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Time-resolved cryogenic modulation reveals isomer interconversion profiles in dynamic chromatography

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Abstract

The dynamic chromatographic study of interconversion of *E* and *Z* forms of oximes has been investigated by using a novel cryogenic modulation method in a two-dimensional gas chromatographic array. The primary column is a conventional capillary GC column on which the molecular interconversion proceeds. In this case, the molecular dynamical process leads to a peak profile describing the kinetics and thermodynamics of the interconverting molecules during its chromatographic elution. Thus an interconversion region intercedes the elution of the individual stereoisomers of the reaction. Since the molecules are isomers, classical molecular identification methods such as gas chromatography–mass spectrometry are unable to study the individual instantaneous amounts of each of the compounds. Hence the infinitesimal profiles of interconversion along the entire column have never been experimentally observed; rather the total profile is normally subjected to mathematical modelling studies in order to match experiment with theory, and to gain the kinetic parameters of the process. In the present study, an instantaneous ratio of the individual isomers can be found during the chromatographic elution by direct measurement. This is achieved by using a cryogenic zone focussing process, with rapid longitudinal modulation of a cold trap and continual pulsing of collected zones into a fast-analysis high-resolution capillary column on which isomer interconversion is minimized. The data can be displayed as a two-dimensional contour plot to demonstrate the individual isomer profiles. The two-dimensional analysis also allows easy measurement of the peak ratios of the two isomers which is an indicator of the extent of interconversion that has taken place. Two model systems, acetaldoxime and butyraldoxime, were chosen to illustrate the use of the cryogenic modulation procedure. It is anticipated that the procedure could be applied to other molecules which exhibit gas-phase isomerizations or reactions.

Keywords: Gas chromatography, comprehensive two-dimensional; Molecular dynamics; Thermal interconversion; Oximes

1. Introduction

Dynamic chromatography is a well-established method to investigate analytes undergoing molecular reactions, e.g. enantiomerization, epimerization, isomerization and decomposition, during a chromatographic separation. Elution profiles of binary interconversions obtained by dynamic chromatography are generally characterized by plateau formation or peak broadening. Yet the prerequisite is the quantitative on-column separation of the binary mixture into the two stereoisomers in the respective chromato-

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graphic setup. For instance, chair–chair forms of mono-substituted cyclohexanes have a very small conformational energy difference (≈25 kJ/mol), and so in a GC study they will be chromatographically identical with only a single averaged peak being found. This coalescence phenomenon is despite any likelihood that the two forms might have different retention factors. In the chromatography reactor, the peak profile no longer represents that of a unique single non-reacting compound, and it can be used to either demonstrate that some reaction has taken place, or used to derive kinetic data for the reaction under consideration. The literature provides examples of a range of interconversion processes occurring during chromatography, covering almost all the major areas of separation techniques including GC [1–18], supercritical fluid chromatography [19], HPLC [20–32], planar chromatography methods [33], and capillary electrophoresis [34–37].

A schematic dynamic molecular description of the chromatographic reactor is shown in Fig. 1. Each of the equilibria will be controlled by their kinetic and thermodynamic parameters considering that the kinetic step may not be reversible. If the energy barrier of interconversion (A⇌B) is diminishingly small compared to the energy input into the system a reasonable equilibrium for the interphase transfer process $A_M \rightleftharpoons A_S$ or $B_M \rightleftharpoons B_S$ is allowed, consequently only a single narrow averaged peak will be found (peak coalescence phenomenon of the third kind) [38]. Under circumstances where there is a reasonable similarity in the energy requirements for these processes, then the characteristic plateau formation of the elution profile can be observed which reflects the molecular interconversion. Oximes (Fig. 2) are configurationally labile compounds during the time-scale of the GC measurement, and will be the model compounds for the present study.

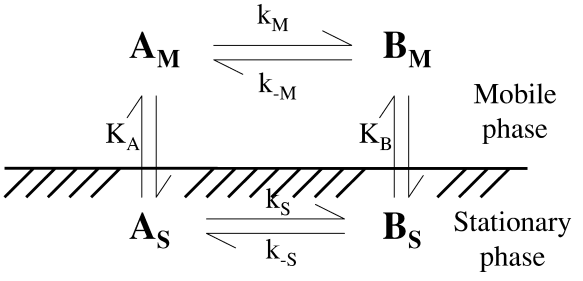

Fig. 1. Schematic diagram of the molecular interconversion and molecular distribution processes describing dynamic secondary equilibria and molecular separation of the isomers respectively. K_A and K_B are the distribution constants for isomers A and B controlling the separation. k_S, k_M and k_{-S}, k_{-M} are the interconversion rate constants which determine the extent of reaction in the mobile (M) and stationary (S) phases, for the forward (A→B) and reverse (B→A) reaction respectively.

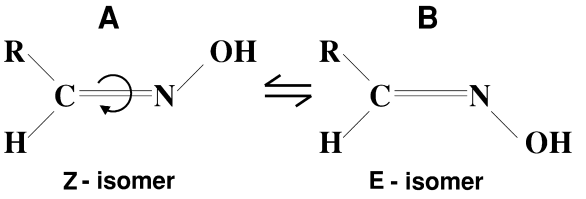

Fig. 2. Representative reaction for interconversion of the isomer forms of the oxime compounds. A = *Z* isomer; B = *E* isomer.

