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DETERMINATION OF TRACE AMOUNTS OF CYCLOHEXANONE IN CYCLOHEXANONE OXIME BY LIQUID–LIQUID CHROMATOGRAPHY AND COLORIMETRIC DETECTION

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SUMMARY

Qualitative and quantitative determinations of separated constituents in effluents from liquid chromatographic columns can be carried out by continuously adding a suitable colorimetric reagent and monitoring the absorbance of the reaction mixture. The method can result in a highly selective and sensitive analytical procedure. However, a considerable amount of band broadening usually arises from the use of such reaction systems and this prevents a general application to fast liquid chromatography. A method is described for the determination of trace amounts of cyclohexanone in concentrated solutions of cyclohexanone oxime in toluene. The separation is achieved by liquid–liquid chromatography using a phase pair obtained from a mixture of 2,2,4-trimethylpentane, ethanol and water. In an on-line reaction system, carbonyl-containing constituents are converted into 2,4-dinitrophenylhydrazones, which turn into red-coloured compounds on the addition of an alcoholic solution of potassium hydroxide; the absorbance of the resulting mixture is measured at 430 nm. All reactions are carried out in a gas-segmented liquid stream. The contributions to band broadening of the various parts of the reaction system were evaluated and minimized. A delay time of 3 min in the reaction system involves a standard deviation of the residence time distribution of 6 sec. The detection limit for cyclohexanone corresponds to an injected amount of about 0.1 μg.

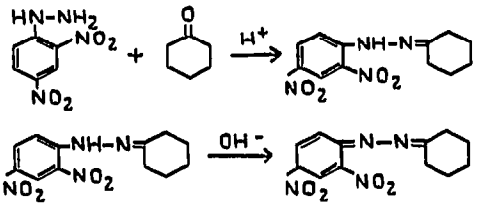
INTRODUCTION

A number of methods of chemical analysis are based on specific colour reactions followed by photometric measurements. However, the specificity of the reaction is frequently unsatisfactory and prior separations by extraction or chromatography may be required in order to eliminate interfering substances.

Direct coupling of a chromatographic column to a chemical reaction system not only makes possible the elimination of interfering substances but also permits the determination of individual compounds if a reagent is used that is specific to a group of compounds.

Automatic on-stream detection by measuring the colour developed when a suitable reagent is mixed with the column effluent is currently applied in the analysis of biologically important mixtures by ion-exchange chromatography. The considerable amount of band dispersion that usually arises from these detection systems hampers application in fast liquid–liquid chromatography because of the adverse effect on the column performance, especially with narrow elution peaks.

Quality control of cyclohexanone oxime, which is used for the production of caprolactam, requires a sensitive method to determine low concentrations (0.01 % w/w or less) of cyclohexanone. The classical methods for the direct determination of ketones cannot be used because of the presence of large amounts of the oxime, which also contains trace amounts of coloured substances. The application of gas chromatography presents serious difficulties owing to the rapid decomposition of cyclohexanone oxime at higher temperatures. The problem was solved by the combination of liquid–liquid chromatography and a colorimetric detection system. The system relies upon the reaction between carbonyl compounds and 2,4-dinitrophenylhydrazine. The hydrazones formed in an acidic medium subsequently turn into red-coloured compounds on the addition of an alcoholic solution of potassium hydroxide.



An automatic method for the determination of low concentrations of carbonyl compounds (total carbonyl content) has been described earlier[1].

THEORETICAL

The colorimetric detector consists of a reaction system and a measuring cell. Concentration peaks leaving the chromatographic column will undergo an additional dispersion in the reaction system.

Let the variance, expressed in time units, of the peak passing from the column into the reaction system be $\Delta\sigma_{tc}^2$, the variance of the residence time distribution function in the reaction system $\Delta\sigma_{td}^2$ and the variance of the peak at the end of the system σ_t^2. As the time variances are additive, then

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

The increase in band width due to the dispersion phenomena in the reaction system adversely affects the separation between the components A and B. This separation is characterized by the resolution R_{BA}:

$$R_{AB} = \frac{\Delta t_R}{2(\sigma_{t,A} + \sigma_{t,B})} = \frac{\Delta t_R}{2\sigma_{t,B}(n+1)} \qquad (2)$$

where Δt_R represents the difference in retention time, $\sigma_{t,A}$ and $\sigma_{t,B}$ are the standard

deviations of the elution function and $n = \sigma_{t,A}/\sigma_{t,B}$. The maximum value of R_{BA}, $R_{BA_{max.}}$, will be obtained if $\Delta\sigma_{td} = 0$.

