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## Direct Mixture Analysis by Mass-Analyzed Ion Kinetic Energy Spectrometry Using Negative Chemical Ionization

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**Abstract:** Negative ions, generated by chemical ionization and isolated by mass analysis, can be dissociated by collision to yield either positively or negatively charged fragments both of which are characteristic of particular functional groups. These MIKE spectra have comparable S/N characteristics to those of selected positively charged ions and they form the basis for the characterization of specific components in complex mixtures (such as glucose in urine) without any sample pretreatment. Isomeric compounds can frequently be distinguished and it is shown that the charge stripping reaction ( $m_1^- \rightarrow m_1^+$ ) can be more sensitive to different isomers than is collision-induced dissociation. Sensitivity is better than  $10^{-10} \text{ g}$  with the highest sensitivity being obtained in the single reaction monitoring mode in which a selected reaction of a chosen ion is monitored as a function of time. The detection limit is lower in the MIKES method than in conventional mass spectrometry and this is accounted for by the decreased chemical noise in MIKES.

A recently developed alternative to combined chromatography/mass spectrometry for the analysis of mixtures is direct analysis in a two-stage mass spectrometer.<sup>1</sup> The first stage functions as an ion separator and the second as an ion identifier. The best developed<sup>2-5</sup> of the various instrumental permutations which fall within this protocol is that in which ionization is by chemical ionization (CI), separation is by a sector magnetic field, fragmentation is by collision-induced dissociation (CID), and fragment ion analysis is by a sector electrostatic analyzer. This specific set of choices<sup>6</sup> represents the use of a reversed-sector MIKE spectrometer (mass-analyzed ion kinetic energy

spectrometer). Hallmarks of this analytical procedure are that little or no workup of complex samples is required, while good sensitivity with specificity of compound identification is obtained.

All previously reported work on the direct two-stage analysis of mixtures has employed positive ions.<sup>1,3</sup> Our objectives here include an investigation of the potential of negative ions in such analyses. In addition, we have sought to introduce more selectivity and specificity into the direct analysis protocol at two points: (1) the ionization process; (2) the types of ion/molecule reactions used to characterize the selected ion. It is well known

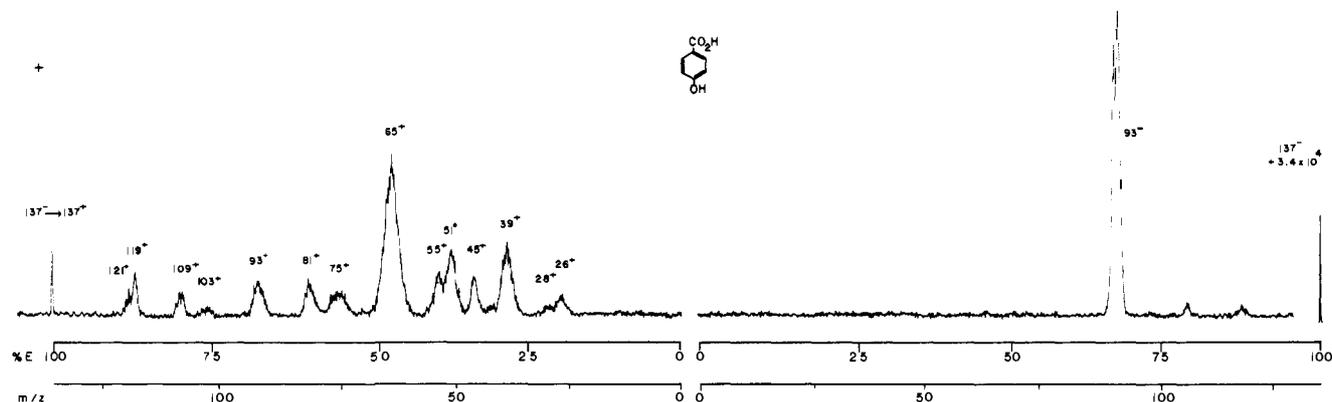


Figure 1. MIKE spectrum of the  $(M - H)^-$  ion of *p*-hydroxybenzoic acid showing both positively and negatively charged product ions. The abscissa is calibrated both in terms of the measured ion kinetic energy and the mass calculated therefrom. Note the stripping peak ( $137^- \rightarrow 137^+$ ) which is recorded simply by reversing the potentials applied to the energy analyzer.

that negative ions are generated in large abundance for certain classes of compounds and not for others.<sup>14</sup> This has been exploited, for example, in analyzing hydrocarbon mixtures by electron impact for thiols<sup>15</sup> and suggested as a means of examining pesticide residues.<sup>16</sup> The advantages of negative chemical ionization (NCI) should be augmented with the ability to characterize ions in the second stage of the MIKE spectrometer.

