mobile phase concentration of KHP increases the amount of IIR adsorbed onto the stationary phase and leads to an increase in the number of anion-exchange sites. This did not, however, lead to an increase in anion retention since competition was increased for the anion-exchange sites due to the higher concentration of counter-anions present.

Comparison of conductivity and indirect visible detection

Detection of the inorganic anions was done by using conductivity and indirect visible detection. The conductivity of the mobile phase was low enough so that sensitive detection (< 1 ppm) of the anions could be done. Indirect visible detection at a wavelength of 620 nm provided for the analysis of phosphate which was poorly detected when using conductivity. Fig. 5 shows the separation of Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}, I^-, and $H_2PO_4^-$ on a PLRP-S column using conductivity (I) and indirect visible detection (II).

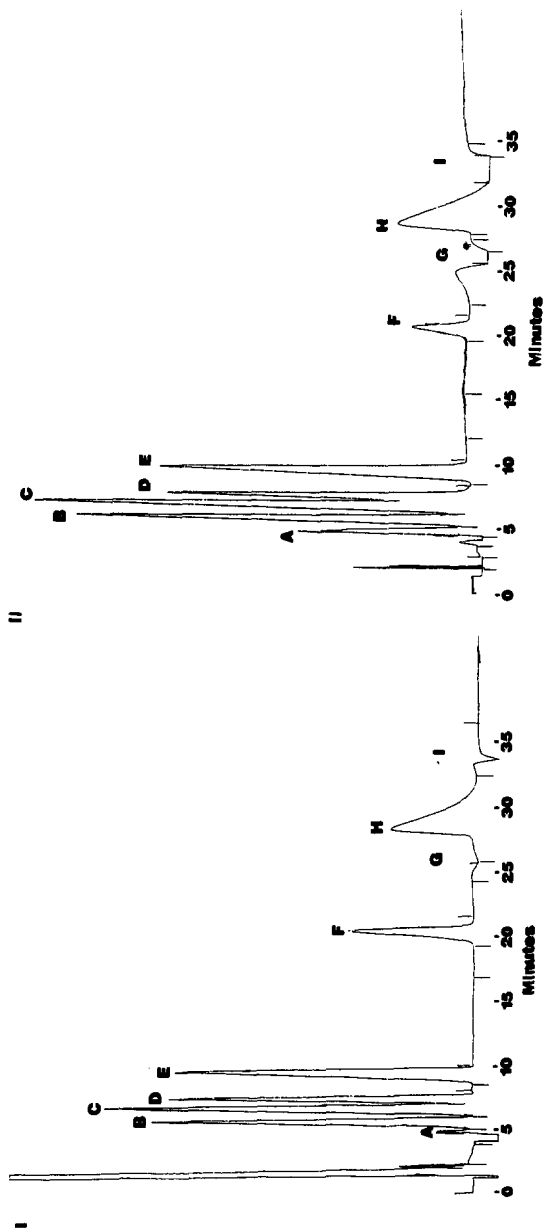

Fig. 5. The separation of several inorganic anions using (I) conductivity and (II) indirect visible detection. Mobile phase conditions: 0.1 m*M* ethyl violet, 1.0 m*M* KHP, pH 4.5, acetonitrile–water (25:75). Peaks: A = $H_2PO_4^-$; B = Cl^-; C = NO_2^-; D = Br^-; E = NO_3^-; F = I^-; G = system peak; H = SO_4^{2-}; I = system peak.

Other counter anions studied

Different counter anions were also used for the separation of inorganic anions. The counter anions that were studied and found to be acceptable were sodium benzoate, *p*-hydroxybenzoic acid (pHBA), salicylic acid, succinic acid and citric acid. The counter anions that provided the best separations were KHP, pHBA and sodium benzoate. A higher concentration of sodium benzoate was required when compared to KHP since sodium benzoate is a weaker eluant. pHBA was found to be a stronger counter anion than either KHP or sodium benzoate.

Separation on an ODS column

A silica-based ODS column was also coated with the dye and used for the separation of the inorganic analyte anions. The peaks obtained on the ODS column were broad and resolution was poor. Several disadvantages were apparent with the silica-based column when compared to the polymer-based column. First, it took a significant amount of acetonitrile–water to strip the IIR off the ODS column. The ethyl violet was found to adsorb very strongly onto the stationary phase. The polymer column, however, could easily be cleaned by passing 100 ml of an acetonitrile–water (80:20) eluent through it. Secondly, the results were not as reproducible as the polymer-based column. This may be due to ethyl violet that remained adsorbed on the column, as well as degradation of the silica-based packing. Studies have indicated that free silanol sites within the silica backbone can participate in cation exchange. Some of the cationic ethyl violet is retained as a cation and thereby eliminating it as a source for anion exchange [18]. Thirdly, the polymer column is stable throughout the pH range of 1 to 13, whereas the silica based column is not.

Calibration curve

Calibration curves using a 20-μl sample loop were made covering the range of 1 to 1000 ppm for Cl^-, Br^-, and NO_3^-, as well as 5 to 1000 ppm for SO_4^{2-}. The mobile phase consisted of 0.1 m*M* ethyl violet, 1.0 m*M* KHP, pH 4.5, acetonitrile–water (20:80). Correlation coefficients of 0.999, 1.00, 1.00 and 1.00 were obtained for Cl^-, Br^-, NO_3^-, and SO_4^{2-}, respectively. The limit of detection for each anion was found to be 0.5 ppm, except for SO_4^{2-} which was found to be 1.0 ppm.

CONCLUSIONS

It was found that an ethyl violet-coated stationary phase provided acceptable separations of the inorganic anions studied. The mobile phase variables that affect anion retention were identified and studied. Both conductivity and indirect visible detection could be used for the analysis of the anions. Correlation coefficients were better than 0.999 with detection limits of 1.0 ppm or less were found.

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