Ion Chromatography Studies of Quaternary Ammonium Halide Solutions and the Determination of Pharmaceuticals

Lai-Hao Wang
Department of Applied Chemistry, China Nan University of Pharmacy and Science, Tainan, Taiwan, 71743, R.O.C.

Abstract

The feasibility of using ion chromatography (IC) in solutions containing quaternary ammoniums is studied. The results establish the applicability of IC for the determination of halide ions in a pharmaceutical sample without interferences by various excipient ions. For IC measurements with suitable methodical parameters, linearity is maintained over the range of 20 to 200 mg/L and 10.0 to 250 mg/L for the chloride and bromide of quaternary ammoniums, respectively. A comparison of the anion results obtained from differential pulse voltammetry shows good agreement.

Introduction

Quaternary ammonium compounds such as benzethonium chloride (BZC), cetylpyridium chloride (CPC), chlorhexidine digluconate (CHXG), cetrimonium bromide (CTB), and domiphen bromide (DPB) are cationic surfactants with antimicrobial and bacterial activities that are used in disinfectant and pharmaceutical preparations. The structures of BZC, CPC, CHXG, CTB, and DPB are shown in Figure 1.

The literature presents several methods for the determination of quaternary ammonium compounds. Most spectrophotometric methods (1–4) are based on the formation of ion-association complexes with anionic dyes. These methods have difficulties and inaccuracies resulting from incomplete extraction. A direct potentiometric titration method (5–8) was employed with a silver–mercury sulfate electrode system. Gas chromatography (GC) of the quaternary ammonium salt (which is nonvolatile) has previously been carried out by a thermal decomposition method, but it is not quantitative because of the appearance of multiple peaks and poor response (9–11). In a thin-layer chromatographic (TLC) method (12), identification and quantitation consist of a color reaction with KIO_3 and a densitometry at 400 nm. High-performance liquid chromatographic (HPLC) methods previously described (13,14) are either based on separation by cation exchange or a combination of ion-pair and reversed-phase chromatography using acetate or perchlorate as the counter ion and C_{18} material as the stationary phase. In the most extensive work to date a combination of HPLC, TLC, and GC–MS is preceded by a cleanup using both silica gel and an ion-exchange resin. However, to our knowledge, there is no single-column system suitable for the simultaneous determination of the chloride and bromide of quaternary ammoniums, particularly in the presence of fluoride and sulfate ions in oral hygiene products. This study dealt with the separation of the chloride and bromide of quaternary ammoniums. These are often present in samples containing many other organic components that can interfere with their separation. The method presented in this study was based on ion-exchange chromatography because this can be a specific separation for these compounds. The influence of the various compounds of the mobile phase that can affect the separation of the quaternary ammoniums was studied.

Experimental

Apparatus

The liquid chromatograph was a Shimadzu (Kokyo, Japan) LC-10 AD chromatography module containing a pump and conductivity detector (Shimadzu CDD-6A). The analytical column used a Shim-Pack IC-A1 (10-cm × 4.6-mm i.d.) polymethacrylate-based anion exchanger (10 µm). Chromatographic data were collected and analyzed with a Chromatopac C-R6A. All electrochemical experiments were performed using an EG&G Princeton Research (Princeton, NJ) Model 253 Versatat connected to an EG&G Model 616 Rotating Electrode system. A three-electrode system was employed consisting of an Hg^{2+}–Au working electrode, a platinum counter, and a saturated calomel electrode (SCE) reference electrode.

Reagents and materials

BZC (Hyamine 1622) and CHXG were obtained from Aldrich (Milwaukee, WI). CPC, cetyltrimethylammonium bromide (cetrimide), and DPB were purchased from E. Merck (Darmstadt, Germany), Sigma (St. Louis, MO), and Lancaster Chem.

Co. (Eastgate, White Lund, Morecambe, U.K.), respectively. All other chemicals were of analytical-reagent grade. The eluent (containing 0.94 mmol/L sodium carbonate) was adjusted to pH 9.85 with 0.31 mmol/L sodium hydrogen carbonate (Na_2CO_3–$NaHCO_3$) and 2.5 mmol/L phthalic acid (PA)–2.4 mmol/L tris(hydroxymethyl)aminoethane (THA) (pH 4.31), respectively. Samples of disinfectants, mouse rinses, and cleanthroat troches were bought from a number of retail outlets in the south of Taiwan.

