Journal of Chromatography, 218 (1981) 45–50
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,004

EXTRA-COLUMN EFFECTS IN POLAROGRAPHIC *VERSUS* UV DETECTION IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

W. KUTNER*, J. DĘBOWSKI and W. KEMULA
Institute of Physical Chemistry of the Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw (Poland)

SUMMARY

A comparison of extra-column effects of chromatographic peaks in high-performance liquid chromatography recorded using a flow-through polarographic detector and a typical UV (254 nm) detector is presented. The diameter of the detection channel of the polarographic detector was 2 mm and the volume of the detection channel of the UV detector was 9.2 μl. When extra-column effects due to injection, tubing and the electronic system were considered, it was found that within experimental error the values of the peak variances are comparable for UV and polarographic detection and equal to $(2.7 \pm 1.0) \cdot 10^3$ μl^2.

INTRODUCTION

One of the most important features of a good detector for high-performance liquid chromatography (HPLC) is a minimal detection volume in order to eliminate broadening of the chromatographic peaks. This extra-column effect of peak broadening, which in practice should not exceed one tenth of the peak volume[1,2], becomes important for narrow bands in high-performance size-exclusion chromatography and also when small-volume analytical columns with a diameter of the support particles smaller than 5 μm are used. For integrating detectors in which changes of some physico-chemical bulk property of a flowing liquid is measured, *e.g.*, a UV detector, a decrease in extra-column effects due to the detector may be achieved by making its detection volume smaller. However for non-integrating detectors in which detection is performed from a thin layer of solution directly contacting a sensor, *e.g.*, a wall jet detector, the problem is more complicated. Then, the shape of the detection channel and the hydrodynamics of the mobile phase flow are responsible for peak broadening due to detection. Therefore, it is impossible to predict which of these two types of detectors causes a larger extra-column effect if one simply compares solely the geometrical volumes of the detection channels.

We have mentioned this problem previously[3,4] and, for polarographic detectors it has also been considered by other workers[5]. For the determination of washing out, *i.e.*, the "active" volume of the detector, a frontal method in a flowing system in the absence of a column was used[4]. In this work we have considered this problem in

0021-9673/81/0000–0000/$02.50

detail using methods more relevant to the usual operating conditions in HPLC. A comparison of extra-column effects of a flow-through polarographic detector with those of a commonly used UV (254 nm) detector is presented.

The total variance of a gaussian chromatographic peak expressed in volume units, $\sigma^2_{v,tot}$, can be presented as a sum of independent variance components[1,2,6–9] of column $\sigma^2_{v,col}$, and extra-column, $\sigma^2_{v,ext}$, effects of peak broadening.

$$\sigma^2_{v,tot} = \sigma^2_{v,col} + \sigma^2_{v,ext} \tag{1}$$

where

$$\sigma^2_{v,ext} = \sigma^2_{v,det} + \sigma^2_{v,inj} + \sigma^2_{v,tub} + \sigma^2_{v,other} \tag{2}$$

Here $\sigma^2_{v,det}$, $\sigma^2_{v,inj}$, $\sigma^2_{v,tub}$ and $\sigma^2_{v,other}$ denote, respectively, peak variances due to detection, injection, tubing and other possible factors, *e.g.*, electronic apparatus delay, $\sigma^2_{v,el}$. The peak variance due to detection can be calculated when other components of eq. 2 are evaluated.

Column peak variance is related to the retention volume, V_R, by the number of theoretical plates, N:

$$N = \left(\frac{V_R}{\sigma_{v,col}}\right)^2 \tag{3}$$

So, from eqns. 1 and 3 it follows that the dependence of $\sigma^2_{v,tot}$ on V^2_R for a mixture of substances with similar diffusion coefficients separated on the column should be linear and, when extrapolated to zero, one could obtain the value of the extra-column effects $\sigma^2_{v,ext}$.

For the estimation of the values of $\sigma^2_{v,inj}$, eqn. 4 can be applied:

$$\sigma^2_{v,inj} = kV^2_{inj} \tag{4}$$

where V_{inj} is the volume of sample injected and k is a constant for the given system. For a plug type of injection $\sigma^2_{v,inj}$ is independent of flow-rate.

For sufficiently long and narrow straight tubes, the value of $\sigma^2_{v,tub}$ can be calculated from the dependence[1,10–15]

$$\sigma^2_{v,tub} = \frac{\pi r^4 lf}{24\,D} \tag{5}$$

where r and l are the radius and length of the connecting tubing, respectively, f is the mobile phase flow-rate and D is the diffusion coefficient of the substance in the mobile phase. Eqn. 5 holds in the absence of mixing in tubes.

