Determination of Benzalkonium Chloride by Chemical Ionization Mass Spectroscopy

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Abstract □ A new specific and sensitive method of analysis for samples of benzalkonium chlorides is presented. Chemical-ionization mass spectroscopy has been used to identify and determine the proportions of various alkyl chain lengths in commercial mixtures of benzalkonium chlorides. This method allows the direct and simultaneous determination of individual benzalkonium chlorides.

Keyphrases □ Benzalkonium chloride—determination by chemical-ionization mass spectroscopy, alkyl chain lengths □ Chemical-ionization mass spectroscopy—determination of benzalkonium chloride, alkyl chain lengths

Benzalkonium chlorides (I) USP are a mixture of benzalkonium chlorides, where $R = n$-alkyl and n can vary from C_{10} to C_{18}. The relative amounts of these different species greatly affects both the antimicrobial spectrum and

$$\left[C_6H_5CH_2\text{—}\overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{N}}\text{—}R \right]^+ Cl^-$$

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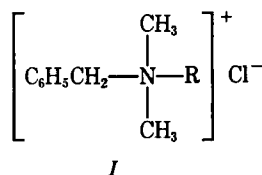
activity of the mixture (1–3). The current methods of identification of benzalkonium chloride solutions according to the USP (4), rely upon titrimetric analysis of total alkyl benzyldimethylammonium chloride based upon potassium iodate equivalents. Additionally, the ratio of the various n-alkyl components of benzalkonium chlorides can be determined by microhydrogenation followed by solvent extraction and GLC. The ratio of alkyl components is then calculated and must meet specific USP requirements. These requirements state that the C_{12} homologue must comprise at least 40% of the total benzalkonium chloride content and that the C_{14} homologue must be at least 20%. Furthermore, these two homologues must comprise together 70% of the total content. Other methods have been used for the determination of benzalkonium chlorides: ion-pairing techniques (5–8), direct titration involving tetraphenylboron (9) and iodate (10), reverse-phase HPLC (11), and pyrolytic GLC (12).

The present method directly determines the chemical composition and alkyl chain length ratios of benzalkonium chloride species in commercial samples.

EXPERIMENTAL

Apparatus—Measurements were conducted upon a spectrometer[1] fitted with an electron-impact–chemical-ionization (EI–CI) source and a DS-55 data system.

Reagents and Solvents—Individual benzalkonium chloride standards were prepared with $R = C_1–C_{20}$, by a previous method (13) and characterized by spectroscopic and elemental analysis. Purity was in all cases

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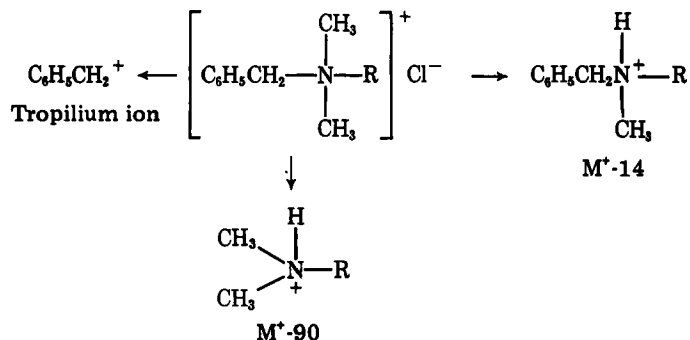
Scheme 1—*Chemical-ionization mass fragmentation pathways for benzalkonium chloride (1).*

>99.6%. The commercial benzalkonium chloride mixtures were obtained from three different manufacturers. Samples for analysis were prepared as millimolar solutions in methanol[2].

