

CHROM. 21 333

REPELLER EFFECTS IN DISCHARGE IONIZATION IN COMBINED LIQUID OR SUPERCRITICAL-FLUID CHROMATOGRAPHY–MASS SPECTROMETRY USING A THERMOSPRAY INTERFACE

I. CHANGES IN THE REAGENT GAS SPECTRUM

W. M. A. NIESSEN*, R. A. M. VAN DER HOEVEN, M. A. G. DE KRAA, C. E. M. HEEREMANS, U. R. TJADEN and J. VAN DER GREEF

Division of Analytical Chemistry, Center for Bio-Pharmaceutical Sciences, Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden (The Netherlands)

SUMMARY

The effects of the repeller voltage on the discharge-on mass spectra of the reagent gas and of analytes has been investigated systematically. This first part describes in detail the influence on the reagent gas spectra produced from methanol–water mixtures in liquid chromatography–mass spectrometry and from methanol–carbon dioxide mixtures in supercritical-fluid chromatography–mass spectrometry. Significant changes in the reagent gas spectra are observed as a function of the repeller voltage, especially in supercritical-fluid chromatography–mass spectrometry, where the most abundant species are protonated methanol (clusters), protonated carbon dioxide, or carbon dioxide molecular ions. This in principle offers the opportunity to select the ionization conditions by setting an appropriate repeller voltage.

INTRODUCTION

The thermospray interface and ion source for combined liquid chromatography–mass spectrometry (LC–MS) were introduced by Blakley and Vestal in 1983¹. The system is now widely available from several instrument manufacturers. Several hardware changes have been made to the initial source design. The most important changes are the addition of a filament, a discharge electrode, and a repeller or retarding electrode.

The discharge electrode, like the rhenium filament, has been added to the thermospray source to induce ionization when the thermospray buffer ionization is ineffective, *e.g.* with non-aqueous solvents or when no buffer salts are present in the mobile phase^{2,3}. A repeller electrode was initially introduced to increase the sampling efficiency of the ion source^{4,5}, resulting in better detection limits. It is placed either slightly downstream (retarding electrode)⁴ or directly opposite to the sampling cone (repeller electrode)⁵. The influence of the repeller voltage, placed opposite to the ion sampling cone, in thermospray ionization of some model compounds has been studied

systematically by Lindberg and Paulson⁶ in order to optimize the sensitivity, and by Robins and Crow⁷ in order to improve the high-mass sensitivity and the ion current stability. The influence of the voltage of a retarding electrode, placed slightly downstream, has been studied systematically by Bencsath and Field⁸. In general, it appears that similar effects can be observed with a repeller electrode and with a retarding electrode, although a particular effect is observed at lower voltages for a repeller electrode than for a retarding electrode. Surprisingly, little systematic research has been performed on the influence of the voltage of repeller or retarding electrode in the other thermospray modes, *i.e.* filament-on and discharge-on⁹. The repeller electrode used at higher voltages in the discharge-on mode has been claimed to induce fragmentation comparable with collisionally induced dissociation (CID), the so-called repeller-CID effect^{2,10}.

This paper describes the influence of the repeller voltage on the reagent gas spectrum both in LC-MS using methanol in water as mobile phase and in supercritical-fluid chromatography-mass spectrometry (SFC-MS) using methanol in carbon dioxide as mobile phase. The use of the discharge-on mode in SFC-MS has only recently been described, but without demonstrating repeller effects¹¹. The results of the investigations reported in this paper indicate that the explanation of the repeller effects in analyte spectra as given by McFadden and Lammert¹⁰ is not the main process.

EXPERIMENTAL

Equipment

(Tandem) mass spectrometry was performed on a Finnigan MAT TSQ-70 instrument (San José, CA, U.S.A.), equipped with a Finnigan MAT thermospray interface. The discharge voltage was set at values between 800 and 1200 V. In the LC-MS experiments the vaporizer tube was treated as usual, while in the SFC-MS experiments the vaporizer capillary must act as the restrictor; this was achieved by pinching the last 2 mm of the tube until a stable back-pressure of typically 30–36 MPa was achieved.