Studies on negative ions also provide an opportunity to utilize reactions other than collision-induced dissociation to characterize the selected ion. Charge stripping is such an alternative which has proved valuable in ion structural studies on positive ions,<sup>17,18</sup> although it has not been used in chemical analysis. Stripping of negative ions (to yield positively charged ions) is even easier to effect than stripping of positive ions. It displays a large cross section and has found use in mechanistic studies in organic mass spectrometry.<sup>19</sup>

### Experimental Section

The MIKE spectrometer<sup>20</sup> and the chemical ionization source<sup>21</sup> have previously been described. Operating conditions follow: accelerating voltage  $-7$  kV, source temperature  $150$ – $250$  °C, collision gas indicated pressure  $3$ – $5 \times 10^{-5}$  Torr. Negative ions were extracted and studied by simple reversal of accelerating and magnetic sector voltage polarities. No changes were made in the detection system. MIKE spectra were recorded by scanning the electric sector potential. Scans of both the positively and negatively charged fragments were obtained by appropriate choice of the electric field direction. Single reaction monitoring was accomplished by appropriately setting the electric field for continuous transmission of a collision-induced fragment ion derived from a mass-selected precursor ion.

Most experiments were done using as the primary ion  $(M - H)^-$ ; however,  $(M + Cl)^-$  was employed in some cases. These chloride attachment experiments involved saturating the isobutane reagent gas with methylene chloride and mass selecting the appropriate species. In all other cases isobutane alone was used as reagent gas for both negative and positive primary ion formation. Nitrogen was generally employed as collision gas but benzene was also used for lactic acid and *o*-hydroxybenzoic acid. No significant differences in the resulting  $(M - H)^-$  MIKE spectra could be detected in either the positively or negatively charged fragment ions. All data refer to MIKE spectra taken in the presence of collision gas; metastable ions were not studied.

Negative ions were observed in the CI mass spectrum with comparable intensity to positive ions generated by isobutane chemical ionization. Negative ion formation was, however, found to be more sensitive to the ion source conditions. In particular, sensitivity was noted with respect to the ion source pressure as well as to impurity and background species, such as halogenated compounds, which divert the ion current into undesirable channels.

All samples were used as obtained without treatment or purification. The only exception occurred in the analysis of ascorbic acid in urine.

In this case,  $200 \mu\text{L}$  of urine was freeze dried in order to obtain enough sample for a complete MIKE spectrum.

### Results and Discussion

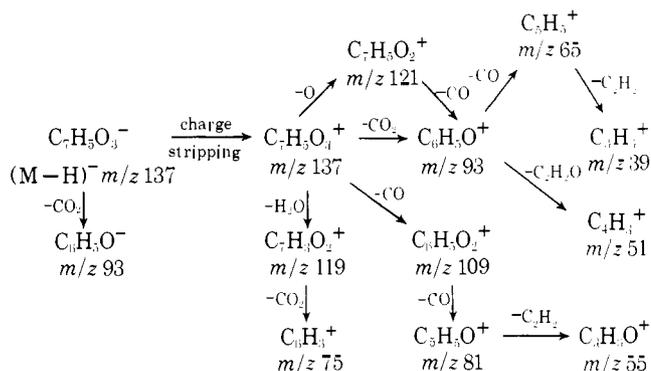
**Fundamental Studies.** Negative ions are characterized in this work by three types of high-energy ion/molecule reactions: (1) charge stripping  $m_1^- + N \rightarrow m_1^+ + N + 2e^-$ ; (2) collision-induced dissociation  $m_1^- + N \rightarrow m_2^- + m_3 + N$ ; (3) dissociative stripping  $m_1^- + N \rightarrow m_2^+ + m_3 + N + 2e^-$ . For polyatomic ions of given kinetic energy, reaction 1 gives rise to products of a single mass to charge ratio and thus kinetic energy to charge ratio while 2 and 3 give a spectrum of products distinguished by their various masses and thus energy to charge ratios.

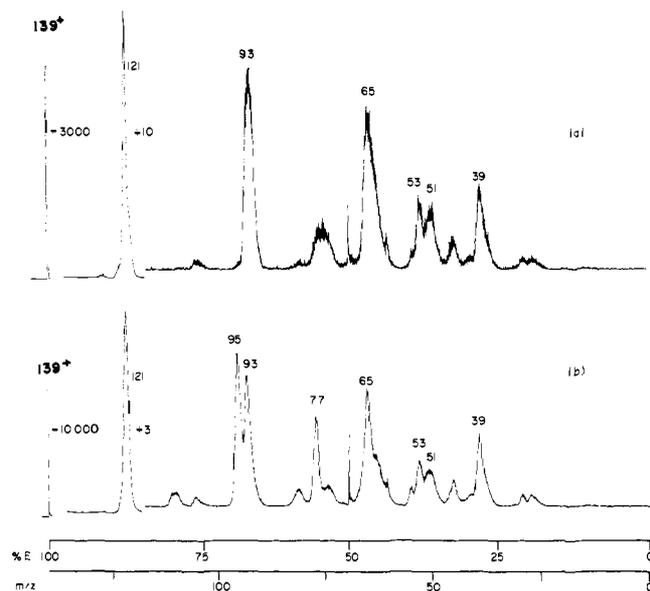
Three points arise: (1) Can the fragment ions be accounted for in terms of established principles of ion chemistry? (2) Are these reactions representative of the functional group(s) in question? (3) Is the method applicable to the analysis of complex mixtures? It will be shown that all three questions can be answered in the affirmative.

