

Effect of Analogue Ions in Normal-Phase Ion-Pair Chromatography of Quaternary Ammonium Compounds

Louis H. Bluhm and Tingyu Li*

Department of Chemistry, Box 1822-B, Vanderbilt University, Nashville, TN 37235

Abstract

The effect of analogue ions of ion-pair reagents in the normal-phase ion-pair chromatographic behavior of quaternary ammonium compounds is shown. Different analogue ions can have a significant impact on the chromatographic behavior of quaternary ammonium compounds. Among the ion-pair reagents investigated (sodium bromide, tetramethylammonium bromide, tetraethylammonium bromide, tetrapropylammonium bromide, and tetrabutylammonium bromide), tetramethylammonium bromide proves to be the most effective reagent for both reducing retention time and eliminating peak tailing. The influence of analogue ions on the chromatographic behavior of quaternary ammonium compounds correlates well with their affinity for normal-phase silica gel.

Introduction

Quaternary ammonium compounds are an important class of chemicals, because they are widely used as cationic surfactants, fabric softeners, and antimicrobials (1,2). Because of the formal positive charge of the nitrogen atom, these compounds possess many unique properties that have been utilized in numerous other applications. For example, a number of molecular recognition systems for nucleotide triphosphates are based on quaternary ammonium compounds (3,4,5). Because quaternary ammonium compounds can interact with many polar and even some nonpolar functional groups of proteins (6), they show promise as useful drug candidates (7).

In terms of chromatographic separations, ion-pair reagents are often required as mobile phase additives for quaternary ammonium compound analysis, especially with silica gel-based stationary phases (8). These additives can eliminate the severe peak tailing and irreversible adsorption of quaternary ammonium compounds on silica gel-based stationary phases. In addition, these ion-pair reagents modulate the retention time of the

cationic analytes. A number of theories have been proposed to explain the effect of ion-pair reagents. For example, theories such as ion pairing, dynamic ion exchange, dynamic complex exchange, and the electrostatic model have been frequently discussed in the context of reversed-phase ion-pair chromatography (IPC) (9). In comparison, relatively little discussion of the mechanism of normal-phase IPC exists in the literature. In these discussions, the importance of counter ions is often emphasized, whereas the role of analogue ions receives less attention (when NaBr is used as the mobile phase additive for the separation of cations, Br⁻ is considered to be the counter ion and Na⁺ serves as the analogue ion). In a related study of the chromatographic behavior of certain aromatic amines, the effects of certain buffer cations were found to have a significant influence on the chromatographic behavior of these basic analytes (10). The influence of buffer cations on the chromatographic behavior of various oligopeptides has also been studied (11). In this article, observations concerning the effects of analogue ions are discussed for the retention of 2 representative quaternary ammonium ions in normal-phase IPC (12,13).

Experimental

General supplies and equipment

All chemicals and solvents were purchased from Aldrich (Milwaukee, WI), Fluka (Milwaukee, WI), or Fisher Scientific (Pittsburgh, PA). All solvents were high-performance liquid chromatography (HPLC) grade, and solutions prepared with each additive were filtered using FP-Vericel membrane filter paper (0.2 μ m, Gelman Sciences, Ann Arbor, MI). HPLC-grade Allsphere silica gel (5- μ m particle size, 80- \AA pore size, 220 m²/g surface area) was purchased from Alltech (Deerfield, IL). Thin-layer chromatography (TLC) was completed using EM silica gel 60 F-254 TLC plates (0.25 mm). A standard column packer was purchased from Alltech. Empty stainless steel HPLC column hardware was purchased from Separation Technologies (Hopedale, MA). HPLC analyses were carried out on a Beckman

* Author to whom correspondence should be addressed: e-mail tingyu.li@vanderbilt.edu.

(Fullerton, CA) analytical gradient system (System Gold) with ultraviolet (UV) detection at 254 nm.

Column packing

The normal-phase silica gel HPLC column required for this study was packed in-house using the standard slurry packing method at 7000 psi with ethanol as both the slurry and pressurizing solvents. The Allsphere silica gel (5 μm , 80 \AA) was acid-washed following the procedure in the literature (14,15) prior to its use.

Dead time t_0 was measured with 1,3,5-tri-*t*-butylbenzene as the void volume marker according to literature procedure (16).

