The Role of Analogue Ions in the Ion-Pair Reversed-Phase Chromatography of Quaternary Ammonium Compounds

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Abstract

The choice of analogue ion of the mobile phase additive is shown to significantly affect the analysis of quaternary ammonium compounds (QACs) in ion-pair reversed-phase high-performance liquid chromatography. A series of bromide-containing and dodecyl-sulfate-containing mobile phase additives are investigated using two QAC probe analytes. In all instances, the quaternary-ammonium-containing mobile phase additives perform better than the corresponding sodium-containing additives for effective QAC elution. These results indicate that the structure of the analogue ion, not just its formal charge, is important in the reversed-phase ion-pair chromatography of these compounds. The relative elution order of the QAC probe analytes is also influenced by the counter ions of the mobile phase additives, with bromide and dodecyl sulfate offering opposite elution orders.

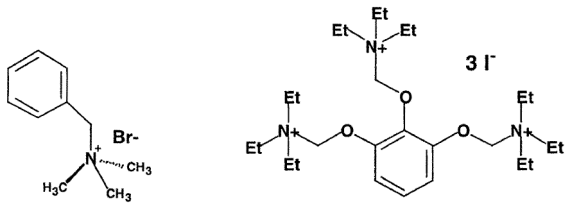
Introduction

Quaternary ammonium compounds (QACs) are an important class of organic chemicals that are used in large quantities worldwide (1). These compounds have applications as cationic surfactants (1), pharmaceuticals (2–4), herbicides (5), and antimicrobials (6–8). QACs consist of four alkyl groups attached to a nitrogen atom, thus giving the nitrogen atom a formal positive charge.

One important difficulty in the chromatographic analysis of QACs involves the strong interaction between the analyte and silica-gel-based stationary phases. The general consensus is that electrostatic interactions between the cationic analyte and anionic surface silanol groups are responsible (9–12). The result is that QACs often elute with poor peak efficiency and severe peak tailing or, in some instances, not at all.

In order to minimize the impact of these surface silanol groups and to modulate QAC retention, reversed-phase ion-pair chromatography is often employed. For example, methods using octadecylsilica (ODS)-based columns (9,10) as well as polymeric stationary phases (13) are present in the literature. The solvent system is typically a low to moderate pH aqueous buffer in conjunction with an organic modifier such as methanol or acetonitrile (9). With respect to the choice of ion-pair reagent, both inorganic additives [such as $NaClO_4$ (10,14)] as well as organic additives [such as alkanesulfates (9) or perfluoronated alkylcarboxylates (15)] are commonly used.

The ion-pair reagent consists of two ions: an anion (counter ion) that possesses an opposite charge to the QAC and a cation (co-ion, or analogue ion). Although the role of co-ion or analogue ion has been modeled, many experiments do not consider the effect of the co-ion or analogue ion (16). In a previous publication, we have shown that the choice of analogue ion is of considerable importance in the normal-phase ion-pair analysis of QACs (17). In that study, the use of a quaternary ammonium bromide (such as tetramethylammonium bromide) showed decreased retention and increased peak symmetry of QAC analytes compared with chromatograms obtained using a corresponding inorganic bromide (NaBr) under identical chromatographic conditions. In this study, a systematic study of the behavior of two representative quaternary ammonium analytes [benzyltrimethylammonium bromide and gallamine triethiodide (GTI) (Figure 1)] under reversed-phase conditions is given, with various ion-pair reagents serving as mobile phase additives. Specifically, the influence of quaternary ammonium halides and quaternary ammonium alkylsulfates on the elution of these two QAC probe analytes as compared with the corresponding sodium halides and sodium alkylsulfates is presented.



Benzyltrimethylammonium bromide ($BnMe_3NBr$)

Gallamine triethiodide (GTI)

Figure 1. Structures of the two quaternary ammonium probe analytes.

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Experimental

Chemicals and equipment

Chemicals were purchased from Aldrich (Milwaukee, WI) or Lancaster (Windham, NH). Alltech (Deerfield, IL) provided the silica gel used for all of the column chromatography experiments, having a pore size of 80 Å, a particle size of 5 µm, and a surface area of 220 m^2/g. Aldrich supplied Amberlyst 36 (wet) ion-exchange resin, a strong cation exchanger used to prepare the quaternary ammonium dodecyl sulfates. All of the hardware for preparing the HPLC columns was from Isolation Technologies (Hopedale, MA), and Alltech supplied the column packer. Atlantic Microlab (Norcross, GA) completed all of the elemental analysis work.

Preparation of the ODS stationary phase

HPLC-grade silica gel from Alltech was subjected to an acid-washing procedure (18) in order to remove impurities before further modification.

The modification of silica gel with octadecyl functionalities was performed according to literature (18).

The resulting stationary phase was packed into a 50- × 4.6-mm-i.d. column with ethanol as both slurry and pressurizing solvents using a standard slurry packing technique. Elemental analysis of the stationary phase showed a ligand surface coverage of 1.7 µmol/m^2 (8.15% C). The dead time of this column was found to be 0.5 min using $NaNO_3$ as a void volume marker (19).

