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Dynamic coating ion-exchange chromatography of cations on an octadecyl-bonded silica stationary phase

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Abstract

It was found that common cations (Na^+ , NH_4^+ , K^+ , Mg^{2+} and Ca^{2+}) could be strongly retained on an ODS stationary phase when aqueous solutions of carboxylic acids were used as eluents. The chromatographic conditions used in this work were the same as in common cation-exchange chromatography on a cation-exchange resin and the retention behavior of the above-mentioned cations on the ODS column was quite similar to that on a cation-exchange column. The retention behavior and mechanism have been investigated using a number of carboxylic acids as eluents. The retention mechanism of the cations in these experiments was considered to be a dynamic coating ion-exchange mechanism. The carboxylic acids in the mobile phase were coated onto the surface of the ODS stationary phase and formed a dynamic carboxylic acid functional layer which could act like the functional group layer of a carboxylic group cation exchanger. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Mobile phase composition; Dynamic coating ion-exchange chromatography; Ion-exchange chromatography; Carboxylic acids; Inorganic cations

1. Introduction

Octadecylbonded silica (ODS) stationary phase, which is the most common reversed-phase liquid chromatographic stationary phase, can be used for the separation of ionic species [1–4]. Traditionally, two methods have been reported for the separation of cations using ODS columns. One method is ion-pair chromatography (IPC); first the cations or their complex anions formed hydrophobic ion-pair compounds with a counter ion offered by the ion-pair reagent, and then the ion-pair compounds could be

retained and separated on the ODS stationary phase by the hydrophobic distribution mechanism. However, alkali metal and alkali earth metal ions do not readily form complexes with any other reagents, so separations of these ions using IPC were very rare. The other method for the separation of cations on the ODS stationary phase is based on coating the stationary phase. The usual coating reagents are anion surfactants and micellar taurine-conjugated bile salts [5–7] or crown ether compounds [8–10]. Usually, the coating reagents were coated on the surface of the ODS stationary phase permanently and formed a functional layer to retain cations. The coating methods with micellar taurine-conjugated bile salts and crown ether compounds would be harmful to the column because the coating reagents

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may be adsorbed on the ODS stationary phase permanently making regeneration of the column very difficult. In addition, an ODS column has a cation-exchange ability under acidic eluent conditions due to the presence of metal oxides such as Al_2O_3 and Fe_2O_3 as impurities in the silica gel. But an ODS column with a low content of Al_2O_3 and Fe_2O_3 as impurities in the silica gel is not enough to be applied to analysis of cations.

In this work, we found that common cations (Na^+ , NH_4^+ , K^+ , Mg^{2+} and Ca^{2+}) were retained on an ODS stationary phase when aqueous solutions of carboxylic acids were used as eluents. The chromatographic conditions used in this work were the same as in normal cation-exchange chromatography with a cation-exchange resin. The retention behavior of the cations on the ODS column was different from IPC and the coating stationary phase chromatography reported while quite similar to that on a cation-exchange column. Therefore, a dynamic coating ion-exchange chromatography (DCIEC) was considered. In our study, the carboxylic acids were used as both the coating reagents and eluents, and no other additives were used in the mobile phase. The carboxylic acid in the mobile phase could be adsorbed on the surface of the ODS stationary phase to form a dynamic coating carboxylic acid layer which is of functional carboxylic group. The cations could retain on the dynamic coating ODS stationary phase like as on cation-exchange resins. The carboxylic acid layer coated dynamically on ODS stationary phase could be easily washed out by water after use.

2. Experimental

2.1. Apparatus

The ion chromatograph used in this study was HIC-6A (Shimadzu, Japan), consisting of a LP-6A liquid delivery pump, a CTO-6AS column oven and a CCD-6A conductance detector. The column oven was maintained at 40 °C. Samples were injected using a 20- μl loop injector. A Shimadzu chromatographic workstation (C-R4A) was used for data processing.

2.2. Chemicals

All chemicals used were of analytical-reagent grade and deionized water was used to prepare all solutions and mobile phases. Stock solutions of cations were prepared as an aqueous solution at a concentration of 1000 mg/l using their chloride salts for NH_4^+ , K^+ , Mg^{2+} and Ca^{2+} and sodium nitrate for Na^+ . Potassium chloride and sodium nitrate were purchased from Beijing Hongxing (China), calcium chloride from Beijing Jingqiu (China), and ammonium chloride and magnesium chloride from Beijing Yili (China). The standard cation mixtures were prepared using their stock solutions.