The typical GC result given by the chromatography reactor can be seen in Fig. 3. Species A and B are *E*,*Z*-stereoisomers, and the degree or extent of interconversion is demonstrated by the shape of the interconversion region. The two additional peaks (internal standard and the peak at ca. 14.5 min) represent the expected peak shape of a single regular non-reacting solute in the GC experiment. The greater the extent of interconversion, the less will be the height of the terminal stereoisomer peaks A and B. This allows study of the kinetics of the reaction

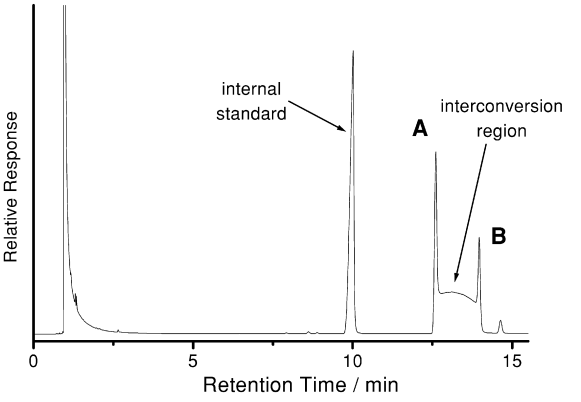

Fig. 3. Typical chromatogram of a dynamic secondary equilibrium (interconversion) process for molecules A and B (for butyraldoxime in this instance), illustrating the plateau region between the two ''limiting'' species. The unconverted molecules of A and B still define a peak shape equivalent to a non-interconverting species, as indicated by the internal standard peak.

by using appropriate experiments involving variation in temperature of the oven or time of interconversion (by variation in carrier flow velocity). Another method is to simulate the elution profile with the theoretical plate model or stochastic model until the simulated chromatogram fits the experimental. This latter approach enables calculation of a theoretical abundance–time distribution of both A and B underlying the total distribution given by the overall peak shape. In either case, there are only a few experimental and theoretical papers [26,27,36] that can separately confirm the actual abundances of molecules A and B in the experiment, or their separate profiles in the interconversion region. This is precisely because, in almost all cases, A and B are isomers that have very similar detection responses and so unique instantaneous amounts cannot be found. For instance, mass spectrometry will give the same spectra for A and B and only the total summed abundance of the two compounds will be obtained rather than the individual amounts of the isomers. The few potential spectroscopic detection methods that could be used for this (in GC and/or HPLC) will be Fourier transform infrared, non-dispersive infrared (NDIR) and NMR, however it is not apparent in the literature if these tools were used in chromatography reactor studies.

A novel cryogenic modulation system for general use in multidimensional GC has been pioneered by one of the present groups, which has a range of advantages in high-resolution studies of complex samples and in routine GC analyses. The broad applicability of the method to generate novel information from chemical separations has been reviewed recently [39,40]. The essence of the procedure is to use a small CO_2-cooled trap near to the junction of two columns, and by oscillating the trap along the column segment it is possible to sequentially cryofocus and then rapidly evaporate any volatile solutes which enter the cold region. The capillary column is inserted through the trap, so there are no additional connections to affect the carrier gas flow or solute migration. Further applications of the method have recently been demonstrated, such as targeted multidimensional analysis [41], and the cryo-modulator has been used for a comprehensive two-dimensional gas chromatography experiment for enhanced separation and sensitive analysis of a variety of sample types such as essential oils [42]. The present study is an extension of these basic principles to the specific objective of analysing coeluting isomers throughout the chromatographic reactor interconversion profile, which is a major study area of the other of the present groups. Since the modulated cryogenic method works by zone-compressing the volatile solute and then pulsing the band to the second column, the only requirement is to be able to separate the co-trapped solutes on the second column. The second column is a very fast elution column, so it is valid to assume that there is insufficient time to undergo any interconversion within the few seconds residence on this column. The two peaks recorded at the detector are a precise measure of the relative proportions of the two isomers at that point in the progress of the chromatographic elution.

Horvath and Melander, who worked with HPLC of proteins and peptides that contained the proline group, which was known to undergo a structural change during the separation process, developed a modelling procedure for the *cis–trans* isomerization data [43,44]. The first simulation program for the evaluation of enantiomerization experiments based on the theoretical plate model was developed by Schurig et al. [3] and later extended for simulations up to 120 000 theoretical plates (SIMUL) [6–9,14]. Other researchers modified and advanced this first computer simulation program such as König and co-workers [10,12,13,15,19], Gasparrini et al. [31], Oxelbark and Allenmark [32], and Boyer [45]. The stochastic model [46] has also been applied to determine reaction rate constants from interconversion peak profiles by Veciana and Crespo [27] followed by Mannschreck and co-workers [24–26]. Recently, a new computer program (ChromWin), based on both the theoretical plate and the stochastic model, was developed by Schurig and co-workers [16–18,36,37]. These studies present underlying profiles of the two compounds participating in the interconversion, based on the simulated data. In none of these instances are these profiles supported by direct experimental evidence for abundances of the individual compounds. In the slightly different case of irreversible thermal dissociation, as reported by Langer and co-workers [47,48] for dicyclopentadiene (DCP) dissociation to the monomer, the asymmetric

peak distribution comprises only the product of dissociation, and so the individual molecule profile is available for the chromatogram. However, overlapping the original DCP peak is some trace cyclopentadiene (CP), and the actual shape of the CP contribution under the DCP peak can only be mathematically predicted.

The aim of the present study is to demonstrate that by using the cryogenic modulation procedure, reliable isomer profiles can be found over the total dynamic chromatography peak distribution.

