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Part I. Field desorption mass spectrometry of the isomeric N,N,N-trimethylammoniophenolates. Part II. Field desorption mass spectrometry of cyclodextrin inclusion complexes.

Taras William Obal
University of Windsor

PART I

FIELD DESORPTION MASS SPECTROMETRY OF THE ISOMERIC N,N,N-TRIMETHYLAMMONIOPHENOLATES

PART II

FIELD DESORPTION MASS SPECTROMETRY OF CYCLODEXTRIN INCLUSION COMPLEXES

by

Taras William Obal

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ABSTRACT

PART I

The isomeric N,N,N-trimethylammoniophenolates and their trimethyl-d(9) analogs were submitted for FDMS analysis. Their behaviour under FD conditions was studied and is discussed. In general, the title compounds yield FD spectra which do not correspond to those obtained by EIMS. Structural effects, such as substituent position on the benzene ring, are considered. The ortho- isomer is characterized by a base peak corresponding to the $[C-1]^{+\cdot}$ species, whereas the meta- and para- isomers exhibit base peaks corresponding to the intact cation, $[C]^{+}$. Based on FD studies using the labelled analogs, modest amounts of intermolecular methyl group transfer are observed for the meta- and para- isomers, while the ortho- isomer showed no indication of this type of behaviour. However, intramolecular methyl transfer in the ortho- isomer is considered and discussed. The appearance of the FD spectra for these compounds is shown to be dependent upon anode temperature.

PART II

Cyclodextrin inclusion complexes of a series of substituted naphthalenes, fluorenes and anthracenes are studied by FDMS. Inclusion of the guest compounds yields a decrease in

volatility such that FDMS is possible on these compounds. The guest/host ratio affects the desorption profile of the compound studied, to the extent that one can manipulate the desorption of the guest. Based on differential effects on volatility and/or desorption, the potential for distinguishing closely related species by FDMS is studied. Differentiation of positional isomers by FDMS of their inclusion complexes yields inconclusive results. However, different desorption behaviour is observed between the inclusion complexes of the enantiomeric R- and S-(1-naphthyl)ethylamines.

To My Family

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A, A^-	anion
BAT	best anode temperature
C, C^+	cation
CD	cyclodextrin
cm^{-1}	reciprocal centimetre
DMF	dimethylformamide
ehc	emitter heating current
EI	electron impact
FAB	fast atom bombardment
FD	field desorption
FI	field ionization
GC	gas chromatography
HPLC	high performance liquid chromatography
IR	infrared
M, $M^{+\cdot}$	molecular ion
mA	milliampere
MS	mass spectrometry
m/z	mass to charge ratio
NMR	nuclear magnetic resonance
ppm	parts per million
TLC	thin layer chromatography

PART I

FIELD DESORPTION MASS SPECTROMETRY OF THE ISOMERIC N,N,N-TRIMETHYLAMMONIOPHENOLATES

CHAPTER I

INTRODUCTION

Methyl group transfer during the mass spectrometric analyses of zwitterionic species containing a quaternary ammonium group and a negatively charged oxygen has been observed in several instances, by a variety of ionization techniques. These include electron impact (EI) [1], pyrolysis gas chromatography/mass spectrometry (GC/MS) [2], laser ionization mass spectrometry (LIMS) [3], secondary ion mass spectrometry (SIMS) [4] and field desorption mass spectrometry (FDMS) [5]. In all cases, characteristic mass spectra containing peaks corresponding to the products of alkyl group, usually methyl group, transfer from the quaternary ammonium to an oxygen group within the molecule were observed.

Reported herein are the results obtained for the FDMS analyses of the isomeric N,N,N-trimethylammoniophenolates[a] and their d-9 analogs (Figure 1). As positional isomers, these compounds provide a more subtle test of structural effects than the homologous aliphatic zwitterions previously studied by FDMS [5].

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a) It should be noted that throughout this dissertation, the zwitterion and its hydroiodide salt are referred to as the N,N,N-trimethylammoniophenolate.

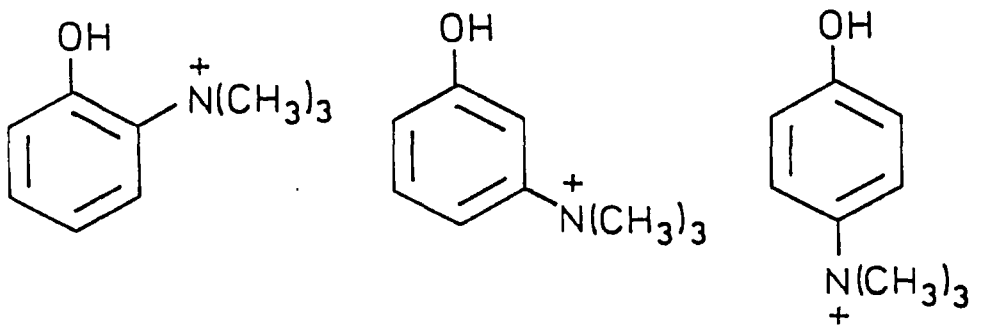


Figure 1: o-, m- and p-N,N,N-trimethylammoniophenolates.

Structurally, this group of molecules belongs to the larger class of compounds referred to as betaines, by virtue of the quaternary ammonium and negatively charged oxygen which they possess as the zwitterions.

Betaines are of interest industrially because of their application in detergent, soap and shampoo formulations [6-9]. Certain betaines are useful as surfactants in aqueous media. Other characteristics which make them important components of industrial formulations include their relative insensitivity to the presence of certain metal ions in hard water, such as Ca^{2+} and K^{+}, their increased biodegradability and their low irritancy in such products as shampoos, etc..

As organic salts, the N,N,N-trimethylammoniophenolates are characteristically non-volatile and as such, are not appropriate candidates for the more conventional modes of mass spectrometric ionization which require a gaseous sample. The temperatures required for volatilization of these species are generally quite high, and their analysis by EI techniques is

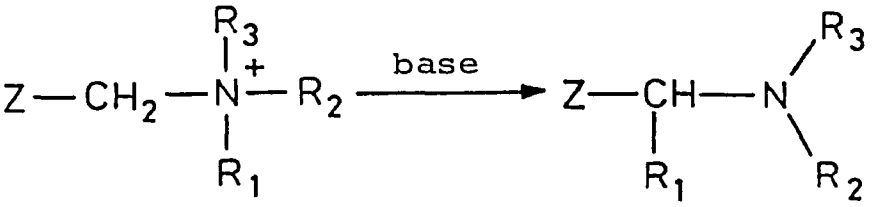
often accompanied by pyrolysis of the sample as well as extensive fragmentation of the parent cation. This results in a complicated spectrum comprised of the fragmentation pattern for the molecule of interest, as well as the fragmentation patterns for any products of thermal decomposition. Thus a softer form of ionization is required in order to distinguish between the intact molecular ion and any artifacts resulting from reactions which may have occurred.

CHAPTER II

BACKGROUND

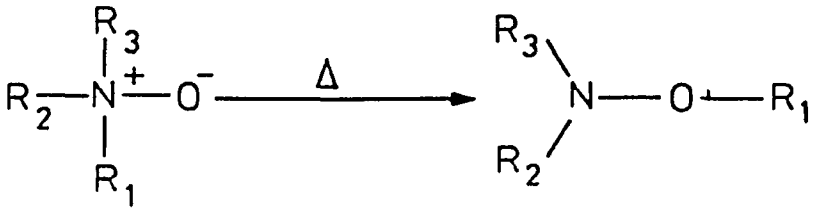
A. Alkyl Transfer Reactions - General.

One of the most prevalent migrations by an alkyl group from a quaternary ammonium functionality occurs during the Stevens Rearrangement [10].



In this reaction, a quaternary ammonium salt containing an electron withdrawing group attached to an α-carbon rearranges to give the tertiary amine when treated with a strong base. Based on crossover experiments with ^{13}C labelled precursors [11, 12], the mechanism of this reaction was determined to be intramolecular.

Another reaction which occurs via a similar mechanism is the Meisenheimer rearrangement [13].



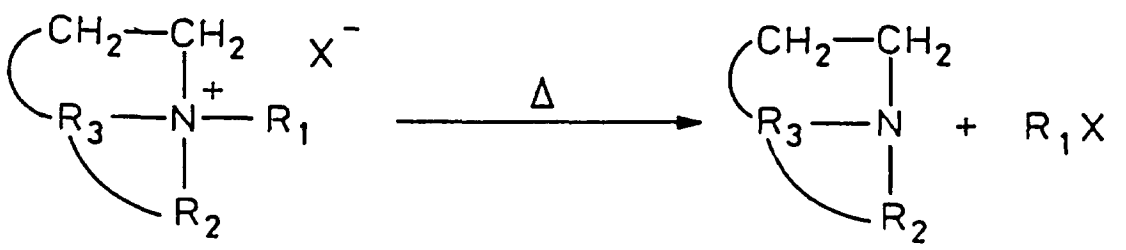
In this case, it was found [14,15] that heating of N,N-alkylaniline oxides in strongly alkaline solutions afforded the isomeric O-alkyl-N-alkylphenylhydroxylamine. In further studies by Cope and Towle [16], it was observed that simple heating of the fairly dry amine oxides alone at 85-165° C effected a smooth rearrangement to the amine.

B. Alkyl Transfer Studies by Mass Spectrometry.

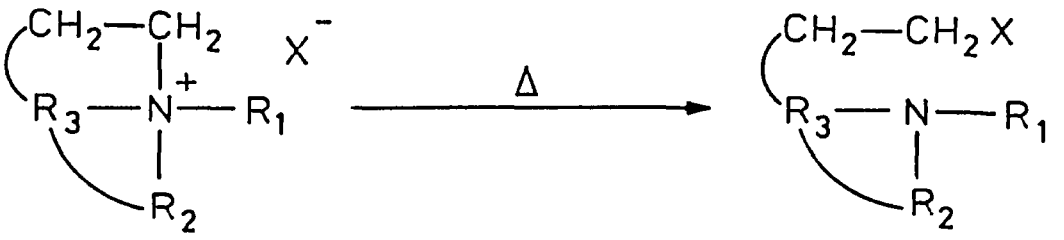
1. Electron Impact Mass Spectrometry (EIMS).

Because of low volatility and thermal instability, the EI mass spectra of quaternary ammonium compounds are characterized by peaks corresponding to the products of thermal degradation. Generally, three types of degradation have been observed [17]: dealkylation, attack by the anion on another N-substituent and Hofmann degradation.

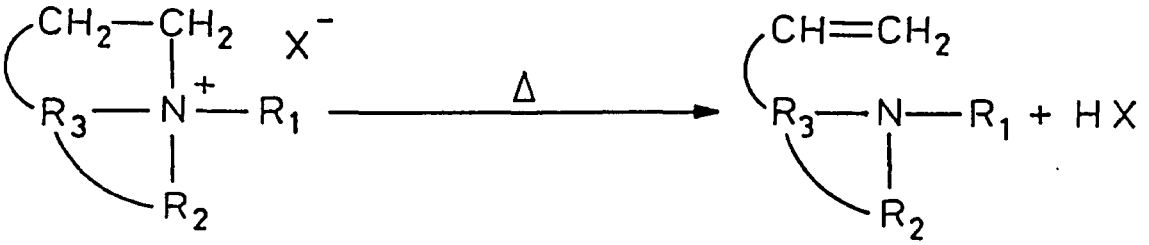
The most prevalent of these reactions is dealkylation of the quaternary ammonium functionality yielding the nor-base and the alkyl halide. The products of this dealkylation are then vaporized and ionized yielding the corresponding mass spectrometric data.



Attack by the anionic species on another N-substituent results in products and mass spectra corresponding to the tertiary amine with substitution by the halide. Clearly, a peak corresponding to the formula weight of the salt may be observed. However, the fragmentation pattern of the compound will differ vastly from that of the nor-base.



Finally, a common thermal degradation process which occurs during the vaporization of a quaternary ammonium compound is Hofmann degradation resulting in the hydrogen halide and the unsaturated Hofmann product.



In general, these degradation processes do not account for all of the ions observed. Thus, with more complex compounds, complicated mass spectra may be observed [18]. Other factors,

such as the type of anion present, have been shown [19] to influence the degradation processes and the extent to which they occur. Iodides tend to favor dealkylation, while fluorides yield mass spectra corresponding to the Hofmann elimination products.

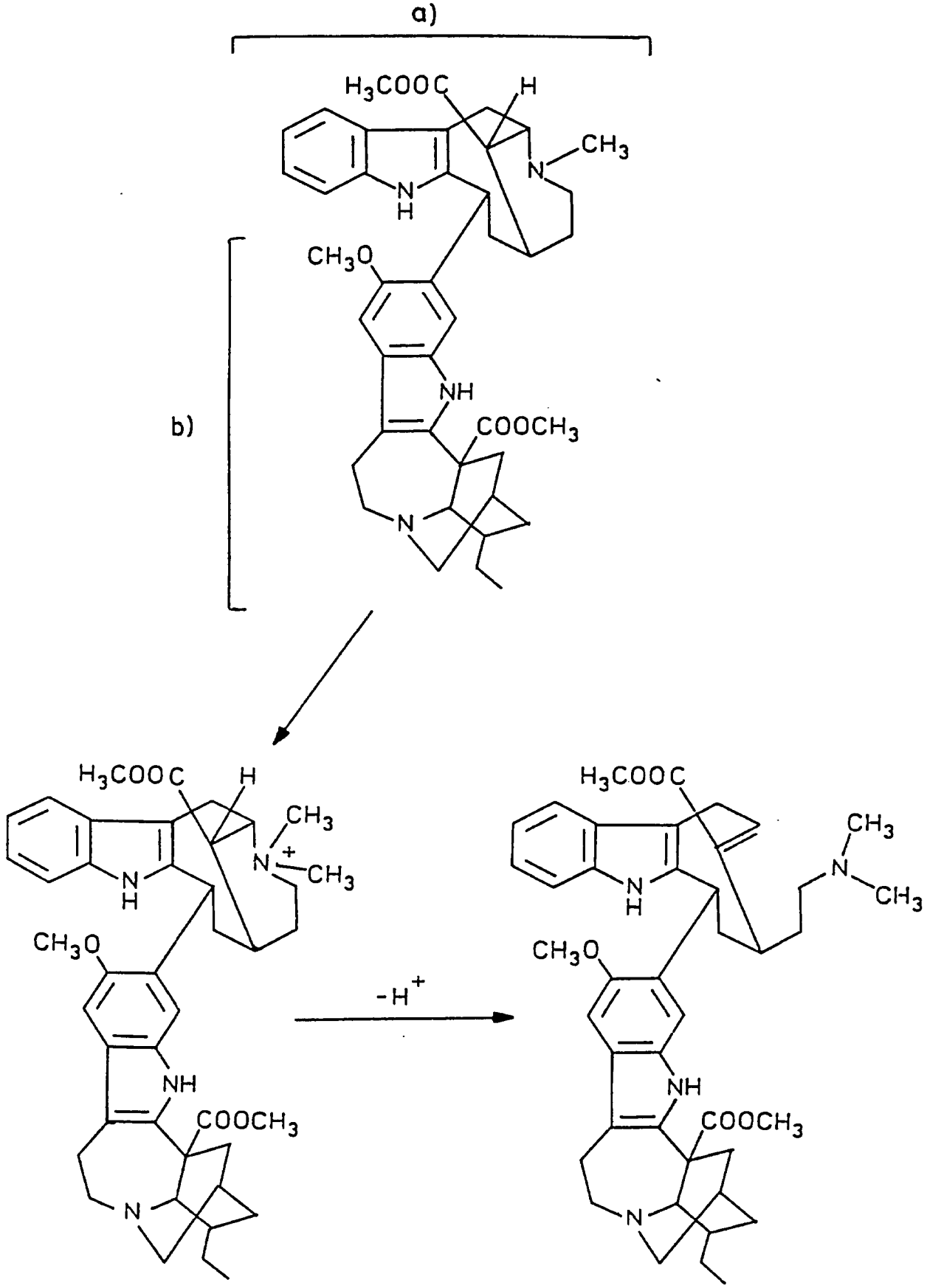
One of the first examples of alkyl transfer among quaternary ammonium salts was observed during the EIMS analysis of voacamine (Figure 2) [20,21]. In this case the authors, while attempting to elucidate the structure of this indole alkaloid, observed an anomalous peak at m/z = 718 (calcd. mol. wt. = 704), whereas the products of hydrogenolysis (m/z = 678), and the primary acetate (m/z = 660) prepared from decarbomethoxy voacamine by hydride reduction and acetylation, respectively, yielded molecular ion peaks corresponding to that expected for the predicted structure. It was therefore presumed that intermolecular methyl transfer had occurred when the sample was directly evaporated into the ion source, and the peak at m/z = 718 actually corresponded to the mass of the methine base.

Further studies on voacamine and its d-3 analog [23] confirmed that this process occurs via an intermolecular mechanism. Upon evaporation in the ion source, peaks corresponding to the molecular ion (m/z = 704; 707 (d-3)) were observed, as well as peaks corresponding to intermolecular transmethylation (m/z = 718; 721 (d-3); 724 (d-6)) from the voacangine carbomethoxy group to the amino group of the voabasinol residue (Figure 2). The thermal nature of this

Fig. 2: Methyl Transfer in Voacamine [20,21,23].

a) vobasinol

b) voacangine



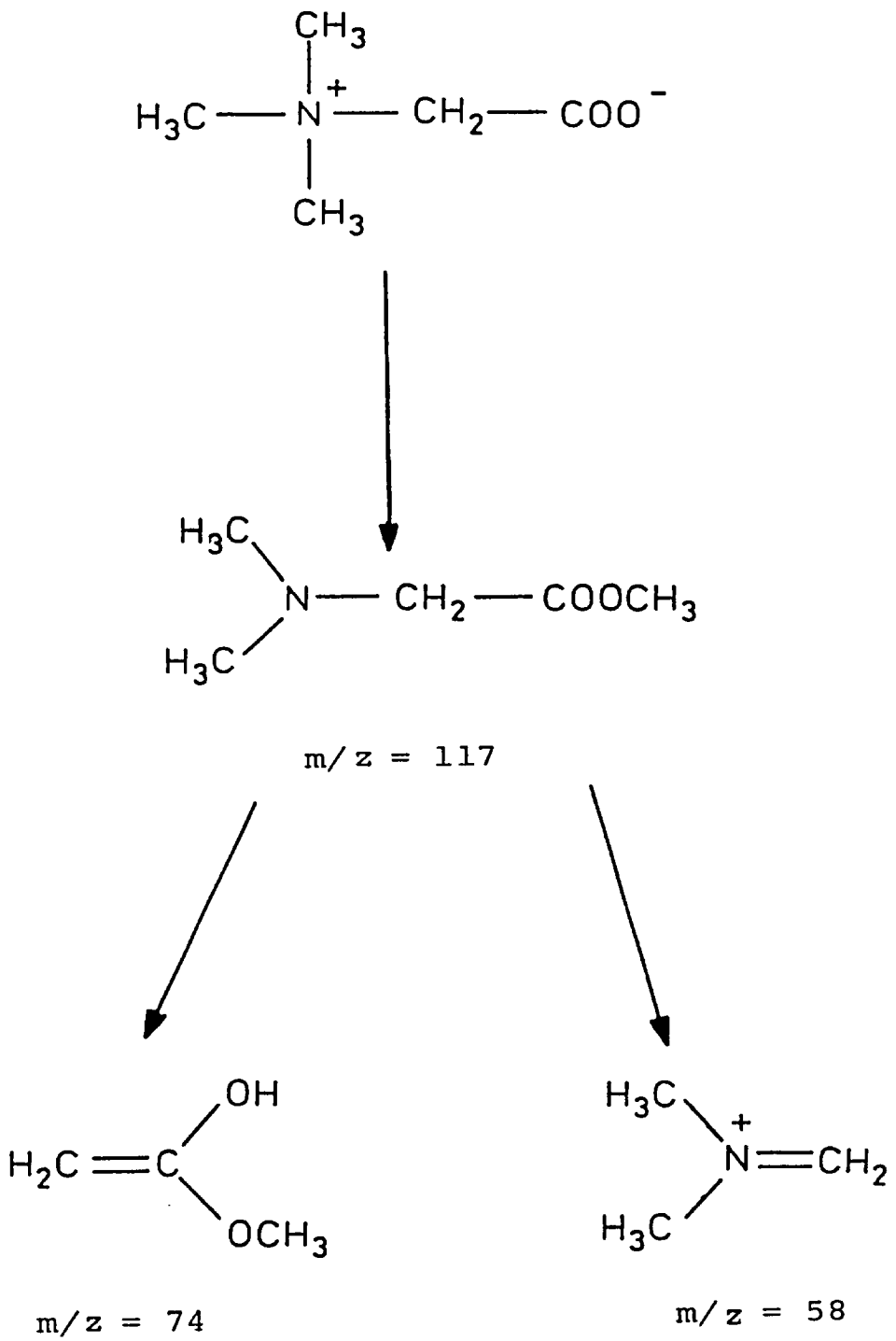
reaction is indicated by an increase in the relative abundance of the $[M + 14]^+$ peak with increased vaporization temperatures [23].

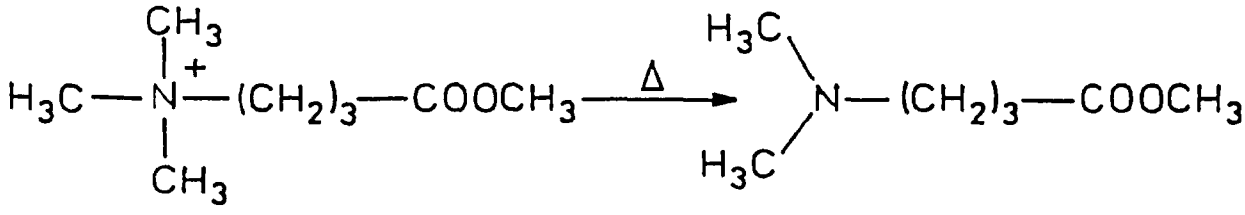
Almost concurrently, the EIMS data obtained for vinblastine (mol. wt. = 810) [24] exhibited similar ions corresponding to the products of transmethylation. Peaks occuring at m/z values 14 and 28 mass units above the expected molecular weight were observed and shown to arise similarly.

The class of compounds referred to as the amino acid betaines exhibit similar behaviour under EI conditions [25-27]. The glycine betaine [25, 26], because of its low volatility, undergoes transalkylation between the quaternary ammonium functionality and the negatively charged oxygen of the carboxylate group to form the volatile methyl ester prior to evaporation into the ion source. Based on studies with deuterium labelled samples, it was found that this rearrangement occured via an intermolecular process, yielding spectra which are characterized by a molecular ion corresponding to the esterified acid, a peak corresponding to the trimethylamine fragment (m/z = 58) formed by β-cleavage with respect to the amine, and an ion corresponding to the McLafferty product of the ester at m/z = 74 (Figure 3).

While studying the pyrolytic behaviour of other amino acid betaines, Ohya, et. al. [28] found that capronium chloride upon heating yielded N,N-dimethyl-γ-butyrate with methyl group exchange.

Fig. 3: Rearrangement and Fragmentation Products of Glycine Betaine under EI Conditions [25].





Upon further analysis using deuterated derivatives [27] it was found that the starting material exhibited intermolecular methyl transfer under pyrolytic conditions. Pyrolysis GC/MS analysis of a mixture of capronium chloride and its perdeuterated analog yielded peaks corresponding to the products of transmethylation (Figure 4).

Extensive studies have been carried out on the title compounds under EI conditions [1, 29-31]. It was found [29] that the meta- and para-trimethylammoniophenolates underwent transalkylation to the more volatile methyl ethers, prior to evaporation in the ion source. This was confirmed by comparison of the spectra obtained for the zwitterionic species with those obtained for authentic samples of the ethers. However, the ortho- isomer exhibited anomalous behaviour, in that the EIMS results differed from those obtained for the authentic sample of the methyl ether. To gain further insight into the process of transmethylation among these compounds, homogeneous mixtures of each isomer and its d-9 analog were prepared and analyzed by EIMS. Exclusive intermolecular methyl transfer was observed for the meta- and para- isomers, as indicated by the presence of peaks corresponding to the mixed transmethylation products at m/z = 151, 154, 157 and 160 (Figure 5). All of

Fig. 4: Intermolecular Methyl Transfer in Capronium Chloride and its d-9 Analog [27].

