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FLOW INJECTION ANALYSIS
Part X. Theory, Techniques and Trends†

J. RŮŽIČKA* and E. H. HANSEN

Chemistry Department A, Building 207, The Technical University of Denmark, 2800 Lyngby (Denmark)

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SUMMARY

The dispersion of sample zones in Flow Injection systems is described by analogy with the mixing of fluids in large-scale chemical reactors. This comparison has resulted in a definition of the sample zone dispersion, its mathematical description, and the identification of the main parameters which can be used to effect the desired degree of mixing. The miniaturized Flow Injection system, designed with the aid of the rules derived on the basis of the theory of dispersion, uses only 5—30 μl of sample solution and gives the analytical readout within 5—30 s after sample injection. The most recent Flow Injection methods, such as pH measurements at limited dispersion, voltammetry (including anodic stripping at trace levels), solvent extraction, and a new approach to enzymatic assays, are briefly reviewed to illustrate the trends towards future developments in continuous analysis in unsegmented streams.

It might appear that the sole reason for automation of chemical analysis is the need to analyse a large number of samples rapidly with minimum expense of labour. This is indeed so in most clinical and many industrial laboratories, where the increased precision, inherent to automated handling, is also appreciated. From a research or academic viewpoint, automation is, unfortunately, often considered as a mere mechanization of existing procedures which does not offer much opportunity for innovation. This is true for most batch analysers (with the exception of the centrifugal analysers), but does not apply to continuous flow systems which are more flexible, and where the flow of liquids and the mixing patterns at the interfaces between regions of different concentrations offer many new aspects for performing chemical analysis. Yet, within the past 20 years, during which the air-segmented continuous flow systems based on Skeggs's concept [1] have been in use, only a small fraction of the several thousand published papers on Auto-Analyzer systems has dealt with the theory, the most significant contributions being those of Thiers et al. [2, 3], Begg [4, 5] and of the Technicon group [6—8]. Even these authors, however, have focussed their attention entirely

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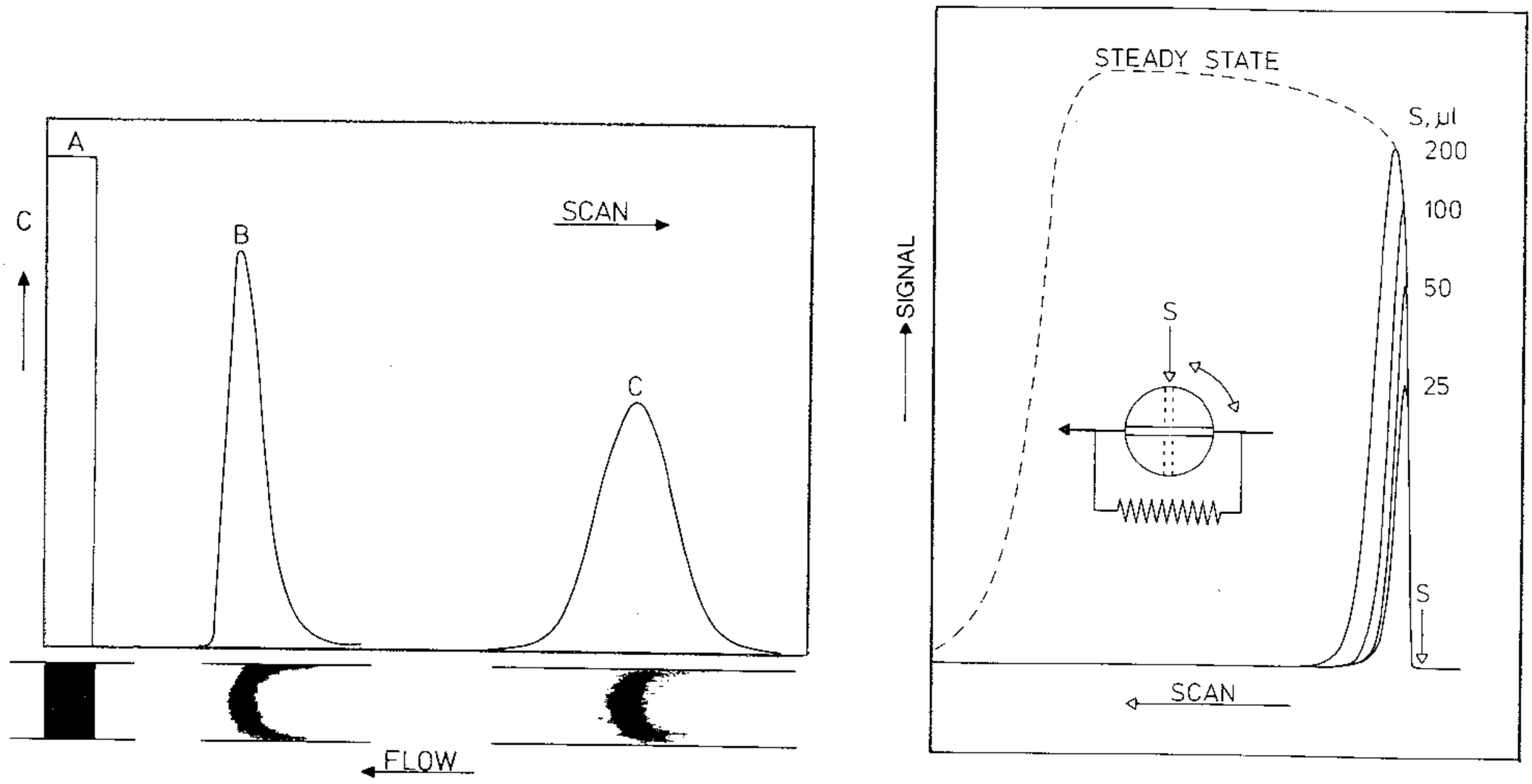


Fig. 2. Typical peak forms and corresponding concentration profiles observed: (A) at the point of injection; (B) shortly after injection; and (C) after passage through an open narrow tube.

