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Ion-mobility spectrometry as a detection method for packed-column supercritical fluid chromatography

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ABSTRACT

Initial investigations are reported for the use of an ion-mobility spectrometer as a detector for packed-column supercritical fluid chromatography. The spectrometer was coupled to the supercritical fluid chromatography system through a post-column split, and a small portion of the chromatographic flow was introduced directly into the ion-mobility spectrometer using either a frit restrictor or an integral restrictor. The effect of a mobile phase modifier, methanol, on the gas phase ion chemistry of the detector was studied using the reactant ions normally present in the drift tube and the response of the detector to benzoquinone and benzophenone. Separations were performed for a series of Triton X oligomers to demonstrate the various operational modes of the detector. Product ions captured for these compounds had reduced mobilities, a dimensionless measure of mobility, in the range of 1.00 to 0.405, which would make these some of the largest compounds ever introduced, individually, into an ion-mobility spectrometer.

INTRODUCTION

During the last ten years, the use of supercritical fluids has matured from an experimental technique, used mainly in research laboratories, to a useful analytical tool for the extraction and separation of non-polar and moderately polar compounds. As the development has progressed a number of subdisciplines have emerged, such as capillary supercritical fluid chromatography (cSFC), packed column supercritical-fluid chromatography (pSFC), and supercritical fluid extraction (SFE). Each of these techniques has its own strengths and particular applications.

Currently, cSFC is probably the best established and most commonly used. It is a high-resolution technique capable of separating complex samples. An additional advantage of cSFC is the adaptability of the technique to a wide variety of detectors, universal and selective, particularly detectors commonly used for gas chromatography (GC). There are two basic reasons for this advantage: (1) Flow-rates in cSFC are relatively low, less than 10 ml/min of gas. Carbon dioxide, the most common mobile phase, shows no response in flame ionization detection (FID), therefore the chromatography may be accomplished with a mobile phase that neither causes a high background nor extinguishes the flame. Also, the low flow-rate is manageable for direct introduction of the chromatographic effluent into a mass spectrometer. (2) Fused-silica columns are extremely inert, with few active sites. Carbon dioxide is a relatively non-polar solvent. To increase the range of compounds which are amenable to SFC

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small amounts of organic solvents, called a modifier, are added to the carbon dioxide. However, it seems that the main effect of the modifiers is to cover up active sites on the column material [1,2]. Since fused-silica capillary columns are already inert, there is little advantage in using a modifier. In most separations organic modifiers, which would show an FID response, are not needed to elute compounds of interest. The disadvantages of cSFC are the long analysis times and the low sample-loading capacity of the columns.

Packed columns are more useful for separations which require high speed or high sample capacity, particularly for samples containing only a few components. In general, the resolution in pSFC is not as high as in cSFC. There are several characteristics of pSFC which make detection more difficult than for cSFC. The mass flow of the mobile phase is much higher. For conventional packed columns, 2–4 mm I.D., the gaseous flow-rate may be several hundred millilitres per minute. If the detector is sensitive to the mobile phase flow, such as a mass spectrometer, it is necessary to split the column effluent after the column, which means that some of the sample is lost. And, while a great deal of progress has been made recently producing inert column packings, in most cases an organic modifier is still necessary to improve separation, so that FID is difficult or impossible.

Previsous publications have described a detector based on the principles of ion-mobility spectrometry (IMS), which might be suitable for pSFC [3–5]. IMS is an instrumental technique for the separation of gas phase ions. Organic species are introduced into the spectrometer, by either direct injection or chromatography, ionized through a series of ion–molecule reactions, and separated according to their time of flight through an inert atmosphere. From this drift time the mobility of particular ion may be calculated, which provides information about the ion's size. Chromatographic detection may be accomplished by monitoring the formation of ions in the spectrometer, in a manner analogous to selected ion monitoring in mass spectrometry. Because IMS is rapid and sensitive it has been used in applications as diverse as continuous monitoring, analytical spectrometry and chromatographic detection. IMS has been successfully coupled to GC [5] and cSFC [6], but it has not yet been used with pSFC. IMS combines some of the best features of the other detection systems commonly used for these separation techniques. The sensitivity of the detector is in the picogram range. It is not necessary that analytes contain a chromophore to be detected. Selectivity is based on the size and shape of an analyte molecule, rather than on the presence of a hetero-atom. Qualitatative data about analytes may be determined in terms of drift times and moblities. And finally, IMS shows some promise for being compatible with SFC mobile phases which contain an ionizable organic compound.

