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DETERMINATION OF TRACE AMOUNTS OF HYDROPEROXIDES BY COLUMN LIQUID CHROMATOGRAPHY AND COLORIMETRIC DETECTION

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SUMMARY

The sensitive and selective determination of separated compounds in effluents from liquid chromatographic columns can be carried out by continuously adding a suitable colorimetric agent to the column effluent and continuously monitoring the absorbance of the reaction mixture. However, a considerable amount of additional broadening of the chromatographic peaks may occur in these systems, especially if slower reactions make it necessary for longer residence times to be used in the reactors.

It is shown how this additional broadening can be reduced to an acceptable level by using packed reactors. Some general characteristics of these reactors are discussed and rules for the optimal design are given.

A method is described for the determination of hydroperoxides in reaction mixtures from the oxidation of hydrocarbons. Separations are carried out by adsorption chromatography. In an on-line packed reactor, iodine is formed by hydroperoxides in an acidic solution of sodium iodide, and the absorbance of the reaction mixture is measured at 362 nm. A delay time of 1.5 min at a temperature of 70° in the reactor involved a standard deviation of the residence time distribution of less than 1 sec. Nanogram amounts of hydroperoxides can be determined by means of this colorimetric detector.

INTRODUCTION

Photometric flow detectors are probably the most widely used detection devices in modern liquid chromatography (LC). They are operated mainly in the UV region of the spectrum, where numerous compounds of chemical or biological interest show absorption. Sometimes, non-absorbing molecules are converted into absorbing products by appropriate derivatization reactions ("labelling") prior to separation. However, the sensitivity of the UV detector to a wide range of substances implies a low selectivity, which may be troublesome in the chromatographic analysis of complex mixtures.

The selectivity of LC photometric detectors can be substantially increased by coupling the chromatographic column to a chemical reaction system in which the column effluent is continuously mixed with a colour reagent that is specific for the group of compounds to be determined and by measuring the absorbance of the reaction mixture. These on-stream reaction–detection systems are currently used in the analysis of biologically important mixtures by means of ion-exchange chromatography. However, the reactor will inevitably cause additional broadening of the chromatographic band, which may have an adverse effect on the column performance, especially with narrow peaks.

It is shown in the present study that additional band broadening and the consequent loss of resolution can be reduced to an acceptable level by careful design of the reaction system, even if high-performance columns are used for separation. Hydroperoxides are important intermediates in a number of industrial oxidation processes and the study and control of these processes require sensitive methods for determining the individual hydroperoxides in the reaction mixtures. Because of their thermal instability, these compounds should preferably be separated by column liquid chromatography.

Direct UV absorption detection has been used in the liquid chromatography of hydroperoxides. However, this approach proved to be unsuitable for use with complex reaction mixtures containing high concentrations of other reaction products. A well-known sensitive and selective method for the colorimetric determination of hydroperoxides is based upon the reaction with sodium iodide in a mixture of 2-propanol, acetic acid and water; the absorbance of the reaction mixture due to I_3^- is usually measured at 360 nm. The direct objective of this work was the construction of an on-stream system for this reaction which could be used in combination with the chromatographic separation of hydroperoxides.

THEORETICAL

A colorimetric detector consists of a reactor and a flow cell, and a chromatographic peak eluted from the column will undergo additional broadening in the reactor. Let the variance, expressed in time units, of a solute band leaving the column be $\Delta\sigma_{tc}^2$, the variance of the residence time distribution function in the reactor $\Delta\sigma_{td}^2$, and the variance of the peak at the end of the colorimetric detector system σ_t^2. The variances are additive, and therefore

$$\sigma_t^2 = \Delta\sigma_{tc}^2 + \Delta\sigma_{td}^2 \qquad (1)$$

It is assumed that the variance $\Delta\sigma_{td}^2$ in the reactor can be approximated by the variance of the residence time distribution function of an inert non-reacting component.

The separation between two peaks that differ by Δt_R in their retention times is characterized by the resolution, R:

$$R = \frac{\Delta t_R}{4\sigma_t} \qquad (2)$$

Where σ_t represents the standard deviation of the second peak. The maximum value

of the resolution, $R_{max.}$, will be obtained with $\sigma_t = \Delta\sigma_{tc}$ and $\Delta\sigma_{td} = 0$. The following equation can then be derived:

$$\frac{R}{R_{max.}} = \left[1 + \left(\frac{\Delta\sigma_{td}}{\Delta\sigma_{tc}}\right)^2\right]^{-\frac{1}{2}} \tag{3}$$

If a 5% decrease in resolution is considered acceptable, then

$$\Delta\sigma_{td} = 0.33\,\Delta\sigma_{tc} \tag{4}$$

Now, $\Delta\sigma_{tc}$ can be calculated from the plate number, N, of the column used and the retention time, t_R, of the solute.

