Analytica Chimica Acta, 78 (1975) 145–157

FLOW INJECTION ANALYSES

PART I. A NEW CONCEPT OF FAST CONTINUOUS FLOW ANALYSIS

J. RŮŽIČKA and E. H. HANSEN

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

(Received 10th February 1975)

The ever-increasing demand for analyses in clinical, agricultural, pharmaceutical, industrial and other types of analytical control has led to the development of a large number of different instruments for automated analysis[1]. Developments in this field have been further stimulated by the additional advantages of automation, such as increased precision, decreased cost of individual assay, and the satisfactory reliability of automated equipment. It has been estimated[2] that the market for automated instruments for wet chemistry will achieve an annual growth rate in excess of 15% up to 1980, compared with the growth rate of 9% for all other types of analytical instrumentation. The demand for the type, complexity, multiplicity and rate of analyses to be performed is very diversified; consequently, the major manufacturers tend to concentrate on specific areas, like clinical analysis, which offer a large market, and relatively similar types of material to be analysed. The present trend is aimed at increased sampling rates of both single and multipurpose analysers, which thus become more complex to devise and more expensive to manufacture. The numerous instruments manufactured and suggested for analysis of a large number of individual sample solutions can be divided into two groups: batch analysers and continuous flow analysers.

In the batch analyser, each sample is placed in its individual container in which it remains for at least most of the analytical procedure which comprises discrete additions of buffers and reagents dilution, heating, *etc.* at predetermined points of the analytical cycle until the treated sample reaches the colorimetric cell or other detector. Up to that point the individual character of each sample is strictly preserved as it moves within the instrument in an individual container. Therefore, cross-contamination is minimal, and this allows a high sampling rate, up to 150 samples per hour. Even higher sampling rates can be achieved in the so-called fast parallel analysers based on the transfer of samples and reagents by centrifugal force. This most interesting system, originally developed at the Oak Ridge National Laboratory[3], employs a rotor containing cavities to hold samples and reagents. By spinning at speeds of about 1200 rev min^{-1}, the solutions are mixed and transferred into set of fifteen cuvettes arranged radially; the cuvettes spin past the stationary beam of a light of a photometer and the absorbances are displayed as a series of peaks on an oscilloscope. To assist the transfer of liquids, a variable underpressure is applied at the rotor chamber. Thus, fifteen analyses are executed simultaneously, within 1–2 min, depending on the reaction rate, but the washing of

the rotor and pipetting of the samples and reagents into their cavities also take some time. Currently, at least three different manufacturers are marketing slightly different versions of this machine. The disadvantage of such machines, as well as of more conventional batch analysers, which contain moving belts to transport the sample containers, is the complexity of their moving parts, which eventually become worn during use and are expensive to manufacture. Yet, batch analysers find wide application, especially for one-component determinations in clinical laboratories.

Another type of system, the stop-flow analyser[4], is by concept and construction close to batch analysers. In this system, both sample and reagent are rapidly mixed by injection into a mixing chamber from which the reacting mixture is forced into an absorption cell. There the flow of solution is abruptly stopped and the absorbance of the idle solution is measured, before the cell is flushed out. Recently, these analysers have been successfully used in reaction-rate methods of analysis[5].

In the continuous flow analysers, the samples are successively aspirated from their individual containers into a tube through which they move until the whole analysis is completed. Thus the samples become a part of a continuously moving stream, into which, at predetermined points, reagents are added at fixed flow rates. The processed stream finally flows through the cell of a spectrophotometer (or another measuring device) where the quantitative measurement is executed and the signal continuously recorded. The movement of all liquids within the conduits of continuous flow analysers is controlled by a peristaltic pump which also takes care of aspiration of the samples. It is the versatility of continuous flow analysers which causes their widespread use: samples can be split for multiple analyses, and the flowing stream can be dialyzed, extracted, filtered, heated, decanted and even distilled. The greatest disadvantage of the continuous flow concept is that each sample is liable to contamination from the preceding sample; moreover, contamination increases as the rate of sampling is increased because of closer spacing of the samples. By alternate aspiration of sample solution and distilled water, cross-contamination can be suppressed, and in a simple module, sampling rates up to 10–15 samples per hour can be expected under laminar flow conditions. It was, however, the introduction of air-segmented streams by Skeggs[6] in 1957 which made continuous flow analysis practicable. Today, Autoanalysers, which are based on Skegg's idea and produced by Technicon, form an indispensable part of any clinical laboratory and find an increasing number of applications in all other types of routine laboratory. Over five thousand papers have been devoted to Autoanalyser techniques, but only about 1% of these deal with the theoretical aspects of the air-segmented, continuously moving stream.