There are a number of questions which must be answered concerning IMS as a detection method for pSFC: (1) How much carbon dioxide can the detector tolerate? (2) What are the effects of mobile phase modifers on the sensitivity of the detector? (3) What types of samples can be detected? This paper is an attempt to answer these questions and evaluate the use of IMS as a detection method for pSFC.

EXPERIMENTAL

A schematic of the combined SFC–IMS instrument is shown in Fig. 1.

Although the pSFC system used for this work has been described in greater detail in a previous publication [7], a brief description is presented here as an aid to the reader. Carbon dioxide, instrument grade, was supplied from a 30-kg tank equipped with a siphon tube. A 2-μm filter was installed in the carbon dioxide line shortly after the tank. The mobile phase was delivered by a pair of piston pumps (Gilson, Model 303), one for carbon dioxide and the other for the modifier. The carbon dioxide pump head was cooled to − 10°C with a cooling bath (MGW Lauda, Model RC 6) and special fitting. In addition, the carbon dioxide transfer line was coiled and immersed in the cooling bath prior to the pump, in order to precool the carbon dioxide. Modifier was delivered directly to the second pump. All organic solvents used as modifiers were high-performance liquid chromatograph (HPLC) grade. The two flows were mixed in a three-stage dynamic mixer (Gilson, Model 811). A second particle filter, 0.5 μm (Rheodyne), was placed in the transfer line prior to the injector. Injection was made with an autosampler (Gilson, Model 231) equipped with a four-port, internal loop injector (Rheodyne, Model 7413), 1 μl volume. The column temperature was controlled in a GC oven (Carlo Erba, GC 600 Vega Series). All columns were 100 mm × 2 mm I.D., packed with 3-μm particles (Stagroma, Switzerland).

The post-column flow was split using a tee with zero dead volume (Valco). The majority of the flow exited the oven, passed first through a heat exchanger, and then passed through a UV–VIS detector (Kratos, Spectraflow 783) equipped with a high-pressure flow cell. The column pressure was controlled after the UV–VIS detector by a pressure valve (HI-TEC, valve Type F-032, controller Type P-532; Bronkhorst Hi Tec, Ruurlo, Netherlands). Pressure programming was accomplished by micropro-

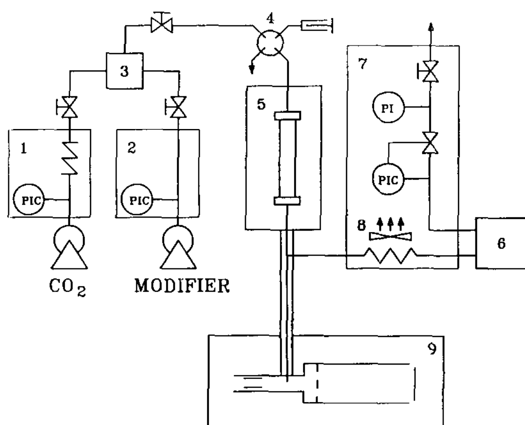


Fig. 1. Schematic diagram of the packed-column SFC system. 1 = Carbon dioxide pump and pump control; 2 = modifier pump and pump control; 3 = dynamic mixing chamber; 4 = autosampler and injection valve; 5 = column oven; 6 = UV detector; 7 = pressure control unit; 8 = heat exchanger; 9 = ion-mobility spectrometer.

cessor control of the pressure control valve. Chromatographic data were collected with a Hewlett-Packard Series 300 computer and software (Xtra Chrom II) from Nelson Analytical.

