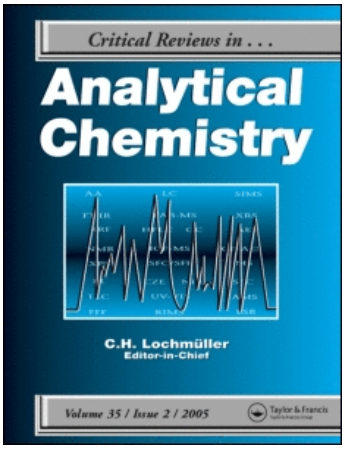

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THEORETICAL BACKGROUNDS OF FLOW ANALYSIS

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I. INTRODUCTION

A. What Is Flow Analysis?

To most workers in the field of flow analysis it appears to be self-explanatory that what they are doing is flow analysis (FA), and they will easily quote many advantages of their system. Nevertheless, it is not easy to properly define what flow analysis is. Even more difficult is defending its advantages against other semiautomatic or automatic analysis methods, in general, because of the wide variety and the fast development of both FA and other instrumentation. A very broad definition of FA might be that in flow analysis the transport of the sample from the place of sampling to the waste (or back to the sampling area in case of recirculation) through stages of various kinds of manipulation like separation, chemical reaction, heating or cooling, detection, etc. is done by imparting the sample or an aliquot of it into a flowing stream. The flow moves the sample through the spatially separated stages of the analysis process in an automatic way. FA is not the only way of automating the sample transport process; one alternative is, e.g., to keep each sample in a separate container and to move many such containers on a chain belt to the various stages. The specific feature and most crucial problem of FA is the interaction of the sample with the stream carrying it.

There are two widely used techniques which are commonly regarded as FA: continuous flow analysis (CFA), best known in its realization as the Auto Analyzer® by Technicon, and flow injection analysis (FIA) (see, e.g., the References[1-3]). One definition of FA given above covers both techniques, but it is not restricted to these; it obviously covers other techniques like liquid chromatography or gas chromatography, and the operation of a simple flame photometer is described by it, too.

There have been some disputes about names and definitions of CFA and FIA. It appears that a definition of FA which would cover only these two techniques would not serve any useful end. Viewing these two techniques separately from others had, in fact, retarded taking over useful elements from the theory and practice of liquid chro-

matography into FIA. Our discussion will be limited, however, and chromatographic separation will not be dealt with at all, because that aspect is very well documented. The technique of air segmentation will be treated only marginally for the same reason.

This review will then mainly cover what is commonly known as FIA. We avoided, however, use of this name for our subject, because both the name and the concept behind it appear to be misleading. The name FIA was apparently first used by Ruzicka and Hansen[1] for their measurements consisting of sample injection into a nonsegmented flowing stream of inert or reacting carrier and downstream detection of the sample or a reaction product. Recent historical reviews[4,5a] have shown that a number of such systems had been in use before the name FIA appeared. The original FIA concept differs from our general definition of FA in mentioning the use of an injection device and nonsegmented flow. A more recent definition of FIA given by the originator of this name[6] regards, however, the method of injection and the use of nonsegmented flow less important, and emphasizes only the controlled dispersion of solutes in the flow channel. Thus, the name flow injection analysis does not completely cover what it is used for. On the other hand, any kind of FA is meaningful only if dispersion is kept under control in one way or another, since otherwise the results would not be reproducible. So "controlled dispersion flow analysis" is equivalent to saying just flow analysis.

What name should then be given to the couple of techniques which belong to FA, but differ from chromatography, flame photometry, continuous flow analysis, etc.? It appears to us that, regarding the wide variety and the recent rapid development of our field, it is not meaningful to forcefully draw rigid borderlines through it. This view is similar to that of Mottola[5b] reached after an in-depth examination of various definitions of FIA. For the reasons presented we prefer speaking of the broader category FA. The name FIA will be used occasionally to denote nonsegmented FA in a straight or coiled open tube reactor with sample introduction by an injector. The name CFA will be used in its traditional meaning, although this name is also misleading, since it appears to cover a much broader principle than it really does.

B. The Theory of Flow Analysis

Flow analysis has emerged from analytical laboratories as a practical tool rather than a scientific principle. One can easily and successfully operate an FA system using only a couple of empirical or semiempirical rules. However, there have been many attempts to mathematically describe what is going on in such a system, with the aim to relate the output parameters, like peak height, peak width, etc., to the input parameters like flow rate, injected volume, reaction rate, etc. None of these attempts appears to have had enough success to be generally accepted by all workers in the field. Moreover, most such studies attack only a part of the problem, i.e., they are devoted to the study of a subsystem, like the injection device, the transport line, or a certain kind of detector. Thus, we cannot speak of a unique theory of flow analysis; rather, we have to gain insight into the mechanism of FA devices by reviewing some of the most interesting theoretical contributions found in the literature. By the nature of FA, transport phenomena will have an eminent role.

C. Transport Phenomena in Flow Analysis

In flow analysis sample is transported through the various stages of analysis by flow. While moving downstream the sample interacts with the carrier solution. This interaction may be purely physical (e.g., mixing) or physical and chemical (e.g., mixing plus reaction). The analysis line may include a number of various units designed to interact physically (e.g., heat exchangers) or in a physicochemical process (e.g., separation,

electrolysis, etc.) with the sample. These interactions involve transport phenomena: transport of heat, momentum, and mass. This review is devoted to the most important transport phenomenon in flow analysis: the dispersion of solute in the flowing stream. An excellent review of this subject was published in 1983.[7]

II. DISPERSION OF SOLUTE IN A FLOWING STREAM

Most flow analysis equipment uses flow conduits with circular cross sections and with dimensions assuring laminar flow at practical flow rates. In contrast to turbulent plug flow, where a sample zone can move downstream without considerably mixing with the carrier stream, laminar flow causes spreading of the sample into the carrier and vice versa. One might anticipate that this apparently simple phenomenon which has been known for about a century can be easily described by mathematical formalism. This is, however, not the case. Although Taylor[8] has found the solution for two important limiting cases of the laminar dispersion problem, a general solution does not exist. The range of parameters typically used in flow analysis falls into an area where the differential equation for laminar dispersion can only be solved numerically. This complicates the derivation of quantitative relations. To render the situation even more complicated it turns out that the peak shapes normally observed in flow injection analysis are quite different from those calculated from the numerical solution of the differential equation of laminar dispersion in a straight tube. This discrepancy can be attributed, at least partly, to the curvature in the coiled tubes used in FIA. Careful experimental work with straight tubes yields, however, results consistent with the numerical solutions.[9-13]

Laminar dispersion has still more peculiarities. For example, introduction of a sample into a laminar stream can occur in various ways (see later) and subtle differences in detector geometry may also cause considerable differences in the detector output. The role of connections between elements of the flow line appears to be rather critical. It has been found, for example, that the same length of straight tubing behaves quite differently if it is made up of several shorter pieces with connections than if it is in one piece.[11] No wonder, then, that experimental observations of different authors or sometimes even of the same author are contradictory. There have also been very few attempts to treat the even more complicated case of laminar dispersion combined with chemical reaction. Segmentation of the flow by a second phase (gas or liquid) requires a completely different treatment, because there the laminar flow pattern is broken up by the segmentation.

It will be attempted below to give an extended account of some theoretical and experimental results concerning solute dispersion in flow analysis. It should be stressed in advance, however, that a number of questions will have to remain open.

Different approaches have been used to characterize dispersion. Useful semiempirical rules of thumb have been found to relate peak height to different system parameters like tube length, flow rate, etc.[2] The conceptually very simple tanks-in-series model has also been used occasionally. The most rigorous approach has been to set up adequate physical models and solve these analytically or numerically. The discussion to follow will concentrate on the latter approach.

A. Unsegmented Flow without Chemical Reaction

The most simple flow analysis equipment does not apply any chemical reaction; the carrier is only used to carry individual samples from the sampling unit (which is often an injector) to the detector. Sample parameters measured by the detector may be, e.g., light absorption at a suitably chosen wavelength, pH, conductance, etc. Liquid chro-

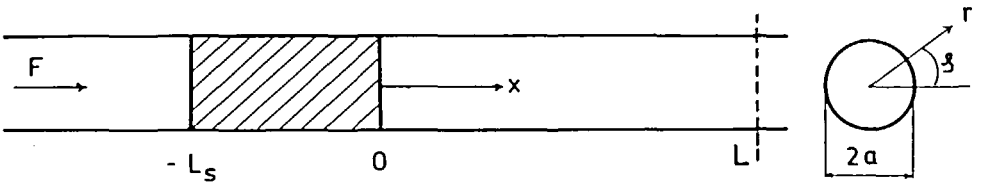

FIGURE 1. Distribution of solute in a straight tube immediately after a square wave-type (slug) injection.

matography can be thought of as a combination of this simple flow analysis system with on-line separation. In fact, much impetus to the study of the simple FA system comes from studies dealing with extracolumn band broadening in LC. Moreover, suggested geometries for the connecting line between injector and detector in this simple FA system do not only include narrow tubes of circular cross section, but also columns packed with inert material[14] like glass beads and a hybrid of empty tube and packed column: the single bead string reactor (SBSR)[15] consisting of a tube filled with inert spheres of slightly smaller diameter than the tube itself. A further possibility is to include a mixing chamber into the flow line between the injector and detector.[16] All the above flow line geometries can be used and have been used for determinations including chemical reactions. For the purpose of describing solute dispersion, it is, however, more convenient to regard the case of no chemical reaction first. As in actual experimental dispersion studies the sample can be thought of as a colored substance injected into a colorless stream.

1. Dispersion in a Straight Tube

Let us consider first the very simple flow system shown in Figure 1. It consists of a long piece of straight tube with a circular cross section of radius a. The carrier solution is streaming from left to right with flow rate F. The situation depicted in the figure is that at time zero, when in a section of length L_s, the carrier stream is instantaneously replaced by a solute slug of concentration c_0. It is advantageous to now set up a polar coordinate system with the x axis along the tube axis, and the radius r and the angle ϱ as shown in the figure. The zero of the x axis is at the right-hand end of the solute slug. A detector, sensitive to solute concentration, is positioned at x = L.

The flowing stream now carries the solute particles towards the detector. Let us assume that laminar flow prevails in the tube. It is well known that in laminar flow particles near the x axis will move faster, while those near the tube wall will move very slowly. The local velocity is described by:

$$u = u_{max}\left(1 - \frac{r^2}{a^2}\right) \tag{1}$$

where u_{max} is the velocity at the x axis (Figure 2). The whole slug will be deformed by this velocity distribution (Figure 3). This deformation causes, however, a secondary phenomenon which acts against the deformation: molecular diffusion. At the front side of the deformed slug there will be a considerable concentration gradient towards the tube wall, whereas at the rear side there will be a gradient towards the axis. Molecular diffusion will, therefore, cause the solute to move as indicated by the arrows in Figure 3 and thus decrease the nonuniformity of the concentration distribution. By the time the front of the solute slug arrives at the detector, the original "rectangular" shape of the slug will be considerably distorted. The detector will record the passing of a peak-shaped concentration distribution. The task of the analytical chemist is then to

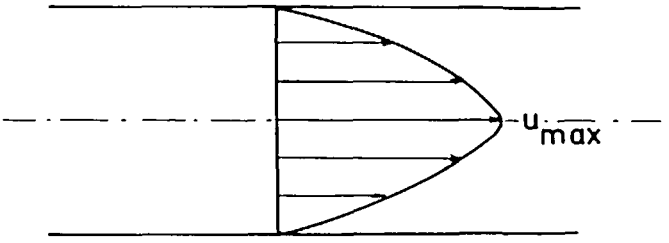

FIGURE 2. Velocity distribution in laminar flow (the arrows are local velocity vectors).

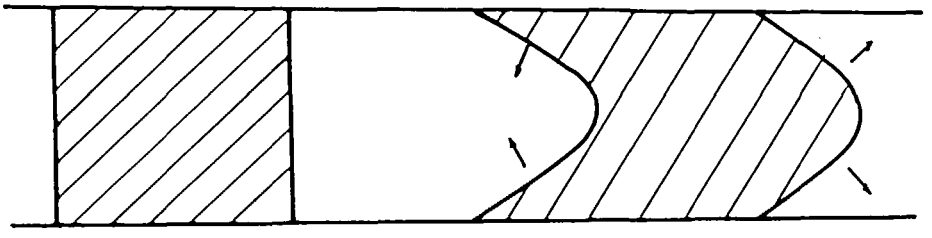

FIGURE 3. The distortion of a "rectangular" solute slug by laminar flow ($t = 0$ and $t > 0$).

conclude on the original solute concentration c_o from the recorded signal. This task can be solved in a very practical manner by correlating an experimentally determined peak parameter, like peak height or peak area, to c_o. Linear correlations have often been observed and used efficiently for concentration measurement.