To establish the groundwork for the study of negative ions, attention was focused on aromatic carboxylic acids. The first results discussed are those for benzoic acid and *p*-hydroxybenzoic acid. Figure 1 shows the complete MIKE spectrum (positively and negatively charged ions) of  $(M - H)^-$  generated by chemical ionization of *p*-hydroxybenzoic acid. Benzoic acid yields a similar spectrum with one major negative-ion fragment,  $77^-$ , and several positive ion fragments:  $105^+$  (11% relative abundance),  $77^+$  (base peak),  $63^+$  (8%),  $51^+$  (60%), and  $39^+$  (10%).

The spectra of both compounds can be readily rationalized by simple collision-induced dissociation to yield the fragment anions and dissociative stripping to yield the fragment cations.

#### Scheme I. Dissociation and Dissociative Stripping of $(M - H)^-$ Ions of *p*-Hydroxybenzoic Acid





**Figure 2.** MIKE spectra of the  $(M + H)^+$  ions of (a) *o*- and (b) *p*-hydroxybenzoic acid showing that selection of *positive* ions allows isomers to be distinguished and gives similar spectral characteristics to negative ion selection. Note the stripping peak ( $139^+ \rightarrow 139^{++}$ ) observed at 50% E.

The third type of ion/molecule reaction noted above also occurs; it yields  $(M - H)^+$  and, although this ion is only observed in *p*-hydroxybenzoic acid, it is believed to be the precursor for all fragment cations (see below).

The decarboxylation reaction is the dominant process leading to negatively charged fragment ions for both acids. This result is representative of all the aromatic acids studied. It thus serves as a characteristic of the functional group. The positively charged fragments, on the other hand, clearly indicate the presence of the aryl units ( $77^+$ ,  $51^+$  in benzoic acid), and they are diagnostic of the particular carboxylic acid.

Scheme I summarizes the reactions observed for *p*-hydroxybenzoic acid. Most of the observed processes are straightforward and have well-established precedents in conventional mass spectrometry. Virtually all fragment ions result from the elimination of one or more stable neutral molecules. A noteworthy exception to this is the formation of the benzoyl ions by loss of 16 mass units (atomic oxygen) from the precursor benzoate ion.<sup>22</sup> The species,  $ArCO_2^+$ , is coordinately unsaturated and is not usually encountered in mass spectrometry. Its high enthalpy makes fragmentation by O atom loss with acylium ion formation or  $CO_2$  loss with aryl ion formation accessible pathways. There is a further factor which causes extensive fragmentation in the positive ion sequence. Charge stripping of the benzoate anion occurs by a vertical electronic transition under the conditions used here.<sup>23</sup> This requires that the nascent cation be generated with the same geometry as the anion and this will be different than its equilibrium (ground state) configuration. It will therefore have considerable internal energy and dissociate extensively, as observed.

The sequence of events involved in production of the fragment cations is shown in Scheme I to occur by stripping (to yield  $ArCO_2^+$ ) followed by fragmentation. The alternative sequence, fragmentation of the parent anion followed by charge stripping of the fragment ions, is considered less likely since it would generally require two collisions. A linear pressure dependence was observed for the formation of  $Ar^+$  from  $ArCO_2^-$  confirming the single collision process. Moreover, close correspondence exists between the major positively charged ions seen in the MIKE spectrum and in the EI mass spectra of benzoyl derivatives.<sup>24</sup> In these EI spectra the major

portion of the fragmentation occurs via the benzoyl or substituted benzoyl cation. We take this as evidence that the lower mass fragment ions in the MIKE spectra arise, at least in part, by stripping followed by dissociation. The proposed mechanism is also consistent with observations<sup>19,25</sup> that polyatomic anions can be dissociated upon collision to yield a set of fragment cations which closely mirrors that observed when the corresponding parent cations are generated directly and then dissociated. Alternative mechanisms, such as that in which the anion yields an excited neutral molecule which fragments to give a cation and anion pair,<sup>26</sup> are probably not important under the present conditions. The fact that the intact cation  $(M - H)^+$  is observed in at least some cases makes the proposal unnecessary; moreover, the complementary fragment is not observed. Thus, there is no detectable  $O^-$  which would accompany  $ArCO^+$  formation from the benzoate anion (see, for example, Figure 1).

