



Theoretical and experimental study of recycle capillary gas chromatography with carrier gas propelled by a peristaltic pump

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Abstract

A new technique of recycle capillary gas chromatography (RCGC) characterized by a very high separation efficiency of more than 10^6 theoretical plates has been developed to solve the problem of separation of isomers with similar physico-chemical properties. The technique replaces the recycle valve by a peristaltic pump that propels the carrier gas. A general model has been developed for description of RCGC characteristics and experimentally verified on the retention behaviour of methane and the separation of a test pair of 3-methyl-1-butene and 2-methylbutane.

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1. Introduction

Recycle chromatography improves the separation efficiency by repeated passage of the effluent through one or more columns, the separation efficiency being given by the product of the single column efficiency and the number of passages. Recycle high-performance liquid chromatography (HPLC) has become an established method for both analytical and preparative separations, however, the principle has not found much use in gas chromatography (GC) [1].

Recycle GC (RCGC) was first proposed by Martin [2] for effective increasing of the column length. Porter and Johnson [3–5] described the first practical

applications of the technique utilizing two packed columns and a mobile phase driven by a recycling pump. Maxwell [6] suggested a system comprising two columns interconnected through a six-port valve. Deford [7] attained up to six effluent passes in preparative recycle GC. Chizhkov and co-workers [8–18] thoroughly studied various theoretical and experimental aspects of recycle gas chromatography. Recycle gas chromatography with capillary columns has been studied less extensively. Ševčík [19] experimented with systems of both packed and capillary columns utilizing an eight-port valve and concluded that capillary columns were to be preferred, but that the extra-column effects of the valve and of the carrier gas pressure changes during the valve operation severely limited the practical use of the technique. Jennings et al. [20,21] described a system employing an in-line switching valve with short

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capillary columns that generated 2×10^6 plates. Miller [1] used a recycle system with off-line flame ionization detection for separation on non-polar mixtures. Extra-column effects in capillary column recycle gas chromatography were discussed by Bertsch and co-workers [22,23].

Successful operation of recycle gas chromatography requires that the switching valve does not contribute to the peak broadening and that an in-line detector is capable of maintaining the chromatographic efficiency without using a make-up gas. In dual column designs, detector cells must be placed in both the column loops in order to monitor the chromatographic process and to determine the instant of the valve actuation [1]. To overcome these problems of valve switching, Kubinec et al. [24] described a system employing a peristaltic pump for propelling the carrier gas in recycle capillary gas chromatography and applied it to mixtures of enantio- and diastereoisomers of 3,4-dimethylhexane that were not separated previously.

The present work is aimed at investigation of the possibilities of recycle gas chromatography with a single capillary column, using a peristaltic pump for propelling the carrier gas, and at development of a general model for characterization of RCGC in terms of the retention time, the elution profile and the optimum number of cycles.

2. Theoretical

2.1. Characteristics of recycle gas chromatography

A sample flow diagram and variables of a recycle system operated by a peristaltic pump are shown in Fig. 1. A recycle chromatography system can quantitatively be described by chromatographic parameters such as the retention time and the elution profile width within all the parts of the chromatographic system.

2.2. Retention time in recycle chromatography

The retention time t_R of analyte a moving from an injector to a detector (see Fig. 1) is given by:

$$t_{Ra(1)} = t_{in} + t_{ex} + t'_{Ra} + t_{det} \quad (1)$$

For the retention time of a recycled sample part it holds that:

$$t_{Ra(2)} = t_{Ra(1)} + (t_{ex} + t'_{Ra}) \quad (2)$$

and for the retention time after i loops it holds that:

$$t_{Ra(i)} = t_{Ra(1)} + (i - 1) \cdot (t_{ex} + t'_{Ra}). \quad (3)$$

Therefore, the retention time between two consecutive cycles is equal to the sum of the analyte