2.3. Chromatographic conditions

A TianHe C_{18} column (250 mm \times 4.6 mm I.D., 10 μm , Tianjin, China) was used. The mobile phases used in this study were aqueous solutions of carboxylic acids with concentrations ranging from 0.2 to 5.0 mM. The carboxylic acids used were formic acid, acetic acid, propionic acid, *n*-butyric acid, *n*-valeric acid, *n*-capronic acid, citric acid, oxalic acid, salicylic acid, maleic acid, D-tartaric acid, benzene-1,2,3,5-tetracarboxylic acid, malonic acid, glutaric acid, adipic acid, maleic acid and fumaric acid. The flow-rate was 1.0 ml/min. The column oven temperature was set at 40 °C. The sensitivity of the conductance detector was 1 $\mu\text{S}/\text{cm}$.

3. Results and discussion

3.1. Retention behaviors of cations on ODS column

The retention behaviors of alkali metal and alkali earth metal ions were investigated using the above-named carboxylic acids as eluents. Both alkali metal and alkali earth metal ions could be retained strongly on the ODS column (TianHe C_{18}). Alkali metal and alkali earth metal ions were eluted in two groups. The resolution between groups was fairly high whereas the separation of the cations in the same group was difficult, and the resolution of the cations was different when the different eluents were used. The separation pattern of the cations was the same as

on a cation-exchange resin. As an example, the chromatogram of four cations (K^+ , Na^+ , Ca^{2+} and Mg^{2+}) obtained using TianHe C_{18} column and oxalic acid eluent is shown in Fig. 1. Although this chromatogram suggests that the column performance and the plate counts of the column are not comparable to a cation-exchange resin possessing carboxylic acid functions, this technique offer a new method for simple and convenient analysis of ions. In the obtained results, peak tailings led to the poor separate efficiency. Subsequent work would focus on the improvement of separation and the application of this new technique.

Running the mobile phase for about 30–60 min, the carboxylic acid in the mobile phase is coated onto the surface of ODS stationary phase to arrive a dynamic equilibrium. The equilibrium time decreases with increasing the concentration of the carboxylic acids in the mobile phase because the concentration of the carboxylic acids will affect the rapidity of adsorption and desorption of the carboxylic acid molecules in the process of dynamic coating equilibrium. In our experiment, dynamic coating equilibrium could be judged by the smoothness and steadiness of the base line.

The retention behavior of the cations on the ODS column was different from IPC and the reported coating stationary phase chromatography while it was quite similar to the retention behavior of cations

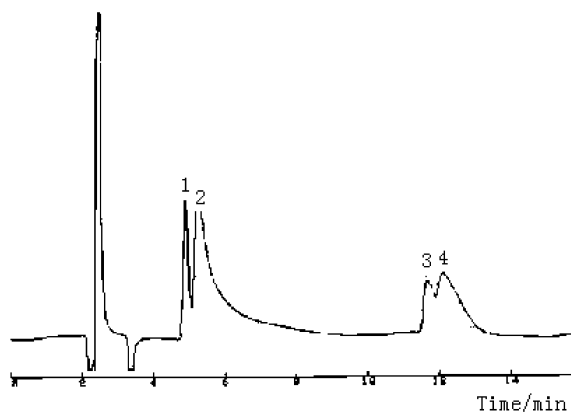


Fig. 1. Separation of cations on ODS column. Column: TianHe C_{18} (250 mm \times 4.6 mm I.D.); mobile phase: 1.1 mM aqueous solution of oxalic acid; flow-rate: 1.0 ml/min; detection: conductivity; column temperature: 40 °C; sample size: 20 μ l; peaks: 1= Na^+ , 2= K^+ , 3= Mg^{2+} , 4= Ca^{2+} .

on a cation-exchange column. Therefore, a DCIEC method was considered. When the aqueous solution of carboxylic acids were used as eluents, the carboxylic acid molecules were adsorbed on the surface of an ODS stationary phase by the hydrophobic interaction to form a carboxylic acid molecular layer. The adsorption equilibrium of the carboxylic acid molecules on the ODS stationary phase was in a dynamic equilibrium. The carboxylic acid molecules adsorbed on the surface of ODS stationary phase could contribute anionic functional groups (carboxylic groups) on which the cations were adsorbed by the electrostatic interaction. H^+ ionized from carboxylic acid in the mobile phase could act as the eluent ion. The eluting ability of the mobile phase increases with increasing H^+ concentration in the mobile phase, and the increase of H^+ concentration in the mobile phase will cause a decrease of the retention time of the cations.