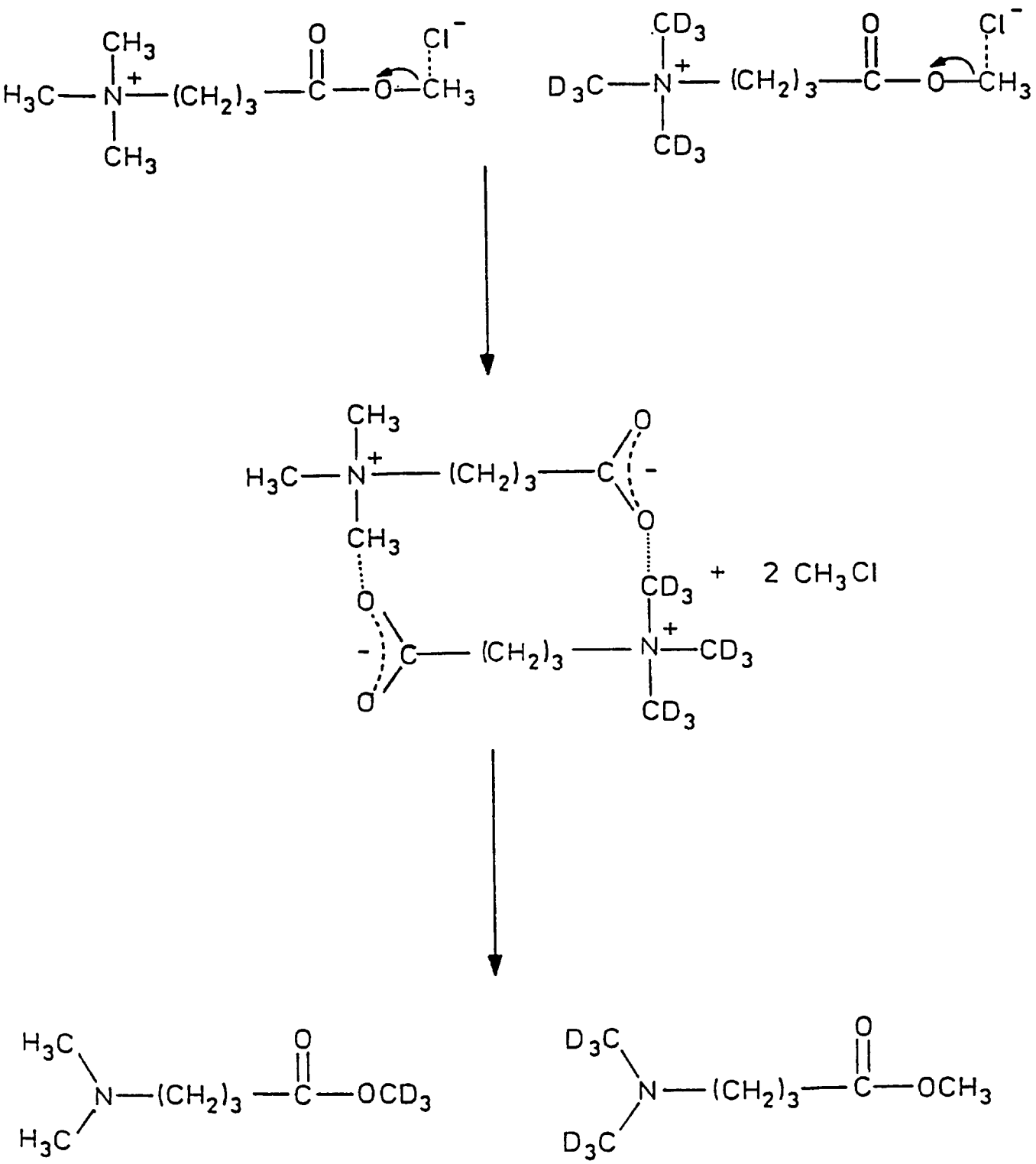
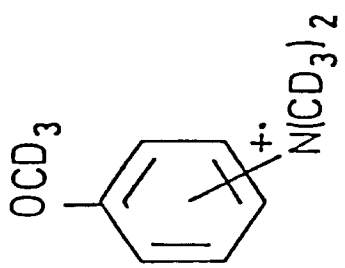
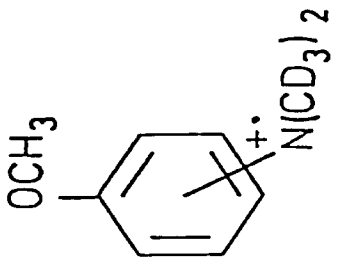


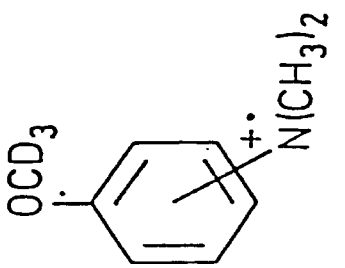
Fig. 5: Mixed Products Resulting from Intermolecular Methyl Transfer [29].



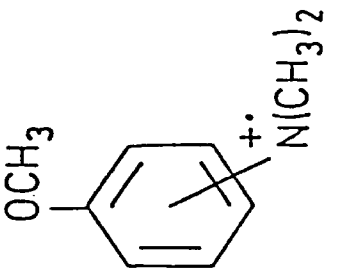
m/z = 160



m/z = 157



m/z = 154



m/z = 151

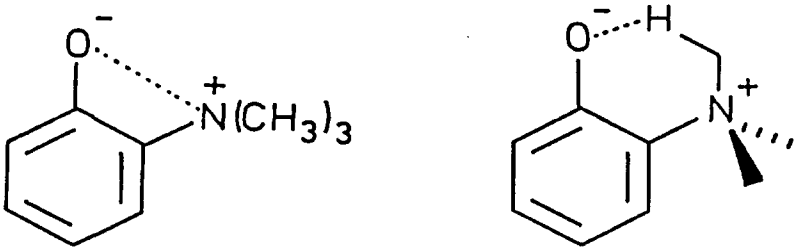
these peaks were of equal intensity, indicating exclusively intermolecular methyl transfer. Analogous studies on the ortho- isomer yielded only 10% intermolecular transalkylation. The remaining 90% yielded a fragmentation pattern which differed from that of the corresponding ether. It was found that prior pyrolysis of the sample at reduced pressure resulted in a complete conversion of the sample to the volatile methyl ether. Thus it was postulated that, by the direct insertion technique, the ortho- isomer evaporated mainly as the zwitterion without any structural changes.

Two possible rationales were given to account for this behaviour (Figure 6). It was considered that the close proximity of the oppositely charged groups leads to an internal partial charge compensation, which in turn results in decreased electrostatic attraction between molecules and consequently an increase in the volatility of the zwitterionic species. Intramolecular hydrogen bonding may also play a role in increasing the vapour pressure of the sample.

Further evidence favouring the postulate that the ortho- isomer evaporates structurally unchanged was obtained by comparison of the semi-empirically derived ionization potentials for the zwitterion and the corresponding methyl ether [30]. It was found that that the zwitterion had an ionization potential of 6.88 ± 0.05 eV, compared to 7.59 ± 0.05 eV for the ether.

Based on these studies, it has been concluded that o-N,N,N-trimethylammoniophenolate is evaporated in the ion source predominantly as the zwitterion admixed with the methyl

ether. These processes are competing [31], and the degree to which transmethylation occurs is dependent on such factors as temperature and substituent effects.



a) b)

Fig. 6: Rationale for the Increased Volatility of the Structurally Unchanged o-N,N,N-trimethylammoniophenolate [29]:

a) internal partial charge compensation

b) intramolecular hydrogen bonding.

At a given sample temperature, the relative amount of each component in the gas phase is determined by the rate of transmethylation compared to the rate of evaporation of the zwitterion. Increases in sample temperature tend to favour ether formation.

Bulky substituents tend to decrease the degree of transmethylation by virtue of steric effects. Electron withdrawing groups decrease the amount of alkyl transfer as well, by decreasing the nucleophilicity of the oxygen moiety. It must be noted that the degree of transmethylation is only substituent dependent in the case of the ortho- isomer.

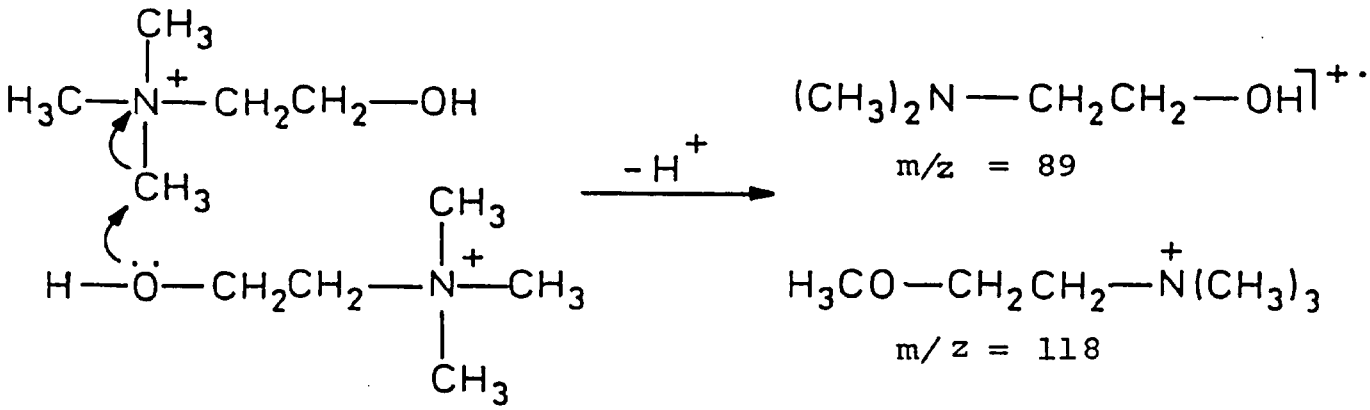
2. Field Desorption Mass Spectrometry (FDMS).

The application of FDMS techniques to the analysis of onium salts has been well documented [32-35]. FDMS data on these compounds are characterized by peaks corresponding to the cationic species, C^+, and various artifactual peaks caused by a number of ion forming processes observed in FD.

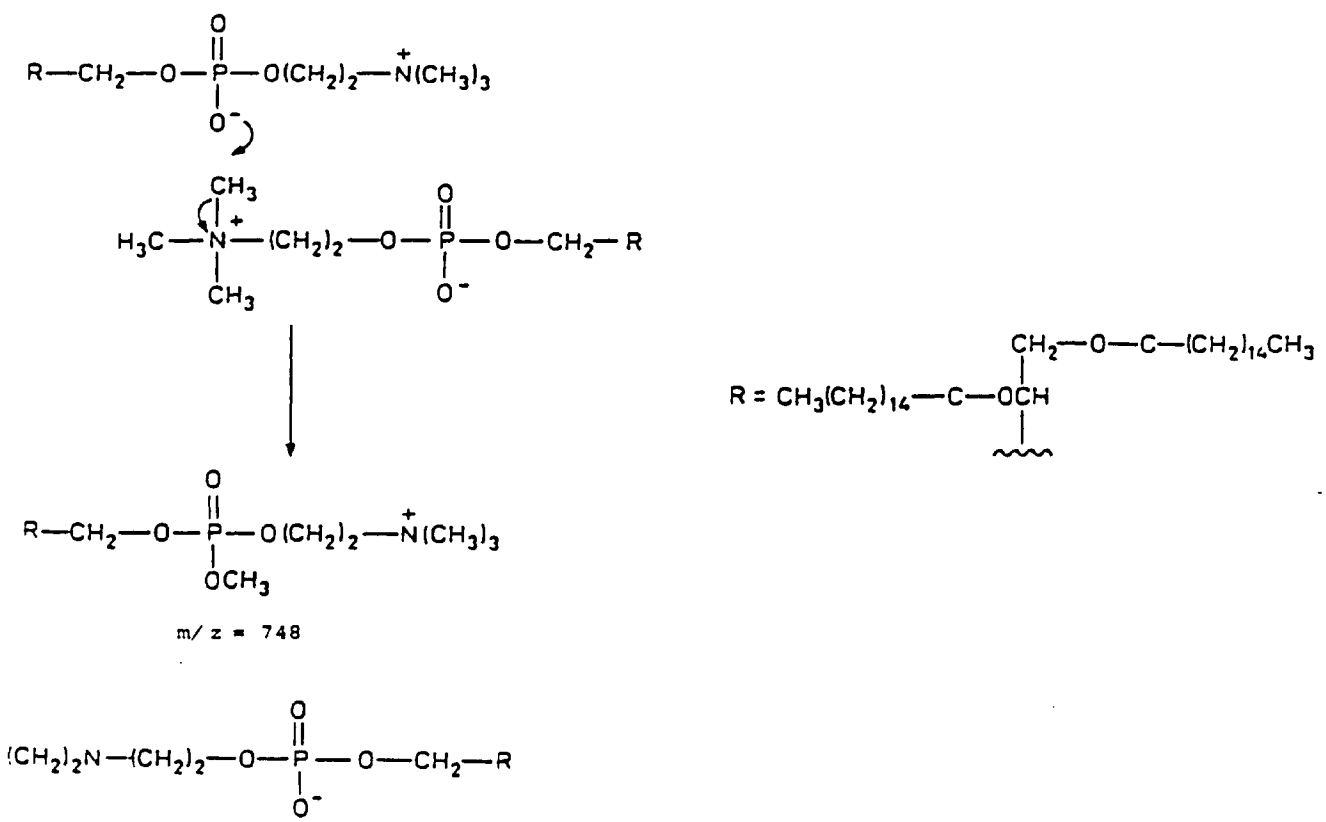
Because FD is a softer form of ionization (see Appendix I) compared to EI, the spectra are characteristically less complex, generally containing mainly peaks corresponding to the molecular ion and any products of reactions or rearrangements occurring within the system. Thus FDMS is ideally suited for the study of alkyl transfer processes among the betaine class of compounds, as one should observe mainly the cation, and any products due to rearrangement of the cation.

FDMS techniques involve gentle desorption of the sample with concomitant ionization, or more frequently, desorption of the charged species formed in the condensed phase, thus reducing any interferences in the spectra caused by thermal degradation of the sample. As well, FDMS allows analysis of non-volatile samples without having the high sample temperatures required by EIMS.

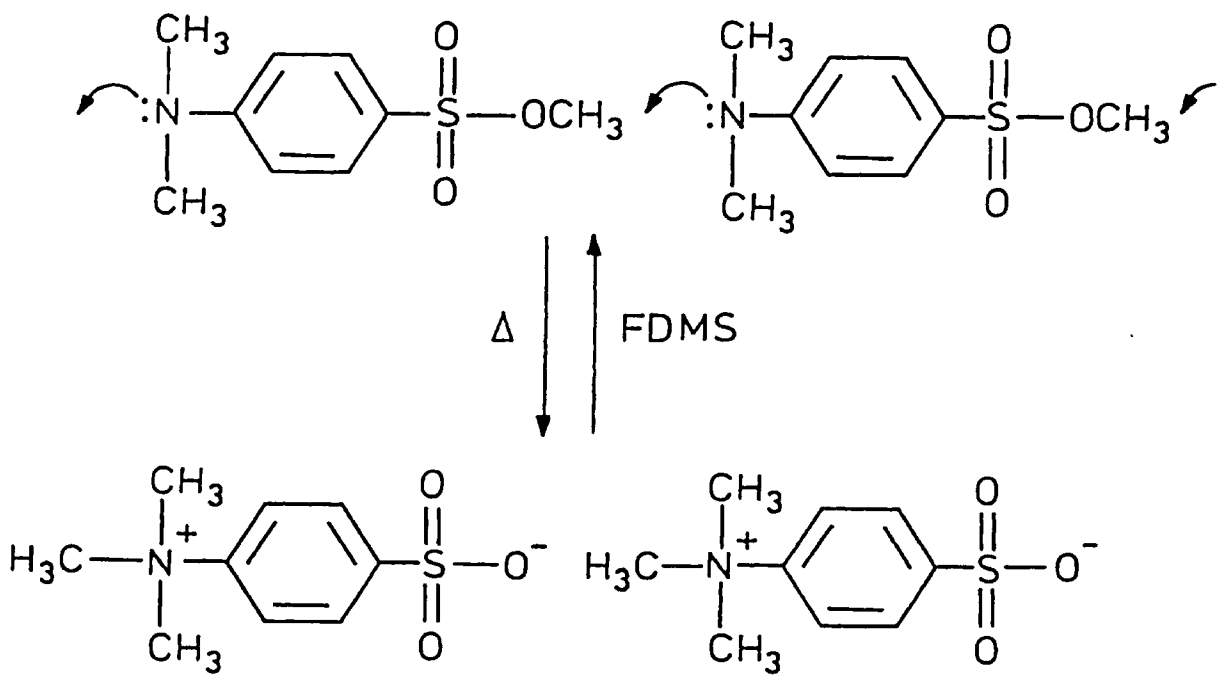
Anomalous peaks occuring 14 mass units higher than the mass of the cationic species were first observed in FDMS by Brent, et. al. [32] and later by Wood, et. al. [36] during the analysis of choline chloride and synthetic phosphatidylcholines, respectively. Further studies on a series of choline halides [37], led to the conclusion that this peak was caused by an intermolecular methyl transfer between the quaternary ammonium functionality and the hydroxyl moiety, as illustrated. Thus the FD spectra of these compounds were characterized by peaks corresponding to the product of demethylation (m/z = 89), the product of methyl transfer (m/z = 118), as well as the cation (m/z = 104). Based on labelling studies, it was found that it is in fact the hydroxyl proton which is lost in this rearrangement process, and that the reaction occurs via an intermolecular mechanism.



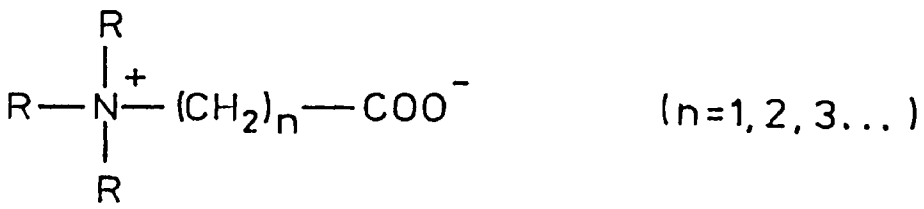
Subsequent studies on the FD behaviour of dipalmitoylphosphatidylcholine, and its d-4 and d-9 analogs yielded similar results [38]. Spectra contained base peaks corresponding to $[M+H]^+$ (m/z = 734 (d-0); 738 (d-4); 743 (d-9)) attributed to thermal rearrangement of a methyl group between the quaternary ammonium group of choline and the negatively charged phosphoryl oxygen. It should be noted that peaks were not observed consistently, corresponding to the demethylated compound. No explanations were given regarding this phenomenon. However, small peaks at m/z = 724 and m/z = 726 were observed on occasion for the d-4 and d-9 compounds, respectively. It was presumed that these peaks were caused by the products $[M+2-CH_3]^+$ and $[M+2-CD_3]^+$.



An interesting reaction proceeding in the opposite sense was observed during solid-state studies on <u>para</u>-N,N,N-trimethylammoniobenzenesulfonate [39]. In this case, it was observed that the ester of interest underwent an intermolecular methyl transfer from the sulfonate substituent to the tertiary amine yielding the zwitterionic species. Labelling studies have shown that this reaction is at least 76% intermolecular, based on the intensities of the mixed product peaks observed upon submitting homogeneous mixtures of the d-0, d-3, d-6 and d-9 zwitterionic species to FDMS analysis. The results cited for the FD analyses were in fact a reversal of the indicated solid-state reaction (i.e., zwitterion to methyl ester), thus corresponding to the trends observed among the FD data obtained for the previously studied compounds [35-38].



Probably the most widely studied class of compounds exhibiting alkyl transfer under FD conditions are the amino acid betaines [5, 40, 41] having the general formula:



In general, these compounds yield FD spectra characterized by peaks corresponding to the intact cation and to the products of intermolecular methyl transfer.

Sanders, et. al. have shown [40] that quaternary ammonium hexanoates readily undergo alkyl transfer under FD conditions to form volatile, as well as non-volatile quaternary ammonium esters. It was observed that compounds containing N-methyl, N-ethyl and N-propyl groups yielded peaks corresponding to $[M+14]^+$, $[M+29]^+$ and $[M+43]^+$, indicating intermolecular transfer of the alkyl groups attached to the quaternary nitrogen. This process was shown to be dependent on the emitter heating current (ehc). At low ehc values, the spectra were dominated by peaks corresponding to the $[M+H]^+$ ion as well as clusters corresponding to $[2M+H]^+$ and $[3M+H]^+$. At moderate ehc values, one observed a decrease in intensity among the cluster ions and the appearance of a peak corresponding to $[M+alkyl]^+$. At high ehc values, the spectra were dominated by the $[M+alkyl]^+$ peaks, while the cluster ions showed very low intensities. In some cases, the alkyl group transferred was as

large as $C_{20}H_{21}$, yielding a peak at $[M+141]^+$. The authors thus concluded that by FDMS one may identify all of the N-substituents present in a particular amino acid betaine.

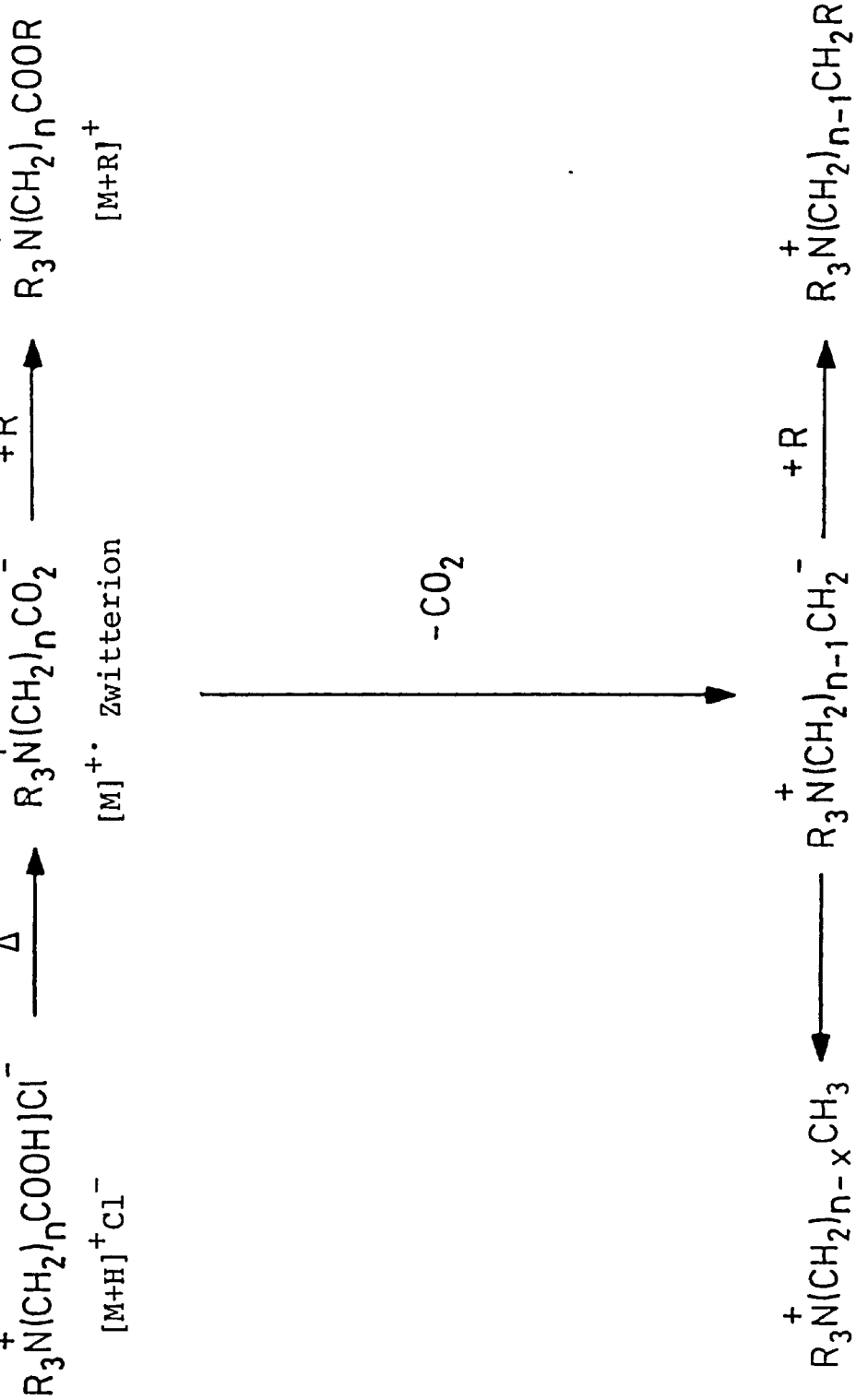
FD studies on less complex ammonioalkanecarboxylate hydrochloride salts and their d-9 analogs [5, 41] yielded similar results. As with the ammonioalkanehexanoates previously studied by FDMS [40], the spectra were dominated by peaks corresponding to $[M+H]^+$ and clusters of the general formula $[nM+H]^+$ (n = 1, 2, 3) at low ehc values. As the temperature of the emitter was increased, the intensities of these peaks decreased while the intensities of the peaks corresponding to $[M+alkyl]^+$ and $[M]^+$ increased. It was thought that $[M]^{+\cdot}$ corresponded to the product of isomerization from the zwitterionic species to the more volatile methyl ester. The identity of the peak corresponding to $[M+15]^+$ as $[M+CH_3]^+$ was demonstrated by the FD data obtained for homogeneous mixtures of the non-deuterated compounds and their d-9 analogs. Four peaks corresponding to the mixed methyl transfer products were observed:

$$[M+CH_3]^+,\ [M+CD_3]^+,\ [M(d-9)+CH_3]^+,\ [M(d-9)+CD_3]^+$$

As well, these results confirmed that the mechanism of this rearrangement is intermolecular. Decarboxylation was also found to occur among these compounds, yielding peaks corresponding to $[M-44]^{+\cdot}$. An interesting aspect of the FD behaviour of the decarboxylated zwitterions is that they also participate in

the methyl transfer process, giving rise to peaks corresponding to the general formula $[M-44+R]^+$ (Figure 7). This was also shown to occur via an intermolecular mechanism, based on FD studies of the labelled and unlabelled compounds. Four peaks were observed corresponding to $[M-29]^+$, $[M-26]^+$, $[M(d-9)-29]^+$ and $[M(d-9)-26]^+$. This reaction presumably occurs through a Stevens type of rearrangement.

Fig. 7: Methyl Transfer Reactions Observed in N,N,N-trimethyl-ammonioalkanecarboxylate Salts [41].



CHAPTER III

OBJECTIVES OF THE STUDY

Electron impact studies on the N,N,N-trimethylammoniophenolates require high temperatures for sample volatilization, and electron energies on the order of 70 eV for ionization. This results in pyrolysis and extensive fragmentation of the molecular ion. Thus, a soft ionization technique is required in order to detect intact molecular ions. FDMS is the method of choice due to its ability to ionize non-volatile salts without the transfer of excess heat or energy.

As there has been no report in the literature regarding the FDMS analysis of the N,N,N-trimethylammoniophenolates, these compounds have been submitted for FDMS in an effort to further clarify the process of methyl transfer among these types of compounds and to provide data for comparison with the EIMS results. The mechanism of the rearrangement was studied by the FDMS analysis of their N,N,N-perdeuterated derivatives. As positional isomers, these compounds were selected to provide a more subtle indication of any structural effects dictating the process of transmethylation.