Thiers *et al.*[7] were the first to make general observations on mutual sample interactions; their work, which was confirmed and extended by Walker *et al.*[8], remains the basis of quantitative considerations in determining the highest practicable sampling rate. To increase the sampling rate, which is usually around 60 samples per hour, two approaches have been used: (a) modification of the original Autoanalyser design; and (b) introduction of an appropriate correction factor[9] or even computer regeneration[10] of the recorded curve. The most significant changes in the design have been electronic rather than mechanical timing of the sampler, a rapidly moving sampling probe[11], a bubble-gating flow cell[12] or a computer-watched flow

cell through which the air bubbles are allowed to pass. These innovations, together with computerized curve regeneration[10], or even more recently rising curve slope computing techniques, lead to complex and inevitably more expensive instrumentation which may have only doubled or perhaps quadrupled output.

The purpose of the present work is to introduce a new concept of continuous flow analysis based on injecting the sample into a rapidly flowing carrier stream[13] which has not been segmented by air.

THEORY

The peaks recorded by a continuous flow analyser are made of rise and fall curves which represent the transition between different steady-state conditions[7,8] (A, B, Fig. 1). These curves are quantitatively described by two parameters, the half-wash time $W_{\frac{1}{2}}$ and the lag phase a. The half-wash time, which has been shown[8,14] to originate in non-segmented parts of the stream, describes the exponential part of the transition curve. The lag phase, which is said to be mainly due to air segmentation[14,15], precedes the exponential part. Thus, in the absence of lag phase, the rise curve is described by the equation $y=E(1-e^{-t/b})$, and the fall curve by the equation $y=Ee^{-t/b}$; here y is the absorbance at time t, E is the absorbance at the plateau (B, Fig. 1), and t is the time elapsed from the start of the rise curve or the fall curve. The half-wash time equals $0.69b$. In order to minimize irregularities in the sampling time, which are due to uneven pumping, different levels of sample liquid in the sampler cups, and slightly uneven timing of the sampler, 95% or better achievement of the plateau is used in AutoAnalyser methodologies. This obviously requires sampling times of 4.5 $W_{\frac{1}{2}}$ or $3b$. Consequently, with a typical lag phase of 20 s and a half-wash time of 10 s, a total of 65 s is needed to reach 95% of peaking. Thus