Fig. 3. The principle of the injection valve, furnished with a bypass of higher hydrodynamic flow resistance; and peaks obtained by injecting, at point S, increasing volumes of sample solutions (S, μl).

also requires a reproducible travel pattern of the sample from the point of introduction to the detector. Yet, because of the inherent compressibility of air, any segmented stream pulsates and therefore neither the tube diameter nor the tube length can be decreased beneath a certain value if these pulsations are not adversely to affect the reproducibility of the timing required by the pertinent chemistry. Additional adverse factors are the addition and removal of air from systems and the irregularity of the bubble pattern.
In contrast, the only source of pulsation in an unsegmented stream can be attributed to the use of an imperfect pump. Moreover, because the sample does not pass through a pump on its way to the detector, its path through the system is well defined, and the dispersion of the sample zone and the residence time can be chosen at will to suit exactly the requirements of the chemistry involved. Not only long but extremely short residence times can be maintained reproducibly.

The controlled dispersion of the sample zone which occurs during its passage through the system towards the detector results in a response curve (Fig. 2) which has a peak shape characteristic of the Flow Injection system. Naturally, the sample zone broadens as it moves downstream and changes from the original asymmetrical shape to a more symmetrical and eventually Gaussian form. By changing the flow parameters, the dispersion can be easily manipulated to suit the requirements of a particular analytical procedure so that optimum response is obtained at minimum time and reagent expense. Furthermore, the concentration gradients in the interfacial regions

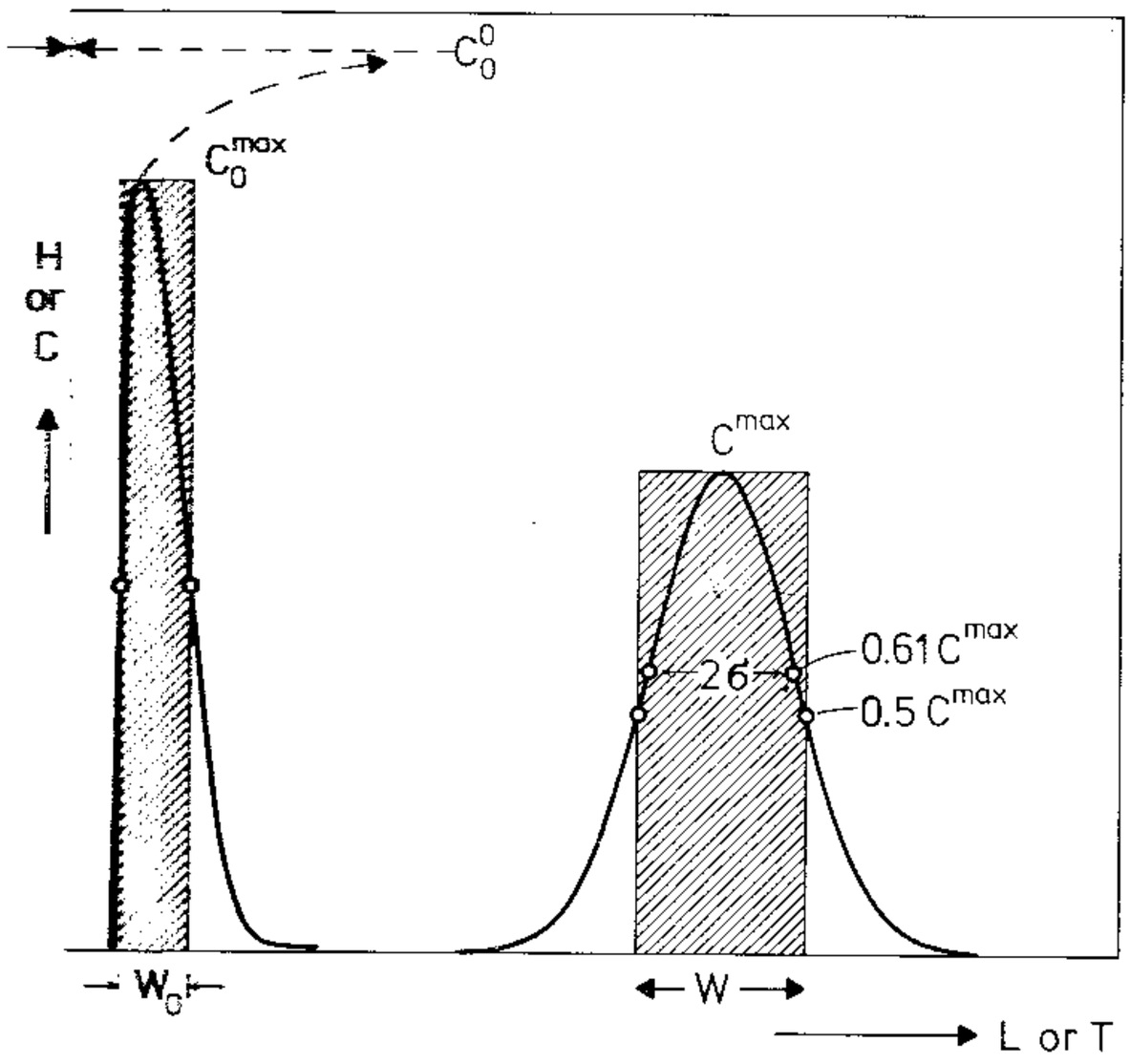


Fig. 8. The key parameters for defining dispersion in the Flow Injection method. Note that for $C = C_0^0$, $D_t = 1$.

obtained by calibration. In colorimetry — provided that the Lambert—Beer law holds for the concentration range chosen — H can be expressed as the recorded peak height (e.g., in mm) and H_0^0 as the distance between the base-line and the signal recorded with the flow cell filled with the dye of the original concentration.