A small part of the flow was routed to the IMS system. A 1-m × 60 μm I.D. fused-silica tube was used as the transfer line. The temperature of the transfer line was maintained using the inlet temperature of the ion-mobility spectrometer. Flow into the detector was controlled by a restrictor at the end of the transfer line. As usual, the choice of restrictor type was important. Frit restrictors (Lee Scientific) were reliable, but did not efficiently transfer high-molecular-weight compounds from the high pressure of the SFC to the atmospheric pressure in the ion-mobility spectrometer. Guthrie, or integral, type restrictors (J&W Scientific) were more successful, but there were problems with plugging and the reproducibility of retention times.

The ion-mobility spectrometer, Model GHT 100, maximum operating temperature 250°C, was purchased from PCP (West Palm Beach, FL, USA). Mobility spectra were collected with a computer (IBM AT) equipped with an analog-to-digital (A/D) board and digital storage oscilloscope emulation software (Graseby Analytics, UK). Nitrogen was used as the drift gas for all experiments; the flow-rates of the drift gas and make-up gas were 400 and 150 ml/min, respectively. A 0- to 5-V output of the ion-mobility spectrometer could be used to collect chromatographic data on the Nelson system.

Qualitative data about the ions in the spectrometer may be gathered from the drift times and mobilities of the ions. Since drift times are dependent on the strength of the electric field, they are usually expressed as a reduced mobility, which is independent of the electric field. The drift time data must then be further corrected for the number density conditions of the drift gas. To compare ion mobilities under equivalent conditions of number density, the mobility must be corrected for temperature and pressure differences, and expressed as a reduced mobility, K_0. The reduced mobility may be calculated from eqn. 1 [8]:

$$K_0 = (L/t_d E)\,(273.16/T)\,(P/760) \quad (1)$$

where T is the temperature of the drift tube in K, P is the atmospheric pressure in mmHg, E is the electric field gradient in V/cm, t_d is the drift time in s and L is length of the drift tube in cm.

An alternative method of computing reduced mobilities using an internal standard has been proposed [9]. Following the suggestion by Karpas [9], 2,4-lutidine was used as the standard and assigned a reduced mobility of 1.95. The mobilities of unknowns could then be calculated from eqn. 2. This method of calculating K_0 eliminates the need to measure the barometric pressure, eliminates errors due to inexact measurement of any of the variables in eqn. 1, and should eliminate some of the disagreement in K_0 values due to design differences in ion-mobility spectrometers:

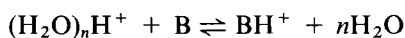
$$K_{0,2} = (1.95)\, t_{d,1}/t_{d,2} \quad (2)$$

where $K_{0,2}$ is the reduced mobility of the analyte, $t_{d,2}$ is the drift time of the analyte and $t_{d,1}$ is the drift time of the internal standard, 2,4-lutidine.

RESULTS AND DISCUSSION

If carbon dioxide is not efficiently swept from the detector, it may contaminate the drift region and affect the drift times of ions in the detector. It has been shown that drift times are lengthened and sensitivity is lost in a carbon dioxide drift gas [10]. It has also been shown that carbon dioxide can interfere with drift time measurement in a bidirectional flow instrument [11]. It was found that for this particular model up to about 40 ml/min of carbon dioxide did not interfere with the detector. At higher flow-rates the drift times of the reactant ions changed with changes in the carbon dioxide flow, indicating that carbon dioxide contaminated the drift region of the spectrometer, and the intensity of the reactant ions decreased, until finally, at flows over 100 ml/min, the reactant ions completely disappeared. In general the flow of gas into the ion-mobility spectrometer was kept to approximately 20 ml/min. For a 2-mm packed column, the SFC system was operated at a flow of 2 ml/min of liquid carbon dioxide; the split after the column was about 40:1.