Determination of quaternary ammonium compounds by ion chromatography

A stock solution of standards was prepared by dissolving the appropriate amount of quaternary ammonium compounds in ethanol and deionized water (1:1, v/v). A set of standard solutions were produced by diluting aliquots of the stock solutions with ethanol and deionized water to 10 mL in volumetric flasks. Taking into account the content of quaternary ammonium compounds in mouth rinses, cleanthroat troches, and disinfectants, approximately 0.01 to 0.30 g of the latter were weighed accurately in a 15-mL beaker, diluted to approximately 5 mL with ethanol and deionized water, and dissolved and transferred into a 25-mL volumetric flask. The beaker was rinsed twice with 3-mL portions of deionized water, and the rinsings were combined in the volumetric flask. The solution was diluted to volume with deionized water. The chloride of CHXG was extracted from the disinfectants and liquid from the mouse rinses by flask combustion. The extraction was performed on approximately 0.1 to 1.0 g of liquid exactly weighed, which was dissolved with 10 mL of deionized water for 3 min. This liquid mixture was placed in the flask, which was itself a few milliliters of absorbing solution. Next, combustion of the flask was performed with a stream of oxygen that was from 30% hydrogen peroxide until the sample had completely evaporated. The flask was left to cool, and the absorbing solution was swirled to ensure complete absorption of all volatile oxidation products. Then, the flask was opened, the supernatant was transferred to a 25-mL volumetric flask, and it was diluted to volume with deionized water. An aliquot of the solution was filtered through a 0.45-µm and 0.20-µm membrane filter prior to ion chromatographic (IC) analysis. A Shim-Pack IC-A1 (10-cm × 4.6-mm i.d.) polymethacrylate-based anion exchanger (10 µm) was used for IC. The mobile phases were Na_2CO_3–$NaHCO_3$ and PA–THA, respectively. The following chromatography conditions were used: the eluent flow rate was 1.5 mL/min, the conductivity temperature 40°C, the sensitivity 1.0 µs/cm, the injection volume 50 µL, and the recorder chart speed 5 mm/min. By means of the injection value, 50 µL of the prepared sample solution and standard solution were chromatographed under the operating conditions described previously. Quantitation was based on the peak area of the sample.

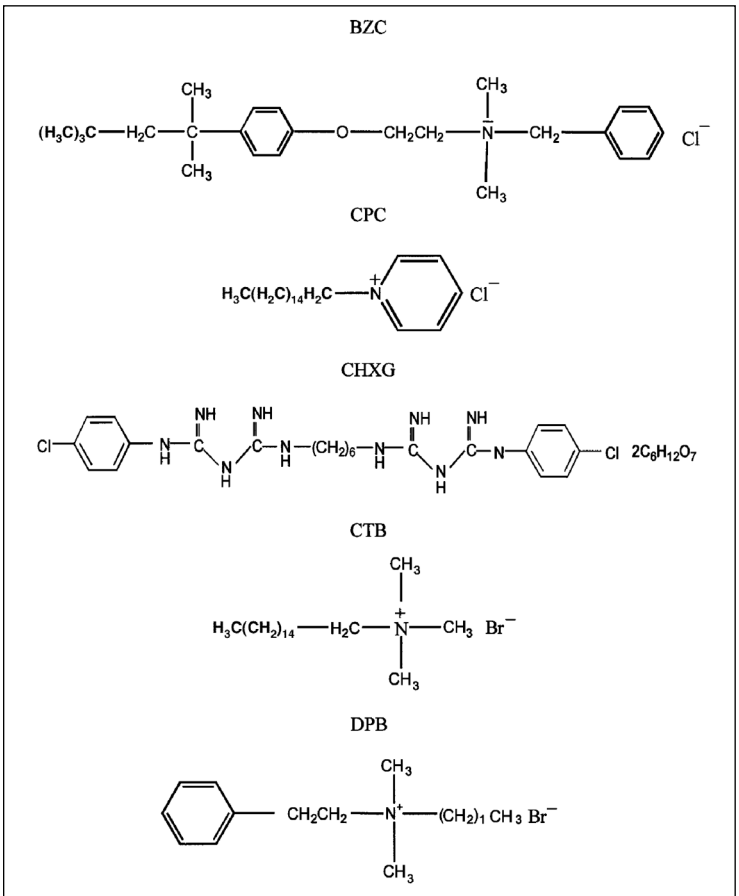

Figure 1. The structures of various quaternary ammonium compounds.