Similarly, if it is necessary to measure the contribution of an individual component in a system, one can take its input condition, e.g. H_n, as a basis. Therefore $D_{n+1} = H_n/H_{n+1}$, and for the total dispersion: $D_t = (H_0^0/H_n)\cdot(H_n/H_{n+1})$, or

$$D_t = D_1 \cdot D_2 \cdot D_3 \ldots D_{n+1} \qquad (15)$$

Thus by injecting, for example, a coloured solution into a colourless stream and by measuring the respective peak heights obtained colorimetrically, the influence of an additional coil, a confluence arrangement, or a mixing chamber can be rapidly estimated.

It is self-evident that the dispersion defined and measured in this manner describes not only the degree of dilution of the original sample at any point along its way from the injection port to the detector, but also the ratio in which the sample has been mixed with the reagent in the carrier stream. For $D = 1$, there is no mixing in the critical volume of fluid, while, e.g., for $D = 5$ the sample volume constitutes 20% of the critical volume.

The next important choice is the parameter to which D should be related, i.e., to line length L or residence time T of the sample in the system. It might appear that the line length is the better choice, as a certain length of tube is easy to associate with a certain degree of dispersion. Moreover, manifolds in continuous flow analysis have been traditionally and most easily described by coil length. Also, residence time T and line length L are related through

required to reach 50% of C_0^0 ($S_{1/2}$) will be four times smaller. This is why th tube radius should be kept small if low or medium dispersion is required a sample solution is scarce. Additionally, reagent economy is improved whe narrow tubes are employed: for the same linear velocity F, the pumping rate Q in a tube of linear radius r is only one quarter of that required for a tube of radius $2r$.

It follows from the foregoing and especially from eqns. (17) and (18) a Fig. 9, that an increase in the sample volume has a gradually decreasing effect on the decrease of the total dispersion in the system, and that rega of which laws D_f and D_i are governed by, these two values must be kept close to unity if limited dispersion ($D_t \leqslant 3$) is to be obtained in a Flow Injection system. Therefore:

Rule 4: Limited dispersion is obtained by injecting a sample volume corresponding to a minimum of one $S_{1/2}$ into a carrier stream, pumped at the minimum practical flow rate, and by having the shortest possible line length of i.d. 0.4 mm between the injection port and the detector.

For illustration, reference is made to the experimental part (Figs. 10 a 12), from which it appears that $S_{1/2} = 28$ µl for $Q = 0.5$ ml min^{-1} and $L = 22$ cm of 0.4-mm i.d. ($D_i = 1.3; D_d = 1$). In other words, the sample zone should always pass through less than half its own length in all syste with limited dispersion ($L < l_i < l_s$).

From an analytical viewpoint, systems with medium dispersion are mu more interesting as they can accommodate various chemical reactions. T