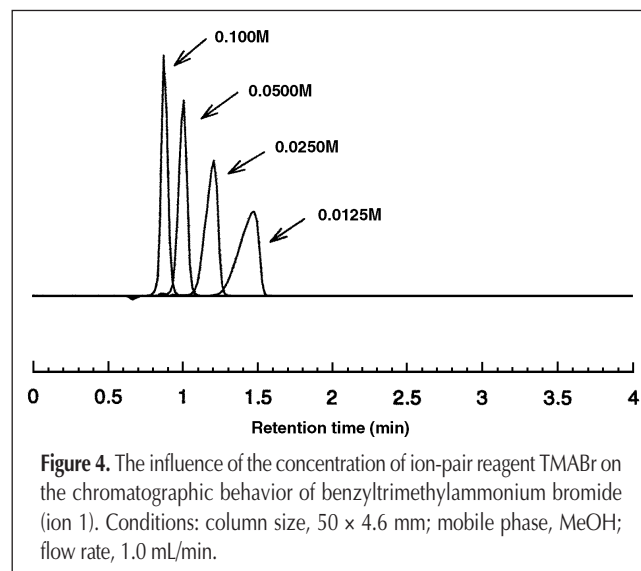
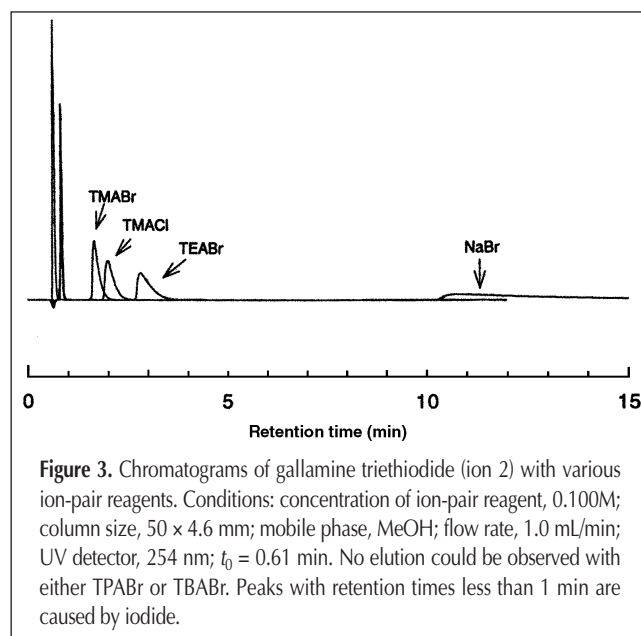
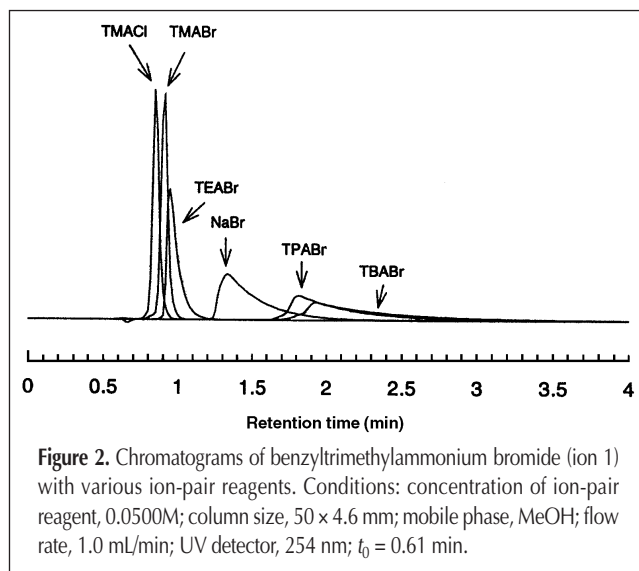
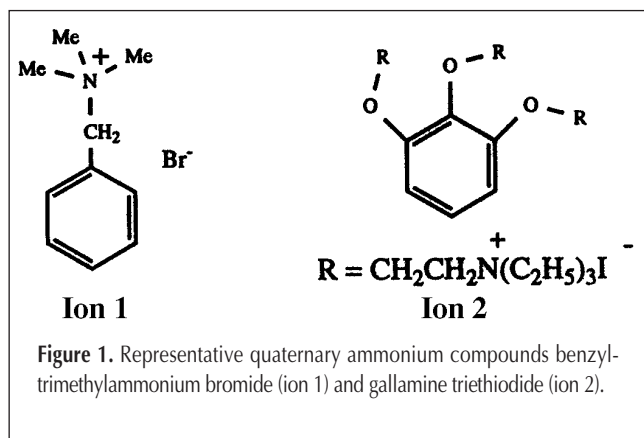
Results and Discussion