On the other hand, one can try to express the detector signal (peak) parameters as a function of the physical quantities describing the system, notably, a, L, F, L_s, and carrier viscosity η, carrier-specific gravity ϱ, and solute diffusion coefficient D. Before proceeding to such a solution we shall turn our attention to the many difficulties in realizing the simple FA system we have discussed above.

a. Problems Associated with the Model Assumptions

We recall first that the solute slug was assumed to replace instantaneously a carrier slug of equal size. It was tacitly assumed that the carrier flow was not stopped for this moment; otherwise we should have had to discuss the effect on solute dispersion by the acceleration after restarting the flow.

Many variations of real sample introduction exist and none of these conforms completely with the ideal model. Figure 4 shows a simple slide valve which closely approaches the model injector, but flow is stopped here during switching and is restarted thereupon. Connection of tubing to the injector is never as smooth as shown in the figure, and this may cause disturbance in the flow pattern and hence also in the solute distribution sensed by the detector. Chromatographic loop injectors are even farther from our model than the slide valve shown before. They have a curved instead of straight loop and the internal diameter of the loop and of the bores of the injector body may differ considerably from the internal diameter of the connecting tubing. They may also have a few 90° bends and these may again disturb the dispersion pattern.

Septum injectors (Figure 5) are very far from our original model. The sample is introduced from a syringe into the flowing carrier stream. Depending on the geometry and the injection rate various models can be constructed for this kind of injection. A

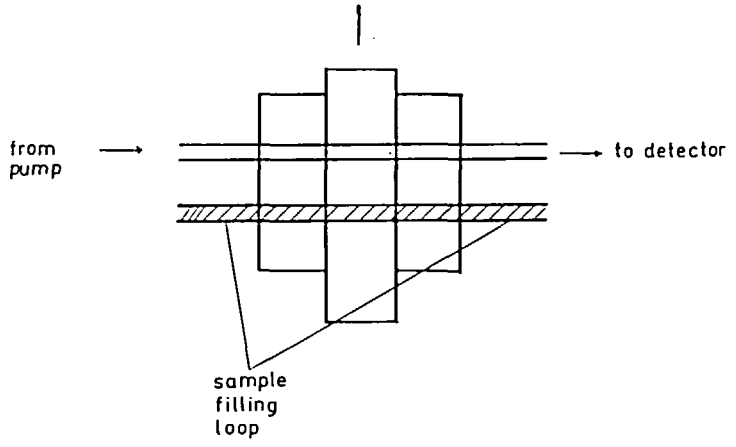

FIGURE 4. Slide valve for sampling (sample shown by dashing).

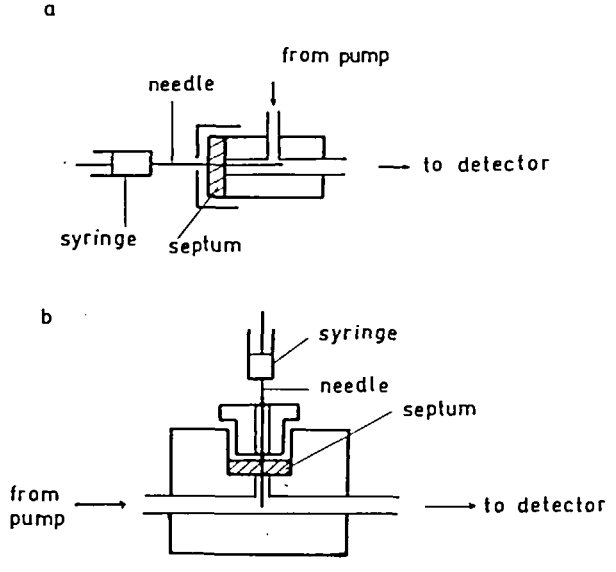

FIGURE 5. Septum injectors.

sudden fast stroke may introduce the sample as a slug, the shape of which is difficult to establish. If the syringe shaft is moved at a constant low speed, sample may be introduced exclusively into those streamlines which lie at the orifice of the syringe needle. Even if the injected sample were distributed over the whole cross section of the tube in some way, the relative quantities swept away by streamlines of different speed might vary, e.g., proportionally with the local flow velocity.

Septum injectors were only used in the early days of FA. The term sample injection has been retained, however, by many workers, although sample intercalation would be a more appropriate expression.

A further way of sampling is to suck samples from sample vials (Figure 6). Again in this case the introduced quantities of solute may vary from streamline to streamline.

Sampling is only one source of complexities in creating a valid dispersion model. Detection is about as intriguing as sampling. As we have pointed out above, in laminar

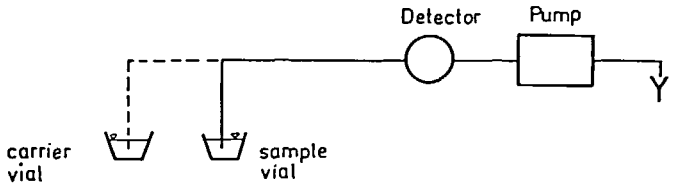

FIGURE 6. Sample introduction by suction.

flow solute can be unevenly distributed both along the x axis and within any cross section of the tube. Thus, a detector located at distance L from the injector will either measure some kind of average concentration in the cross section at L or it will measure a local (point) concentration somewhere in the cross section, e.g., at the tube wall or in the center of the tube. Besides this, practical detectors have a finite length in the x direction, so they will average the concentration over a certain length of the tube, too.

Apart from some electrochemical detectors, most detectors measure an average concentration over the detector volume. Although it may appear surprising at first, one can define — and measure — different averages. Two important kinds of cross-sectional average concentration are the mean concentration, c_m, and the bulk concentration c_b. These are defined as:

$$c_m = \frac{\int_q c\, dq}{\int_q d\, q} = \frac{\int_q c\, dq}{q} \tag{2}$$

and

$$c_b = \frac{\int_q uc\, dq}{\int_q u\, dq} = \frac{\int_q uc\, dq}{F} \tag{3}$$

where q is the cross section of the tube (at x = L), u and c are the local (point) values of the velocity and the solute concentration, respectively, and F is the flow rate. In other words, c_m is the usual average of the local values over the cross section, whereas c_b is the cross-sectional average concentration weighted by the local velocity. To make the importance of c_b clear, it should be noticed that the integral in the nominator of Equation 3 is equal to the amount of solute (in moles) passing across the detector during 1 sec.

In a flow injection experiment one obtains a peak-shaped signal starting from a baseline and returning to it after the sample zone has passed the detector. Based on chromatographic experience, one may assume that with a detector responding proportionally to concentration, the peak area is proportional to the total amount (moles) of sample injected. This expectation will be fulfilled for c_b, but not for c_m. We have seen that c_b is proportional to the flux of solute across the detector section per 1 sec. Hence, its integral with respect to time will be proportional to the total amount of solute passing the detector. The situation is quite different with c_m. To understand why, let us think of a flow injection experiment with the flow stopped for some time during the detection of the peak (Figure 7). c_b will drop to zero, because the flow velocity is zero (Equation 3 is not meaningful in this case), but c_m will remain constant at the value it

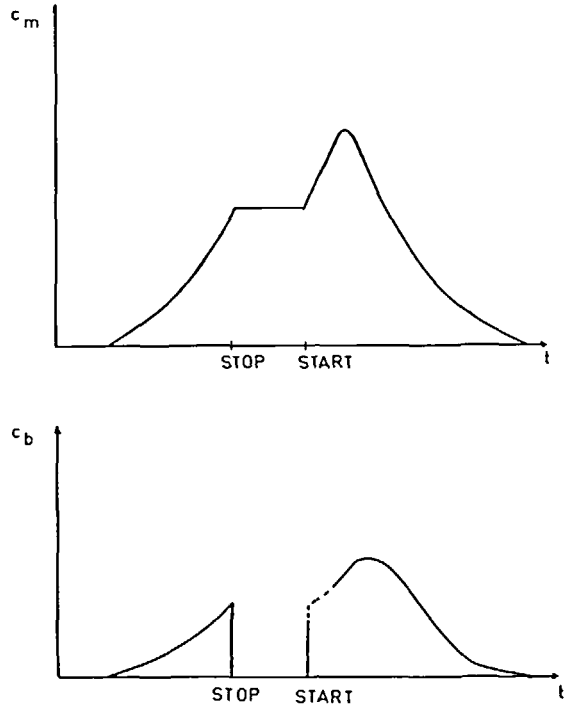

FIGURE 7. Effect of stopping the flow on c_m and c_b.

had assumed just before the flow was stopped. We can see from Figure 7 that the peak area under c_m has been increased by stopping the flow, although the amount of solute has not changed.

Focusing again on uninterrupted laminar flow in a straight tube, we recognize that the fluid layers near the tube wall are almost stagnant, so that solute in these layers will be perceived by a c_m-sensitive detector for a long time compared to solute passing in the fast core near the axis. Thus, c_m is an inappropriate measure of the amount of solute passing the detector. It is, however, worth mentioning that if there were no cross-sectional variation in c, the local (point) concentration, then c_m and c_b would again be equal and the peak area proportional to sample mass. This last remark is cross-sectional variation in c, the local (point) concentration, then c_m and c_b, would again be equal and the peak area proportional to sample mass. This last remark is extremely important for practical FA, since in practical systems the cross-sectional variation of c is effectively diminished by various factors, most notably by the coiling of the flow line and by disruption of the flow pattern at the entrance of the detector, by connectors (fittings), and sharp bends.

If the cross-sectional inhomogeneities of concentration and velocity are not swamped out at the detector entrance, the commonly used detectors will measure different averages. For example, if the solute emits radiation (radioactive solute or fluorescence) the total amount of radiation leaving a short section of the flow line can be measured by shielding the rest of the tube. The concentration average measured in this way is c_m. If a similar short section of the flow line is illuminated transversally by an external light source (Figure 8) and the absorbance of the solute is measured, the concentration average will differ both from c_m and c_b.

The most often used detector cell is the longitudinally illuminated spectrophotometric cell (Figure 9). By its nature it must have a sharp bend at the entrance. This

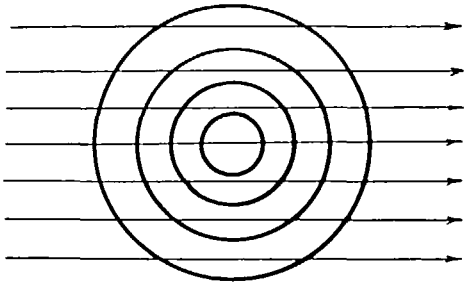

FIGURE 8. A parallel light beam illuminating the flow line from the side. The concentric circles are lines of constant concentration.

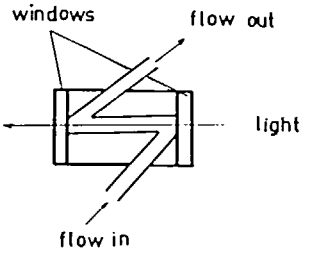

FIGURE 9. Longitudinally illuminated spectrophotometric flow cell (Z cell).

bend acts as a transversal mixing device and thus distributes solute uniformly over the cross section "seen" by the light sensor. If this were not the case, i.e., the laminar pattern remained intact, the detector would measure an average concentration which can be shown to differ both from c_m and c_b.

The bulk concentration c_b, i.e., the ratio of solute flux to carrier flux, can be measured, for example, by cutting the experimental tube at $x = L$ and collecting small aliquots of the eluted solution, and analyzing each aliquot — after thorough mixing — for the solute concentration in it. It is this measurement process which led to an alternative name of the bulk concentration: the cup-mixing value. Alternatively, one can use practically any of the earlier mentioned averaging detectors provided that a perfect cross-sectional mixing takes place right before the solute enters the detector. A sharp break in the flow direction may be sufficient. A third possibility is to directly determine the total amount of solute passing the detector per second. This can be done, for example, by coulometric detectors. In this case, Fc_b is measured.