The LC-MS experiments were performed in the flow-injection analysis (FIA) mode with 20–80% of methanol in water at a flow-rate of 1–1.5 ml/min. The solvent was delivered with two Model 2150 LC pumps (LKB, Bromma, Sweden) controlled by a Model 2152 LC controller (LKB). A block temperature of 200°C and a vaporizer temperature of 90–110°C, depending on the solvent composition, were used.

The packed column SFC-MS experiments were performed on a laboratory-built instrument consisting of slightly modified commercially available modules. The system is described in detail elsewhere¹². The mobile phase was carbon dioxide modified with 0–15% of methanol at a flow-rate of 1.5–2 ml/min. The laboratory-packed column (150 mm × 4.6 mm I.D.) was filled with either Nucleosil C₁₈ material (5 μm, Macherey-Nagel, Düren, F.R.G.), or Rosil aminopropyl material (7 μm, Alltech, Deerfield, IL, U.S.A.). The block temperature was kept at 150°C, and the vaporizer temperature was 50°C unless stated otherwise.

In order to study systematically the influence of the repeller voltage on the reagent gas spectra a procedure was written in the Instrument Control Language of the TSQ-70, which automatically starts data collection, increase the repeller voltage from

0 up to 195 V in 10-V steps every 10 scans, and stops data acquisition after completion of the successive steps. The reconstructed total ion current (RIC) and (relative) intensity values reported here are averaged values of the scans 3 to 8 at each repeller voltage.

RESULTS

Liquid chromatography-mass spectrometry

In the source design used in the experiments described here the repeller electrode is placed opposite to the sampling cone. With all mobile phases used so far in both thermospray buffer ionization and filament-on and discharge-on ionization modes, a distinct influence of the repeller voltage on the intensity and the appearance of the reagent gas spectrum is observed. An automatic procedure in which the repeller voltage is varied from 0 to 200 V (in positive ion mode) and the resulting mass spectra are acquired, is used as a diagnostic tool to ascertain good performance of the interface and ion source¹³. Investigation of the repeller effect is also helpful in optimizing the sensitivity in particular applications, and may help in the understanding of the mechanisms of thermospray ionization. In this paper the effects of the repeller voltage on the reagent gas mass spectra obtained in the discharge-on mode are described.

The intensities of the most abundant ions in the reagent gas spectrum resulting from 20% methanol in water are plotted as a function of the repeller voltage in Fig. 1. The plot in Fig. 1 is a profile that can be shifted along the repeller voltage axis as a result of various experimental parameters, which are discussed below. The mass spectrum at low repeller voltages is dominated by protonated methanol clusters (ions of the type $[x\text{CH}_3\text{OH} + \text{H}]^+$ with $x = 1, 2, 3, \dots$), decreasing in size with increasing repeller voltages. At repeller voltages above 80 V, protonated methanol (m/z 33) is the most intense ion in the spectrum. Other abundant ions in the latter region are protonated water (m/z 19), $[\text{CH}_2=\text{OH}]^+$ at m/z 31 and $[2\text{CH}_3\text{OH} + \text{H} - \text{H}_2\text{O}]^+$ at m/z 47. The vaporizer temperature, varied between 90 and 140°C, does not influence the (relative) intensities of the various protonated clusters.