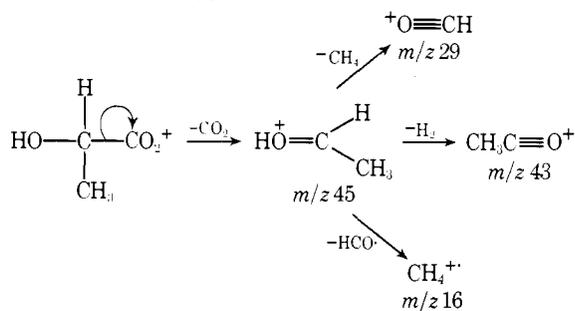
The fact that  $(M - H)^-$  ions can be used to characterize benzoic acid and *p*-hydroxybenzoic acid raises questions regarding the relative merits of analysis schemes based on mass-analyzed negative ions vs. positive ions. To provide information on this point the  $(M + H)^+$  ion derived by protonation of *p*-hydroxybenzoic acid was selected and its MIKE spectrum obtained (Figure 2b). Both the primary ion current, viz.,  $(M + H)^+$  vs.  $(M - H)^-$ , and the fragment ion intensities are comparable in the two sets of experiments. The MIKE spectrum of  $(M + H)^+$  is readily rationalized in terms of the structure of the protonated molecule. The losses of  $H_2O$ ,  $CO_2$ , and  $(CO + H_2O)$  and the formation of  $C_3H_5^+$ ,  $CO_2H^+$ , and  $C_3H_3^+$  are observed and are associated with expected modes of cleavage. Thus, the MIKE spectra of  $(M + H)^+$  and  $(M - H)^-$  ions embody similar structural information. However, NCI offers the advantage of specificity in the ionization process, thus leading to selective ionization of those compounds having a propensity for negative ion formation.

To examine the scope of the NCI/MIKES methodology some representative carboxylic acids and phenols were selected for study. The aliphatic carboxylic acids showed features in common with the aromatic acids, although the decarboxylation reaction was less important for the compounds studied because many alternate fragmentation pathways are available to these ions. These processes are again characterized by a simple ion chemistry centered around the loss of stable neutral molecules. Thus, citric acid yields an  $(M - H)^-$  species which fragments upon collision to yield the following major negatively charged ions:  $173^-$  ( $H_2O$  loss, base peak),  $131^-$  ( $CH_3COOH$  loss, 20% relative abundance), and  $111^-$  ( $H_2O$ ,  $H_2O$ , and  $CO_2$  loss, 60%). Glutaric acid can be characterized by fragment anions due to loss of  $H_2O$  (base peak) and  $CO_2$  (90% relative abundance) from the  $(M - H)^-$  ion, while the lactate anion yields fragments for which the stable structures  $CH_3CH_2O^-$  ( $m/z$  45, 12% relative abundance),  $CH_2=CHCO_2^-$  ( $m/z$  71, 68%), and  $CH_2=CHO^-$  ( $m/z$  43, base peak) can be written. This is typical of the simple chemistry underlying the NCI/MIKES methodology.

The positively charged fragments observed in the MIKE spectrum of the  $(M - H)^-$  ions of the above aliphatic acids are also the result of simple reactions, and they yield complementary information on the identity of the carboxylic acids. For example, the  $(M - H)^-$  ion of lactic acid yields the positively charged fragment ions shown in Scheme II.<sup>27</sup>

To further extend the scope of the investigation, a series of substituted benzoic acids was studied. Only a few highlights of these results will be commented upon here. The negatively charged fragment ions need, in principle, be neither less numerous or abundant than their positively charged counterparts, although this is often the case. Where a number of negatively charged fragment ions occur their origin is explicable via simple losses of neutral fragments from  $(M - H)^-$ . *p*-Nitro-

Scheme II. Positively Charged Fragment Ions Generated by Dissociative Charge Stripping of the  $(M - H)^-$  of Lactic Acid



benzoic acid shows negatively charged fragments which reflect the nature of both substituents. The loss of  $\text{CO}_2$  (base peak) characterizes the carboxylic acid, while loss of  $\text{NO}$  (5% relative abundance) is representative of the nitro function. Both types of reaction also occur in sequence (20%). Similar observations pertain to the positively charged fragment ions (derived by charge stripping) of the *p*-nitrobenzoate anion, prominent among which are the *p*-nitrobenzoyl and *p*-nitrophenyl cations, and products due to further loss of  $\text{CO}$ ,  $\text{NO}$ , and  $\text{NO}_2$ .

The strongly electron-donating substituent in *p*-methoxybenzoic acid provides interesting parallels. The negative fragments are again dominated by  $\text{CO}_2$  loss, while both  $\text{CH}_3$  and  $\text{CH}_2\text{O}$  eliminations occur and can be ascribed to reactions of the methoxy substituent. The positively charged fragments also indicate both the presence of the carboxylic acid and that of the methoxy substituent (loss of  $\text{O}$ ,  $\text{O} + \text{CH}_3$ ,  $\text{CO}_2$ ,  $\text{CO}_2 + \text{CH}_3$ ,  $\text{CO}_2 + \text{CH}_2\text{O}$ ).

