Research Article

Investigation of Cesium Ions as Silanol-Blocking Additives in Reverse-Phase Liquid Chromatographic Separations

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Abstract

In this work, the silanol-blocking ability of cesium ions (Cs^+) in reverse-phase liquid chromatographic separations with different characterizing methods for silanol activity has been investigated. A comparison of the blocking ability of Cs^+, barium ions (Ba^{2+}) and amine silanol blockers, including triethylamine and octylamine, on the ion-exchange interaction, was performed using six basic probe solutes at pH 7 on a conventional C_{18} phase. The effect of blocking the hydrogen bonding interaction of different additives was also characterized by using toluidine isomers, p-ethylaniline, aniline and phenol as probes. The results demonstrate that the ability to block silanophilic interactions is greater for Cs^+ than for Ba^{2+}. Considering the blocking ability of Cs^+ on both ion-exchange and silanophilic interactions, Cs^+ exhibits a better blocking effect with respect to tailing compared to Ba^{2+}. When using Cs^+ as an additive in the mobile phase to analyze four alkaloids, protopine, allocryptopine, sanguinarine, and chelerythrine, a better silanol-blocking effect was obtained using a slightly acidic mobile phase.

Keywords: Alkaloids; Barium; Cesium; RPLC; Silanol-Blocking Additives

Introduction

Silica is the most used support for Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) stationary phases because of its versatility. However, silica supports have some disadvantages, especially in the analysis of basic compounds, because of the strong interaction between bases and the acidic residual silanols of the support. This interaction results in asymmetric peaks, low column efficiency, poor reproducibility and larger retention times than expected. Many studies have been carried out to block, remove or decrease the number of residual silanols on the silica surface. To block the interaction between silanols and amines on silica, the addition of mobile phase additives, e.g., triethylamine and trifluoroacetic acid, has been regularly employed. In addition to organic additives, the blocking effects of different alkaline earth metal cations including calcium (Ca^{2+}), magnesium (Mg^{2+}) and barium (Ba^{2+}) have also been demonstrated [1]. The results concluded that Ba^{2+} was the best blocker among the alkaline earth metal ions.

Ba^{2+} can be a good choice for use as a silanol-blocking agent if amine additives cause problems such as column instability, slow equilibration, or if they present other disadvantages, such as chemical reactivity with the analyte. However, Ba^{2+} easily forms precipitates with anions such as SO_4^{2-} and PO_4^{3-}, so its applicability as a tailing-blocking additive is limited when certain buffers are components of the mobile phase. Therefore, if an alkaline metal ion had a similar blocking ability, the limitation caused by precipitation will be reduced. With the development of a constructed micro-apparatus, the interaction between the surface of silica and alkaline metal ions has been investigated [2-9]. Dishonet al. [7] have investigated the

interaction between silica surfaces and aqueous electrolytes in 1:1, 2:1, and 3:1 ratios using Atomic Force Microscopy (AFM) [9]. They obtain the following ranking of monovalent and divalent cation adsorbability to silica: $Mg^{2+} < Ca^{2+} < Na^+ < Sr^+ < K^+ < Cs^+$. The results imply that Cs^+ could have silanol-blocking ability. In this paper, the silanol-blocking ability of Cs^+ was investigated using different characterizing methods for silanol activity. A comparison of the blocking abilities of Cs^+, Ba^{2+} and amine silanol blockers was also completed on a conventional C_{18} phase.

Experimental

Apparatus

HPLC analysis was carried out on a Shimadzu LC-20A HPLC system (Shimadzu, Japan) consisting of two LC-20AT HPLC pumps, an SPD-M20A detector (190-800 nm) and a column temperature controller-20AC. Data processing and the calculation of peak parameters were performed with an LC-solution chromatographic workstation (Shimadzu, Japan). LC separations were accomplished on a Spherigel C_{18} column (5 μm, 200 mm × 4.6 mm, specific surface area 270 m^2/g, carbon loading 12.5%, end capping; Dalian Johnsson Separation Science & Technology Corporation, China) at 25°C. The injection volume was 20 μL. DAD detection was set with a range of 200-450 nm.