Organic analytes which enter the detector are ionized by a series of ion–molecule reactions with background ions, called reactant ions, which exist in the spectrometer. When nitrogen is used as the drift gas these ions have been identified as $(H_2O)_xNH_4^+$, $(H_2O)_yNO^+$ and $(H_2O)_zH^+$, where x, y and z are equal to 0, 1, 2, 3 and depend on the temperature of the drift tube [12–14]. If a neutral analyte entering the ion source has a greater gas-phase basicity than water, the analyte will become protonated through the following general reaction:



$$(H_2O)_nH^+ + B \rightleftharpoons BH^+ + nH_2O$$

The identity and quantity of the reactant ions then determine the sensitivity of the detector. The integrity of the gas phase chemistry in the drift tube can be determined by the ions present, the most obvious clue to identity being the drift time.

Since most pSFC separations require a modifier, it was necessary to determine the effect of the modifier on the identity of the reactant ions. Because methanol is the most commonly used modifier, initial experiments were done with a methanol modifier. The changes in the reactant ions as the amount of methanol in the mobile phase is increased are shown in Fig. 2. Moderate amounts of carbon dioxide by itself had no effect on the mobilities and intensities of the reactant ions. As the concentration of methanol is increased the hydrated water ion is depleted and a new reactant ion, with a longer drift time, is seen in the detector. A summation of reactant ion drift times for different methanol concentrations is given in Table I. This is also true when the pressure of the system is increased for a given concentration of methanol, since the increase in pressure increases the flow through the restrictor.

The effect of a methanol modifier on the IMS sensitivity can be seen in Figs. 3 and 4, the analyses of benzoquinone and benzophenone, respectively. In each case a probe molecule was separated from the solvent by SFC at 80°C, pressure programmed from 125 to 250 bar in 10 min. IMS conditions are given in the figure captions. In Fig. 3 the chromatographic response of the detector to benzoquinone is plotted for conditions of no modifier and 1% methanol as a modifier. Because benzoquinone is a relatively strong base, the modifier has only a small effect on the sensitivity of the detector. Minimum detection limits for both cases are very similar,

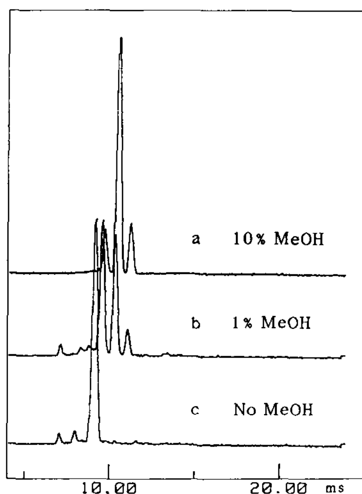


Fig. 2. Reactant ion-mobility spectra. IMS conditions: 225°C, 196.5 V/cm, entrance gate 0.2 ms, total scan time 24 ms, average of 64 scans. Methanol (MeOH) modifier added to mobile phase: (a) 10%; (b) 1%; (c) none.

approximately 0.0020 mg/ml when no methanol is present and approximately 0.0025 mg/ml when 1% methanol is added as a mobile phase modifier. Detection limits were estimated from twice the peak-to-peak noise level.

In Fig. 4 it can be seen that the methanol modifier significantly reduces the IMS response to benzophenone, which is a weaker base, especially at high analyte concentrations of modifier. While the minimum detectable amount of compound is very similar to that found for benzoquinone when only 1% methanol is present, for a 10% concentration of methanol the minimum detectable amount is reduced to approxi-