Further clarification of the processes accompanying mass spectrometric analysis of these relatively simple compounds will assist in the interpretation of mass spectrometric data of more complex compounds.

CHAPTER IV

EXPERIMENTAL TECHNIQUE AND PREPARATION OF MATERIALS

A. Synthesis of the N,N,N-trimethylammoniophenolates[a].

The isomers of N,N,N-trimethylammoniophenolate were prepared according to the procedure outlined by Pfleger and Waldmann [42, 31]. The general synthesis is given below.

To 15 mL of absolute methanol were added aminophenol (0.005 mole), methyl iodide (0.018 mole) and anhydrous sodium carbonate (0.005 mole). The solution was heated under reflux for 15 hours. The reaction mixture was then concentrated and refrigerated overnight, effecting precipitation of the reaction product. The crude product was recrystallized once from methanol.

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a) The syntheses of the title compounds and their d-9 analogs, as well as the IR spectrophotometric analyses were carried out by Dr. D. V. Ramana. His contribution to this project is gratefully acknowledged.

B. Synthesis of the N,N,N-perdeuterated Derivatives of the N,N,N-trimethylammoniophenolates[a].

The d-9 analogs of the compounds of interest were prepared using the procedure outlined above. However, methyl iodide (d-3) was substituted for the unlabelled methyl iodide as the quaternizing agent.

C. Characterization of the Synthetic Products.

The products obtained from the indicated syntheses were characterized by ^{1}H-NMR spectroscopy and IR spectrophotometry. ^{1}H-NMR chemical shift data were obtained using a Bruker WP80 CW NMR spectrometer equipped with a 80 MHz magnet. All isomers analyzed were dissolved in DMSO (d-6).

IR absorption data were obtained with a Beckman IR20A spectrophotometer. Potassium bromide disks containing the sample of interest were prepared and submitted for analysis.

D. Mass Spectrometric Analyses.

The mass spectrometric analyses of the isomeric N,N,N-trimethylammoniophenolates and the analogous d-9 compounds were carried out on a Varian MAT CH-5 double focusing mass spectrometer equipped with a combined EI/FI/FD ion source as well as FAB capabilities. The system was interfaced to a Nova 4 computer employing an INCOS data system.

Samples analyzed by mass spectrometry included each isomer of N,N,N-trimethylammoniophenolate as well as the analogous d-9 compounds. In order to elucidate the mechanism of methyl transfer, samples containing homogeneous mixtures of approximately equal amounts of each isomer and its corresponding d-9 analog were submitted for mass spectrometric analyses.

Samples submitted for FDMS analyses were dissolved in deionized water at concentrations of approximately 10 mg/mL. Samples were adsorbed onto benzonitrile activated FD emitters via the standard dipping technique, and introduced into the ion source. High vacuum was maintained at less than 10^{-6} torr, and high voltage was applied (+3 kV to the emitter and -8 kV to the extraction plate). Focusing of the ion source was carried out in the FI mode using acetone, introduced from the batch inlet, as the standard. The magnetic scan (the magnet was scanned quadratically from m/z = 900 to m/z = 10 at 12 second intervals) was then started, and the emitter was heated at a steady rate, varying from 1 - 5 mA/scan depending on the analysis. Due to low instrument sensitivity and the erratic desorption behaviour characteristic of these compounds, the resolving slits were left open, yielding a nominal resolution of approximately 265.

After each analysis was completed, the emitter was heated to approximately 50 mA in order to remove any remaining sample as well as any impurities exhibiting higher desorption temperatures.

CHAPTER V

EXPERIMENTAL RESULTS AND DISCUSSION

A. Synthesis of o-, m- and p-N,N,N-trimethylammoniophenolate and Their N,N,N-perdeuterated Derivatives.

The syntheses of the different isomers of N,N,N-trimethylammoniophenolate and their d-9 analogs yielded products of varying appearance and yields (Table 1). The products appeared as colourless to faint pink powders with some indication of plate formation.

The synthetic products were characterized by ^{1}H-NMR spectroscopy and IR spectrophotometry. The ^{1}H-NMR chemical shift data for each isomer and its d-9 analog are outlined in Table 2. Appropriate singlets were observed for the methyl protons at resonances of 3.58-3.68 ppm. Clearly, no peaks were observed for the deuterated analogs. Aromatic protons exhibited chemical shifts between 6.90-7.76 ppm with appropriate AB quartets being observed in the cases of the para- isomers. Finally, peaks corresponding to the hydroxyl protons were not observed. One might account for this by considering the rapid exchange between the acidic hydroxyl proton and the solvent. This phenomenon might cause broadening of the NMR peak to the extent that it is not observed.

Table 1

Appearance and Yields for Synthetic Isomers of N,N,N-trimethylammoniophenolate and their N,N,N-Perdeuterated Derivatives.

ISOMER	APPEARANCE	YIELD
ortho-	beige crystals	42%
meta-	light pink powder	75%
para-	colourless powder	63%
ortho- (d-9)	light grey crystals	35%
meta- (d-9)	off-white powder	70%
para- (d-9)	off-white plates	80%

Table 2

^{1}H-NMR Chemical Shift Data for
o-, m- and p-N,N,N-trimethylammoniophenolate
and their N,N,N-perdeuterated Derivatives.

ISOMER	δ (ppm)	
ortho-	3.68 (s, 9H)	7.29 (m, 4H)
meta-	3.58 (s, 9H)	7.18 (m, 4H)
para-	3.58 (s, 9H)	6.90 (d, 2H)\ AB quartet 7.76 (d, 2H)/
ortho- (d-9)	-	7.00 (m, 4H)
meta- (d-9)	-	7.17 (m, 4H)
para- (d-9)	-	6.90 (d, 2H)\ AB quartet 7.75 (d, 2H)/

The IR spectra for these compounds (Tables 3 and 4) are characterized by broad absorption bands in the range of 3400-3000 cm^{-1}, indicative of hydrogen bonded hydroxyl stretching. Frequencies corresponding to aromatic C=C stretching were observed between 1460-1615 cm^{-1}. Characteristic C—O and C—N stretching frequencies were observed at 1340-1360 cm^{-1} and 1220-1230 cm^{-1}, respectively. C—H out of plane bending frequencies were observed between 780-835 cm^{-1}, characteristic of aromatic C—H bonds. Absorption frequencies characteristic of N—CH_3 deformations were observed at approximately 1415-1420 cm^{-1}.

Table 3

IR Absorption Frequencies (cm^{-1}) for o-, m- and p-N,N,N-trimethylammoniophenolate.

ortho-	meta-	para-	Type of Frequency
3400-3000	3400-3000	3400-3000	H-bonded -OH str.
1615, 1520, 1465	1615, 1500, 1475	1605, 1520, 1460	Benzenoid ring breathing
1360	1340	1350	C—N str.
1230	1225	1220	C—O str.
780	760	835	C—H OOP bending
1415	1420	1420	N—CH_3 deform.

Table 4

IR Absorption Frequencies (cm^{-1}) for o-, m- and p-N,N,N-trimethylammoniophenolate (d-9).

ortho- (d-9)	meta- (d-9)	para- (d-9)	Type of Frequency
3400-3200	3400-3000	3400-3000	H-bonded -OH str.
1610, 1500	1620, 1505	1630, 1610 1525	Benzenoid ring breathing
1325	1345	1370	C—N str.
1255	1250	1225	C—O str.
740	760	840	C—H OOP bending

B) FDMS of o-, m- and p-N,N,N-trimethylammoniophenolate and their N,N,N-perdeuterated Derivatives.

As mentioned earlier (Section II.B.1), Undheim and Hvistendahl [29] have studied the EI behaviour of the title compounds. Because of the high temperatures required for volatilization of the salts, as well as the large amounts of energy transferred in the ionization process, the EI mass spectra of these compounds are dominated by fragmentation of the molecular ion and the products of pyrolysis (Figure 8).

Depending on the orientation of the two benzene ring substituents, the major ions obtained by FDMS correspond to one

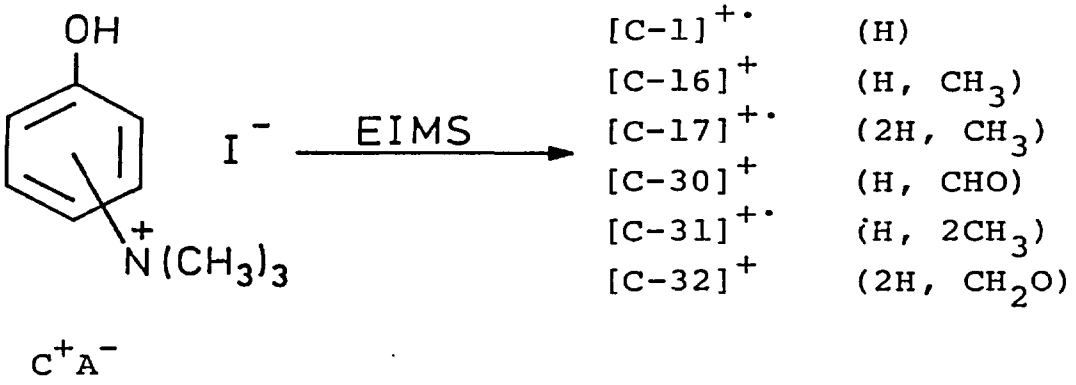


Fig. 8: Major Ions Formed by EIMS of o-, m- and p-N,N,N-trimethylammoniophenolate [29].

or more of the ions illustrated in Figure 9. It should be noted that none of the distinctive FD spectra for these isomers corresponds to the EI spectra reported earlier [29].

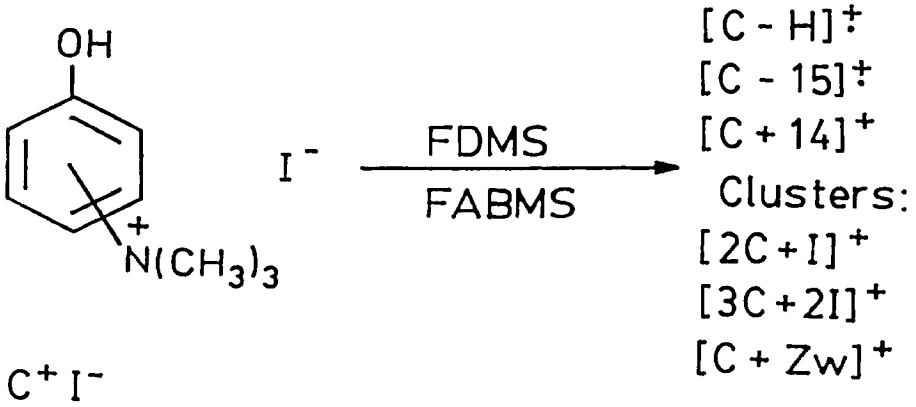
The structures attributed to the FDMS data observed are illustrated in Figure 10. The peaks corresponding to $[C+14]^+$ and $[C-15]^{+\cdot}$ have been assigned to the tetramethyl species and the demethylated compounds, respectively. The tetramethyl species arises from the replacement of the hydroxyl proton by a methyl group, presumably donated by the quaternary ammonium group of another molecule. It is not clear if the demethylated species arises from attack by the anion on an N-methyl substituent or if it is due to the parent cation having participated in the methyl transfer process.

The peak corresponding to $[C-1]^{+\cdot}$ has been assigned the structure of the product of rearrangement from the cationic species to the methyl ether. This process is accompanied by the loss of the hydroxyl proton. In the case of the ortho-isomer, this peak is attributed to the zwitterion.

A variety of cluster ions were observed at lower anode temperatures. These clusters were generally of the formulae $[nC+(n-1)I]^+$ (n = 1, 2) and $[nC+nZwitterion]^+$ (n = 1, 2) (Figure 11). It was noted, that at increased emitter temperatures, the intensities of the cluster ion peaks decreased considerably.

In general, the spectra for each of the isomers were dependent upon the emitter temperature. At low to moderate emitter temperatures, the spectra were dominated by peaks

Fig. 9: Major Ions Formed by FDMS of o-, m- and p-N,N,N-trimethylammoniophenolate.



$C^+ I^-$

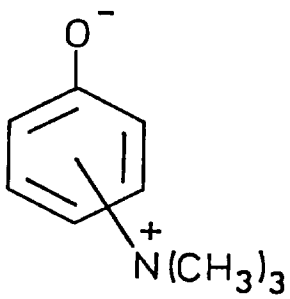
Fig. 10: Structures Attributed to the Major FD Ions.

a) zwitterion

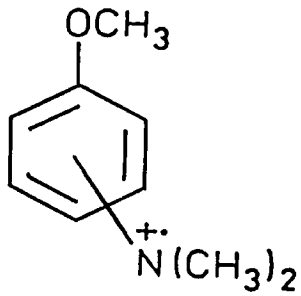
b) $[C-1]^{+\cdot}$

c) $[C+14]^{+}$

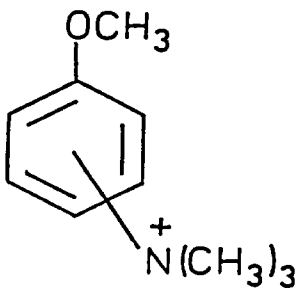
d) $[C-15]^{+\cdot}$



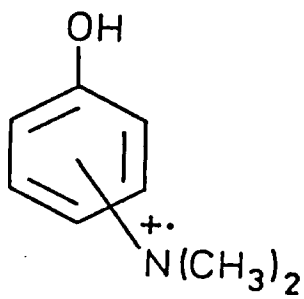
a)



b)

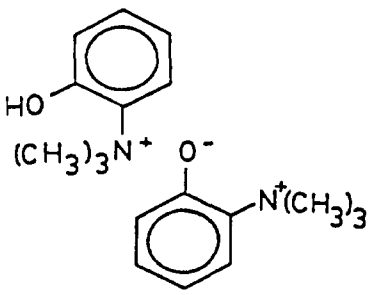


c)

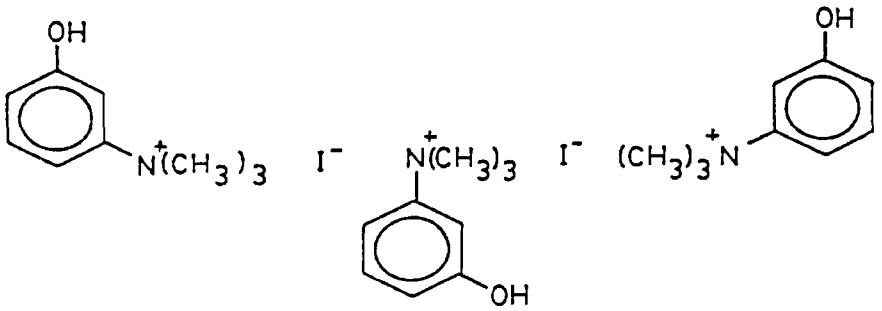


d)

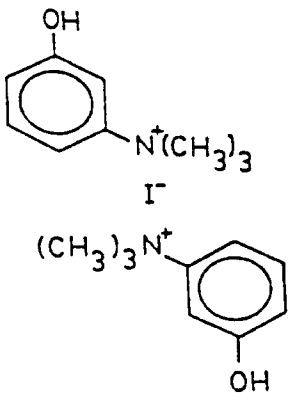
Fig. 11: Cluster Ions Formed by FDMS of N,N,N-trimethyl-ammoniophenolate Salts.



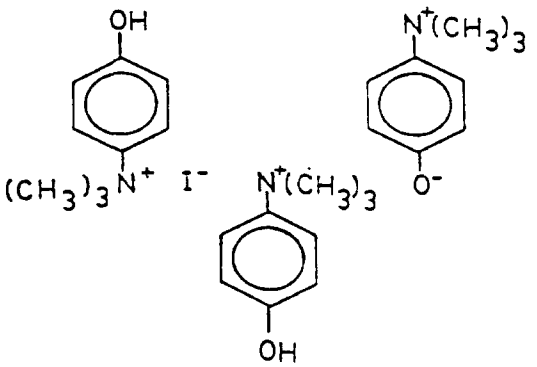
m/z = 303
$(2C - H)^+$



m/z = 710
719 (d_9)
728 (d_{18})
737 (d_{27})



m/z = 431
$(2C + I^-)^+$



m/z = 582
$[(2C + I^-) + \text{zwitterion}]^+$

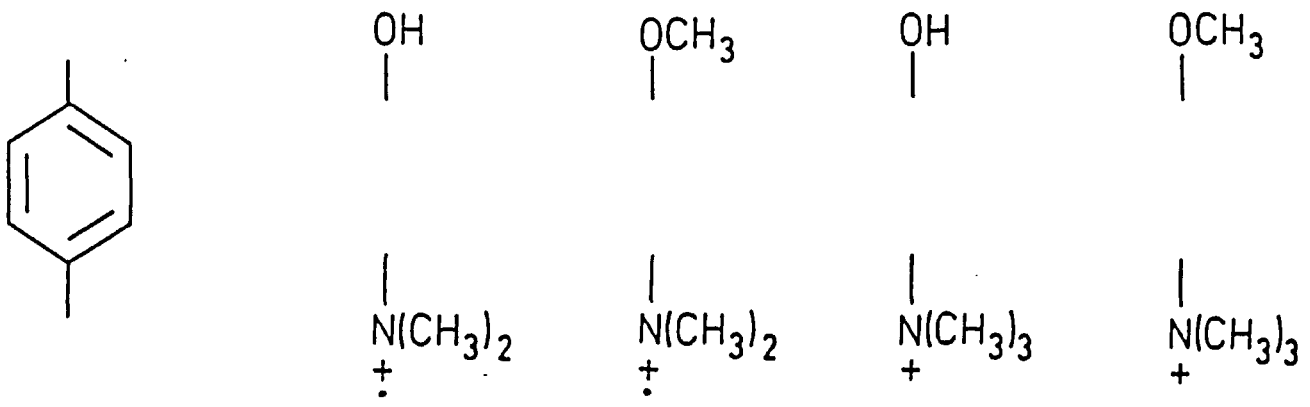
corresponding to $[C]^+$ or $[C-1]^{+\cdot}$. Peaks resulting from the products of transmethylation were observed, as well as the cluster ions. As the emitter temperature was increased, one observed a decrease in the intensities of these peaks, and an increase in the intensities of the peaks corresponding to the demethylation products, i.e. $[C-CH_3]^{+\cdot}$.

It is clear from the results of the study, that isomeric structure plays an important role in the outcome of the FD analyses. More specifically, the FD spectra for the para- isomer are characterized by base peaks corresponding to the intact cation at m/z = 152 (m/z =161 for the d-9 analog) (Figure 12). In some instances, peaks were observed which corresponded to the tetramethyl species. This would imply some modest degree of intermolecular transmethylation. As well, minor peaks were observed at m/z = 151 and m/z = 160 (d-9) which indicate the presence of the methyl ether.

FD analysis of a homogeneous mixture of the para- isomer and its d-9 analog yielded no conclusive results regarding the nature of the methyl transfer occuring. The results (Figure 13) show major peaks corresponding to the intact cations (m/z = 152; 161 (d-9)) at low ehc values, and the demethylated species (m/z = 137; 143 (d-6)) at high emitter temperatures. No peaks were observed which correspond to the mixed methyl transfer products. One would have expected the occurence of peaks corresponding to the mixed tetramethyl species at m/z = 169 and m/z = 175. However, these species were not observed during the analyses of the para- and meta- isomers. This may imply a

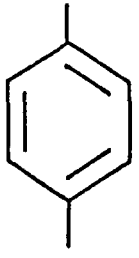
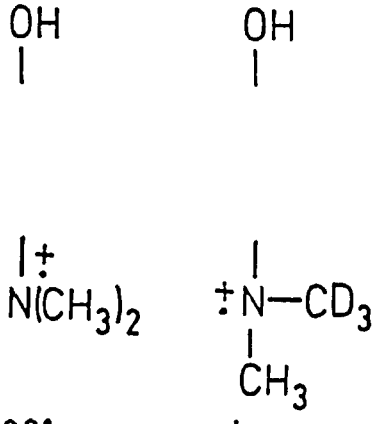
Fig. 12: FDMS Data for p-N,N,N-trimethylammoniophenolate and its d-9 Analog.

ex d0	137	151	152	166
ex d9	143	160	161	178



FD	minor	minor	100%	trace?
FAB	minor	trace	100%	trace

Fig. 13: FDMS Data for a 1:1 Mixture (d-0:d-9) of p-N,N,N-trimethylammoniophenolate.

d0/d9	137	140	143	152	161
					
FD	100%	minor	major	major	major
FAB	minor	NP	minor	major	100%

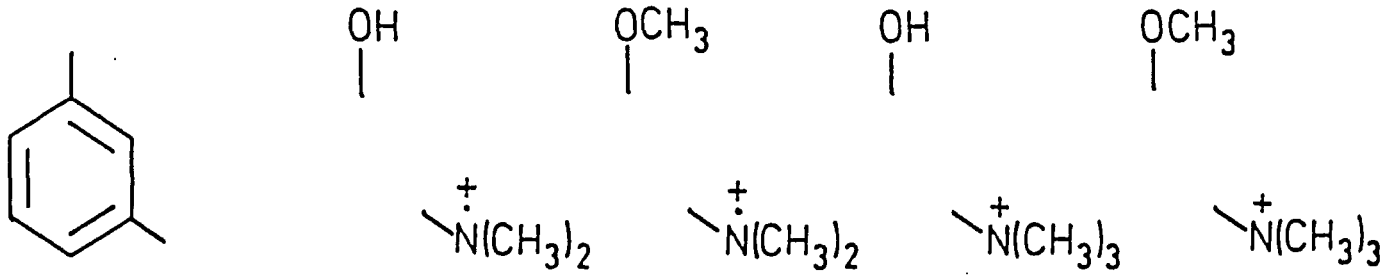
segregation of the d-0 and d-9 species perhaps arising through dimerization between cations having the same composition, allowing formation of only the d-0 and d-12 transmethylation products.

At low ehc values, the meta- isomer exhibited similar results compared to the para- isomer (Figure 14). The spectra were dominated by the intact cation peaks. As well, peaks were observed at $[C+14]^+$ and $[C(d-9)+18]^+$ indicating rearrangement to the tetramethyl species. As the emitter temperature was increased, one observed a substantial decrease in the $[C]^+$ and $[C+CH_3]^+$ peak accompanied by increases in the intensities of the $[C-15]^{+\cdot}$ and $[C-1]^{+\cdot}$ peaks. This implies that demethylation is the predominating reaction at higher emitter temperatures. These data may provide evidence that the peak corresponding to the demethylated species had participated in the methyl transfer process.

FDMS analysis of an equimolar mixture of the meta- isomer and its d-9 analog yielded considerably more information than that observed for the para- isomer (Figure 15). As was shown with the individual isomers, the spectra are dominated by the intact cations at m/z = 152 and 161 (d-9). As well, the tetramethyl species (m/z = 166; 178 (d-12)) were observed at low to moderate ehc values. It should be noted that the demethylated species (m/z =137; 143 (d-6)) were not observed. However, as the ehc values were increased, one observed an increase in the intensities of the peaks corresponding to the demethylated species, to the extent that they dominated the spectra.

Fig. 14: FDMS Data for m-N,N,N-trimethylammoniophenolate and its d-9 Analog.

ex d0	137	151	152	166
ex d9	143	160	161	178



FD	major	trace?	100%	trace
FAB	minor	trace	100%	minor

Fig.15: FDMS Data for a 1:1 Mixture (d-0:d-9) of m-N,N,N-trimethylammoniophenolate.

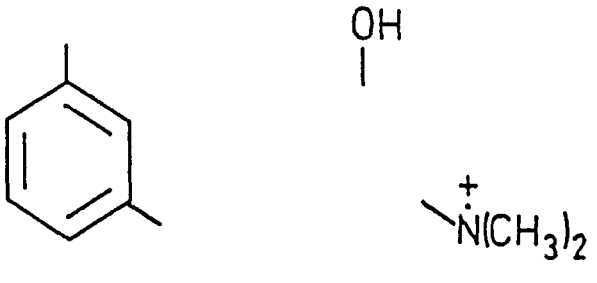
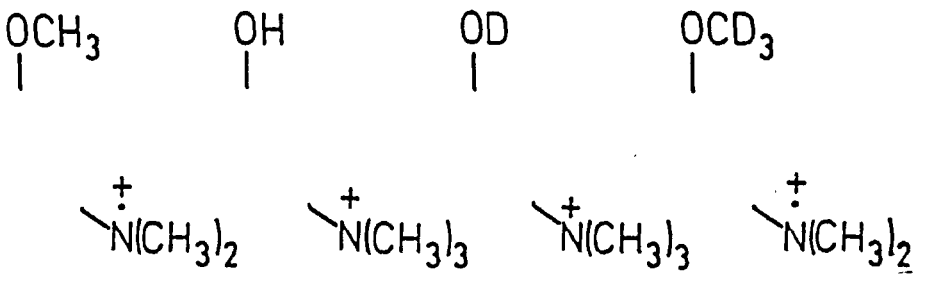
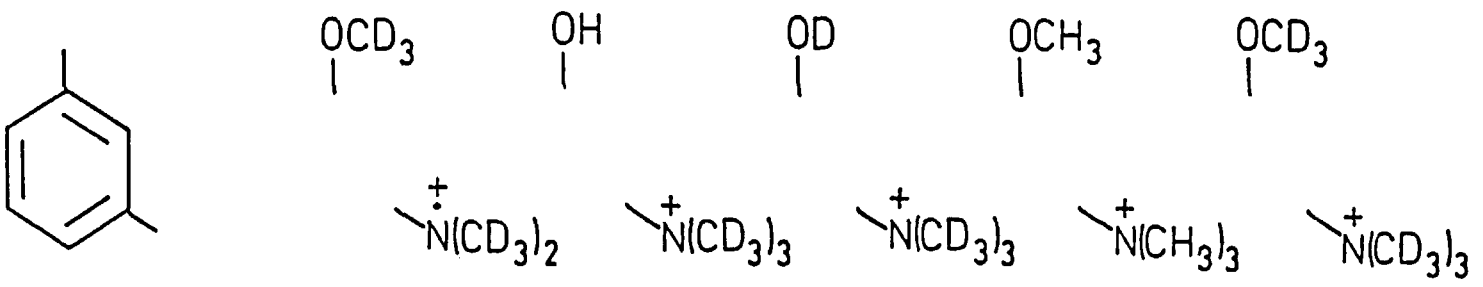
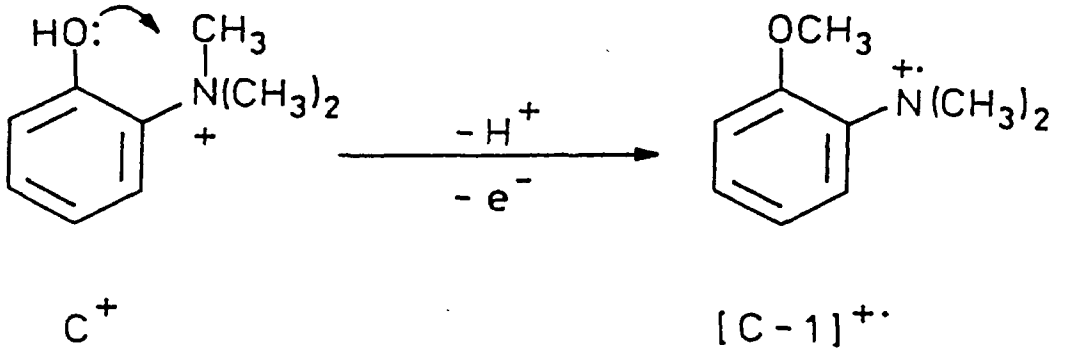
d0/d9	137/143	151	152	153	154
					
FD	NP	minor	major	major	minor
FAB	minor	minor	100%	minor	NP

Fig. 15 (cont.): FDMS Data for a 1:1 Mixture (d-0:d-9) of m-N,N,N-trimethylammoniophenolate.

d0/d9	160	161	162	166	178
					
FD	minor	100%	major	major	major
FAB	minor	major	NP	minor	minor

Increases in the intensities of the peaks corresponding to $[C-H]^{+\cdot}$ were also observed at higher anode temperatures, while the intensities of the $[C]^{+}$ and $[C+14]^{+}/[C(d-9)+18]^{+}$ peaks diminished. It is interesting to consider the occurrence of a peak at m/z = 154 implying intermolecular reactivity, i.e., one of the expected mixed products. An interesting peak observed during the FD analysis of the meta- isomer was that presumed to arise from the exchange of deuterium at the phenolic oxygen, yielding m/z = 153 and m/z = 162 (d-10).