To characterize the fragmentations which occur in simple phenols, phenol itself and 1,4-dihydroxybenzene were studied. The phenoxide anion fragments only to a limited extent when negatively charged ions are monitored. On the other hand, it undergoes charge stripping to produce an  $(M - H)^+$  ion. The most abundant positively charged fragments (relative to  $m/z$  93<sup>+</sup>) are  $m/z$  77 (22%), 65 (65%), 64 (50%), 63 (48%), 51 (27%), 50 (30%), 39 (32%), and 38 (28%). The  $(M - H)^-$  ion of 1,4-dihydroxybenzene loses  $\text{CO}$  to form the only negatively charged fragment ion, 81<sup>-</sup> (0.1% of main beam 109<sup>-</sup>). Charge stripping produces a larger abundance of  $(M - H)^+$ , 109<sup>+</sup> (0.2% of 109<sup>-</sup>), than positively charged fragments derived from it. Paralleling the fragmentation of  $(M - H)^-$ ,  $\text{CO}$  is lost from  $(M - H)^+$  in the dissociative charge stripping spectrum. These observations on  $\text{CO}$  loss tally with the behavior of phenols in EI mass spectra;<sup>28</sup> they also help explain the fragmentation of *p*-hydroxybenzoic acid (Figure 1) where  $\text{CO}$  loss is associated with the formation of several major ions.

### Isomers

Since mass analysis does not separate isomers, special attention has been given them since the inception of the MIKES technique.<sup>9</sup> For example, it has been shown previously that there are large differences in the collision-induced dissociations of isomeric ketone molecular ions generated by electron impact, making their analysis possible.<sup>9</sup> In the present study, positional isomers of substituted benzoic acids were investigated through anions extracted from the CI source.

*o*-Hydroxybenzoic acid and its para isomer showed similar reactions with regard to both positive and negative fragment ions. Some differences in relative ion abundances occur and the minor ion 103<sup>+</sup> occurs only in the para isomer. A more significant difference between the isomers is found in the yield of the charge stripping ion,  $(M - H)^+$ . The efficiency of charge stripping for *o*- to *p*-hydroxybenzoic acid, normalized to the  $(M - H)^-$  abundance, is 20:1. It has been predicted<sup>17</sup> that ion/molecule reactions with large energy defects, such as stripping compared to collision-induced dissociation, should

be relatively less sensitive to interconversion of two isomers. The entire ion population, including those species with insufficient internal energy to isomerize, is more uniformly sampled in stripping than in CID. Differences in charge stripping for isomeric ions are thus accounted for from two lines of argument. First, stripping reactions, with their relatively large endothermicities, are expected to be more representative of the total ion population than are lower activation energy reactions such as most collision-induced dissociations. This implies that isomerization should be less complete and distinctions between isomeric ions maximized in stripping. The second point concerns the stable configurations of positive and negative ions. If these differ greatly then the nascent cation, generated by a vertical transition, will possess considerable internal energy. Thus, fragmentation cross sections are expected to be sensitively dependent upon details of molecular structure including differences between positional isomers. The present results are concordant with these arguments.

The results given in Figure 2, for mass-analyzed positive ions, show that the pair of isomers just discussed can be distinguished via MIKE spectra of  $(M + H)^+$  as well as  $(M - H)^-$ . The major point of interest in these positive ion spectra is an ortho interaction which channels fragmentation exclusively via  $\text{H}_2\text{O}$  loss. The para isomer, and benzoic acid itself,<sup>29</sup> fragment by primary loss of  $\text{CO}_2$  as well as  $\text{H}_2\text{O}$ . It is proposed that  $\text{CO}_2$  loss yields the protonated phenol ion and that strong intramolecular hydrogen bonding in the ortho isomer stabilizes a form of the molecular ion from which  $\text{CO}_2$  loss is not possible. Hence, the ortho isomer should not yield protonated phenol ( $m/z$  95) or fragments arising from it. The spectrum of protonated phenol itself shows the major collision-induced fragment to be due to loss of water to yield  $m/z$  77. In agreement with the above proposal the major differences between the hydroxybenzoic acids (Figure 2) is the absence of  $m/z$  95 and 77 in the spectrum of the ortho compound.

Returning to our main theme of NCI/MIKES, another pair of isomers investigated in some detail was 3,4- and 2,5-dihydroxybenzoic acid. In this case the differences in the collision-induced dissociations (Figure 3) are substantial and can