EXPERIMENTAL

The high-performance liquid chromatograph, columns and packings, chromatographic procedure and detectors, *i.e.*, UV (254 nm) with a detection channel of

length 10 mm and diameter 1.08 mm and a polarographic flow-through detector with a detection channel of diameter 2 mm, have been described previously[4].

Polarographic detection was performed using an LP-7 polarograph and an EZ-7 *y–t* recorder (Laboratorni Přistroje, Prague, Czechoslovakia). For recording chromatograms with UV detection a TZ 21 *y–t* recorder (Laboratorni Přistroje) was used. Samples were injected with a Rheodyne Model 7120 injection valve with a 10-μl sample loop unless stated otherwise. The dropping mercury electrode capillary characteristics in dynamic (flow-through) experiments were $m = 3.005$ mg sec^{-1}, $t_1 = 0.9$ sec at $h_{Hg} = 150$ cm and $E = -1.0$ V *vs.* Hg pool, and in steady-state experiments for the determination of diffusion coefficients they were $m = 3.56$ mg sec^{-1} and $t_1 = 1.875$ sec at $h_{Hg} = 50$ cm. Current oscillations due to the mercury dropping were eliminated using electronic damping of the polarograph. The time constant of this damping at $1 - 1/e = 0.632$ of full scale of the recorder was 2.2 sec and for UV detection it was 0.5 sec. A Hewlett-Packard Model 9830 A minicomputer was used for linear regression analysis calculations.

Analytical-reagent grade chemicals and doubly distilled water were used for the preparation of solutions. The mobile phase was methanol–1/15 *M* phosphate buffer (pH 6) (2:3) according to Michaelis. A mixture of substances that were easily detectable by UV and polarographic methods was used to represent model compounds, *i.e.*, *p*-, *m*- and *o*-nitroaniline and β-(5-nitrofuryl-2)acrylic acid.

RESULTS AND DISCUSSION

For the calculation of extra-column effects, high-performance liquid chromatograms of the mixture of model compounds were recorded independently with UV and with polarographic detectors (Fig. 1). It can be seen that nitrate, the ion often used as a marker of column dead volume, V_0, (peak 1) can be detected by the UV method whereas it is polarographically inactive, and oxygen dissolved in a sample is polarographically active (peak 3) but is UV inactive. The retention volume of potassium nitrate was 2.2 ml, whereas the theoretical value of V_0 for a cylindrical column closely packed with spheres was 1.04 ml. The mean value of the peak asymmetry factor measured at 10% of the peak height[2,16] was 2.7 for oxygen and 1.7 ± 0.1 for all nitro compounds.

In Fig. 2, the dependence of $\sigma^2_{v,tot}$ on V_R^2 is plotted for all substances being separated using linear regression analysis at flow-rates of 0.2, 0.5, 1.0 and 2.0 ml min^{-1}. It can be seen that the plots recorded with both UV and polarographic detectors are linear with a mean correlation coefficient for all curves close to 0.999. The value of $\sigma^2_{v,tot}$ for nitrate and oxygen were not taken into account in this calculation as they deviated markedly from this linear dependence, presumably owing to some other retention mechanism resulting in higher peak asymmetry factor for oxygen and peak splitting for nitrate. The intercepts of the plots in Fig. 2 represent $\sigma^2_{v,ext}$, with a mean value for both polarographic and UV detection of 2900 ± 1000 μl^2 at a significance level of 90%.

For the elucidation of the extra-column effect due to injection, eqn. 4 was tested. Fig. 3 shows the dependence of $\sigma^2_{v,tot}$ on the square of the injection volume, V^2_{inj}, for *o*- and *m*-nitroaniline for a flow-rate of 0.2 ml min^{-1} using the UV detector. The mean value of k (eqn. 4) calculated from the slopes of these curves was 1.36.