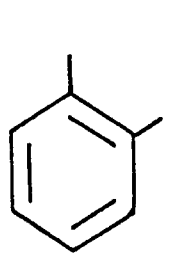
The ortho- isomer exhibited FD behaviour markedly different from that observed for the other isomers (Figure 16). At low ehc values, although the $[C]^{+}$ peak was present, a peak corresponding to $[C-1]^{+\cdot}$ was found to dominate the spectra. The structure to which this peak is assigned is the zwitterionic species as suggested by the previous EI studies [29, 30]. However, the intensity of this peak may be due in part, to an intramolecular methyl transfer and ionization (shown here on N).

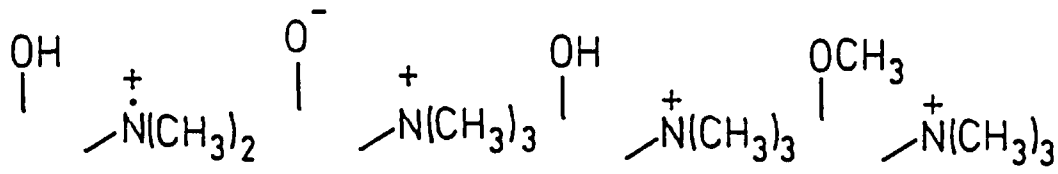


C^{+} $[C-1]^{+\cdot}$

Fig. 16: FDMS Data for o-N,N,N-trimethylammoniophenolate and its d-9 Analog.

ex d0	137	151	152	166
ex d9	143	160	161	178





FD	100%	major	minor	NP
FAB	minor	trace	100%	NP

A likely mechanism for this rearrangement involves a nucleophilic attack by the phenolic oxygen on an N-methyl, accompanied by the loss of the hydroxyl proton. It must be noted that at no emitter temperatures were any peaks corresponding to a tetramethyl species observed. This may provide evidence that the mechanism for the methyl transfer is intramolecular, as compared to the behaviour of the meta- and para- isomers whose FD spectra show evidence of intermolecular rearrangement.

Analysis of an equimolar mixture of the ortho- isomer with its d-9 analog, under FD conditions yields further evidence in favour of direct desorption of the zwitterion (Figure 17). However, intramolecular methyl transfer cannot be completely ruled out. The peaks corresponding to $[C-H]^{+\cdot}$ (m/z = 151; 160 (d-9)) dominate the spectra, and no mixed methyl transfer products (m/z = 154, 157) were observed.

The common feature among the spectra obtained for each isomer was the predominance of the demethylated species, $[C-15]^{+\cdot}$ (m/z = 137; 143 (d-6)) at high emitter temperatures (> 24 mA). As illustrated in Figure 18, the base peak at low emitter temperatures corresponded to $[C-H]^{+\cdot}$ for the ortho- isomer and $[C]^{+}$ for the meta- and para- isomers. However, at high emitter temperatures, the base peak in each spectrum is $[C-CH_3]^{+\cdot}$ (m/z = 137; 143 (d-6)). At this point, one may be observing attack by the anion yielding the dealkylated species, as might be expected at higher temperatures [18].

Fig. 17: FDMS Data for a 1:1 Mixture (d-0:d-9) of o-N,N,N-trimethylammoniophenolate.

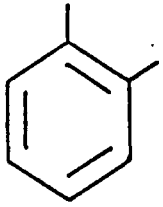
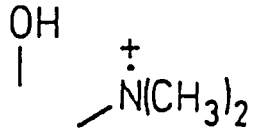
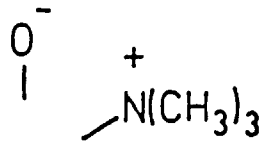
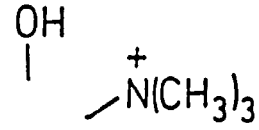
	d0/d9	137/143	151	152
				
FD		minor	100%	minor
FAB		minor	trace	major

Fig. 17(cont.): FDMS Data for a 1:1 Mixture (d-0:d-9) of o-N,N,N-trimethylammoniophenolate.

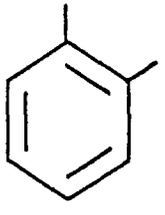
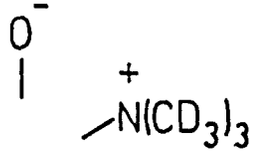
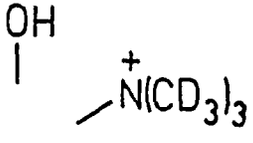
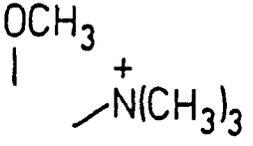
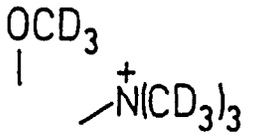
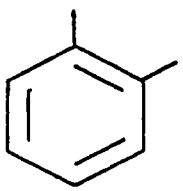
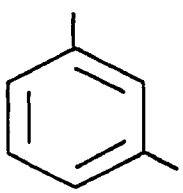
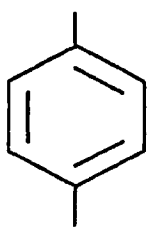
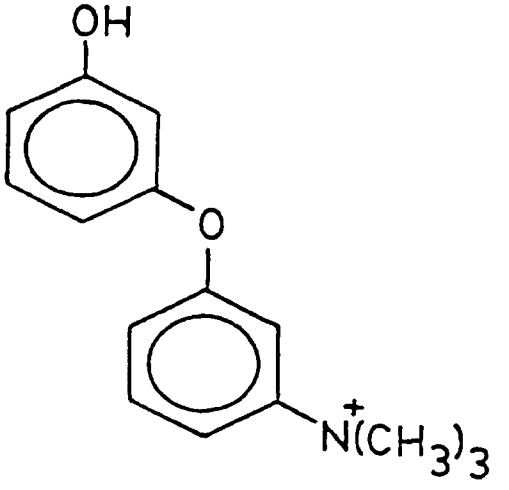
d0/d9	160	161	166	178
				
FD	major	minor	NP	NP
FAB	minor	100%	trace	trace

Fig. 18: Base Peaks Observed at 21 mA and 24 mA.

COMPOUND	BASE PEAK 21 mA	24 mA
	151	137
	152	137
	152	137

One of the surprising artifacts observed during the FD analyses of these compounds was a peak at m/z = 244; 253 (d-9). This peak is presumed to arise from a reaction between two cations. A possible structure accounting for this peak is illustrated below. However, the mechanism resulting in this product has not been studied.



m/z = 244; 253 (d_9)

For the purpose of comparison, these experiments were repeated using fast atom bombardment mass spectrometry (FABMS). In all cases, the base peaks observed (not including matrix related peaks) were those corresponding to the intact cations. Peaks corresponding to the rearrangement products were observed only at trace levels, as compared to the analogous results under FD conditions.

From the data, one might predict similar behaviour under FD conditions for compounds having comparable functional groups. The positions of the two reacting groups with respect to each other may have an effect on the type of species formed

in FDMS at low to moderate emitter temperatures. At increased ehc values, the behaviours of these types of compounds are dictated by temperature rather than structure. The dominant decomposition being dealkylation of the quaternary ammonium substituent.

CHAPTER VI

CONCLUSIONS

The behaviour of the isomeric N,N,N-trimethylammoniophenolates under FD conditions indicates a positional effect dictating the ions observed. The ortho- isomer exhibits the loss of a proton, presumably from the phenolic oxygen. It is difficult to determine if this species is the product of direct evaporation of the zwitterion or of intramolecular transmethylation. One might consider further studies on these compounds such as FD-collision induced dissociation (CID) experiments. The results from this type of study may indicate the identity of the $[C-1]^{+\cdot}$ peak based on the daughter ion spectra. The meta- and para- isomers exhibit the intact cation as the base peak, with less intense peaks indicating a modest degree of intermolecular methyl transfer.

The FD spectra observed for all of the isomers studied are dependent upon the ehc. At low to moderate ehc values, the spectra are dominated by either $[C]^{+}$ or $[C-1]^{+\cdot}$ peaks. At higher emitter temperatures, demethylation is the dominating process. This process is presumably caused by attack of the anion on an N-methyl group. However, in some instances evidence was obtained that implied participation by the demethylated species in the methyl transfer process.

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PART II

FIELD DESORPTION MASS SPECTROMETRY OF CYCLODEXTRIN INCLUSION COMPLEXES

CHAPTER I

INTRODUCTION

The general interest in inclusion compounds has increased considerably over the past several decades. Of particular interest is the information regarding non-covalent intermolecular forces derivable from the study of these compounds. They serve as models for studying topochemical reactions and the mode of action of enzymes. Inclusion compounds are also used in the laboratory as ion-exchangers, catalysts and microencapsulators of sensitive, active and aromatic compounds. A large number of inorganic and organic host compounds exist [1], ranging from small molecules such as urea, to larger biological macromolecules such as starch and various enzymes.

The chemistry of the cyclic oligomers known as cyclodextrins, and their inclusion complexes has been documented in several monographs [2-4] and review articles [5-9]. Probably the most important characteristic of cyclodextrins (CDs) is their ability to form inclusion compounds with a wide variety of guest molecules. It is this property which has prompted their use in enzyme modelling [5, 10], stabilization of unstable compounds [11], decreasing the volatility of volatile compounds [12], increasing the solubility of compounds [13] and catalytic applications [14].

Since CDs are ring-shaped, they are suited for the "host-

guest" interactions involved in inclusion complex formation. Inclusion of a "guest" in the hydrophobic cavity of the CD "host" is thought to arise via a space-filling mechanism. The main binding forces in these complexes are thought to be hydrogen bonding, Van der Waals forces and hydrophobic interactions.

These inherent properties and the low volatility of the cyclodextrin molecule itself prompted this study of cyclodextrin inclusion complexes and their potential applications in field desorption mass spectrometry (FDMS).

CHAPTER II

BACKGROUND STUDIES

A. Structure and Nomenclature of Cyclodextrin.

Cyclodextrins (CDs), also known as Schardinger dextrins, cycloamyloses or cycloglucans are composed of D-(+)-glucopyranose units connected by α-(1,4)-linkages, yielding a doughnut shaped molecule. The most common forms of CD are comprised of 6, 7 or 8 glucose units. These forms of CD are labelled α-, β- and γ- respectively. Rings having less than six glucose units are not known to exist, most likely due to steric stress [15].

In general, CDs are torus shaped molecules with all of the glucose units in substantially undistorted C1(D) (chair) conformations (Figure 1) as shown by NMR studies [16, 17]. The cavity of CD is slightly "V" shaped with the secondary hydroxyls (on the C-2 and C-3 carbons of the glucose units) located on the more open rim, while the primary hydroxyls are located on the opposite, less open side of the torus. The bonds are preferentially directed away from the ring opening. However, a certain freedom of rotation allows the primary hydroxyls to partially block one end of the CD cavity. The locations of the primary and secondary hydroxyls give rise to the hydrophilic exterior of the CD molecule. Intramolecular hydrogen bonds exist between the secondary hydroxyl groups of adjacent glucose

Fig. 1: α-, β- and γ-cyclodextrin.

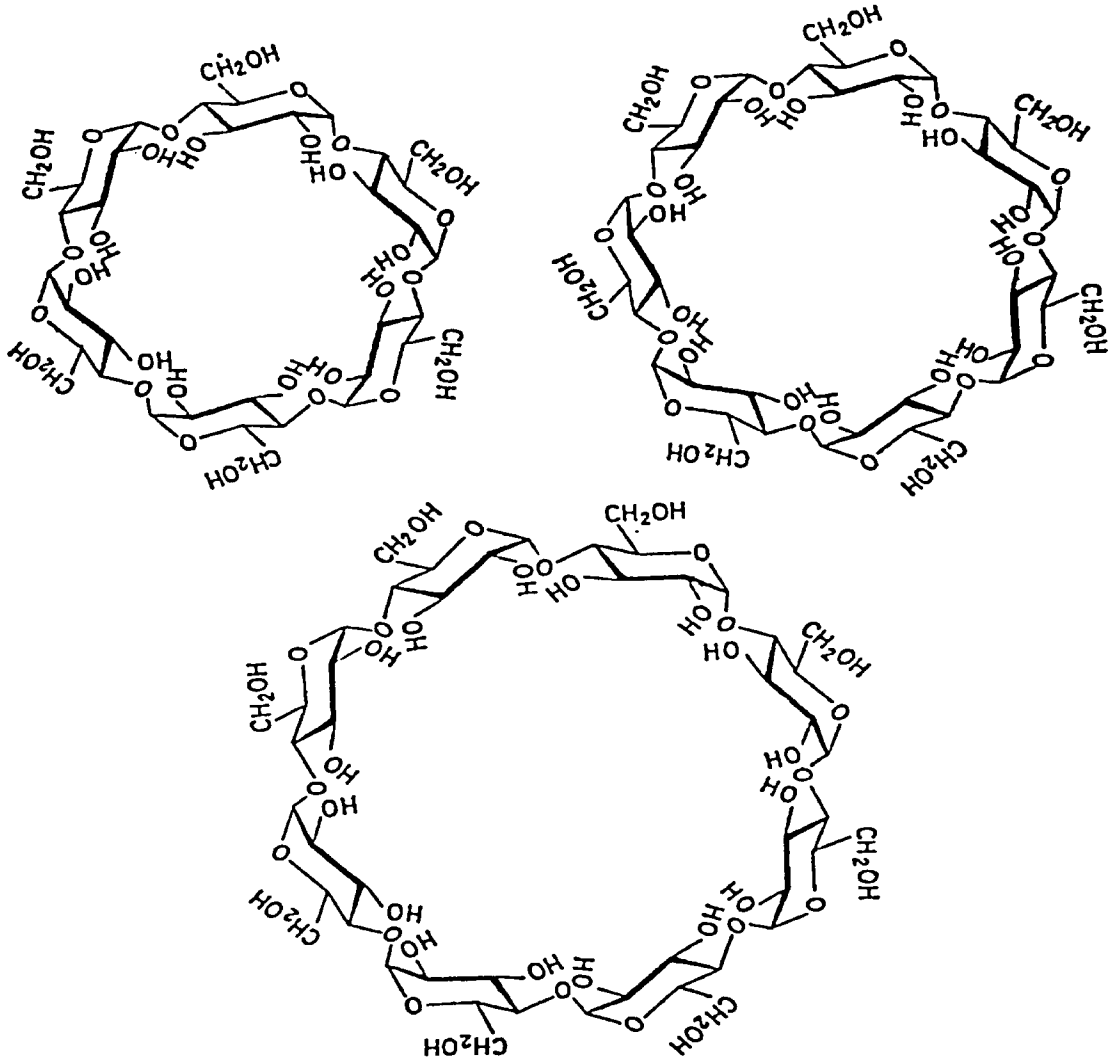


Table 1

Dimensions of Cyclodextrins [2, 9, 18]

CD	Number of Glucose Units	Mol. Weight[a]	Int. Diameter (Å)	Depth (Å)
α-	6	972	4.5	6.7
β-	7	1134	6.0-6.4	7.0
γ-	8	1296	7.5-8.3	7.0

a) Anhydrous weight calculated using nominal masses for isotopes.

units (i.e., O(3)-H....O(2) and O(3)....H-O(2)) stabilizing the CD macrocycle. The interior of the CD is lined with aliphatic C-H groups and ether-like oxygens, yielding a hydrophobic inner cavity. Table 1 outlines some of the dimensions of the more common CDs.

B. Historical Aspects.

CD was first isolated in 1891 by Villiers [19] as a by-product obtained during the degradation of starch. While growing Bacillus amylobacter on a starch medium, he noted a small amount of crystalline material which he characterized by its physical properties, including solubility, etc. The results he obtained were in good agreement with those found by Schardinger in 1904 [20, 21] for β-CD, who first characterized this crystalline material as a cyclic oligosaccharide.

In 1938, Freudenberg, et. al. [22] reported that CDs were constructed from α-(1,4) linked glucose units. The molecular weights of the individual CD molecules were determined much later, however [23-25]. It was French and coworkers [26], as well as Freudenberg, et. al. [22], who developed the procedure for synthesizing the pure CDs by the enzymatic degradation of starch, employing a type of amylase, glycosyl transferase.

Freudenberg, et. al. [25] first recognized the ability of CDs to form inclusion complexes. This capacity for inclusion of a guest molecule by the CD host was studied systematically by Cramer, et. al. [27-29].

For the most part, it was Cramer and coworkers who were initially at the forefront of the development of the applications of CD inclusion complexes. The catalytic action of CDs on some chemical reactions was discovered by Cramer, et. al. in 1953 [30]. It was Cramer as well who first noted the ability of CD to discriminate between and consequently resolve racemic mixtures by selective precipitation [31].

C. The Chemistry of Cyclodextrins.

1. Physical and Chemical Properties.

The cyclodextrins are non-reducing sugars, showing no sensitivity to alkalis. In general, they have no well defined melting points. At temperatures above 200° C the CD molecules decompose to the more basic glucose units. The most striking properties of CDs are their abilities to form inclusion complexes with a wide range of substrate molecules, and their solubilizing and catalytic activity.

The aqueous solubilities of the most common cyclodextrin molecules are outlined in Table 2. β-cyclodextrin shows limited solubility in water as compared to α- and γ-CD, however in bridging solvents such as dimethylsulfoxide (DMSO) and dimethylformamide (DMF), it shows excellent solubility [9]. The temperature dependence of β-CD·12H_2O in water, as determined by refractometry [32] is given by

Table 2

Physical Properties of Cyclodextrins [24].

CD	Water Solubility (g/100ml)	No. of water molecules taken up by cavity	Specific Rotation $[\alpha]_D^{25}$
α-	14.5	6	150.5 ± 0.5
β-	1.85	11	162.5 ± 0.5
γ-	23.2	17	177.4 ± 0.5

$$2.303 \times \log m = (3.88 \pm 0.02) - \frac{3583 \pm 18}{T}$$

where m = the molar ratio of β-CD in a saturated solution and T = the absolute temperature. Generally, in the presence of organic molecules the solubilities of CDs decrease, owing to complex formation.

2. Inclusion Complex Formation.

It appears that the major requirement for inclusion of a guest within the CD cavity is compatibility of dimensions, as all types of guest molecules, ranging from molecular to ionic in nature, are accepted [33, 34]. That is to say, the guest must fit within the dimensions of the cavity. However, the extent to which complex formation occurs is also dependent upon the polarity of the guest molecule [6]. As a rule [4], the included molecules are oriented in such a way that their position achieves the maximum possible contact between the apolar part of the guest and the hydrophobic cavity of the CD host. The hydrophilic portion of the guest remains, as far as possible, at the outer face of the complex ensuring maximum contact with the solvent and the hydroxyl groups on the rim of the CD torus.

Based on X-ray crystallographic analyses [33], the predominant host-guest interactions, as determined by the interatomic distances, are Van der Waals type forces. This

would imply that, in general, specific host-guest interactions do not occur. The forces stabilizing the CD inclusion complex include those described by London dispersion attraction, dipole-dipole interaction and in some cases, hydrogen bonding.

The driving forces, that is, the intermolecular interactions responsible for complex formation have been discussed in a number of articles [2, 8, 27, 35-37]. It has been shown that several forces act simultaneously during complex formation. The extent to which each is involved is generally substrate related. The dependence of inclusion on substrate polarizability indicates [38, 39] that Van der Waals forces are the dominant interactions in complex formation. As well, the formation of hydrogen bonds between the guest and the C-6 hydroxyl groups of CD have been demonstrated crystallographically.

Hydrophobic interactions are also involved in the inclusion process [35, 40, 41]. In order to be included in the CD cavity, the guest must expel water molecules already present within the cavity, as well as strip off its own solvation sphere. The liberated water molecules are subsequently assimilated with the bulk of the solvent. By doing so, they gain degrees of freedom and contribute to the stability of the complex due to an increase in entropy.

It appears that inclusion is largely independent of the chemical properties of the guest molecule. It is suggested that forces inherent in the CD contribute to association. An example of such forces is the water enclosed in the "empty"

CD. The water molecules are in an unfavourable hydrophobic environment and cannot satisfy their hydrogen bonding potentials. As such, these water molecules are deemed "activated" [41, 42]. Upon expulsion of these "activated" water molecules, complex formation is favoured by a gain in entropy as well as a gain in potential energy [38, 43].

Summarizing the above, one can subdivide the process of inclusion into several steps [35]:

i. Approach of the substrate and CD.
ii. Elimination of water molecules from the CD cavity and the immediate vicinity of the portion of the substrate to be included.
iii. Assimilation of the water molecules by the surrounding solvent (gain in entropy).
iv. Interaction between the CD and the substrate as a result of Van der Waals forces and interaction of the substituents of the substrate molecule with groups on the rim or inside the CD.
v. Formation of possible hydrogen bonds.
vi. Reconstitution of the solvated structure around the finished complex.

3. Preparation of Cyclodextrin Inclusion Complexes.

To form inclusion complexes with CDs, the guest molecules apparently need only to satisfy the steric requirements of the

CD cavity [9]. That is, they must fit entirely or at least partially into the CD cavity.

The preparation of inclusion complexes is a simple process [44]. The most commonly employed procedure is to stir an aqueous solution of CD with the guest molecule or its solution. This may be carried out in a common solvent, different but miscible solvents, different immiscible solvents, or no solvent at all.

Preparation of crystalline cyclodextrin inclusion complexes can follow the same format, but the method of choice, is generally dependent upon the properties of the guest components. If the guest is water-soluble, equimolar amounts or up to a ten-fold excess of this substance can be dissolved directly in a concentrated aqueous solution of CD. The inclusion compound should crystallize out immediately or upon removal of the solvent.

Substrates which are not water-soluble can be dissolved in a suitable organic solvent (e.g., ether, chloroform, benzene, etc.), then shaken with or layered with a concentrated aqueous CD solution. Crystals should then form at the solvent interface or as a precipitate.

Large scale preparation of CD inclusion complexes involves a process known as "kneading". The liquid or dissolved guest compound is added to a slurry of the CD and 2 to 5 times as much water. On stirring, the viscosity generally increases yielding a paste. This paste is subsequently dried, washed and powdered. Lyophilization may be substituted for drying,

causing the remaining inclusion complex to retain its form as a finely dispersed powder, which is advantageous in such areas as the pharmaceutical industry, as it facilitates dissolution of the complex.

D. Applications of Cyclodextrin Inclusion Complexes.

1. Catalysis and Enzyme Modelling.

As a consequence of inclusion complex formation, several of a guest compound's physical and chemical properties are altered in a reversible manner. In this section, one considers the effects of these changes and their importance from a practical standpoint, i.e., their application in research, and in the pharmaceutical, food and chemical industries.

The ability of CDs to catalyze certain reactions, in many cases stereospecifically, was first recognized by Cramer [30, 45]. It was this catalytic activity that led to the concept of using CDs as possible enzyme models. Enzyme-substrate interactions are generally established by covalent bonding or by some form of non-covalent bonding (eg. hydrogen bonding, Van der Waals forces, etc.). If one considers CD catalysis, the complex formed often mimics the behaviour of the enzyme-substrate complex. In covalent catalysis, CD establishes a covalent bond with some entering or leaving component of the guest molecule. In the case of non-covalent catalysis, one observes simple inclusion complex catalysis [46]. That is,

this effect may be the result of either a microsolvent effect or a conformational effect [47].

