Journal of Chromatography, 602 (1992) 97–102
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2493

Separation and indirect visible detection of inorganic and organic analyte cations on dye-coated stationary phases

Thomas A. Walker

Pharmaceutical Sciences, Marion Merrell Dow Inc., P.O. Box 9627, Kansas City, MO 64134 (USA)

ABSTRACT

The separation and indirect visible detection of inorganic and organic analyte cations on dye-coated stationary phases was studied. The dye employed in this study, Thymol Blue, is typically used for pH titrations and is composed of hydrophobic groups and a fixed charge site. The mobile phase variables that were found to affect analyte cation retention, resolution and selectivities are: concentration of dye, concentration of organic modifier, mobile phase pH, type and concentration of countercation and/or ligand, and ionic strength. Two different types of reversed-phase packings were used in this study: polymer-based stationary phases and silica-based ODS stationary phases. Detection of the analyte cations was accomplished by indirect visible detection at 428 nm.

INTRODUCTION

The addition of a hydrophobic counterion to the mobile phase for the separation of inorganic and organic analyte ions has been studied over the past several years, and models have been developed to explain the interactions that take place between an analyte ion, the counterion, and the stationary phase [1–4].

One model that has been successfully used to describe the interactions between an analyte ion and the mobile phase counterion is ion-interaction chromatography [4–15]. A hydrophobic ion (ion-interaction reagent) that contains a fixed charge site is added to the mobile phase. The ion-interaction reagent (IIR) is sorbed on the stationary and forms a charged double layer. The primary layer is composed of the sorbed hydrophobic counterion while the co-ion occupies the diffuse secondary layer. The analyte ions of interest are then separated in the diffuse secondary layer, based on selectivity differences between ions.

Several recent reports deal with the separation of inorganic and organic ions and mobile phases that contain a dye. The dyes that were studied are commonly used for pH titrations. A Brilliant Green-coated stationary phase was used for separating aliphatic acids [16], while another study dealt with the separation of organic and inorganic anions, where Methylene Blue was added to the mobile phase [17]. Inorganic anions were separated on a Methyl Green-coated column [18], while the separation of metal ions was studied with several different dyes [19]. One paper described the separation and indirect invisible detection of inorganic and organic anions with a mobile phase that contains Ethyl Violet [20]. Both polymer- and silica-based stationary phases were studied, using Ethyl Violet mobile phases.

In the present study, the hydrophobic dye is used as both the IIR for separating the inorganic and organic analyte cations and for their indirect visible detection. This paper describes the mobile phase variables that affect the separation of inorganic and organic analyte cations on the dye-coated stationary phases. The results obtained are discussed.

EXPERIMENTAL

Chemicals

HPLC-grade acetonitrile was obtained from Baxter Scientific Products (McGraw Park, IL, USA).

HPLC-grade water was obtained by passing de-ionized water through a Nanpure water purification unit. Thymol Blue, citric acid, tartaric acid, inorganic salts, guanidines, and metal salts were obtained from Aldrich (Milwaukee, WI, USA). All chemicals were of reagent grade.

Apparatus

The instrumentation used in this study consisted of a Hewlett-Packard liquid chromatography system, Model 1090. The columns used were: a 150 × 4.1 mm I.D. Hamilton (Reno, NV, USA) PRP-1 column, a 150 × 4.6 mm I.D. PLRP-S column from Polymer Labs. (Amherst, MA, USA), and a 5 μm, 150 × 4.6 mm I.D. B&J OD5 column from Baxter Healthcare Corp. (McGraw Park, IL, USA). The PRP-1 column contains a spherical, 10-μm poly(styrene–divinylbenzene) packing. The PLRP-S column is composed of a spherical, 5-μm poly(styrene–divinylbenzene) packing. Flow-rates of 1.0 ml/min and aqueous analyte samples of *ca.* 500 μg/ml and sample aliquots of 50 μl were used. Inlet pressures of 500–1500 p.s.i. were observed. A wavelength of 428 nm was used for the indirect visible detection.

Mobile phase preparation

The Thymol Blue dye was quantitatively transferred (appropriate volume of a 0.01 *M* Thymol Blue 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 dye adsorbed on the stationary phase was calculated from the breakthrough volume [7]. The column was then allowed to equilibrate for an additional 30–60 min.