Assuming $n = 1$, we can derive the equation

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

This relationship is graphically represented in Fig. 1.

The column length, L, giving a resolution R_{BA} at the column outlet can be calculated from the equation

$$L = 4\,(n+1)^2\,R_{BA}^2\left(\frac{r_{BA}}{r_{BA}-1}\right)^2\left(\frac{k_B'+1}{k_B'}\right)^2 H_B \tag{4}$$

where $r_{BA} = K_B/K_A$ (the selectivity factor, which is greater than unity), K_B and K_A are the distribution coefficients of the components B and A, respectively, $k_B' = Kq$, where q is the ratio between the volumes of the stationary and the mobile phase, and H_B represents the plate height for the slower migrating component B.

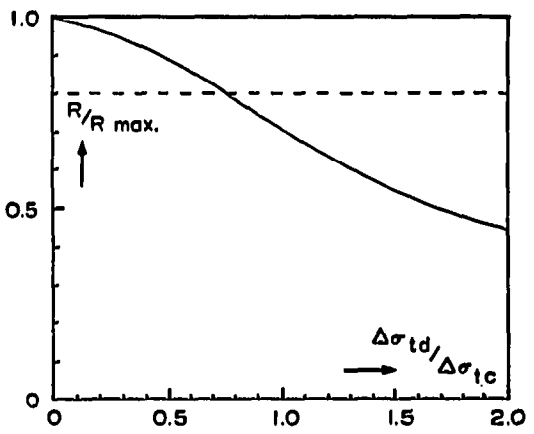


Fig. 1. Relationship between $R/R_{max.}$ and $\Delta\sigma_{td}/\Delta\sigma_{tc}$.

The reaction section of colorimetric detection systems described in the literature consists essentially of a narrow glass or plastic tube. The internal diameter and length should be chosen so as to give the required reaction time. In open tubes with continuous flow, a residence time distribution originates from the laminar flow profile[2,3].

If it is assumed that the variance $\Delta\sigma_{td}^2$ in such a reactor can be approximated by the variance of the residence time distribution of an inert, non-reacting component, the following equation can be used:

$$\Delta\sigma_{td}^2 = \frac{r^2\,t_v}{24\,D_m} \tag{5}$$

where r is the radius of the tube, t_v the mean residence time and D_m the molecular diffusion coefficient of the trace compound in the reaction mixture.

When a reaction time of several minutes is required, $\Delta\sigma_{td}$ can be maintained at an acceptable level only by using extremely low and, therefore, impractical values

of r. The peak broadening in the reactor can be considerably reduced by using a gas-segmented liquid flow. This type of reactor is used in the Technicon AutoAnalyzer systems. A number of applications of these reaction systems coupled with chromatographic columns have been developed, but in spite of the segmentation a considerable amount of dispersion persists and hampers application in fast liquid–liquid chromatography. For instance, a value of 500 sec^2 was found for $\Delta\sigma_{td}^2$ in a ninhydrin detection system of an amino acid analyzer[4].

In these detection systems, the column effluent is fed into a continuous series of short slugs of reagent, separated by air bubbles. A leakage between the segments of the reaction mixture is brought about by fluid from a segment wetting the wall of the tube and mixing with the next segment. An effective longitudinal dispersion arises from this leakage and can be quantitatively described if it is assumed that complete and instantaneous mixing occurs in every segment and that the concentration of the component in the liquid film on the wall equals the concentration in the donor segment. The following expression can be derived for the variance of the residence time distribution in the reaction system[4,5]:

$$\Delta\sigma_{td}^2 = \frac{2\pi^2 dLl r^3}{Q} \tag{6}$$

where d represents the uniform thickness of the liquid layer on the wall of the tube, L is the length of the tube, l the length of a liquid segment and Q is the liquid flow-rate (see Fig. 2).

It should be noted that the residence time distribution is formally described by a Poisson function. In practice, however, it can be approximated by a Gauss function.

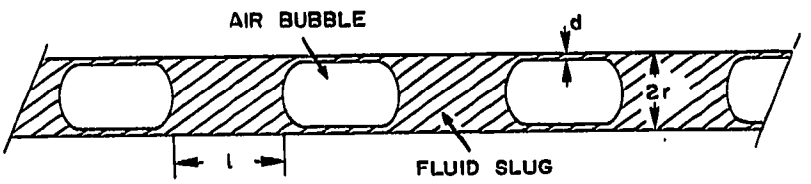


Fig. 2. Schematic representation of a gas-segmented liquid flow. l = Length of fluid slug; d = thickness of layer on tube wall; r = radius of tube.