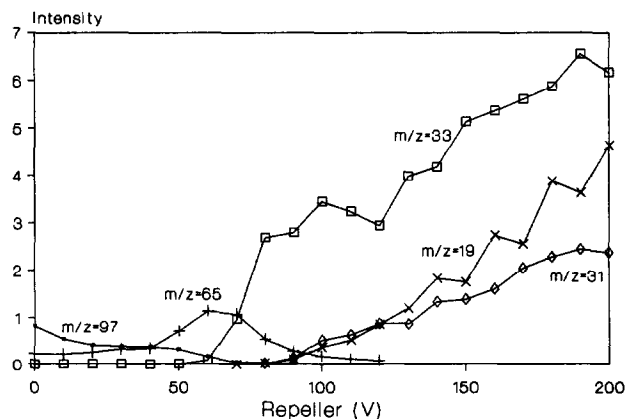


Fig. 1. Intensities (arbitrary units) of the most abundant ions in the reagent gas spectrum of 20% methanol in water in the discharge-on mode, as a function of the repeller voltage. Conditions: discharge, 1000 V; vaporizer temperature, 110°C; flow-rate, 1.2 ml/min; for other conditions see text.

A closer look at the mass spectra obtained at lower repeller voltages reveals the presence of several series of protonated clusters from water, methanol, and combinations of water and methanol with a general formula $[x\text{CH}_3\text{OH} + y\text{H}_2\text{O} + \text{H}]^+$. The various ions observed in the reagent gas spectrum of 20% methanol in water are given in Table I. Most of the protonated clusters are found with low intensity, *i.e.* below 1% of the intensity of the base peak. Several other species are also observed. Clusters with the general formula $[x\text{CH}_3\text{OH} - \text{H}_2\text{O} + \text{H}]^+$ with $x = 1, 2$ and 3 are observed at m/z 15, 47 and 79, respectively.

The reagent gas spectra of methanol–water mixtures in discharge-on mode have been studied over a broad range of solvent compositions. Increasing the percentage of methanol in the mobile phase results in a gradual shift of the profile given in Fig. 1 (for 20% of methanol in water) towards higher repeller voltage. The intensity of the protonated clusters containing water molecules decreases with decreasing water content of the mobile phase, while the protonated methanol clusters become more dominant.

Supercritical-fluid chromatography–mass spectrometry

Discharge-on ionization is also possible in high-pressure ion sources in combined packed-column SFC–MS with either pure carbon dioxide or a few percent of methanol in carbon dioxide as the mobile phase. As in LC–MS, the discharge potential has no influence on the total ion current of the reagent gas spectrum above the onset value of a stable discharge, which lies at *ca.* 700 V in the experiments reported here. Discharge potentials between 800 and 1200 V are used in practice.

In the reagent gas spectrum of pure carbon dioxide the two most intense ions are observed at m/z 44, corresponding to the carbon dioxide molecular ion, and at m/z 88, corresponding to the carbon dioxide cluster ion. The latter ion is observed only at repeller voltages below 40 V, and is the base peak of the reagent gas spectrum at repeller voltages below 20 V. The repeller voltage also exerts a marked influence on the RIC of the reagent gas spectrum: an eight-fold increase in the RIC is observed when the repeller voltage is increased from 30 to 120 V. The molecular ion of carbon dioxide has also been observed by others^{14,15}, working with either packed or capillary column SFC. At high pressures the carbon dioxide molecular ion becomes involved in charge-

TABLE I

THE m/z VALUES OF THE PROTONATED METHANOL–WATER CLUSTERS OBSERVED IN THE DISCHARGE-ON MASS SPECTRA OF 20% METHANOL IN WATER AT LOW REPELLER VOLTAGES

x	y					
	0	1	2	3	4	5
0	—	19	37	55	73	91
1	33	51	69	87		
2	65	83	101	119		
3	97	115				
4	129					

exchange processes. For many analytes the observed charge-exchange mass spectrum closely resembles the electron impact (EI) spectrum.