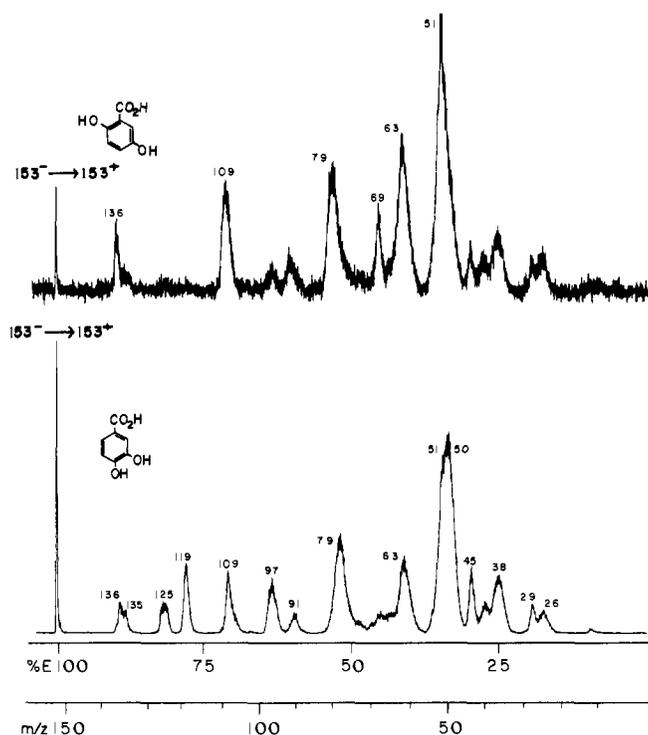


Figure 3. MIKE spectra of the  $(M - H)^-$  ions,  $m/z$  153, of the isomers indicated. Only positively charged fragments are shown.

**Table I.** Direct Analyses of Mixtures by Negative Chemical Ionization/MIKES

component	mixture analyzed	precursor ion	collision-induced fragments		concn of component
			-ions	+ions	
benzoic acid	shampoo	121 ( $M - 1$ ) <sup>-</sup>	77	105, 77, 63	0.25% (w/w)
	root beer			51, 39	
salicylic acid	aspirin	137 ( $M - 1$ ) <sup>-</sup>	93	121, 119, 109, 93	
				81, 75, 65, 55, 51,	
				45, 39	
BHA	yeast	179 ( $M - 1$ ) <sup>-</sup>	164, 134, 89	45	0.10% (w/w)
hippuric acid	urine	178 ( $M - 1$ ) <sup>-</sup>	134, 121, 73	105, 77, 51	32 $\mu\text{g}/\mu\text{L}$
ascorbic acid	urine	175 ( $M - 1$ ) <sup>-</sup>	157, 146, 145,	60, 44, 31	10 ng/ $\mu\text{L}$
			143, 131, 127,		
			115, 87, 71		
glucose	root beer	179 ( $M - 1$ ) <sup>-</sup>	161, 149, 143,	73, 62, 44, 31	
	Gatorade		131, 119, 113,	30	
	strawberry		107, 101, 89		
	jelly				

be rationalized simply from the structures involved. For example, assuming that ring positions remain intact, the 3,4 isomer can be predicted and is found to show many reactions<sup>30</sup> in common with *p*-hydroxybenzoic acid (Figure 1). It is also noteworthy that the prominence of H<sub>2</sub>O eliminations in the breakdown of the 3,4 isomer correlates with the vicinal dihydroxy functional, while OH loss in the 2,5 isomer may be the result of an ortho interaction.

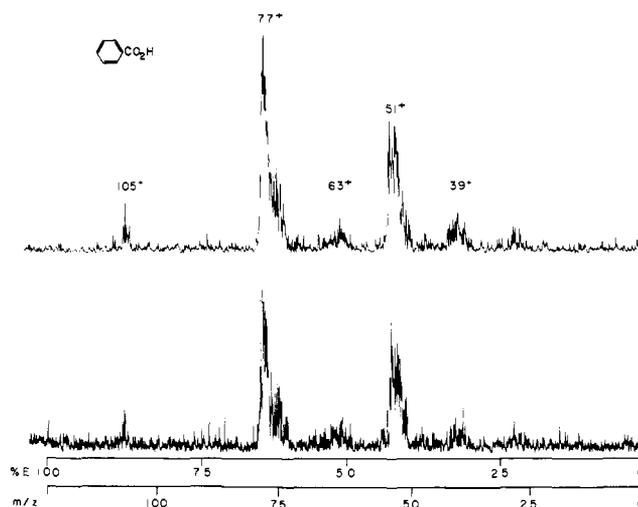
Several other pairs of isomeric substituted benzoic acids were examined including the *o*- and *p*-methyl, *o*- and *p*-nitro, and *o*- and *p*-methoxy compounds. In most cases differences in the MIKE spectra were observed but they were restricted to differences in relative ion abundances. The methylbenzoic acids gave indistinguishable spectra. Ions due to simple stripping were not observed in this set of compounds and in their absence, MIKE spectra apparently reveal no further information than EI mass spectra in studies on isomeric compounds.

### Applications

Some of the most promising applications of direct mixture analysis mass spectrometry lie in the examination of samples of biological origin. Examples of direct analysis of plant material for alkaloids,<sup>4,31</sup> urine for steroids and other components,<sup>32</sup> and drug preparations for barbiturates<sup>33</sup> have been reported using positive ions. Here this approach is broadened by generating negative ions and using their reactions to characterize the components of complex mixtures.