Chromatography

All of the HPLC analyses were completed using a Beckman (Fullerton, CA) analytical gradient system equipped with System Gold Nouveau software. Each mobile phase combination was allowed to equilibrate thoroughly with the column for at least 30 min before QAC analysis. All of the chromatograms were acquired using monochromatic UV detection (254 nm) at a flow rate of 1.0 mL/min.

Synthesis of quaternary ammonium dodecyl sulfates

Ion-exchange resin served to convert sodium dodecyl sulfate (SDS) to the desired quaternary ammonium dodecyl sulfate. Amberlyst 36 (wet) (20 mL), a strongly acidic cation-exchange resin (Aldrich), was loaded into a glass flash chromatography column with a coarse frit and rinsed with copious amounts of water. The appropriate quaternary ammonium hydroxide (~ 50 mL) was passed through the column as an aqueous solution, and the eluent was monitored with pH paper to determine the transition from neutral to basic pH. After rinsing excess quaternary ammonium hydroxide from the column using water, an aqueous solution of SDS was loaded onto the column. The molar amount of SDS loaded was tenfold less than the molar amount of quaternary ammonium ions estimated to be present on the resin as determined by the exchange capacity (1.9 meg/mL). The column was flushed with water until the eluent no longer tested positive for QACs on an undeveloped TLC plate using Dragendorff's reagent (~ 100–125 mL total eluent) (21). The solvent was removed by rotary evaporation in order to yield a white solid. Methanol was added to the solution periodically during solvent removal to prevent solution foaming and bumping. After placing the dried solid on high vacuum overnight, elemental analysis confirmed the quantitative displacement of sodium with the intended quaternary ammonium ion. This procedure was used to convert SDS to tetramethylammonium dodecyl sulfate (Me_4NDS), tetraethylammonium dodecyl sulfate (Et_4NDS), and tetrapropylammonium dodecyl sulfate (Pr_4NDS).

Results and Discussion

In this study, a systematic comparison of the choice of analogue ion of the mobile phase additive was completed under reversed-phase chromatographic conditions for the analysis of QACs. The two analytes (Figure 1) were chosen because of their ready availability and UV activity. Also, one was a monoquaternary ammonium ion and the other a tris-quaternary ammonium ion. Mobile phase additives were chosen because of their simplicity and their similar chemical properties as the analytes.

The elution of GTI was compared using different mobile phase additives under reversed-phase conditions (as shown in Figure 2). Tetrabutylammonium bromide eluted the QAC analyte with the shortest retention factor and highest peak symmetry of the series, followed by Pr_4NBr, Et_4NBr, and Me_4NBr. No elution was observed using NaBr at this concentration. In this instance, the most effective additive was the most hydrophobic analogue ion, Bu_4N^+.

A similar situation became apparent in using $BnMe_3NBr$ as a QAC probe analyte. Figure 3 shows the trend exhibited by these

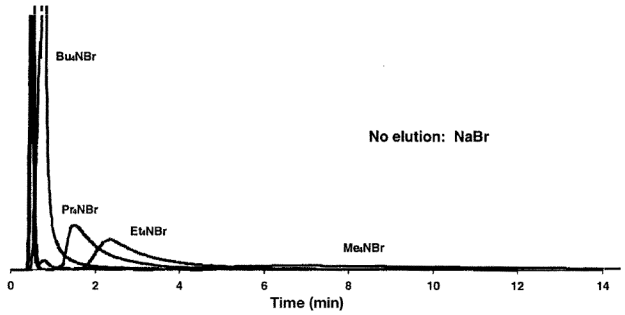

Figure 2. Analysis of GTI under reversed-phase conditions using different mobile phase additives. The mobile phase was an additive (12.5mM concentration) in 1:1 MeOH–H_2O using an ODS column with a flow rate of 1 mL/min. The detection was UV at 254 nm.

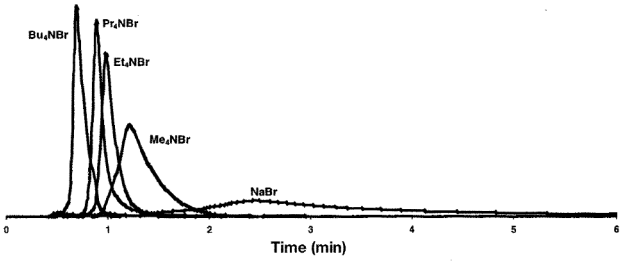

Figure 3. Analysis of $BnMe_3NBr$ under reversed-phase conditions using different bromide-containing mobile phase additives. The mobile phase was an additive (12.5mM concentration) in 1:1 MeOH–H_2O using an ODS column with a flow rate of 1 mL/min. The detection was UV at 254 nm.

analogue cations to be identical to that found with GTI. Tetrabutylammonium was the analogue cation that allowed the most rapid and symmetrical elution of the analyte, followed successively by analogue cations of decreasing hydrophobicity.