Inclusion complex catalysis shares several characteristics of enzyme catalyzed reactions. These include saturation limits, competitive inhibition and unproductive bonding. From the standpoint of reaction kinetics, inclusion complex formation may be either productive or unproductive. In the case of productive inclusion complex formation, the reactivity of the guest molecule is enhanced. Conversely, unproductive complexation stabilizes the substrate from transformation. As well, inclusion complex catalysis is subject to competitive inhibition in the presence of other complexing agents. In this instance, the inhibition constant is nothing more than the dissociation constant of the CD with the inhibitor.

Cramer, et. al. [48] found that racemic mandelic esters hydrolyzed 1.38 times more rapidly in the presence of CDs with partial stereospecificity. Thus, covalent CD catalysis has features common to enzymic hydrolytic reactions. For example, the substrate binding sites in both cases are apolar cavities, the active sites are aliphatic hydroxyl groups, and in both cases a covalent intermediate is formed.

In the case of ester hydrolysis, if the active center is accomodated within the CD cavity, the rate of hydrolysis decreases. But, if the ester functionality is within the proximity of the hydroxyl groups on the rim of the CD torus, then the rate of hydrolysis increases owing to an "alkoxide" catalytic effect [49]. This implies that orientation plays an

important role in CD catalysis. As an example, Hennrich and Cramer [50] found that the rate of hydrolysis of diarylpyrophosphates was promoted in the presence of CDs by a factor as high as 400. It was found [51] that this reaction proceeds in such a fashion that only half of the pyrophosphate is transferred to the hydroxyl. Thus, it was concluded that in this case, CD plays a dual role. It brings the substrate into a specific position by Van der Waals forces, and cleaves it by covalent catalysis. A list of other reactions catalyzed by CDs is given in Table 3.

2. Industrial Applications

In a more practical sense, CDs have found widespread application in the chemical, pharmaceutical and foodstuff industries. The vast majority of these applications are based on the ability of CDs to micro-encapsulate a particular guest molecule. Micro-encapsulation is the process where each guest molecule is individually surrounded by a CD molecule. This may lead to advantageous changes in the chemical and physical properties of the guest. Cyclodextrins are used extensively as stabilizers of light- or air-sensitive substances. Many insecticides are impractical to use because of their reactivity in the presence of light or air. For example, pyrethins and their synthetic analogs are very toxic toward insects, but harmless toward warm-blooded animals. They occur as yellowish, light-sensitive oils and as such are of limited use.

Table 3

Reactions Catalyzed in the Presence of CDs [9].

Reaction	Substrate	f(max.)[a]	Nature[b]
Ester hydrolysis (asymmetric induction)	Phenyl esters	300	C
	Mandelic esters	1.4	U
Amide hydrolysis	Penicillins	89	C
	N-Acylimidazoles	50	C
	Acetanilides	16	C
Cleavage of phosphoric and phosphonic esters	Pyrophosphates	>200	C
	Diaryl methyl-phosphonates	66	C
Cleavage of carbonates	Aryl carbonates	19	N
Intramolecular acyl migration	2-hydroxymethyl-4-nitrophenyl pivalate	6	N
Decarboxylation	Glyoxylate ion	4	N
	Cyanoacetate ion	44	N
Oxidation	-hydroxyketones	3	N

a) f = maximum acceleration factor relative to the uncatalyzed reaction.

b) C: Mechanism involves a covalent intermediate

N: Mechanism invovles a non-covalent intermediate

U: Unknown mechanism

However, in the presence of CDs, they are precipitated as a yellowish powder which is very stable and easy to handle. The important point is that the pyrethin/CD complex remains toxic to insects long after its application [52]. Similar results were obtained [9] after complexation of the insecticide DDVP (O-(2,2-dichlorovinyl)-O,O-dimethylphosphate) which is unstable in its pure form.

CD has also been found to stabilize certain pharmaceuticals such as benzocaine [53], procaine, atropine, aspirin and phenylbutazone [54], among others. This stabilizing ability is employed extensively in the foodstuff and toiletries industries to stabilize various aromatic substances.

CDs have the ability to modify the chemical activity of certain guest molecules. Reactive substances are protected by inclusion and can be mixed with other substances without risk. Based on this property as well, reactions can be made selective by the inclusion of certain functional groups within the CD cavity. One also has the capacity to promote or supress certain reactions. Nitroglycerine is stabilized upon complexation with β-CD [53]. The crystalline adduct is easily formed into tablets and is no longer explosive.

As previously mentioned, CDs are used for the fixation of very volatile substances. This leads to improved storage and handling capabilities, especially where toxic substances are concerned. This property also allows for the quantity of the volatile substance required to be reduced, since little or no vaporization occurs. Similarly, particular quantities of

aromatic or physiologically active substances can be more accurately measured out.

Certain modifications to the physicochemical properties of guest molecules, upon complexation, can be used to advantage. Substances sparingly soluble in water are made more soluble by the addition of CD. As well, CDs can be used as emulsifying agents. Colours of substances may be altered upon complexation, as inclusion generally produces changes in the spectrum of a particular molecule. Of particular interest in the foodstuff industry is the ability of CDs to supress unpleasant tastes and odours.

An interesting application of CDs is in the agricultural industry. CDs have been shown [9] to improve the yield of grain harvests. Upon treatment of the seeds with an aqueous solution of β-CD, the amylases which degrade starch deposits in the seeds are at least partially inhibited, thus delaying their germination. Although the young plants are at first smaller than those from untreated seeds, they soon grow to produce a 20-45% larger harvest.

CDs have recently been applied in environmental analyses [55] as an alternative "clean-up" technique, in place of typical methods such as liquid-liquid partition or column chromatography. This technique consists of the formation of CD-guest compound inclusion complexes, precipitation of the complexes and release of the guest molecules. This method was applied to a variety of organic trace contaminants. The recoveries ranged from 0-56.3%, using α-CD, 5.9-66.7%, using

β-CD,and 21.9-100%, using γ-CD.

3. Resolution of Racemates.

The ability of CDs to resolve racemic mixtures was first recognized in 1959 [31]. Because CDs are themselves optically active molecules, they form diastereomeric pairs with each included racemate. Consequently, the two components exhibit different physical properties.

The separation of enantiomeric pairs was initially based on preferential precipitation of one of the diastereomers formed. Up to 84% enantiomeric purity was obtained when this process was employed in the separation of racemic isopropyl methyl phosphinates and their ethyl analogs [56]. As well, racemates of chiral sulfinyl compounds (eg. sulfoxides, sulfinates and thiosulfinate S-esters) could be resolved upon inclusion with β-CD, yielding enantiomeric purities up to 68%, usually achieved in one separation step [57-59].

Most recently, cyclodextrins have been applied to the separation of a variety of mixtures, including racemic mixtures, by thin layer [60-63], gas [64-66] and high performance liquid chromatography [67-71] (TLC, GC and HPLC, respectively). In TLC, or more appropriately "pseudophase" TLC, an aqueous solution of CD is employed as a mobile phase modifier. The process is analogous to the formation of micelles in the mobile phase, as CDs bind aromatic molecules in their hydrophobic cavity. The use of CDs in the mobile phase offers

the advantage over other TLC techniques, that pure or mixed solvent systems cannot duplicate both the electrostatic and hydrophobic interactions observed with CDs [72]. CDs also offer the advantage of selectivity on the basis of size, i.e., the diameter of the hydrophobic cavity physically limits the size of molecule which can be complexed. Solutions of CDs, therefore, can act as mobile molecular sieves in TLC [60]. In general, it has been observed [61, 62] that the Rf values of various aromatic species were dependent upon both the structural features of the compounds and the concentration of CD in the mobile phase. In further studies [63], Armstrong, et. al. developed β-CD bonded TLC plates and used them to separate several enantiomers, diastereomers and structural isomers.

Cyclodextrins have been employed as a tool for separating mixtures of isomers of alkyl derivatives of benzene [64, 65]. As well, mixtures of cis- and trans-decalin are resolvable by GC analysis employing columns packed with DMF solutions of β-CD on Celite [66]. Examination of the data led to the conclusion that resolvability of mixtures by this technique was dependent upon the differences in stabilities of the corresponding CD complexes formed. Thus, increased stability of the CD inclusion complex yielded an increased retention time for the guest compound.

Considerable interest has been shown toward chiral stationary phases in HPLC [67], in particular CD bonded stationary phases. Up to eighty compounds have been separated

from their isomers by HPLC using CD bonded columns [69]. These include a variety of structural isomers (including polycyclic aromatic hydrocarbons and prostaglandins), geometric isomers and steroid epimers. As with other chromatographic techniques, the separation mechanism is based on inclusion complex formation. In general, all isomers could be completely resolved except for 3- and 5-methylindole, and o- and p-aminobenzoic acid which were only partially resolved. Among the racemates separated by this technique are the naphthyl esters of various amino acids [70]. In comparison to other chiral stationary phases, CD bonded columns offer advantages in terms of flexibility and versatility. The solvent and experimental requirements of a CD stationary phase are somewhat more relaxed compared to those for other chiral stationary phases. Highly polar mobile phase combinations can be used, whereas more common chiral stationary phases are typically not very stable when used with comparable mobile phases. As well, CD bonded stationary phases can be used in the HPLC separation of other classes of compounds such as structural and geometric isomers, as mentioned previously.

CHAPTER III

OBJECTIVES OF THE STUDY

Cyclodextrins have been shown to form inclusion complexes both in solution and in the solid state, with a wide variety of guest compounds. This phenomenon is caused by a variety of factors, most notably the presence of a hydrophobic inner cavity. An interest in the chemistry of cyclodextrin inclusion complexes has led to the evaluation of their behaviour under FD conditions.

In an effort to apply FDMS to the analysis of compounds having subtle structural differences, the possibility of selective desorption of the guest molecules at the emitter surface was explored. Based on differential effects on their volatility and/or desorption behaviour, an attempt was made to identify closely related compounds. As well, because CD is in itself a chiral molecule, the selective desorption of enantiomers, by virtue of the diastereomeric differences in the complexes, was considered.

The model guest compounds selected for this study included a series of substituted naphthalenes, fluorenes and anthracenes.

CHAPTER IV

EXPERIMENTAL TECHNIQUE AND PREPARATION OF MATERIALS

A. Chemicals.

β- and γ-cyclodextrin, 2-naphthylamine, 2-acetonaphthone, 1- and 2-naphthoic acid, 1-naphthaldehyde, 2-methylnaphthalene, 2- and 9-aminofluorene, 9-hydroxyfluorene, 9-fluorene carboxylic acid, 9-fluorene methanol, 9-anthracene carboxylic acid, 9-anthracenecarbonitrile, R- and S-2,2,2-trifluoro-1-(9-anthryl)ethanol, and 2,2,2-trifluoro-1-(9-anthryl)ethanol (racemic mixture) were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin.

α-cyclodextrin, 9-fluorene methanol, dl-phenylalanine (racemic mixture), and R- and S-(1-naphthyl)ethylamine were purchased from Sigma Chemical Company, St. Louis, Missouri.

1-naphthylamine, 1- and 2-naphthol, and naphthalene were obtained from Fisher Scientific Company, Fair Lawn, New Jersey.

1- and 2-anthramine were purchased from Pfaltz & Bauer Inc., Waterbury, Connecticut.

Anthracene was obtained from J. T. Baker Chemical Company, Phillipsburg, New Jersey.

Fluorene was purchased through Caledon Laboratories Ltd., Georgetown, Ontario.

All chemicals were used as purchased, without further purification. All reagents and solvents were ACS grade, and were used without further purification.

B. Instrumentation.

All samples were analyzed with a Varian MAT Model CH-5 DF mass spectrometer equipped with an EI/FI/FD ion source and operated in the FD mode. The system was interfaced to an INCOS data system. The practical aspects of the mass spectrometric analyses and the operating parameters used are outlined in detail in Part I, Chapter IV. D..

C. Sample Preparation.

Because of the limited solubility of β-CD in water [26] and its insolubility in organic solvents, DMF was chosen as the solvent for sample preparation. The substrates studied, as well as the CDs, all showed excellent solubility in DMF.

Solutions of each substrate and CD were prepared at concentrations of 0.1 M. Samples having varying molar ratios of guest to CD were prepared by simply mixing aliquots from each stock solution to give the desired ratios.

Samples submitted for FDMS analyses included the solutions of the guest molecules alone and solutions containing differing ratios of guest to host. These molar ratios ranged from 1:1 to 40:1 (guest/host).

In two instances, solid inclusion complexes were prepared and analyzed by FDMS. To 10 mL of a 0.01 M aqueous solution of β-CD was added 10 mL of a 0.01 M methanolic solution of the guest compound. The resulting mixture was stirred for 1 hour and refrigerated overnight. The resulting precipitate was filtered with suction and air-dried. Samples of the solid inclusion complexes were dissolved in DMF and submitted for FDMS analysis.

CHAPTER V
EXPERIMENTAL RESULTS AND DISCUSSION

A. FD Behaviour of Cyclodextrin Inclusion Complexes.

Inclusion complex formation appears to be mainly dependent upon the "fit" of the guest compound in the hydrophobic inner cavity of the CD torus. However, substituent and hydrophobic interactions seem to affect the stability of the complex formed. As illustrated in Figure 2, the hydrophobic portion of the guest molecule is positioned within the CD torus and in most cases the substituents if present, remain at the outer rim of the torus where they may interact with the secondary hydroxyls lining the rim.

The intent of this project was to determine any potential applications of CD inclusion complexes in FDMS. The strategy of the study, therefore, was to develop a rapid, simple method of forming the inclusion complexes and submitting them for FDMS analyses in the hope that a slow release mechanism would occur upon gentle heating of the field anode, followed by ionization of the guest molecule (Figure 3).

The FD effects to be considered upon inclusion of the guest molecules were the desorption range, the integrated ion flux and the ehc at which maximum desorption occured. The desorption range is the range of ehc values in which one

Fig. 2: The Process of Inclusion Complex Formation.

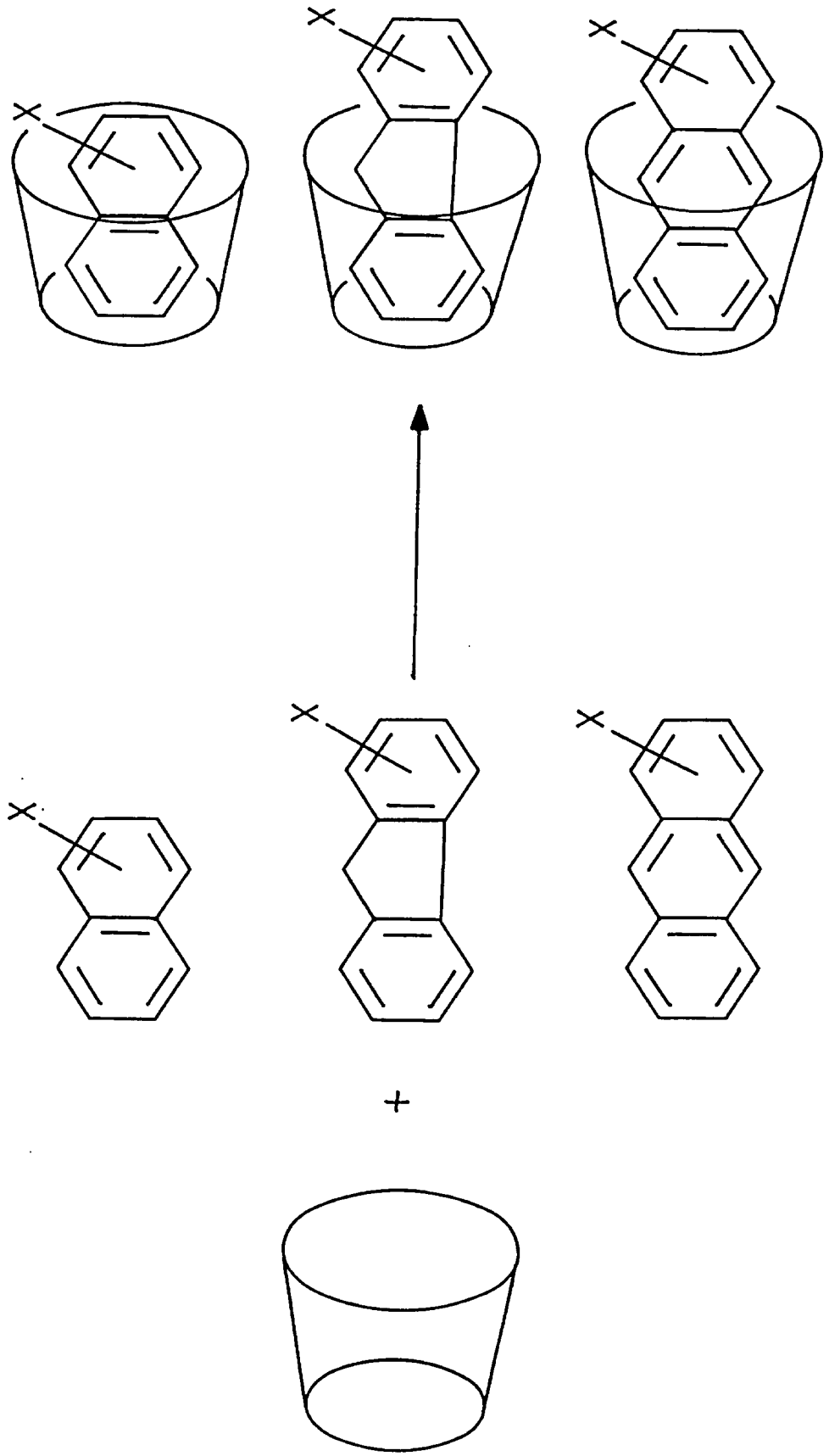
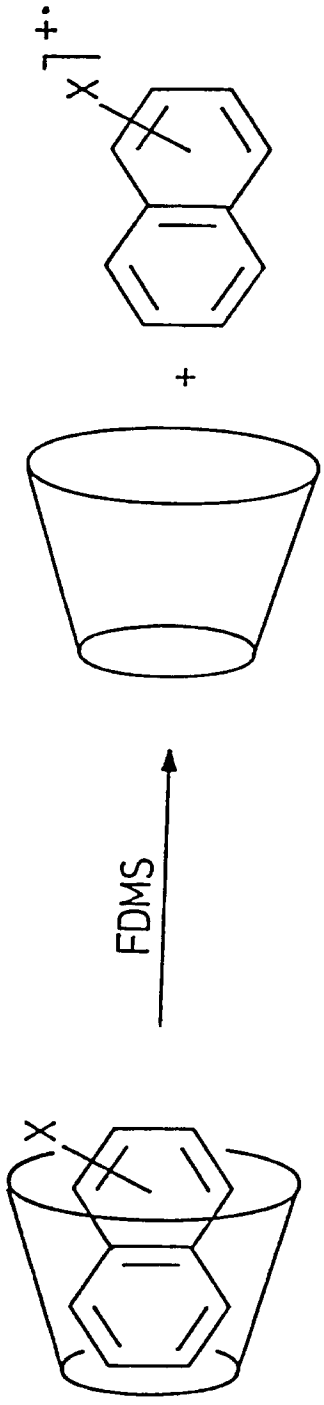


Fig. 3: Proposed FD Behaviour of CD Inclusion Complexes.

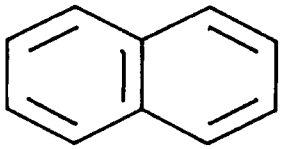


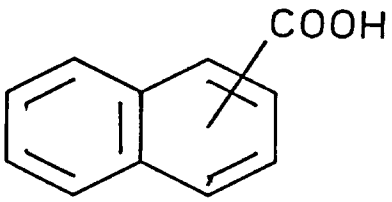
observed peaks corresponding to the molecular ion of the guest molecule. The integrated ion flux is an indication of the sensitivity of the technique toward the guest compound, or of the proportion of the guest detected. Finally, the emitter temperature at which maximum desorption occurs is the point in the desorption profile where the molecular ion shows the greatest intensity. This value is somewhat analogous to the best anode temperature (BAT).

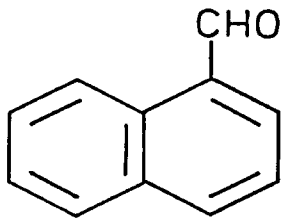
The choice of model guest compounds was largely dependent upon volatility. That is, if inclusion of the guest were to decrease its volatility, one would consider guest molecules whose volatility precludes their analysis by FD techniques. Thus, the first group of guest compounds studied were naphthalene and a series of its substituted derivatives (Figure 4).

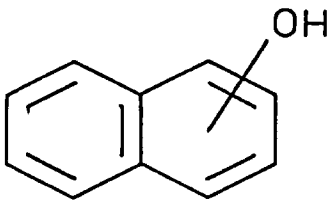
In most cases, the volatilities of the model compounds were such that only trace amounts were observed under FD conditions, at very low or no ehc. Upon complexation with β-CD, one observed distinct increases in the desorption ranges, integrated ion fluxes and ehc values where maximum desorption occured (Figure 5). The desorption envelopes for all of the substituted naphthalenes showed increases ranging from 7.0 mA for 2-methylnaphthalene to 15.0 mA for 2-naphthylamine. The integrated ion fluxes for each compound showed definite increases for each of the substituted naphthalenes. These ranged from a factor of approximately 3 for 2-methylnaphthalene to an increase by a factor of approximately 80 for 2-naphthylamine. One of the most drastic effects of inclusion complex

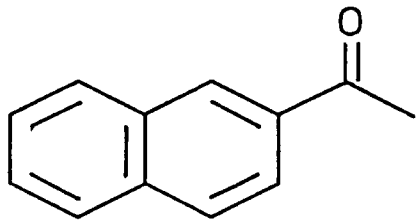
Fig. 4: Substituted Naphthalenes Studied.

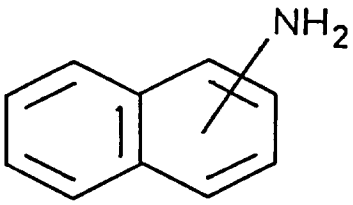












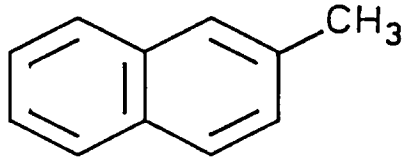
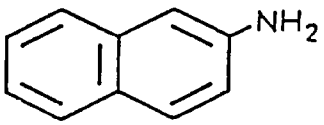
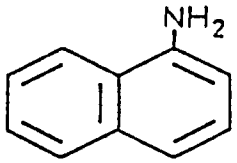
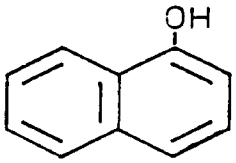
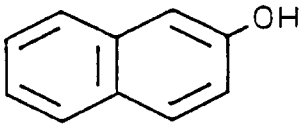
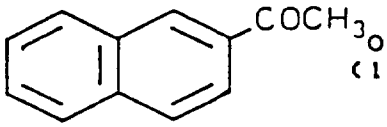
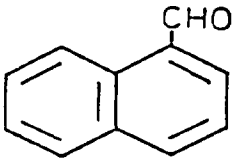
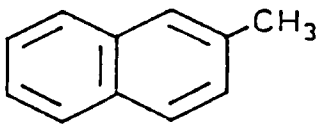


Fig. 5: FD Data for β-CD Inclusion Complexes of Substituted Naphthalenes (4:1 guest/host; values in brackets indicate data for samples containing guest compound only).

COMPOUND	DESORPTION RANGE (mA)	INTEGRATED ION FLUX	ehc @ MAXIMUM DESORPTION (mA)
	0.0 - 23.0 (0.0 - 8.0)	26016 (338)	13.0 (0.0)
	0.0 - 21.0 (0.0)	6696 (29)	18.0 (0.0)
	0.0 - 21.0 (0.0)	6248 (15)	19.0 (0.0)
	0.0 - 20.0 (0.0)	4320 (18)	12.0 (0.0)
	0.0 - 20.0 (1.0 - 8.0)	31136 (130)	12.0 (3.0)
	0.0 - 20.0 (0.0 - 15.0)	7272 (1096)	19.0 (0.0)
	0.0 - 11.0 (0.0 - 4.0)	1066 (391)	0.0 (0.0)

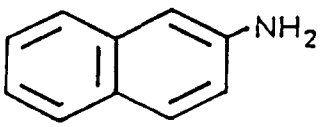
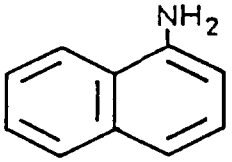
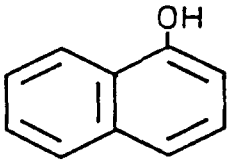
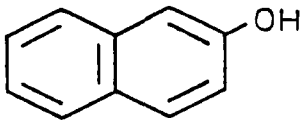
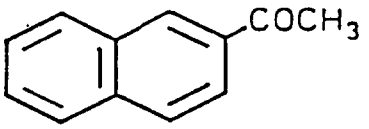
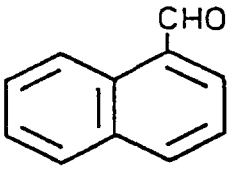
formation was that on the emitter temperature of maximum desorption. In most cases, the value was increased by 12-19 mA.

The implications of the above data were that by simply mixing with a solution of β-CD, a volatile sample is easily analyzed by FDMS techniques. Upon decreasing the guest/host ratio from 4:1 to 1:1, one observes higher desorption temperatures and narrower desorption envelopes (Figure 6). However, one also observes a general decrease in the integrated ion flux for each of the guest compounds. This behaviour might be accounted for in part by day to day fluctuations in instrument sensitivity. A more likely cause for this behaviour, however, has to do with the nature of CD inclusion in the condensed phase.

It has been pointed out [73], that the structure of CD inclusion complexes differ significantly in solution and in the crystalline state. In solution, the guest molecule resides within the CD cavity, and the entire complex exists as a solvated species. In the crystalline state, the guest molecules can be accomodated not only in the CD cavities, but also in the intermolecular cavities of the crystal lattice. Therefore, solid CD complexes are seldom of stoichiometric composition, whereas in solution the molar ratio is 1:1.

