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Comparative study of divalent metals and amines as silanol-blocking agents in reversed-phase liquid chromatography

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Abstract

In this work we compare the silanol-blocking ability of different alkaline earth metal cations, including calcium, magnesium and barium, and strong amine silanol blockers, such as triethylamine and octylamine, using six basic probe solutes at pH 7 on a conventional octadecylsilane phase. Some amines are better blocking agents than the metal cations but this varies with the amine and analyte structure. Among the metals, barium is the best blocker. For certain solutes barium is as effective at blocking silanols as some of the amine blockers. It produces short retention times and good peak shapes with satisfactory peak symmetry factors. However, amines with long alkyl chains, such as octylamine, are better blocking agents than barium. Peak symmetry is still poor for some solutes even in the presence of the strongest blocking agent in the eluent. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Silanol-blocking agents; Mobile-phase composition; Amines; Calcium; Magnesium; Barium

1. Introduction

The analysis of amines by RPLC is of considerable interest due to the large number of important compounds such as pharmaceuticals which possess basic functionalities. Such analyses, which use silica-based columns, show several different, if not problematic features, which are not as yet fully resolved including severely tailed peaks, no elution of strongly retained bases, low column efficiency, and a strong dependence of retention on sample size. Silanol interactions are one of the most important

reasons for such behavior [1–3]. These interactions with basic compounds can be weakened at very low pH where both the silanol groups and the bases are protonated or at very high pH where both the silanol groups and the bases are unprotonated [3]. However, the deleterious consequences of using extreme pHs are well known [3–5].

The retention of organic bases in RP-HPLC can be influenced by hydrophobic, ion-exchange, and hydrogen-bonding interactions [2,4,5]. Ion-exchange is the predominant mode of interaction of basic compounds at intermediate ranges of pH, where most of the undesirable effects of the surface silanols become apparent [5].

For protonated bases at pHs between 3 and 8, where a measurable to large fraction of the surface

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silanol groups are ionized [2], the most important silanol-sample interactions are due to ion-exchange between the analyte and the cation associated with the ionized silanol group [6,7].

Silanol effects can be further reduced [8] by using quaternary ammonium salts with long alkyl tails as eluent additives [4]. However, these salts have proven to be highly corrosive towards silica-based stationary phases [9]. The use of tertiary amines such as triethylamine (TEA) or amines with longer hydrocarbon chains such as hexylamine (HA) or *N,N*-dimethyloctylamine (DMOA) may solve this problem because these reagents have proven to be very good silanol blockers both at neutral and acidic pHs [10,11]. For some solutes, TEA did not provide acceptable efficiency and peak symmetry [12,13]; DMOA is often more effective than TEA but it can be difficult to remove from the column upon switching to another eluent [3]. Another disadvantage of using amine additives is that they can react chemically with analytes containing carbonyl groups [14].

In this work, we compared barium (II), magnesium (II) and calcium (II) with strong amines as silanol blockers in 30:70 (v/v) acetonitrile–MOPS (3-[*N*-morpholine]-propanesulfonic acid) buffer at pH 7. The buffer per se serves to some extent as a silanol blocker. Nonetheless, a comparative study of different silanol blockers as additives is still possible if the buffer concentration is kept constant in all cases. Because the alkali earths metals are divalent cations their interactions with ionized silanol groups will be much stronger than that of the univalent alkaline earths and thus they should be better silanol blockers. Additionally, it is well known that the solubility of alkali silicates is higher than alkali earth silicates [15]. This means that the adsorption energy of alkaline earth metals on silanol groups should be higher as compared to alkaline metals. We used benzylamines as the probe solutes since they have high pK_a s so they are fully protonated at pH 7 and thus ion-exchange interactions predominate.