Determination of quaternary ammonium compounds by differential pulse voltammetry

The thin-film metal electrode was produced by the following method. Prior to analysis, the gold electrode (4-mm diameter) was mirror-polished sequentially with an aqueous suspension of 1.0, 0.5, and 0.05 µm alumina, respectively. The gold electrode was rinsed with deionized water and electrolytically plated with mercury from 25 mL of an acetate buffer (pH 4.23) that was 1.0×10^{-4} to 4.0×10^{-3}M mercury (II). Plating times were 4 min with a potential scan from –0.8 to 0.0 V at 1500 rpm.

A 1.0-g amount of sample was accurately weighed, dissolved in 10 mL of a mixture of methanol and water (1:1, v/v), and mixed with vertex treatment for 20 min. After centrifuging, the supernatant was transferred into a 10-mL calibrated flask and diluted to volume with the methanol and water mixture. In order to obtain a calibration graph for the quaternary ammonium compound, 10 mL of the supporting electrolyte (a 1+1 mixture of lithium chloride and lithium hydroxide) was pipetted into a voltammetric cell and deaerated with nitrogen for 4 min before voltammetric measurement. By using a micropipette, aliquots of a 1000-ppm quaternary ammonium compound solution were added. After each addition voltammograms were obtained; the solution deaerated for 1 min after each addition before obtaining the voltammogram. Quantitative analyses were performed in the differential pulse mode. The potential was set at –1.0 to –2.0 V versus an SCE for reduction. The pulse height was 50 mV and the scan rate was 10 mV/s with a film electrode. For sample solution analysis, 1 mL of the solution was pipetted to volume with a 1+1 mixture of a lithium chloride and lithium hydroxide solution. This solution

was analyzed by differential pulse voltammetry (DPV) using the same condition as for the calibration graph.

Results and Discussion

Choice of eluent

In general, the eluent should have a similar affinity for the stationary phase as the ions to be analyzed. Two mixtures of Na_2CO_3–$NaHCO_3$ and PA–THA solutions were used as mobile phases. Eluent concentration and pH are two of the most important factors that determine the retention times of the ions being chromatographed. Thus, when considering changes in the selectivity of a system by varying the ratio of Na_2CO_3–$NaHCO_3$ or PA–THA and the pH of the eluent, they must be treated simultaneously. As can be seen from Figure 2, F^-, Cl^-, Br^-, NO_3^-, and SO_4^{-2} can be separated using a PA–THA eluent. The retention times were 2.68, 5.45, 7.26, 8.95, and 11.39 min for F^-, Cl^-, Br^-, NO_3^-, and SO_4^{-2}, respectively, using

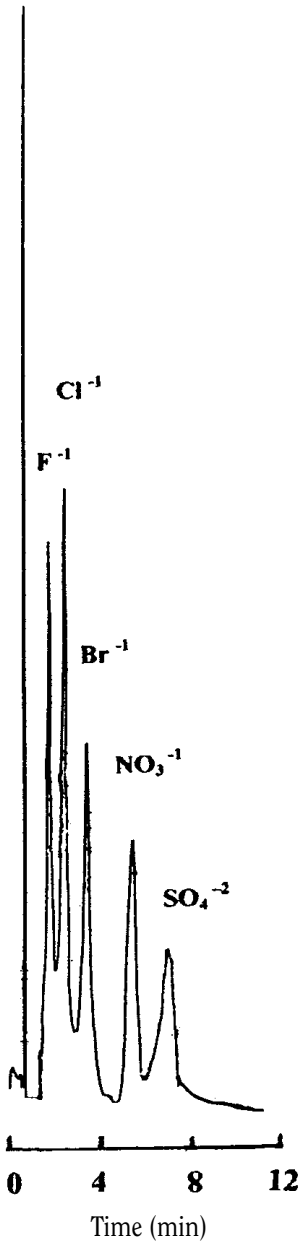

Figure 2. A chromatogram of various inorganic mineral acids.