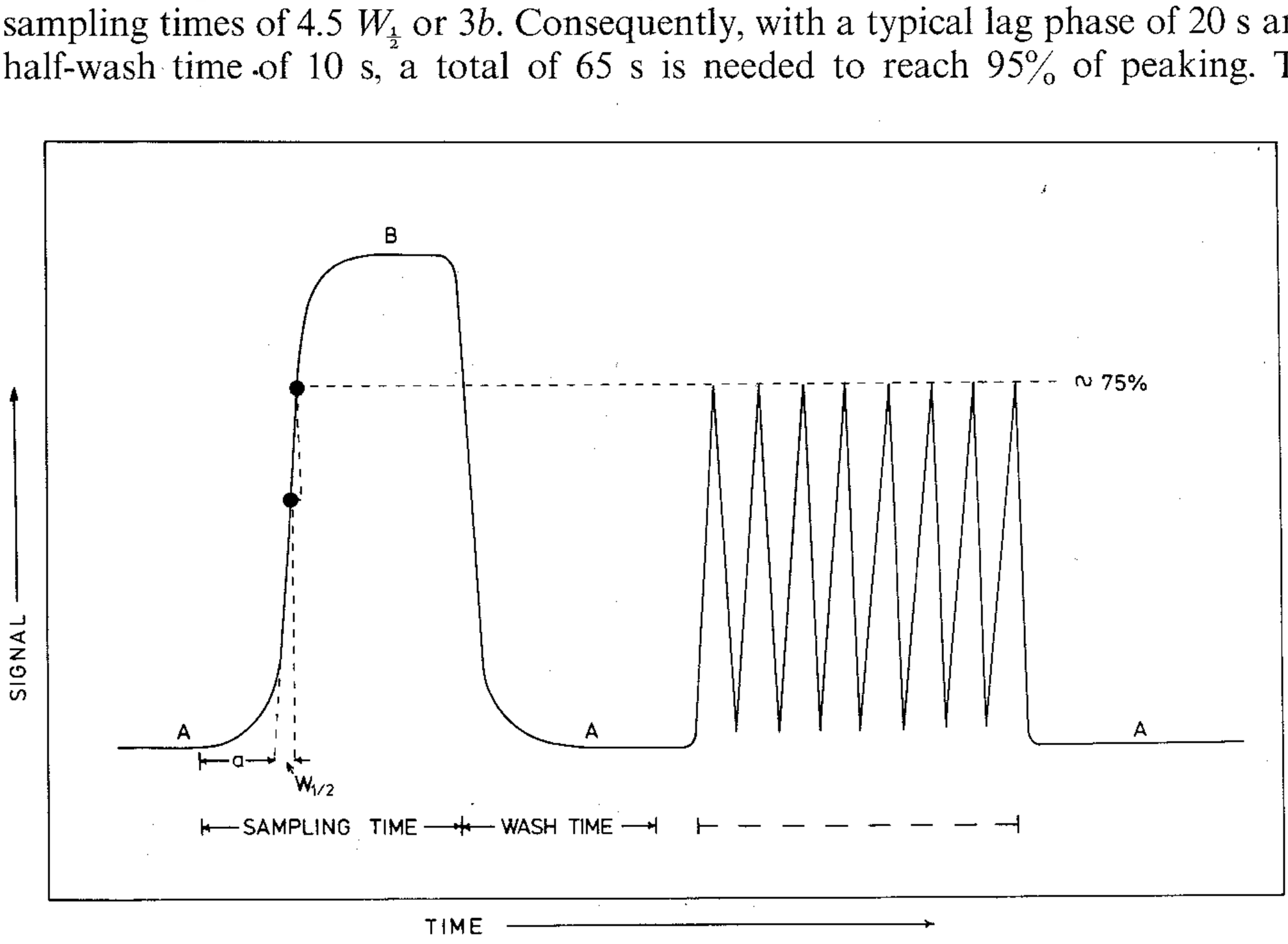

Fig. 1. Relation between the "steady-state" peak, as obtained in an AutoAnalyser, and a peak obtained by injecting the sample for two half-wash times. A and B are the steady states, a is the lag phase and $W_{\frac{1}{2}}$ is the half-wash time.

with a sampling/wash ratio of 2:1, 100 s are needed for one sampling cycle, which gives an analysis output of 40 samples/h. Higher sampling rates lead to significant carry-over, and unless some sort of correction factor can be worked out, the precision and accuracy of the determination deteriorate.

It is, however, known[7,8] that: (a) the rise and fall curves are almost identical reversals of each other; (b) the lag phase and half-wash time are independent of E; (c) any peak which does not reach E is the arithmetic mean of the rise and fall curve.