An overall ten-fold increase in the RIC is observed when carbon dioxide modified with a low percentage of methanol is used. The influence of the repeller voltage on the RIC and on the appearance of the reagent gas spectrum has been investigated in detail for a mobile phase consisting of 2% of methanol in carbon dioxide. Fig. 2 shows the intensities of the most abundant ions in the reagent gas spectrum as a function of the repeller voltage. The plot in Fig. 2 is a profile that can be shifted along the repeller voltage axis as a result of various experimental parameters, which are discussed below. Three regions based on the most intense ions can be distinguished. At low repeller voltages the reagent gas spectrum is determined by protonated methanol (clusters) of the general structural formula $[x\text{CH}_3\text{OH} + \text{H}]^+$ with $x = 1, 2, 3$ and 4. The number of methanol molecules present in the most abundant cluster decreases with increasing repeller voltages. At repeller voltages above 80 V, protonated carbon dioxide becomes the base peak, and at repeller voltages above 140 V the carbon dioxide molecular ion at m/z 44 is the base peak. Four typical reagent-gas spectra obtained with 2% of methanol in carbon dioxide at four different repeller potentials are given in Fig. 3.

The repeller voltages reported here are typical values under the conditions commonly used. However, the precise values are effected by two parameters, *i.e.* the vaporizer temperature and the degree of contamination of the ion source.

Increasing the vaporizer temperature results in a decrease of the intensities of the protonated clusters, resulting in a shift of the complete profile given in Fig. 2 to lower repeller voltages. For example, the maximum abundance of the protonated methanol cluster at m/z 65 is found at a repeller potential of 30 V at a vaporizer temperature of 50°C, but it shifts to 10 V when the vaporizer temperature is increased to 100°C.

The effect of ion-source contamination is more difficult to quantify. Increased contamination of the ion source, and especially of the repeller electrode, results in a shift of the profile of Fig. 2 to higher repeller voltages, which change can probably be

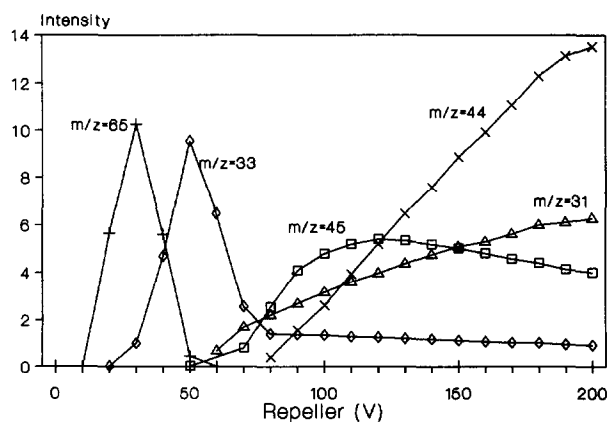


Fig. 2. Intensities (arbitrary units) of the most abundant ions in the reagent gas spectrum of 2% methanol in carbon dioxide in the discharge-on mode, as a function of the repeller voltage. Conditions: discharge, 800 V; flow-rate, 2 ml/min; for other conditions see text.