2. Experimental

The stationary phase used here was a Zorbax SB ODS 5 μm (5 cm \times 0.46 cm I.D.). It was packed by the upward-slurry technique at 5000 p.s.i using

isopropanol as the packing solvent. The performance of the column was tested by injecting non-silanol solutes (benzene and anisole) prior to all other work. The peaks were very symmetrical. All measurements were made at $30.0\pm 0.1^\circ\text{C}$. The buffer MOPS (3-[*N*-morpholine]-propanesulfonic acid) (Sigma) was 10 mM adjusted to pH 7.02 ± 0.01 after adding organic solvent. Changes in the pH after adding the organic solvent were small and the effect on retention and peak shape is negligible. The amount of solute in each injection was 1.5 μg , all dissolved in pure acetonitrile. We injected some solutes dissolved in a mixture of acetonitrile (ACN) and water (30:70) and the retention times and peak shapes were exactly the same. Retention factors, k' , were averages of at least duplicate determinations. The dead time, t_o , was measured with uracil. Asymmetry factors, A_s , were calculated at 5% of the peak height from the width ratio of the rear and front of the peak. This is procedure recommended by the U.S. Pharmacopeia [3]. Usually these measurements are made at 10% of the peak height but conclusions should not be affected. All data were collected with a Hewlett Packard 1090 LC with an UV detector set at 254 nm. The column was flushed with 60 column volumes of mobile phase after each change in eluent. After flushing, the k' values did not change with time. Retention times were taken as the peak maxima as reported by a Hewlett Packard 3396 data system. The column was operated at flow rate of 1 ml/min. HPLC-grade water and acetonitrile (Mallinckrodt) were used throughout this work. All amines were from Aldrich and were analytical grade.

3. Results and discussion

The chief advantage of using alkali earth metals as silanol blockers in the mobile phase is that they allow the use of neutral pH. Other ions such as iron (III) [16] were tried as silanol blockers but it became evident that acidic pHs must be used to avoid precipitation of the salt. The reason for using test solutes with high pK_a s is to assure that they are nearly completely protonated at pH 7. In this way, the ion-exchange interactions are maximized and competition between the probe solutes and the blocking agents for silanol sites can be better sensed.

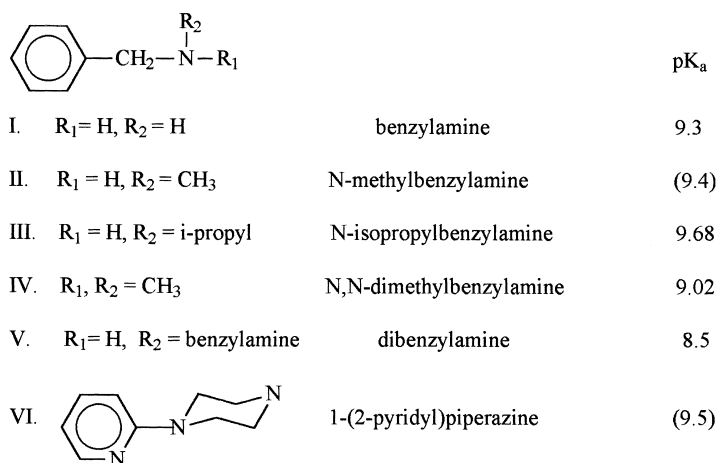


Fig. 1. Amines used in this work and their pK_a values. Numbers shown in parenthesis were estimated from similar compounds.