2. Experimental

2.1. Gas chromatography system

A Model 6890GC system (Agilent Technologies, Burwood, Australia) with a Chemstation data system was used in these studies to obtain the cryogenic modulation data, with flame ionization detection operated at a data collection frequency of 100 Hz. The Chemstation event control is used to instruct the modulation control system to commence modulation at a precise time. Split injections were used throughout. For preliminary studies, a HP5890GC system was used with a single column (the same BP21 column reported below).

2.2. Column set and GC conditions

The column set used for the two-column multidimensional system incorporated a primary column of 20 m×0.25 mm I.D., 0.5 μm film thickness (d_f) BP21-coated column (polyethylene glycol terephthalate terminated; polar column), directly coupled to a second, BP20-coated column of 0.8 m×0.1 mm I.D., 0.1 μm d_f (polyethylene glycol; polar column). Both columns were from SGE International (Ringwood, Australia). Preliminary studies were conducted using a Hewlett-Packard 5890 GC, with flame ionization detection (FID) and a single column (the primary column noted above). The 5890 FID does not operate at the fast data acquisition rate required for the pulsing studies, which were all carried out using the 6890 instrument. The GC oven was operated under a variety of conditions depending on the objective of the particular study. Both isothermal and temperature program conditions were employed as required. Generally the isothermal temperature range was from 60 to 150°C, with an injector pressure setting of 2.5–25 p.s.i. (1 p.s.i. = 6894.76 Pa). The temperature program rates varied from 5 to 20°C/min, again depending on the study. Split injection was employed, with a split ratio of ca. 20:1–50:1.

2.3. Cryogenic modulator

The GC×GC experiment is achieved here by use of a cryogenic modulator as described elsewhere [49]; the pneumatic system outlined therein is replaced with a motor drive in the present model. The first demonstration of the principles of rapid longitudinal cryogenic modulation was reported in 1997 [50], and a summary of its unique capabilities was recently outlined [39]. The present unit, the LMCS Everest model, was from Chromatography Concepts (Doncaster, Australia). The cryogenic system was retrofitted to the 6890GC system. It was operated under various modulation timing periods from 2 to 4 s cycle time and 0.5 s hold time in the release position. The CO_2 cryogen coolant maintains the temperature of the modulation trap to at least 100°C below the prevailing oven temperature. Ref. [49] should be consulted for details of the instrument setup.

2.4. Data conversion

Chemstation data (100 Hz; time–response area and height) may be exported in ascii file format (*.csv files) for subsequent data display. The original exported file was read into Origin (Microcal Software, Northampton, MA, USA) to present the chromatographic traces, with contour displays presented by using the Transform program (Fortner Research, VA, USA).

2.5. Samples

The acetaldoxime and butyraldoxime samples were obtained from Aldrich and Tokyo Kasei respectively, and used as received. Samples of ca 1% concentration were prepared in acetone solvent.

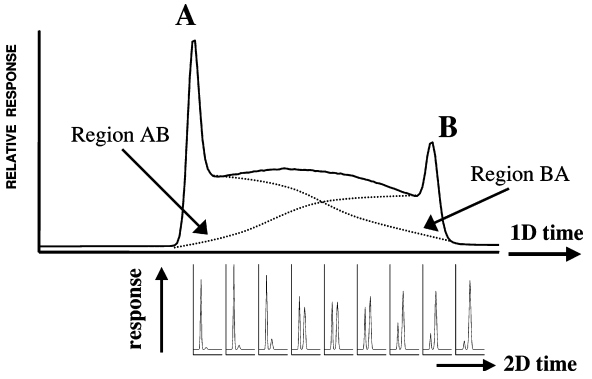

Fig. 4. Hypothetical curves (dotted lines) for the underlying isomers A and B which arise from reaction of original B and A molecules. Region AB shows the distribution of product B arising from A undergoing reaction; region BA shows the distribution of product A arising from B undergoing reaction. The plot below the main chromatogram illustrates what should be obtained if isolation and measurement of the isomers can be achieved instantaneously and continuously during the chromatographic analysis.

3. Results and discussion

3.1. The approach to obtaining modulated data

Fig. 4 is a schematic representation of the concept of rapid separation of zone compressed segments of the overlapped chromatographic profiles of the interconverting isomers A and B. Here there are only nine separate second dimension chromatograms describing the peak profile. Each second dimension analysis shows narrow fully resolved peaks of A and B. Their relative amounts (areas) will be equivalent to the total amounts of A and B collected during the zone compression (trapping) step, of 2–4 s duration here. It can be seen in this hypothetical case that component A is the major component in the early second dimension analyses, whilst B dominates later in the distribution.

A modulation frequency suitable for the time duration of the interconversion process is required. This is determined by the retention factors of the stereoisomers, and their consequent retention times. A sufficient number of data points is required in order to fit the modelling data and this will in turn suggest a maximum pulsing time interval to use. For instance, if 30 data points are deemed necessary, and the terminal peaks are 2 min apart, then a modulation period of at least 120 s/30=4 s is needed. This is easily achieved. A second consideration is the time of elution on the second column, and the relationship this has with the conditions which cause or lead to interconversion on the first column. If the pulses are effected every 4 s, then it is preferable that both isomers elute from the second column within 4 s. This is not an absolute requirement, but is preferable; peaks from one pulsing event should not overlap those from a subsequent pulse. For the column set used, it can be approximated (using the formula $K = k\beta$) that the later compound should elute in about 2.1 s. This is well within the 4 s modulation period, and is confirmed by experiment. A frequency of 0.33 s^{-1} will still be within the experimental peak elution limitation and will provide more data points.