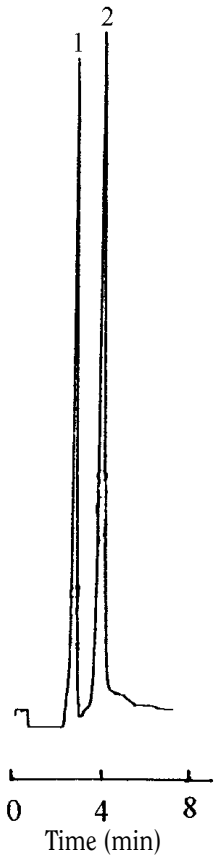

Figure 3. Separation of a mixture (250 mg/L) of (1) chloride and (2) bromide.

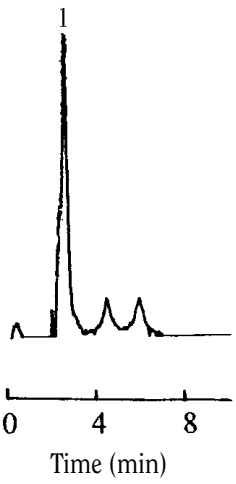

Figure 4. Chromatogram of chloride in the absorption after the flask combustion of (1) chlorhexidine diglyconite.

Na_2CO_3–$NaHCO_3$ and sulfate elutes before bromide in the presence of a higher concentration of chloride without suppression techniques (15). Although carbonate buffers with pH 9.85–11.0 gave good separations of the anions F^-, Cl^-, Br^-, NO_3^-, and SO_4^{-2}, the overall analysis time was considered too long. At a lower pH of 4.30, PA–THA was found to be ideal for resolving bromide and chloride in a shorter time. Therefore, the mixture of PA–THA was chosen for use in the determination of quaternary ammonium compounds in pharmaceuticals. Figures 3 and 4 show chromatograms of a standard solution mixture containing quaternary ammonium compounds using an eluent of PA–THA.

Precision and recovery

The calibration graphs obtained by plotting the peak height against the concentration of chloride and bromide of the quaternary ammoniums show good linearity over the range of approximately 20 to 200 mg/L and 10.0 to 250 mg/L for the chloride and bromide of quaternary ammoniums, the regression equations being $y = 17339 + 2509x$ (correlation coefficient (r) = 0.9996) and $y = 27448 + 4729x$ (r = 0.9990), respectively. Recovery tests were carried out on pharmaceutical products to evaluate the reproducibility and accuracy of the proposed IC method. Quaternary ammonium halogenide mixtures for fortification were prepared by mixing the stock solution and diluting it with deionized water. A 125- to 2500-µL aliquot of the mixture was added to 25 mL of commercial pharmaceutical samples that contained known amounts of endogenous quaternary ammonium halogenides, and extraction was carried out as described previously. In order to calculate the percent recovery, the amount of quaternary ammonium halogenides was subtracted from the measured total amount, divided by the added amount, and multiplied by 100. Tables I and II show the IC–conductivity traces obtained for commercial pharmaceutical samples spiked with CPC, DPB, BZC, CHXG, and CTB, respectively. Excellent recoveries and precision were observed, and recoveries ranged from 97% ± 5.5% to 104% ± 3.9%.