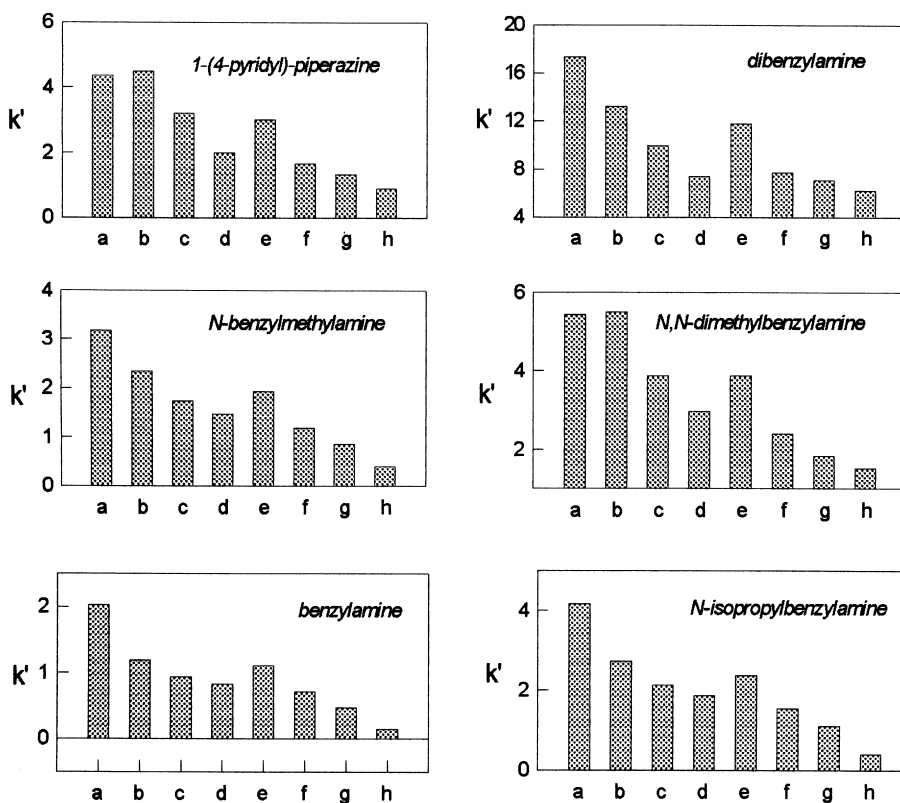


Fig. 2. Effect of mobile phase additives on retention of the indicated test solutes: (a) 10 mM buffer MOPS, pH 7.02 alone; (b) 10 mM magnesium + buffer; (c) 10 mM calcium + buffer; (d) 10 mM barium + buffer; (e) 5 mM barium + buffer; (f) 10 mM DTA + buffer; (g) 10 mM TEA + buffer; and (h) 10 mM OA + buffer.

This is the typical scenario in the chromatography of highly basic compounds ($pK_a > 8$) on silica bonded phases because it is not usually possible to work at pHs higher than 8 or 9 due to the problems discussed above. Most k' values in 30/70 (v/v) ACN–water, with small amounts of blocker, were greater than 1.

In Fig. 1 we show the structures of the solutes studied and their pK_a values. Related with the amounts of solute injected, McCalley [17] has shown that overloading on silica based ODS columns can occur with sample masses as low as 0.5 μg at pH 3 but as much as 10 μg can be injected at pH 7 without any serious effect on peak shape. This was attributed to the increase in the number of dissociated silanols at neutral pH [17].

In Figs. 2 and 3 we show the effect of the different additives on the k' and A_s values compared to those

observed with the buffer alone. As an example, in Fig. 4 we show the effect of OA and TEA and 5 mM and 10 mM barium solutions compared with the buffer alone. In this example, we used a newly packed column and we ran the additives in the following order: buffer alone, 5 mM barium, 10 mM barium, TEA, and OA. This is because reequilibration times upon changing the additive increase in the same order. Before adding a new additive to the mobile phase, the retention time for dibenzylamine was measured until we could reproduce the initial retention time with the buffer alone. In order to reproduce the initial retention time for benzylamine upon changing from OA to the buffer alone, almost 2.5 h were needed at 1 ml/min flow rate. However, only 20 min was needed for reequilibration after use of 10 mM barium. We must point out that just adding