Low values of $\Delta\sigma_{td}^2$ will be obtained for short liquid segments which are close together in a reaction tube with an internal diameter that is as large as possible without impairing the stability of the slugs. In our experiments, the distance between these liquid slugs was about 1 cm and the radius of the reaction tube was 1.2 mm.

The chromatographic separation process always involves a dilution of the sample components, which adversely affects the detection limit of any chromatographic method as compared with the direct concentration measurement. The dilution effect in liquid chromatography can be described as the ratio of the maximum of the elution peak leaving the column, $C_{max.}$, to the concentration of the component in the sample, C_0 (ref. 6):

$$\frac{C_{max.}}{C_0} = \frac{V_0}{A(1+k')\sqrt{2\pi LH}} \tag{7}$$

where V_0 is the injected volume and A the free cross-sectional area of the column. The

product C_0V_0 corresponds to the amount of the component injected. If a reaction system is used as detector, further dilution occurs owing to the addition of the reagent and the band broadening in the reaction system:

$$\frac{C_{max.}}{C_0} = \frac{Q_c}{Q_c + Q_R} \cdot \frac{A\sigma_{tc}}{\sigma_t} \cdot \frac{V_0}{A(1+k')\sqrt{2\pi LH}} \quad (8)$$

where Q_c and Q_R represent the flow-rate of the eluent and the reagent, respectively.

EXPERIMENTAL

The liquid chromatograph (Fig. 3) was constructed in our laboratory, and has been described in detail elsewhere[7]. A ternary liquid–liquid system for partition chromatography[8] was prepared from a mixture of 2,2,4-trimethylpentane, ethanol and

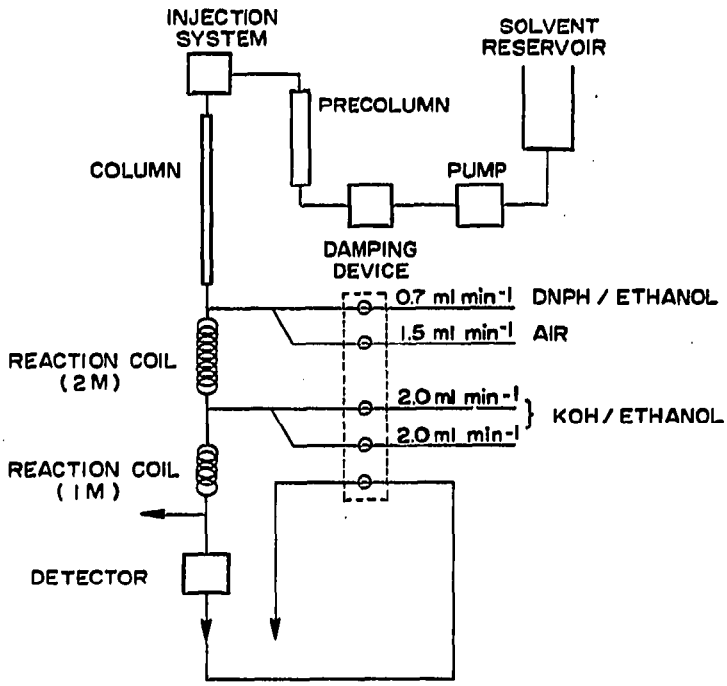


Fig. 3. Schematic diagram of the chromatographic system.

water (85:12.5:2.5, w/w). The less polar upper layer was used as the eluent and the polar lower layer as the stationary phase. A diatomaceous material, Hyflow Super Cel, particle size 7–11 μm, was used as the solid support.

Glass columns with an I.D. of 4 mm were prepared by a slurry-packing procedure and the packing was coated by an *in situ* technique[9]. A precolumn was used for the exact equilibration between the mobile and the stationary phase. Both columns were thermostatted at 25.0°. Samples were injected by using an appropriate syringe or an injection valve (Chromatronix HPS).