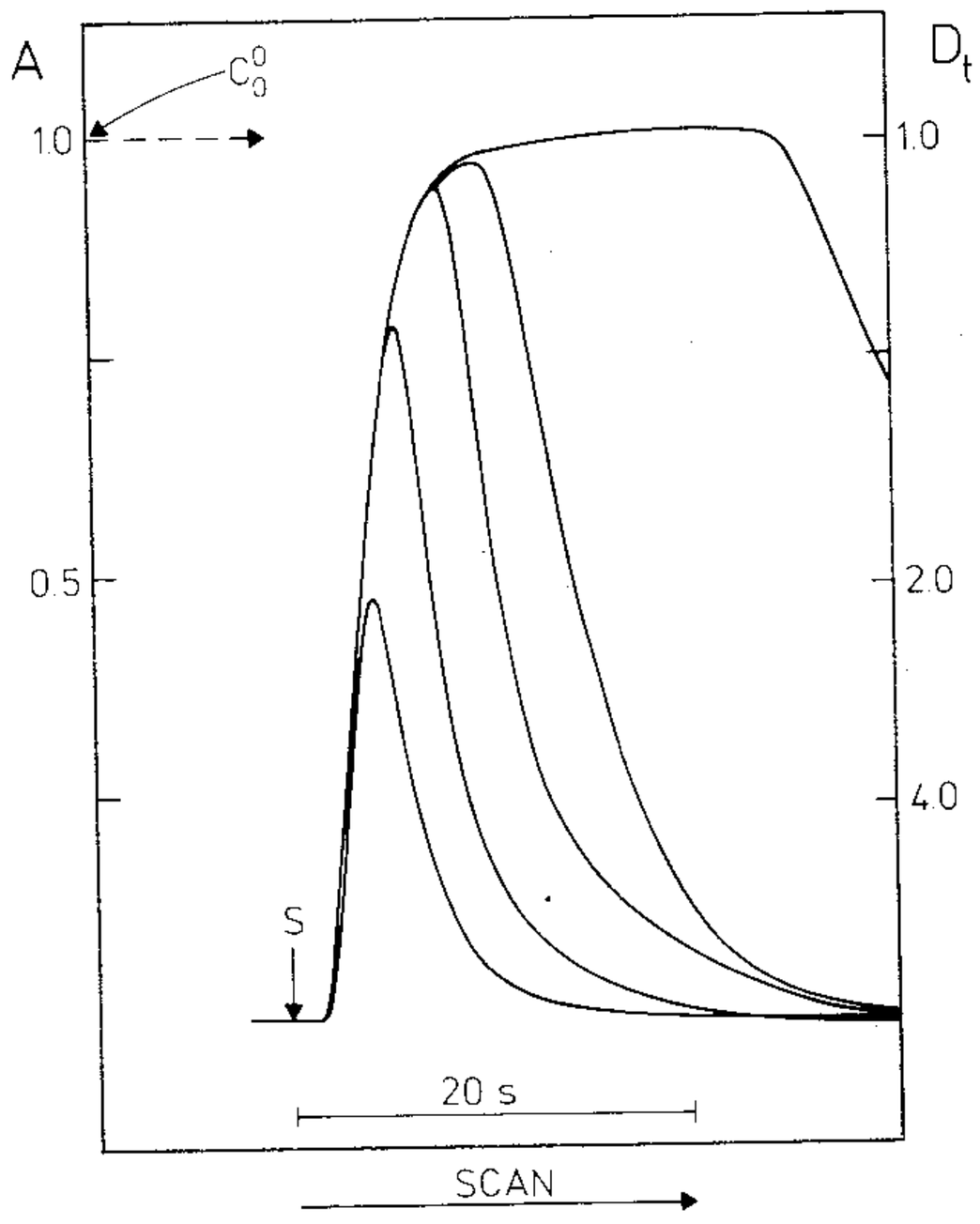


Fig. 9. Flow Injection response curves obtained by injecting 59, 108, 206, 403, and 795 µl, respectively, of a dye solution into a colourless carrier stream. S is the point injection, while