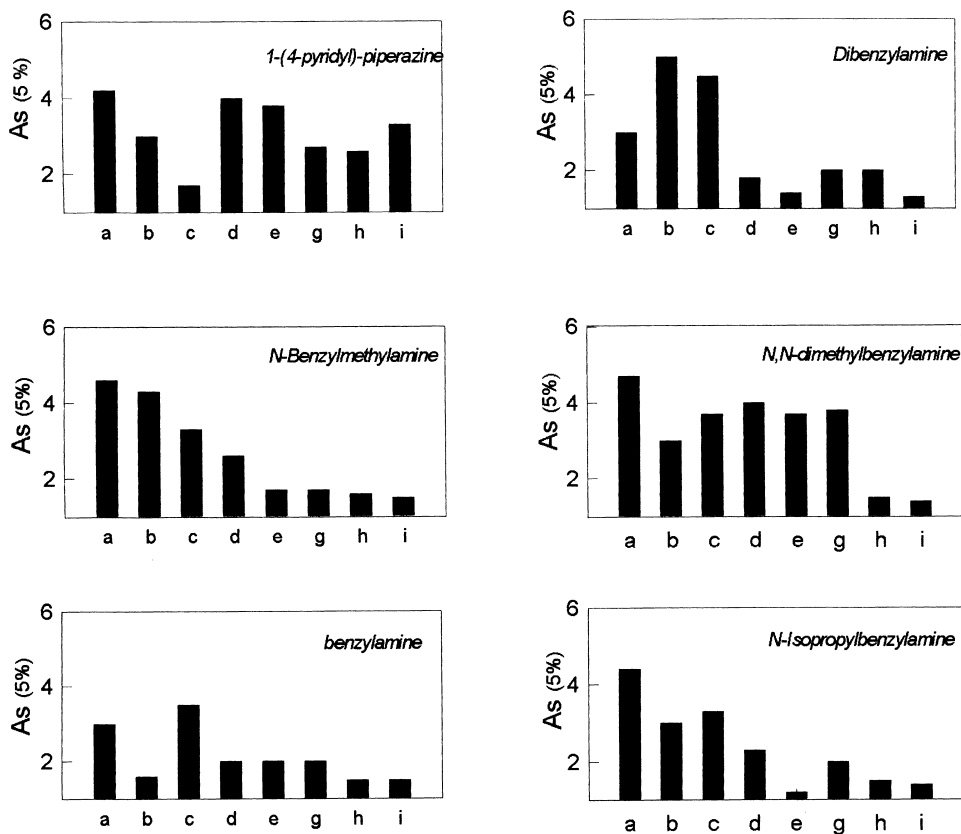


Fig. 3. Effect of mobile phase additives on peak asymmetry of the indicated test solutes: (a) 10 mM buffer MOPS, pH 7.02 alone; (b) 10 mM magnesium + buffer; (c) 10 mM calcium + buffer; (d) 10 mM barium + buffer; (e) 5 mM barium + buffer; (f) 10 mM DTA + buffer; (g) 10 mM TEA + buffer; and (h) 10 mM OA + buffer.

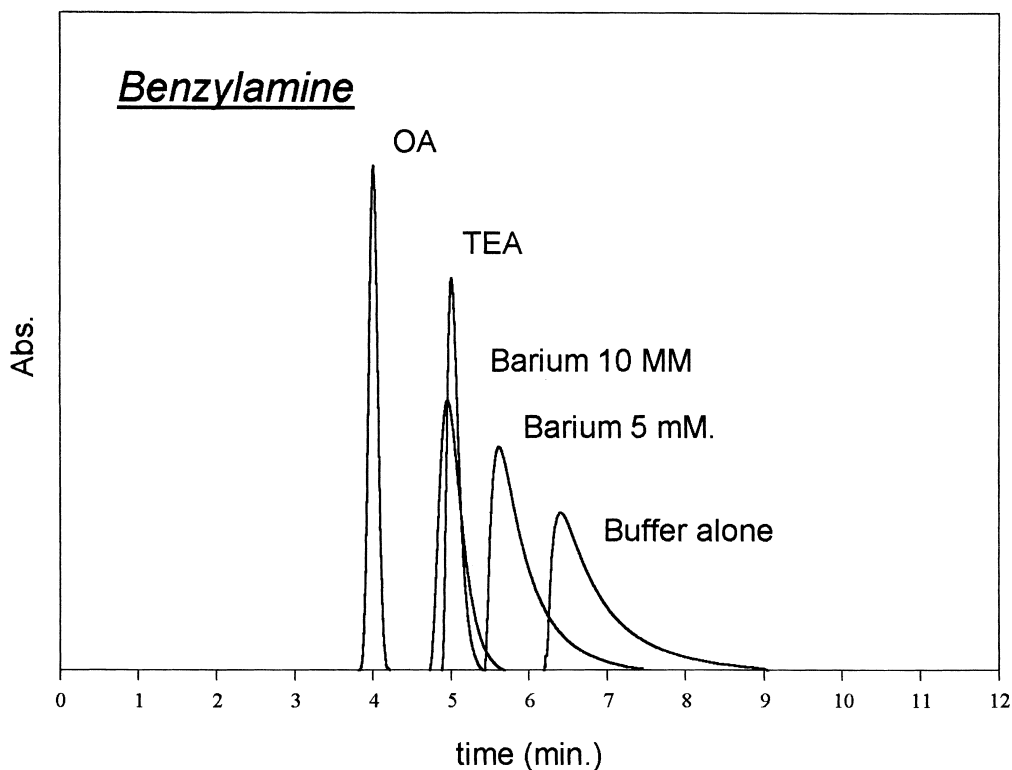
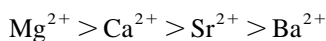