The reagents were added to the column effluent by means of a Technicon pro-

portioning pump. The flow-rates of the reagents indicated in Fig. 3 produced a column flow-rate varying from 0.5 to 1.5 ml min^{-1}. Practical considerations in the choice of the make-up of the reagent solutions are the need to prevent de-mixing of the final solvent mixture and to avoid precipitation of salts in the reaction system. The presence of water in the reaction mixture is essential. The formation of the hydrazones requires the presence of hydrochloric acid. On the addition of the alcoholic potassium hydroxide solution, salts are formed, which should be kept in solution, and therefore, because of its greater miscibility with the eluent and water, ethanol was substituted for the methanol used in the original procedure[10]. The absorbance of the reaction mixture was continuously measured at 430 nm with a Zeiss PMQ II spectrophotometer equipped with a low dead-volume flow-cell. Additional peak broadening in the non-segmented liquid flow in the couplings and connection tubes was reduced as much as possible by using short tubes of an I.D. of 0.4 mm.

The reaction conditions were investigated extensively. Both reactions are relatively fast. Finally, two reaction coils, each of 2 m length and I.D. 2.4 mm, were selected. The mean residence time in the reaction system was about 3 min. No additional reaction could be observed either by longer delay times or by heating the reaction mixture.

The contribution of the various parts of the detection system to the overall variance, $\Delta\sigma_{td}^2$, was investigated by removing the component in question from the system and measuring the decrease in the variance. The experiments were carried out by the injection of toluene ($k' \approx 0$). The absorbance of the reaction mixture was measured at 270 nm. Carbonyl components are not suitable for these experiments as the coloured products are not formed or are formed incompletely if the reaction coils are omitted. Toluene, as an almost unretarded component, has the additional advantage of a low value of $\Delta\sigma_{tc}$, which facilitates the determination of the extra-column peak broadening effects. The values given in Table I were found at an eluent flow-rate of 0.8 ml min^{-1}, which corresponds to a linear velocity, u, of 0.15 cm sec^{-1}.

TABLE I
CONTRIBUTION OF THE VARIOUS PARTS OF THE REACTOR SYSTEM TO $\Delta\sigma_{td}^2$

Component of the reactor system	$\Delta\sigma_{td}^2$ *(sec²)*
Reaction coil I	16
Reaction coil II	6
Mixing tees and de-bubbler	11
Total dispersion in the reaction system	33

From the value of $\Delta\sigma_{td}$ found by these experiments and the curve given in Fig. 1, it can be concluded that for peaks of equal width leaving the column with $\Delta\sigma_{tc} > 7$ sec, the loss of resolution will be less than 20%.

The chromatographic conditions for the separation of cyclohexanone and cyclohexanone oxime were chosen so as to reduce the loss of resolution to 20% and to have a short time of analysis and a low detection limit (minimum sample dilution), which was effected in the following way. Firstly, the dependence of H on u for the two components was determined for various column loadings and, therefore, different k' values. In these experiments, the components were detected directly in the column effluent by measuring the absorbance at 220 and 280 nm. From these results,

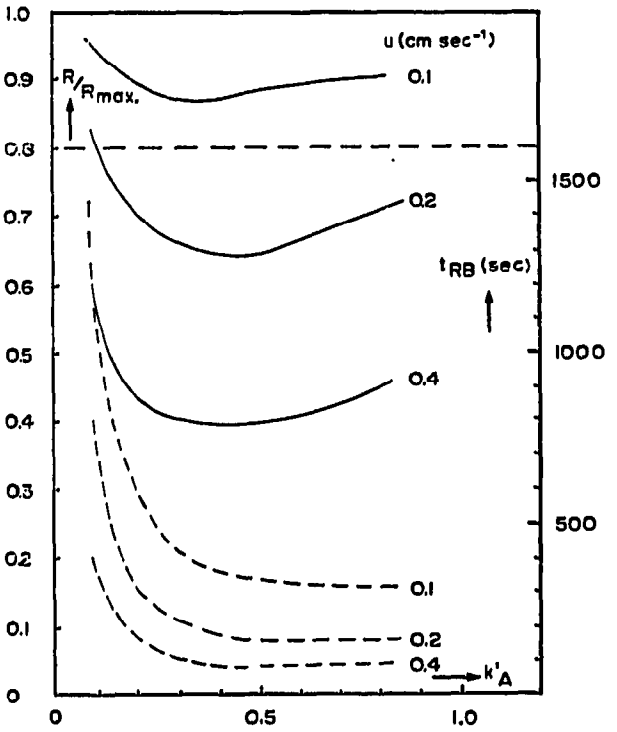


Fig. 4. Influence of the reaction system on resolution and time of analysis.