The applicability of the NCI/MIKES methodology to the analysis of a variety of common crude mixtures is demonstrated in Table I. These experiments were done on milligram samples of the intact products with the component of interest being present in concentrations of ~0.1% of total sample weight. The results thus represent typical analyses rather than testing the ultimate sensitivity of the method.

Benzoate, often used as a preservative, was identified in samples of shampoo (Figure 4) and root beer by the characteristic loss of CO<sub>2</sub> upon collision-induced dissociation to form 77<sup>-</sup> and 77<sup>+</sup>, as well as the positive ion fragments 51<sup>+</sup>, 39<sup>+</sup>, 63<sup>+</sup>, and 105<sup>+</sup>. Given mixtures of increasing complexity, impurities are expected to be present in the mass-selected ion beam. As a consequence of the dual filter feature of the method (i.e., mass filter followed by energy filter) they cause minimal interference in the MIKE spectrum. This is illustrated by the root beer analysis for which the positively charged fragments match an authentic sample of benzoic acid, while those with a negative charge include extra peaks at *m/z* 97, 94, and 89 which are of extraneous origin. This example illustrates that incomplete compound separation in the mass-analysis step will



**Figure 4.** Identification of benzoic acid in a household product by comparison of the MIKE spectra of the benzoate anion generated from benzoic acid (upper) and from the sample (lower).

not, in general, prejudice the identification (or quantitation, see below) of a component.

Salicylic acid, an impurity in aspirin, was easily detected in the drug by its characteristic loss of CO<sub>2</sub> from the ( $M - H$ )<sup>-</sup> ion of *m/z* 137. This example demonstrates the versatility of the technique even for an acidic component in an acidic sample.

Dry yeast was analyzed for the preservative BHA (butylated hydroxyanisole) by selecting mass 179<sup>-</sup> from the CI source. Upon collision several fragment ions are formed including 164<sup>-</sup>, 137<sup>-</sup>, and 109<sup>-</sup> which were used to characterize BHA in this mixture. Comparison with an authentic sample confirmed the identification. Thus, the NCI/MIKES methodology is equally applicable to phenolic components as it is to carboxylic acids.

The specificity of the NCI/MIKES methodology was further demonstrated on an untreated sample of normal human urine which is known to contain a complex mixture of carboxylic acids. Just 2  $\mu\text{L}$  of urine was sufficient to obtain a positive (Figure 5) and negative MIKE spectrum of 178<sup>-</sup>. By comparison to an authentic sample it was shown definitely that hippuric acid was present. Typically normal urine (2  $\mu\text{L}$ ) contains about 65  $\mu\text{g}$  of hippuric acid.<sup>34</sup>

In addition to carboxylic acids and phenols, alcohols such as carbohydrates can be readily identified in complex mixtures. Ascorbic acid was detected in urine (3  $\mu\text{L}$ ) by monitoring a single reaction, that in which the mass-selected 175<sup>-</sup> ion was collisionally dissociated to 115<sup>-</sup>. Alternatively, ascorbic acid

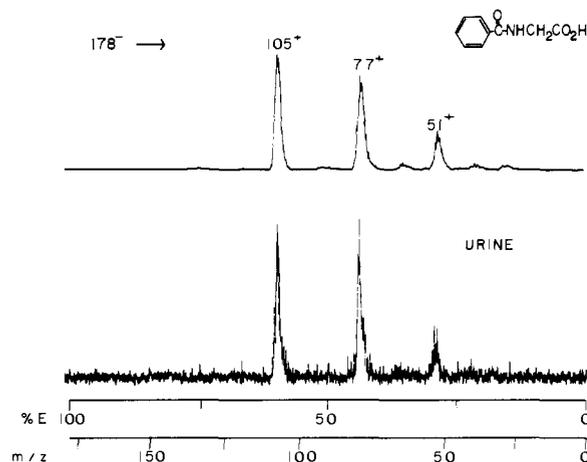


Figure 5. Identification of hippuric acid in urine by negative chemical ionization/MIKES. The analyses employed an untreated 2- $\mu$ L urine sample.

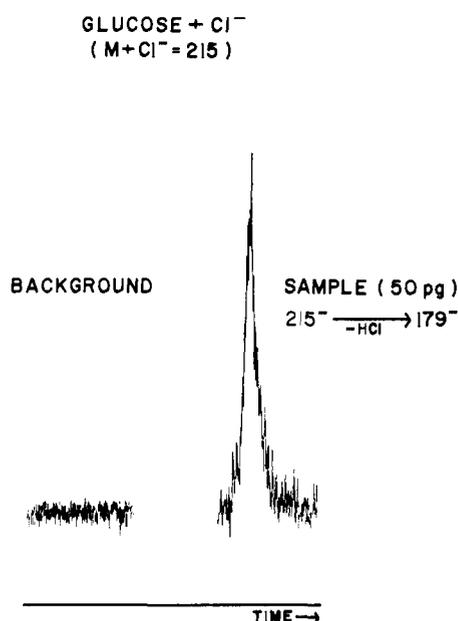


Figure 6. Detection of 50 pg of glucose (total sample loaded into instrument) by monitoring the loss of HCl from the  $(M + Cl)^-$  ion. The peak has a width (fwhm) in time of 25 s.

was detected by recording an entire negative ion MIKE spectrum of the  $175^-$  ion obtained from freeze-dried urine.