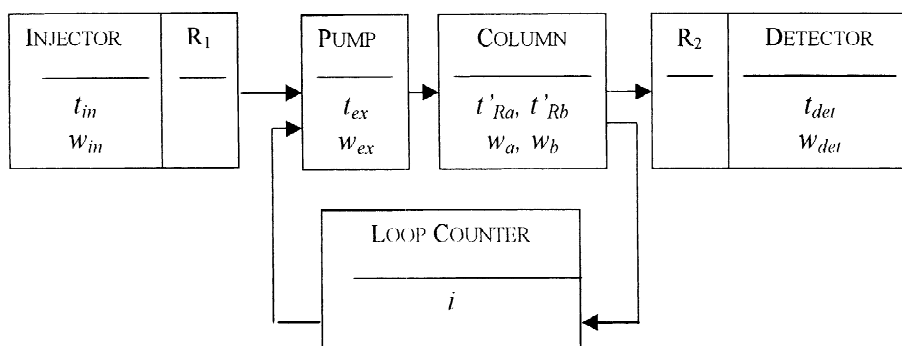


Fig. 1. A sample flow diagram and variables of a recycle system operated by peristaltic pump. t_{in} —injector dead time contribution, t_{ex} —interface dead time contribution, t'_{Ra} —adjusted retention time of compound a, t'_{Rb} —adjusted retention time of compound b, t_{det} —detector dead time contribution, w_{in} —injector extra-column contribution (starting peak width), w_{ex} —interface extra-column contribution, w_a —peak width of compound a, w_b —peak width of compound b, w_{det} —detector extra-column contribution (time constant), R_1 , R_2 —restrictors, i —number of cycles.

retention time in the interface (t_{ex}) and the adjusted retention time in the column (t'_{Ra}). The increase in the retention time between the cycles is thus constant. It holds that:

$$[t_{\text{Ra}(i)} - t_{\text{Ra}(i-1)}] = (t_{\text{ex}} + t'_{\text{Ra}}) = \text{const.} \quad (4)$$

The retention times t_{in} and t_{det} can be obtained by subtracting Eq. (4) from Eq. (1).

2.3. Elution profile width in recycle chromatography

The elution profile width given in terms of the standard deviation is determined by the sum of the variances of the partial contributions. For variance w^2 of the peak width of analyte a moving between an injector and a detector (see Fig. 1) it holds that:

$$w_{\text{a}(1)}^2 = w_{\text{in}}^2 + w_{\text{ex}}^2 + w_{\text{a}}^2 + w_{\text{det}}^2 \quad (5)$$

The variance of the elution profile width of a recycled sample part is then given by:

$$w_{\text{a}(2)}^2 = w_{\text{a}(1)}^2 + (w_{\text{ex}}^2 + w_{\text{a}}^2) \quad (6)$$

and for the variance of the peak width after i loops it holds that:

$$w_{\text{a}(i)}^2 = w_{\text{a}(1)}^2 + (i - 1) \cdot (w_{\text{ex}}^2 + w_{\text{a}}^2) \quad (7)$$

The variance of the elution profile width between two consecutive cycles is thus equal to the extra column effects within the interface (w_{ex}^2) and the peak broadening in the column (w_{a}^2). The increase in the variance of the peak width between cycles is thus constant. It holds that:

$$[w_{\text{a}(i)}^2 - w_{\text{a}(i-1)}^2] = (w_{\text{ex}}^2 + w_{\text{a}}^2) = \text{const.} \quad (8)$$

On the basis of the above equations for the retention time and the retention profile width, all the other chromatographic characteristics can be derived (the separation efficiency, resolution, etc.).

2.4. Number of cycles

The number of cycles is of essential importance for application of RCGC, as it is a key factor in

attaining a high separation efficiency and thus obtaining a high resolution system. The number of cycles can be calculated in various ways, in dependence on the optimization criterion (e.g., the maximum attainable resolution, the number of cycles required for resolution $R=1$, the maximum time difference to the front peak, the attaining of the same time difference to the front and to the rear peak, etc.).

Our approach is based on the solution of Eq. (9) which to some extent corresponds to the selectivity coefficient (for large retention factors k'). For the optimization criterion it holds that:

$$\frac{t_{\text{Rb}(i)}}{t_{\text{Ra}(1)}} = \frac{t_{\text{Ra}(i+1)}}{t_{\text{Rb}(i)}} \quad (9)$$

Assuming that the extra-column dead time is much smaller than the retention time within the loop it holds that:

$$t_{\text{in}} + t_{\text{det}} \ll t_{\text{ex}} + t'_{\text{Ra}} \quad (10)$$

On introducing the analyte mixture into the recycle loop, the retention time difference between the peaks increases and, after i cycles, is given by Eq. (11):

$$t_{\text{Rb}(i)} = [t_{\text{Ra}(i)} + it_{\Delta(b-a)}] \quad (11)$$

where $t_{\text{Ra}(i)}$ is obtained from Eq. (3).

The distance between the first and the last peak of the retention interval increases stepwise and thus the first peak can finally coelute with the last one and the resolution be lost. Thus there is an optimum number of loops, corresponding to the optimum retention times of analytes a and b, $t_{\text{Ra}(i_{\text{opt}})}$, $t_{\text{Rb}(i_{\text{opt}})}$ and $t_{\text{Ra}(i_{\text{opt}}+1)}$, attained after the optimum number of loops (i_{opt}).