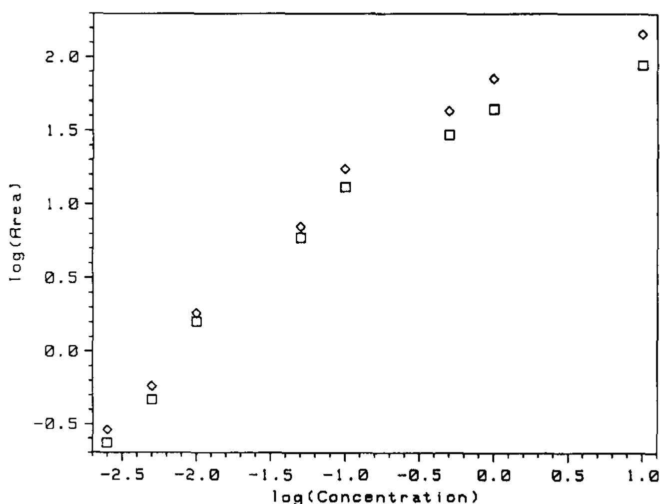


Fig. 3. Effect of methanol modifier on IMS response to benzoquinone. IMS conditions as in Fig. 2; drift times monitored from 12 to 18 ms. ◇ = No methanol; □ = 1% methanol.

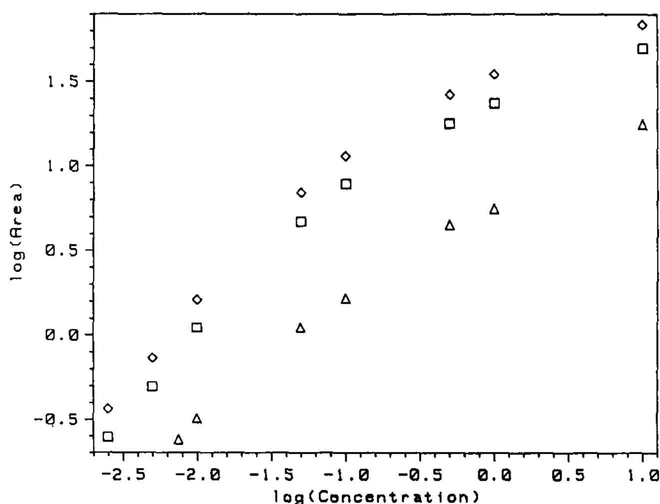


Fig. 4. Effect of methanol modifier on IMS response to benzophenone. IMS conditions as in Fig. 2; drift times monitored from 12 to 18 ms. ◇ = No methanol; □ = 1% methanol; △ = 10% methanol.

mately 0.0079 mg/ml. This may be expected from the table of gas phase basicities compiled by Kebarle [15]. The response of the detector then will depend on the amount of methanol entering the ion-mobility spectrometer, which is dependent on the modifier concentration and the column pressure. It should be kept in mind that these figures also reflect the split of the mobile phase. The detection limits reported are for concentrations introduced into the chromatograph. If the entire column effluent was introduced into the spectrometer, there would of course be more sample in the detector, but there would also be more interference from the methanol.

pSFC separation of a Triton X-114, an octyl phenol polyethylene glycol ether non-ionic surfactant (average monomeric subunits, n = 7–8), is shown in Fig. 5. Although the concentration of the methanol is relatively high, detection could be accomplished by monitoring drift times outside the range of the reactant ions. The sample, a 2.5% (w/w) solution of Triton X-114 in methanol was separated on a C_{18} column at a temperature of 150°C. The carbon dioxide flow was 2 ml/min and the

TABLE I

REDUCED MOBILITIES OF REACTANT IONS

K_0 ($cm^2\ V^{-1}\ s^{-1}$)

Ion	No methanol	1% Methanol	10% Methanol
1	3.12	3.08	2.28
2	2.74	2.63	2.11
3	2.38	2.49	1.96
4	–	2.29	–
5	–	2.11	–
6	–	1.96	–