C_0^0 marks the absorbance (A) corresponding to the undiluted dye so placed in the flow cell under static conditions. See also points (○) in Fig. 10.

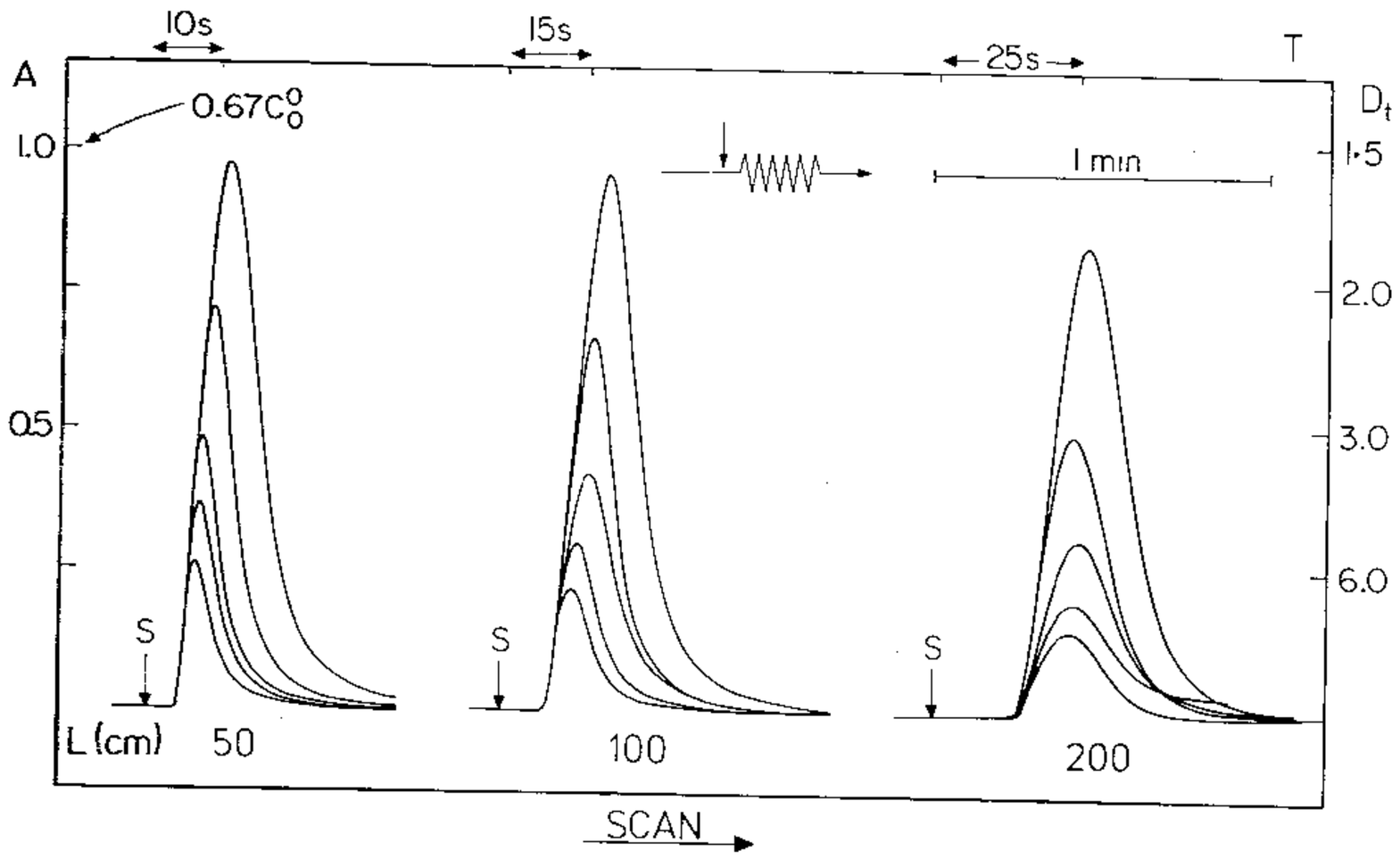
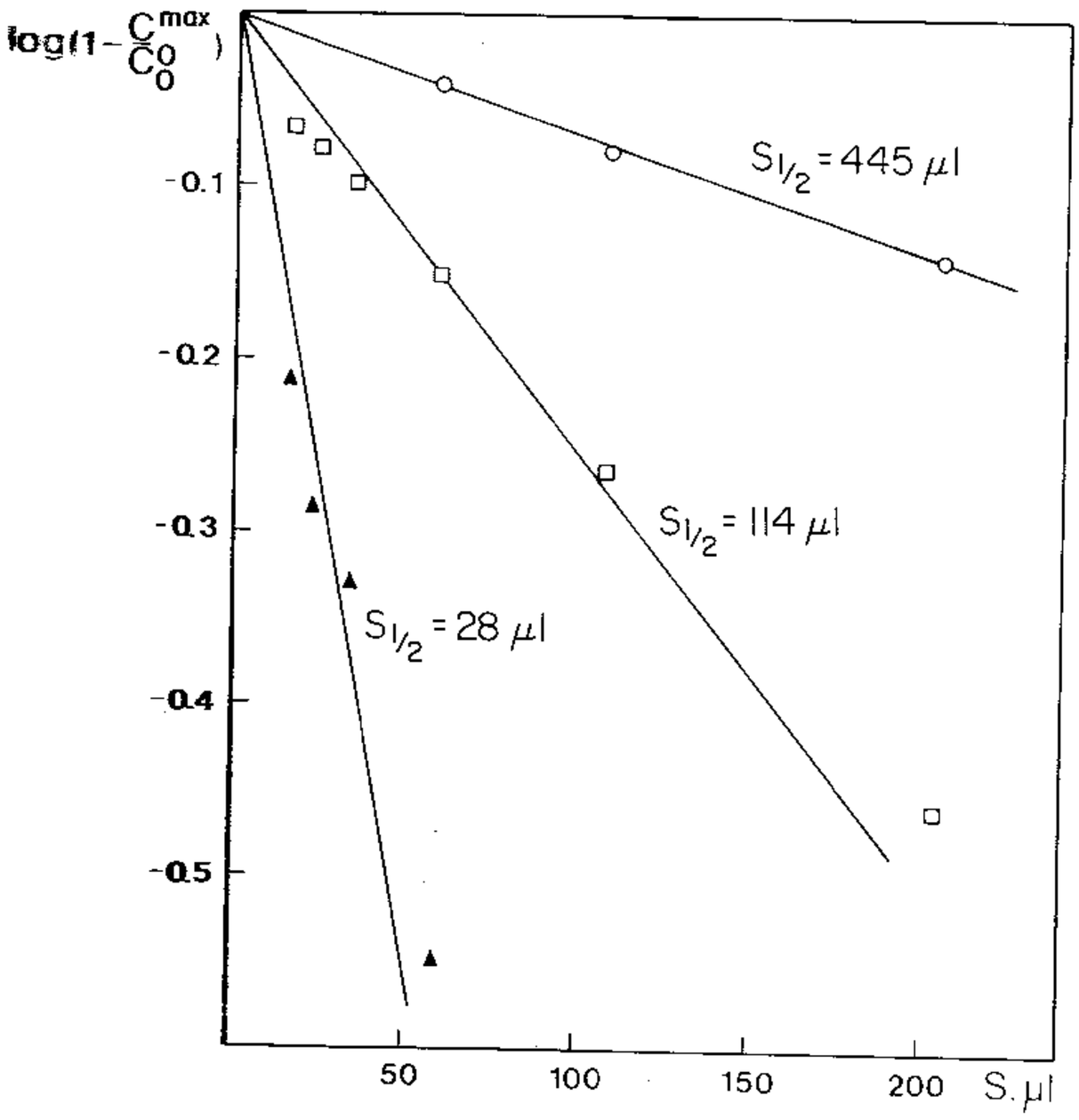


