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FLOW CELL AND DIFFUSION COEFFICIENT EFFECTS IN FLOW INJECTION ANALYSIS

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Summary. The effects of flow cell geometry and nature of the solute on peak shape and dispersion/flow rate relationship are described. Flow cells produce significant effects caused by the finite volume sampled by the light beam and the disruption of laminar flow conditions. At low flow rates, the larger the molecules, the greater the dispersion.

Since the introduction of flow injection analysis (f.i.a.) by Růžička and Hansen in 1975 [1], several theoretical approaches have been used in attempts to provide a quantitative description of the dispersion processes that occur in flow-injection manifolds. These include the use of numerical techniques for solving the diffusion-convection equation [2], and the use of flow models borrowed from chemical engineering theory [3]. The reason for using such approaches is that it is not possible to obtain an exact solution to the diffusion-convection equation under the conditions employed in practical f.i.a.

Fundamental to the methods mentioned, is the concept of an ideal system or theoretical manifold. This concept arises from the assumptions commonly made, namely that (i) conditions of laminar flow exist, (ii) the flow is undisturbed by the injection process, (iii) flow occurs down a long, straight tube of circular cross-section, and (iv) the solute concentration is measured in a plane perpendicular to the direction of flow.

The problem in usefully applying such theoretical treatments to real flow-injection manifolds is that such assumptions are not valid. For example, although in principle laminar flow occurs in a manifold under the conditions of f.i.a., connections, valves and tight bends will all disrupt this primary flow pattern and introduce a variety of secondary flow patterns. Also, practical detectors have a finite volume, and may well give rise to major disruptions of the flow pattern as, for example, occurs in the nebulizer of a flame atomic absorption spectrometer, or in the path through a spectrophotometric flow-cell. Another problem in applying theory to practice is that manifolds for practical analyses are often more complicated than the simple, single-line case. A practical manifold may contain confluence points, segmentors, phase separators, dialysis units and so on.

With these problems in mind, it seems unlikely that a rigorous theoretical treatment of flow-injection manifolds will be successful in producing accurate equations describing dispersion behaviour. A completely empirical approach, however, would be time-consuming, and would have no predictive power. Thus the use of flow models to describe the dispersion processes would seem to be an attractive proposition.

The single well-stirred tank model has been described for f.i.a./a.a.s [4], and this, together with other models, are currently being studied in this laboratory. As part of this study, an extensive investigation of the factors affecting dispersion is being conducted. In this communication, the results of studies of the effects of the type of flow cell and the solute used are presented.

Experimental

Apparatus. The same basic apparatus was used for all the experiments. This comprised of a peristaltic pump (Gilson Minipuls 2) fitted with an air-column depulser, an injection valve (Rheodyne 5020) fitted with a 113- μ l sample loop, and one of four flow cells, connected via a 110-cm length of 0.58 mm i.d. PTFE tubing (RS Components). The flow cells used are shown in Fig. 1. Two of the cells were standard absorbance flow cells, having volumes of 8.0 and 60 μ l (Pye-Unicam). The third cell was a combined absorption/fluorescence cell, having a volume of 25 μ l (Hellma). The fourth cell was constructed from a piece of drawn glass capillary tubing (approximately 0.6 mm i.d.), mounted in a black perspex block at right angles to the light path of the instrument. The volume of this cell was estimated to be approximately 0.6 μ l. The detector was a Pye-Unicam SP6-250 visible spectrophotometer, connected to a chart recorder (W + W Tarkan 600).

Materials. Materials used as tracers were potassium permanganate (1.33 g l^{-1}), cobalt(II) chloride (36.3 g l^{-1}), tartrazine (0.25 and 0.020 g l^{-1} ; C.I.

Fig. 1. The flow cells examined. F indicates flow path; L indicates light path; shading indicates optical volume. Cell volume: (a) 0.6 μ l; (b) 8.0 μ l; (c) 25 μ l; (d) 60 μ l.

19140, Pointing Ltd.), ponceau S (0.020 g l^{-1} ; C.I. 27195, Fisons) and vitamin B₁₂ (0.135 g l^{-1} ; Sigma). Absorbances were monitored at wavelengths of 526, 513, 426, 532 and 550 nm, respectively. All the materials gave linear calibrations up to the concentrations given above.

Effect of flow cell. Solutions of tartrazine were used. The peak shapes obtained for flow rates of 0.84, 2.0 and 6.0 ml min^{-1} were recorded for each cell in turn at high chart speeds (10, 20 or 30 cm min^{-1}). Steady-state absorbance values were also measured, so that the peaks could be normalised for direct comparison, values being read directly from the chart recorder output.