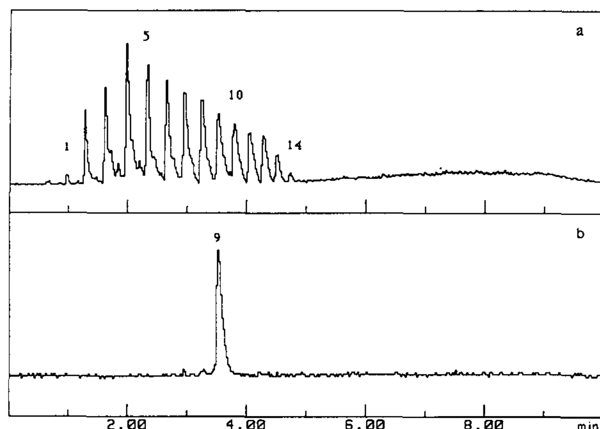


Fig. 5. Chromatograms of Triton X-114. IMS conditions: 150°C, 250 V/cm, 60-ms scan, entrance gate 0.5 ms. (a) Drift times monitored 14–50 ms. (b) Drift times monitored 27.7–28.7 ms.

methanol modifier flow was 0.15 ml/min. The pressure was programmed from 125 to 320 bar in 8 min. IMS conditions are given in the figure caption. Non-selective detection of the polymer mixture is shown in Fig. 5a. It was also possible to demonstrate selective detection of this compound, as shown in Fig. 5b. A list of the reduced mobilities for the ions captured is given in Table II. Reduced mobility values were calculated from an assigned reduced mobility of 1.95 and observed drift time of 10.60 msec for 2,4-lutidine for the IMS conditions given in the figure caption.

A heavier version of the same compound, Triton *X*-305, average monomeric subunits, $n = 25$, is shown in Fig. 6. In this case the column temperature was 165°C,

TABLE II

REDUCED MOBILITY VALUES FOR TRITON X-114 OLIGOMERS

Peak No.	Retention time t_r (min)	Drift time t_d (ms)	Reduced Mobility ($cm^2 V^{-1} s^{-1}$)
1	0.95	14.8	1.40
2	1.14	14.7	1.41
3	1.61	21.3	0.970
4	1.96	22.4	0.923
5	–	23.5	0.880
6	2.66	24.6	0.840
7	2.94	25.8	0.801
8	3.24	27.0	0.766
9	3.52	28.2	0.733
10	3.79	29.4	0.703
11	4.04	30.5	0.678
12	4.27	31.7	0.652
13	4.51	32.8	0.630
14	–	33.9	0.610
15	–	34.9	0.592
16	–	36.0	0.574

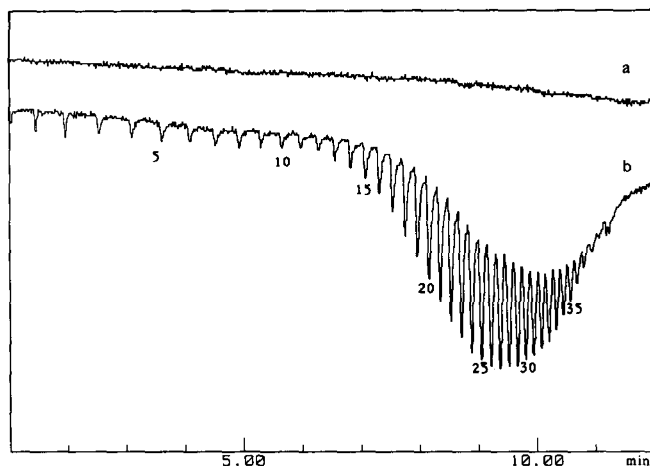


Fig. 6. Chromatograms of Triton X-305. IMS conditions: 100°C, 268 V/cm, 60-ms scan, entrance gate 0.5 ms, drift times monitored 8–12 ms. (a) Methanol injection. (b) Triton X-305 in methanol [10% (w/w)].