DCIEC is very different from IPC. In reversed-phase IPC, solute ions form hydrophobic ion-pair compounds with an ion-pair reagent, and then the ion-pair compounds are adsorbed on the stationary phase by hydrophobic interaction of the ion-pair reagent. The elution of solutes is controlled by the distribution of the ion-pair reagent used between the surface of the ODS stationary phase and the mobile phase. In IPC, the stronger the hydrophobic interaction of ion-pair reagent, the longer the retention of solutes. The experimental results shown in following section validate the DCIEC mechanism.

3.2. Effects of the properties of carboxylic acids on the retention of cations

Aqueous solutions of carboxylic acids are the most usual eluents for cation-exchange stationary phases. The ODS stationary phase has been widely applied to the separation and analysis of the carboxylic acids in food, environmental and medication samples [11–13] in HPLC because carboxylic acids not only can be retained, but can be separated on an ODS stationary phase. Therefore we attempted to apply an ODS column to the separation of cations in ion chromatography (IC). In our experiments, alkali metal ions (Na^+ , NH_4^+ and K^+) and alkali earth metal ions (Ca^{2+} and Mg^{2+}) were retained and separated on ODS stationary phases using aqueous

solutions of carboxylic acids as eluents. The retention behaviors of the cations should answer for the cation-exchange mechanism if the DCIEC mechanism assumed in this paper were true. In IEC, the key parameters influencing the retention behaviors of solute ions are the pH of the mobile phase and the concentration of the eluent ion. The retention time of solutes decrease with increasing eluent ion concentration. In this experiment, the eluent ion is H^+ ionized from the carboxylic acids in the mobile phase. The dissociation constant of the carboxylic acids affects the concentration of H^+ . The H^+ concentration and the eluting ability of the mobile phase were increased with the increase of dissociation constants of the carboxylic acids. In this work, some studies were undertaken to compare DCIEC with IPC, because DCIEC is easily mistaken for IPC.

Firstly, the effects of carboxylic acid structure on the retention time of cations were investigated under the same H^+ concentration. Aqueous solutions of short chain carboxylic acids including acetic acid, propionic acid, *n*-butyric acid, *n*-valeric acid and *n*-caproic acid were used as eluents. The longer the carbon chains in these carboxylic acids, the stronger is their hydrophobic interaction. In IPC, the retention ability of cations is based on the hydrophobic interaction ($\log P$) of cations [14,15]. A higher value of $\log P$ means a stronger retention of the cations. The $\log P$ of the carboxylic acids could be calculated using the following formula [14]:

$$\log P = f(\text{CH}_3) + nf(\text{CH}_2) + f(\text{COOH})_{al}$$

where n is the amount of $-\text{CH}_2$ including in the carboxylic acid molecule. The values of $f(\text{CH}_3)$, $f(\text{CH}_2)$ and $f(\text{COOH})$ were 0.702, 0.530 and -0.954 , respectively. The values of $\log P$ for acetic acid, propionic acid, *n*-butyric acid, *n*-valeric acid and *n*-caproic acid were -0.252 , 0.278 , 0.808 , 1.338 and 1.868 , respectively. If an IPC mechanism is assumed, the anionic parts of the carboxylic acids would form ion-pair compounds with the cations, the retention of the cations would be based on the hydrophobic interaction of carboxylic acid anion part, so the retention time of the cations would increase with increasing the carbon chain length of the carboxylic acids. However, the retention times of the cations were almost the same when different

chain-length carboxylic acids were used as eluents. This could not be explained with the IPC mechanism, but could be well explained by the DCIEC mechanism. In cation-exchange chromatography, the retention time of the cations would decrease with increasing the H^+ concentration in the mobile phase. The short chain carboxylic acids used in this study have similar dissociation constants and concentrations (1.0 mM), so similar retention times for the cations were obtained (Table 1). Elution of Mg^{2+} and Ca^{2+} was difficult under these eluent conditions. In IEC, divalent ions have much higher retention times than mono-valent ions on ion-exchange resins. It is also proved that the ion-exchange mechanism is reasonable on a dynamic coating ODS stationary phase.