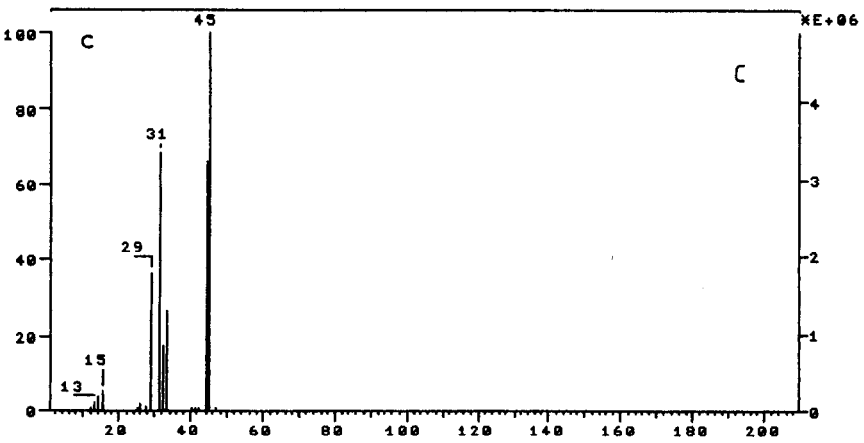
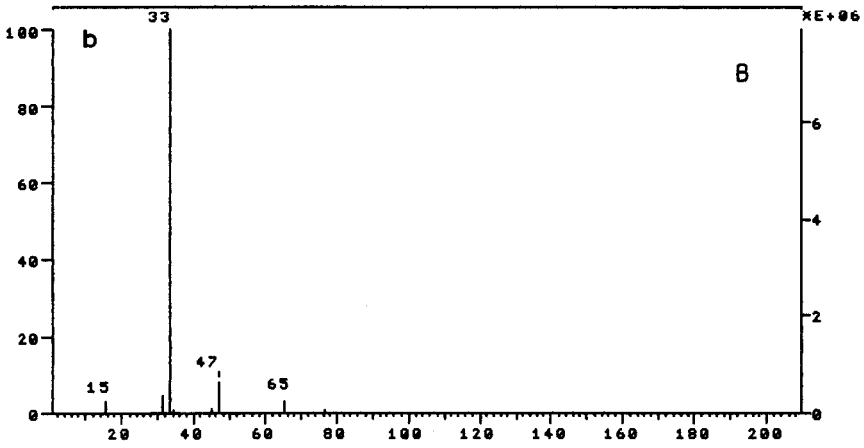
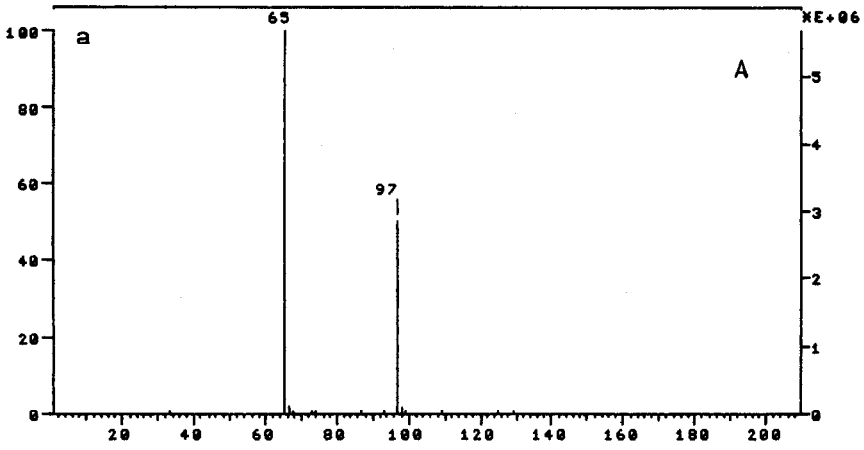


Fig. 3.

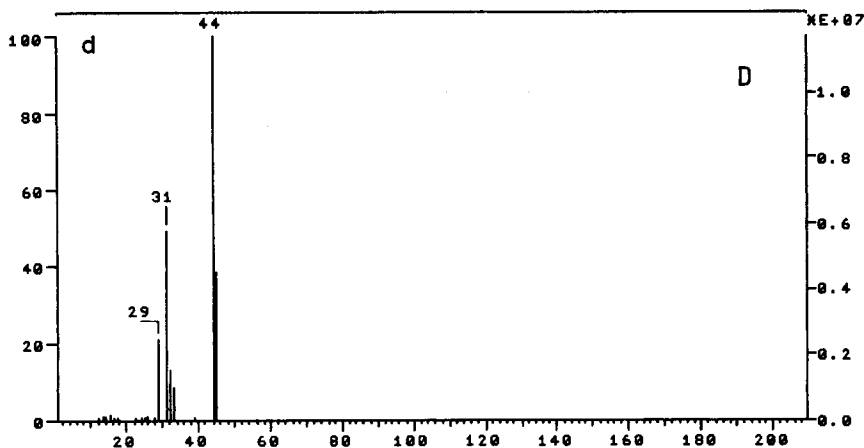


Fig. 3. Typical reagent gas spectra obtained at repeller potentials (a) 20 V, (b) 50 V, (c) 100 V, and (d) 180 V. Conditions as in Fig. 2.