RESULTS AND DISCUSSION

Two major equilibria can be used to describe the retention of inorganic and organic analyte ions on reversed stationary phases with mobile phases that contain a hydrophobic ion of opposite charge [7,8,12–15]. Eqn. 1 describes the first equilibrium that takes place, where the hydrophobic counterions sorbs on the stationary phase. The second equilibrium (eqn. 2) describes the interaction that takes place in the diffuse secondary layer between the analyte ion and the co-ion that is associated with the retained hydrophobic ion.

$$A + TB^- + C^- + M^+ \rightleftharpoons A \cdots TB^- \, {}^+M + C^- \quad (1)$$

$$A \cdots TB^- \, {}^+M + X^+ + C^- \rightleftharpoons A \cdots TB^- \, {}^+X + C^- + M^+ \quad (2)$$

A represents the stationary phase, TB^- represents an ion-interction reagent (UV-active counteranion) in the mobile phase, M^+ is the counteraction associated with the IIR, the buffer and/or added inert electrolyte, C^- is an anion associated with the countercation and/or the analyte cation, and X^+ is the analyte cation. The variables that have been found to affect the separation of ions are: the reversed stationary phase, the type and concentration of the IIR, the concentration of organic modifier, the type and concentration of countercation and/or buffer in the mobile phase, and the mobile phase pH. The inorganic analyte cations that were studied in this paper exhibited little or no retention on the stationary phases in the absence of the IIR, whereas the organic analyte cations show some retention, depending on the hydrophobicity of the organic analyte cation.

An advantage of the IIR used in this study is that it contains a chromophoric group which allows the indirect visible detection of the analyte cations. Care must be taken to keep the absorbance in the UV–visible detector below 0.8 A.U.F.S. when using indirect visible detection. If the detector absorbance exceeds 0.8 AUFS, the detector is outside of its linear working range. How indirect visible detection works can be explained by differences in the relative concentrations of the IIR in the effluent. As an analyte cation travels down the column (eqns. 1 and 2), the concentration of the UV-absorbing IIR band changes relative to the background absorbance. The concentration of the IIR in the band either increases, due to its removal from the column and provides a positive chromatographic peak, or it de-

creases, due to its uptake on the column, in which case a negative chromatographic peak is produced. The IIR in the mobile phase is responsible for both the retention of the analyte cations and in the indirect visible detection of the analyte cations.

Effect of Thymol Blue concentration

The first mobile phase parameter studied was the concentration of Thymol Blue and its effect on analyte cation retention. The amount of Thymol Blue adsorbed on the stationary phase was found to increase as its concentration in the mobile phase increased. The amount of Thymol Blue adsorbed on the stationary phase was found to be similar to that of low-capacity cation exchangers [12–14,21] and to mobile phases that contain ion-interaction reagents, such as alkylsulfonate salts [12–14].

As the concentration of Thymol Blue in the mobile phase increased, a corresponding increase in the amount of Thymol Blue adsorbed on the stationary phase was observed. This, in turn, leads to a higher number of cation exchange sites available on the stationary phase and should lead to higher analyte cation retention. A comparison of two different mobile phase concentrations of Thymol Blue is shown in Fig. 1. Chromatogram I shows the separation when 0.1 m*M* Thymol Blue was used, while chromatogram II shows the separation when the concentration of Thymol Blue was increased to 0.2 m*M*. Although the higher concentration of Thymol Blue provided longer retention times, the lower concentration of Thymol Blue provided better separation and better sensitivity. Therefore, lower concentrations of Thymol Blue were used.

Mobile phase variables: effect on Thymol Blue adsorption

The concentration of organic modifier was found to affect the amount of Thymol Blue adsorbed on the stationary phase. The amount of Thymol Blue adsorbed on the stationary phase decreased as the mobile phase concentration of organic modifier was increased. This, in turn, leads to a decrease in the number of cation-exchange sites present on the stationary phase and a corresponding decrease in analyte cation retention.

Ionic strength will also affect the amount of Thymol Blue adsorbed on the stationary phase [3,7,12, 13,15]. As the mobile phase ionic strength was increased, a corresponding increase in the amount of Thymol Blue adsorbed on the stationary phase was observed. The increase in the amount of Thymol Blue adsorbed showed an increase in the apparent number of cation-exchange sites present. The amount of Thymol Blue adsorbed on the stationary phase increased until an ionic strength of about 0.120 (0.075 m*M* NaCl) was reached, when the number of cation-exchange sites leveled off. Even though more cation-exchange sites are present at higher mobile phase ionic strengths, analyte cation retention decreased due to increased competition for the cation-exchange sites from the higher concentration of countercations (see eqn. 2).