Chemicals

All chemicals, such as Cesium Nitrate ($CsNO_3$), Barium Nitrate ($Ba(NO_3)_2$), 3-[N-Morpholine]-Propanesulfonic Acid (MOPS), Triethylamine (TEA), Octylamine (OA), formic acid, 1-(2-pyridyl)piperazine, dibenzylamine, N-isopropylbenzylamine, benzylamine, N-methylbenzylamine N,N-dimethylbenzylamine, phenol, aniline, p-ethylaniline, and toluidine isomers, were analytical grade and purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Acetonitrile (ACN) was HPLC grade and purchased from Tedia Company, Inc. (Fairfield, OH, USA). Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA). Standards of Protopine (PRO), Allocryptopine (ALL), Sanguinarine (SA), and Chelerythrine (CHE) were prepared by preparative HPLC in our laboratory. Structures of the alkaloids are given in (Figure 1). Their identity was verified by ESI/MS, ^{1}H NMR and ^{13}C NMR. Their purity was higher than 96.5% (HPLC).
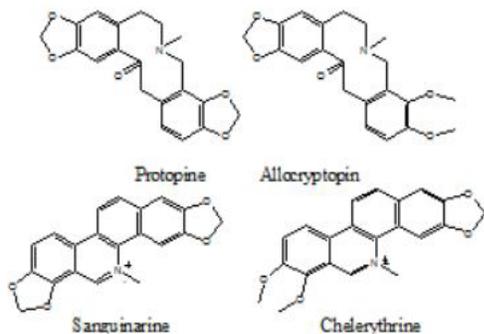

Figure 1: Structures of four alkaloids.

Preparation of probe solutions and standard solutions

The probe solutions, i.e., 1-(2-pyridyl)piperazine, benzylamine, dibenzylamine, N-isopropylbenzylamine, N-methylbenzylamine N,N-dimethylbenzylamine, phenol, aniline, p-ethylaniline, and toluidine isomers, used to characterize silanol activity were prepared in aqueous methanol solution (1:1 v/v). The concentrations were all 5 μg/mL. Stock solutions of PRO, ALL; SA and CHE were prepared in methanol. The concentration of all solutions was 1.0 mg/mL and they were stored at -4°C. One milliliter aliquots of each stock solution were transferred into a 50 mL volumetric flask, mixed and diluted to volume to yield a mixed working standard solution (each 20 μg/mL).

Results and Discussion

Competition between the probe solutes and the blocking additives for silanol sites under the maximized ion-exchange interaction

As described in Mario's work [1], when the pH of the mobile phase is 7, ion-exchange interactions are maximized and competition for silanol sites between the probe solutes and blocking additives can be best sensed. Therefore, we used the same probe system to characterize the blocking ability of agents on the ion-exchange between the analyte and the cation associated with the ionized silanol group. LC separations were accomplished on a Spherigel C_{18} column. The mobile phase for isocratic elution was ACN + aqueous solution (pH=7.0) containing 10 mM of the buffer MOPS and 10 mM of the blocking additives (30 + 70, v/v). The flow rate was set at 1 mL/min. The injection volume was 20 μL. Using 1-(2-pyridyl) piperazine (pKa=9.5) as an example probe, the effect of 10 mM Cs^+, Ba^{2+}, OA, and TEA solutions compared with buffer alone is shown in (Figure 2). From the chromatogram, it can be seen that Cs^+ has a significant blocking effect on silanol groups. Although the retention time using Cs^+ is slightly longer than that with the mobile phase containing Ba^{2+}, the peak is more symmetrical.
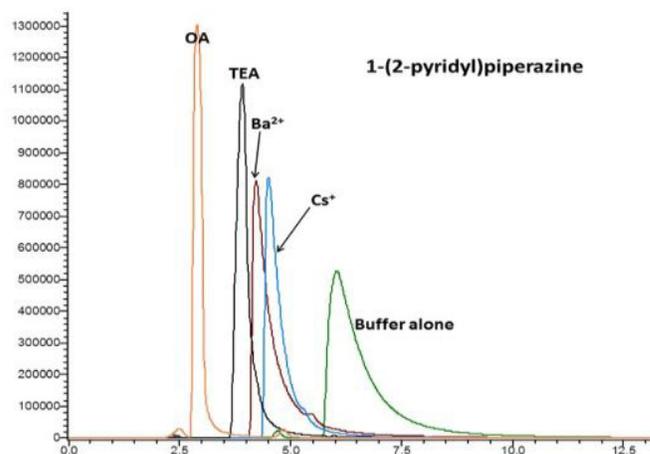

Figure 2: Effect of silanol-blocking additives on 1-(2-pyridyl) piperazine peak shapes.