After having learned to appreciate the complexities of realizing an apparently simple model experiment for studying dispersion in a straight tube under laminar flow, we can turn our attention to predicting the detector signal from the system input parameters, i.e., tube radius a, injector-detector distance L, flow rate F, sample slug length L_s, carrier viscosity η and specific gravity ϱ, and solute diffusion coefficient D. It will be assumed that a "rectangular" sample slug is injected without stopping the flow.

b. Prediction of the System Output

Taylor[8] was the first to carry out a quantitative treatment of this problem. He gave the solution of two important special cases: case A, when the concentration changes due to convective transport along the tube take place in a time which is so short that

the effect of molecular diffusion can be neglected, and case B, when the time necessary for appreciable effect to appear, owing to convective transport, is long compared with the time during which radial variations of concentration, caused by the parabolic velocity distribution, are reduced to a fraction of their initial value through the action of molecular diffusion. For case A, a mean value detector would observe the following concentration vs. time dependence:[17]

$$c_m = c_o\left(1 - \frac{L}{u_{max} \cdot t}\right) \text{ for } \frac{L}{u_{max}} \leq t \leq \frac{L + L_s}{u_{max}} \tag{4}$$

and

$$c_m = c_o \frac{L_s}{u_{max} \cdot t} \text{ for } t \geq \frac{L + L_s}{u_{max}} \tag{5}$$

The bulk concentration in case A will vary with time according to:[17]

$$c_b = c_o\left[1 - \left(\frac{L}{u_{max}\, t}\right)^2\right] \text{ for } \frac{L}{u_{max}} \leq t \leq \frac{L + L_s}{u_{max}} \tag{6}$$

and

$$c_b = c_o \frac{L_s(L_s + 2L)}{u_{max}^2\, t^2} \text{ for } t \geq \frac{L + L_s}{u_{max}} \tag{7}$$

In contrast to case A, which can be solved by relatively simple calculus, case B leads to the following partial differential equation:

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial r^2} + \frac{1}{r}\frac{\partial c}{\partial r} + \frac{\partial^2 c}{\partial x^2}\right) - u_{max}\left(1 - \frac{r^2}{a^2}\right)\frac{\partial c}{\partial x} \tag{8}$$

together with suitable boundary conditions expressing the concentration distribution at time zero, the impermeability of the tube wall and the axial symmetry of the dispersion, and fixing the concentration values at infinity. For the "rectangular" slug input the solution reads:[9]

$$c_m = \frac{c_o}{2}\left[\text{erf}\frac{(u_{max}t/2) - L}{\left(\frac{a^2 u_{max}^2}{48\, D}\, t\right)^{1/2}} + \text{erf}\frac{L + L_s - (u_{max}t/2)}{\left(\frac{a^2 u_{max}^2}{48\, D}\, t\right)^{1/2}}\right] \tag{9}$$

According to the Taylor theory of case B, the center of the solute slug will move along the tube at the average flow velocity $u = u_{max}/2$, and the slug will spread out symmetrically around the center plane into a Gaussian-type distribution. This spreading is very similar to the well-known distribution pattern caused by molecular diffusion, but it is a much faster process with an effective diffusion coefficient

$$D_{eff} = \frac{a^2 u_{max}^2}{192\, D} \tag{10}$$

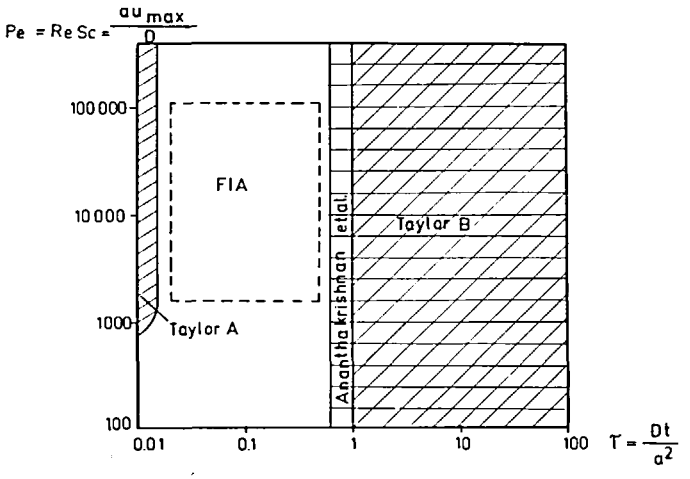

FIGURE 10. Range of validity for various solutions of the laminar dispersion problem. In the unshaded region only numerical solutions exist. Pe — Peclet number, Re — Reynolds number, Sc — Schmidt number. (Adapted from Vanderslice et al.[13,18,19])

When Taylor's condition B is valid, the detector signal will be a slightly distorted Gaussian peak as given by Equation 9.

Having seen the mathematical solutions of Taylor's case A and B, the question now is how we can establish whether a certain FA experiment falls under case A or B. The surprising answer is that most FA experiments described in the literature do not fall into either category. Figure 10 shows the range of validity of various solutions of the laminar dispersion problem. In the shaded areas explicit mathematical solutions exist. The majority of FA (mainly, FIA) experiments fall into the unshaded area where only numerical solutions (of Equation 8) exist.

Before turning attention to these numerical solutions, we should observe the variables on Figure 10. On the horizontal axis dimensionless time $\tau = Dt/a^2$ is displayed, while on the vertical axis another dimensionless number, the Peclet number $Pe = au_{max}/D$, is used. The Peclet number also equals the Reynolds number multiplied by the Schmidt number, since $Re = au_{max}/\nu$, where ν is the kinematic viscosity, $\nu = \eta/\varrho$, and $Sc = \nu/D$. Dimensionless numbers like these are often used in chemical engineering. They are very useful in reducing the number of necessary variables when we are looking for interrelations between a large number of system parameters. In the present case we can read from the graph that in FA Taylor's conditions cannot be applied, because Dt/a^2 is not sufficiently small for case A to hold, and not sufficiently large for case B to be valid. If any of the three variables, D, t (in our case t_L, the time for reaching the detector), or a, the tube radius were changed suitably, Taylor's conditions would apply and we could use the exact mathematical solutions given by him.

The range of parameters in Figure 10, where solute dispersion in the straight tube can be calculated only numerically, has been of vivid interest to a number of researchers, not only in the field of FA. Chemical engineers needed the solutions, as well as physiologists studying circulation. When carrying out numerical solutions it is expected

1. To find agreement with experiments
2. To find pseudo-empirical correlations between the system parameters by comparing the results of a large number of numerically simulated experiments; dimensionless numbers are again very useful in reducing the number of necessary simulations

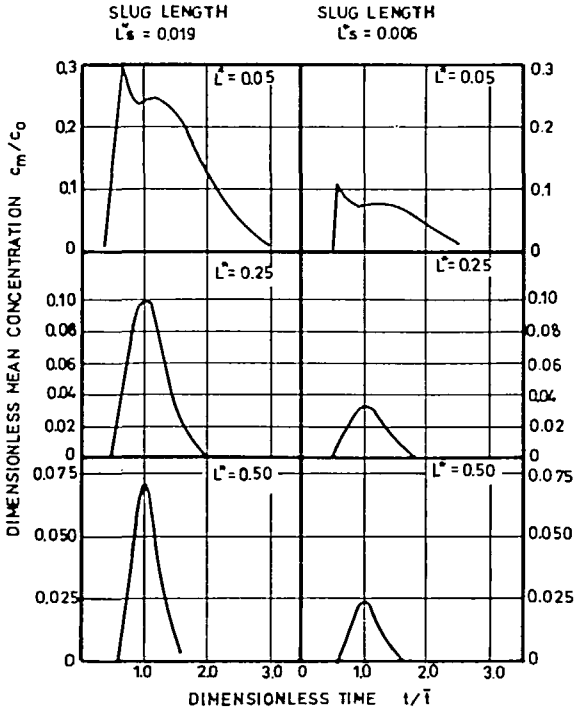

FIGURE 11. Comparison of the time distribution of c_m/c_0 for different slug lengths at Pe = 1000. $L^* = \frac{L}{a \cdot Pe} = \frac{LD}{a^2\mu_{max}}$ dimensionless location of detector, $L_s^* = \frac{L_s}{a \cdot Pe} = \frac{L_sD}{a^2\mu_{max}}$ dimensionless slug length, and $\bar{t} = \frac{2L}{\mu_{max}}$ mean residence time. The dimensionless values of the mean residence time are $\tau_L = 0.1$, 0.5, and 1.0 for L* = 0.05, 0.25, and 0.50, respectively. (From Gill, W. N. and Ananthakrishnan, V., *AIChEJ*, 13, 801, 1967. With permission.)

Gill and Ananthakrishnan[9] apparently were the first to carry out numerical analysis of the laminar dispersion equation for the intermediate τ values in the unshaded area of Figure 10. Their results for Peclet number 1000 are shown in Figures 11 and 12. Figure 11 shows the mean concentration (divided by the original sample concentration c_0) as registered by hypothetical detectors located at three different distances from the injector. The effect of injecting different sample volumes (sample slugs of different lengths) is also shown. Next to each curve is shown the injector-detector distance in dimensionless length units. For the same tube, flow rate, and solute, dimensionless length is proportional to real length. The solute slug length has been given in the same units, so that $L^*_s/L^* = L_s/L$. The horizontal axis has been scaled in a time unit often used in chemical engineering: the mean residence time, $\bar{t}$. This is the average time spent by a solute molecule in the tube section between x = 0 and x = L. This time is often (but not in any situation) equal to the ratio of the distance L to the mean velocity of flow u = $F/a^2\pi$. (In laminar flow in a straight tube, also, $\bar{u} = \bar{u}_{max}/2$.) $\bar{t}$ Will be identified here with $L/\bar{u}$ regardless if it is the true mean residence time of the solute or not. It is only because of the reduced time scale that the peaks belonging to an observation point further downstream from the detector appear to be slimmer than those belonging to a

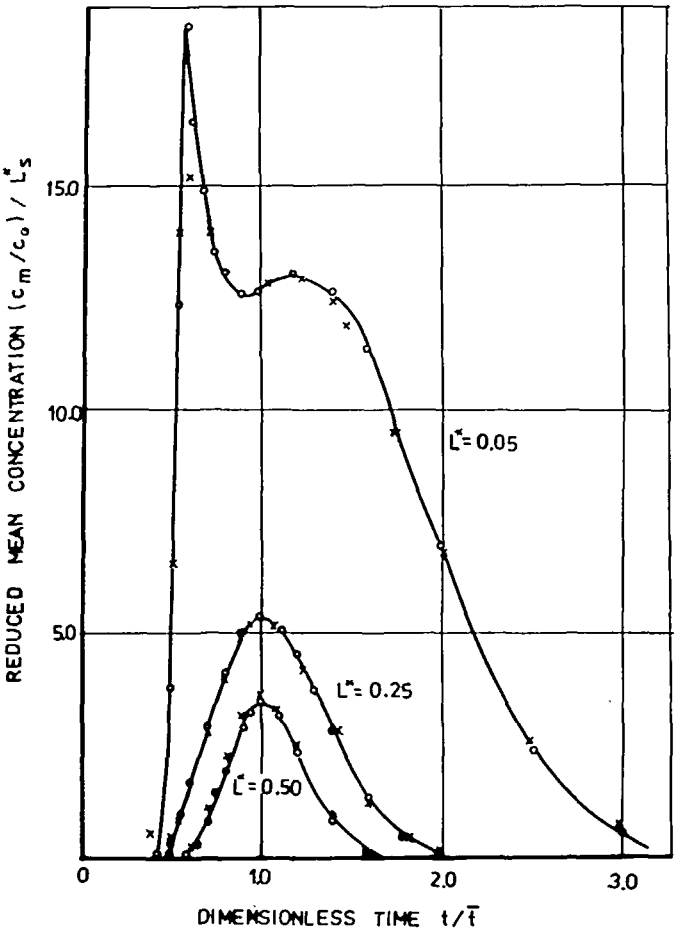

FIGURE 12. The curves of Figure 11 redrawn with reduced concentrations. X and o are numerical results for $L_s = 0.006$ and 0.019. (From Gill, W. N. and Ananthakrishnan, V., *AIChEJ*, 13, 801, 1967. With permission.)

shorter distance. On a nonreduced time scale and taking the first row of Figure 11 as scaled to time, the figures in the second row would be five times broader and in the third row ten times broader than shown.

One can observe two striking effects in these curves: one is their peculiar shape, especially in the first row, and the other is that the slug length appears to influence only the height of each figure, but neither its shape nor its width. These observations are confirmed by Figure 12 where the curves of Figure 11 have been redrawn, but this time the concentration scale of the curves has been divided by the slug length. The curves belonging to the same distance, L, but different slug lengths are now practically indistinguishable. This means that — at least as long as the slug lengths are small compared to the length traveled — the width and shape of the dispersed slug are independent from the original width of the slug. Obviously, this cannot be true for very long slugs.