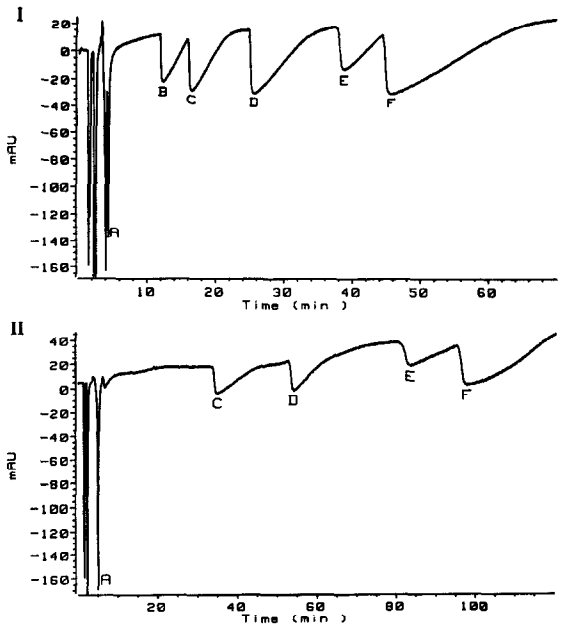

Fig. 1. Separation of several metal cations on a PLRP-S stationary phase at different mobile phase concentrations of Thymol Blue. Mobile phase: (I) 0.1 m*M* Thymol Blue, 15.0 m*M* tartrate (pH 3.7), acetonitrile–water (10:90); (II) as I except 0.2 m*M* Thymol Blue. A = Cu^{2+}; B = Zn^{2+}; C = Ni^{2+}; D = Co^{2+}; E = Fe^{2+}; F = Mn^{2+}.

Effect of organic modifier on cation retention

Fig. 2 illustrates how analyte cation retention was affected by the concentration of acetonitrile that covered a range of 7.5% to 20%. As the mobile phase concentration of acetonitrile was increased, the amount of Thymol Blue adsorbed on the stationary phase decreased. This, in turn, leads to a

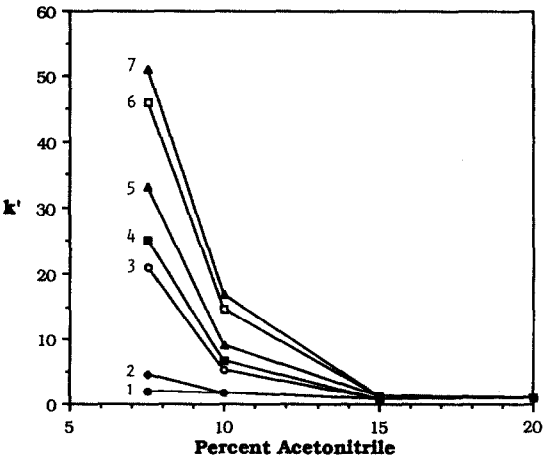

Fig. 2. Effect of acetonitrile concentration on analyte cation retention (k' = capacity factor). Mobile phase conditions: 0.1 mM Thymol Blue, 10.0 mM tartrate (pH 3.7) in acetonitrile–water. Curves: 1 = Fe^{3+}; 2 = Cu^{2+}; 3 = Zn^{2+}; 4 = Ni^{2+}; 5 = Co^{2+}; 6 = Fe^{2+}; 7 = Mn^{2+}.

lower number of apparent cation-exchange sites present and lower analyte cation retention. Resolution of the analyte cations was better at lower concentrations of acetonitrile due to the higher number of apparent cation-exchange sites present on the stationary phase. At lower concentrations of acetonitrile, breakthrough volumes for Thymol Blue were found to be extremely high. A mobile phase that contained 5% acetonitrile required over 2 l of eluent to be passed through the column before the breakthrough occurred.

