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SOLVENT SEGMENTATION IN LIQUID CHROMATOGRAPHY —APPLICATION TO PHOTOCHEMICAL REACTION DETECTORS[*]

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SUMMARY

On the basis of a theoretical model, a comparison of solvent-segmented and non-segmented flow systems for post-column reaction detectors in liquid chromatography is made. From band broadening calculations, one can conclude that the application of the segmentation principle should be favoured even for relatively short reaction times, *e.g.* approx. 2 and 25 sec for 0.8- and 0.25-mm I.D. capillaries, respectively. This conclusion only holds true if phase separators of proper configuration are used; band broadening due to the capillaries is shown to be negligible. The dependence of proper system performance on carrier-stream composition and choice of segmentation solvent is also discussed.

INTRODUCTION

In recent years post-column reaction detectors have gained more widespread acceptance in liquid chromatography[1]. As for theoretical aspects, several studies have been published which deal with air-segmented[2–4], and with coiled tubular[5–7] and packed-bed[5–7] reactions. Tijssen[5] has stated that for reaction times of up to 20 min small-diameter (r = approx. 20 μm) coiled tubing should be used as post-column reactor. For reasons of convenience (back pressure, clogging, material cost, etc.), however, many workers prefer the use of larger-diameter, say 0.2 to 2.0 mm I.D., tubing. For systems involving their use, Van den Berg *et al.*[6] prefer packed-bed reactors for reaction times of up to 5 min; for more prolonged times, they favour segmented systems. Because of the known large contribution to band broadening of tee-pieces and phase separator (a much quoted[8] value being $\sigma_t^2 = 8$ sec^2, which corresponds to $\sigma_v^2 = 7000$–9000 μl^2) it must be stated that segmentation, indeed, should only be used for long reaction times. In the present study, it will be shown, however, that with the improved versions of tee-pieces and phase separator nowadays available

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the performance of a segmented-flow system becomes comparable to that of a non-segmented tubular reactor for even relatively short reaction times.

As an application, the results of our theoretical discussions have been verified with a chromatographic system involving the use of a post-column photochemical reaction detector. Up until now, this promising device has been used for relatively fast photochemical reactions only[9-11]; consequently, non-segmented flow was used in all instances. Since it is our aim to expand the capability of the photochemical reactor to longer reaction times, the obvious solution is the adoption of segmented-flow systems to suppress band broadening during irradiation. Here, solvent segmentation[12,13] seems to be of particular interest due to its advantages of non-compressibility and ease of handling over air segmentation.

EXPERIMENTAL

Fig. 1 shows the Technicon (Tarrytown, NY, U.S.A.) AutoAnalyzer system as set up for segmentation. The Technicon pump was used to deliver the carrier stream (φ_1), and segmentation solvent (φ_4), and to control the flow going to waste ($\varphi_3 + \varphi_4$).
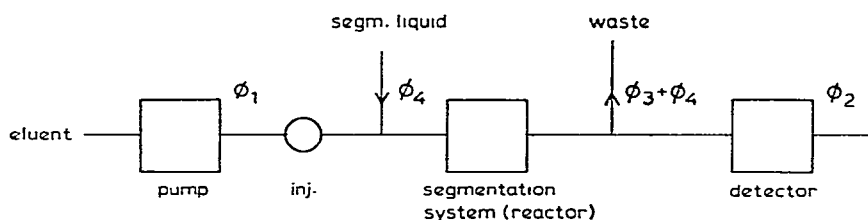

Fig. 1. Flow scheme of the segmentation system.

A Rheodyne six-port injection valve, a Technicon A-10 glass tee-piece and, as phase separator, a home-made tee-piece of 1.52 mm I.D. were used. Into the phase separator PTFE coils of 1/16 in. O.D. and 0.35, 0.5 or 0.8 mm I.D. were inserted in such a way that its inner volume was only 10–15 μl. Detection was done at 254 nm with a variable-wavelength UV detector (LC-3, Pye Unicam, Cambridge, Great Britain). The signal was recorded on a Moseley (Pasadena, CA, U.S.A.) Model 2DR-2AM recorder at a chart speed of 20 sec/cm.