The shape of the mean concentration curve shown for the shortest tube length is very striking, because it shows a double peak, which looks quite unreasonable at first sight. It should also be noted that the reduced distance belonging to this curve is quite similar

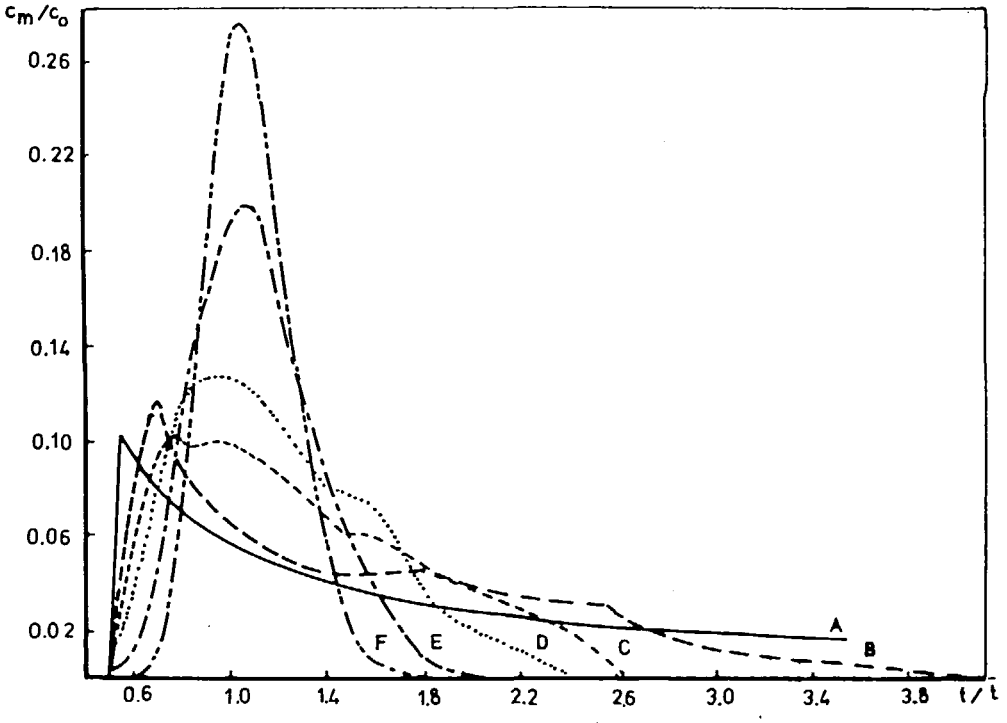

FIGURE 13. Numerical solutions for solute dispersion vs. $t/\bar{t}$ given by Bate et al.[10] Dimensionless values of the mean residence time, τ_L: A = 0, B = 0.054, C = 0.14, D = 0.29, E = 0.71, F = 1.76. L* is always half of the above τ_L value. L_r/L is 0.12 for each curve.

to that of many FA systems (e.g., a = 0.25 mm, L = 1 m, and F = 1.5 m*l*/min would give about L* = 0.06 or τ_L = 0.12, and although the Peclet number is about 6.10^4 in this case, Gill and Ananthakrishnan[9] remark that their results are independent of Peclet number changes above Pe = 1000, to which Figures 11 and 12 belong). In practical flow analysis, however, such double peaks are usually not observed. The most probable explanation of this contradiction is apparently that in practical analysis no care is exercised to use strictly straight tubes; rather, the tubes are coiled or at least slightly curved. Authors experimenting with straight tubes often notice that the observed dispersion pattern becomes irreproducible unless the tube used is carefully straightened. We shall also see later that coiling has a profound influence on laminar dispersion.

Double peaks and other irregularly looking peak shapes have been observed and numerically deduced from the dispersion equation by a number of authors. Bate et al.[10] calculated the peak shapes shown in Figure 13 for dimensionless tube lengths between 0.027 and 0.78. Their curve A corresponds to Taylor's case A (L* = 0), and curve F to Taylor's case B. Bate et al. observed reasonable agreement between the calculated and experimental peak shapes. They used a radioactive tracer as the solute and detected the radiation leaving a short section of the tube at x = L. Thus, they may have indeed measured c_m for which the curves have been calculated. This appears to be important, because already Gill and Ananthakrishnan[9] have pointed out that the shape of the dispersion curve may largely differ for c_m and c_b. Bate et al. also noticed that the mean residence time calculated from the simulated or the experimental c_m vs. time curves with small dimensionless residence time ($\tau_L < 0.4$) was significantly longer than $L/\bar{u}$, so that tracer experiments cannot be used in a straightforward way for measuring the flow rate in laminar flow. If, however, Taylor's condition B applied ($\tau_L > 0.8$), the measured mean residence time equaled $L/\bar{u}$.

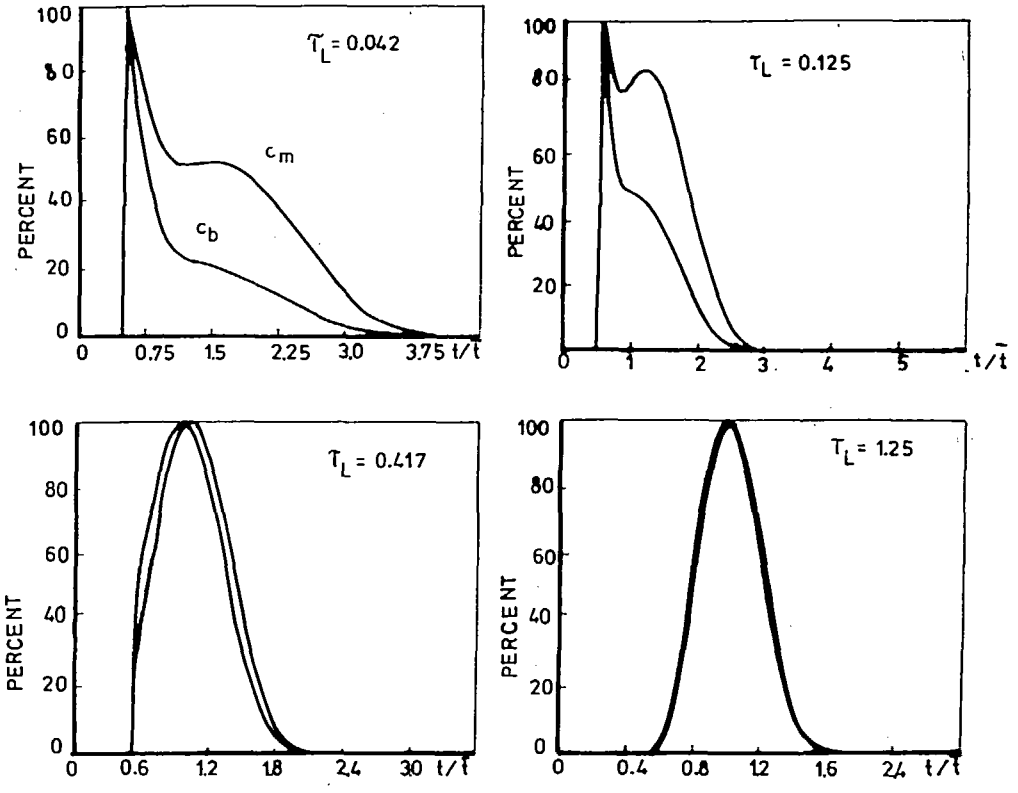

FIGURE 14. Simulated c_m and c_b vs. $t/\bar{t}$ curves.[11] The c_b curves always precede the c_m curves in time. Concentration on the vertical axis has been given as the percent of the maximum concentration.

Gill and Ananthakrishnan[9] and Bate et al.[10] all conclude that peak shapes can be described by Taylor's case B formula if τ_L is greater than about 0.6 to 0.8. This is in good agreement with Figure 10. Also, the difference between the c_m and c_b curves disappears at this limit.

Golay and Atwood[11,12] again obtained similar numerical and experimental results. Their interest was related to chromatography and, therefore, they used chromatographic units. By converting these into the units we have used throughout this review, we find that double peaks on their c_m vs. time curves were most pronounced at τ_L = 0.125. Since these authors calculated both c_m and c_b curves, their results show very nicely how much for low τ_L values the two curves differ (Figure 14). The peaks observed for τ_L less than about 1 show a behavior which is quite removed from well-behaved chromatographic peaks. Neither the location of the peak maximum nor the mean residence time coincide with $L/\bar{u}$, and usual measures of peak width depend on the residence time in an unexpected manner.

Atwood and Golay[12] also carried out a very interesting experiment to see if the peak variances (centralized second moments, σ^2) were additive for short pieces of straight tubing. In chromatography it is generally accepted that the total system variance is the sum of the variances attributed to each successive element of the system, i.e.,

$$\sigma^2_{tot} = \sum_i \sigma_i^2 \tag{11}$$

Atwood and Golay determined the variance of the elution peak detected at the end of a straight tube 1 m long, then cut the tube into smaller pieces and measured the vari-

ance separately for the pieces. Finally, they coupled the pieces by unions to again give a straight tube of 1 m length and measured the variance again. Surprisingly, the variance caused by the single-piece 1-m tube was larger than that caused by the 1-m tube joined together from pieces, while the latter was equal to the sum of the variances measured with its pieces. This observation has been attributed to the cross-sectional mixing effect of the unions. The necessary condition for variances of successive components in a flow path to add is, namely, that the concentration of sample entering and leaving each component must be describable by a single-valued function of time. This is not the case for short segments of open tube smoothly joined together. Union pieces, on the other hand, may act as localized mixers and redistribute the solute uniformly over the entrance of the following segment, without adding significant peak spreading of their own. Therefore, variances of tube segments joined by such mixing unions should add.

The numerical study by Mayock et al.[20] also revealed the existence of double peaks at about $\tau_L = 0.2$. These authors gave an interesting explanation for the observed highly asymmetric peak shapes. They calculated not only the elution curve c_m/c_o vs. time, but also gave separate elution curves for three different regions of the tube cross section: one at the axis, one for $r/a = 0.7$, and one for the vicinity of the tube wall (Figure 15). From this figure it is apparent that solute near the axis, which traverses the same length much faster than solute near the wall, has less time to be affected by diffusional processes and elutes as a sharply ascending (but also considerably tailing) peak. Solute layers farther away from the axis spend more time in the tube and are, therefore, better homogenized by diffusion. Since c_m is the average of the point concentrations weighted by the respective radii (Equation 2), it is no more surprising that double peaks are calculated and observed. It has also been found that the radial differences in the point elution curves gradually disappear if the solute has more time to spend in the tube (τ_L is increased) until the Taylor B region is attained. Vanderslice et al.[13] carried out a similar numerical analysis to the earlier ones, but this time FA applications were in the foreground. They have found relatively simple correlations between the *a priori* known system parameters and the time for peak onset and the width of the peak at the baseline, respectively. The time for the first solute segment to arrive at the detector was found to be very close to L/u_{max} (which would be only expected if there were no diffusion effect). The width of the peak measured at the baseline was found to be almost independent of the original solute slug length, below a certain limit, of course. This is in agreement with Gill and Ananthakrishnan.[9] On the other hand, peak width was found to correlate very well with tube length when the logarithms of the respective dimensionless quantities were plotted (Figure 16). This is quite interesting, given the irregular shapes of the peaks and the somewhat vague definition of $\Delta\tau_{base}$. It is interesting to notice that "base width" as used by Atwood and Golay[12] was similarly well behaved. A further partial confirmation of these results comes from Gerhardt and Adams[21] who successfully used the correlation given by Vanderslice et al. for $\Delta\tau_{base}$ to measure diffusion coefficients of various compounds. It should be noted, however, that Gerhardt and Adams used the relation $\Delta t_{base} = \text{const.}D^{-0.36}$ which is only a consequence of the Vanderslice et al. equation. They have also used a coiled tube (5 to 10 cm coil diameter), whereas the original work dealt with straight tubes.[22] A pictorial presentation of the bolus shapes calculated by Vanderslice et al. has recently become available.[23]

2. Dispersion in Curved Tubes

In the foregoing section we have seen that dispersion in a straight tube under laminar flow conditions is a rather complicated process, because the parabolic flow pattern and