The second column has two functions. The first is to elute the isomers rapidly so that there is almost negligible interconversion within this time and at the column temperature used, and secondly to ensure there is adequate separation so that precise areas for the separated components might be measured. The first depends on the phase ratio variation in the columns, the distribution constant of the later eluted isomer (determined by the choice of phase), the column length and the carrier flow-rate. The second is related to the efficiency of the second column (having a small I.D., it will show greater efficiency than the first column, per metre) and the absolute difference in the isomer retention factors. In this present experiment, different columns for the first and second dimensions have been chosen. This served the purpose of the study undertaken. It is entirely possible here that the same column can be used in both dimensions, provided the above requirements are met. It is only necessary that the cryogenically collected isomers are separated on the second column.

The two primary variables which affect the chromatographic presentation of the interconverting process are temperature (which changes the rate of reaction), and flow-rate (which alters the time allowed for the conversion and hence extent of the interconversion that has taken place). The overall ''dynamic chromatogram'' shape will also depend on the absolute difference in retention times of the two isomers with respect to their peak widths. Thus two experiments may be conducted to obtain varying

degrees of interconversion. By increasing the oven temperature, less of the unconverted isomer exists, and the interchange or plateau region increases. By increasing flow-rate at constant temperature, less time is available for reaction so less interconversion occurs.

3.2. Typical results

By way of further exemplifying the conceptual framework of the chromatography approach, Fig. 5 is actual data recorded for butyraldoxime with Fig. 5A showing the non-modulated profile, Fig. 5B is the pulsed result (a 4 s pulse duration was used), and Fig. 5C is an expanded trace of three sequential pulses shown by the circle in Fig. 5B. The baseline resolution in the latter case should be noted. The effect of zone compression on peak sensitivity is demonstrated by the relative responses of Fig. 5A (22 pA for the larger isomer peak) and Fig. 5B (1040 pA) — a factor of some 45-fold better. It can be discerned that the two pulsed peaks swap their relative magnitudes at about 8.25 min. The peak basewidths are about 0.125 s, indicative of the fast analysis on the short second column. The peak ratio of the butyraldoxime isomers shows that the first eluted isomer is the more abundant in the original mixture. In accord with previous GC analyses of these compound types [51], the *Z* conformation isomer, labelled ''A'', is taken as eluting first from the GC column.

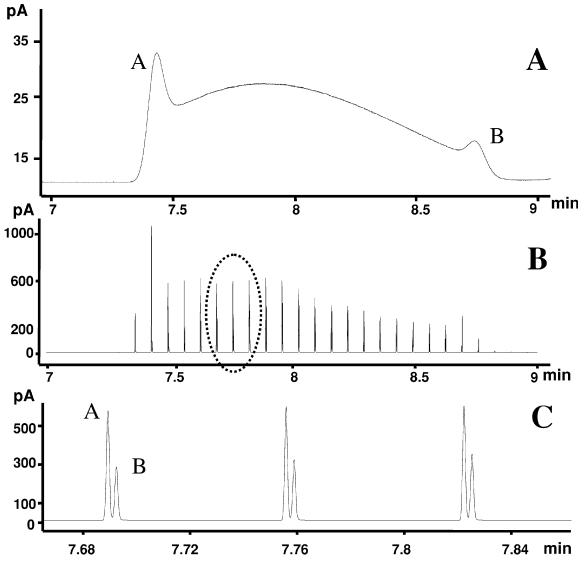

Fig. 5. Demonstration of cryogenic modulation two-dimensional analysis of butyraldoxime at 90°C, and column head pressure 20 p.s.i. (A) Normal GC result. (B) Pulsed chromatogram with modulation duration of 4 s, showing the excellent correspondence of the overall chromatography ''envelope'' shape. Note that the pulse response is about 50 times greater than the response in the normal GC trace in (A). (C) Expansion of the circled region in (B) indicating that the two isomers are collected and analyzed on the second fast column (with an elution time of ca. 1.33 and 1.55 s after the modulation step for isomers A and B, respectively). The two peaks are 0.22 s apart, and are essentially baseline resolved. Peak basewidths are approximately 125 ms.

Fig. 6 shows chromatograms of acetaldoxime at 80°C. This sample has a more abundant later-eluted isomer. In comparison with the above butyraldoxime result, acetaldoxime will have a lower activation energy for interconversion because a lower temperature and faster carrier flow-rate is used in Fig. 6 compared with Fig. 5, but similar extents of interconversion are apparent. The expanded trace (Fig. 6B) shows an interesting cross-over pattern for relative isomer abundance (they swap at about 8.22 min) and almost baseline separation.

By reducing the flow-rate by using lower carrier pressure (5 p.s.i.), the elution profile is dominated by the interconversion profile. This means that all of the injected mixture of A and B has statistically undergone at least one interconversion process. There is nothing to prevent a particular molecule undergoing conversion from A to B, then back to A and so forth. This happens more rapidly as the temperature increases, or is more likely to occur as time increases. Fig. 7 illustrates this for acetaldoxime. In comparison to Fig. 6, now the molecular distribution starts to resemble a Gaussian packet of pulsed peaks, with two interleaved envelopes apparent. Note that the first envelope is of smaller response magnitude than the later eluting envelope, and has a smaller total area than the second envelope. The lower flow-rate for Fig. 7B compared with Fig. 6B means that the second column is now more efficient, and the pulsed peaks are better resolved as seen for the inset expanded peaks. The two interleaved envelopes of isomer peaks have distribution maxima that are relatively close together, but do not coincide. They will only coincide if the rate of conversion is so fast that neither of the isomers can be individually