The chromatographic system consisted of an Altex (Berkeley, CA, U.S.A.) Series 100 pump, a Valco injection valve, a photochemical reactor[11] with a 200-W Xe–Hg high-pressure lamp, a fan as cooling device and a mirror as reflection material, and a Perkin-Elmer (Norwalk, CT, U.S.A.) Model 204 A fluorescence spectrophotometer. The signal was recorded on a Kipp (Delft, The Netherlands) BD-8 recorder.

All chemicals were of analytical-grade quality.

THEORETICAL ASPECTS

The total variance, $\sigma_{v,tot}^2$, of a reaction detector system is given by

$$\sigma_{v,tot}^2 = \sigma_{v,inj}^2 + \sigma_{v,con}^2 + \sigma_{v,cell}^2 + \sigma_{v,react}^2 \qquad (1)$$

where the terms on the right-hand side of the equation denote the variance contribution due to injection system, connective tubing, detector cell and post-column reactor, respectively.

The variance of an injected block function of volume V_{inj} is expressed as

$$\sigma^2_{v,inj} = V^2_{inj}/K \tag{2}$$

where K is a proportionality constant. In the present work, plotting $\sigma^2_{v,inj}$ *versus* V^2_{inj} yielded $K = 5$, which satisfactorily agrees with the experimental value of 4 (ref. 14).

In a segmented-flow system, a fraction φ_3/φ_1 (see Fig. 1) of the carrier stream, or mobile phase, goes to waste in the phase separator and, consequently, so does a portion of the injected volume. The contribution to variance of the injection in a segmented-flow system should therefore be rewritten as

$$\sigma^2_{v,inj,seg} = 0.2\left(V_{inj} - \frac{\varphi_3}{\varphi_1} V_{inj}\right)^2 \tag{3}$$

From Fig. 1 one reads that

$$\varphi_3 = \varphi_1 - \varphi_2 \tag{4}$$

Combining eqns. 1, 3 and 4 now yields for the total variance, $\sigma^2_{v,seg}$, of a segmented system

$$\sigma^2_{v,seg} = 0.2 \frac{\varphi^2_2}{\varphi^2_1} V^2_{inj} + \sigma^2_{v,con} + \sigma^2_{v,cell} + \sigma^2_{v,react,seg} \tag{5}$$

Substituting the total variance of a non-segmented system (which is short-circuited) into eqn. 5 gives

$$\sigma^2_{v\ seg} + \left(\frac{\varphi^2_1 - \varphi^2_2}{\varphi^2_1}\right) \frac{V^2_{inj}}{5} = \sigma^2_{v,react,seg} + \sigma^2_{v,n-seg,s} \tag{6}$$

That is, the variance contribution of the reactor cannot simply be calculated by subtracting $\sigma^2_{v,n-seg,s}$ from $\sigma^2_{v,seg}$: an additional term $(\varphi^2_1 - \varphi^2_2) V^2_{inj}/5\varphi^2_1$ has to be included.

If a non-segmented reaction detector, *i.e.* a coiled tubular reactor, is compared with a segmented one, the ratio of the peak areas, A, can be written as

$$A_{seg}/A_{n-seg} = \varphi_2/\varphi_1 \tag{7}$$

With

$$A = \sqrt{2\pi} \cdot h \cdot \sigma \tag{8}$$

where h is the peak height, eqn. 7 becomes

$$h_{seg}/h_{n-seg} = (\varphi_2/\varphi_1) (\sigma^2_{v,n-seg}/\sigma^2_{v,seg})^{1/2} \tag{9}$$

If one arbitrarily states that the use of a segmented-flow system, instead of a non-segmented one, should be favoured if the height of the peak in the segmented-flow system is at least twice as high as that in the non-segmented one, it follows from eqn. 9 that

$$\varphi_2^2 \cdot \sigma_{v,\text{n-seg}}^2 > 4\varphi_1^2 \cdot \sigma_{v,\text{seg}}^2 \tag{10}$$