In order to estimate $\Delta\sigma_{td}$ in practical liquid chromatography, we consider a 30-cm column. The application of a 5-μm packing enables plate numbers of about 15,000 to be attained in such a column. Suppose that the column is operated slightly above its optimal linear velocity, for instance at 2 mm·sec^{-1}. In practical separations, the capacity ratio, k', will be at least 1, which corresponds to a retention time of 300 sec. Using these values for t_R and N, we find $\Delta\sigma_{tc} = 2.45$ sec ($k' = 1$) and, hence, $\Delta\sigma_{td} = 0.81$ sec.

In the simplest design, flow reactors consist of narrow glass or plastic tubing. However, as the laminar flow profile in the tubes causes considerable band broadening, these reactors can be used with very fast reactions only[1]. Although this additional peak broadening can be considerably reduced by using a gas-segmented liquid flow, it still exceeds the limits imposed by modern liquid chromatography, even in carefully designed systems[2]. Jolley *et al.*[3] used a packed tubular reactor in a colorimetric detection system for a carbohydrate analyzer, with glass beads serving as the packing material. The reactor design was not critical here because of the long retention times and the rather broad elution peaks.

As in chromatographic columns, peak broadening, $\Delta\sigma_{td}$, in packed tubular reactors depends on the mean residence time, t_v, in the reactor and the plate number, N. In Table I the plate numbers that meet the requirements of the typical chromatographic system described above are given for various reaction times, t_v. It can be seen that the successful use of colorimetric detection in modern column liquid chromatography is possible only with relatively fast reactions and, consequently, at low values of t_v, *i.e.*, 2–3 min at most. The problems of reactor design are similar to those encountered in choosing the optimal conditions for LC separations[4]. In fact, a suitable

TABLE I

REACTOR PLATE NUMBER FOR VARIOUS REACTION TIMES, t_v, ASSUMING $\Delta\sigma_{td} = 0.81$ sec

t_v (sec)	N
60	5,500
90	12,500
120	22,000
180	50,000
300	137,000

choice of reactor length, L, particle size of the glass-bead packing, d_p, and pressure drop over the reactor, Δp, should be made for a given combination of reaction time, t_v, and additional band broadening, $\Delta\sigma_{td}$. This can be done by starting from the following set of equations:

$$N = \frac{L}{H} \tag{5}$$

$$H = \frac{2\gamma D_m}{u} + \frac{\lambda_1 d_p}{1 + \lambda_2 \left(\frac{D_m}{u d_p}\right)^{\frac{1}{2}}} \tag{6}$$

$$t_v = \frac{L}{u} \tag{7}$$

$$u = \frac{k_0 d_p^2 \Delta p}{\eta L} \tag{8}$$

Where γ is the tortuosity factor, D_m is the diffusion coefficient of the component in the moving fluid, u is the linear velocity of the reactor fluid, λ_1 and λ_2 are constants characterizing the geometry of the reactor bed, η is the viscosity of the reaction mixture and k_0 is the permeability constant of the reactor. Eqn. 6 was taken from Hiby[5].

In fact, this set can be reduced to only two independent equations, which implies that of the five parameters L, d_p, Δp, t_v and $\Delta\sigma_{td}$, three can be chosen at will. As t_v and $\Delta\sigma_{td}$ are fixed otherwise, only one of the three remaining parameters need be chosen.

In Fig. 1, Δp and L are plotted against d_p for various reaction times, t_v, and for

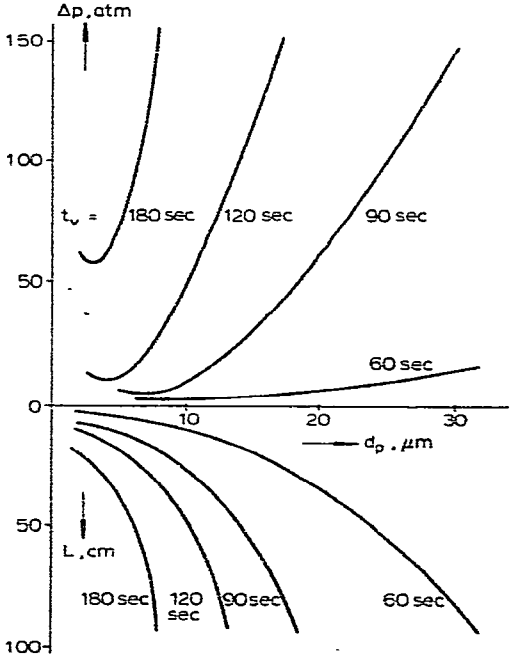

Fig. 1. Reactor length and reactor pressure drop as a function of the particle diameter, for various reaction times, t_v, and $\Delta\sigma_{td} = 0.81$ sec.