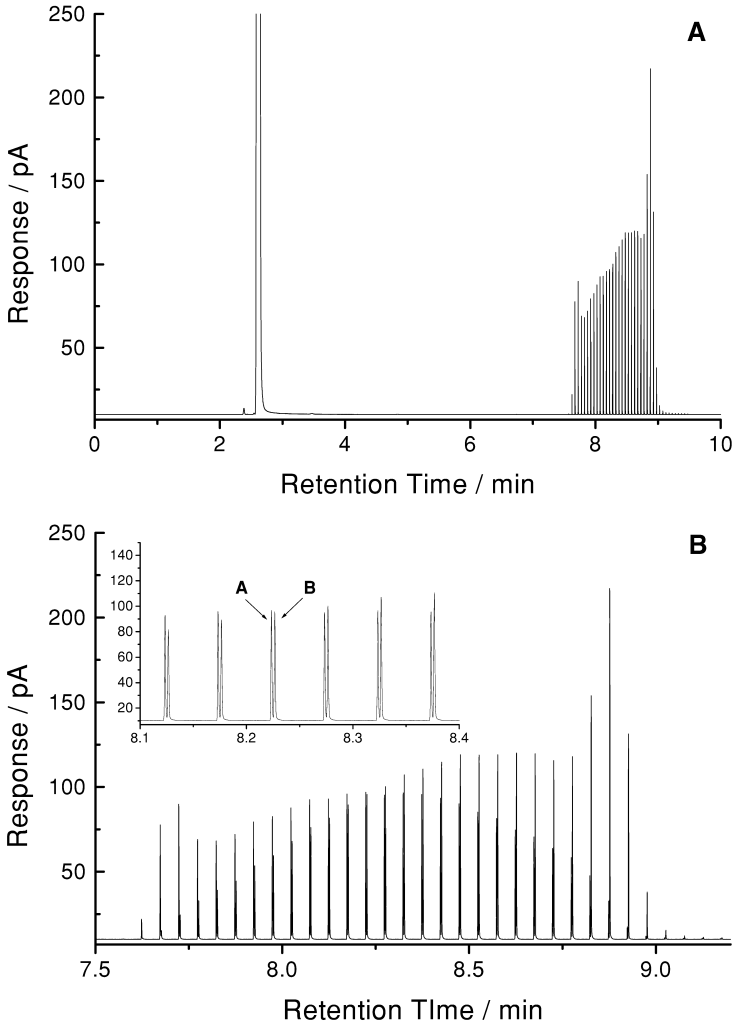

Fig. 6. Cryogenic modulation two-dimensional analysis of acetaldoxime at 80°C, and column head pressure 10 p.s.i. (A) Full chromatogram (note, modulation commences after the solvent passes through the cryogenic zone). (B) Expansion of the pulsed peak region with an inset showing almost baseline resolution of the two isomers.

recognized. The peak envelopes look very broad compared with an internal standard comparison peak, but again this is only due to the peak range still being defined by the hypothetical elution of isomer A and B, which will be at about 14.5 and 17.5 min if they were conformationally rigid. An even better example of faster interconversion is shown in Fig. 8, which shows the expanded chromatogram of butyraldoxime under conditions of almost completely overlapping distributions (90°C and 5 p.s.i.). In this case the mean, median and modes of the two isomer peaks can be expected to be quite close, and resemble even more closely a Gaussian distribution.

More data points are obtained by modulating faster. Fig. 9 is a more extreme case of very well separated isomers (stereoisomers) whilst only allowing limited interconversion, achieved by a low oven temperature and high flow-rate for butyraldoxime. A modulation period of 2 s means many data points are available to allow very close scrutiny of the shape of the normal chromatogram and the modulated one. They correspond very well, though a 2 s collection