the pressure was programmed from 125 to 350 bar in 12 min, and the methanol modifier was programmed from 0.15 to 0.25 ml/min in 10 min. IMS conditions are given in the figure caption. Detection was accomplished by monitoring the depletion of the reactant ions, this is a non-selective method of detection which in this case resulted in better sensitivity, and explains why negative peaks are seen in the chromatogram. Although the reactant ions change, due to the presence of methanol and pressure programming, because the entrance gate was open for a long time, lowering the resolution of the instrument, the reactant ions appeared unchanged as the chromatography progressed. The detector baseline for these conditions is shown in Fig. 6a. This sample was unusual in that the depletion of the reactant ions was associated with the disappearance of any product ion. The drift times and reduced mobilities for some of the product ions captured are shown in Table III. While product ions were captured for all the peaks in the chromatogram, only a few are reported. The peak numbers were assigned, as accurately as possible, by matching the peak retention time with the time elapsed in the chromatographic run. Product ions were observed with

TABLE III

REDUCED MOBILITY VALUES FOR TRITON X-305 OLIGOMERS

Peak No.	Retention time t_r (min)	Drift time t_d (ms)	Reduced Mobility ($cm^2 V^{-1} s^{-1}$)
1	1.51	20.1	1.063
5	3.80	24.5	0.872
10	5.84	30.1	0.710
15	7.22	35.2	0.607
20	8.31	39.9	0.536
25	9.17	44.2	0.484
30	10.08	48.4	0.442
35	10.52	52.8	0.405

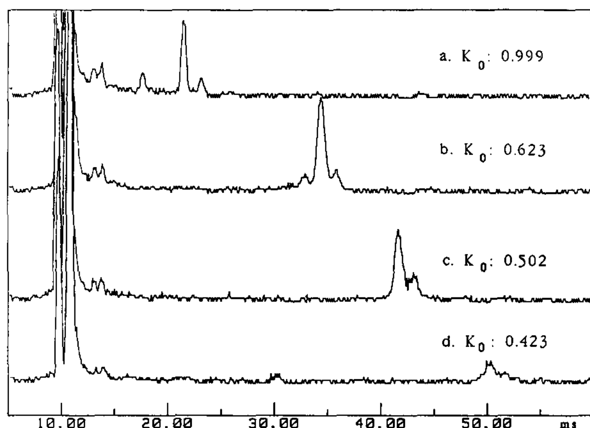


Fig. 7. Ion-mobility spectra of Triton X-305 oligomers. IMS conditions: 100°C, 268 V/cm, 60-ms scan, entrance gate 0.5 ms, average of 32 scans. (a) Peak 2, drift time 21.4 ms, K_0 = 0.999. (b) Peak 14, drift time 34.3 ms, K_0 = 0.623. (c) Peak 23, drift time 42.6 ms, K_0 = 0.502. (d) Peak 32, drift time 50.2 ms, K_0 = 0.423.

drift times as long as 52.8 ms and reduced mobilities as low as 0.405. Examples of the product ions captured are shown in Fig. 7.

The introduction to this paper posed three questions concerning detection by IMS after pSFC. The first related to the amount of carbon dioxide which could be introduced into the detector. Our experience showed that the limit appeared to be approximately 40 ml/min of gaseous carbon dioxide. At flow-rates much above this the drift time of the reactant ions began to change and the intensity of the reactant ions decreased, until, at about 100 ml/min, the reactant ions disappeared altogether. It would seem then that the entire flow from capillary columns [16] and micropacked columns may be introduced into the spectrometer, but that for larger columns the mobile phase flow must be split.

The mobile phase modifier was seen to have an effect on the performance of the spectrometer. This is in contrast to earlier work [16]. However, this work was done on capillary columns, where the flow of modifier into the spectrometer is much smaller. A second difference is that the piston pumps used in this work leave no possibility for the modifier to remain in the pump.

Lastly, the types of samples used in this paper showed good response, in some cases superior to UV–VIS detection currently in use. However, a broad study of high-molecular-weight compounds which respond in the ion-mobility spectrometer has not yet been thoroughly performed.

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