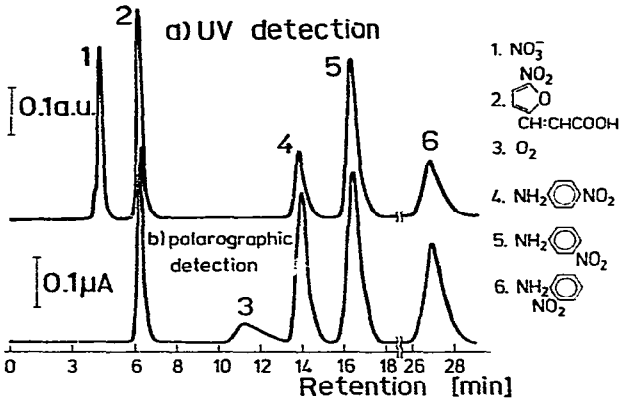

Fig. 1. HPLC traces recorded with (a) a UV (254 nm) detector and (b) a polarographic flow-through detector. Separation conditions: 250 × 4 mm I.D. column filled with LiChrosorb RP-18 (10 μm); mobile phase, methanol–1/15 *M* phosphate buffer (pH 6) (2:3) according to Michaelis; flow-rate, 0.5 ml min^{-1}; sample size, 10 μl; $E = -1.0$ V *vs.* Hg pool. Peaks: 1 = 1 *M* KNO_3; 2 = $7.5 \cdot 10^{-4}$ *M* β-(5-nitrofuryl-2)acrylic acid; 3 = O_2; 4 = 10^{-3} *M* *p*-nitroaniline; 5 = 10^{-3} *M* *m*-nitroaniline; 6 = 10^{-3} *M* *o*-nitroaniline.

which is much higher than 1/12, the theoretical value for plug-type injection[6]. Hence, for a 10-μl injection volume $\sigma^2_{v,inj} = 136$ μl^2.

Extra-column effects originating in the tubing were calculated using eqn. 5. Diffusion coefficients of nitro compounds in the mobile phase were calculated from the polarographic steady-state diffusion limiting currents using the Ilkovič equation.

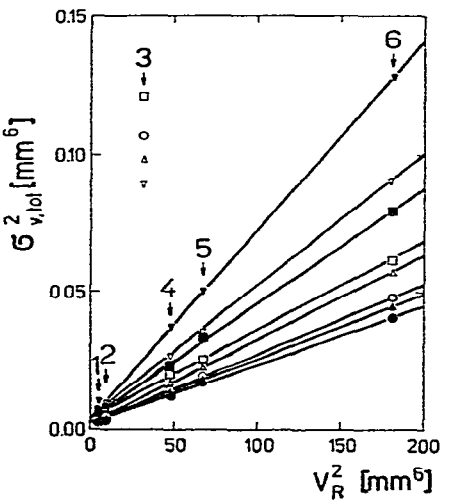

Fig. 2. Dependence of total peak variance, $\sigma^2_{v,tot}$, on square of retention volume, V_R^2. Flow-rate: (○, ●) 0.2; (△, ▲) 0.5; (□, ■) 1.0); (▽, ▼) 2.0 ml min^{-1}. Open symbols represent the polarographic detector and closed symbols the UV detector. Separation conditions as in Fig. 1. Numerals indicate separated compounds as in Fig. 1.

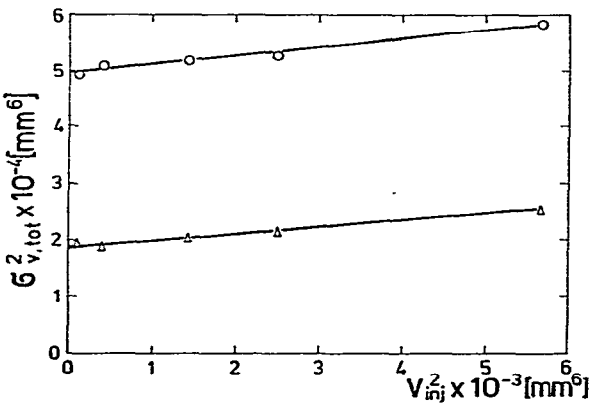

Fig. 3. Dependence of total peak variance, $\sigma^2_{v,tot}$, on square of sample volume injected, V^2_{inj}, with UV detection. (○) $4 \cdot 10^{-4}$ *M* *o*-nitroaniline; (△) $7 \cdot 10^{-4}$ *M* *m*-nitroaniline. Flow-rate, 0.2 ml min^{-1}. Other separation conditions as in Fig. 1.