Effect of solute. With the 8.0- μl cell in the spectrophotometer, the peak dispersion, D (defined as the ratio of injected to peak concentrations), was measured as a function of flow rate for each of the five solutes. Ten replicate injections were made for each sample at various flow rates in the range 0.3–10.0 ml min^{-1} . Each set of injections was followed by a measurement of the steady-state absorbance. The mean, standard deviation and 95% confidence interval about the mean were calculated for each measurement.

Results and discussion

Effect of flow cell. The peak shapes recorded for the different cells are shown in Fig. 2, plotted as normalised signal against time. For each flow rate, the curves for the 0.6-, 8.0- and 25- μl cells lie closely together. The slight differences in peak height and shape between these curves may indicate real differences between the cells, but could equally be attributed to the variation (evaluated in a separate experiment) produced by the breaking and making of connections required to change cells (see later). Although these

Fig. 2. Peak shapes obtained at different flow rates. Flow rate: (a) 6.00 ml min^{-1} ; (b) 2.00 ml min^{-1} ; (c) 0.84 ml min^{-1} . Cell volume: A, 0.6 μl ; B, 8.0 μl ; C, 25 μl ; D, 60 μl .

three cells appear very different in size, the 0.6- and 25- μl cells represent similar measuring conditions because, for the 25- μl cell, detection also occurs across the flow, whilst only a small part of the cell coincided with the light path of the spectrophotometer, thus reducing the effective optical volume. The biggest difference in peak shape is observed between these three cells and the 60- μl cell. Peak height is significantly reduced, whilst peak width is correspondingly greater. The peak also appears significantly later in time, even though the connecting tubes were carefully cut to keep the distance between the injector and the optical volume constant. Similar results were obtained from calculations of the effect of different measuring volumes on peak shapes, in the absence of any additional mixing processes, computed from a well-stirred tank model. It was also found that the way in which the dispersion varies with flow rate can be strongly influenced by the flow cell used. These results suggest that the flow cell contributes to the observed dispersion coefficient for any manifold in two ways. One is the effect of measuring solute concentration within a finite volume; the other is the effect of bends, edges and changes in bore through the cell, giving rise to regions of turbulent mixing.

Effect of solute. The variation of the dispersion coefficient, D , with flow rate is shown for the five different solutes in Fig. 3. With the apparatus used, relative standard deviations for ten replicate injections were typically less than 1%. However, poorer precision was found for the value of D obtained under apparently identical conditions on a day-to-day basis. For a flow rate

Fig. 3. Variation of the dispersion coefficient, D , with flow rate for different solutes: (○) vitamin B₁₂; (□) ponceau S; (×) tartrazine; (+) cobalt(II) chloride; (◇) potassium permanganate.

of 5.0 ml min^{-1} , the mean of ten individual measurements of D was found to be 1.95 with a 95% confidence interval of ± 0.012 . When these measurements were repeated over a period of several days, D was found to be 1.90 with 95% confidence interval ± 0.089 . These measurements involved the breaking and making of connections within the manifold.

Thus, some care needs to be exercised in interpreting the results obtained. Generally, the dispersion/flow rate curves for the same solute recorded on different days were in good agreement, some differences being observed at the lower flow rates. For all the solutes, the value of D tended to become constant above a flow rate of about 6 or 7 ml min^{-1} . This may indicate a limiting of the extent of dispersion by increased turbulent mixing at connections, valve and flow cell.

The biggest differences in behaviour occurred at low flow rates. Comparison of the confidence intervals for individual points for any given solute revealed that the observed changes in the value of D with flow rate represent a real effect, although the curves for each solute were not defined with great accuracy, given the poor reproducibility of the dispersion coefficient on a day-to-day basis. Differences in the value of D between the different solutes were tested by injecting each in turn at fixed flow rates, and were found to be real differences. That the diffusion coefficient of the solute should have an effect on the observed dispersion behaviour is to be expected from the diffusion-convection equation, a fact which has been exploited by Gerhardt and Adams [5] in the determination of the diffusion coefficients of molecules of biological interest.

It is proposed that the reason for the observed behaviour is that in regions of flow where the diffusion/convection equation is applicable, bigger molecules move only slowly between streamlines and are therefore subject to greater dispersion.

Conclusions

It has been shown that both the type of flow cell and the diffusion coefficient of the solute used have an influence on the observed dispersion for any given flow-injection manifold. Clearly, then, careful attention must be paid to the selection of the cell used, the cell volume and type being optimized for a particular analysis. Further, any solute used as a tracer for optimizing a flow manifold, especially for applications involving large molecules, should have similar physical properties to the analyte being determined. The results obtained also indicate that, with the injector, manifold and detector used here, the flow pattern is a function of flow rate; diffusion-convection mechanisms predominate at low flow rates but give way to a greater contribution from turbulent patterns at higher flow rates.

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