The effect of the mobile phase additives on retention factor and peak asymmetry of probe analytes is shown in (Figure 3). The results of amine additives are consistent with those described in reference [1]. However, the tailing factors using Cs^+ are all smaller than those using Ba^{2+} for different probe solutes.
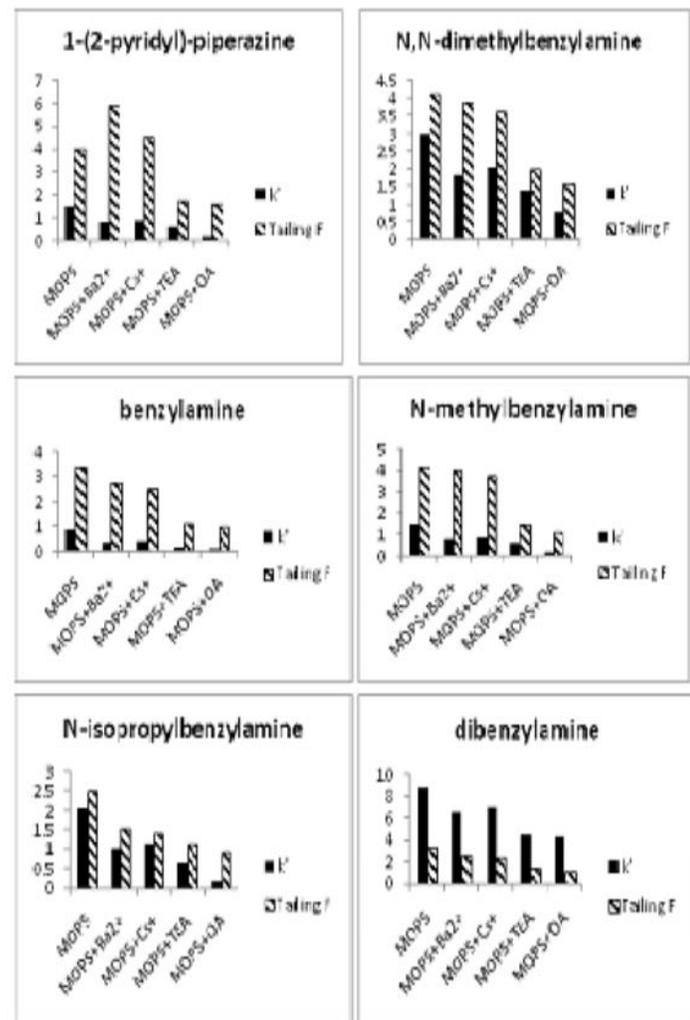

Figure 3: Effect of the mobile phase additives on retention factor and peak asymmetry of probe analytes.

Characterization of silanophilic interactions when using blocking additives

Silanol activity is normally evaluated through two different properties: hydrogen bonding ability and ion-exchange properties [10]. One of the most prominent tests to date was proposed by Engelhardt et al. [11,12]. To investigate the causes of the improved peak symmetry using Cs^+ compared to Ba^{2+}, aniline and phenol,

toluidine isomers, and p-ethylaniline were used as probe solutes to investigate the silanophilic interactions in the presence of different additives. The results are listed in (Table 1).

Additives	Resolution of aniline and phenol	Toluidine isomers (o-, m-, p-)		Tailing Fof p-ethyla-niline
		Peak number	Tailing F	
-	1.12	3	-	1.66
10 mM BaCl₂	1.67	2	-	1.64
10 mM CsNO₃	1.78	1	1.07	1.20
10 mM TEA (pH=7.0 adjusted by formic acid))	1.72	1	1.01	1.04
10 mM OA (pH=7.0 adjusted by formic acid)	1.77	1	1.01	1.01

Table 1: Characterization of silanophilic interactions in the presence of different additives.

Note: The mobile phase was $MeOH+H_2O$ (49+51, v/v), 1 mL/min. Detection: 254 nm.

According to Engelhardt et al. [11] silanophilic interactions can be determined by the asymmetry value and elution order of the toluidine isomers, p-ethylaniline, aniline and phenol. First, when aniline is eluted before phenol, silanophilic interactions can be considered negligible. Second, if the toluidine isomers are not separated and are eluted with symmetric peaks, silanophilic interactions are also considered negligible. Third, the asymmetry value at 10% of the p-ethylaniline peak can be used to characterize silanophilic properties [11,12]. If the additives block silanophilic interactions, the results on the same column with different additives should be similar to those on different columns without additives. From (Table 1), it can be seen that the blocking ability for silanophilic interactions is better for Cs^+ than Ba^{2+}, which is almost the same as that of TEA and OA. Therefore, considering the blocking ability of Cs^+ on both ion-exchange and silanophilic interactions, Cs^+ exhibits a better blocking effect with respect to tailing than Ba^{2+}.