$\Delta\sigma_{td} = 0.81$ sec, the latter condition corresponding to 5% additional band broadening in the reactor system for the typical chromatographic situation described earlier. The curves were calculated from eqns. 5–8 with $\gamma = 0.7$, $D_m = 2 \cdot 10^{-5}$ cm$^2 \cdot$sec^{-1}, $\lambda_1 = 11$, $\lambda_2 = 18$, $k_0 = 2 \cdot 10^{-3}$ and $\eta = 0.5$ cP. The values of the Hiby constants λ_1 and λ_2 and the permeability constant k_0 were taken from results of separate experiments with glass-bead columns[6]. Favourable conditions (short reactors and low pressure drops) are found near the minimum of the Δp *versus* d_p curves. Fig. 1 also confirms that the use of colorimetric detectors in HPLC is possible only with relatively fast reactions.

The length and internal diameter, d_r, of the reactor tube should be chosen so as to fit in with the volume flow, Φ_t, through the reactor. The internal diameter (d_r) can be found from the equation

$$d_r = \left[\frac{4\, t_v\, (\Phi_c + \Phi_r)}{\pi\, \varepsilon_T\, L}\right]^{\frac{1}{3}} \tag{9}$$

Where Φ_c and Φ_r denote the eluent flow and the reagent flow, respectively, and ε_T is the total void fraction of the reactor column. The chromatographic process always involves dilution of the sample components. This dilution effect can be described as the ratio of the maximum of the concentration peak leaving the column, $c_{max.}$, to the concentration of the component in the sample, c_0 (ref. 2):

$$\frac{c_{max.}}{c_0} = \frac{V_0}{A_c\,(1 + k')\,(2\,\pi\, L_c\, H_c)^{\frac{1}{2}}} \tag{10}$$

where V_0 is the sample volume, A_c the cross-sectional area of the separation column, L_c the length and H_c the plate height of the column. In the reactor, further dilution takes place owing to the addition of the reagent and the extra band broadening:

$$\frac{c_{max.}}{c_0} = \frac{\Phi_c}{\Phi_c + \Phi_r} \cdot \frac{\Delta\sigma_{tc}}{\sigma_t} \cdot \frac{V_0}{A_c\,(1 + k')\,(2\,\pi\, L_c\, H_c)^{\frac{1}{2}}} \tag{11}$$

If small amounts have to be detected, Φ_r should be made as small as possible.

EXPERIMENTAL

Chromatography

The liquid chromatograph (Fig. 2) was constructed in our laboratory and has been described elsewhere[2]. Chromatographic columns (30 cm × 4 mm) were made from stainless-steel tubing. The columns were filled with 5-μm silica gel (Merckosorb SI-60, Merck, Darmstadt, G.F.R.) by a slurry packing procedure. The plate numbers achieved in these columns under normal operating conditions appeared to range from 12,000 to 15,000. A 50% water-saturated mixture of 2,2,4-trimethylpentane and ethanol (95:5) was used as the eluent. Normally, the columns were operated at a liquid flow-rate (Φ_c) of about 1.1 cm$^3 \cdot$min^{-1}, which corresponds to a linear eluent velocity of *ca.* 0.2 cm$\cdot$sec^{-1}.

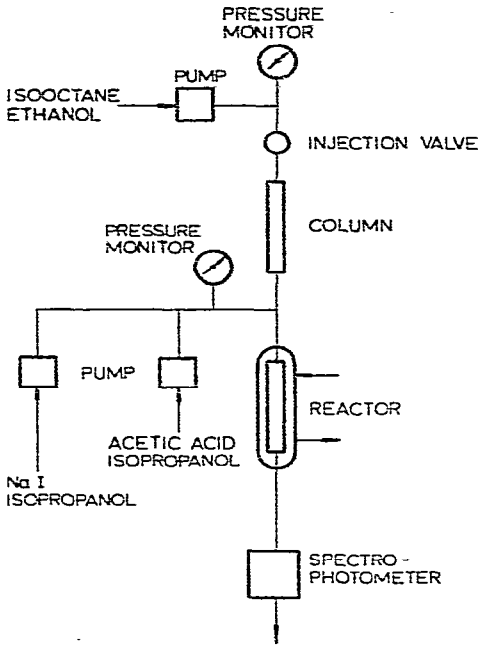

Fig. 2. Schematic diagram of the chromatographic system.