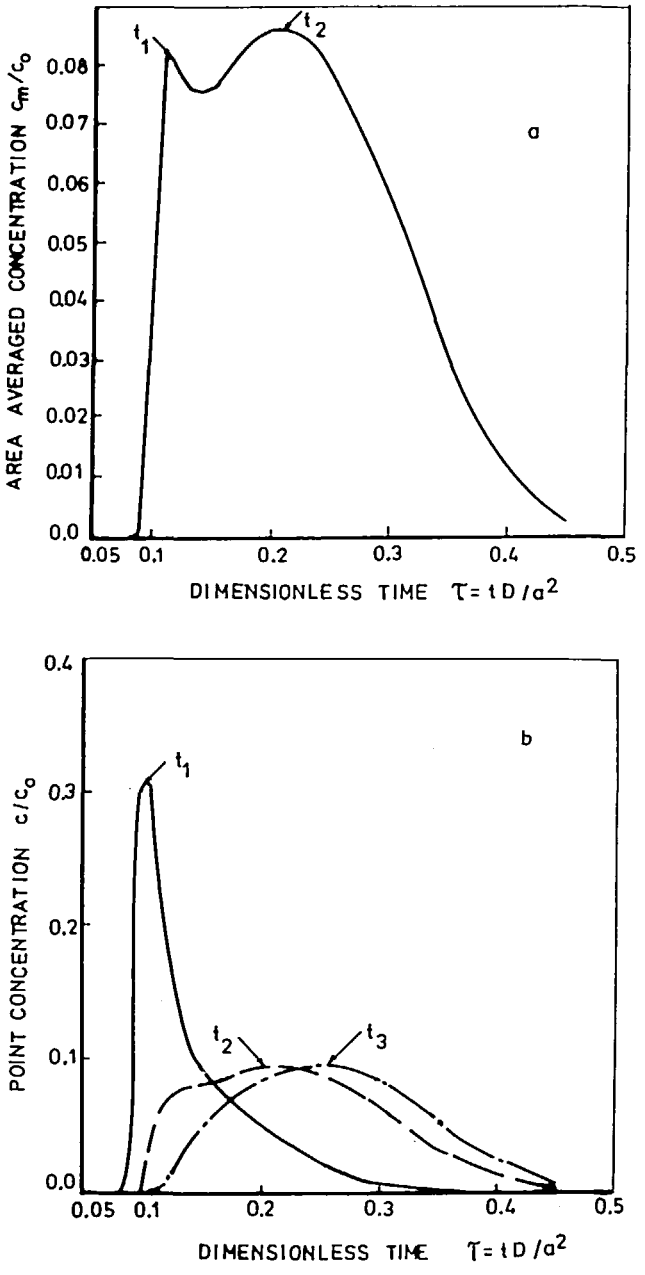

FIGURE 15. Elution from a short straight tube ($L^* = 0.1$). (a) Cross-sectional average concentration; (b) point concentration at various distances from the tube axis. ———, r/a = 0; — — — —, r/a = 0.7; —·—·—, r/a = 1.0. (From Mayock, K. P., Tarbell, J. M., and Duda, J. L., *Sep. Sci. Tech.*, 15, 1285, 1980. With permission.)

diffusion interact to produce the experimentally observable irregular peak shapes. Coiling of the tube makes the dispersion problem almost intractable by theory, because inertial forces appear which make the flow pattern rather complex. On the other hand, however, coiling appears to make the experimental results more reproducible and the peaks become narrower and more symmetrical in coiled tubes under otherwise un-

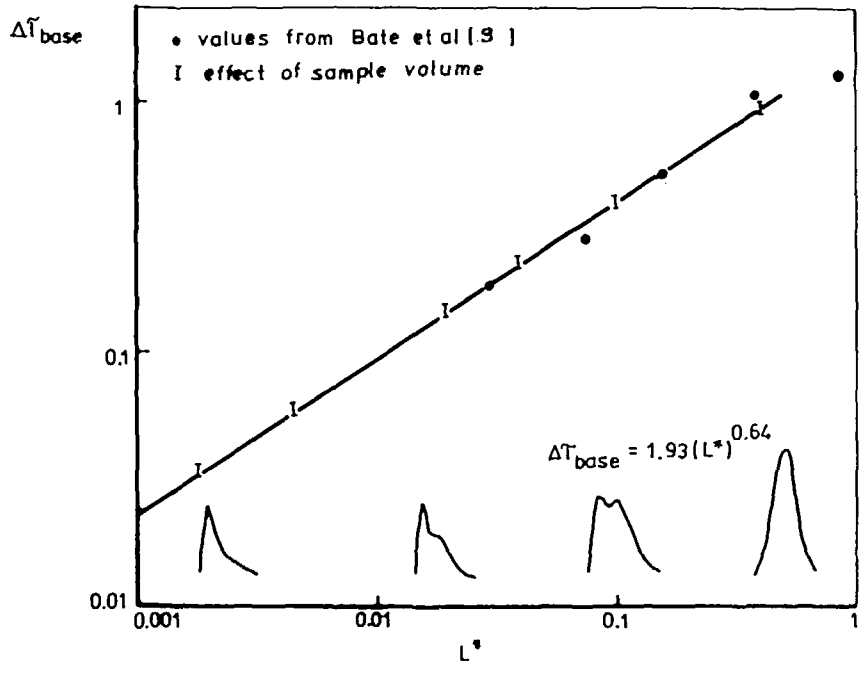

FIGURE 16. Baseline-to-baseline time dispersion vs. distance in dimensionless units.[13] Numerically obtained curve shapes as a function of L* are illustrated. The error bars represent differences due to changes of sample size from 5 to 20% of the total volume of the system. $\tau = \frac{tD}{a^2}$, $L^* = \frac{LD}{a^2\mu_{max}}$.

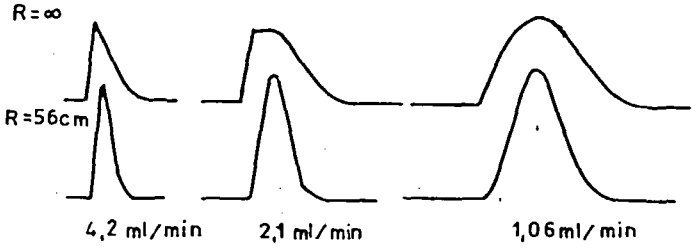

FIGURE 17. Effect of coiling.[12] The originally straight tube (366 cm × 0.38 mm I.D.) was coiled on a very wide radius (R = 56 cm, R/a ≈ 3000); the effect is still quite considerable, particularly at high flow rate.

changed circumstances (Figure 17, Table 1). This effect is more pronounced the higher the flow rate and the tighter the coiling (Figure 18).

Irregular peak shapes cannot be completely avoided by coiling. Approximately Gaussian peak shapes are observed, however, for much smaller dimensionless time values τ than with straight tubes. Trivedi and Vasudeva[26] established by experiments that elution curves from coiled tubes can be characterized to a good approximation with an apparent diffusion constant if $\tau_L > 6.0\ Re^{-1}$. This result was found for coil diameter-to-tube i.d. ratios between 10 and 280. Taking a typical Re of 60 in FA, $\tau_L > 0.1$ is obtained as the condition for nearly Gaussian peaks. This should be conferred with the approximate condition $\tau_L > 0.8$ for Taylor's case B to be valid for straight

Table 1
DISPERSION WITH COILING A 1-M PIECE OF 0.010-IN. CAPILLARY TUBE[a]

	0.4 m*l*/min	1.0 m*l*/min
Straight tube	2.42 sec	2.06 sec
Coiled on 1 in. diam	1.52	1.01
Coiled on $^1/_2$ in. diam	1.71	0.85
Coiled on $^1/_4$ in. diam	1.62	0.76

Note: The peak standard deviation is given in seconds.

Data of Scott and Simpson.[24]

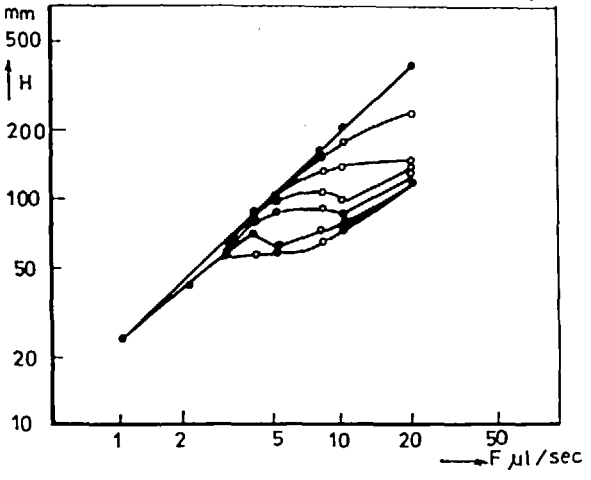

FIGURE 18. Theoretical plate height as a function of flow rate and coiling.[25] The theoretical plate height is proportional to peak variance, σ^2, at constant flow rate. Tube length 3 m, i.d. 0.8 mm. •, Straight; O, coiled, with coil diameters (from top to bottom): 900, 400, 255, 180, 135, and 75 mm; (1 m*l*/min = 16.67 µ*l*/sec).

tubes or with $0.1 < \tau_L < 0.3$ for the appearance of double peaks in straight tubes. Similar to these results, Hofmann and Halász[27] observed with a 0.75-mm i.d., 10-m-long copper tube that peaks became very asymmetric when τ_L was less than 0.44 with the tube straight; when coiling the tube on 12 cm diameter, this limit became only 0.06.

A comprehensive theory of laminar dispersion in coiled tubes has been elaborated by Tijssen.[28,29] The theory is based on the approximate mathematical description of the involved flow pattern. In coiled tubes the flow velocity vector is not parallel to the tube axis, but it has also a component perpendicular to the axis. Thus, a secondary flow arises which mixes the solute transversally while the main stream moves longitudinally. All this can be visualized by injecting a thin stream of dye into a colorless solvent flowing along the coiled tube. The dye stream will move downstream on a helical path either in the upper or in the lower half of the tube cross section. The flow in the tube is seen to be divided into two more or less independently circulating streams. The streamline patterns and velocity distributions are reproduced here for low and high

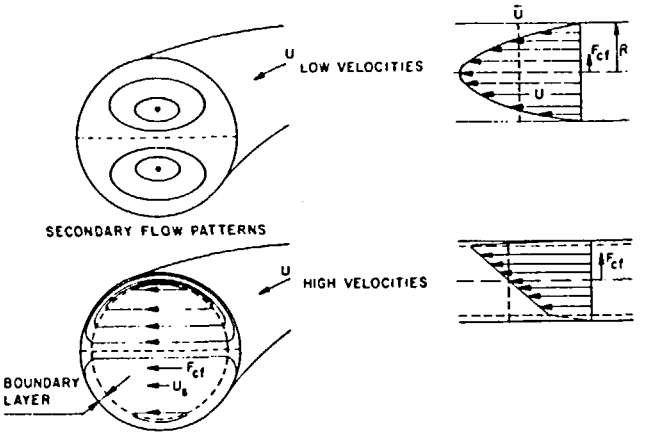

FIGURE 19. Secondary flow patterns and velocity profiles in coiled tube flow at low and high velocities.[29]

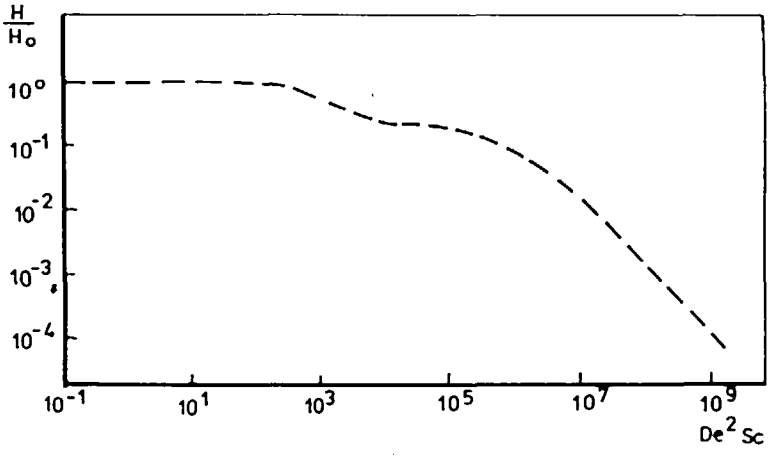

FIGURE 20. Relative axial dispersion in liquid flow-through coiled tubes.[29] The broken line has been fitted to the large number of experimental points shown in the original.

velocities, respectively (Figure 19), and a more detailed explanation can be found in the original work. A further peculiarity of this flow pattern is that it can keep up laminar flow up to much higher Reynolds numbers than in straight tubes.