Therefore, provided that the Lambert–Beer law holds, the y value for any given time t is as good a measure of the concentration of the analyte as the final steady state reached. For optimal sensitivity and readability, y should be read at the top of a peak resulting from a partial rise and fall curve (Fig. 1). The peak height, rather than its area, would have analytical significance, provided that the timing of sample introduction is sufficiently reproducible. However, the timing must be the more precise, the shorter the sampling period, because the rate of change of the absorbance, $dy/dt = -b^{-1} E e^{-t/b}$, is faster at the beginning of the rise (and fall) curve. Provided that exact instant sampling can be achieved, sampling times of one or two $W_{\frac{1}{2}}$ should allow a substantial increase in the sampling rate (Fig. 1).

The beneficial effects of air segmentation have been so obvious that the necessity of introducing air bubbles was never really doubted, although the drawbacks of its presence in the flowing stream are well known: (a) because of the compressibility of air, the stream tends to pulsate rather than flow regularly; (b) streams have to be debubbled before they enter the flow cell or before repumping; (c) the size of the air bubbles has to be controlled for faster sampling rates; (d) the pressure drop—and flow velocities—vary in the presence of air for different tubing materials[16]. Although it might appear that the role of the bubble is to divide the stream into a number of slugs which then do not mix, the main function of the air segments is in fact to cause wall friction with resulting turbulent, rather than laminar flow even at low pumping velocities[17]. Yet it is known that at higher pumping rates, and with decreased diameters of tubing, sufficient turbulence will be produced to avoid the laminar flow which is responsible for carry-over. At conventional sampling rates, however, the consumption of the sample may exceed its availability, and the consumption of the reagents would become uneconomical. Instant discrete sampling, however, with five-fold higher rates of analysis would still give the same reagent consumption even at five-fold faster reagent flows.

It is therefore suggested here that a small volume of sample be injected rapidly into a turbulently flowing carrier stream of reagent. Thus, at the point of injection, the reagent solution would be pushed aside and in this manner a slug or zone of sample would be formed, which would then be further processed and carried into the flow cell of a detector. The feasibility of this idea would depend on the following factors: (a) reproducibility of the volume of the sample and of the speed of the injection; (b) a reproducible movement of the sample zone within the conduits of the analyser, and a reproducible degree of disturbance arising from travel and addition of further reagents downstream; and (c) sufficient and reproducible mixing of each sample with the reagent contained in the carrier stream, yet minimum carry-over between samples.

While the first requirement is not difficult to fulfil, as the piston-type devices deliver easily reproducible volumes, the last two requirements are, to some extent,

contradictory. Therefore, all the test experiments described here were devised so that they involved a chemical reaction between the carrier solution and the reagent stream.

EXPERIMENTAL

Sample injection

Samples were injected from 1-ml disposable plastic syringes (B-D-Plastipac, Ireland) supplied with a standard hypodermic needle (thickness 0.5 mm, length 10 mm). The sample solution (0.7–0.6 ml) was aspirated into the syringe, care being taken to avoid air bubbles, and then the piston was pressed so that its edge reached exactly the 0.5-ml mark. The actual injection was done manually, at maximum, but still convenient, rate. The early experiments were made with an injector which was made of a rubber tube (wall thickness 3 mm) situated in a perspex block, furnished with precisely bored holes, which allowed controlled piercing of the wall of the rubber tube with an hypodermic needle. As the tube had a tendency to bleed after multiple piercing, an injection block (Fig. 2) was made of two perspex blocks, screwed together by two screws (not shown) which passed through the top block freely and fitted a threaded hole in the bottom one; a silicone rubber disc (Beckman spare part no. 567026, for Gas Chromatograph 65) was squeezed between the blocks and served as a septum. The dimensions of the needle guide in the top block were chosen so that the orifice of the inserted hypodermic needle was situated in the carrier stream at the moment of injection. As the septum is compressed, and can be easily repositioned, several hundred injections can be made through one disc before the carrier liquid starts to bleed through or air leaks into the system.