values of n and, by means of eqn. 4, the column length required for a resolution $R_{max.} = 3$ were calculated for different values of u and k' for cyclohexanone. The retention time for cyclohexanone oxime was taken as the time of analysis, t. The values of σ_t for the two components were derived from $\Delta\sigma_{tc}$ and $\Delta\sigma_{td}$ according to eqn. 1, and the values of R and the loss of resolution expressed as $R/R_{max.}$ could then be calculated. In Fig. 4, $R/R_{max.}$ and t are plotted against k' values for cyclo-

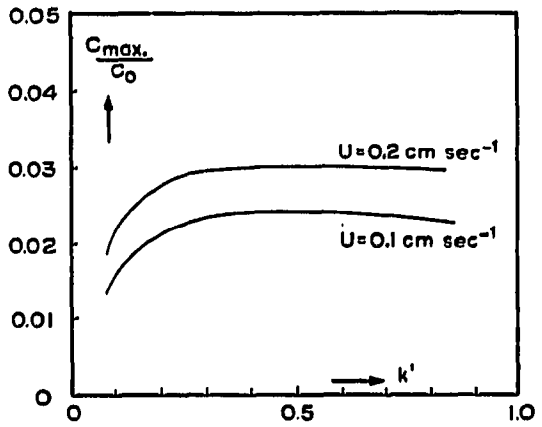


Fig. 5. Sample dilution *versus* capacity ratio for cyclohexanone.

hexanone for various values of u. It was assumed in these calculations that $\Delta\sigma_{td}$ did not depend on the flow-rate of the eluent, which is a reasonable simplification, as the reagent flow-rate is much greater than the eluent flow-rate. Finally, the values of the dilution factor, $C_{max.}/C_0$, for cyclohexanone were calculated from eqn. 8; the results are plotted in Fig. 5.

From Figs. 4 and 5, it can be concluded that a reasonable compromise between speed of analysis, sensitivity and resolution can be found for $k' \sim 0.5$ and $u \approx 0.15$ cm sec.$^{-1}$

For routine analysis, a 40-cm column was used. At a linear eluent velocity of 0.15 cm^{-1} and $k' = 0.3$ for cyclohexanone, a resolution $R_{max.} = 3$ was obtained. The resolution decreased to 2.4 when the reaction system was used. The influence of the reaction is shown in Fig. 6; the lower chromatogram was obtained by direct UV detection in the column effluent (see above), and in the upper chromatogram the reaction system was used.

The absorption spectra of dinitrophenylhydrazones in alcoholic solutions of potassium hydroxide show molar extinction coefficients of about $2 \cdot 10^4$. The standard

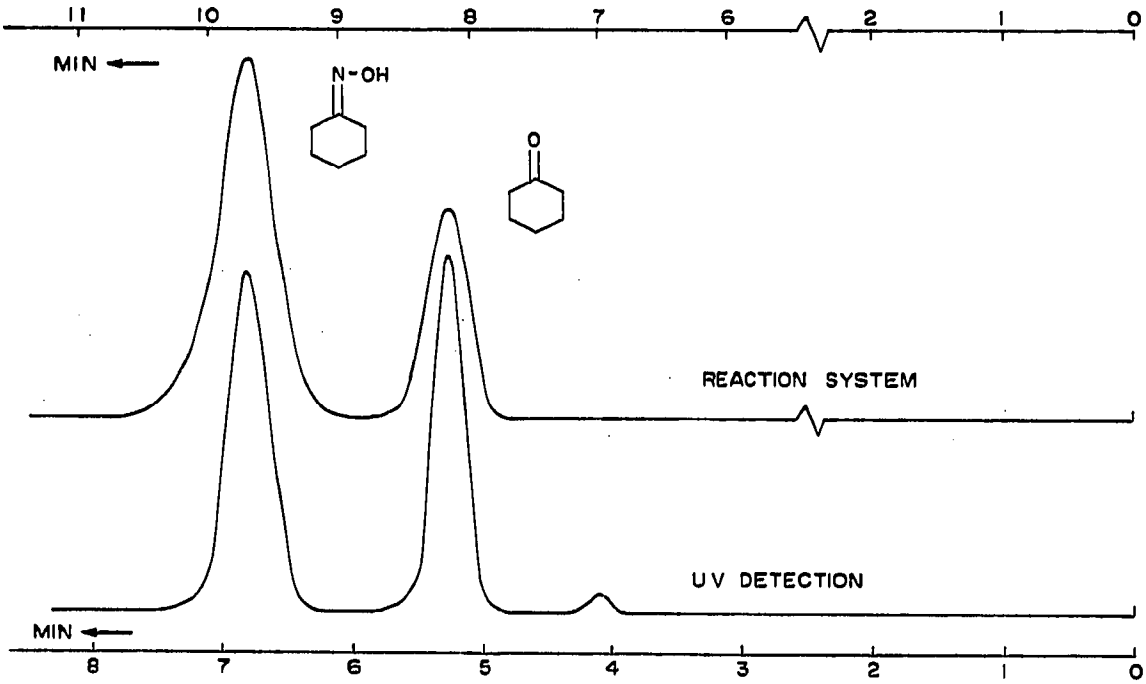


Fig. 6. Separation of cyclohexanone and cyclohexanone oxime.