Fig. 11. Response curves obtained by injecting 16, 34, 59, 108, and 206 μl of a dye solution (S) into a carrier stream pumped at a rate of 1.5 ml min^{-1} (Q). The curves were recorded after the sample zone had passed through 50, 100 and 200 cm, respectively, of a tube having 0.5-mm i.d. (See also points (□) in Fig. 12.)



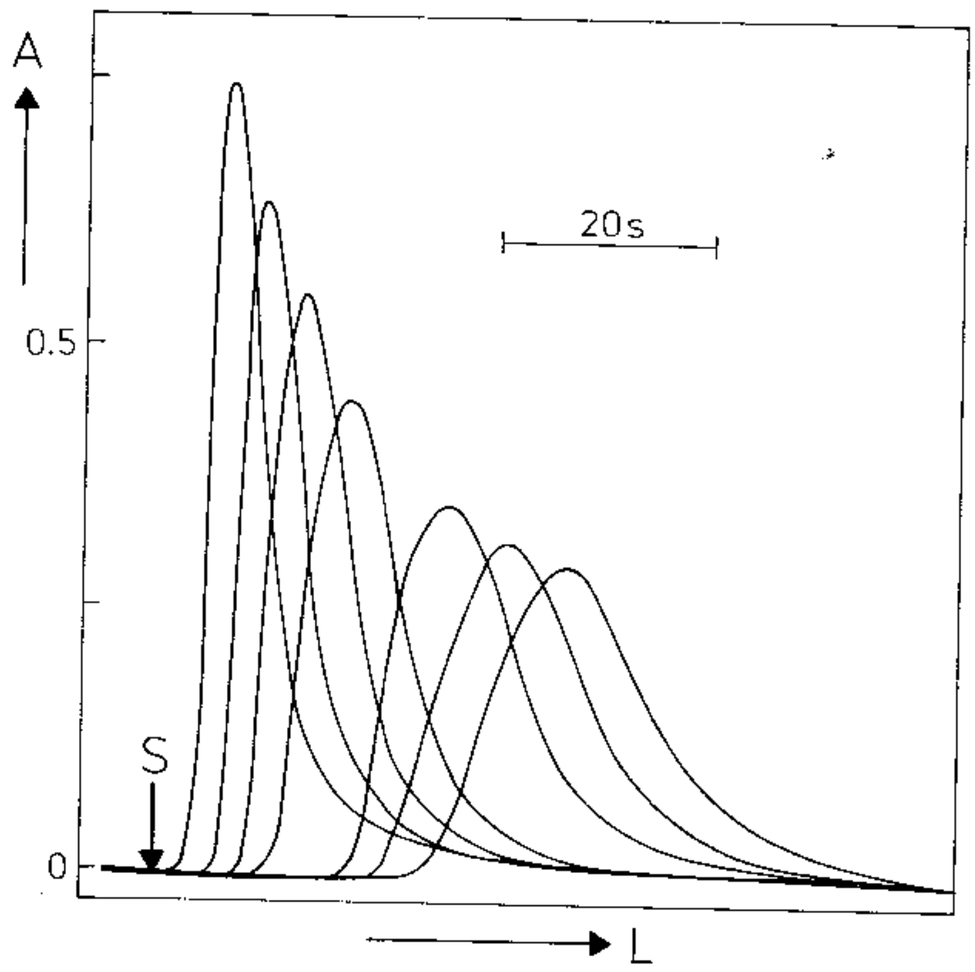
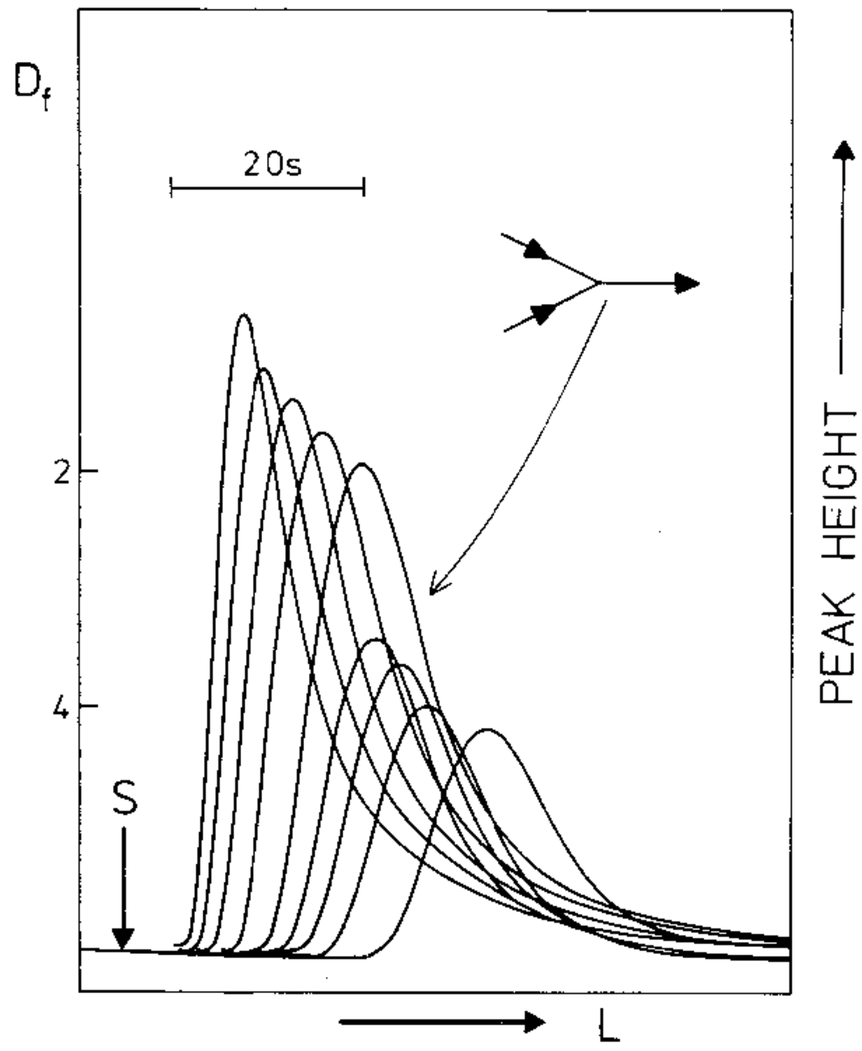


Fig. 12. The dependence of the $S_{1/2}$ value on the flow rate and diameter of the tube carrying **the sample to** (and through) the detector. (○) 1-mm i.d. tube with measurement at L = 100 cm; **(□) 0.5-mm** i.d. with measurement at L = 100 cm; (▲) 0.4-mm i.d. with measurement at L **= 22 cm. Pumping** rate Q for unfilled symbols, 1.5 ml min^{-1}; and for filled symbols 0.5 ml min^{-1}.

Fig. 13. Response curves obtained by injecting 50 μl of a dye solution into a colourless **carrier stream** pumped at a rate of 1.5 ml min^{-1} through a tube of 0.5-mm i.d. The tube **lengths (L) were**: 25, 75, 125, 175, 250, 300, and 350 cm, respectively, and the sample **was always** injected at point S. Compare with the filled circles in curve A of Fig. 14, where **measurements** at L = 50, 100, 150 and 200 cm are also included.

S = 16—206 μl) varies from 1.6 to 10 within line lengths of 50 to 200 cm **with residence** times of 10 to 25 s (Fig. 11). This alone shows how flexible **the system** is in terms of sample/reagent mixing ratio within a very short span **of time. It is,** however, interesting to determine whether the relation between