The optimum number of loops is obtained on substitution for the retention time from Eq. (3) into Eq. (9):

$$i_{\text{opt}} = \frac{1}{\left(\frac{t_{\text{Rb}(1)}}{t_{\text{Ra}(1)}}\right)^2 - 1} = \frac{1}{\left(\frac{t_{\text{Rb}(i)}}{t_{\text{Ra}(i)}}\right)^2 - 1} \quad (12)$$

Further cycles decrease the resolution between

retention times $t_{Rb(i)}$ and $t_{Ra(i+1)}$ and thus decrease the system resolution.

3. Experimental

3.1. Materials

Three fused-silica capillary columns were used for the investigations. Uncoated columns of 5 m×0.32 mm I.D. and 20 m×0.32 mm I.D. (Caco, Bratislava, Slovak Republic) were used for investigation of transport phenomena, and a 50 m×0.32 mm I.D., 0.4 μm column (Caco) coated in our laboratory by a static method with the OV-1 stationary phase for the study of the separation behaviour. Restrictors of 0.7 m×0.04 mm I.D. and 0.4 m×0.04 mm I.D. were used, respectively, for connection to an MS detector and to the sampling part (Caco). 3-Methyl-1-butene and 2-methylbutane were obtained from Aldrich (USA).

3.2. Instrumentation

Gas chromatographic measurements on model compounds (methane and a mixture of 3-methyl-1-butene and 2-methylbutane) were performed on a Trace GC 2000 series Thermoquest CE Instruments and Voyager GC–MS Thermoquest Finnigan system. The injected sample volume was 0.2 μl of the pure compound, with a split ratio of 1:5000 at the temperature of the injection port 200 °C and pressure 100 kPa. Helium was used as the carrier gas at a linear velocity of about 20 cm s⁻¹. The carrier gas was propelled by a peristaltic pump (Labeco, Spišská Nová Ves, Slovak Republic), with a pump head from Viton tubing, 50 mm×0.9 mm I.D. (Masterflex, USA) at 25 to 40 rpm. The pump head, the column and the restrictors were interconnected by polyimide (Supelco, USA). The MS system was operated in the selected ion monitoring (SIM) mode, monitoring the m/z 15, 70 and 72 ions. The transfer line temperature was 280 °C. Quadrupole conditions were: electron energy 70 eV, emission current 150 μA and ion source temperature 200 °C. Each GC peak was inspected for constancy of MS pattern in order to detect possible overlapping compounds and to measure their retention data.

4. Results and discussion

A one-column RCGC system has been developed that contains no recycle valve but employs a peristaltic pump (Fig. 2). It comprises a gas chromatograph, a peristaltic pump, restrictors (R_1 and R_2), a split/splitless injector (I) and an MS detector. Fig. 3 shows details of the column and restrictor connection in the peristaltic head fittings.

4.1. RCGC transport behaviour

A peristaltic pump with a head volume of 15 μl, operating at a temperature equal to that of the GC oven, was used to propel the mobile phase. Various materials were tested for the pump head, e.g., Tygon, silicone rubber and Norprene, but the Viton tubing (see Experimental) was found to be most suitable. The system allows separation of up to C₁₀ hydrocarbons. For analysis of higher hydrocarbons and polar compounds it will be necessary to deactivate the pump head or to use some other inert materials.

The carrier gas velocity through the column was regulated by controlling the peristaltic pump rate. Samples were injected by a syringe through a regular GC injection port and entered the pump head via restrictor R_1 which guaranteed a constant pressure of the mobile phase in the pump head during the analysis. The sample was split between the detector and the column using restrictor R_2 . The split ratio could be adjusted by varying restrictors R_2 and R_1 and the pressure in the injection port.

The zone broadening caused by the peristaltic pump was examined using an uncoated capillary column and methane as the analyte that is not retained in the separation system; the chromatogram is shown in Fig. 4.