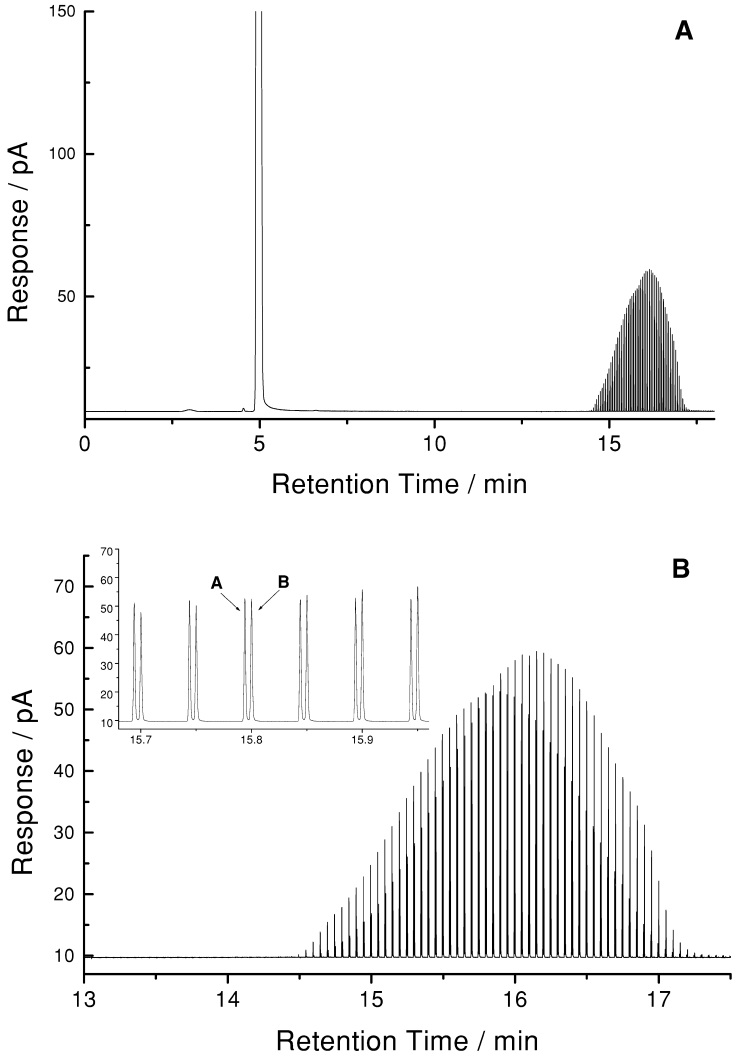

Fig. 7. Cryogenic modulation two-dimensional analysis of acetaldoxime at 80°C, and column head pressure 5 p.s.i. and 3 s pulsing. Note that under these conditions no original unconverted isomer can be recognized. (A) Full chromatogram. (B) Expansion of the pulsed peak region with an inset now showing baseline resolution of the two isomers.

of solute compared with 4 s used earlier means the sensitivity improvement is less in this case. By studying the shape of the individual profile of the pulsed minor isomer (e.g., B) underlying the major peak A (see the dotted circle region), it can be seen that there is considerable change in B at this location, but relatively less change in this isomer at some intermediate point in the distribution. This agrees with the general observation found for simulated chromatographic profiles [44].

3.3. Two-dimensional separation space

Comprehensive gas chromatography of complex mixtures, which is achieved using precisely the modulation process described here, usually requires data presentation in a two-dimensional separation space format. The pulsed data are converted into a matrix, which is then displayed in accord with the times in each dimension. A contour plot is preferred, with peak height represented by color or contour

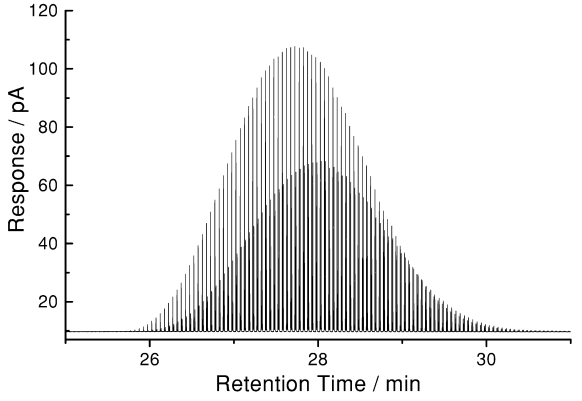

Fig. 8. Cryogenic modulation two-dimensional analysis of butyraldoxime at 90°C, and column head pressure 5 p.s.i. and 3 s pulsing. Note that under these conditions no original unconverted isomer can be recognized and each isomer envelope is almost a symmetric distribution.

lines. Fig. 10 is a presentation of the data from Fig. 9A. The major, early-eluted isomer A, elutes faster on the second column and so it can be seen at a time of about 0.6 s. Isomer B elutes later, at about 0.9 s. Isomer A has a long tail extending to a first dimension retention of about 17 min, i.e. equal to that of original isomer B. This tail can only be formed by isomer B converting into A. Since more B is present initially, more of it undergoes conversion, however this will be confounded by isomer A (the more abundant isomer) likewise converting to B, which can then convert back to A. The higher intensity contour lines are seen for the zone where the original isomer is present in high abundance. Since these correspond to high concentrations of solute, then the contour lines at the lower response sections of the peak will appear slightly ''bloated'' in the $^{2}t_{R}$ dimension. Just considering these original isomer regions which approximate circular zones, they bear close resemblance in size to the impurity peak which is present in the mixture. This shows that the unconverted isomer does elute in a nicely formed peak shape — it is only when a conversion step happens that the peak shape then becomes distorted.

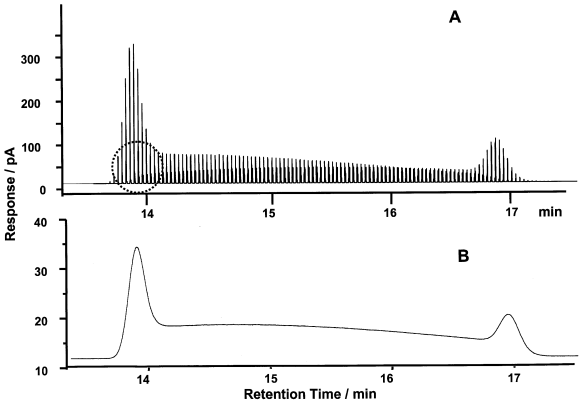

Fig. 9. Cryogenic modulation two-dimensional analysis of butyraldoxime at 70°C, and column head pressure 25 p.s.i. and 2 s pulsing. The greater number of pulses, using a faster modulation period, gives a closer correspondence of the shape of the normal (B) and pulsed trace (A). The shorter pulse duration (2 s) with only 2 s zone compression leads to a smaller response enhancement than observed above for 4 s pulses.

By comparison, the two-dimensional separation space for a situation where more rapid conversion takes place is shown in Fig. 11, for data from Fig. 7. Here, there is no localized high contour response at the end of the peak zone. Rather, the distribution appears to be more symmetric in both dimensions, with a maximum at the centre.