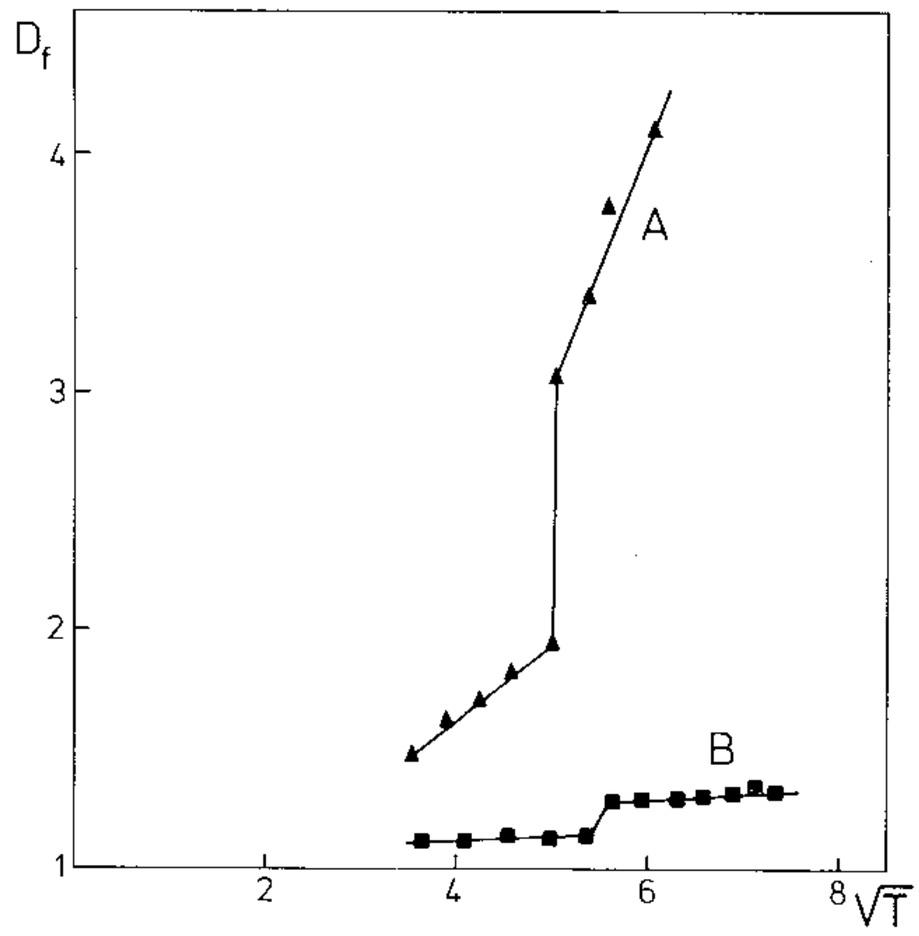


Fig. 18. Response curves obtained by injecting 50 μl of a dye solution at point S into a colourless carrier stream pumped at a rate of 0.75 ml min^{-1}, showing the influence of addition of a second colourless carrier stream also pumped at 0.75 ml min^{-1}. The individual curves (left to right) were recorded at L = 25, 50, 75, 100 and 125 cm; then the second stream was added, and after the confluence point curves for L = 150, 175, 200, and 250 cm were recorded. (Compare also line A, Fig. 19.)

Fig. 19. The dispersion values obtained in a confluence manifold by injecting 50 μl of sample into a system where two streams merge. Curve A corresponds to the curves shown in Fig. 18, while curve B was obtained with tubes of 0.4-mm i.d. and slower pumping rates, i.e. 0.25 ml min^{-1} in the main line and 0.12 ml min^{-1} in the additional line merging with the main stream at the confluence point at T = 30 s.

as long as the mixing process is reproducible, this short delay in complete mixing of merging streams has no practical consequences.

The influence of the pumping rate on the D_f values is profound. First, the slopes of the two dispersion lines (Fig. 19, curve A) before and after the confluence point agree roughly with the slopes of the lines obtained at the respective pumping rates in a simple manifold (Fig. 16). Then, when the pumping rate and the tube diameter are decreased (Fig. 19, curve B), the slope of the dispersion line both before and after the confluence point decreases dramatically, yet the merging streams (1 + 2) are still sufficiently mixed for the purpose of promoting a chemical reaction.

A),

The mixing chamber

The dispersion of the sample zone in a mixing chamber was investigated by injecting 50 μl of the dye solution into a carrier stream pumped at a
B). rate of 0.75 ml min^{-1}, and by recording the peak in the usual manner. Three experiments are shown in Fig. 20. For curve A, the sample zone was
s, carried to the detector through 50 cm of 0.5-mm i.d. tube, so that this experiment corresponds to the third curve shown in the first group of peaks in Fig. 11. For curve B, a mixing chamber (V_m = 1.9 ml) was placed

stopped-flow injection principle. A commercially available System Glucose enzyme set (Merck, Germany) was used, in which the coenzyme nicotinamide adenine dinucleotide (NADH) serves as a chromogen which can be measured at 340 nm. Details on the reactions and reagent compositions, and some experiences with one- and two-point kinetic measurements have already been reported [32]. The manifold used in the present experiment, and two series of response curves are shown in Fig. 28.

In the first series of experiments, the concentration of glucose in the injected sample (S = 30 μl) was increased from 1 to 20 mmol l^{-1}, and the sample zone was stopped in the flow cell as soon as the recorded curve reached its maximum, i.e. when $t = T$ (Fig. 28b). As the pump was then stopped for 9 s, the measuring cycle consisted of three parts: (1) sample injection, dispersion and transport into the detector (4.5 s); (2) the measuring period with stopped flow (9 s); and (3) the washing period (10 s) at the end of which the next sample was injected. Further details of this method, which allows a multipoint kinetic determination to be performed at a rate of 150 samples/hour, will be published shortly [50].