Effect of pH

Mobile phase pH plays a very important role in the retention and separation of metal cations. Complexation takes place between the ligand in the mobile phase and the metal cations. The metal–ligand complexation was found to be affected by the mobile phase pH. Fig. 3 shows the effect of pH on transition-metal retention. Retention times changed dramatically over the pH range of 3.0 to 5.0 (ionic strength held constant). In this study, citric acid was the ligand used. Similar results were also obtained when tartrate was used. As the pH of the mobile phase was increased, metal retention decreased. This is attributed to complexation taking place between the ligand and the metal cation. Optimal conditions for metal cation retention and resolution were found between pH 3.5 and 4.0.

A similar study was also made for the alkaline-earth metals and for several simple guanidines. Retention of the alkaline-earth metals was found to be affected in the same way that the transition metals were: increasing the mobile phase pH led to lower retention times. As the pH of the mobile phase was increased from 3.5 to 7.0, retention of the alkaline-

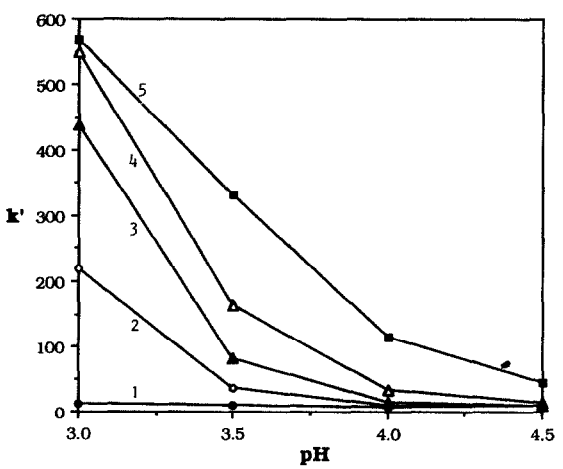

Fig. 3. Effect of mobile phase pH on analyte cation retention. Mobile phase conditions: 0.1 mM Thymol Blue, 3.0 mM citrate, acetonitrile–water (10:90). Curves: 1 = Fe^{3+}; 2 = Cu^{2+}; 3 = Ni^{2+}; 4 = Zn^{2+}; 5 = Fe^{2+}.

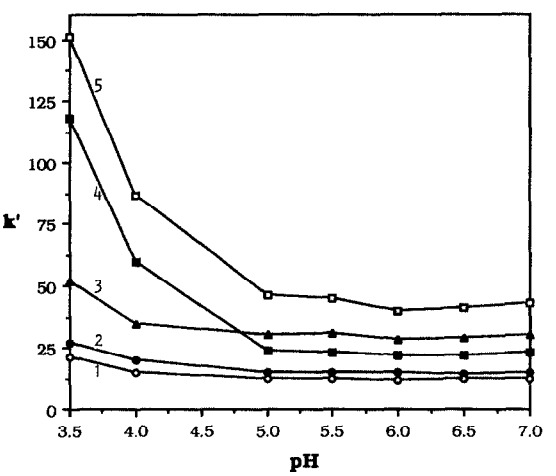

Fig. 4. Effect of mobile phase pH on analyte cation retention. Mobile phase conditions: 0.1 mM Thymol Blue, 15.0 mM tartrate, acetonitrile–water (10:90). Curves: 1 = guanidine; 2 = 1-methylguanidine; 3 = 1-ethylguanidine; 4 = Ca^{2+}; 5 = Mg^{2+}.

earth metals decreased; however, selectivities did not change. The simple guanidines initially decreased in retention with increasing pH and then leveled off at pH 5.0. The effect of mobile phase pH on the retention of the alkaline-earth metals and simple guanidines, is shown in Fig. 4.

Effect of ligand concentration

The concentration of ligand in the mobile phase plays a key role in the separation of the metal cations. The complexation that takes place between the metal cation, the adsorbed IIR, and the mobile phase ligand control retention and resolution. If a ligand were not added to the mobile phase, the metals would have very high retention due to the strong complexation with the sorbed IIR. If IIR were absent but the mobile phase ligand was present, the metal cations would show little or no retention.