Since under normal operating conditions $\sigma_{v,\text{con}}^2 + \sigma_{v,\text{cell}}^2$ will be negligible compared to the variance of the reaction detector, the variance can be written as[5,6]

$$\sigma_{v,\text{n-seg}}^2 = \frac{V_{\text{inj}}^2}{5} + \kappa \frac{\pi r^4 L}{24 \, D_\text{m}} \cdot \varphi \tag{11}$$

for a non-segmented coiled tubular reactor with length L and inner radius r —where D_m is the diffusion coefficient of the solute in the carrier stream— provided the so-called diffusion term can be neglected. The value of the proportionality constant κ depends on the flow profile in the reactor[5,6]. Substituting eqns. 5 and 11 into eqn. 10 and setting $\varphi_2 = 0.9 \, \varphi_1$ (which means that 90 % of the carrier stream is sucked through the detector cell) yields

$$0.8 \, \kappa \cdot \frac{\pi r^4 L}{24 \, D_\text{m}} \cdot \varphi_1 > 0.5 V_{\text{inj}}^2 + 4 \, \sigma_{v,\text{react,seg}}^2 \tag{12}$$

If V_{inj} is small —as will often be true in practice (cf. ref. 15 for a detailed discussion)— eqn. 12 can be simplified to

$$r^4 L > \frac{120 \, D_\text{m}}{\kappa \pi \varphi_1} \cdot \sigma_{v,\text{react,seg}}^2 \tag{13}$$

Introducing the equation for the residence time, t_r, in the post-column reactor

$$t_r = \pi r^2 L / \varphi \tag{14}$$

eqn. 11 can be rearranged to give

$$\sigma_{v,\text{n-seg}}^2 = \kappa \frac{t_r r^2}{24 \, D_\text{m}} \cdot \varphi_1^2 + \frac{V_{\text{inj}}^2}{5} \tag{15}$$

Conversely, combining eqns. 10 and 15, one can express eqn. 13 as

$$t_r r^2 > \frac{120 \, D_\text{m}}{\kappa \varphi_1^2} \cdot \sigma_{v,\text{react,seg}}^2 \tag{16}$$

In the next section, the usefulness of the above relationships in comparing the merits of non-segmented- and segmented-flow systems will be demonstrated.

RESULTS AND DISCUSSION

In order to make the performance of a segmented-flow system comparable to that of a non-segmented one, the variance contribution of the reactor, $\sigma_{v,\text{react,seg}}^2$

obviously should be as small as possible. This is especially true, if reaction times are short (*cf.* eqn. 16). For a post-column reactor used in the segmented-flow mode, the variance is made up from contributions from the capillary and the phase separator plus tee-piece:

$$\sigma_{v,react,seg}^2 = \sigma_{v,ph.sep}^2 + \sigma_{v,cap}^2 \tag{17}$$

Variance contribution of the capillary

The data demonstrating the influence of the length and the diameter of PTFE coils on peak shape presented in Table I show that with water as mobile phase, all σ_t values (and, thus, also all σ_v values, because the same flow-rate was used in all experiments) are virtually the same; in other words, $\sigma_{v,cap}^2$ is negligible under the conditions used in our study for residence times of between 9 and 265 sec. For a more conventional mobile phase, *viz.* methanol–water (1:1), the same conclusion holds true, although the peaks appear to be somewhat broader and less symmetrical in this case than they are with water as solvent.

TABLE I

INFLUENCE OF LENGTH AND INNER DIAMETER OF PTFE CAPILLARIES ON PEAK SHAPE

Conditions: segmentation liquid, hexane; injection, 20 μl sodium nitrate; flow-rates: eluent, 0.88 ml·min^{-1}; hexane, 0.32 ml·min^{-1}; through flow-cell, 0.53 ml·min^{-1}.