Aqueous solutions of *meso*-tartaric acid, citric acid and malonic acid were also used as eluents. These three carboxylic acids are of similar dissociation constants (k_{a1}), but since their structures are different, their hydrophobic interactions are also different. However, the retention times of the cations were similar in this experiment (Table 2). This phenomenon could be explained using the DCIEC (but not the IPC) mechanism.

Secondly, the effect of dissociation constants of the carboxylic acids on the retention of the cations was investigated using carboxylic acids with similar structures as eluents. Maleic acid and fumaric acid are a pair of isomeric compounds, of similar structure and hydrophobic interaction, but their dissociation constants are different. Maleic acid is a stronger acid than fumaric acid; so the eluting ability of maleic acid would be greater than fumaric acid under the same concentration in IEC. Table 3 shows the retention time of the cations using the same concentration (1.0 mM) of maleic acid and fumaric acid

Table 1
Retention times of cations obtained using carboxylic acids possessing similar dissociation constants as eluents

Carboxylic acid	Dissociation constant, pK_a	Retention time (min)		
		Na^+	K^+	NH_4^+
Acetic acid	4.86	12.82	13.00	12.90
Propionic acid	4.87	12.86	13.14	12.95
<i>n</i> -Butyric acid	4.83	12.76	12.94	12.89
<i>n</i> -Valeric acid	4.84	12.80	12.95	12.89
<i>n</i> -Caproic acid	4.88	12.91	13.20	13.06

Table 2
Retention times of cations obtained using the carboxylic acids possessing similar dissociation constants as eluents

Carboxylic acid	Dissociation constant			Retention time (min)				
	pK_{a1}	pK_{a2}	pK_{a3}	Na^+	K^+	NH_4^+	Mg^{2+}	Ca^{2+}
D-Tartaric acid	2.98	4.34		6.62	7.36	7.12	28.44	29.01
Malonic acid	2.85	5.69		6.46	7.19	7.15	24.77	26.70
Citric acid	2.96	4.76	6.40	6.95	7.67	7.57	30.00	31.72

Table 3
Retention times of cations obtained using the carboxylic acids possessing similar structure as eluents

Carboxylic acid	Dissociation constant		Retention time (min)				
	pK_{a1}	pK_{a2}	Na^+	K^+	NH_4^+	Mg^{2+}	Ca^{2+}
Maleic acid	1.92	6.23	5.55	5.99	6.02	17.92	17.44
Fumaric acid	3.02	4.38	6.89	7.45	7.45	29.58	28.49

as eluents. The retention times of the cations using maleic acid was shorter than with fumaric acid as eluent—a result that is also in keeping with the dynamic coating cation-exchange chromatography mechanism.

3.3. Effect of organic solvent in mobile phase

In this experiment, aqueous solutions of carboxylic acids were used as mobile phases and the polarities of the mobile phases were greater than that of the stationary phase. The polarity of the mobile phases should decrease with the addition of organic solvent in the mobile phase. When the content of methanol in mobile phase increased from 5 to 20%, the polarity of the mobile phase varied. Because the dissociation of methanol is very poor compared to that of the acid used in the mobile phases, addition of methanol to the mobile phase could not affect the pK_a value of carboxylic acids in the mobile phase. In IPC, the retention of solutes was obviously affected by the content of organic solvent in the mobile

phase, and the retention time should decrease with increasing the concentration of organic solvent. In our experiment, 5–20% of methanol was added to the oxalic acid eluent, but there was no obvious decrease of the retention times of the cations (Table 4). This result proved that the retention of the cations did not submit with the IPC mechanism. In cation-exchange chromatography, adding methanol to the mobile phase could not affect the dissociation of eluents (carboxylic acids), so the eluting ability of the eluents was hardly changed. When the concentration of methanol in mobile phase was increased, carboxylic acids coated on the surface of ODS stationary phase would be more easy to desorb from the stationary phase, so the equilibrium process of adsorption and desorption of carboxylic acids would be faster.