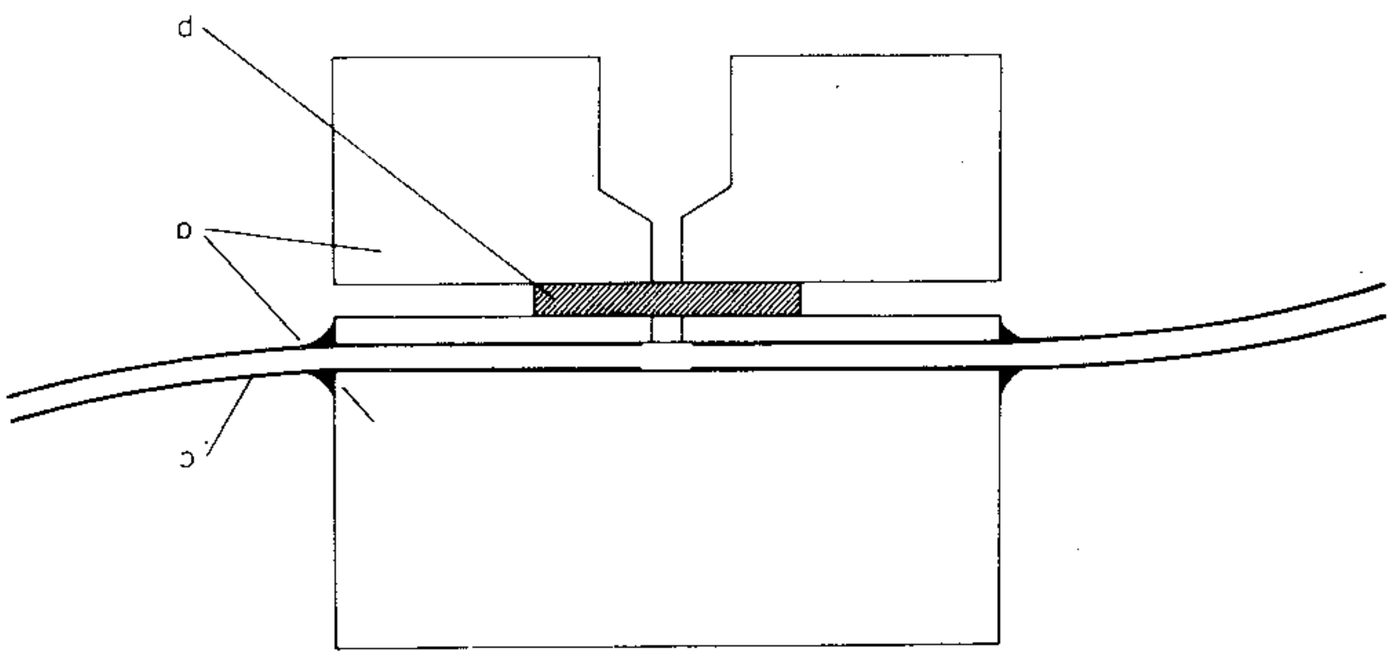

Fig. 2. Injection block. (a) Perspex blocks; (b) silicone rubber septum; (c) polyethylene tubing. (For details, see text).

Pumping system

The carrier stream was pumped at a rate of 18 ml min^{-1}, and additional reagents were added downstream, by peristaltic pump. Two types of pump were used: a standard Technicon AutoAnalyser pump Mark II (where several pumping tubes were joined to pump the carrier stream) and a Polymetron (CH-8634, Hombrechtikon, Switzerland) pump model 8511 (60 r.p.m.) in which 3-mm silicone rubber tube was used to pump the carrier stream. Unlike the Technicon pump, the Polymetron pump does not use rollers to squeeze the pump tube, but a series of

closely spaced plungers, located on the same crankshaft. As the plungers press the plastic tubing and do not pull it at the same time, the pump tubes withstand two months of full-time operation before they have to be renewed. Moreover, the absence of friction during pumping avoids any build-up of static electricity, which is undesirable when ion-selective electrodes are used as detectors.