Applying this argument to the data for the inclusion complexes of the substitued naphthalenes, one might postulate that in the case of the 1:1 (guest/host) samples, a greater amount of the guest molecules were included in the CD cavit-

Fig. 6: FD Behaviour of β-CD Inclusion Complexes of Substituted Naphthalenes (1:1 guest/host).

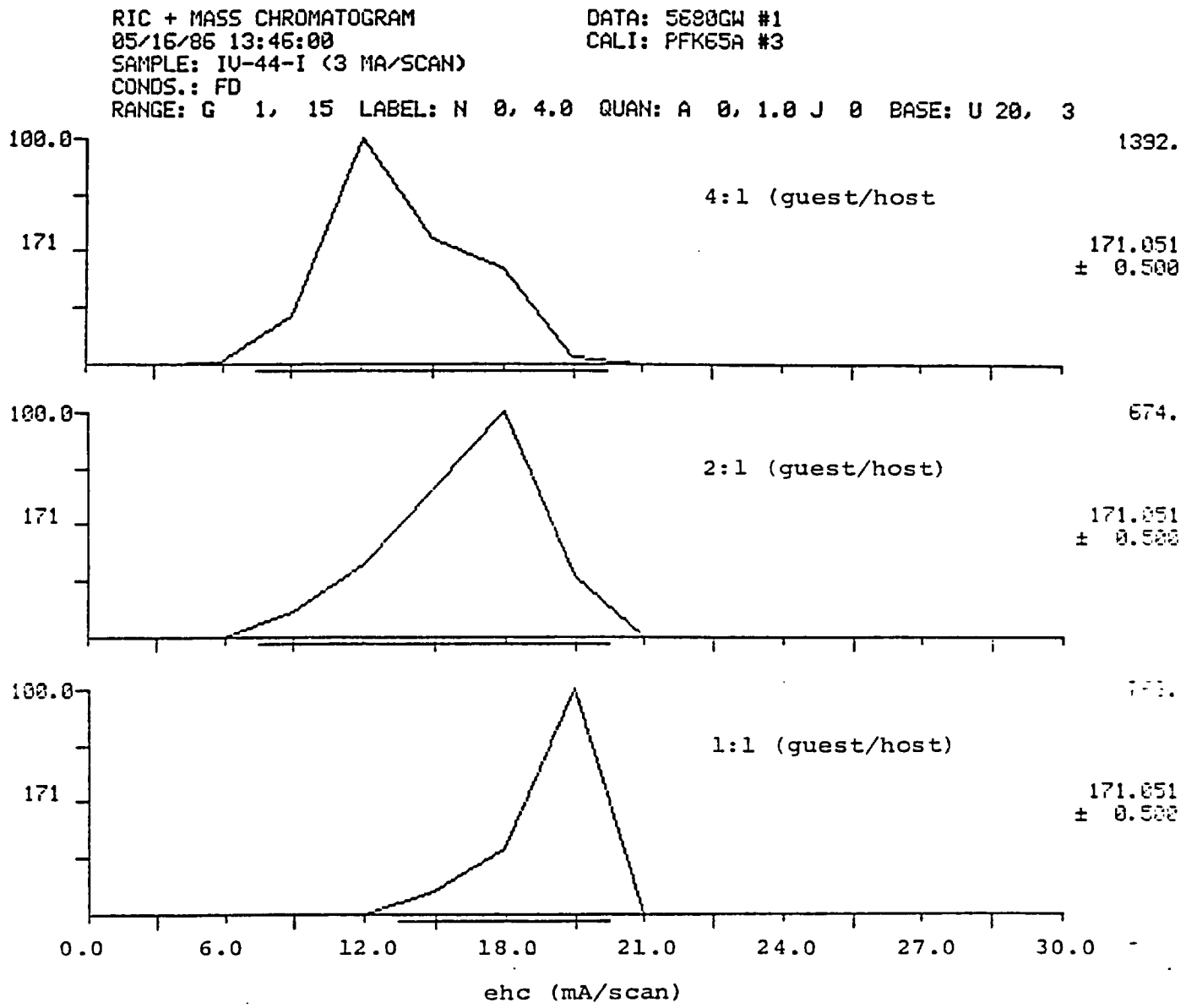
COMPOUND	DESORPTION RANGE (mA)	INTEGRATED ION FLUX	ehc @ MAXIMUM DESORPTION (mA)
	11.0 - 26.0	2344	21.0
	9.0 - 18.0	5832	16.0
	11.0 - 21.0	3452	19.0
	4.0 - 18.0	3076	16.0
	0.0 - 19.0	2312	17.0
	17.0 - 23.0	351	20.0

ies. Presumably, the binding strength of the hydrophobic cavity is greater than that of the intermolecular cavity of the crystal lattice. Thus in the 1:1 case one would expect the observed results for the desorption ranges and desorption maxima. One might also expect a decrease in the integrated ion fluxes as less of the guest compound is released due to tighter binding. In other words, a large portion of the guest molecules in the 4:1 (guest/host) samples are trapped in the intermolecular cavities and as such, are more readily released. As well, due to the limited mass range of the instrument, one cannot account for possible desorption of the intact complex. A large portion of the guest molecules in the 1:1 samples may have been desorbed as the intact complex yielding decreases in the integrated ion flux related to free guest molecules.

What these data indicate is that one can manipulate the desorption behaviour of volatile compounds by varying the molar ratio of CD present in the sample. The desorption envelope can be broadened by decreasing the amount of CD relative to the amount of guest compound. The desorption maximum may be shifted to higher anode temperatures by increasing the amount of CD present, up to the ehc where the CD molecule decomposes (approximately 24-26 mA). This decomposition is indicated by peaks at m/z = 162, 144 and 126, corresponding to the glucose monomer fragment and its dehydration products.

A more dramatic representation of this behaviour is illustrated in Figure 7. The desorption behaviour of R-(1-

Fig. 7: Desorption Profiles for R-(1-naphthyl)ethylamine at Different β-CD Concentrations.



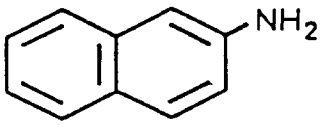
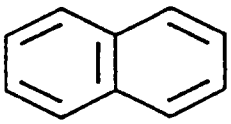
naphthyl)ethylamine in the presence of β-CD at ratios of 4:1, 2:1 and 1:1 (guest/host) clearly indicate that one can effectively manipulate the desorption profile of the guest compound by varying the concentration of β-CD in the mixture.

An exception to the above observations was the unsubstituted naphthalene. Mixing of a solution of naphthalene with a solution of β-CD in DMF showed no effect on its desorption behaviour. This might imply that inclusion complex formation had not occurred completely, or that the stability of the CD inclusion complex is strongly substituent-dependent and thus low in the case of the unsubstituted naphthalene. In other words, complexation may have occured, but the stability of the complex was low and the guest was released rapidly. Therefore there was no discernable difference in desorption behaviours between the complexed and uncomplexed forms.

In an effort to assess whether differing behaviour among these samples was related to "completeness" of complex formation, two of the complexes were isolated and submitted for FDMS analysis (Figure 8).

The isolated naphthalene/β-CD complex desorbed at 19.0 mA with moderate ion abundance, a result in sharp contrast to the behaviour of the compound alone, where its volatility precluded detection of any molecular ion. For 2-naphthylamine, the behaviour of the isolated complex did not differ significantly from that observed for the 4:1 (guest/host) mixture. When a solution of naphthalene was mixed with a solution of β-CD, it is apparent that complexation had not reached completion. This

Fig. 8: FD Behaviour of Isolated β-CD Inclusion Complexes.

COMPOUND	DESORPTION RANGE (mA)	INTEGRATED ION FLUX	ehc @ MAXIMUM DESORPTION (mA)
	0.0 - 21.0	44096	9.0
	0.0 - 22.0	3876	19.0

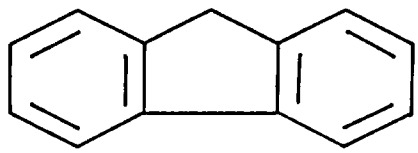
may imply that the degree to which complexation occurs is not only dependent upon hydrophobicity, but also on substituent interactions.

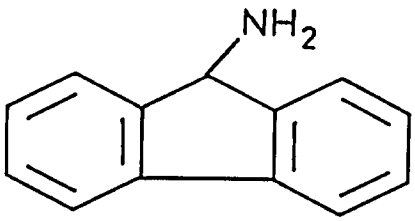
Attempts were made to reproduce the type of behaviour outlined above using α-CD as the host compound. The resulting FD spectra differed very little from those obtained for the guest compounds alone. These results were expected based on observations made using CPK "space-filling" models. The naphthalene portion of the molecule is too bulky to fit in the cavity of α-CD. These results are an indication of the role of "fit" in determining whether complexation occurs or not.

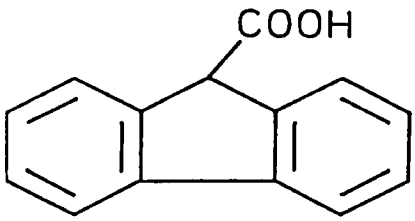
A series of substituted fluorenes was studied under the same conditions to determine any effects on their FD behaviour. The fluorenes studied are illustrated in Figure 9.

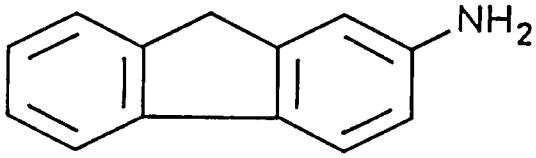
The FD behaviour of the fluorenes was affected in much the same way as the series of substituted naphthalenes upon complexation with β-CD (Figure 10). Alone, only the fluorenes containing amino substituents were observed to be suitable candidates for FDMS analyses, as can be seen from their desorption profiles in the absence of CD. However, upon complexation with β-CD, these compounds exhibited broader desorption envelopes and moderately higher desorption maxima. The integrated ion flux for 9-aminofluorene exhibited a decrease which might be attributed to a decrease in the amount of free guest compound, due to an increase in the binding strength of the 9-aminofluorene/β-CD complex. This might also imply desorption of the intact complex.

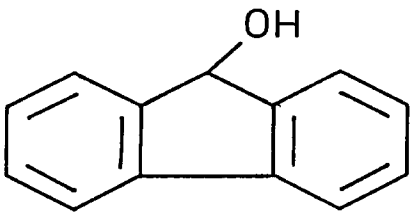
Fig. 9: Substituted Fluorenes Studied.











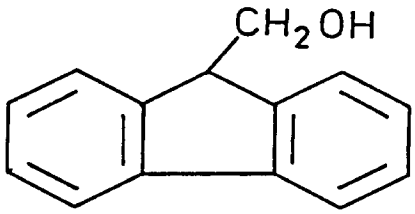
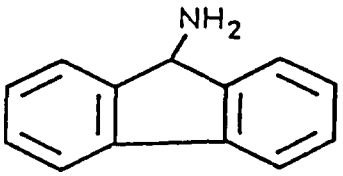
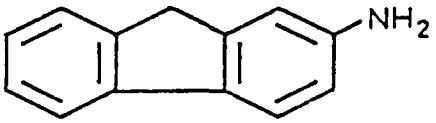
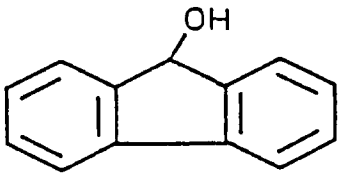
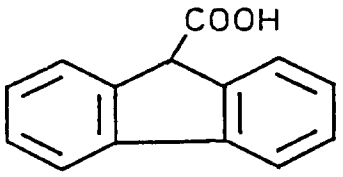


Fig. 10: FD Behaviour of β-CD Inclusion Complexes of Substituted Fluorenes (4:1 guest/host; values in brackets indicate data for samples containing guest compound only).

COMPOUND	DESORPTION RANGE (mA)	INTEGRATED ION FLUX	ehc @ MAXIMUM DESORPTION (mA)
	10.0 - 21.0 (8.0 - 17.0)	12512 (23680)	19.0 (15.0)
	0.0 - 23.0 (0.0 - 9.0)	34624 (37312)	9.0 (7.0)
	0.0 - 21.0 (0.0 - 10.0)	24864 (755)	10.0 (2.0)
	0.0 - 21.0 (0.0 - 5.0)	5088 (135)	18.0 (1.0)

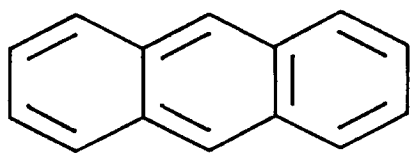
9-hydroxyfluorene and 9-fluorene carboxylic acid both exhibit desorption behaviour very similar to their analogous naphthalene derivatives. That is, increases were observed in all aspects of the desorption profile.

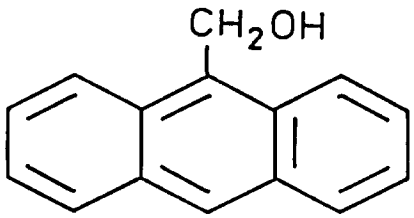
In an effort to compare the effects of complexation with β-CD to those observed with larger CDs, the series of substituted fluorenes were complexed with γ-CD and submitted for FDMS analysis. The results obtained by this experiment were virtually identical to those observed with β-CD. This may indicate that the size of the fluorene moiety is small enough to fit in the β-CD cavity, yet large enough to interact with the larger cavity of the γ-CD.

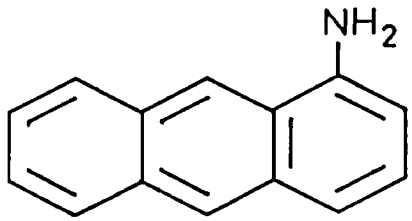
Based on these data, it appeared that the most versatile and practical of the CDs studied was β-CD. Thus, subsequent experiments were carried out using β-CD as the complexing agent. It is readily available commercially and relatively inexpensive. The size of its internal cavity appears to make it the most versatile of the host compounds, in terms of the variety of compounds with which its inner cavity is compatible.

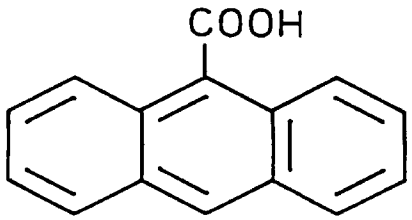
As a final experiment in assessing the effects of CD complex formation on the desorption behaviour of various guest molecules, a series of substituted anthracenes (Figure 11) were complexed with β-CD and submitted for FDMS analysis. As was anticipated, the series of substituted anthracenes exhibited desorption behaviour (Figure 12) similar to that observed with the previously studied compounds, upon complexation. In most

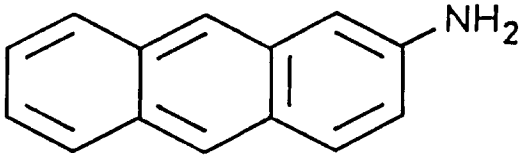
Fig. 11: Substituted Anthracenes Studied.











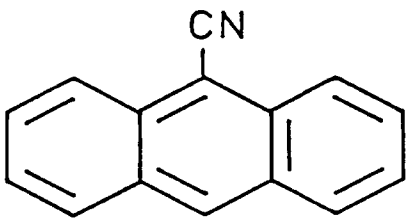
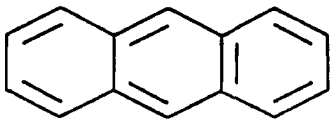
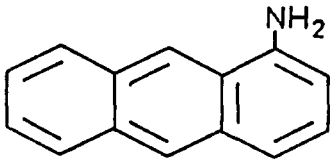
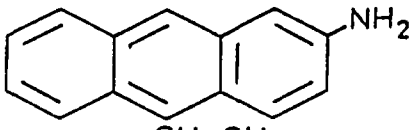
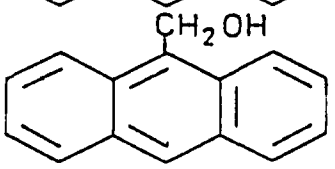
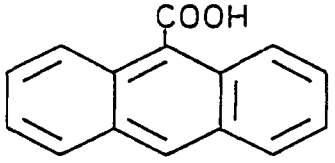
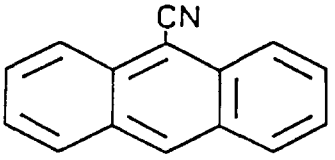


Fig. 12: FD Behaviour of β-CD Inclusion Complexes of Substituted Anthracenes

COMPOUND	RATIO (guest/β-CD)	DESORPTION RANGE (mA)	ehc AT MAXIMUM DESORPTION (mA)
	1:0	0.0 - 23.0	6.0
	1:0.25	0.0 - 21.0	6.0
	1:1	0.0 - 10.0	7.0
	1:0	0.0 - 13.0	6.0
	1:0.25	5.0 - 22.0	12.0
	1:1	17.0 - 23.0	21.0
	1:0	0.0 - 14.0	12.0
	1:0.25	5.0 - 19.0	14.0
	1:1	13.0 - 22.0	19.0
	1:0	0.0 - 13.0	11.0
	1:0.25	0.0 - 20.0	11.0
	1:1	11.0 - 21.0	19.0
	1:0	5.0 - 19.0	14.0
	1:0.25	5.0 - 19.0	13.0
	1:1	10.0 - 22.0	20.0
	1:0	0.0 - 16.0	9.0
	1:0.25	0.0 - 21.0	10.0
	1:1	0.0 - 22.0	13.0

cases, the desorption ranges showed increase, as did the desorption maxima. In general, the integrated ion fluxes showed no change upon inclusion of the guest molecules.

Based on these observations, CD inclusion complex formation decreases the volatility of the guest compound to the extent that it may be analyzed by FDMS techniques. This effect is observed with a number of guest molecules having different sizes and and a variety of functional groups. A more striking example of the potential of CD inclusion complex formation in FDMS is the capacity to manipulate the desorption behaviour of a given guest compound.

B. Separation of Closely Related Compounds by the FDMS Analysis of Their CD Inclusion Complexes.

1. Positional Isomers.

Having shown that inclusion complex formation yields differing desorption behaviour for each of the model guest compounds, an attempt to apply this to the identification of closely related isomeric compounds was made. The positional isomers 1- and 2-naphthylamine, 1- and 2-naphthol and 1- and 2-naphthylene carboxylic acid were studied.

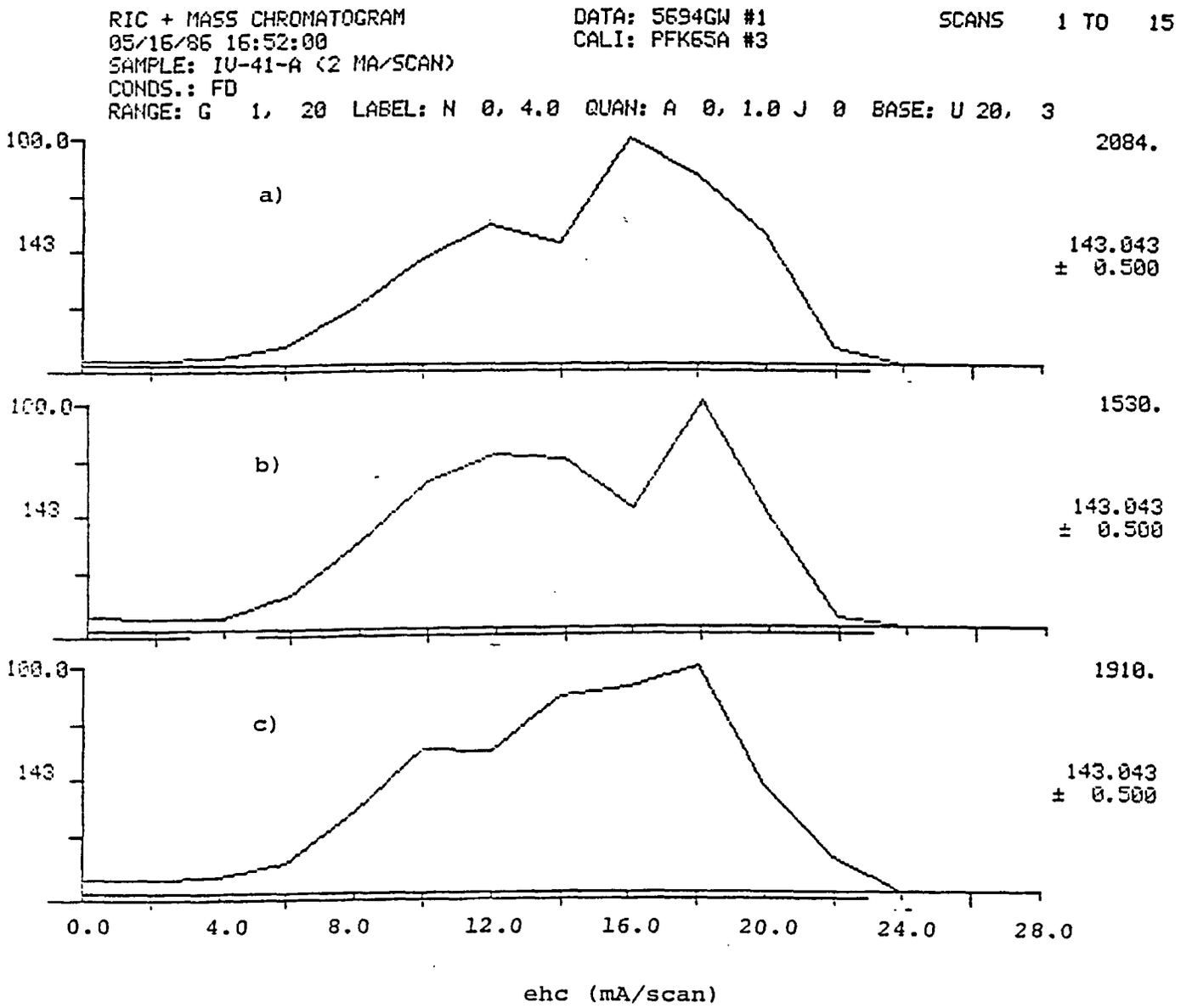
The desorption profile for a mixture of 1- and 2-naphthylamine (Figure 13) is broadened, as compared to that observed for for the complexes of the individual isomers. However, distinct peaks corresponding to any particular isomer could not

Fig. 13: Desorption Profiles for 1- and 2-naphthylamine/β-CD Inclusion Complexes and Their Mixture.

a) 1-naphthylamine

b) 2-naphthylamine

c) 1-, 2-naphthylamine mixture



be attributed with any degree of confidence.

In the case of the 1- and 2-naphthalene carboxylic acid derivatives (Figure 14), two peaks are observed in the desorption profile. Because of the anomalous behaviour of the complexes of the individual isomers, though, one cannot determine the identities of these peaks.

The situation involving 1- and 2-naphthol begins to show separation of the isomers (Figure 15). The 1-isomer exhibits its maximum desorption at approximately 18 mA, as would be expected, based on the previous studies of the complexes of the individual isomers. The desorption maximum of the 2-substituted isomer is observed at approximately 12 mA. When a mixture of the two isomers was complexed and submitted for FDMS analysis, two desorption maxima were observed, corresponding approximately to the maxima observed for the complexes of the individual isomers.

These data imply that selective desorption of positional isomers may be a difficult procedure. It appears that the difference in stability between the inclusion complexes formed between two positional isomers is too small to allow for selective desorption of one isomer over the other.