Based on the analysis of the flow pattern Tijssen worked out a theory for the laminar dispersion in coiled tubes. Again adhering to chromatographic conventions, the results have been given as theoretical plate heights $H_x = \sigma_x^2/L$, where the subscript x denotes that the peak variance refers to the concentration distribution along the tube axis (in contrast to the time variance of an elution peak). For comparison, the theoretical plate height for a straight tube, with Taylor's condition B valid, would have the value

$$H_o = \frac{a^2 u_{max}}{48\ D} \tag{12}$$

Tijssen correlated H_x/H_o with the dimensionless number De^2Sc (Figure 20). De is the Dean number, $De = Re\lambda^{-1/2}$, where λ is the ratio of coil diameter to tube diameter

(Tijssen uses the inverse definition). Sc is the Schmidt number, $Sc = \nu/D$. For dilute aqueous solutions and diffusion coefficients around 10^{-5} cm^2s^{-1}, the Schmidt number is in the order of 10^3. One can read from Figure 20 that band broadening in a coiled tube is equal or less than in a straight tube with the same dimensions (and assuming Taylor's condition B to hold for the latter). The band-contracting effect of coiling increases with flow rate (Re) and the tightness of coiling $\lambda^{-1/2}$). Under extreme conditions band broadening can be very small. The usual range of parameters in FA, however, does not allow achieving a very great decrease in band broadening. Let us consider the following example:

tube length L = 150 cm

tube i.d. 2a = 0.05 cm

flow rate F = 1 $m\ell$ min^{-1}

coil diameter 2R = 1 cm(λ = 20)

diffusion coefficient D = 10^{-5} cm^2s^{-1}

kinematic viscosity ν = 10^{-2} cm^2 s^{-1}

Then

Re = 42.5

Sc = 10^3

De = 9.50

$De^2Sc = 9.10^4$

and from Figure 20, $H/H_o \approx 1/4$ and therefore the peak width is decreased about twofold by coiling. It should be noted, however, that in the same example $\tau_L = 0.28$. This means that in a straight tube with the above data and flow rate Taylor's condition B does not hold and double peaks can be expected to appear, unlike the case with the coiled tube. The above example also shows that comparison of the dispersion in the coiled tube with that in the straight tube under Taylor's condition B is not completely correct, because this condition may not be valid. From Atwood and Golay's work,[12] however, we can see that despite the unusual peak shapes obtained with straight tubes, the peak variances are quite near to the variance calculated, assuming the validity of Taylor's condition B. As a matter of fact, even in straight tubes when $\tau_L < 0.8$, dispersion is somewhat less than predicted by Taylor's formulas.

Recently, three-dimensional coiling of a plastic tube[30] has been found to be very efficient in producing secondary flow and thereby decreasing peak spreading.

3. Dispersion in Packed Columns and Tubes

As alternatives to open tubes, packed columns[14] and packed tubes[15] have been suggested for use in FA. In the first case, the diameter of the inert packing (e.g., glass beads) is much less than the column diameter. Van den Berg et al.[14] have used a 4.6-mm i.d. column packed with 40-μm beads. This technique had been used earlier as postcolumn reactor by the same group. Reijn et al.[15] have also followed earlier chromatographic practices[31] when they filled a 0.6-mm i.d. plastic tube with glass beads 0.4 mm in diameter. When packed correctly, the beads exhibit a regular zigzag pattern (Figure 21). The authors named this device the single bead string reactor (SBSR). A

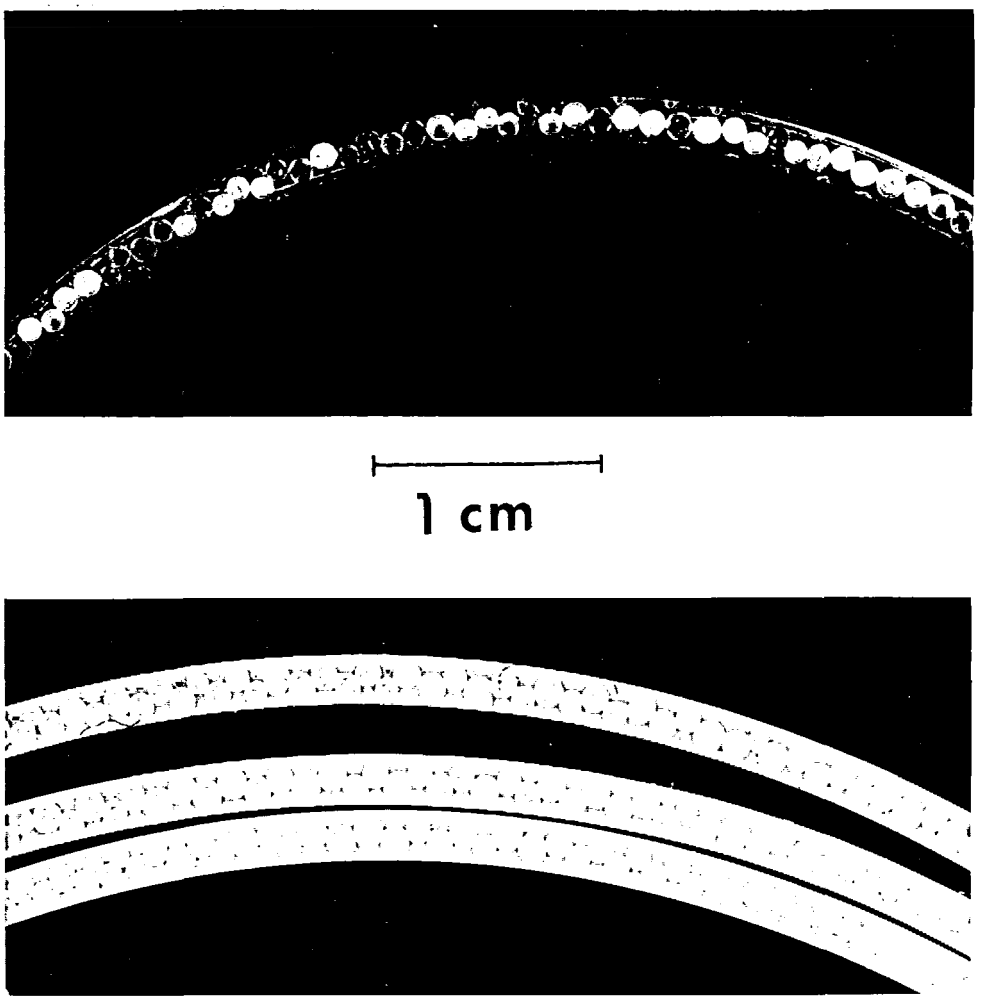

FIGURE 21. Regular bead pattern in an SBSR. (By courtesy of H.A. Mottola.)

similar arrangement has been used more recently in hollow fiber suppressors for ion chromatography.[32] Both packed columns and the SBSR have been found to be effective in decreasing peak broadening. Peak broadening in the packed column has been characterized by various approximate equations. Van den Berg et al.[14] used Hiby's[33] formula. This has been criticized, however, by Tijssen,[29] who prefers the relations given by Snyder and Kirkland[34] and by Done and Knox.[35] The last named equation reads:

$$H/d_p = (\bar{u}d_p/D)^{1/3} + 0.1(\bar{u}d_p/D) \quad (13)$$

where H is the plate height, d_p is the particle diameter of the column packing, and $\bar{u}$ is the linear flow rate. (Recall that according to Equation 12, for a straight open tube $H/a = \bar{u}a/24D$). For the SBSR no rigorous plate height equation is known yet. Experiments[15] have shown the dispersion to be quite independent of flow rate in the SBSR. Columns and tubes packed with inert beads have been used little in FA, but they have been analyzed as alternatives to the open tubes. We shall come back to this subject in the paragraph on optimization.

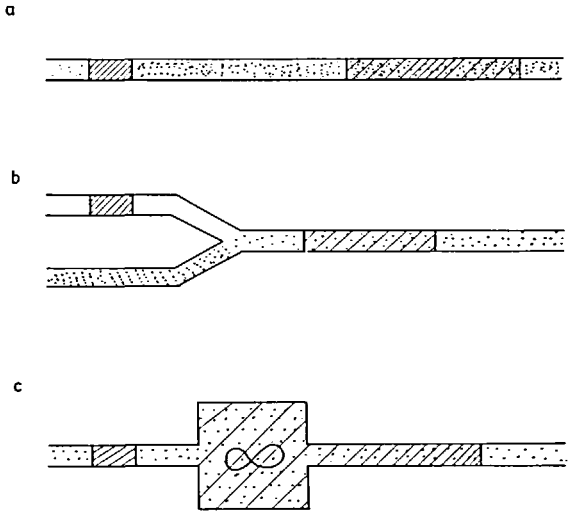

FIGURE 22. Various mixing devices. (a) Open tube (or packed tube, column); (b) merging streams; (c) mixing chamber.

4. Dispersion in Mixing Devices

Mixing in FA is needed to homogenize the sample with a diluent or a conditioning solution or with a reagent. In the early days of unsegmented FA,[16] mixing was also necessary to counterbalance the radial inhomogeneities caused by simple injection devices and wide-bore straight tubing. Mixing can be affected by active or passive mixers, i.e., mixers with or without moving parts.

As discussed earlier, flow in an open or packed tube or column more or less causes spreading of the sample slug into the carrier solution. Obviously, the carrier solution in front of and behind the sample slug also disperses into the slug. Thus, the sample and the carrier solution are mixed to some extent during the transportation process. Since the carrier may itself be the diluent, conditioning solution, or reagent, the sought for mixing can be achieved simply by flow dispersion. This passive mixing process has been proven experimentally to be rather reproducible. It should also be clear, however, that this kind of mixing can be described only in a rather complex way and that the mixing ratio of sample to carrier is varying both as a function of the distance from the moving center of the sample slug and as a function of the travel time in the tube or column (Figure 22a). The extent of mixing is also influenced by the sample viscosity and solute diffusivities.

A second way of passive mixing is to let two streams, one containing the sample slug and one consisting of the other solution to be admixed to the sample, merge at a suitably designed confluence point. This mixing assures a constant mixing ratio (Figure 22b), but care should be exercised that radial inhomogeneities do not arise (in an extreme situation two separate parallel flows may exist after the confluence point). Coiling the tube after the confluence point improves radial mixing as discussed earlier. The confluence point can also be built into a dripping vessel (Figure 23). Rotation in the developing drop and the splashing of the drop efficiently mix the two streams.[36]

Active (or mechanical) mixing in FA has been done mainly in mixing chambers (Figure 22c). The solute concentration at the output of the mixing chamber is in a good approximation[16]

$$c = c_o\left(1 - e^{-\frac{F}{V_r}t}\right) \text{ for } o < t < \frac{V_s}{F} \qquad (14)$$

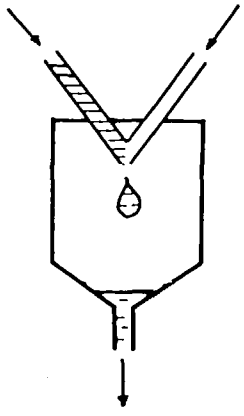

FIGURE 23. Dripping vessel as mixer.

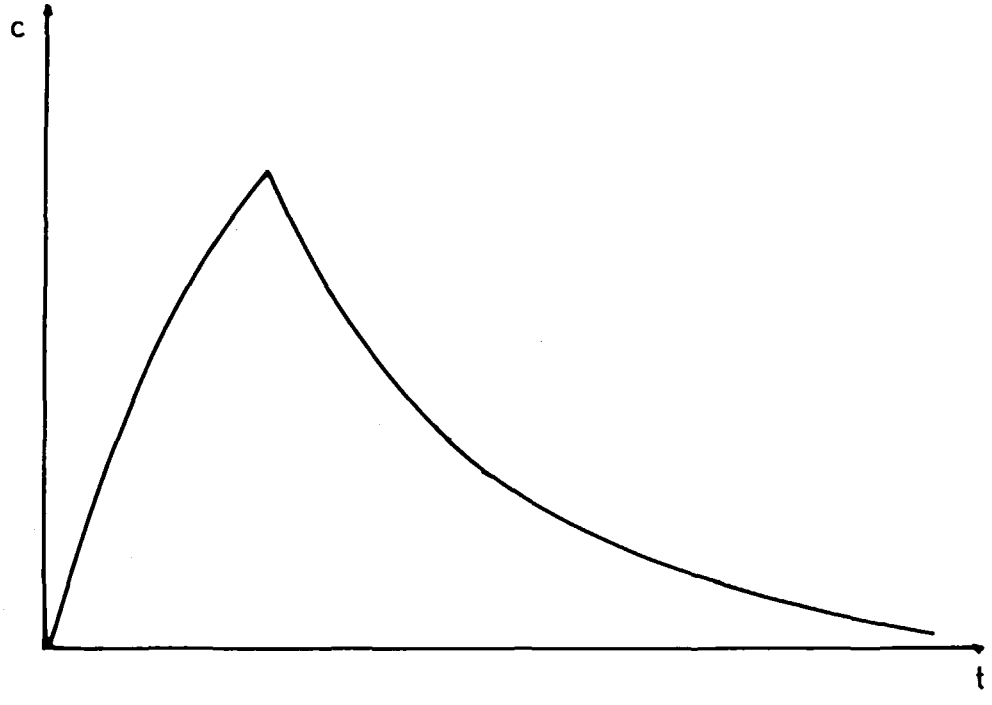

FIGURE 24. Change of solute concentration with time in a mixing chamber (rectangular slug input).

and

$$c = c_o\left(e^{\frac{V_s}{V_r}} - 1\right) e^{-\frac{F}{V_r}t} \text{ for } t > \frac{V_s}{F} \quad (15)$$

where V_r and V_s are the volume of the mixing chamber and the sample, respectively, and c_o, c, F, and t have their usual meaning (Figure 24). More detailed mathematical descriptions have been given by Pardue and Fields and by Tyson.[37-40] The obvious advantage of active mixing is that it does not depend on sample viscosity, etc. and it produces a simple predictable exponential concentration change. Thus, it has been used

for detector calibration in gas chromatography[41,42] and for the calibration of ion-selective electrodes[43-45] where the simple linear relation between time and log concentration (Equation 15) has special benefits. Essentially, the same relation can be used to carry out concentration determinations through time measurement.[44,46-49] The time needed to pass a given concentration level by the falling curve is linearly related to the logarithm of c_o, the sample concentration. The time span between the rising and falling curve passing the same concentration level can also be used under suitable conditions. The attainment of the given concentration level can be established by direct measurement or by titration, i.e., admixing reagent of suitable constant concentration to the exponentially perturbed sample.