Analysis of alkaloids using Cs^+ as a blocking agent

Using Cs^+ as a blocking agent, four alkaloids, PRO, ALL, SA, and CHE, were analyzed. Better adsorption of cations to silica has been observed at slightly acidic pH values [13,14], implying that the silanol-blocking ability of Cs^+ should be higher in an acidic mobile phase than in a neutral mobile phase. (Figure 4) shows the corresponding chromatograms. The tailing factors and retention times of alkaloids were decreased with an increase in the concentration of formic acid. When the concentration was higher than 20 mM, the peak parameters were not changed.
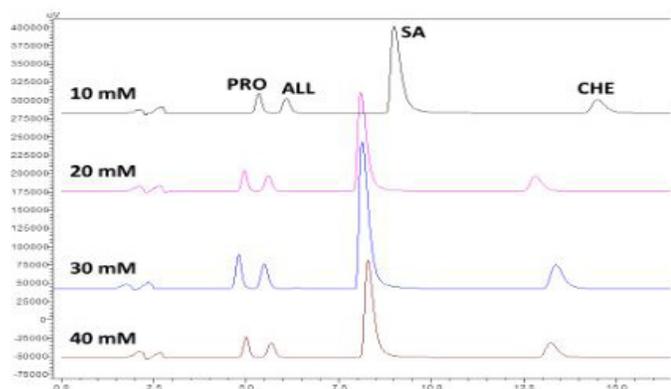
Figure 4: Chromatograms of alkaloids using different concentrations of formic acid in the mobile phase (Mobile phases: 30% ACN + 10 mM $CsNO_3$ + formic acid).

The efficiency of the reduction of peak tailing also varied with changing concentrations of the additives. To obtain optimal conditions, different concentrations such as 1 mM, 5 mM, 10 mM, 20 mM, and 30 mM Cs+ were tested. (Figure 5) shows the corresponding chromatograms. With increasing concentration, the elimination of peak tailing improved.
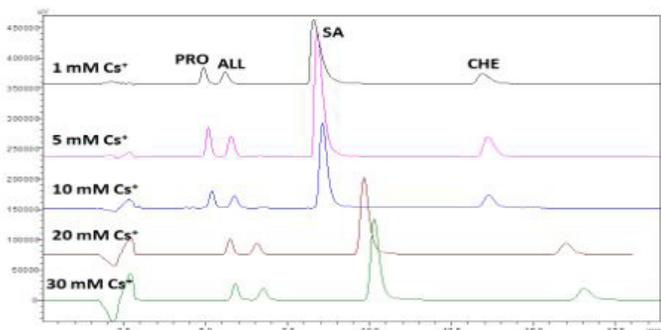

Figure 5 Chromatograms of four alkaloids using different concentrations of $CsNO_3$ in the mobile phase (Mobile phase: 30% ACN + 30 mM formic acid + $CsNO_3$).

However, when the concentration of Cs^+ is higher than 10 mM, there was no additional obvious increase in the reduction of tailing. When the concentration of Cs^+ was higher than 20 mM, the retention times of the alkaloids increased. Dishon et al. [7] have shown that more cations are adsorbed to negatively charged silica when the ion concentration is increased, gradually neutralizing the surface charge and, hence, suppressing electrostatic double layer repulsion and revealing van der Waals attraction. At even higher salt concentrations, repulsion re-emerges due to a surface charge reversal due to excess adsorbed cations. Adsorption grows monotonically with cation radius. At pH 5.5, Na^+ neutralizes the surface at 0.5-1 M, K^+ at 0.2-0.5 M and Cs^+ at ~0.1 M [7]. Thus the

interaction of alkaloids with the stationary phase will vary with the changing Cs^+ concentration in the mobile phase. This will affect the retention factors of the alkaloids. In (Figure 5), we can see that when the concentration of Cs^+ was 1, 5, and 10 mM, the retention factors of the alkaloids were not significantly different. However, the retention factors are obviously higher with 20 and 30 mM Cs^+. These results imply that at a concentration of 20 mM of Cs^+ there is a change in the interaction type.

Conclusions

In this study, the silanol-blocking effect of Cs^+ was demonstrated by investigating the reduction in peak tailing for different probe solutes and alkaloids. Compared to Ba^{2+}, the blocking ability for silanophilic interactions by Cs^+ is better. Considering the blocking ability of Cs^+ on both ion-exchange and silanophilic interactions, Cs^+ exhibits a better blocking effect with respect to tailing than Ba^{2+}. Because Ba^{2+} more easily forms precipitates with anions such as SO_4^{2-} and PO_4^{3-}, its applicability as a tailing-blocking additive is limited when certain buffers are used in the mobile phase. Therefore, the limitations caused by precipitation can be reduced by using Cs^+ as an additive.

Acknowledgments

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