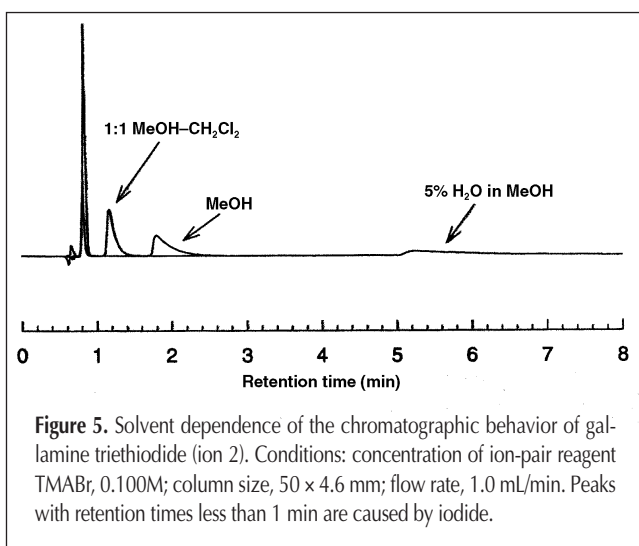
The chromatographic behavior of 2 quaternary ammonium ions, benzyltrimethylammonium bromide (ion 1, a monoquaternary ammonium ion) and gallamine triethiodide (ion 2, a trisquaternary ammonium ion) was studied (Figure 1). Both analytes are commercially available, and their chromatograms can be monitored readily using a UV detector. Five ion-pair reagents were employed for this study: sodium bromide (NaBr), tetra-

methylammonium bromide (TMABr), tetraethylammonium bromide (TEABr), tetrapropylammonium bromide (TPABr), and tetrabutylammonium bromide (TBABr). In these ion-pair reagents, the counter ion (bromide) is kept the same; therefore, any difference in the observed chromatographic behavior must result from the influence of the analogue ions.

The chromatograms of benzyltrimethylammonium bromide (ion 1) with these different mobile phase additives are shown in Figure 2. As can be seen clearly from these chromatograms, using the same solvent and mobile phase additive concentration, TMABr was more effective than TEABr in both eliminating peak tailing and reducing the retention time of this analyte. TEABr was more effective than NaBr, which was in turn more effective than TPABr. Following a similar trend, TPABr was more effective than TBABr as a mobile phase additive. In these experiments, no elution of the analyte could be observed in the absence of these mobile phase additives.

With gallamine triethiodide (ion 2), a similar series of elution





profiles were observed (Figure 3). Once again, TMABr proved more effective than TEABr, which was much more effective than NaBr. For this particular analyte, no elution could be observed when either TPABr or TBABr was used as the mobile phase additive. It is apparent that the chromatographic behavior of gallamine triethiodide is much more strongly dependent on the choice of mobile phase additive than the monoquaternary ammonium compound benzyltrimethylammonium bromide.

As expected, the chromatographic behavior of these quaternary ammonium compounds also depended on the concentration of the mobile phase additive. For example, when the concentration of TMABr was increased from 0.0125 to 0.100M, the retention time of benzyl-trimethylammonium bromide decreased from 1.48 to 0.88 min (Figure 4).

The chosen solvent also has a role in the retention of these compounds. Interestingly, the retention times actually increased in this normal-phase ion-pair experiment with more-polar solvents (Figure 5). One possible explanation for this somewhat surprising result is that in a less-polar solvent, quaternary ammonium compounds have a greater propensity to exist as ion-pair complexes rather than discrete ionic species. Compared with discrete ionic species, the polarity of ion pairs is smaller, thus the retention to normal-phase silica gel might be weaker.

The effect of the counterion on the chromatographic behavior of these 2 quaternary ammonium compounds was also briefly examined. As shown in Figure 2, for benzyltrimethylammonium bromide, tetramethylammonium chloride (TMACl) was slightly more effective than TMABr as a mobile phase additive. However, for trisquaternary ammonium gallamine triethiodide, TMABr was more effective than TMACl (Figure 3). A reasonable explanation for such conflicting phenomena has not been postulated.

The effectiveness of these mobile phase additives correlates well with their affinity toward normal-phase silica gel. On normal-phase silica gel TLC plates with 0.1M LiCl in MeOH as the developing solvent, the R_f values of TMABr, TEABr, NaBr, TPABr, and TBABr are 0.24, 0.29, 0.65, 0.67, and 0.76, respectively. These results clearly indicate that the order of binding strength of these mobile phase additives to silica gel is TMABr > TEABr > NaBr, TPABr > TBABr, an observation that parallels their ability to reduce the retention time and peak tailing of ben-

zyltrimethylammonium bromide and gallamine triethiodide. The TLC plates of TMABr, TEABr, TPABr, and TBABr are visualized with bismuth subnitrate (17), a quaternary ammonium selective stain, whereas TLC plates of NaBr are visualized with tetrahydroxy-1,4-benzoquinone, a sodium-selective stain (18).