Fig. 4. Effect of some silanol-blocking agents on benzylamine peak shapes.

the buffer to the mobile phase very much improved the peak shapes and significantly decreased the k' values compared with the use of a mobile phase comprised solely of ACN and water. This improvement is commonly observed [18] and is attributed to the silanol blocking effect of the amine buffer. Without the buffer some amines did not elute and others showed very bad peak shapes with very high retention. If we compare the k' values for the different additives with those obtained through only the use of buffer, we see that all produce a decrease in retention for all compounds. This means that both the amines and the alkali earth cations act as silanol-blockers. We see a decrease in the retention times as the cation's atomic radius is increased (for the same concentration of the cation in the mobile phase). This can be explained by considering a solubility equilibrium-like reaction between the alkaline earth cations and the ionized silanol groups. It is well known that the solubility of alkali earth silicates is very low [14]

and that the solubility of these cations with large anions decreases in the following order [19]:



This means that barium should block silanol groups more effectively and as a consequence it will decrease retention of the analytes.

In any case, if we compare the capacity factors in 10 mM octylamine or triethylamine with 5 mM barium (the strongest blocking metal cation), the strength of the amine is higher. However, dibenzylamine, which is the most retained compound, shows almost the same k' values with both 10 mM barium and 10 mM TEA (see Fig. 4). Increasing the amount of salt in the mobile phase decreased the retention time. This behavior can be attributed to either the residual unblocked silanol groups or to the Debye-Hückel effect (salting-in effect) [20]. At low concentration of the salt (in this case, the protonated

analyte and chloride anions coming from the barium chloride), the activity coefficients of the ions decrease as salt concentration is increased. As a consequence, the solubility of the analyte increases in the mobile phase and retention decreases.

On the other hand, 5 mM barium seems to be very effective compared to either 10 mM TEA or 10 mM OA in decreasing the asymmetry factor of dibenzylamine, *N*-isopropylbenzylamine, and *N*-benzylmethylamine. For 1-(4-pyridyl)-piperazine neither OA nor TEA seems to be sufficiently effective. For all of these compounds A_s values are higher

than 1. This also suggests that piperazine or piperazine derivatives should be very good silanol blockers.

Among the amines, OA behaves as the best blocking agent since both the capacity factors and asymmetry factors observed in its presence were the lowest. This result has been observed previously with other structurally similar amines such as dimethyloctylamine and quaternary ammonium salts with long alkyl chains and was attributed to strong dispersive interactions with the alkyl bonded phase [6,21,22].