3.4. Effect of the eluent concentration on the retention of cations

In IEC, the retention time of ionic species de-

Table 4
Effect of methanol in the mobile phase on the retention of the cations

Mobile phase	Retention time (min)			
	Na^+	K^+	Mg^{2+}	Ca^{2+}
1.1 mM Oxalic acid	4.94	5.38	11.58	11.71
1.1 mM Oxalic acid with 5% methanol	4.69	5.07	10.65	11.13
1.1 mM Oxalic acid with 10% methanol	4.71	5.52	10.86	11.19
1.1 mM Oxalic acid with 15% methanol	4.82	5.50	11.82	12.10

creases with an increase in the concentration of the eluent and there is a linear relationship between the logarithm of the capacity factor of solute ions ($\log k'$) and the logarithm of the eluent concentration ($\log [E]$):

$$\log k' = -\frac{y}{x} \cdot \log [E] + C \quad (1)$$

where k' is the capacity factor of solute ion, y is the charge of the solute ion, x is the charge of eluent ion, C is a constant and $[E]$ is the concentration of eluent. Fig. 2 shows the relationships between $\log k'$ and $\log [E]$ using aqueous solutions of oxalic acid (Fig. 2A) and maleic acid (Fig. 2B) as mobile phase. The plots

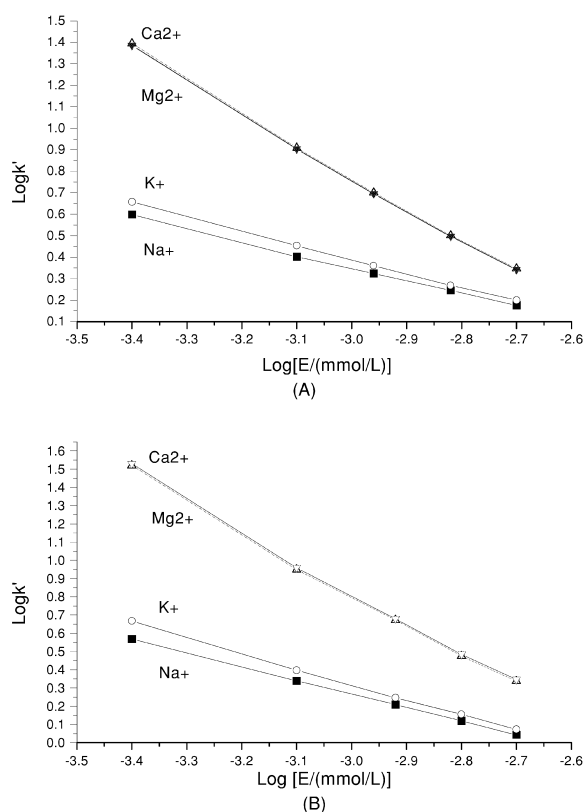


Fig. 2. Graphs of the logarithm of the capacity factor of cations ($\log k'$) against the logarithm of the eluent concentration ($\log [E]$). Column: TianHe C_{18} (250 mm \times 4.6 mm I.D.); mobile phases: (A) aqueous solution of oxalic acid (the concentrations of mobile phase were 0.4, 0.8, 1.1, 1.5, 2.0 mM), (B) aqueous solution of maleic acid (the concentrations of mobile phase were 0.4, 0.8, 1.2, 1.6, 2.0 mM); flow-rate: 1.0 ml/min; detection: conductivity; column temperature: 40 °C; sample size: 20 μ l.

show a good linearity for each cation. This result also proves that the retention mechanism of cations on the ODS stationary phase in these experiments was DCIEC.

4. Conclusions

In this work, it was found that common cations (Na^+ , NH_4^+ , K^+ , Mg^{2+} and Ca^{2+}) could be retained on an ODS stationary phase remarkably strongly when aqueous solutions of carboxylic acids were used as eluents, and a new concept, DCIEC was established to explain the retention behavior of the common cations on the ODS stationary phases. It was proved that the retention mechanism of the cations on the ODS stationary phase is an ion-exchange mechanism, but is not an IPC mechanism. DCIEC has several merits compared with cation-exchange chromatography and IPC. First, DCIEC used the most common ODS column which is very much cheaper than an ion-exchange column. Secondly, DCIEC used a much simpler mobile phase than that used in IPC to which some ion-pair reagent and organic solvent must be added. Thirdly, DCIEC can use conductivity detection for the detection of the cations, so the analysis of cations on ODS columns can be accomplished by employing an ion chromatograph. Under the present conditions, baseline separation of the same valent cations was difficult. In the future, we will perfect the method and improve the resolution.

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