An interesting aspect of this new approach to kinetic analysis is shown in Fig. 28c, which was obtained when the concentration of glucose in the

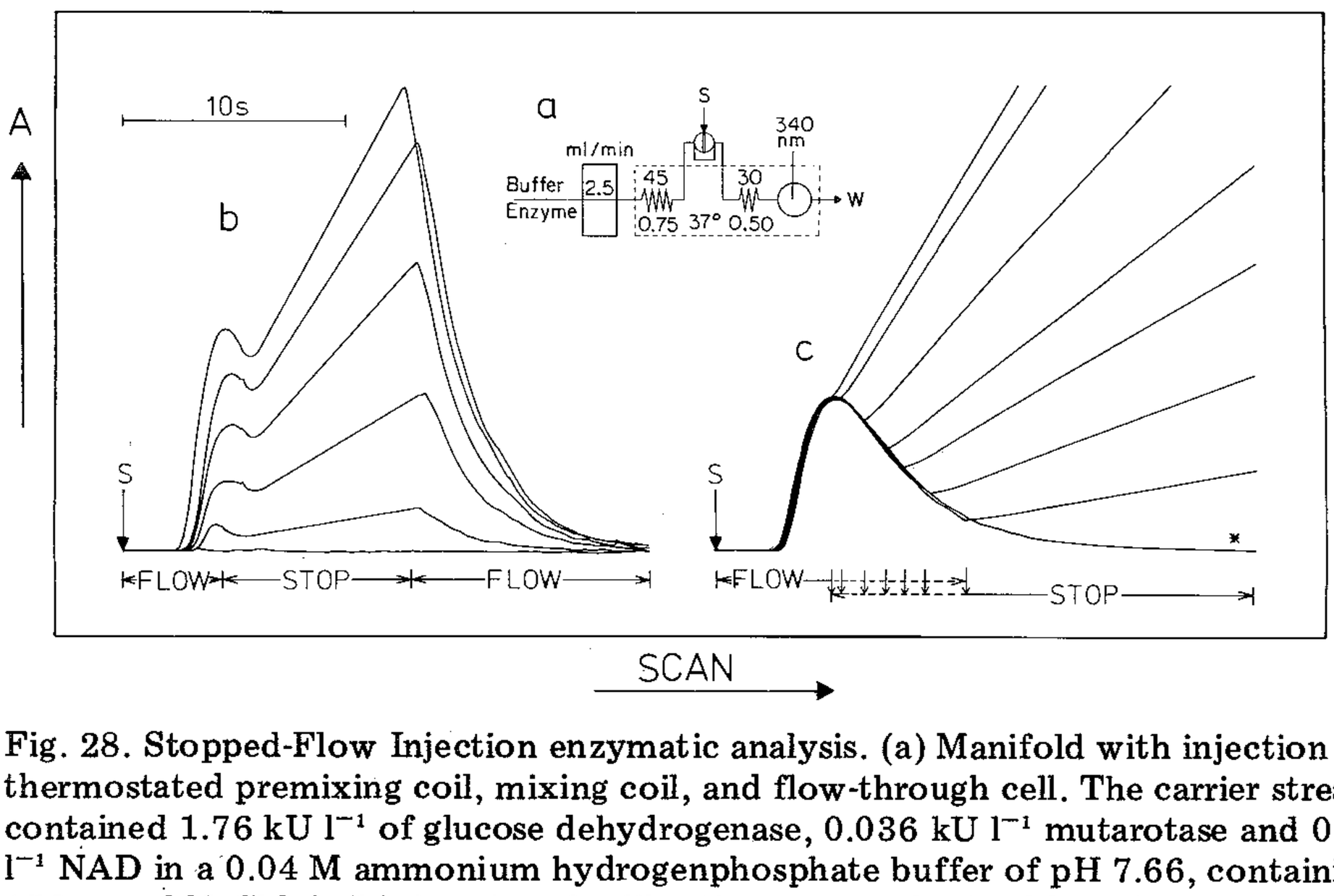


Fig. 28. Stopped-Flow Injection enzymatic analysis. (a) Manifold with injection valve (S), thermostated premixing coil, mixing coil, and flow-through cell. The carrier stream contained 1.76 kU l^{-1} of glucose dehydrogenase, 0.036 kU l^{-1} mutarotase and 0.36 mmol l^{-1} NAD in a 0.04 M ammonium hydrogenphosphate buffer of pH 7.66, containing 150 mmol NaCl l^{-1}. (b) A series of calibration curves obtained by injecting 30 μl of aqueous solutions containing 0.0, 1.0, 5.0, 10.0, 15.0, and 20.0 mmol of glucose per l, respectively. All samples were injected at point S, and each analytical run consisted of a 4.5-s pumping period (FLOW), a 9-s stop (STOP), and a 10-s washout period (FLOW). The slopes of the individual records versus concentration yielded a straight line. (c) A series of curves obtained by injecting 30-μl aliquots of an aqueous solution containing 15.0 mmol glucose per l. The flow was stopped 5.1, 5.5, 6.5, 7.6, 8.4, 9.5, and 11.0 s after the point of injection (S), except for the last experiment (marked *) where the pump was run continuously.

injected samples was kept constant (15 mM), but the flow was stopped at different times. In this way, the kinetics of the chemical reaction were monitored in different sections of the sample zone where — because of concentration gradient profiles — different ratios between sample and reagent solutions were available; consequently, the recorded response curves have different slopes. It is, therefore, important always to perform a kinetic measurement in the same section of the sample zone, as was done in the first series of experiments (Fig. 28b). For this type of analysis, the peak maximum sensor [27], combined with a delay device, will readily allow any section of the dispersed sample zone following the appearance of the peak maximum to be chosen reproducibly.

CONCLUSIONS

The above outline of the theory and the most recent developments in Flow Injection Analysis forms a natural conclusion to this series of ten papers which over the last four years has encompassed the evolution of the method from an initial concept for fast practical application to the design of advanced systems. In this way, a new method of continuous flow analysis has been developed, based on the formation and exploitation of concentration profiles when an injected sample is carried by an unsegmented stream towards a detector.

Although it has been doubted that unsegmented streams could ever become useful in continuous flow analysis, this work does show — as N. G. Anderson once pointed out in a discussion with L. Skeggs [51] — "that there must be some other way to do all that".

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