Reactor system

The reagent used for colorimetric detection of hydroperoxides was formed *in situ* by mixing two streams of equal flow-rates (0.6 $cm^3 \cdot min^{-1}$), one consisting of a solution of sodium iodide in 2-propanol (12.5 $g \cdot l^{-1}$), the other being a mixture of acetic acid and 2-propanol (10:90). *In situ* formation of the reagent is necessary because of its instability. The reagent streams were supplied by reciprocating high-pressure pumps (Type DMP-AE-10.4, Orlita, Giessen, G.F.R.). An effective pulse-damping system is essential because a pulsating reagent flow will disturb the homogeneity of the reaction mixture and, consequently, produce a high detector noise. A pulse-damping circuit consisting of a large volume bourdon tube and capillary tubing proved to be sufficient.

The reagent was added to the column effluent through a 0.1-mm I.D. stainless-steel capillary tube. The connection between the column and reactor consisted of a short piece of 0.25-mm I.D. capillary tubing.

The stainless-steel reactor column (50 cm × 4.6 mm) was filled with glass beads (Sovitec, Charleroi, Belgium) of 16 μm mean particle size. Narrow sieve fractions of these glass beads were prepared by means of an air classifier (Zickzacksichter MZR, Alpine, Augsburg, G.F.R.). An equal-density slurry method[6] was used for packing the columns. The reactor column was thermostated at 70°.

The absorbance of the reaction mixture due to the presence of I_3^- was continuously measured at 362 nm with a PM 2D spectrophotometer (Zeiss, Oberkochen, G.F.R.) equipped with a thermostated low-dead-volume flow cell. Contrary to the manufacturer's instructions, the spectrophotometer was operated with a deuterium lamp, which resulted in a reduction of the detector noise.

RESULTS AND DISCUSSION

The reaction conditions were derived from a standard colorimetric method for the assay of low concentrations of hydroperoxides in hydrocarbons; this method has been in use for several years in our laboratory. It was found that the reaction time for hydroperoxides could be reduced considerably from that in the original procedure by heating the reaction mixture.

In view of the boiling points of the components of this mixture, a temperature of 70° was chosen. At this temperature, the influence of the reaction time on the formation of I_3^- from sodium iodide by cyclohexyl hydroperoxide was investigated; the results are shown in Fig. 3. The measurements were carried out on mixtures containing equal volumes of the reagent mixture sodium iodide, 2-propanol and acetic acid and the eluent 2,2,4-trimethylpentane–ethanol. The curve shows that at a reaction time of 90 sec almost 80% of the maximum absorbance is attained. Therefore, a residence time of 90 sec was chosen for the reaction system.

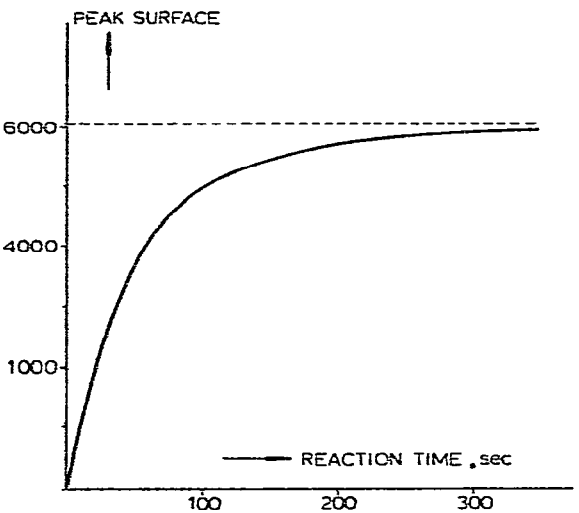

Fig. 3. Influence of reaction time on the formation of I_3^- from sodium iodide by cyclohexyl hydroperoxide.