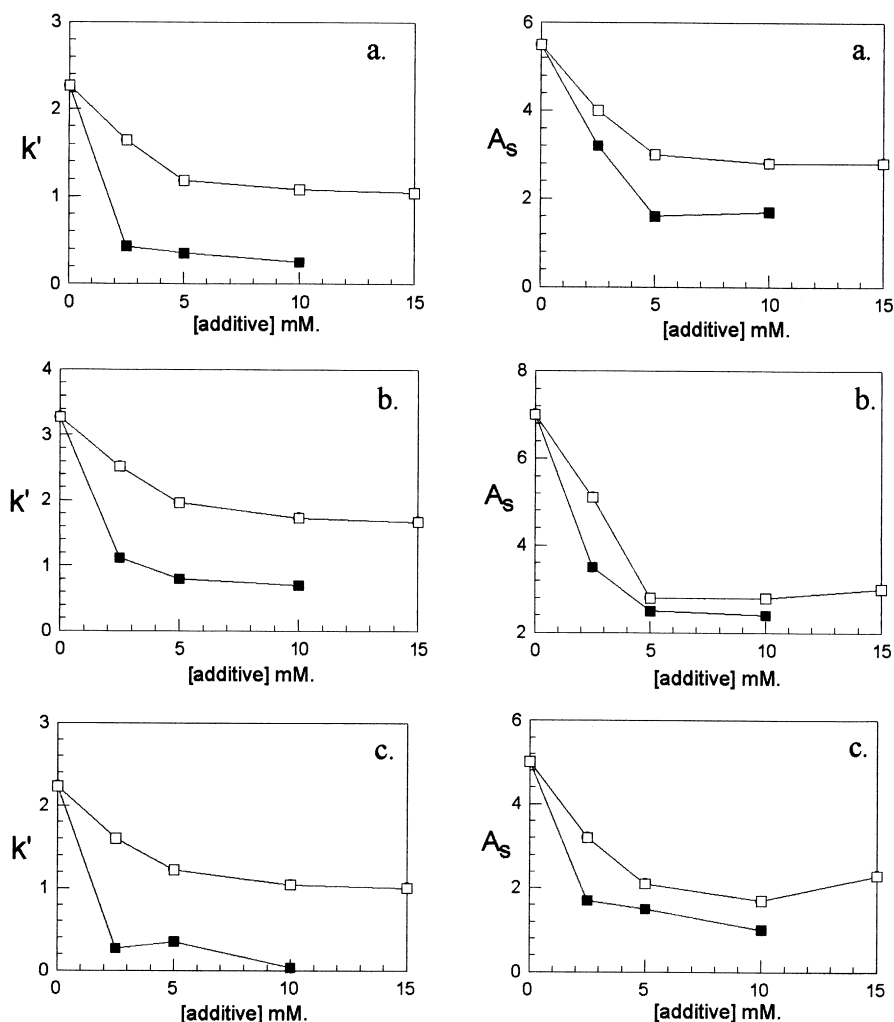


Fig. 5. Effect of concentration of additive on k' and A_s values: (a) 1-(4-pyridyl)-piperazine as solute, (b) *N,N*-dimethylbenzylamine as solute, and (c) *N*-isopropylbenzylamine as solute. (□) Ba^{2+} (■) OA.

In order to corroborate these results, we compared the concentration effect of barium and of OA, the strongest studied blocking agent, on the k' and A_s values. In Fig. 5 we show some results obtained in a new column packed with the same material. Again, we used barium as the additive before using the strongly retained OA. As we said above, the k' values decrease substantially as the concentration of either OA or barium are increased. However, the k' values with OA are much lower than with barium. In spite of this, some A_s values are still higher than unity mainly for *N,N*-dimethylbenzylamine and 1-(4-pyridyl)-piperazine. Nonetheless, for the former compound and for *N,N*-isopropylbenzylamine A_s values are similar at 5 mM and 10 mM for both additives.

On the other hand, TEA is a better silanol blocker than TDA. It is well known that the silanol blocking capability of amines follows the order: tertiary > secondary > primary [2,18] and this is attributed to the electron-donating ability of alkyl groups which increase the basicity of the amine.

4. Conclusions

Alkali earth cations block ionized silanol groups with a strength inversely proportional to their atomic radius. Barium can be a good choice for use as a silanol-blocking agent if amine additives cause problems including column instability, slow equilibration, or if they present some other disadvantages, such as chemical reaction with the analyte. Barium decreases both the retention time and asymmetry factor compared to their values with the buffer alone by an amount that in some cases is comparable to triethylamine. Octylamine is the strongest silanol blocker since it decreases the retention factor substantially compared with the other blocking agents. However, for some solutes it is not able to produce completely symmetric peaks. Moreover, reequilibration times upon changing the type of mobile phase are much higher than with barium.

Whether amines are better blockers than barium seems to depend on their structure. Tertiary amines or amines with long alkyl chains are better than other amines due to their stronger dispersive interactions with the bonded ligands of the stationary phase.

However, they can substantially alter the hydrophobic contribution to retention of the solutes in comparison to barium. Additional studies of the effect on other columns are needed before we can confirm the generality of these results.

Acknowledgements

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