The results observed for the Thymol Blue–citrate or tartrate mobile phases and the metal cations indicated that the ligand concentration had a major affect on metal retention. At low concentrations of ligand, retention of the metals was very high. As the concentration of ligand was increased, metal retention decreased. Elution orders were found to remain the same over the ligand concentration range. Elution orders for the transition metals with either a citrate or a tartrate mobile phase were: $Fe^{3+} < Cu^{2+} < Zn^{2+} < Ni^{2+} < Co^{2+} < Fe^{2+} < Mn^{2+}$. Elution orders for the alkaline-earth metals were different, depending on the ligand used. For a citrate mobile phase the elution order was $Mg^{2+} < Ca^{2+} < Sr^{2+} < Ba^{2+}$, whereas when tartrate was added to the mobile phase the elution order was $Ca^{2+} < Sr^{2+} < Ba^{2+} < Mg^{2+}$. This difference in elution order is apparently due to differences in the complexation between Thymol Blue, alkaline-earth metals, citrate, and tartrate. The complexation between magnesium and citrate is substantially stronger than that of magnesium and tartrate. Fig. 5 shows the separation of the alkaline-earth metals with a mobile phase containing tartrate.

Effect of ionic strength

Sodium chloride was added to the mobile phase in order to determine the effect of ionic strength on cation retention. Retention of the cations decreased as the concentration of sodium chloride was increased. This is attributed to increased competition

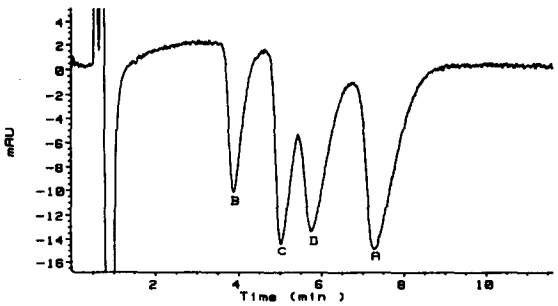

Fig. 5. Separation of alkaline-earth metals on a Hamilton PRP-1 column. Mobile phase conditions: 0.1 m*M* Thymol Blue, 25.0 m*M* tartrate (pH 4.5), acetonitrile–water (10:90). A = Mg^{2+}; B = Ca^{2+}; C = Sr^{2+}; D = Ba^{2+}.

for the cation-exchange sites (eqn. 2). As previously stated, the amount of Thymol Blue adsorbed on the stationary phase increases with increasing ionic strength, and this leads to an increase in the apparent number of cation-exchanges sites. However, this did not lead to an increase in cation retention, since competition for the cation-exchange sites was increased due to the higher concentration of sodium ions present.

Separation on an ODS column

A silica-based ODS column was coated with Thymol Blue and used for the separation of different cations. The separations on the ODS column were better than those on the polymer-based columns. One problem with the silica-based columns, however, was the lack of ruggedness. The columns did not last as long as the polymer-based columns. Fig. 6 shows the separation of several transition metals

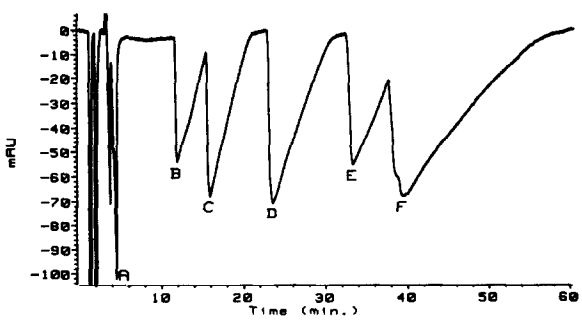

Fig. 6. Separation of several transition metals on a silica-based ODS column (B&J OD5). Mobile-phase conditions: 0.1 m*M* Thymol Blue, 15.0 m*M* tartrate (pH 3.7), acetonitrile–water (12.5:87.5). A = Cu^{2+}; B = Zn^{2+}; C = Ni^{2+}; D = Co^{2+}; E = Fe^{2+}; F = Mn^{2+}.

on a silica-based column. When this is compared with the metal separation on the polymer-based column (Fig. 1), the more efficient silica-based column provided better peak shape and a better separation. The results for the different mobile phase variables on the silica-based column were similar to those observed for the polymer-based columns.

CONCLUSIONS

A Thymol Blue-coated stationary phase provided acceptable separations of the inorganic and organic cations studied. The mobile phase variables affecting cation retention were identified and studied. Good separations of all of the different cations studied were obtained. The elution order of Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} depend on the ligand used. When a tartrate mobile phase was used, Mg^{2+} was eluted after Ba^{2+}. Elution orders for the transition metals did not change when different ligands were used.

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