Negative chemical ionization uniquely allows characterization of certain classes of compound, which include carboxylic acids, phenols, and carbohydrates, by chloride ion attachment.<sup>16</sup> Hydrogen bonding stabilizes a chloride ion in the gas phase by allowing charge delocalization.<sup>35</sup> As a result of the weak nature of a hydrogen bond, loss of hydrogen chloride in a collision-induced detachment reaction occurs as the dominant process.

The attachment/detachment reaction sequence just described was employed in experiments aimed at determining the detection limit for NCI/MIKES. The result was that 50 pg of glucose (total sample introduced) could be detected with S/N 10 using the single reaction monitoring mode and a methylene chloride/isobutane mixture as reagent gas. This limit of detection is an order of magnitude lower than typically observed for positive ions in MIKES.

The utility of chloride attachment in the MIKES methodology was demonstrated by detecting glucose in urine (Figure 6). It can be seen that the mass spectrum of 1  $\mu$ L of urine gives no indication of the presence of glucose as a result of other

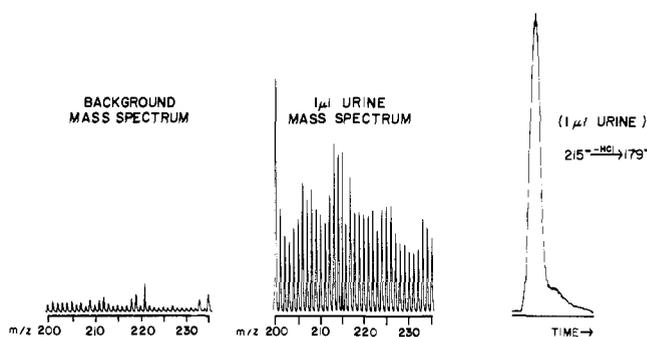


Figure 7. Negative chemical ionization mass spectra showing indiscriminate signal enhancement over background when a complex mixture (urine) is examined. By contrast, the single reaction monitoring mode of MIKES gives a strong signal for glucose,  $m/z$  215 (right-hand figure) but not for ions which do not attach and detach chloride.

components of similar mass. On the other hand, single reaction monitoring indicates quite clearly glucose in urine as characterized by the chloride attachment/detachment sequence. Known quantities of authentic sample indicated that  $\sim 100$  ng of glucose was present in 1  $\mu$ L of urine, in agreement with the expected concentration in normal urine.<sup>36</sup>

### Conclusions

The carboxylic acids, phenols, and alcohols studied here could be characterized and analyzed by MIKES using NCI as the ionization method. The data were obtained on the negatively charged  $(M - H)^-$  ion, although  $(M + Cl)^-$  was also useful. Three types of high-energy ion/molecule reactions (collision-induced dissociation, charge stripping, and dissociative charge stripping) were observed. The dissociation mechanisms leading to both positively and negatively charged fragment ions can be rationalized in terms of losses of stable neutral molecules; there is precedent for many of the observed processes in other areas of mass spectrometry. The  $(M - H)^+$  ion formed from charge stripping of  $(M - H)^-$  is not necessarily observed as a stable species and when it is present, it is accompanied by relatively intense positively charged daughter ions. These and other observations are consistent with a mechanism in which the cation is formed in an excited state by a Franck-Condon transition from the anion.

The MIKE spectra of  $(M - H)^-$  ions show features which pinpoint the functional group and even isomeric compounds are often distinguishable. This allowed the identification of a number of compounds in a variety of complex matrices without prior sample pretreatment. To maximize the sensitivity of the technique a single reaction monitoring mode was employed in which one characteristic reaction was followed as a function of time. Detection limits approaching  $10^{-11}$  g were observed in this way for glucose. A comparison of the detection limit of the MIKES technique with that of mass spectrometry was made under typical instrument conditions in both the static and dynamic modes. Confirming previous results on positive ions,<sup>1,3</sup> it was found that MIKES has a better sensitivity and that this must be due to the more efficient removal of chemical noise in the tandem analyzer configuration of MIKES. Quantitation of the NCI/MIKES assay is possible with minimum effort and moderate accuracy by constructing calibration curves for the compound of interest.<sup>32</sup>

In selected cases a comparison of the utility of the  $(M + H)^+$  ion vs. the  $(M - H)^-$  ion in compound characterization was made. The primary ions were formed in similar abundances and both gave MIKE spectra with similar intensities. The ease of interpretation of the spectra was comparable with negative and with positive ion selection. Isomers could be distinguished by both methods in the case attempted (*o*- vs. *p*-hydroxybenzoic acid). The negative ion selection procedure has advantages