Fig. 4 clearly shows that the proposed system allows more than 130 cycles without considerable zone broadening. A drop in the peak height of methane is caused by material loss, about 1% of analyte over one cycle, in restrictor R_2 directed to the detector. An advantage of the system is continuous monitoring of the analyte throughout the analysis without using switching valves, which would be responsible for pressure pulses in the separation system. The designed system simplifies the RCGC

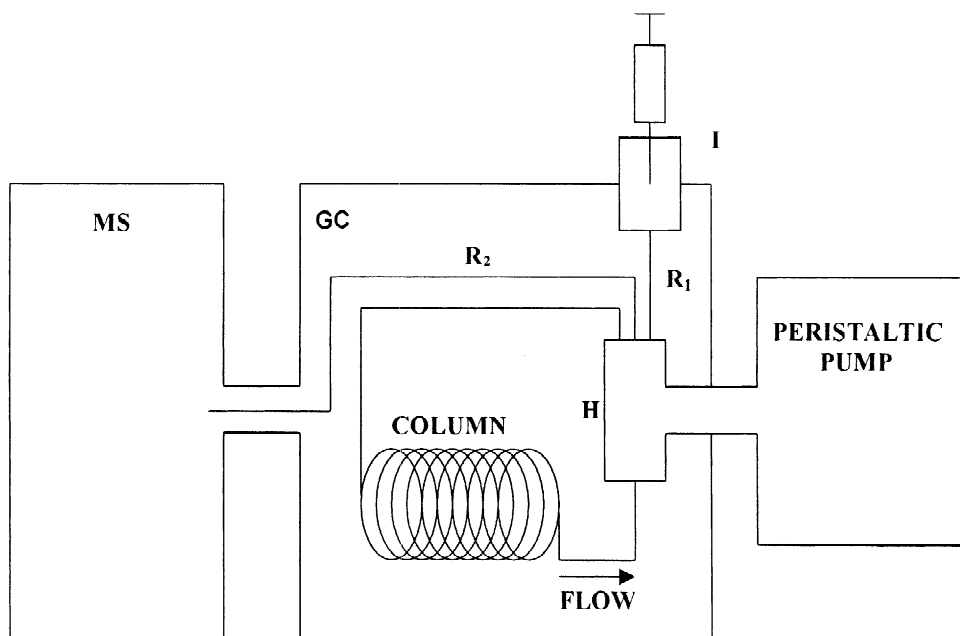


Fig. 2. Schematic of recycle capillary gas chromatographic system. I—Injector, R_1 0.4 m \times 0.040 mm I.D., R_2 0.7 m \times 0.040 mm I.D.—restrictors, H—peristaltic head.

and solves the main problems of the previous recycle systems.

The retention characteristics are statistically evaluated in Table 1 and the elution time increments on a 20 m column are shown in Fig. 5.

The data given in Table 1 and Fig. 5 exhibit validity of Eq. (4) for all the columns used. The negligible difference between the first and second half of the measured retention time differences demonstrates that there is no trend within the measurements and that there is no functional dependence of the retention time increment on the number of

loops (see Fig. 5, the coefficient of determination is close to zero, $R^2=0.001$).

The peak width in RCGC has also been investigated with the three columns. Additivity of peak variances (see Eq. (7)) is demonstrated in Fig. 6, while validity of Eq. (8) (a constant variance increment between adjacent cycles) is shown in Fig. 7. The peak variances are additive for all the three columns over the whole range of cycle number. The coefficients are always close to unity.

On the basis of these results it seems that a longer column with a smaller number of cycles is preferable to a short column with a larger number of cycles. Furthermore, a longer column allows a larger number of cycles compared to a short one.

The column variance increments (Eq. (8)) for the 5, 20 and 50 m columns were 1.212, 2.341 and 7.818 (s^2), respectively. Details on the peak variance behaviour for the 20 m column are shown in Fig. 7. The mean value remains constant over 140 cycles. The data spreading increases with increasing number of cycles because of decreasing precision of the peak width determination caused by decreasing peak height (due to the splitting to the detector and to

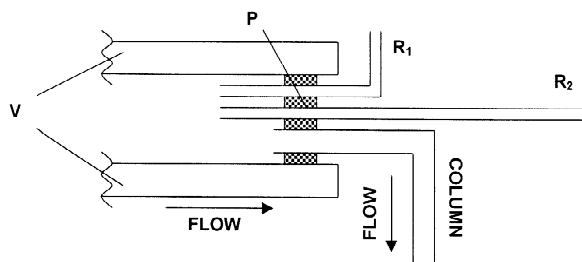


Fig. 3. Schematic of peristaltic head connections: R_1 , R_2 —restrictors, V—Viton tubing 50 mm \times 0.9 mm I.D., P—polyimide.