For *m*-nitroaniline it was $D = 7.05 \cdot 10^{-6}$ cm^2 sec^{-1} and for other nitro compounds it did not deviate more than 15%. The radius of the connecting stainless-steel capillary tubing was 0.015 cm and its length was 20 cm for both detectors. Hence, the value of $\sigma^2_{v,tub}$ for a flow-rate of 0.2 ml min^{-1} was close to 60 μl^2. When the other factors contributing to peak broadening are small enough, $\sigma^2_{v,det}$ for both the polarographic and UV detector is close to $(2.7 \pm 1.0) \cdot 10^3$ μl^2. This result confirms our previous estimation[4] and enables one to conclude that regardless of the great differences in the geometrical volumes and shapes of the detection channels of the two detectors, our polarographic flow-through detector causes practically the same peak broadening as the UV detector.

The comparison of $\sigma^2_{v,det}$ for the two detectors does not require any assumption with regard to the presence or absence of mixing of the sample in the detection channels. In the limiting cases of a non-mixing or ideal mixing behaviour of the detection channel, $\sigma^2_{v,det}$ may be calculated for integrating detectors from eqn. 5 or taken as V^2_{det}[1,6], respectively. For our UV (254 nm) detector these values were 84.64 and 61,816 μl^2, respectively. The deduced value of $\sigma^2_{v,det}$ of $(2.7 \pm 1.0) \cdot 10^3$ μl^2 indicates that some mixing occurs in both detectors. This was confirmed by the dependence of $\sigma^2_{v,tot}$ on flow-rate with V_R kept constant. This dependence of the mean slope of log $\sigma^2_{v,tot}$ on log f is close to 0.5 and 0.25 for the UV and polarographic detector, respectively, for *o*-nitroaniline (Fig. 4). For other nitro compounds this slope for the UV detector is also higher than that for the polarographic detector and the values of these slopes do not differ much from those for *o*-nitroaniline. Hence the greater the mobile phase flow-rate, the greater is the extra-column effect caused by the UV detector compared with the polarographic detector.

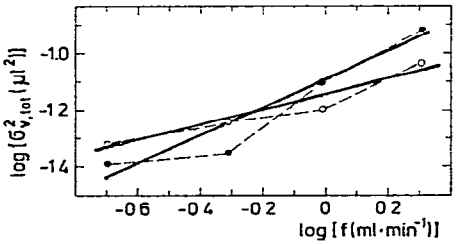

Fig. 4. Dependence of the logarithm of $\sigma^2_{v,tot}$ on logarithm of flow-rate, f, for *o*-nitroaniline. (●) UV detector; (○) polarographic detector. Separation conditions as in Fig. 1.

ACKNOWLEDGEMENTS

We thank to Dr. Peter Markl of the Institute for Analytical Chemistry, University Vienna, Vienna, Austria, for inspiring suggestions. This work was carried out within research project 03.10 of the Polish Academy of Sciences.

REFERENCES

1 M. Martin, C. Eon and G. Guiochon, *J. Chromatogr. Sci.*, 108 (1975) 229.
2 J. J. Kirkland, W. W. Yau, H. J. Stoklosa and C. H. Dilks, Jr., *J. Chromatogr. Sci.*, 15 (1977) 303.
3 W. Kutner, J. Dębowski and W. Kemula, *Polish Pat. Appl.*, No. P 210602, 1978.
4 W. Kutner, J. Dębowski and W. Kemula, *J. Chromatogr.*, 191 (1980) 47.

5 H. B. Hanekamp, P. Bos, U. A. Th. Brinkman and R. W. Frei, *Z. Anal. Chem.*, 297 (1979) 404.
6 J. C. Sternberg, *Advan. Chromatogr.*, 2 (1966) 205.
7 J. F. K. Huber, J. A. R. J. Hulsman and C. A. M. Meijers, *J. Chromatogr.*, 62 (1971) 79.
8 J. J. Kirkland, in S. G. Perry (Editor), *Gas Chromatography 1972*, Applied Science, Barking, 1973, pp. 39–56.
9 B. L. Karger, M. Martin and G. Guiochon, *Anal. Chem.*, 46 (1974) 1640.
10 G. Taylor, *Proc. R. Soc. London, Ser. A*, 219 (1953) 186.
11 G. I. Taylor, *Proc. R. Soc. London, Ser. A*, 223 (1954) 446.
12 R. Aris, *Proc. R. Soc. London, Ser. A*, 235 (1956) 67.
13 I. Halász and P. Walking, *Ber. Bunsenges. Phys. Chem.*, 74 (1974) 66.
14 J. F. K. Huber, R. van der Linden, E. Ecker and M. Oreans, *J. Chromatogr.*, 83 (1973) 267.
15 R. G. Brownlee and J. W. Higgins, *Chromatographia*, 11 (1978) 567.