deviation of the detector noise was found to be $1 \cdot 10^{-3}$ absorbance unit, which, for the 1-cm optical path flow cell, corresponds to a concentration level of $5 \cdot 10^{-6}$ g l^{-1} of cyclohexanone in the reaction mixture.

Samples of cyclohexanone oxime containing low concentrations of cyclohexanone were dissolved in toluene and 50 μl of the solution was injected. No noticeable influence of this sample volume on the plate height could be observed. The di-

lution factor, $C_{max.}/C_0$, was calculated from eqn. 8 and was found to be 0.025 for cyclohexanone under these conditions. It can be seen from these figures that 10^{-7} g in 50 μl of toluene gives a peak top $C_{max.}$ corresponding to 10 times the standard deviation of the detector noise.

DISCUSSION

At an eluent flow-rate of about 1 ml min^{-1}, the value of $\Delta\sigma_{td} = 6$ sec in the colorimetric detector exceeds the values found for low dead-volume flow detectors such as UV absorption cells[11] or specially designed refractometers[12]. However, it compares favourably with conventional refractometers[12] or the moving-wire detector; from recent data given by Scott and Kucer[3], it can be calculated that for this detector $\Delta\sigma_{td}^2 = 100$ sec^2 at a flow-rate of 1 ml min^{-1}. When a reaction system is used, chromatographic conditions, k' values and flow-rate should be chosen carefully in order to suit the characteristics of the detector. The peak width should exceed a limiting value in order to confine the loss in resolution. The limiting value is determined by the sum of variances arising from the various parts of the reaction system. The contribution of the reaction coils can be minimized by selecting suitable conditions according to eqn. 6. From the values given in Table I, it can be concluded that an important contribution to $\Delta\sigma_{td}^2$ arises from the connectors, mixing tees, etc., and great attention should therefore be paid to the construction of these parts of the reaction system. For instance, when a de-bubbler with a capillary outlet (0.4 mm) for the unsegmented liquid flow was used instead of a standard de-bubbler, a reduction in the variance of about 15 sec^2 was observed. The measuring cell should be placed between the de-bubbler and the proportioning pump (Fig. 3). An increase in the variance of at least 100 sec^2 was observed when the pump and cell were interchanged. The dilution of the chromatographic peak by the addition of the reagent adversely affects the sensitivity of the detection system. The ratio between the flow-rates of the eluent and the reagents should be chosen to be as high as possible. The use of columns with a larger internal diameter (*e.g.*, 4 mm) may be advantageous from this point of view.

REFERENCES

1 S. A. Bartkiewicz and L. C. Keynon, *Anal. Chem.*, 35 (1963) 422.
2 G. Taylor, *Proc. Roy. Soc. (London)*, 219 A (1953) 186.
3 R. P. W. Scott and P. Kucer, *J. Chromatogr. Sci.*, 9 (1971) 641.
4 G. Ertinghausen, H. J. Adler and A. S. Reichler, *J. Chromatogr.*, 42 (1969) 355.
5 R. D. Begg, *Anal. Chem.*, 43 (1917) 854.
6 J. F. K. Huber, J. A. R. J. Hulsman and C. A. M. Meijers, *J. Chromatogr.*, 62 (1971) 79.
7 R. S. Deelder, P. J. H. Hendricks and M. G. F. Kroll, *J. Chromatogr.*, 57 (1971) 67.
8 J. A. R. J. Hulsman, *Thesis*, University of Amsterdam, 1971.
9 C. A. M. Meijers, *Thesis*, University of Amsterdam, 1971.
10 D. E. Jordan and F. C. Veath, *Anal. Chem.*, 36 (1964) 120.
11 J. F. K. Huber, *J. Chromatogr. Sci.*, 7 (1969) 172.
12 G. Deininger and I. Halász, *J. Chromatogr. Sci.*, 8 (1970) 499.