Changes in total peak areas of the two isomers, compared with their relative areas when no isomerization takes place, is an indicator of how much conversion has taken place. There are two ways to obtain the total area of isomer A and isomer B. Such direct measures of isomer amounts has not been previously possible in GC reactor studies of this nature. Here, peak pulses, which can be summed over the total elution of the peaks, gives directly the relative peak A and peak B amounts. Alternatively, by importing the matrix data into a spreadsheet, it is a trivial exercise to sum all the response values to get a total response and hence ratio of peak A and B amounts. It is found that, in Fig. 10, the ratio of peaks A:B is 1.428. For Fig. 11, the ratio of peaks A:B is 0.78. These agree qualitatively with the apparent relative amounts in the chromatograms as seen in the pulsed distributions. The same data may also be obtained by summing the areas of each pulsed peak reported in the chromatographic peak report table over the total distribution. In this way, it is possible to obtain the relative isomer ratios under different experimental conditions, which will also relate to the kinetics and thermodynamics of the interconversion.

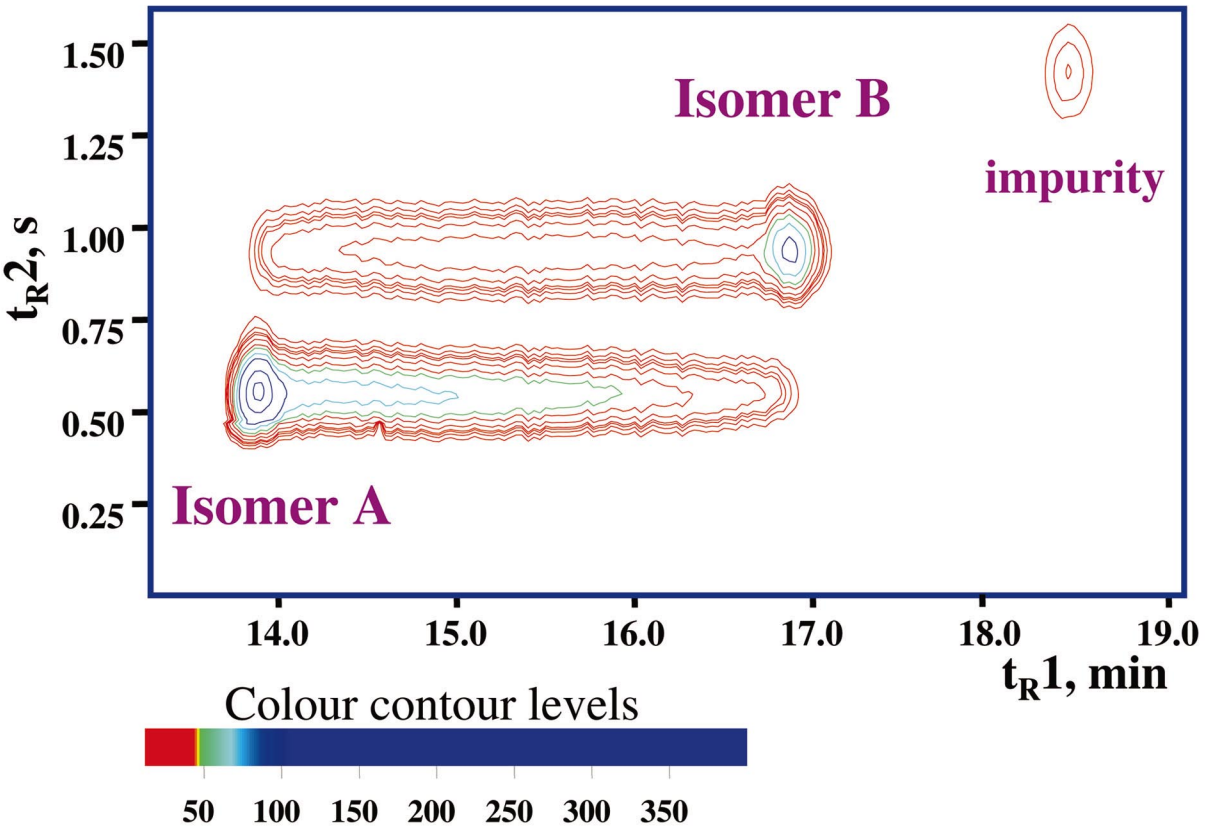

Fig. 10. Two-dimensional contour plot of the data shown in Fig. 9A. Data are presented in the manner normally used for comprehensive gas chromatography, with the two-dimensional separation space shown. Refer to text for details. The impurity peak shown is the same small peak seen after isomer B in Fig. 3. Contour levels refer to response heights in the two-dimensional space; the maximum response of isomer A is greater than that of isomer B, in agreement with the result in Fig. 9.

3.4. Considerations on kinetic data evaluation

Whilst this paper does not discuss derivation of nor describe results for kinetic data for the interconversion process, preliminary details of the interpretation may be given. These will be described more completely in a subsequent paper. The traditional approach to simulation is to fit a theoretical envelope to the observed (non-modulated) interconversion peak envelope based on data such as antipode retentions, peak efficiency, and operation temperature. A general calculation procedure may also incorporate carrier flow-rate, peak widths and column efficiency. This allows ''forward'' and ''reverse'' reaction rates to be calculated at various temperatures and, from these, the activation parameters. This approach gave for acetaldoxime in this study, a simulated k value for the forward reaction (Z to E) of $9.25 \cdot 10^{-3}$ s^{-1} at 90°C and free energy ΔG of 104 kJ mol^{-1} and $k = 5.28 \cdot 10^{-3}$ s^{-1} and $\Delta G =$ 105 kJ mol^{-1} for the reverse reaction. By conducting the experiment at a series of temperatures, activation energy, entropy and enthalpy may be estimated.