2. Resolution of Racemic Mixtures.

As previously mentioned, CDs are capable of discriminating between enantiomers [31]. Formation of diastereomeric pairs upon complexation leads to differing changes in the physical

Fig. 14: Desorption Profiles for 1- and 2-naphthalene carboxylic acid/β-CD Inclusion Complexes and Their Mixture.

a) 1-naphthalene carboxylic acid

b) 2-naphthalene carboxylic acid

c) 1-, 2-naphthalene carboxylic acid mixture

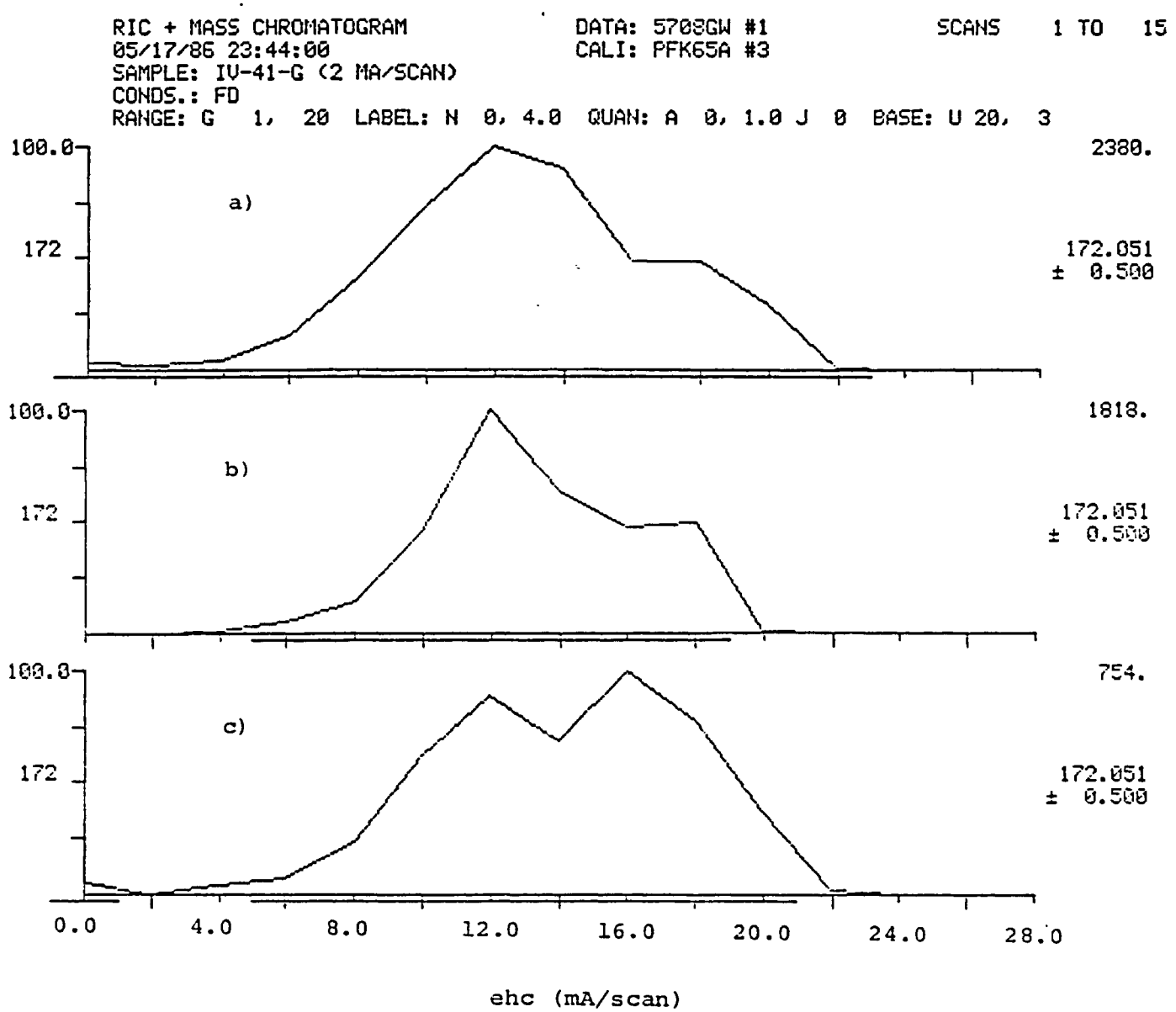
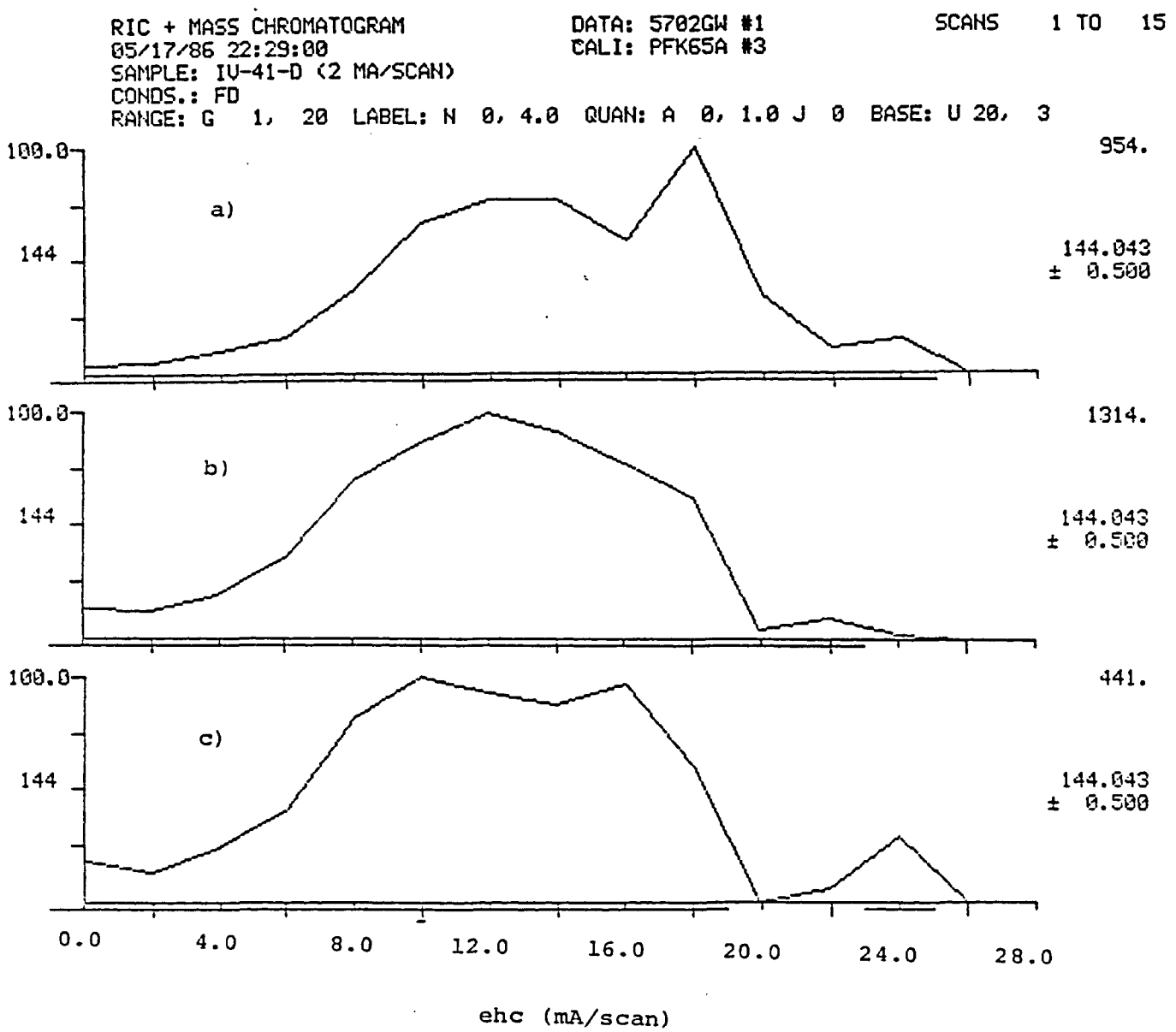


Fig. 15: Desorption Profiles for 1- and 2-naphthol/β-CD Inclusion Complexes and Their Mixture.

a) 1-naphthol

b) 2-naphthol

c) 1-, 2-naphthol mixture



properties of each included component. In an attempt to apply this to FDMS, i.e., selective desorption of the enantiomers by virtue of the diastereomeric differences in their resulting inclusion complexes with β-CD, d,l-phenylalanine was complexed with β-CD and submitted for FDMS analysis. The results of this study proved to be inconclusive. However, it is interesting to note that the host, β-CD diverted the ionization mechanism such that the major ion formed was $[M+H]^+$ rather than $[M]^{+\cdot}$ (Figure 16). This behaviour might be attributed to possible exchange of a proton from one of the secondary hydroxyls lining the outer rim of the CD torus to the negatively charged carboxylate moiety.

A similar attempt was made to distinguish between R- and S-(1-naphthyl)ethylamine. Initial results, using DMF as solvent, showed moderate resolution of the enantiomers (Figure 17). The R-isomer exhibited maximum desorption at approximately 12 mA, while the S-isomer desorbed at approximately 18 mA. A mixture of the R- and S-isomers, when complexed with β-CD yielded two discernible desorption maxima at 12 and 18 mA, corresponding to the maxima observed for the complexes of the individual isomers. Thus, the potential for distinguishing between enantiomers by the FDMS analysis of their CD inclusion complexes is indicated.

When the samples were prepared in a 60% MeOH/water solvent, distinctly different desorption behaviour was observed between the R- and S-isomers (Figure 18). The R-isomer showed maximum desorption at 18 mA and the S-isomer showed maximum

Fig. 16: Ionization Mechanisms for d,l-phenylalanine in the Presence (m/e =166) and Absence (m/e = 165) of β-CD.

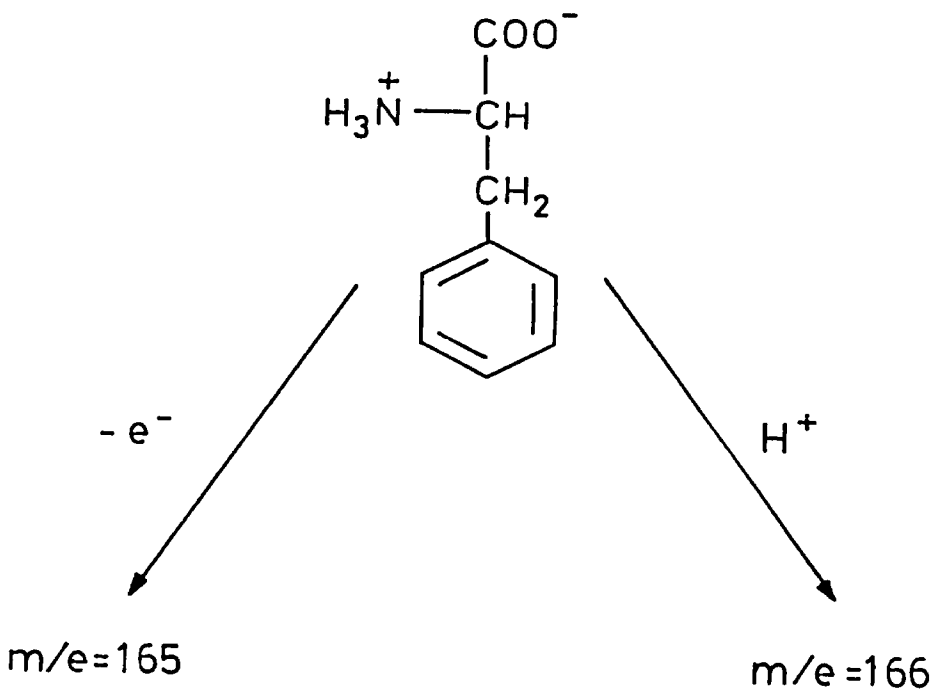


Fig. 17: Desorption Profiles for R- and S-(1-naphthyl)ethylamine Inclusion Complexes with β-CD (2:1 guest/host).

a) R-(1-naphthyl)ethylamine

b) S-(1-naphthyl)ethylamine

c) R-, S-(1-naphthyl)ethylamine mixture

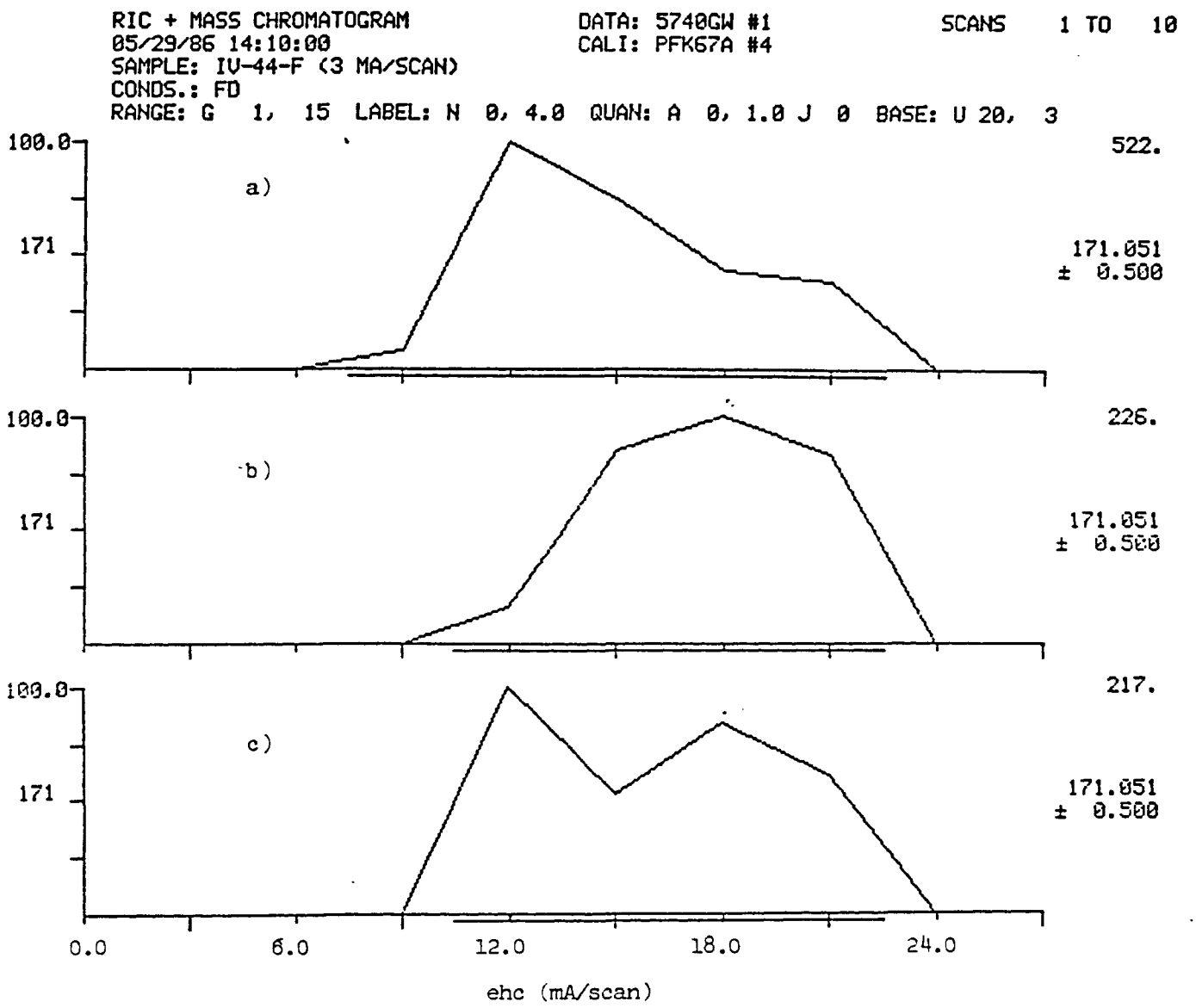
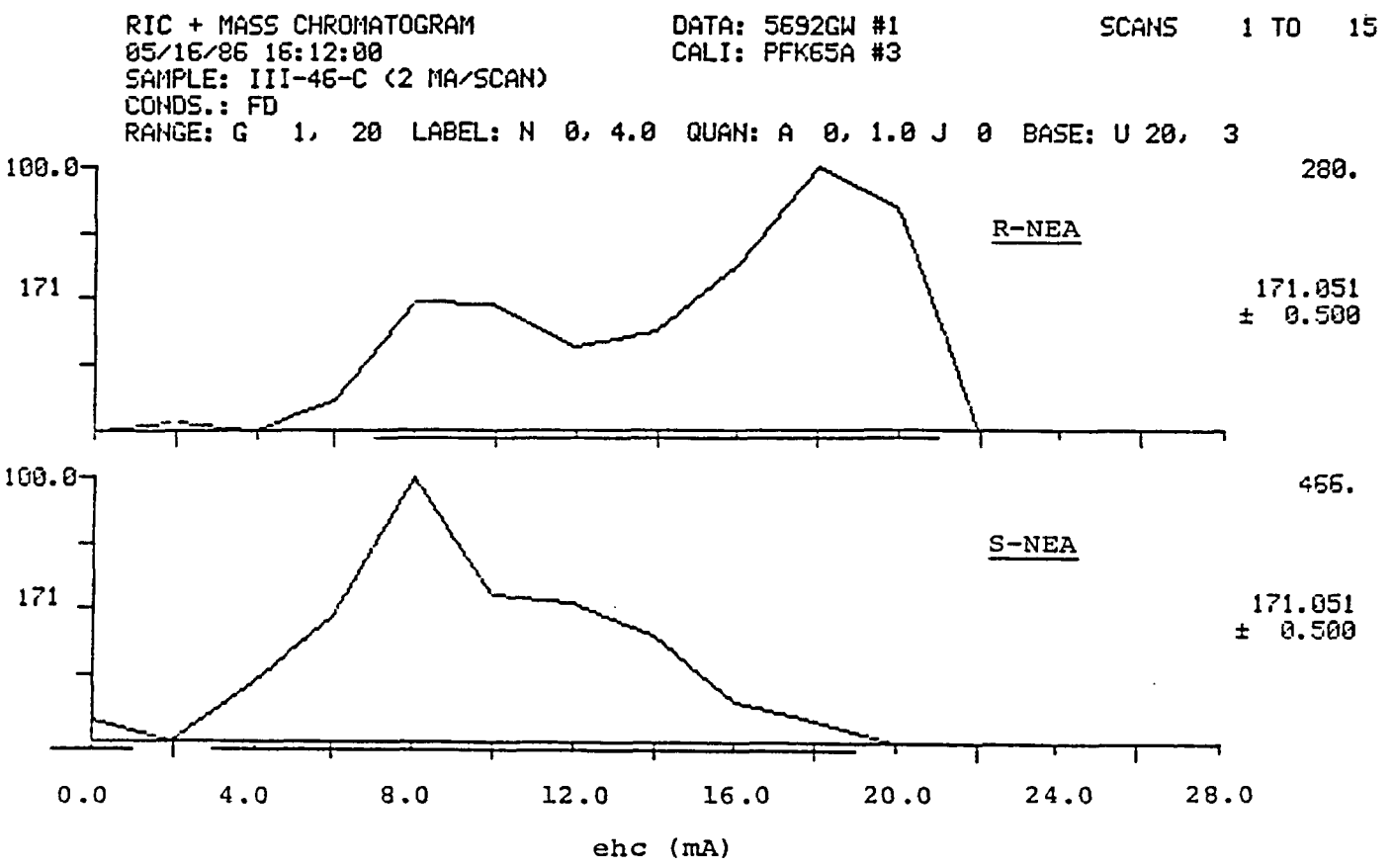


Fig. 18: Desorption Profiles for R- and S-(1-naphthyl)ethylamine Inclusion Complexes with β-CD (2:1 guest/host; 60% methanol/water solvent)

a) R-(1-naphthyl)ethylamine

b) S-(1-naphthyl)ethylamine



desorption at 8 mA. Thus, as well as hydrophobic and substituent effects, a solvent effect is implied, affecting the stabilities of CD inclusion complexes.

By varying the rate of emitter heating, one observes the potential for resolving racemic mixtures, as well as the reproducibility of the ehc for maximum desorption (Figure 19). At an emitter heating rate of 5 mA/scan, one notes that the R-isomer exhibits maximum desorption at 20 mA and the S-isomer at 10 mA. Heating of the emitter at 3 mA/scan yielded desorption maxima at 9 mA and 18 mA for S- and R-(1-naphthyl)ethylamine, respectively. At emitter heating rates of 1 mA/scan, one observes drastic decreases in sensitivity. However, desorption maxima are still discernible at approximately 9 mA, corresponding to S-(1-naphthyl)ethylamine and approximately 16-19 mA, corresponding to R-(1-naphthyl)amine.

As alternative guest compounds, the enantiomers of 2,2,2-trifluoro-1-(9-anthryl)ethanol were complexed with -CD and submitted for FDMS analyses. Samples containing a 4:1 ratio (guest/host) were dissolved in DMF. These complexes showed virtually no differences in their desorption profiles as illustrated in Figure 20. Both enantiomers showed desorption maxima at approximately 10 mA.

Upon changing the solvent species to a 60% methanol/water solution, β-CD inclusion complexes of these compounds showed slight differences in their desorption profiles (Figure 21). The R-isomer yielded a desorption maximum at 8 mA and the S-isomer at 6 mA. The desorption profile for a mixture of the

Fig. 19: Desorption Profiles for R- and S-(1-naphthyl)ethylamine/β-CD Inclusion Complex Mixtures at Varying Rates of Emitter Heating (2:1 guest/host; 60% methanol/water solvent).

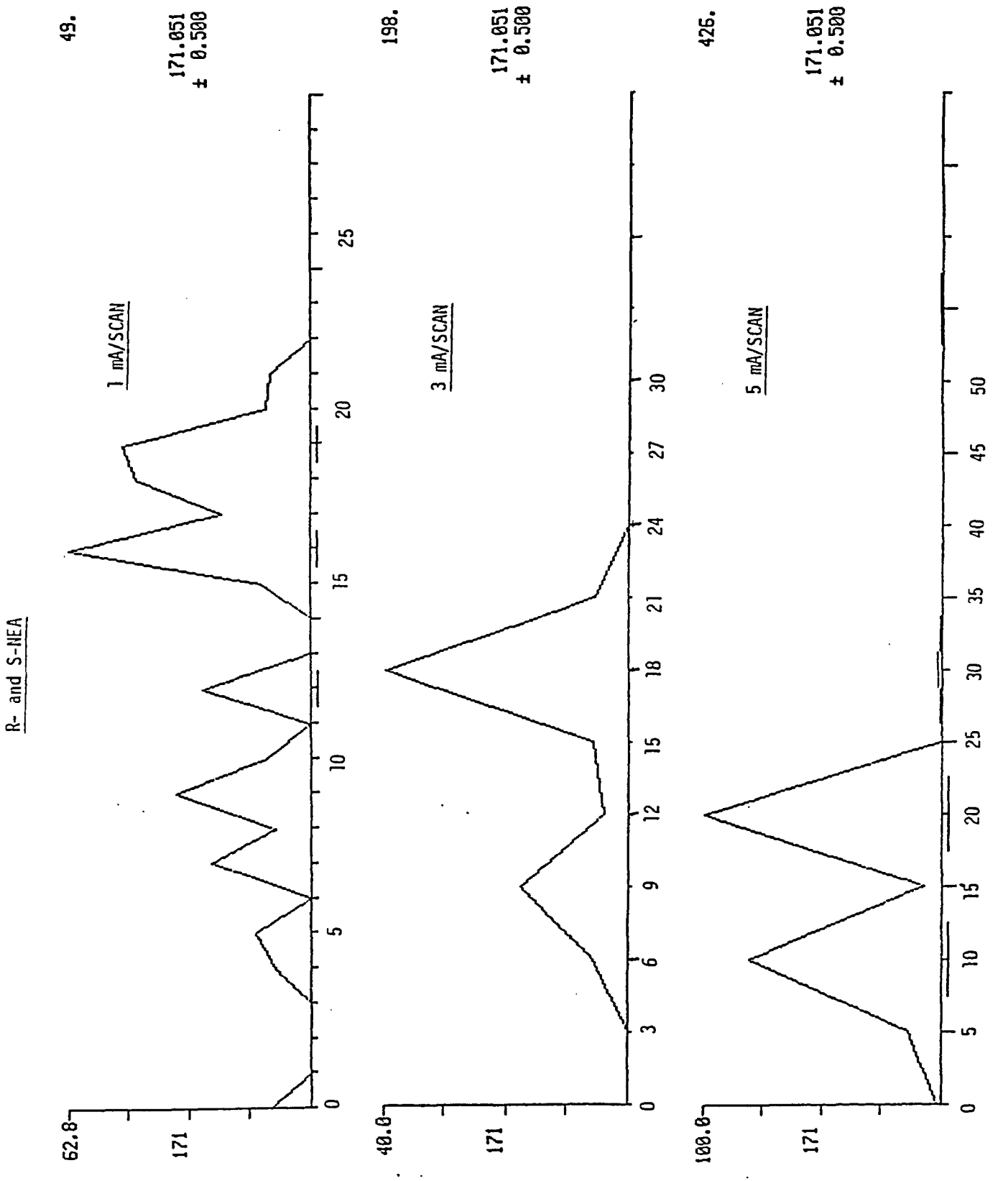


Fig. 20: Desorption Profiles for R- and S-2,2,2-trifluoro-1-(9-anthryl)ethanol/β-CD Inclusion Complexes (4:1 guest/host; DMF solvent).