Capillary		Eluent				Residence time (sec)
L(m)	I.D. (mm)	Water		Water–methanol (1:1)		
		σ_t* (sec)	$w_{0.1h}$** (sec)	σ_t* (sec)	$w_{0.1h}$** (sec)	
1	0.5	2 4	4 4/6.0	2.4	3.3/8.0	9
5	0.5	2.2	4.1/5.7	2.2	3.4/7.5	45
1	0.8	2.3	4.2/5.8	2.5	3.6/8.0	20
5	0.8	2.3	4.5/5.6	2.4	3.8/8.1	97
10.5	0.8	2.4	4.4/5.8	2.6	3.8/9.0	265

  * Measured at front of peak.
 ** Measured at 10% peak height as width of front/back of peak.

Results obtained for PTFE and quartz capillaries using both segmented and non-segmented flow are compared in Table II. The slightly different σ_t values recorded for PTFE tubing in this table and in Table I should be attributed to problems concerning the insertion of connective tubing, phase separator, etc. in exactly the same way in two different series of experiments. For the rest, a comparison between PTFE and quartz capillaries for segmented flow shows only a small difference in peak shape and, thus, in peak height, which again may be due to difficulties encountered with connective tubing which especially occur in the case of quartz capillaries. As is to be expected, in the non-segmented systems, the peaks rapidly become broader, and peak heights decrease with increasing inner diameter of the capillary.

In both Tables I and II sodium nitrate was the model compound. Similar results, which are not shown here, have been obtained with thioridazine and clobazam.

TABLE II

COMPARISON OF PTFE AND QUARTZ CAPILLARIES IN SEGMENTED AND NON-SEGMENTED FLOW SYSTEMS

Conditions: length of capillaries, 1 m; injection, 20 μl sodium nitrate. Flow-rates segmented system: eluent, 0.89 ml·min^{-1}; hexane, 0.32 ml·min^{-1}; through flow-cell, 0.66 ml·min^{-1}. Flow-rate non-segmented system: 0.89 ml·min^{-1}. For $w_{0.1h}$, see Table I.

Capillary	I.D. (mm)	Segmented flow system			Non-segmented flow system		
		Peak height (mm)	σ_t (sec)	$w_{0.1h}$ (sec)	Peak height (mm)	σ_t (sec)	$w_{0.1h}$ (sec)
PTFE	0.35	183	2.0/2.0	3.5/5.5	192	1.9/ 2.0	3.3/ 4.8
	0.5	175	2.0/2.1	3.6/5.8	99	3.2/ 3.8	5.8/ 8.8
	0.8	168	1.9/2.1	3.5/5.7	53	6.7/ 8.0	10.4/17.3
Quartz	0.8	158	2.2/2.3	4.1/6.2	40	9.1/11.0	14.6/26.4
	1.1	168	1.9/2.3	3.7/6.2	24	15.7/19.0	25.8/44.0
Without capillary		212	1.4/1.5	2.3/3.6			

With these two compounds, the peaks were slightly less symmetrical than those recorded for sodium nitrate, especially at high (μg·ml^{-1}) concentration levels. However, as will be demonstrated below (Fig. 2), symmetrical peaks were obtained when using a normal chromatographic system instead of plug injection. It should be added that the peak shapes did not change over the temperature range 20–60°C, which is a matter of some interest with regard to the use of the segmentation principle in a photochemical reactor.

As for segmentation liquids, next to hexane ($\varrho = 0.66$ g·cm^{-3}) chloroform ($\varrho = 1.48$ g·cm^{-3}) also performed well, which is according to expectations[12]. Somewhat surprisingly, even a hexane–chloroform mixture of a density ($\varrho = 0.98$ g·cm^{-3}) similar to water was used successfully after the insertion of a small plug of PTFE wool in the phase separator. The use of such a plug is also recommended for experiments involving carrier streams containing a high proportion of organic modifier, e.g. water–methanol (3:7) and water–acetonitrile (1:1). For the latter two solvent mixtures, band broadening, with sodium nitrate as a model compound, was similar to that observed in the earlier systems ($\sigma_t = 2.0$–2.7 sec).