The GC×GC experiment offers the opportunity to study the mutual variation in each of the isomers throughout the elution process, and the ability to simulate this result, using appropriate kinetic and activation parameters along with chromatographic conditions defining elution on each of the columns, will be a more stringent test of the model developed for simulation of the interconversion. A simple description is beyond the scope of this paper, and

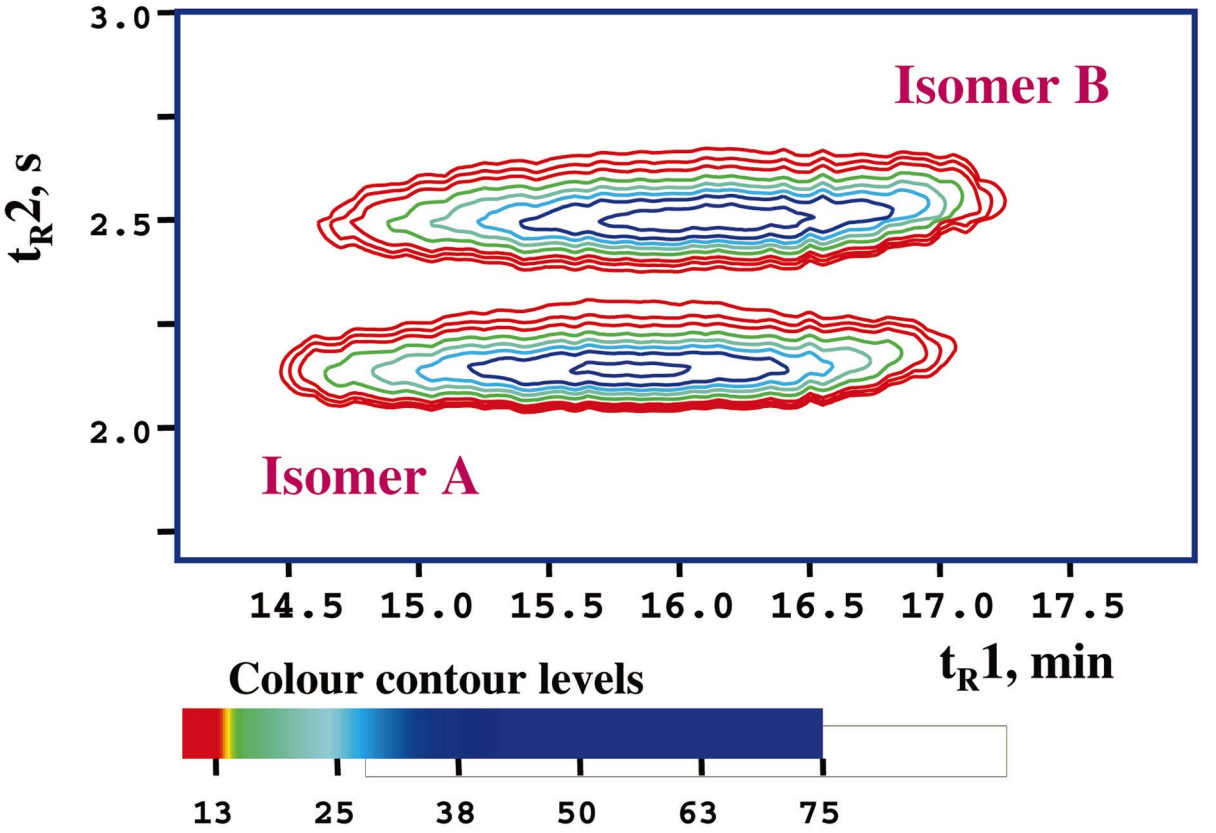

Fig. 11. Two-dimensional contour plot of the data shown in Fig. 7, according to the same procedure as in Fig. 10. Isomer A is of lesser abundance than isomer B as indicated by the contour levels.

must wait until the study is fully discussed in a forthcoming paper.

Since the ratio of interconverting diastereomers, i.e. *E*/*Z* isomers, can only be estimated from a normal gas chromatogram, the evaluation of interconversion rate constants and barriers is more complicated, compared to the determination of enantiomerization barriers in enantioselective dynamic chromatography, where the peak ratio is 1:1 (racemate) and the rate constants are equal in an achiral environment. Therefore the following concept has been elaborated: (i) The exact ratio of the isomers is determined from the temperature-dependent GC× GC experiment and from these data the equilibrium constant $K_{E/Z}$, the Gibbs free energy $\Delta G_{E/Z}$, free reaction enthalpy $\Delta H_{E/Z}$ and entropy $\Delta S_{E/Z}$ can be obtained. The equilibrium constant $K_{E/Z}$ gives the ratio of the forward and backward reaction rate constant. (ii) With these results, the reaction rate constants can be determined by computer simulation of the experimental 1D chromatograms. (iii) To refine these parameters the 2D chromatograms are simulated with 2D simulation module of ChromWin by iterative adaptation of experimental and simulated chromatogram.

4. Conclusions

The recently described cryogenic modulation technique used in a multidimensional separation mode has allowed the individual isomers involved in a dynamic interconversion reaction generated during a chromatography separation to be monitored. The profile of the isomers varied in a manner consistent with those proposed from previous mathematical

modeling studies on similarly dynamic systems. The key to the technique is the ability to resolve, on a very fast time scale, the isomers participating in the interconversion reaction in small contiguous regions over the total peak distribution. These data have not previously been obtained experimentally because single column analysis does not allow unique identification of each isomer. This approach should allow many similar dynamic molecular processes to be studied, including enantiomerizations if a suitable chiral column is used, provided the chromatographic system allows for fast second column separation, e.g. at sufficiently low temperature or rapid elution, thereby giving negligible isomerization.

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