Assay—Standard solutions were prepared as 2 μl methanol solutions containing $R = C_9$ internal standard (0.01 M) together with pure synthetic benzalkonium chloride (0.001–0.01 M) with $R = C_{10}, C_{12}, C_{14}, C_{16}$, and C_{18}. Samples were applied as methanol solutions by the direct-inlet technique at 200° on a glass probe tip and evaporated into the chemical-ionization plasma of ammonia gas. Evaporation profiles were deter-
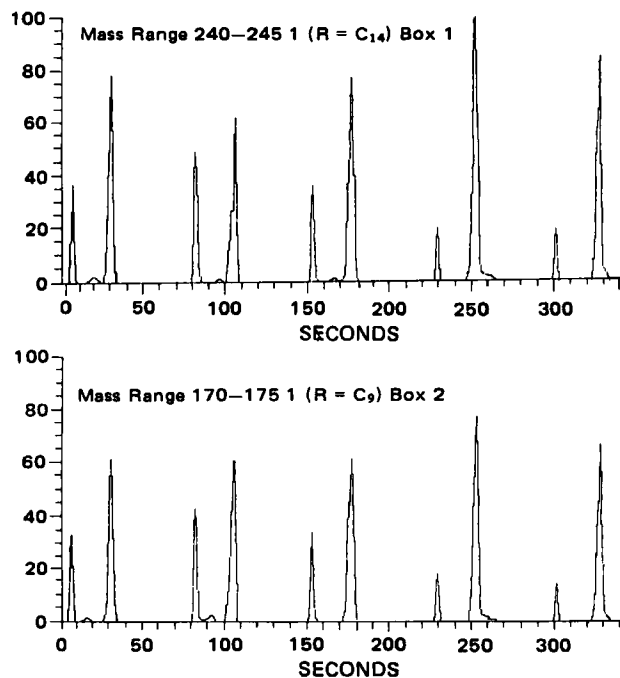
Figure 1—*Evaporation profiles for five repeat 2-μl injections of an R = C_9 + C_{14} mixture (50:50) of benzalkonium chlorides obtained by simultaneous monitoring of the M^+ −90 ions. (The first peak in each evaporation profile is observed as a result of evaporation off the probe before the heater switches in. The second peak is the main evaporation profile. Total ions were collected at one time by continuous scanning.)*

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[1] Kratos MS25.

[2] British Drug Houses, Ltd., London, England.

Table I—Relative Intensities of Diagnostic Ions Obtained for a Series of Synthetic Benzalkonium Chlorides Where R = C_{10} to C_{18}

Diagnostic Ion[a]	R = C_9	R = C_{10}	R = C_{12}	R = C_{14}	R = C_{16}	R = C_{18}
			m/z (RI)			
M^+; $C_6H_5CH_2N(CH_3)_2R^+$	262(9.1)	276(4.6)	304(5.4)	332(0.4)	360(2.8)	388(4.0)
M^+ −14; $C_6H_5CH_2N(H)(CH_3)R^+$	248(61.3)	262(18.6)	290(54.2)	318(10.7)	346(27.3)	374(37.4)
M^+ −90; $(CH_3)_2NHR^+$	172(19.4)	186(73.6)	214(94.5)	242(81.5)	270(91.9)	298(70.1)
M^+ −91; $(CH_3)_2NR^+$	171(14.7)	185(5.1)	213(11.7)	241(1.9)	269(5.4)	297(8.9)

[a] M^+ is equivalent to the mass of the cation and not to the molecular weight of the salt.

Table II—Total Ion-Current Ratios for the M^+ −90 Ions of Five Repeat Injections of Solutions of Synthetic Benzalkonium Chlorides (R = C_{10}–C_{18}) and R = C_9 Internal Standard.

R	Mean	CV^a, %	Mean	CV, %	Mean	CV, %	Mean	CV, %
				Total Ion-Current Ratios C_n/C_9 Internal Standard				
C_{10}	0.735	±3.1	0.411	±7.4	0.185	±4.9	0.088	±4.2
C_{11}	0.867	±3.8	0.369	±2.9	0.198	±5.6	0.130	±9.7
C_{12}	1.145	±2.7	0.603	±3.03	0.246	±8.5	0.117	±6.2
C_{14}	0.969	±1.6	0.424	±4.7	0.179	±6.0	0.084	±4.5
C_{16}	0.808	±2.1	0.312	±4.3	0.137	±5.3	0.060	±9.1
C_{18}	0.852	±2.9	0.271	±6.1	0.107	±6.7	0.019	±9.5
Molar Ratio Cn/C_9	1:1		1:2		1:5		1:10	

[a] Coefficient of variation.

Table III—Relative Intensities of M^+ −90 Ions Obtained in Commercial Mixtures of Benzalkonium Chlorides

Commercial Mixture	Relative intensity of M^+ −90 ion for R						
	C_9	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}
Sample A	0.0	0.0	3.7	5.3	48.4	100.0	0.0
Sample B	0.0	0.0	100.0	60.0	10.6	0.0	0.0
Sample C	0.0	7.6	100.0	23.2	6.6	0.0	0.0

Table IV—Total Ion-Current Ratios for M^+ −90 Ions and Calculated Molar Ratios of Component Benzalkonium Chlorides in Commercial Mixtures and R = C_9 Internal Standard