a) R-2,2,2-trifluoro-1-(9-anthryl)ethanol

b) S-2,2,2-trifluoro-1-(9-anthryl)ethanol

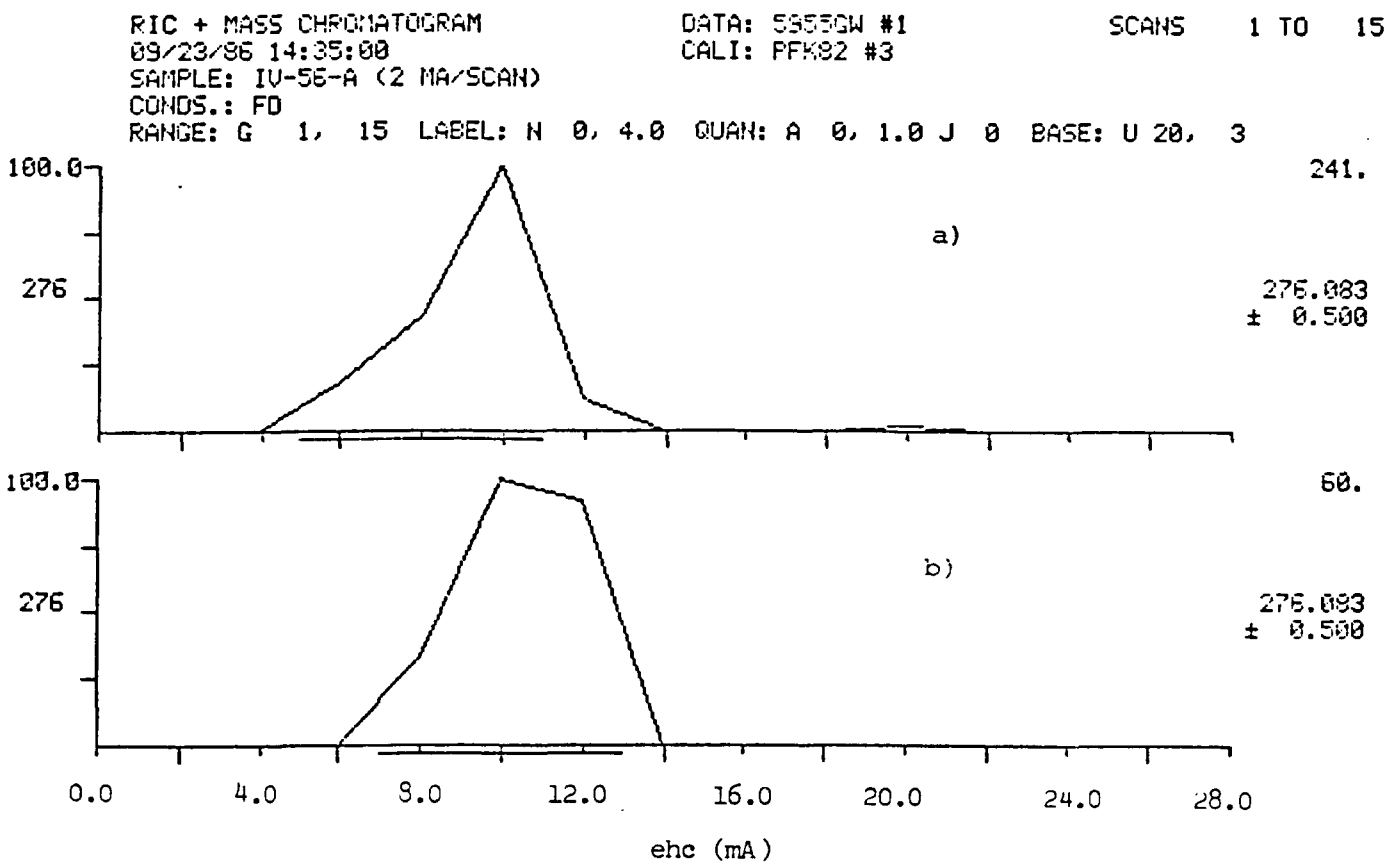
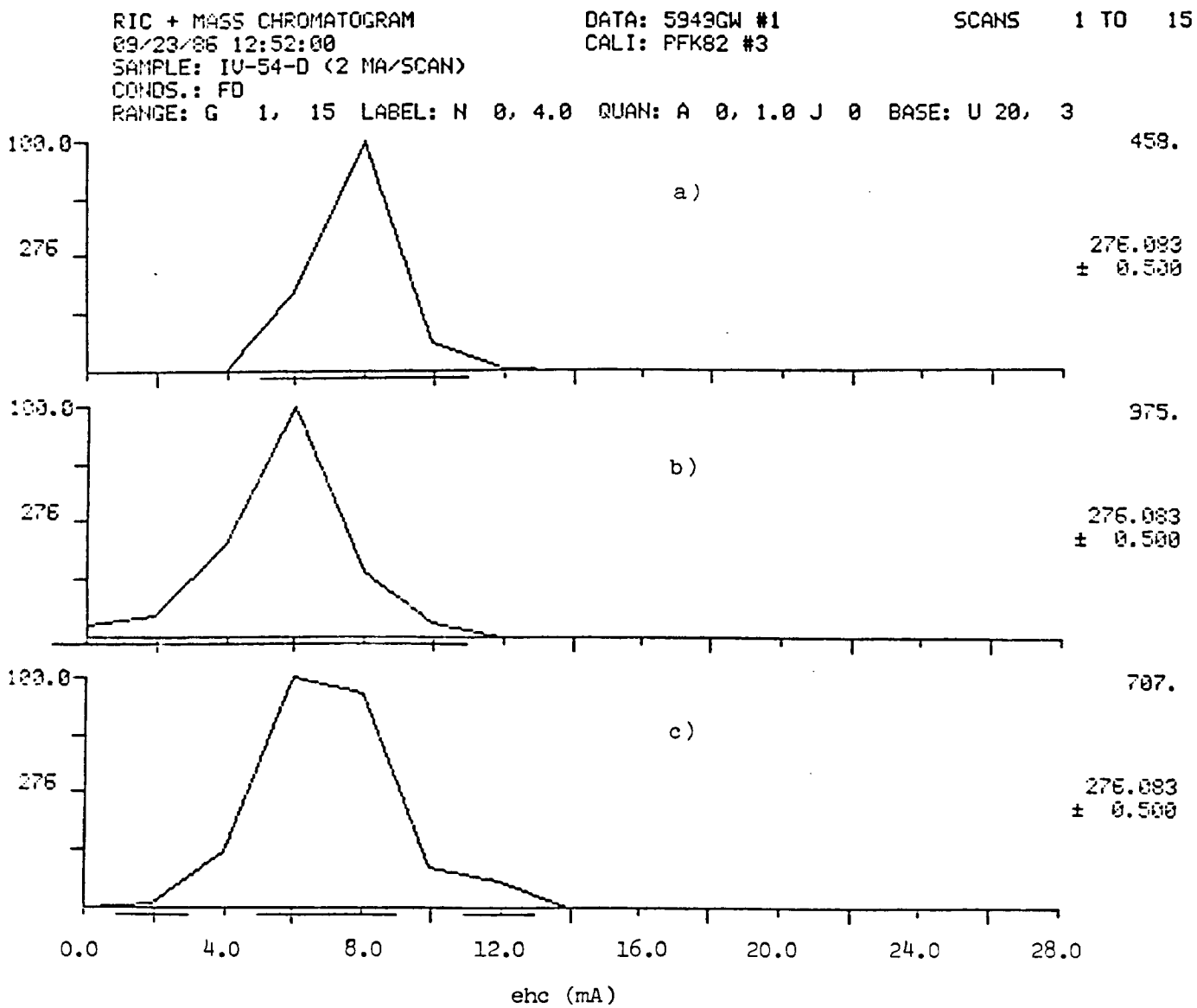


Fig. 21: Desorption Profiles for R- and S-2,2,2-trifluoro-1-(9-anthryl)ethanol/β-CD Inclusion Complexes and their Mixture (2:1 guest/host; 60% methanol/water solvent).

a) R-2,2,2-trifluoro-1-(9-anthryl)ethanol

b) S-2,2,2-trifluoro-1-(9-anthryl)ethanol

c) 2,2,2-trifluoro-1-(9-anthryl)ethanol mixture



complexed enantiomers shows slight broadening, however no resolution of desorption maxima.

Because of the bulkiness of the included portion of the guest molecule, similar experiments were performed using γ-CD in place of the β-CD. The results obtained (Figure 22) show desorption behaviour comparable to that observed for the β-CD inclusion complexes, but at higher ehc values. The R-isomer exhibited maximum desorption at at 18 mA, while the S-isomer showed a desorption maximum at 15 mA. One must note however, that the desorption profile for a mixture of the complexes showed no resolution of desorption maxima, and no broadening of the desorption envelope was observed as in the case of the β-CD inclusion complexes.

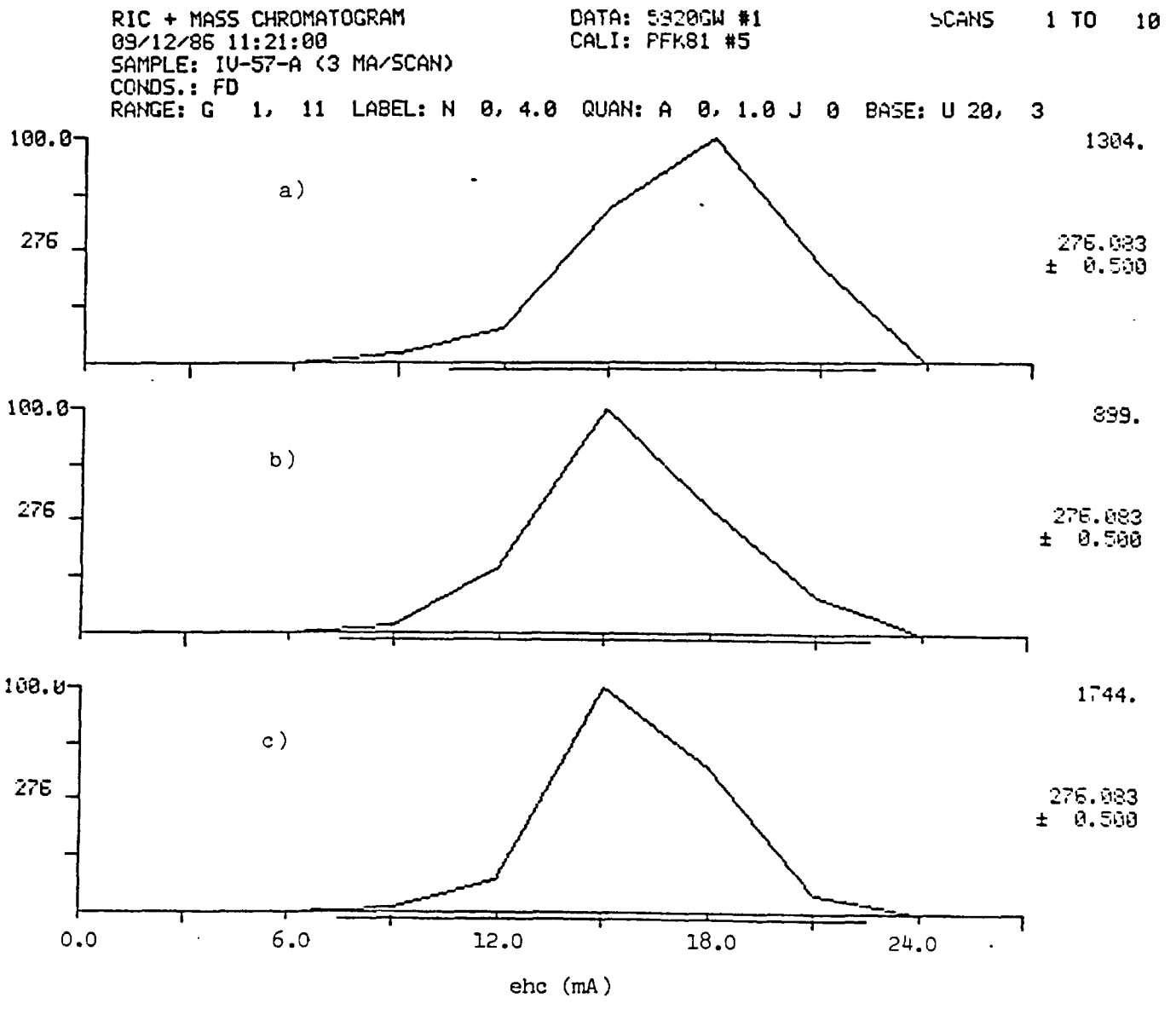
Summarizing these results, one finds that limited resolution of enantiomeric species is conceivable in the case of (1-naphthyl)ethylamine. However, in the cases of the enantiomers of 2,2,2-trifluoro-1-(9-anthryl)ethanol, some slight differences in desorption behaviour are observed among the individual complexes, but resolution of a mixture was not observed. This might imply that the stabilities of the complexes formed between CD and R- and S-2,2,2-trifluoro-1-(9-anthryl)ethanol are too similar to allow for their resolution by these methods. Another consideration is that the CDs are acting as a chiral matrix rather than forming individual guest/host complexes. The differential effects on the FD behaviour of chiral compounds thus may not be as great as would be the case for guest/host complexes. In general, it has not

Fig. 22: Desorption Profiles for R- and S-trifluoro-1-(9-anthryl)ethanol/γ-CD Inclusion Complexes and their Mixture (2:1 guest/host; 60% methanol/water solvent).

a) R-2,2,2-trifluoro-1-(9-anthryl)ethanol

b) S-2,2,2-trifluoro-1-(9-anthryl)ethanol

c) 2,2,2-trifluoro-1-(9-anthryl)ethanol



been possible to demonstrate in this work a clear-cut separation of enantiomers by diastereomeric complexation with cyclodextrins.

CHAPTER VI

CONCLUSIONS

1) CDs decrease the volatility of compounds such as naphthalene and its derivatives, to the extent that FDMS analyses are possible through a slow release of the guest molecules upon anode heating.

2) By varying the guest/host ratio, one can effectively manipulate the desorption behaviour of the guest compound being studied. This might imply that in the condensed phase, CDs act as vapor pressure reducing matrices allowing the FD analyses of volatile samples, rather than providing cavities which lead to the formation of well-defined inclusion complexes.

3) The desorption behaviour of the guest compound appears to be dependent upon one or more of the following:
   i) "Completeness" of complex formation.
   ii) Substituent effects (e.g., H-bonding).
   iii) Structural effects such as substituent position and stereoisomerism.
   iv) Solvent effects.

4) Based on differential effects on the desorption behaviour and/or volatility, the potential exists for differentiation of some positional isomers by the FDMS analysis of their CD inclusion complexes.

5) By virtue of diastereomeric differences in the complexes, it is shown that the potential exists to distinguish between the enantiomers of (1-naphthyl)ethylamine by the FDMS analysis of their CD inclusion complexes. However, the necessity for further studies on a wider spectrum of enantiomeric species is indicated, in order to elucidate any effects dictating the differential stability of the complexes formed. As well, determination of the effect of the solvent species on the differentiation of closely related species is recommended as it appears that the choice of solvent affects the desorption behaviour observed.

6) By forming a CD inclusion complex, it is possible to dictate the ionization mechanism for zwitterionic species such as phenylalanine so that $[M+H]^+$ is formed rather than $[M]^{+\cdot}$.

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APPENDIX I

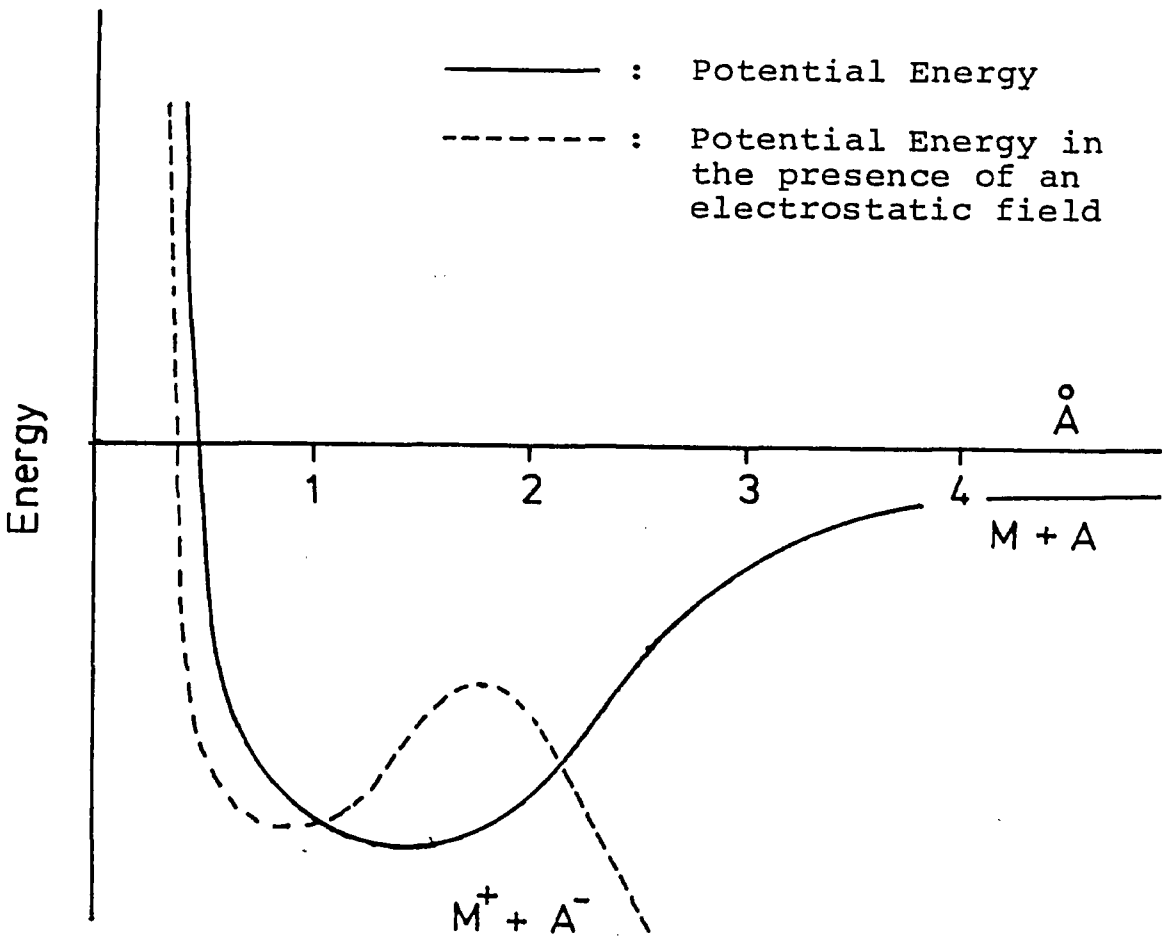
The Theory and Practice of Field Desorption Mass Spectrometry (FDMS).

The basic concept of field desorption was first developed in the mid 1950's by Muller [1]. Its application as an ionization technique for mass spectrometric analyses was pioneered several years later by Beckey [2]. In its 17 year history, FI/FD mass spectrometry has enjoyed widespread application in the analysis of large, non-volatile and/or thermally labile molecules. Since its inception, FI/FD mass spectrometry has been the subject of two monographs [3, 4] and many review articles [5-11]. FDMS acts as an alternative strategy for the analysis of non-volatile or thermally labile compounds [12], because heating of the sample to vaporize it, generally required by other techniques (i.e., EI, CI and FI), often leads to thermal degradation of the compound of interest.

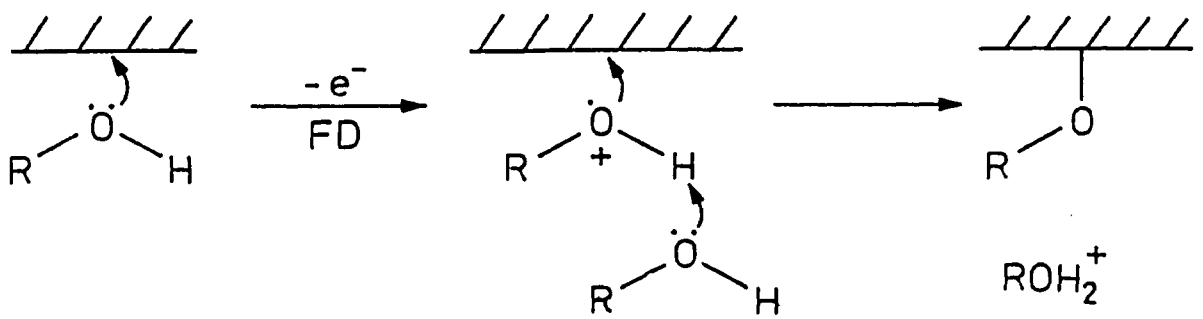
The uniqueness of FD arises from the behaviour of compounds when subjected to high potential fields. Its basis lies in the fact that when an organic layer is adsorbed onto a metal, or more commonly a carbonaceous surface, it experiences an electrostatic force when a high field is applied. If the surface is of the proper geometry, and under high vacuum, this force can be sufficient to induce ionization of the molecules

and eject them as positive ions, allowing for analysis by mass spectrometric techniques.

The exact mechanism of ionization in FD is not known. It has been presumed that the ions were formed via a quantum mechanical concept known as resonance electron "tunneling" or barrier "tunneling". Electron "tunneling" is a quantum mechanical process without a classical mechanical analog. In the FD process, this effect involved a surface substrate such as a metal or carbon. The electrons from the adsorbed organic molecule could only enter the substrate at or above the Fermi level, since below this level there are no vacant quantum states for it to enter at ordinary temperatures [13]. The effect of a high field on the potential energy of an electron is illustrated below.



Recent evidence [14-17] indicates that resonance electron "tunneling" does not account for many of the ions observed in FDMS. However, it is attributed to the first step in some field and temperature induced surface reactions [18]. As an example, alcohols yield dominant [M+H] and [2M+H] peaks under FD conditions [19]. These are rationalized by the processes illustrated below.



$$3\,ROH \xrightarrow[FD]{-e^-} RO^{\cdot} + (ROH)_2H^+$$

The potential field can play a dominant role on the mechanism of many surface reactions [19]. Reactions having low activation energies are found to be field dependent. Other general observations concerning field dependent processes include the following: i) the primary process, excluding emitter surface interactions, is proton transfer; ii) once the ions are formed, they are immediately removed from the reaction zone due to the high potential gradient at the emitter surface; iii) most field induced reactions occur without an applied activation energy; iv) in general, the products of field induced ion-molecule reactions are generated by simple bond rearrangements.

Thermally induced reactions include the formation of dimeric species and "cationization", or the attachment of an ion to the molecule of interest, yielding a "quasimolecular" ion. It has been shown [20] that for saturated and unsaturated carboxylic acids containing 12 or more carbons, the dominant peaks in the FD spectra are: $[M]^{+\cdot}$ and $[M+H]^{+}$ at low emitter temperatures; $[M-17]^{+\cdot}$ and $[M-44]^{+\cdot}$ at moderate emitter temperatures; $[2M+H-H_2O-COOH]^{+\cdot}$ at high emitter temperatures.

"Cationization" is a thermally activated, field induced reaction which results when cations (eg. alkali metal ions) are attached to the adsorbed organic molecules yielding $[M+C]^{+}$ species, where C is the cation. The ions formed by this process tend to show high stabilities due to strong charge localization about the alkali atom. Thus, decomposition of the "quasimolecular" species is prevented because the charge shift required for this decomposition is eliminated by the rearrangement of the bonding electrons [21]. Summarizing, the types of species to be expected in FDMS [10] are illustrated below,

$$\overset{M}{C^{+}A^{-}} \longrightarrow \begin{matrix} M^{+\cdot},\ (M+H)^{+} \\ C^{+},\ (2C+A)^{+},\ \text{etc.} \\ (M+C)^{+},\ (2M+C)^{+},\ \text{etc.} \end{matrix}$$

where M indicates the molecule of interest and $C^{+}A^{-}$ indicates a salt when present.

To effectively carry out FDMS analyses, a large and diverse number of practical aspects need to be considered. In FD, solid organic samples are not vaporized into the ion source [3]. Instead, they are dissolved or suspended in a suitable medium [22], usually at room temperature. An "activated" emitter is then dipped into a concentrated solution of the sample. Under high vacuum, the solvent remaining on the carbon dendrites is removed, leaving an adsorbed layer of the sample to be analyzed. Another method [23] used for applying the sample to the field emitter involves deposition of a droplet of the solution using a microsyringe and a micromanipulator. This method has several advantages over the dipping technique including accuracy in terms of the volume of solution dispensed, use of small amounts of the solution and consequently the sample are required, and finally one can achieve accurate placement of the solution on the emitter.

In the FD source a slotted cathode or extraction plate is separated from the emitter by a distance of 1-2 mm. This separation is sufficient to produce a potential gradient of 10-12 kV. The intense electric fields necessary for FI/FD are produced by the positively charged emitters, having at their surface a region or regions of very great curvature, at which this intense field is established. Clearly, the ionization efficiency is for the most part dependent upon the quality of the of the field emitter used. In general, the ion currents observed in FD are a factor of 10-100 times below that obtained by EI techniques [8]. In most cases, the ionization ability of

the "activated" emitters is dependent upon the structural and morphological composition of the microneedles. The most common [24] emitter now in use is a 10 um tungsten wire, activated with benzonitrile from the vapour phase, under the influence of high electric fields. Subsequent in-situ carbonization occurs followed by deposition of structurally strong carbonaceous needles of approximately 20-40 um in length. The structural ordering determines the chemical and mechanical stability of the microneedles, whereas the morphology determines the efficiency of the microneedles during the ionization process [25, 26].

Temperature dependence (expressed as emitter heating current, ehc) is characteristic of FDMS [4, 10, 27]. The emitter is mounted in the ion source in such a way that a current may be passed through it. This current is usually in the range of 0-50 mA, with the rule of thumb being [10] that ehc values between 10-25 mA correspond approximately to temperatures between 100-250°C.

Organics of low and intermediate polarity are often desorbed at relatively low ehc values with the formation of odd electron $[M]^{+\cdot}$ rather than cationized, "quasimolecular" species [4, 27]. Inorganic salts desorb at considerably higher ehc values (typically >50 mA). Thus, FDMS may be used for the sensitive and specific determination of metal cations [28]. The most important range of ehc values (15-25 mA) is used for the analysis of polar, non-volatile organic and bioorganic molecules with the protonated, $[M+H]^{+}$ species typically formed,

in the absence of metal ions. The dependence of molecular ion desorption on ehc (i.e., best anode temperature, BAT) and the dependence of fragmentation on ehc has been shown [4] to be a most prominent feature for the selectivity of molecular weight determinations by FDMS.

Qualitatively, organic samples suffer less thermal stress in FD than under FI, CI and EI conditions, because the latter require vaporization of the sample. That is, energy equivalent to the sublimation energy of the sample is required. In FD, ionization of the adsorbed molecules and desorption of the ions from the field emitter only require the FD energy which is small at high field strengths. The small amount of energy transferred in the FD process (approximately 0.1 eV [8]) increases the probability of detecting an intact molecular ion. Thus, one of the benefits of FD, leading to its widespread application is the ability to generate intact molecular ions from the condensed phase.

The applications of FDMS are numerous and have been described elsewhere, e.g., [4]. Its application in the analysis of bioorganic molecules has received considerable attention [10].

Summarizing the practical aspects of FDMS, one of the major caveats to be considered is its high instrumentation demands. These include a high field power supply, emitter activation and an ehc control device. Examples of the practical advantages of FD over other ionization techniques include simple sample preparation, the choice of solvent is uncritical

and little or no background is observed.

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VITA AUCTORIS

BORN: August 22, 1958, Jacksonville, Florida, U.S.A.

Son of Mr. and Mrs. B. P. Obal

EDUCATION: 1972-1977 Glenforest Secondary School
Mississauga, Ontario, Canada

1977-1981 McMaster University
Hamilton, Ontario, Canada
Degree: B.Sc. (Chemistry)

1981-1983 University of Windsor
Windsor, Ontario, Canada
Degree: M.Sc. (Chemistry)

1983-1986 University of Windsor
Windsor, Ontario, Canada
Candidate for the Degree of
Doctor of Philosophy (Chemistry).

AWARDS: 1984-1986 University of Windsor
C. P. Crowley Scholarship

PUBLICATIONS:

1. Pugsley, C. W., Hebert, P. D. N., Wood, G. W., Brotea, G. and Obal, T. W. (1985) "Distribution of Contaminants in Clams and Sediments from the Huron-Erie Corridor. I - PCBs and Octachlorostyrene." J. Great Lakes Res., 11(3), 275-279.

2. Obal, T. W., Willis, C. J., Stephan, D. W., Boere, R. T. and Esser, D. (1985) "Complexes of Hybrid Ligands: The Synthesis of a Thioanisole Substituted Fluoro-alcohol and its Complexes with Pd^{2+} and Pt^{2+}; The Structure of a Palladium (II) Complex Containing Alkoxide, Phosphine, Thioether and Chloride Donors." Can. J. Chem. (submitted for publication).