Table I. Analytical Results for CPC and DPB in Mouth Rinses and Cleanthroat Troches*

		Amount			Concentration (w/w,%)	
Sample	Compound	Added (mg/L)	Found (mg/L)	%Recovery	IC method	DPV method
Mouth rinse A	CPC	5.00	5.18	104(3.8%)†	0.088(1.9%)	0.090(2.0%)
	DPB	5.00	4.95	99(3.6%)	0.051(4.9%)	n.d.‡
Mouth rinse B	CPC	10.00	9.70	97(5.5%)	0.102(4.1%)	0.098(4.9%)
	DPB	5.00	5.05	101(4.5%)	0.013(1.5%)	n.d.
Mouth rinse C	CPC	5.00	5.10	102(5.3%)	0.067(1.3%)	0.064(1.0%)
	DPB	n.p.§	n.p.	n.p.	n.p.	n.p.
Cleanthroat troch A	CPC	5.00	5.18	104(3.8%)	0.024(5.0%)	0.023(5.5%)
	DPB	10.00	10.32	103(1.5%)	0.028(3.2%)	n.d.
Cleanthroat troch B	CPC	10.00	9.80	98(5.5%)	0.048(3.1%)	0.049(2.3%)
	DPB	20.00	20.80	104(3.2%)	0.052(0.9%)	n.d.
Cleanthroat troch C	CPC	20.00	20.76	104(3.9%)	0.235(3.3%)	0.243(4.5%)
	DPB	n.p.	n.p.	n.p.	n.p.	n.p.

* $n = 5$.
† Relative standard deviation.
‡ n.d., not detected.
§ n.p., analyte not present in pharmaceutical.

Table II. Analytical Results for BZC, CHXG, and CTB in Disinfectants and Mouth Rinse*

		Amount			Concentration (w/w,%)	
Sample	Compound	Added (mg/L)	Found (mg/L)	%Recovery	IC method	DPV method
Disinfectant A	BZC	10.00	9.95	99.5(4.5%)†	0.103(5.0%)	0.125(1.6%)
Disinfectant B	CHXG	10.00	10.10	101(5.5%)	0.313(5.0%)	0.303(4.3%)
	CTB	15.00	14.70	98(4.6%)	2.971(1.8%)	2.737(0.2%)
Disinfectant C	CHXG	20.00	20.57	103(5.8%)	0.300(5.0%)	0.305(5.5%)
	CTB	20.00	20.40	102(5.5%)	3.165(1.6%)	2.925(5.6%)
Disinfectant D	CHXG	100.00	103.4	103(4.8%)	3.967(4.9%)	3.667(3.3%)
	CTB	n.p.‡	n.p.	n.p.	n.p.	n.p.
Mouth rinse	CHXG	5.00	5.12	102(5.5%)	0.205(3.2%)	0.199(5.1%)

* $n = 5$.
† Relative standard deviation.
‡ n.p., Analyte not present in pharmaceutical.

Determination of commercial samples

The proposed IC method was applied to the determination of quaternary ammoniums in pharmaceutical products. Representative IC chromatograms of a commercial cleanthroat troche and disinfectant are shown in Figure 5. Anions of quaternary ammoniums of analytical results are given in Tables I and II. These results agreed with those cations of quaternary ammoniums obtained by DPV.

Conclusion

The different halogens of quaternary ammoniums can be separated using IC. The method can be used to determine the composition of commercial samples. Other detection modes such as spectrophotometry coupled with a postcolumn derivatization device or elution gradient improvements of the separation of quaternary ammoniums by HPLC method can be used. The direct determination of anions of quaternary ammoniums not only offers higher resistance to organic interferences than indirect determination but also saves more time.