The linear eluent velocity in the chromatographic column corresponds to a volume flow-rate of about 1.1 $cm^3 \cdot sec^{-1}$. The total volume flow-rate for the reagent was set arbitrarily at the same value. This choice is a compromise: on the one hand, high flow-rates cause undesirable dilution of the sample compounds (see eqn. 9), while on the other hand difficulties, such as irreproducible pump settings and irregular flow, will arise from too low to a flow-rate. As can be seen from Fig. 1, the condition $\Delta\sigma_{td} = 0.8$ sec can be met by using short columns (L = 10–20 cm) packed with glass beads in the size range 5–10 μm, at pressure drops under 10 atm.

However, it can be calculated from eqn. 9 that with $\Phi_c + \Phi_r = 2.2$ $cm^3 \cdot min^{-1}$, the internal diameter of the reactor column should be 0.7–0.8 cm. For practical reasons, it was decided to construct the reactor from standard 4.6-mm I.D. stainless-steel tubing. Consequently, standard low-dead-colume end-fittings could be used. From eqn. 9, it is found that $L \approx 50$ cm.

Fig. 1 shows that the column should be packed with 15-μm glass beads in

order to meet the condition $\Delta\sigma_{td} = 0.8$ sec. The viscosity of the reaction mixture is almost equal to the value assumed in plotting Fig. 1 (0.54 compared with 0.5 cP). Therefore, the pressure drop over the reactor was expected to be 25 atm; the experimental result was 20 atm.

Fig. 4 shows the chromatogram obtained for a model mixture of peroxides derived from cyclohexane; the peak of cyclohexyl hydroperoxide ($k' \approx 5$) corresponds to about 1 μg. The minimum detectable amount of this peroxide was 5 ng; injection of this amount produces a peak five times the standard deviation of the detector noise signal.

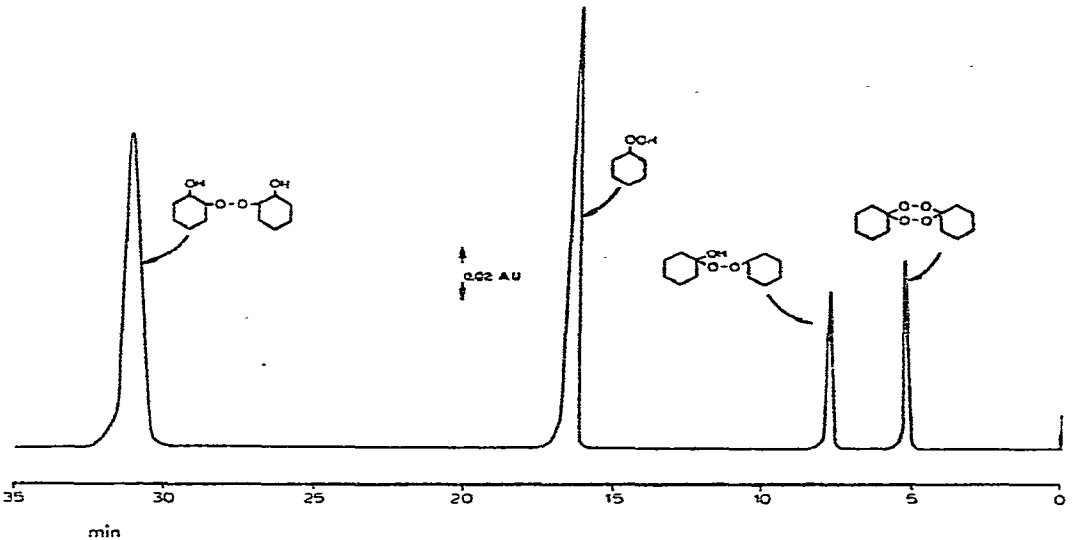

Fig. 4. Chromatogram of a test mixture of peroxides. Column: 30 cm × 4 mm, packed with 5 μm Merckosorb SI-60. Eluent: 2,2,4-trimethylpentane–ethanol (95:5), 50% water saturated. Flow-rate: 1.1 $cm^3 \cdot min^{-1}$. Detection by reactor system.

The additional band broadening in the reaction system, $\Delta\sigma_{td}$, was measured experimentally. For this purpose, a UV-absorbing compound, *m*-nitrophenol ($k' \approx 1.1$), was injected into the chromatographic column, which had been connected directly to the spectrophotometer. The elution peak was observed at 280 nm. The reactor was then placed between the chromatographic column and the spectrophotometer; the reagent flow was replaced by a flow of pure 2-propanol. The same compound was injected again and $\Delta\sigma_{td}$ could be calculated by comparing the band widths of the peaks. The value of 0.9 ± 0.2 sec which was found agrees fairly well with the theoretical value.

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