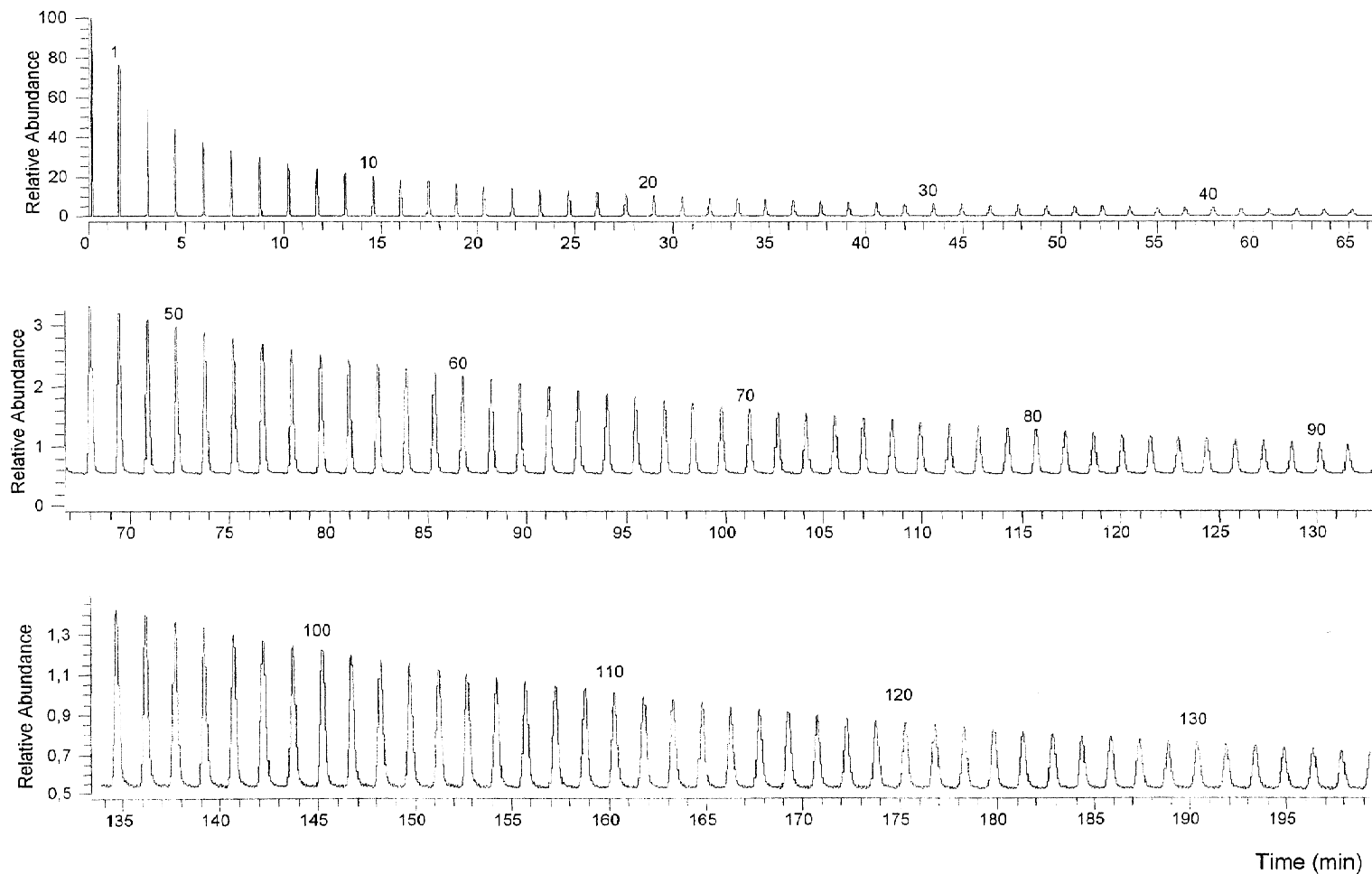


Fig. 4. RCGC chromatogram of a non-retained solute (methane) on a 20 m \times 0.32 mm I.D. uncoated fused-silica capillary column at 40 $^{\circ}$ C and a helium carrier gas flow-rate of 23.1 cm s $^{-1}$. The numbers at peaks correspond to the number of cycles.

Table 1

Statistical evaluation of the retention characteristics on columns of different lengths in the recycle mode; Δt_R (min) is calculated according to Eq. (4)

	5 m		20 m		50 m	
	Cycles	Δt_R (min)	Cycles	Δt_R (min)	Cycles	Δt_R (min)
First half	1 to 39		1 to 69		1 to 21	
Mean		0.322		1.444		4.638
SD		0.004		0.002		0.007
Second half	40 to 78		70 to 138		22 to 41	
Mean		0.322		1.445		4.634
SD		0.004		0.008		0.020
Total	1 to 78		1 to 138		1 to 41	
Mean		0.322		1.445		4.636
SD		0.004		0.006		0.015
\bar{u} (cm s ⁻¹)	25.9		23.1		18.0	

broadening) and decreasing signal/noise ratio. A very good agreement of the experimental data with the theory is demonstrated by the value of the coefficient of determination which is close to zero, $R^2=0.007$.