Commercial Mixture	R	Total Ion-Current Ratio, C_n/C_9	Molar Ratio in Mixture, %
Sample A	C_{12}	0.021 ± 0.0024	2
	C_{14}	0.035 ± 0.0028	2
	C_{16}	0.350 ± 0.0038	26
	C_{18}	1.080 ± 0.0093	70
Sample B	C_{12}	1.812 ± 0.018	71
	C_{14}	0.529 ± 0.020	24
	C_{16}	0.121 ± 0.014	4
Sample C	C_{10}	0.438 ± 0.0157	5
	C_{12}	1.119 ± 0.0488	68
	C_{14}	0.334 ± 0.0640	23
	C_{16}	0.054 ± 0.0223	5

mined and total ions for diagnostic peaks collected by continuous scanning. Average ion currents for particular diagnostic peaks were determined as ratios to that of the R = C_9 internal standard. Calibration curves were constructed for ions of particular interest. Such calibration curves were used in the quantitative analysis of commercial benzalkonium chloride mixtures.

RESULTS AND DISCUSSION

The chemical-ionization mass spectra of pure benzalkonium chlorides have been measured, and predictable, characteristic fragmentation patterns affording diagnostic ions of use in structural identification were determined (Scheme I). Diagnostic ions were observed at M^+ −14 (loss of CH_3 plus addition of H) and M^+ −90 (loss of $C_6H_5CH_2$ plus addition of H). A low-intensity peak was also observed for the benzalkonium cation. A common peak was found at m/z 91 due to the tropilium ion. Table I shows the fragmentation data for a series of pure benzalkonium chloride standards where R = C_{10}, C_{12}, C_{14}, C_{16}, and C_{18}. From these data it was possible to determine the molecular weight of the individual

benzalkonium chloride, since a significant M^+ peak (2–10% relative intensity) in addition to medium intensity ions at M^+ −14 and M^+ −90 was observed. The M^+ −90 peak was the most intense in all the spectra examined and was chosen for quantitative investigations. Replicate 2-μl analytical samples containing varying proportions of the R = C_9 standard and the appropriate benzalkonium chloride were introduced into the mass spectrometer, and the total-ion currents for their M^+ −90 ions, together with their peak heights, were simultaneously determined to give relative evaporation profiles. Evaporation profiles for all the compounds were
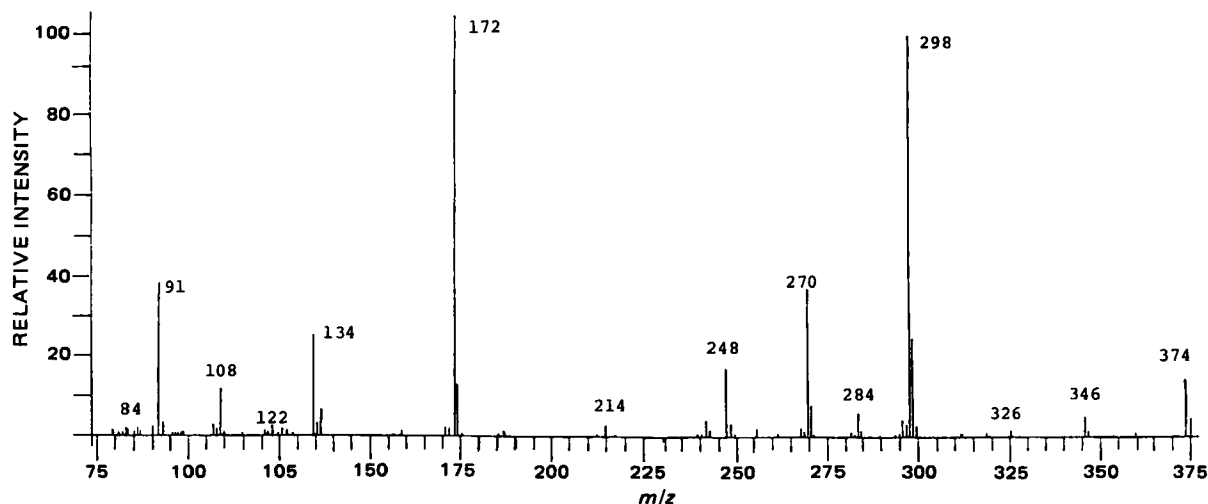

Figure 2—*Chemical-ionization mass spectrum of commercial sample A benzalkonium chloride containing C_9 internal reference from m/z 75–400.*

similar to one another, as illustrated in Fig. 1 for $R = C_{14}$ and $R = C_9$ internal standard. Results were expressed as total ion-current ratios for $R = C_n/C_9$. Table II gives total ion-current ratios together with coefficients of variation for repeat integrations of varying molar proportions of $R = C_9$ and benzalkonium chloride standards $C_{10}-C_{18}$. Interestingly, the integral ratios $R = C_n/R = C_9$ varied with alkyl chain length (Table II); consequently, it was necessary to construct calibration curves for each individual benzalkonium chloride species examined. These were constructed by assay of various dilutions of test compound, spiked with internal standard. Graphs of total ion-current ratios *versus* concentration were linear in nature for all benzalkonium chlorides examined.