Variance contribution of the phase separator

In order to determine the variance contribution of the phase separator (plus tee-piece) —which is equal to $\sigma^2_{v,react}$ (cf. above)— a series of measurements was performed[15] using injection volumes ranging from 19 to 96 μl, and various flow-rates φ_1 (0.6–1.2 ml·min^{-1}) and φ_2 (0.4–1.0 ml·min^{-1}), at φ_2/φ_1 ratios from 0.5 to 0.8. From the experimental data $\sigma^2_{v,react}$ was determined by plotting $\sigma^2_{v,seg}$ versus $V^2_{inj}/5$ (cf. eqn. 6). Values of $\sigma^2_{v,react}$ were consistently found to fall in the region 70–250 μl^2 with an average value of approx. 150 μl^2. The latter value corresponds to a contribution of the phase separator to the variance of less than 1 sec.

Comparison of systems

Next, a comparison between segmented and non-segmented systems was made by inserting suitable numerical values for the various parameters in eqns. 13 and 16.

They were as follows: $\sigma^2_{v,ph.sep} = 150 \ \mu l^2$; $\varrho = 0.9 \ g \cdot cm^{-3}$; $\eta = 0.77 \cdot 10^{-2}$ Poise (mobile phase, methanol–0.01 M sodium acetate (1:1)); $D_m = 1.5 \cdot 10^{-5} \cdot cm^2 \cdot sec^{-1}$; $\varphi_1 = 17 \ \mu l \cdot sec^{-1}$; $\kappa = 0.25$ (average value for capillaries with 60-mm coil diameter and 0.3–0.8-mm I.D.). Eqns. 13 and 16 now yield

$$r^4 L > 2.0 \ mm^5 \tag{18}$$

and

$$t_r r^2 > 0.37 \ mm^2 \cdot sec \tag{19}$$

respectively. To quote some examples, from these equations it can be calculated that for a post-column reactor of length $L = 1$ or 10 m, segmented flow should be preferred to non-segmented flow for an inner radius larger than 0.21 and 0.12 mm, respectively. Or, to use eqn. 19, for a reaction capillary having an inner radius of 0.125 mm, the segmentation principle is to be preferred for reaction times of over 24 sec, while for capillaries with an inner radius of 0.4 mm, this is true for reaction times of over 2 sec. Similar results can be read from the experimental data given in Table II. With the 1 m × 0.35 mm I.D. PTFE capillary, a non-segmented system should be favoured, since $h_{seg}/h_{n-seg} = 0.95$. For the 1 m × 0.5 mm I.D. PTFE capillary, on the other hand, h_{seg}/h_{n-seg} has a value of 1.8. This seems close enough to the arbitrarily chosen value of 2 (*cf.* eqn. 10) —which according to the above theoretical calculations should have been reached for 0.42 mm I.D. tubing— to favour segmented over non-segmented flow.

Application

A comparison of segmented and non-segmented-flow systems under normal operating conditions, *i.e.*, using a chromatographic column plus a photochemical reactor, is shown in Fig. 2; clobazam and desmethylclobazam were used as test compounds. The separation was carried out on a 15-cm long column packed with 5-μm Supelcosil LC-18 (Supelco, Bellefonte, PA, U.S.A.) using methanol–0.01 M sodium acetate (6:4) as mobile phase. Segmentation was done by means of solvent (hexane) or air segmentation. In Fig. 2A, a 0.5-mm I.D. PTFE capillary ($t_r = 19$ sec) was used. It is evident that the peak heights observed in the non-segmented system are distinctly smaller than are those in either the air- or hexane-segmented system, and that resolution is poorer.

The relatively low signal of clobazam in the hexane-segmented system is possibly caused by a change in reaction mechanism due to the presence of hexane and/or the solubility of clobazam or its reaction products in the segmentation solvent. Although the experimental value of h_{seg}/h_{n-seg} for clobazam is somewhat lower than the arbitrarily chosen value of 2 (*cf.* eqn. 10), the chromatograms clearly indicate that the use of a segmented flow system is to be preferred.