4.2. RCGC chromatographic behaviour

On the basis of a quantitative description of the

retention time and peak width variances in RCGC, chromatographic parameters, such as the efficiency, resolution, etc., can be derived.

As the optimization criterion, we selected the number of cycles according to Eq. (12). Its validity is verified by the chromatogram in Fig. 8. The retention time ratio [$t_{Rb(i)}/t_{Ra(i)}$] (Eq. (9)) for the test compounds equals 1.039. The chromatogram indicates an improvement in separation with increasing

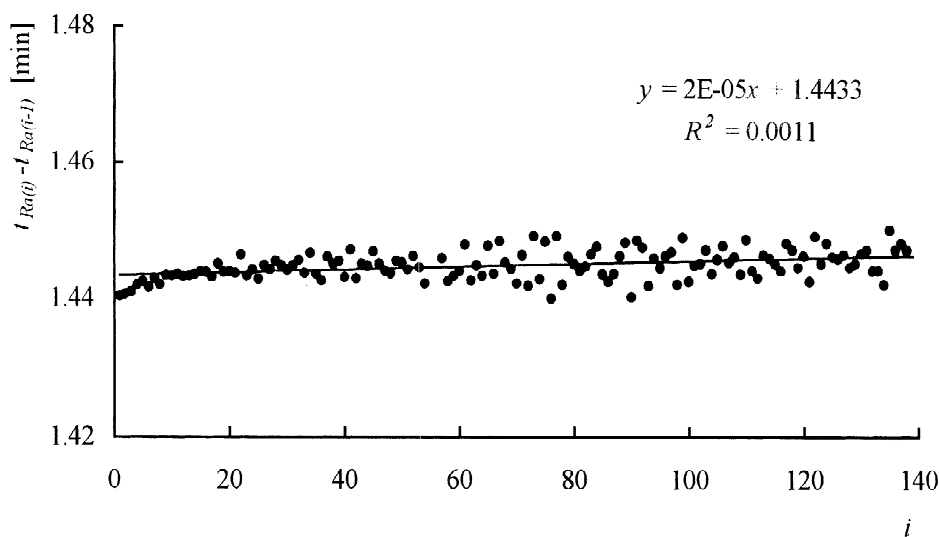


Fig. 5. Difference of [$t_{Ra(i)} - t_{Ra(i-1)}$] versus number of cycles (i) using non-retained solute (methane) on a 20 m×0.32 mm I.D. uncoated fused-silica capillary column at 40 °C and helium carrier gas flow-rate of 23.1 cm s⁻¹. R^2 is the value of the coefficient of determination of the regression equation.

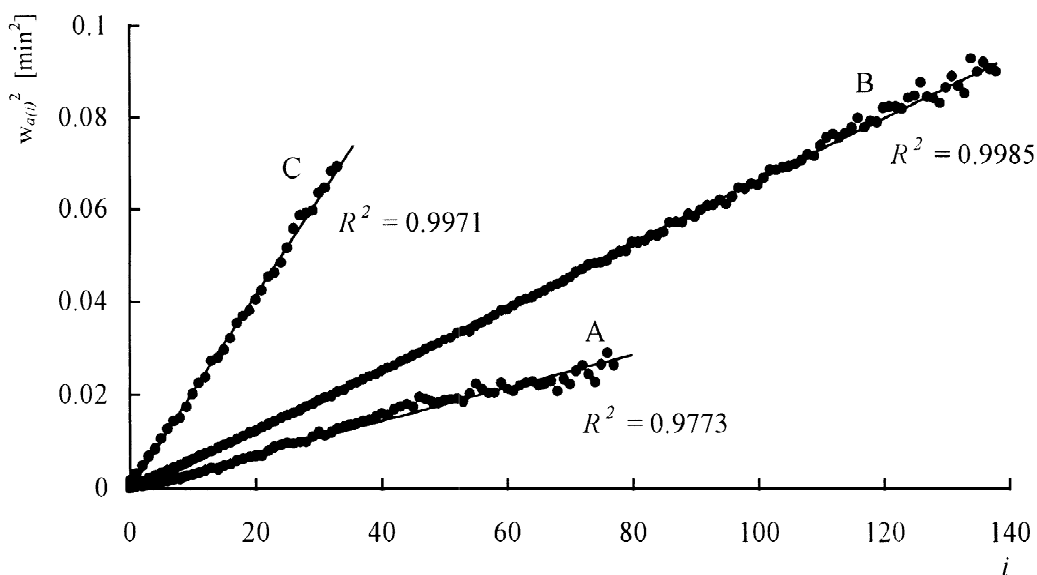


Fig. 6. Dependence of the retention peak variance (Eq. (7)) on the number of cycles (i) for 5 m (A), 20 m (B) and 50 m (C) long column. R^2 is the value of the coefficient of determination of the regression equation.