In addition to using halide salts as ion-pair reagents in reversed-phase chromatography, another common technique was to use organic anions, such as alkylsulfates, to facilitate ion pairing for cationic analytes. To this end, SDS was compared with the corresponding quaternary ammonium dodecyl sulfates Me_4NDS, Et_4NDS, and Pr_4NDS as mobile phase additives. All of the quaternary ammonium dodecyl sulfates were prepared in-house using ion-exchange resin, because none of these compounds were commercially available (see the "Experimental" section).

Figure 4 depicts the elution of $BnMe_3NBr$ using SDS, Me_4NDS, Et_4NDS, and Pr_4NDS as mobile phase additives under otherwise identical chromatographic conditions. Overall, all three quaternary ammonium dodecyl sulfates performed better than SDS in eluting $BnMe_3NBr$. The order of mobile phase additive efficacy however differed from the trend observed in using bromide-containing mobile phase additives under reversed-phase conditions. Me_4NDS eluted the analyte with the shortest retention time followed by Et_4NDS and Pr_4NDS, which eluted the analyte with essentially identical retention times.

The long hydrophobic chain of dodecyl sulfates could be responsible for this observed discrepancy in the elution order. As proposed in the literature, this hydrophobic chain could intercalate with the octadecyl group of the stationary phase, transforming the stationary phase at least partially into an ion-exchange stationary phase (22). Consequently, the QAC analyte has the opportunity to interact with the surfactant via an ion-exchange mechanism, competing with the mobile phase additive cation for electrostatic interaction with the dodecyl sulfate anion at the surface of the hydrophobic ODS layer. In this case, the size, charge, and chemical nature of the analogue ion should dictate the relative ease of which the mobile phase additives may interact with the surface-adsorbed dodecyl sulfate anion. Because all of the analogue ions in this study were monovalent species, the size and chemical nature of the analogue ions were important in determining the relative strength of interaction with the sulfate ions. The formal and hydrated radii of these analogue ions under consideration are shown in Table I (23). Among the three quaternary ammonium ions, tetramethylammonium was expected to interact strongest with the dodecyl sulfate anions because of its small size. This was consistent with the elution order observed with these three quaternary-ammonium-based mobile phase additives. Sodium ion was different; even though the hydrated radius of sodium ion was comparable with that of tetramethylammonium (Table I); it cannot compete effectively with $BnMe_3NBr$ for interaction with the surface sulfate groups, thus resulting in the overall poor elution of $BnMe_3NBr$. We are currently unable to explain this discrepancy.

In the absence of a long hydrophobic chain, as in the cases of using halide salts as ion-pair reagents, such an ion-exchange process may not exist. Instead, the retention of the analyte will be largely determined by its interaction with the octadecyl group of the ODS phase and the surface silanol groups. Effectiveness of the additives depends largely on how these various additives could mask the silanol groups. Because the masking of the surface silanol groups requires the penetration of the additive through the hydrophobic layer, the most hydrophobic analogue ion (Bu_4N^+) proved the most effective.

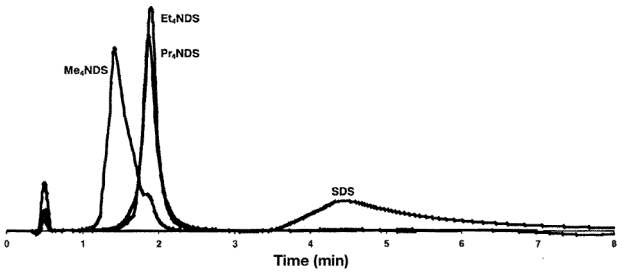

Figure 4. Analysis of $BnMe_3NBr$ under reversed-phase conditions using different dodecyl sulfate mobile phase additives. The mobile phase was an additive (6.7M concentration) in 2:1 MeOH–H_2O using an ODS column with a flow rate of 1 mL/min. The detection was UV at 254 nm.

Table I. Formal and Hydrated Radii (Å) of the Analogue Ions Used with Dodecyl Sulfate*

Analogue Ion	Formal radius	Hydrated radius
Na^+	0.95	3.6
Me_4N^+	3.47	3.67
Et_4N^+	4.00	4.00
Pr_4N^+	4.52	4.52

* Obtained from reference 23.

Conclusion

The chromatographic data presented in this study suggest that the analogue ion is also important in the reversed-phase ion-pair chromatography of QACs. Effects of these analogue ions could be attributed to silanol suppression in some cases. In other cases, one needs to resort to an ion-exchange model. Additives based on QACs are more effective than sodium in eluting other quaternary ammonium analytes. We suspect that a structural–chemical similarity between the quaternary ammonium additives and the quaternary ammonium probe analyte is responsible for such an observation. The relative effectiveness of various quaternary ammonium additives also depends on the counter ions. If the counter ion possesses a long hydrophobic chain, an ion-exchange model is necessary to explain the relative elution order.

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