attributed to changes in the effective potential of the repeller electrode. The protonated methanol cluster at m/z 97 appears to be an appropriate means of detecting the repeller contamination. In a clean source, the maximum abundance of the ion at m/z 97 is found at zero repeller potential, whereas after several days of intensive experiments, sometimes using quite high analyte concentrations, the maximum abundance of m/z 97 is observed at a repeller voltage of 70 V. In our experience the ion source needs cleaning after 2–3 weeks of working with either LC-MS or SFC-MS, to some extent depending on the types of sample investigated. It must be mentioned that the pressure in the ion source, which is a function of the mass-flow of mobile phase into the mass spectrometer, also influences the relative intensities of the peaks in the spectrum at a particular repeller voltage. This effect has not yet been studied systematically; preliminary experiments indicate that increasing the mass-flow results in an increase of the intensity of the protonated species relative to that of the molecular ions (comparable with a shift of the profile of Fig. 2 to higher repeller voltages).

A closer look at the reagent gas spectra obtained with 2% methanol in carbon dioxide indicates several other interesting aspects. At low repeller voltages, especially in the region 20–70 V, many protonated clusters are observed, most of which have only low intensities. Protonated clusters are observed with two general formulae, *i.e.* $[x\text{CH}_3\text{OH} + z\text{CO}_2 + \text{H}]^+$ and $[x\text{CH}_3\text{OH} + z\text{CO}_2 - \text{H}_2\text{O} + \text{H}]^+$. The clusters actually observed are summarized in Table II.

Collision-induced fragmentation of some of the clusters has been performed to check the assignments. When low-energy collisions in a triple quadrupole instrument are used, it does not appear to be possible to induce complete dissociation of the clusters to protonated molecules for some of the clusters studied. The daughter spectra of two of the clusters will be discussed in more detail. In the MS-MS daughter spectrum of the protonated cluster at m/z 153, $[2\text{CH}_3\text{OH} + 2\text{CO}_2 + \text{H}]^+$, the successive losses of one and two methanol molecules or one and two carbon dioxide molecules are observed, resulting in either $[2\text{CO}_2 + \text{H}]^+$ or $[2\text{CH}_3\text{OH} + \text{H}]^+$ at m/z 89

TABLE II

THE m/z VALUES OF THE PROTONATED METHANOL-CARBON DIOXIDE CLUSTERS AND PROTONATED DEHYDRATED METHANOL-CARBON DIOXIDE CLUSTERS OBSERVED IN THE DISCHARGE-ON MASS SPECTRA OF 2% METHANOL IN CARBON DIOXIDE

Cluster	x	z		
		0	1	2
$[x\text{CH}_3\text{OH} + z\text{CO}_2 + \text{H}]^+$	0	—	45	89
	1	33	77	121
	2	65	109	153
	3	97	141	185
	4	129	173	—
$[x\text{CH}_3\text{OH} + z\text{CO}_2 - \text{H}_2\text{O} + \text{H}]^+$	0	—	—	—
	1	15	59	103
	2	47	91	135
	3	79	123	167
	4	111	155	—

and m/z 65, respectively. In the daughter spectrum of m/z 129, corresponding to $[4\text{CH}_3\text{OH} + \text{H}]^+$, consecutive losses of methanol molecules are observed.

At high repeller voltages the reagent gas spectrum has the appearance of an EI spectrum of a mixture of carbon dioxide, with the molecular ion at m/z 44, and methanol, with the molecular ion at m/z 32, a base peak at m/z 31, corresponding to $[\text{CH}_2 = \dot{\text{O}}\text{H}]^+$, and the other peaks normally present in the EI spectrum of methanol.