number of cycles up to 13 cycles, followed by stepwise merging until cycle number 26, when co-elution occurs. Afterwards, the separation of com-

pounds B and A begins again, similar to the first cycle.

An improved resolution R between the first (A_i)

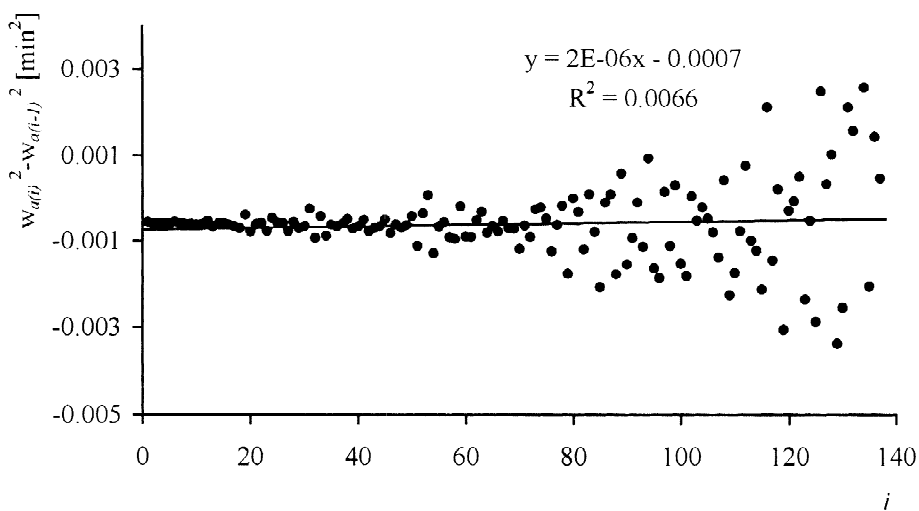


Fig. 7. Dependence of peak variance increment [$w_{a(i)}^2 - w_{a(i-1)}^2$] (Eq. (8)) on the number of cycles (i) using non-retained solute (methane) on a 20 m \times 0.32 mm I.D. uncoated fused-silica capillary column at 40 °C and a helium carrier gas flow-rate of 23.1 cm s⁻¹. R^2 is the value of the coefficient of determination of the regression equation.