In Fig. 2B, the same analysis as depicted in Fig. 2A is shown. In this case however, a capillary of more suitable, *i.e.*, smaller diameter (0.3 mm I.D.) has been selected for the non-segmented flow system. For the segmented flow system, on the other hand, a rather wide-bore capillary of 0.8 mm I.D. has been used. From the chromatograms, it is obvious that even for short reaction times (t_r, 20–30 sec) seg-
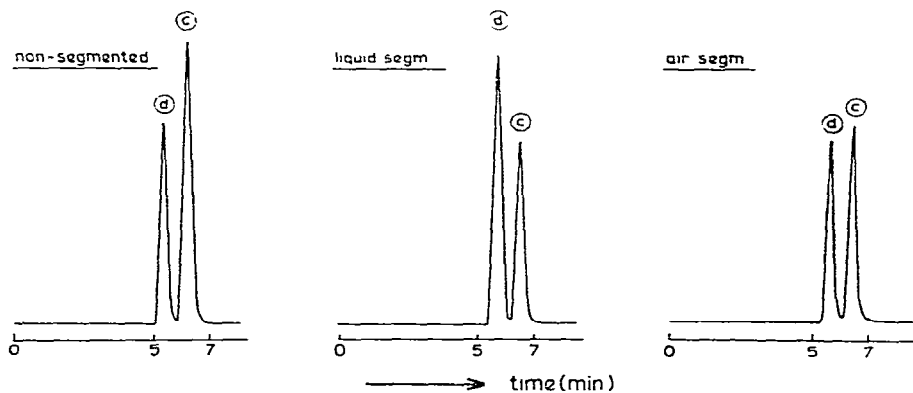
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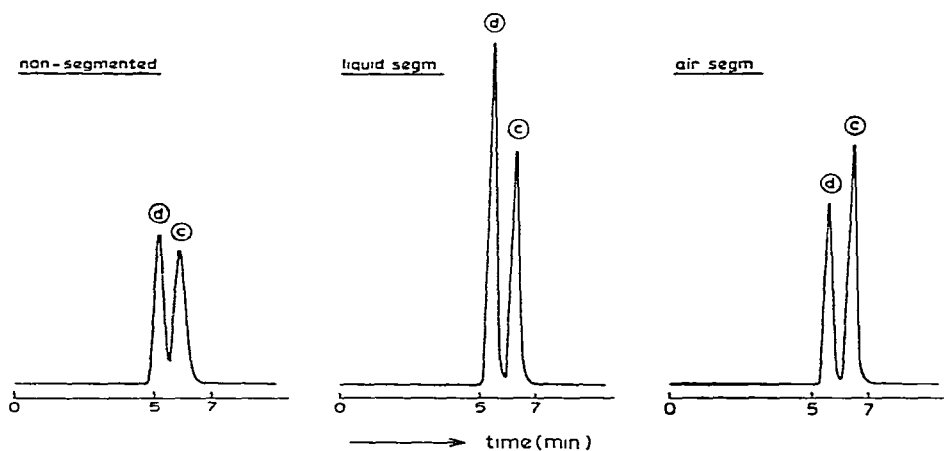
B

Fig. 2. Chromatograms of clobazam (c) and desmethylclobazam (d) after photochemical reaction. (A) Capillary, PTFE of 0.5 mm I.D.; reaction time, 19 sec; flow-rates: φ_1, 0.9 ml/min; φ_2, 0.8 ml/min; φ_4, 0.32 ml/min. (B) Capillary for the non-segmented flow system, 0.3 mm I.D.; for the segmentation systems, 0.8 mm I.D.; other conditions as in (A).

mented flow is competitive with non-segmented flow through relatively small-bore capillaries. Lastly, Fig. 2 demonstrates that air segmentation is a useful alternative to solvent segmentation.

CONCLUSION

It has been demonstrated in this study that with a phase separator of proper configuration the use of solvent-segmented instead of non-segmented flow systems can be favourable with relatively short residence times and capillaries with a relatively large inner diameter in a post-column reaction detector. For photochemical

reactors this is a particularly important conclusion, since the use of packed-bed reactors is not a feasible alternative. The use of capillaries having large inner diameters has the additional advantage of low pressure drop and high energy input. Application of the solvent-, or air-, segmentation principle to photochemical reactions of relatively slow kinetics is currently being investigated.

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