A particular feature of unsegmented flow analysis methods is that concentration gradients can be produced reproducibly. This has led to a number of interesting practical applications. Gisin et al.[50] suggest that the mixing chamber should be preferred in most cases for this purpose, being more precise and having wider dynamic range than capillary tubes. The mixing chamber can also be used successfully to produce a linear concentration gradient.[51,52] This surprising effect has been used for automatic titration, too.

Recently, there have been reports of a pseudo-mixing chamber with very small effective volume V_r.[53-55] It has been realized by connecting an injector to a detector by a short piece of plastic tubing (e.g., L = 23 cm, i.d. = 0.55 mm). The rising and falling parts of the peaks detected upon injection of a tracer were found to be exponential functions. This observation, which can be traced back to the early work by Thiers et al.,[56] has led to the conclusion that an apparent single mixing stage was present. Titrations, similar to those with real mixing chambers, could be done in a much shorter time. Although the usefulness of this observation is beyond doubt, it should be clear that no real mixing chamber is present in this system and no clear explanation for the observed exponential concentration change has been given yet. Dispersion caused by laminar flow and diffusion may be responsible for the observed phenomena, and, indeed, we find that elution curves calculated numerically for short tubes resemble very much an exponential function.[12] However, the connections and diameter changes at both ends of the short tube may also play a role. A point of interest may also be that in such titrations not only the sample concentration changes in an exponential manner, but also the reagent concentration. This point appears to have received little attention until the very recent study by Tyson.[40]

B. The Role of Chemical Reactions on the Dispersion in Unsegmented Flow

In the paragraph on mixing in FA we have seen that a sample slug injected into the carrier stream mixes passively with the carrier. If the carrier is not inert, but it reacts with the sample, the process of mixing is expected to be influenced by this reaction. Mixing is caused by laminar dispersion which depends on diffusion. Diffusion, in turn, depends on solute concentration distribution and this is influenced by chemical reactions of the solute.

We have seen earlier that laminar dispersion without any chemical reaction is a complicated phenomenon itself. Hence, it is not surprising that little work has yet been attempted to describe laminar dispersion with chemical reaction. Haagensen[57] set up a differential equation which should be solved numerically if the necessary data were available. In an extension of their earlier work,[58] Painton and Mottola[59,60] have compared simulated curves for FIA dispersion combined with chemical reaction assuming different reaction rate constants. An increase in the reaction rate constant caused a decrease of the dispersion of the determinand. The peak height of the determinand decreased, at the same time, too, because of the higher reaction rate. A number of

experiments has been carried out by the same author to study the effect of various parameters like sample size, injection method, reactor geometry, etc. It has also been found that conversion could be increased either by increasing the reagent concentration (and thus the analysis cost) or by decreasing the flow rate (and thus the analysis rate). Ruzicka and Hansen[2] point out that in FIA the peak concentration of the reaction product will always have a maximum as a function of residence time. The concentration of the product is increased by the reaction and it is decreased by dispersion. The concentration will be maximum when the two rates become equal. Reijn[19] has remarked that in a single-line FIA system large sample volumes may lead to double peaks, because the middle of the sample plug may not have enough time to mix with reagent. Numerical simulation of the combined processes of physical dispersion and chemical reaction[61] proved to be a useful method for guiding the design of flow analysis experiments. The general trend of the calculated results was in agreement with the expectations based on the earlier workers' findings.

A detailed study is available on the effect of chemical kinetics on sample dispersion in the SBSR.[19,62] This reactor is more easily amenable for a study of this type because the dispersion in the SBSR can be described by the tanks-in-series model with n, the number of tanks, independent of the molecular diffusivity of the solutes and also independent of the flow rate in wide ranges. Reijn concludes from his calculations and experiments that the variance of a FIA curve is virtually independent of the reaction rate for large n. Peak height, on the other hand, depends very much on reaction rate and can be calculated as a function of sample volume, residence time, and rate constant.

If the sample is injected into a nonreacting carrier stream and the reagent stream merges with this carrier downstream from the injector, we may speak of a two-line or premixing system. The role of chemical reaction in the dispersion process can be elegantly described for such systems, at least for first-order kinetics.[63] One should be aware, however, that these results may not apply for systems without premixing.[58]

C. Dispersion in Segmented Flow

In a straight or coiled tube, under nonsegmented laminar flow, an originally rectangular sample plug will be dispersed to a considerable extent as it moves downstream. To avoid this sample zone broadening, Skeggs[64] introduced the air egmented flow system. The fluid stream which consists of a train of samples separated rrom each other by wash liquid is segmented by air bubbles. The bubbles effectively break up the laminar flow pattern, thereby decreasing dispersion. Dispersion is, however, not completely eliminated. A part of the fluid adheres to the tube wall in the form of a liquid film. The mass transfer between this film and subsequent fluid segments creates some axial dispersion (see below). The flow line may also include unsegmented parts (e.g., the sample aspiration line in earlier designs of the Auto Analyzer®) where laminar dispersion takes place. Also, a debubbler may be used to remove the bubbles before detection and such debubblers have been identified as sources of serious band broadening. In more recent designs the last two sources of dispersion have been greatly decreased so that the only important remaining factor to cause zone broadening was the adherent film on the tube wall.[65-67] Snyder and Adler[65,68-70] gave a comprehensive treatment of dispersion in segmented flow. They have extended earlier models, partly by making them completely quantitative, and partly by alleviating a simplifying assumption. The assumptions of the basic model (Figure 25) were the following:

1. Instantaneous mixing of film and segments
2. Constant dimensions of all segments and constant film thickness
3. Negligibly slow longitudinal diffusion in the film

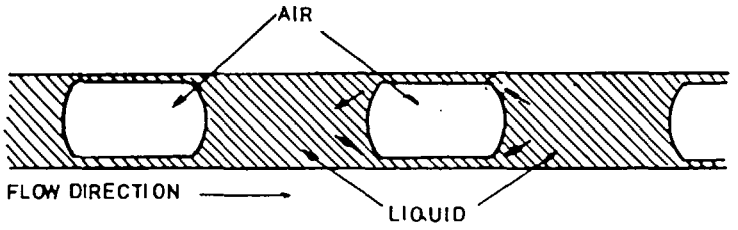

FIGURE 25. Schematic of dispersion in segmented flow.[70]

This model has led to a dispersion pattern described by the Poisson distribution function. This is a one-parameter distribution. For the parameter, q, the following result has been derived:

$$q = 0.67\,\pi\,L\,d_t^2\,(u\,\eta/\gamma)^{2/3}\,V_s \tag{16}$$

where L is the length of the tube traversed by the stream, d is the i.d. of the tube, u is the linear velocity of the segmented stream, η is the liquid dynamic viscosity, γ the surface tension, and V_s the volume of a liquid segment. The basic model was then further developed by rejecting assumption 1 above. The new derivation has led to:[63]

$$\sigma_t^2 = \left[\frac{538\,d_t^{2/3}(F_l + 0.92\,d_t^3\,n)^{5/3}\,\eta^{7/3}}{\gamma^{2/3}F_l D_{w,25}} + \frac{1}{n}\right] \cdot \left[\frac{2.35(F_l + 0.92\,d_t^3 n)^{5/3}\,\eta^{2/3}\,t}{\gamma^{2/3}\,F_l d_t^{4/3}}\right] \tag{17}$$

where σ_t^2 is the time variance of the dispersion curve (concentration vs. time function), d_t, η, and γ have their earlier meaning, F_l is the flow rate of liquid through the tube, n is the segmentation rate or bubble frequency, t is the dwell time of liquid in the tube, and $D_{w,25}$ is an empirical coefficient which characterizes the sample: $D_{w,25}$ varies from 2 to 12 × 10^{-5} depending on the molecular weight. It has been found that only the variables d_t, F_l, n, and t are of major importance. The effects of these calculated for Technicon's SMAC system are shown in Figure 26. d_t is 1.0 mm in the system. The dashed portions of the curves are excluded values which would lead to liquid segments too short for detection in the given system. Considering only the solid curves, the optimum performance, i.e., minimal σ_t, is found at about 0.01 m*l*/sec and 1.5 bubbles per second where the SMAC analyzers actually work (the theory came later than the optimum was found empirically). Snyder[65] also deduced that reducing the tube diameter improves performance. He also admitted, however, that when going below d_t = 1.0 mm the system performance would be limited by other factors than dispersion during segmented flow.

The model of dispersion in segmented flow has been perfected by Pedersen and Horvath[71] to give still closer agreement with experiments. Segmented flow systems may include unsegmented sections which are sources of laminar dispersion. Thiers et al.[56] and Walker et al.[72] have found that the response of an unsegmented section to a step function concentration input can be described by an exponential output concentration function. No explanation has been given for this behavior, but the results are consistent with later work in FIA.[53,55]

Segmented flow is not limited to air segmentation: two immiscible liquids may develop a similar flow pattern if they are fed in alternating segments into a flow line.

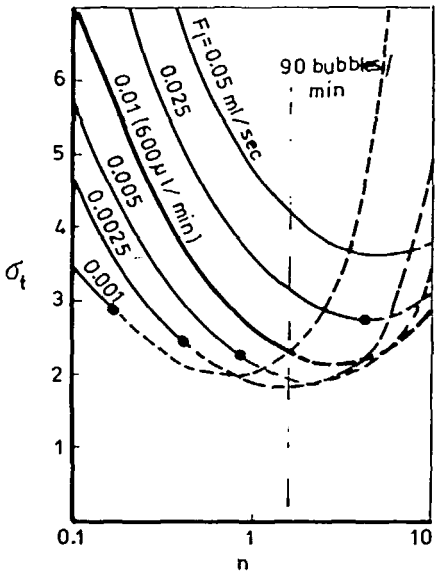

FIGURE 26. Theoretically calculated curves of σ_t vs. bubble frequency for various flow rates.[65] Tube i.d.: d_t = 1.0 mm; n: bubbles per second.

Segmentation of a flowing solution with an immiscible liquid may serve conservation of sample integrity and/or it can be an efficient means for extraction in FA. In the latter application the sample may be injected into the aqueous phase before segmentation occurs. An interesting experimental study of dispersion in liquid-liquid segmented flow is available[73a] and may serve well the design of such systems.

A recently introduced hybrid of air segmentation and concentric streaming of two immiscible liquids apparently eliminates sample dispersion almost completely.[73b] A thin, streaming layer of liquid fluorocarbon separates the tube wall from the segmented stream of analyte and reagent and thereby prevents adhesion on the wall of the tubing. This technique requires sophisticated hardware, but it appears to be very efficient in suppressing dispersion.

D. Dispersion under External Control

In most FA systems sample and/or reagent dispersion are under physical control determined by the flow characteristics of the system. There are, however, also examples when the reagent concentration is kept under external control. Reagent concentration distributions can be produced almost at will by generating the reagent coulometrically at some point in the flow system.[74,75] The same task can be solved by mechanical means, albeit with rather sophisticated mechanics.[76,77]

III. OPTIMIZATION IN FLOW ANALYSIS

Optimization in FA means to find the most suitable FA solution to a given analytical problem. Optimization includes two decisions. First, the basic method (segmented or unsegmented flow; if unsegmented then open tube or packed column, etc.) has to be chosen. Second, the parameters of the method have to be adjusted for optimum solution of the problem.