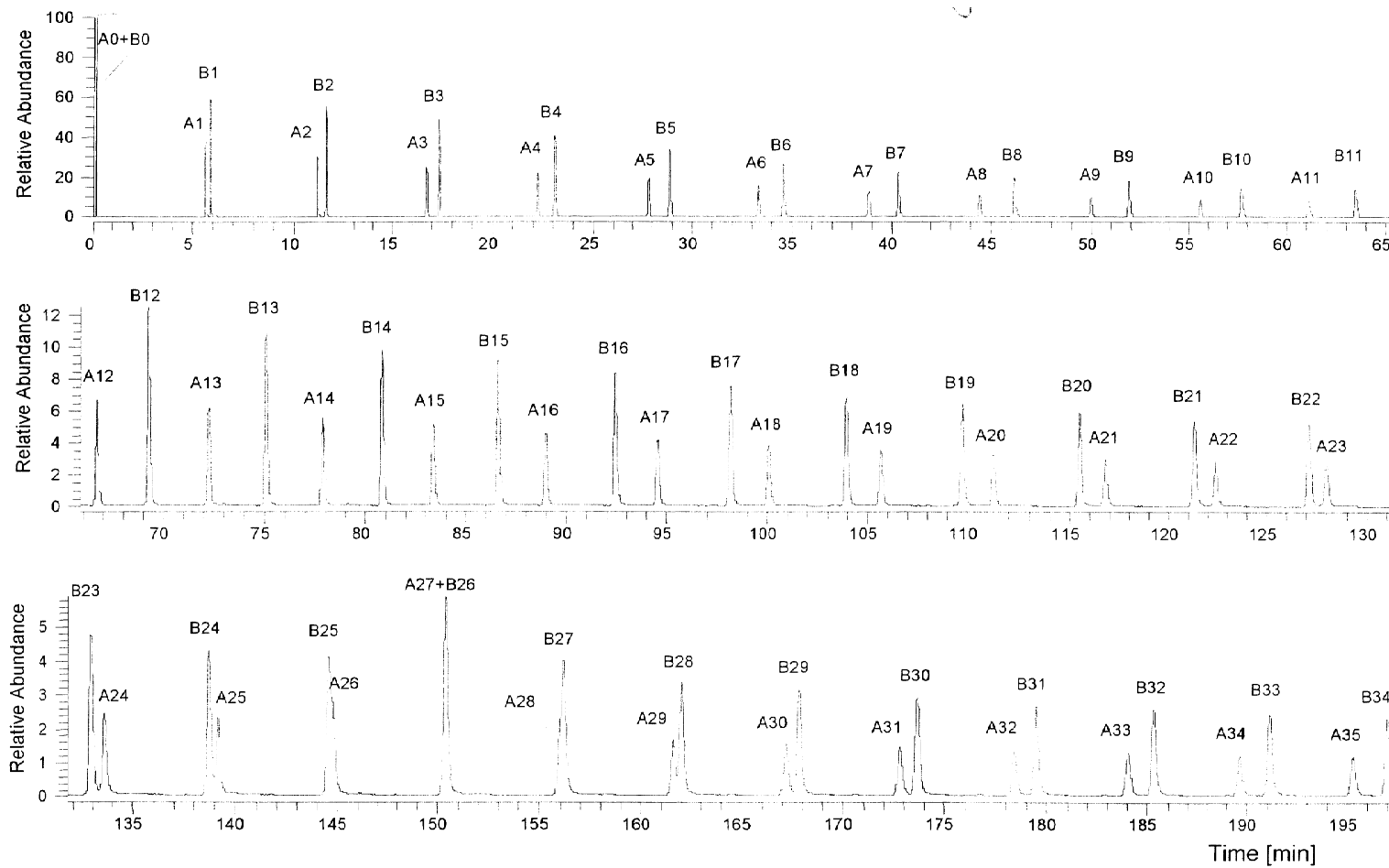


Fig. 8. RCGC chromatogram of separation of 3-methyl-1-butene (A) and 2-methylbutane (B) at 40 °C on a column 50 m×0.32 mm I.D., 0.4 μm coated with OV-1.

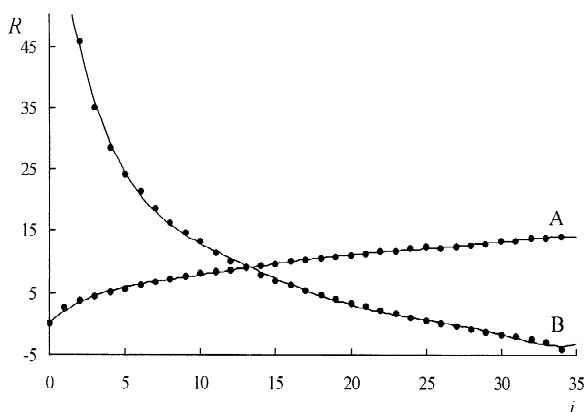


Fig. 9. Dependence of the resolution R on the number of cycles (i). A— $R_{B(i),A(i)}$, B— $R_{A(i+1),B(i)}$.

and second (B_i) peaks is shown in Fig. 9, curve A, while a decreasing resolution between the second (B_i) peak and first (A_{i+1}) peak is demonstrated by curve B. The cross-section of the two curves corresponds to the optimum number of cycles.

The developed RCGC system with a peristaltic pump has been applied to the first successful separation of all the three stereoisomers (two enantiomers and one *meso*-form) of 3,4-dimethylhexane on a chiral stationary phase with a system efficiency of about 2 million plates [24]. In general, the most important application of RCGC can be expected for separation of enantiomers with similar physico-chemical properties, inseparable by the present separation systems.

5. Conclusions

The developed RCGC system is suitable for analysis of mixtures of a limited number of compounds, such as various isomers.

A model has been developed describing the characteristics of a new recycle chromatography with peristaltic pump and its validity has been verified experimentally. It shows that the retention times and the variances of the elution profile contributions are additive. The model permits the calculation of the separation system efficiency and of the number of cycles required for optimum separation.

The peristaltic pump appears to be a good means for achievement of flow-rates required for optimum operation of a broad range of column lengths (from 5 to 50 m).

The RCGC design used, with a fixed end-column restrictor, adjustable by variation of its length and internal diameter, allows continuous monitoring of actual status of the separation process.

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