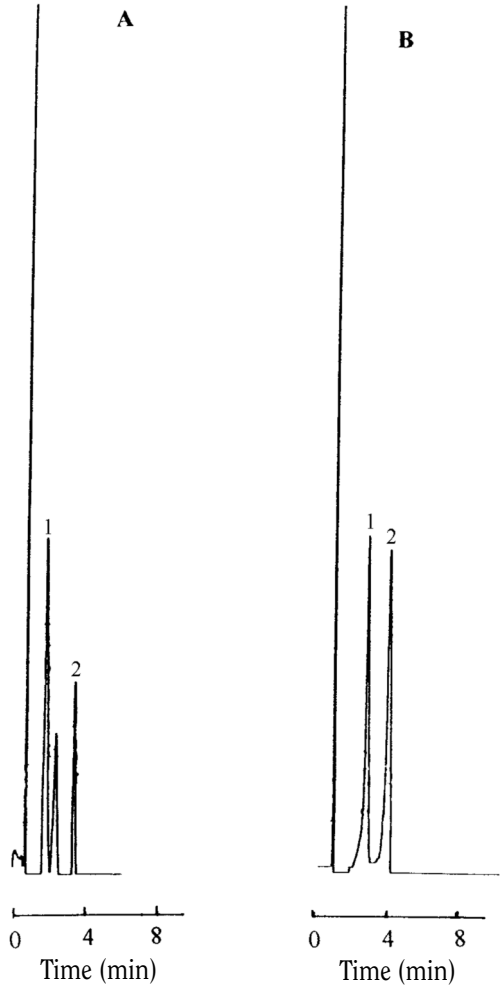

Figure 5. Chromatograms obtained from containing (1) chloride and (2) bromide by (A) cleanthroat troche and (B) disinfectant.

References

1. J.B. Lowry. Direct spectrophotometric assay of quaternary ammonium compounds using bromethlene. *J. Pharm. Sci.* **68:** 110–11 (1979).
2. T. Sakai. Extraction–spectrophotometric determination of benzethonium and benzalkonium salts with bromocresol green and quinine. *Anal. Chim. Acta.* **147:** 331–37 (1983).
3. T. Sakai. Solvent extraction–spectrophotometric determination of berberine and benzethonium in drugs with tetrabromophenolphthalein in ethyl ester by batchwise and flow injection methods. *Analyst* **116:** 187–200 (1991).
4. T. Sakai and N. Ohno. Novel flow injection analysis for selective spectrophotometric determination of cetylpyridinium chloride in pharmaceuticals utilizing thermochromism of ion associates. *Anal. Sci.* **7:** 297–304 (1991).
5. E. Pinzayti and E.L. Porta. Use of silver electrode in the potentiometric determination of quaternary ammonium compounds. *Analyst* **102:** 938–42 (1977).
6. R.C. Talyor and C.Y. Cheng. Potentiometric determination of alkylbenzyldimethyl ammonium chloride in cosmetic preparations. *J. Pa. Acad. Sci.* **66:** 142–45 (1992).
7. S. Pinzauti, G. Papeschi, E. Laporta, P. Mura, and P. Gratteri. Differentical electrolytic potentiometry with twin silver-solver sulphide membrane in micro titration of quaternary ammonium compounds. *J. Pharm & Biomed. Anal.* **6:** 957–61(1988).
8. G.G. Cheng and X.X. Pan. Determination of domiphen bromide by oscillopolarographic titrations. *Gaodeng Xuexiao Huaxue Xuebao* **9:** 1185–86 (1988).
9. S. Kawase, S. Kanno, and S. Ukai. Gas–liquid chromatographic determination of benzethonium chloride. *J. Chromatogr.* **247:** 273–80 (1982).
10. T. Lukaszewski. The extraction and analysis of quaternary ammonium compounds in biological material by GC and GC/MS. *J. Anal. Toxicol.* **9:** 101–108 (1985).
11. P. Bore. *Cosmetic Analysis.* Marcel Dekker, New York, NY, 1985, p 105.
12. J. Pasesen, I. Quintens, G. Thoithi, E. Roets, G. Reybrouck, and J. Hoogmartens. Quantitative analysis of quaternary ammonium antiseptics using thin-layer densitometry. *J. Chromatogr.* **677:** 377–84 (1994).
13. U. Scblegel, C. Hobl, and C. Burgi. Determination of basic and analysis of quaternary ammonium preservatives in cosmetics with HPTLC in combination with HLPC colorimetry. *Mitt. Gebiete Lebensm. Hyg.* **88:** 191–201 (1997).
14. I. Kazuo, A. Takashi, I. Koichi, K. Teruo, N. Hiroshi, and W. Lohya. Determination of benzalkonium chloride and benzethonium chloride in cosmetics by high-performance liquid chromatography. *Kenkyu Nwnpo Tolyo-toristsu Eisei Kenkyusho* **38:** 101–108 (1987).
15. E.L. Johnson. *Handbook of Ion Chromatography*. Dionex Corporation, Sunnyvale, CA, 1986, p 40.

Manuscript accepted March 6, 2002.