The reagent gas spectra have also been studied at higher percentages of methanol (7–12%) in carbon dioxide. The reagent gas spectrum of 2% of methanol in the mobile phase is dominated by protonated methanol (clusters) up to a repeller potential of 80 V (*cf.* Fig. 2), but with higher methanol contents in the mobile phase an even more predominant role of the protonated methanol (clusters) is observed. The most

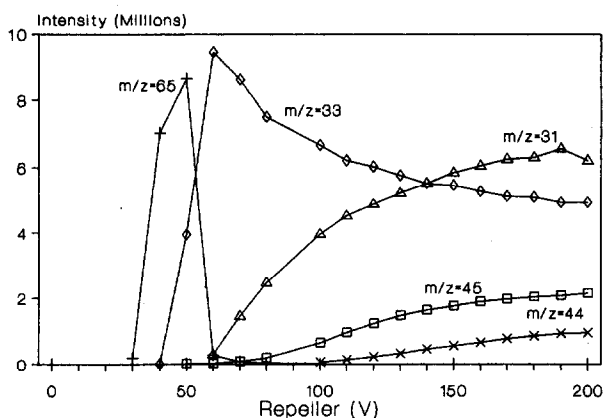


Fig. 4. Intensities (arbitrary units) of the most abundant ions in the reagent gas spectrum of 10% methanol in carbon dioxide in the discharge-on mode, as a function of the repeller voltage. Conditions: flow-rate, 1.5 ml/min; other conditions as in Fig. 2.

abundant species at various repeller voltages in the reagent gas spectra of 10% of methanol in carbon dioxide are shown in Fig. 4. At repeller potentials up to 50 V, protonated methanol clusters are the most abundant species. At repeller voltages above 150 V, the $[\text{CH}_2 = \text{OH}]^+$ ion at m/z 31 becomes the most abundant ion, while in the intermediate region the protonated molecule at m/z 33 is the most intense one. The contributions of the protonated carbon dioxide ion and the carbon dioxide molecular ion to the reagent gas spectrum are greatly reduced compared with the situation at 2% methanol in carbon dioxide (*cf.* Fig. 2). There is some increase in total ion current when comparing the results from 2% and 10% of methanol. It also appears that several of the protonated methanol-carbon dioxide clusters, reported in Table II, are less abundant at 10% of methanol than at 2% of methanol.

DISCUSSION

The reagent gas spectra observed with the methanol-carbon dioxide (or water) mixtures at low repeller potentials can to some extent be predicted from the proton-transfer concepts in the theoretical description of chemical ionization¹⁶. Like in methanol chemical ionization, as found for instance in direct liquid introduction for LC-MS¹⁷, the presence of methanol in the reagent gas results in protonated species due to a series of ion-molecule reactions. Methanol, being the stronger base (proton affinities are 777 kJ/mol for methanol, 727 kJ/mol for water and 541 kJ/mol for carbon dioxide), is expected to dominate the reagent gas spectrum. It is surprising that the potential of the repeller electrode can change the conditions to such an extent that protonated carbon dioxide can become the most abundant species, while the abundance of protonated methanol simultaneously decreases. At even higher repeller potentials, molecular ions and fragments of carbon dioxide and methanol are observed.

From the changes observed in the reagent gas spectra as a function of the repeller voltage in both LC-MS and SFC-MS, significant differences in the analyte mass spectra obtained at different repeller voltages can be predicted. For instance, the protonated methanol clusters are expected to have somewhat different proton affinities from that of the protonated methanol itself. The differences in the ion-molecule reactions between an analyte and protonated methanol (clusters), protonated carbon dioxide, or the carbon dioxide molecular ion and the effects on the analyte spectra, for instance with respect to the degree of fragmentation, can be easily seen. These differences will be discussed in more detail elsewhere¹⁸.