Samples of commercial benzalkonium chloride mixtures were prepared as methanol solutions (1 mg/ml). Spectra were obtained for these mixtures (illustrated in Fig. 2 for sample A), and the relative intensities of their component benzalkonium chloride species were determined (Table III). As can be seen, sample A contains $R = C_{12}$, C_{14}, C_{16}, and C_{18}, with $R = C_{18}$ predominating, whereas samples B and C are predominant in $R = C_{12}$ species but are qualitatively different. Having determined the species content of the mixtures, quantitative measurements were carried out by the simultaneous monitoring of each $M^+ -90$ ion relative to the $M^+ -90$ ion of the internal standard. Concentrations of benzalkonium chloride species were obtained by relating total ion-current ratios (Table II) to those from the calibration data. From these data it can be observed that two of the three commercial samples comply with the USP limits, while the remaining sample varies quite markedly in alkyl chain compositions (See Table IV).

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Solubility in Binary Solvent Systems III: Predictive Expressions Based on Molecular Surface Areas

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Abstract □ The nearly ideal binary solvent model, which has led to successful predictive equations for the partial molar Gibbs free energy of the solute in binary solvent mixtures, was extended to include molecular surface areas as weighting factors. Two additional expressions were derived and compared to previously developed equations (based on molar volumes as weighting factors) for their ability to predict anthracene and naphthalene solubilities in mixed solvents from measurements in the pure solvents. The most successful equation in terms of goodness of fit involved a surface fraction average of the excess Gibbs free energy relative to Raoult's law and predicted experimental solubilities in 25 systems with an average deviation of 1.7% and a maximum deviation of 7.5%. Two expressions approximating weighting factors with molar volumes provided accurate predictions in many of the systems studied but failed in their ability to predict anthracene solubilities in solvent mixtures containing benzene.

Keyphrases □ Binary solvents—solubility, predictive expressions based on molecular surface areas □ Solubility—binary solvent systems, predictive expressions based on molecular surface areas □ Molecular surface area—solubility in binary solvent systems □ Anthracene—prediction of solubility in binary solvent systems based on molecular surface areas □ Naphthalene—prediction of solubility in binary solvent systems based on molecular surface areas

The use of binary solvents for influencing solubility and multiphase partitioning has many potential applications in the pharmaceutical industry. However, maximum realization of these applications depends on the development of equations that enable *a priori* prediction of solution behavior in mixed solvents from a minimum number of additional observations. Ideally, the ability to predict a drug molecule's solubility and partition coefficients based solely on a consideration of molecular structure is desired, but for more practical applications, a less fundamental approach must often suffice.

The nearly ideal binary solvent (NIBS) approach developed previously (1–6) provides a relatively simple method for estimating the excess partial molar properties of a solute, $\overline{Z}_3^{ex}$, at infinite dilution in a binary solvent (components 1 and 2):

$$\overline{Z}_3^{ex} = f_1^0(\overline{Z}_3^{ex})_1 + f_2^0(\overline{Z}_3^{ex})_2 - \Gamma_3(X_1^0\Gamma_1 + X_2\Gamma_2)^{-1}\overline{Z}_{12}^{ex}$$
$$\text{(Eq. 1)}$$

$$f_1^0 = 1 - f_2^0 = X_1^0\Gamma_1/(X_1^0\Gamma_1 + X_2^0\Gamma_2) \quad \text{(Eq. 2)}$$

in terms of a weighted mole fraction average of the properties of the solute in the two pure solvents [$(\overline{Z}_3^{ex})_1$ and $(\overline{Z}_3^{ex})_2$] and a contribution due to the unmixing of the solvent pair by the presence of the solute. This equation leads to accurate predictions of solubilities (3, 5–8), gas–liquid partition coefficients (4, 9), and enthalpies of solution (1, 2) in systems of nonspecific interactions when the weighting factors (Γ_i) are approximated with molar volumes.

A simpler approximation of equating all three weighting factors provides considerably poorer predictions for systems in which the molar volumes of the components differ appreciably. The superiority of expressions based on molar volumes suggests that the relative sizes of the molecules are an important consideration. The use of surface areas as weighting factors may be revealing, since surface area often represents a different measure of molecular size.