Conclusion

Different analogue ions can have a significant impact on the normal-phase IPC separation of quaternary ammonium compounds. Among the 5 ion-pair reagents investigated (TMABr, TEABr, NaBr, TPABr, and TBABr), TMABr proved to be the most effective reagent in reducing the retention time and eliminating peak tailing. With increasing mobile phase polarity, the retention times of quaternary ammonium compounds actually increase under these experimental conditions. Also, the influence of each type of analogue ion on the chromatographic behavior of quaternary ammonium compounds correlates well with the affinity of each analogue ion with normal-phase silica gel.

Acknowledgments

This paper was presented at the 1999 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy in Orlando, FL. The authors appreciate the financial support from Vanderbilt University in the form of a startup fund, research council, and natural science fund. This project was also supported in part with an ACS PRF grant (31692-GI).

References

1. E. Woollatt. *The Manufacture of Soaps, Other Detergents and Glycerine*. Ellis Horwood Limited, New York, NY, 1985, p 105.
2. E. Jungermann. *Cationic Surfactant*. Marcel Dekker, New York, NY, 1969.
3. I. Tabushi, Y. Kobuke, and J. Imuta. Lipophilic diammonium cation having a rigid structure complementary to pyrophosphate dianions of nucleotides. Selective extraction and transport of nucleotides. *J. Am. Chem. Soc.* **103**: 6152–57 (1981).
4. T. Li and F. Diederich. Carriers for liquid membrane transport of nucleotide 5'-triphosphates. *J. Org. Chem.* **57**: 3449–54 (1992).
5. F.M. Menger and K.K. Catlin. Octacationic cyclophanes: binding of ATP and other anionic guests in water. *Angew. Chem. (intl. ed.)* **34**: 2147–50 (1995).
6. D.A. Dougherty and D.A. Stauffer. Acetylcholine binding by a synthetic receptor: implications for biological recognition. *Science* **250**: 1558–60 (1990).
7. Cationic drugs. *Merck Index*, 11th ed., pp 4504, 4540, 4600, 4601, 4628.
8. J. Nawrocki. The silanol group and its role in liquid chromatography. *J. Chromatogr. A* **779**: 29–71 (1997).
9. M.T.W. Hearn. *Ion-Pair Chromatography*. Marcel Dekker, New York, NY, 1985, pp 27–77.
10. E. Papp and G. Vigh. Role of buffer cations in the reversed-phase high-performance liquid chromatography of aromatic amines. *J. Chromatogr.* **282**: 59–70 (1983).

11. W.S. Hancock, C.A. Bishop, J.E. Battersby, D.R.K. Harding, and M.T.W. Hearn. High-pressure liquid chromatography of peptides and proteins. *J. Chromatogr.* **168**: 377–84 (1979).
12. B.-A. Persson and P.-O. Lagerstrom. Ion-pair partition chromatography in the analysis of drugs and biogenic substances in plasma and urine. *J. Chromatogr.* **122**: 305–316 (1976).
13. T.M.P. Chichila and D.M. Gilvydis. Determination of paraquat and diquat in low-moisture food crops using silica column cleanup and liquid chromatography with UV detection. *J. Assoc. Off. Anal. Chem. Int.* **76**: 1323–28 (1993).
14. H. Englhardt and P. Orth. Alkoxy silanes for the preparation of silica based stationary phases with bonded polar functional groups. *J. Liq. Chromatogr.* **10**: 1999–2022 (1987).
15. S.D. Plunkett and F.H. Arnold. Molecularly imprinted polymers on silica: selective supports for high-performance ligand-exchange chromatography. *J. Chromatogr. A* **708**: 19–29 (1995).
16. W.H. Pirkle and C.J. Welch. A convenient void volume marker for several chiral HPLC columns. *J. Liq. Chromatogr.* **14**: 1–8 (1991).
17. A.J. Gordon and R.A. Ford. *The Chemist's Companion*. John Wiley & Sons, New York, NY, 1972, p 379.
18. W. Bock-Werthmann. New universal developing reagent for the paper chromatography of cations. *Anal. Chim. Acta* **28**: 519–23 (1963).

Manuscript accepted June 3, 1999.