A sound explanation for the effects observed in the reagent gas spectra cannot be easily given. The acceleration of electrons from the discharge electrode to the lower pressure region near the high voltage repeller electrode has been suggested by Coutant *et al.*⁹. Those electrons should have reached high enough energies to ionize vaporized compounds. However, the pressure in the ion source is probably too high for the electrons to travel such a large distance in the source. The ionization of analytes can be considered as a secondary ionization process in which charged species present in the reagent gas are involved in proton-transfer and/or charge-exchange reactions. Several factors are of importance in this respect. The repeller electrode can influence the appearance of the reagent gas spectrum as it effectively samples the ions of particular regions of the spray from the vaporizer into the sampling cone. Sampling takes place

from the high-pressure centre and/or the lower pressure exterior regions of the spray. Differences in the concentrations of methanol and carbon dioxide within the spray can be expected as a result of the differences in volatility and of the temperature gradient, which is also expected to be present in the spray. These effects may also influence the composition of the various regions of the spray, the cluster formation, and the types of ionization reaction involved in the secondary ionization process. Higher repeller potentials will also increase the energy of the ion-molecule collisions in the source. Collisionally induced dissociation of protonated clusters in the reagent gas is probably of great importance in the explanation of the reagent gas spectra¹⁹.

In conclusion it can be stated that the repeller electrode can exert a strong effect on the reagent gas spectrum in the thermospray source operated under discharge-on conditions. It appears to be possible, at least in SFC-MS, to select the conditions for the ionization of the analyte by setting the potential of the repeller electrode.

REFERENCES

- 1 C. R. Blakley and M. L. Vestal, *Anal. Chem.*, 55 (1983) 750.
- 2 D. Zakett, G. J. Kallos and P. J. Savickas, presented at the 32nd Annual Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, May 27-June 1, 1984, p. 3.
- 3 F. Poeppel, J. Buchner, H. H. Lo and J. Dulak, presented at the 32nd Annual Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, May 27-June 1, 1984, p. 98.
- 4 I. A. S. Lewis, D. C. Smith and M. Veares, presented at the 32nd Annual Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, May 27-June 1, 1984.
- 5 W. H. McFadden and M. G. Tucker, presented at the 32nd Annual Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, May 27-June 1, 1984, p. 100.
- 6 C. Lindberg and J. Paulson, *J. Chromatogr.*, 394 (1987) 117.
- 7 R. H. Robins and F. W. Crow, *Rapid Commun. Mass Spectrom.*, 2 (1988) 30.
- 8 F. A. Bencsath and F. H. Field, *Anal. Chem.*, 60 (1988) 1323.
- 9 J. E. Coutant, B. L. Ackermann and M. L. Vestal, presented at the 35th Annual Conference on Mass Spectrometry and Allied Topics, Denver, CO, May 24-29, 1987, p. 419.
- 10 W. H. McFadden and S. A. Lammert, *J. Chromatogr.*, 385 (1987) 201.
- 11 J. R. Chapman, *Rapid Commun. Mass Spectrom.*, 2 (1988) 6.
- 12 W. M. A. Niessen, P. J. M. Bergers, U. R. Tjaden and J. van der Greef, *J. Chromatogr.*, 454 (1988) 243.
- 13 C. E. M. Heeremans, R. A. M. van der Hoeven, W. M. A. Niessen, U. R. Tjaden and J. van der Greef, *J. Chromatogr.*, 474 (1989) 149.
- 14 R. D. Smith, H. T. Kalinoski and H. R. Udseth, *Mass Spectrom. Rev.*, 6 (1987) 445.
- 15 J. Cousin and P. J. Arpino, *J. Chromatogr.*, 398 (1987) 125.
- 16 A. G. Harrison, *Chemical Ionization Mass Spectrometry*, CRC Press, Boca Raton, FL, 1983.
- 17 W. M. A. Niessen, *Chromatographia*, 21 (1986) 342.
- 18 W. M. A. Niessen, R. A. M. van der Hoeven, M. A. G. de Kraa, C. E. M. Heeremans, U. R. Tjaden and J. van der Greef, *J. Chromatogr.*, submitted for publication.
- 19 C. E. M. Heeremans, R. A. M. van der Hoeven, W. M. A. Niessen, U. R. Tjaden, J. van der Greef and N. M. M. Nibbering, in preparation.