These two decisions cannot be met one after another, because one should compare the optimum setup for each method. Some possible goals of optimization are

- High rate of determinations
- Low sample and/or reagent consumption
- Short processing time
- High accuracy and/or reproducibility
- High sensitivity in concentration (little dilution)
- Low equipment and operation costs

Further goals may be

- Complete automation of a laboratory (minimum manual interaction)
- Easy operation by less skilled personnel
- Versatility
- Many determinations from a single sample
- Handling of difficult samples (e.g., high viscosity, particles in the sample)

Obviously, one cannot satisfy all these goals at the same time. This is why different FA methods and other automated techniques may have complementary roles.

It is always important to see the goals of the optimization clearly. For example, the use of a mixing chamber has occasionally received strong criticism, because it allows for a lower sampling rate and needs a higher reagent consumption than FIA (unsegmented flow in a coiled tube), and it also introduces considerable dilution of the sample. However, proponents of the mixing chamber have used it mainly in situations where the mentioned parameters were unimportant. The technique was used, for example, in pharmaceutical quality control with electrochemical detection.[78] In this application the analysis of 20 to 60 samples per hour is more than enough, since the number of samples per determination type is not high and sample preparation is the rate-limiting process. On the other hand, a fast change from one kind of determination to the other is important, and in this respect the mixing chamber has no disadvantage. Reagent consumption was again unimportant, since very cheap reagents (usually dilute KCl and HCl) were used. Considerable dilution was an advantage rather than a disadvantage, because the detector working range was usually below the sample concentration range, and thus manual predilution could be avoided. Some particular advantages of the use of a mixing chamber with electrochemical detection were pointed out by Pungor et al.[79a] Reduced reagent consumption may accrue from using the mixing chamber in a closed-loop system.[79b,79c]

Another optimization problem, that has also been subject of a dispute,[80-82] is the effect of flow rate on sampling rate and reagent consumption in FIA. Although it has been pointed out[82] that sampling rate and sample consumption are both important, and a more recent treatment[2] clearly distinguishes the time dispersion σ_t and the volume dispersion σ_v, still the same study[2] points out only that decreasing the flow rate decreases the axial (and hence the volume) dispersion, but does not mention that the time dispersion is increased at the same time. In other words, in a FIA system, by decreasing the flow rate the sample and reagent consumption can be decreased (so that the detector signal at the peak maximum remains the same), but the time necessary for one sample to elute baseline to baseline increases and, therefore, the sampling rate decreases. This is clearly shown by Equations 3.11 and 3.12 in Reference 2:

$$\sigma_t^2 = \frac{\bar{t}\, a^2}{24D} = \frac{\pi\, L\, a^4}{24DF} \tag{18}$$

and

$$\sigma_v^2 = \frac{\pi L a^4 F}{24D} \tag{19}$$

(which are, by the way, only approximately valid for FIA, because they are derived assuming Taylor's condition B to be valid). The same equations indicate that by decreasing the tube radius, sample throughput can be increased and reagent consumption can be decreased at the same time. This has been done in practice, indeed, until a further decrease in tube i.d. became impractical.

The conclusions reached here should be regarded as only qualitative, however. Optimization is a more complicated procedure, and we shall return to this problem later.

Any optimization results should be carefully judged before making practical decisions, since optimization is always subject to stated or unstated constraints and the constraints used by one or another author may reflect biases based on his own experience which do not necessarily apply to other laboratories. Very often, optimum performance of a system is found at the extreme practical limit of at least one parameter (e.g., pressure, flow rate, diameter, etc.). These extremes may depend on the hardware used, the skill of the experimenter, and on the samples studied. There are also situations when practical considerations rule out the use of a system optimal with respect to, e.g., band broadening. The use of aggressive chemicals or high temperature, e.g., may limit one's choice. Scholten et al.[67] have also pointed out that packed bed reactors are secluded as photochemical reactors and extraction in FA makes the use of a solvent segmentation system necessary.

A. Comparison of Flow Analysis Methods

A number of authors have compared various FA methods. Factors that have been compared include sample and reagent consumption, sampling rate, sensitivity, cost of equipment, ease of operation, adaptability to various analytical techniques (separation, detection, special chemistries, etc.), proneness to malfunction, etc. The relations of residence time, sampling rate, and sample and reagent consumption have attracted most interest. In typical FA determinations the sample reacts with a reagent and the reaction product is measured. In some cases a number of further operations is needed, while in others no reaction takes place at all; the flow serves only to transport the sample or to adjust its pH, ionic strength, etc. Apart from the transport-only case, the sample has to spend a certain time in the system. Residence time, defined as the average time spent by a nonreacting tracer molecule between the sample input and the detector, should be long enough to allow sufficient mixing of sample and reagent and to let the reaction proceed to such an extent that a sufficiently high detector signal is obtained. Sample and reagent can meet at a confluence point or the sample may be intercalated into the reagent stream. In the first case good radial mixing is needed, while in the second, sample and reagent have to be mixed axially. In both cases further time is needed for the reaction to proceed.

Reijn et al.[83] derived theoretical equations to characterize straight and coiled open tubes, packed columns, and the SBSR. On this basis they have calculated the theoretically obtainable highest sampling frequencies for each method as a function of residence time. In the calculations two constraints were used: the pressure drop should not exceed 1 bar and the assumed detector volume of 1 $\mu\ell$ should not be the main source of dispersion. The authors have found that all four reactor types allow for sampling frequencies over 100 h^{-1} at residence times of about 100 sec and at a reagent consumption of 8 $\mu\ell$ per determination at a pressure drop of 1 bar. It should be noted immedi-

ately that the conditions under which this performance can be (theoretically) achieved are not equally practical with all four techniques. The tubular reactors, for example, have an i.d. between 0.1 and 0.2 mm, while the i.d. of the SBSR is near to 1 mm. The packing of the packed column should be of about 10 μm diameter.

Huber et al.[25] experimentally compared the volume variances observed with various coiled open tubes and packed columns. The authors conclude that packed columns should be preferred, as they give less peak broadening and require less pressure drop than small diameter tubular reactors with the same residence time.

Snyder[84] compared air segmented and nonsegmented flow analysis in open tubes. His calculations showed that for long residence times (e.g., 500 sec) higher sampling rates can be achieved by segmented than by unsegmented flow. Only for rather short residence times (a few seconds) do maximum sampling rates appear to become equal.

Scholten et al.[66] compared solvent segmented vs. unsegmented flow in open tubes for chromatographic postcolumn reaction detectors. They also found that segmented flow should be favored unless the residence time is very short (e.g., 2 sec for a 0.8-mm i.d. tube). The same authors later[67] compared segmented flow, unsegmented flow, and packed bed reactors. Their final conclusion is that if very narrow (less than 0.3 mm i.d.) capillaries are not regarded for practical reasons, solvent (or gas) segmented tubular reactors should be used for reaction times of over 15 to 20 sec. For shorter reaction times packed bed reactors should be preferred, with nonsegmented tubular reactors coming in only for extremely rapid reactions ($\bar{t}$ of, typically, 0 to 5 sec). They believe, however, that for reasons of convenience many workers will prefer the use of a tubular (segmented or nonsegmented) reactor to that of a packed bed reactor, even for residence times of 5 to 20 sec, and recently they suggested[85] that packed bed reactors should be preferred only for reaction times between 0.5 and 1.0 min.

The same group[86] again compared the performance of 0.1 to 0.2 mm i.d. open tubes (coiled) and 1 mm i.d. columns packed with 5 or 10 μm glass beads for the purpose of reaction detectors in micro-HPLC. Although the packed bed reactors showed significantly better performance (lower σ_r^2/V_r) than the open tubular reactors, this advantage could hardly be used because of large variance contributions from other parts of the system.

Tijssen[29] saw more scope for future developments with unsegmented than with segmented flow systems. He advocated the use of very narrow bore coiled tubes which should outperform packed columns and segmented flow, also at high residence times.

Van den Berg et al.[14,87] found that for slow reactions ($\bar{t}$ above 5 min) gas segmented liquid flow is better than open tubes or packed columns. For shorter reaction times they preferred the packed column.

From the foregoing review we can see that there is no complete agreement between various authors about the ranges of application for various reactors. This is apparently caused by different assumptions in determining the optimal system. In addition to the cited opinions, it should also be noted that the chemistries for various determinations may also be changed or modified so that the reaction times can be considerably decreased. Progress in this direction would favor nonsegmented methods.

Considering the overall performance of the discussed flow methods their respective ranges of practical interest may be summed up in the following. Unsegmented flow in a coiled tube (FIA) or with a small mixing chamber is a relatively inexpensive and simple technique. It has apparently found best acceptance where the number of samples per day or the number of determinations per sample is relatively small, i.e., when automation would be welcome, but CFA or other high workload automated analyzers are not cost effective. An important condition is, of course, that the required determinations are adaptable to FIA (short residence time). Reagent economy can be en-

hanced by the merging-zones technique. FIA has also become quite popular as a sample preparation/sample introduction device for techniques like AAS or ICP, and it has found its way into on-line process control.

Unsegmented flow analysis with a packed column or SBSR may be used under the same conditions as FIA, but first of all for reactions requiring longer residence time. Air segmented flow is an alternative to packed columns or SBSR if long reaction times are encountered. For laboratories with high sample numbers per day and many determinations per sample, this is probably the best FA alternative, particularly with respect to availability of sophisticated commercial realizations and extended applications in literature. Liquid segmented flow appears to be a rather convenient technique for analytical liquid-liquid extraction.

Special problems may require special solutions. So, for example, very high determination rates may be achieved in coiled tubes of rather small i.d. (say below 100 μm), even if long reaction times are needed. Such a technique would be, however, not without technical problems (sampling, clogging, etc.). Another example might be a routine analytical laboratory with tasks that have been found ideally suited to CFA (see above), but the laboratory is in a developing country where working power is not very expensive, but sophisticated instruments are both expensive and more prone to breakdowns. In such laboratories a good number of determinations could be automated by using unsegmented flow techniques (FIA, SBSR, suitably dimensioned packed columns).

In this section on optimization we have dealt only with those techniques where the sample either does not undergo any chemical reaction or it is reacted with a reagent in a more or less fixed ratio, and the product concentration (or the concentration change of one of the reactants) is measured. There are many other FA techniques (e.g., titrations, kinetic determinations, etc.) which can also be implemented in different ways. It appears, however, that it would be still too early to attempt a useful comparison of such methods.

Finally, it should be emphasized that all FA techniques are still in development, and technical innovations may modify their judgment. Also, we should not overlook the fact that FA is not the only way of automation in the analytical laboratory.

B. Parametric Optimization in Flow Analysis

Choosing the most suitable FA protocol includes the determination of the optimal conditions of the analysis by any technique. The parameters that can be more or less freely adjusted in any laboratory are the chemistry of the determination, diameter and length of flow line, diameter of packing material (if used), sample size, and flow rate. Other parameters like maximum pump pressure, detector dead volume, etc. will be usually given and be constraints for the optimization. (The situation is different, of course, with laboratories or manufacturers working in FA development.) As pointed out by Reijn et al.,[83] one will usually find optimum performance at the practical limits set by the constraints. In other words, we usually have such a wide range available in the adjustable parameters that the system can be operated at the limit dictated by the performance of the (rest of the) hardware.

Useful equations and diagrams for guiding the optimal choice of adjustable experimental parameters can be found in the references quoted above with respect to methods comparison and also in other works specialized with respect to each technique. It appears to be little reason to attempt to give general rules for assessing the effects of changing one parameter or the other separately, because changing one parameter will usually also change others, and what parameters will be affected depends on the actual system and its constraints. For example, increasing the length of the flow line increases

the necessary pressure drop if the flow rate should be kept constant, or it decreases the flow rate if the system works at constant pressure. If the residence time has to be kept constant and either the flow rate or the pressure drop is also constant, then an increase in line length has to be compensated by a change in tube diameter. In the last situation the constraints are such that changing the line length "changes" the tube diameter — an absurd statement which is, however, mathematically reasonable. The above examples show that system constraints often make it impossible to change a single parameter at a time with all others unchanged. Changing one parameter, say, the line length, may have different effects on another parameter, say, maximum sampling rate, depending on the actual constraints. Since there are so many combinations of possible optimization goals and constraints, a careful analysis of the analytical problem has to be left to the individual analyst.

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