Manifold and mixing coils

The manifold was made entirely from polyethylene tubing (diameter 1.5 mm, wall thickness *ca.* 0.25 mm). The pumping tubes of the Polymetron pump, which is supplied with 1.0, 2.0 and 3.0-mm internal diameter tubing (wall thickness *ca.* 2.5 mm), served not only as the pumping tube but also in short pieces as elastic connectors between mixing coils, T-fittings, flow cell inlets and polyethylene transmission tubings.

Mixing coils were made of the above polyethylene tubing by winding an appropriate length of it round a glass tube (length 10 cm, diameter 5 cm) and securing it at the ends with tape. T-pieces were made from perspex blocks ($3 \times 3 \times 1.5$ cm) into which T (or Y)-shape connections were drilled (1.9-mm bore). Short pieces of polyethylene tubing were glued into the holes by "araldite" so that their ends within the block left practically no dead space.

Flow-through cells

The flow-through cell for the spectrophotometer (Hellma type 178-QS) had a light path of 10 mm and a volume of 0.080 ml. The flow-through cell for the air-gap electrode[18] was made of perspex and was cylindrical in shape with an internal diameter of 2 cm and a height of 2 cm. The inlet to the cell situated close to its bottom was made as described for the T-piece, while the outlet (internal diameter 3 mm) was situated *ca.* 6 mm above the bottom on the opposite side of the cell. The air-gap electrode body served as a lid, and the electrode itself protruded from it so that its gas-sensitive surface was *ca.* 5 mm above the level of the streaming liquid. The level of the liquid in the cell was maintained by differential pumping, *i.e.* the input was kept at 10 ml min^{-1} and the output at 12 ml min^{-1}. Air was let into the cell by a vent (diameter 1.5 mm), the orifice of which was situated as close as possible to the cell outlet. To avoid condensation of water vapours on the electrode surface (which would eventually form a drop and thus cause a sluggish response), the electrode was always kept at least 2°C warmer than the solution passing through the cell.

Instrumentation

A Beckman Model DB or Model DB-GT Spectrophotometer was used. In both cases, a dual-beam mode was used, the carrier stream liquid serving as a blank in a 10-mm stationary cuvette. In the DB model, a mask with a 3-mm aperture had to be used in the reference beam to compensate for the small window of the flow cuvette, but in the DB-GT model this compensation was made electronically.

The pH meter used in conjunction with the air-gap electrode was a digital one (PHM 64, Radiometer) with a readibility of 0.001 pH.

The recorders used were the Servograph REA 310 (Radiometer), Beckman Ten Inch Laboratory Recorder (Model 1005) or Servogor RE 511 (Goertz, Austria). It is important that the full scale response of the recorder be less than 0.5 s, as

otherwise the high readings would be distorted. For this reason, the Servogor recorder was used only in potentiometric measurements, where the larger dead volume of the flow-through cell allowed the use of a slower responding recorder.

RESULTS AND DISCUSSION

Model system

A model system based on spectrophotometric determination of the red acidic form of methyl orange was chosen to investigate the reproducibility and maximum sampling rates achievable by Flow Injection Analysis. It was decided to inject the yellow basic form of this indicator into an acidic carrier stream, so that the streaming liquid not only provided the transport but also served as a reagent for colour development. Therefore, samples of methyl orange (25.0, 12.5, 6.25, 3.125 and $1.563 \cdot 10^{-4}$%) in $1 \cdot 10^{-3}$ *M* sodium hydroxide were injected into a carrier stream of 2.10^{-1} *M* hydrochloric acid. The volume of the samples was 0.50 ml, the carrier stream was pumped at a rate of 18 ml min^{-1}, and the wavelength of the spectrophotometer was adjusted to 510 nm. The total length of tubing between the injection point and the flow cell was approximately 2.5 m, of which *ca.* 1.5 m was the length of the mixing coil. The continuous recording of absorbance *versus* time is shown in Fig. 3; the calibration graph, obtained by plotting the height of the recorded peaks against the concentration of methyl orange in the samples, was rectilinear over the above-mentioned range. The maximum sampling rate that can be achieved with manual injection is seen in Fig. 4; this actually corresponding to an output of 270 samples per hour.

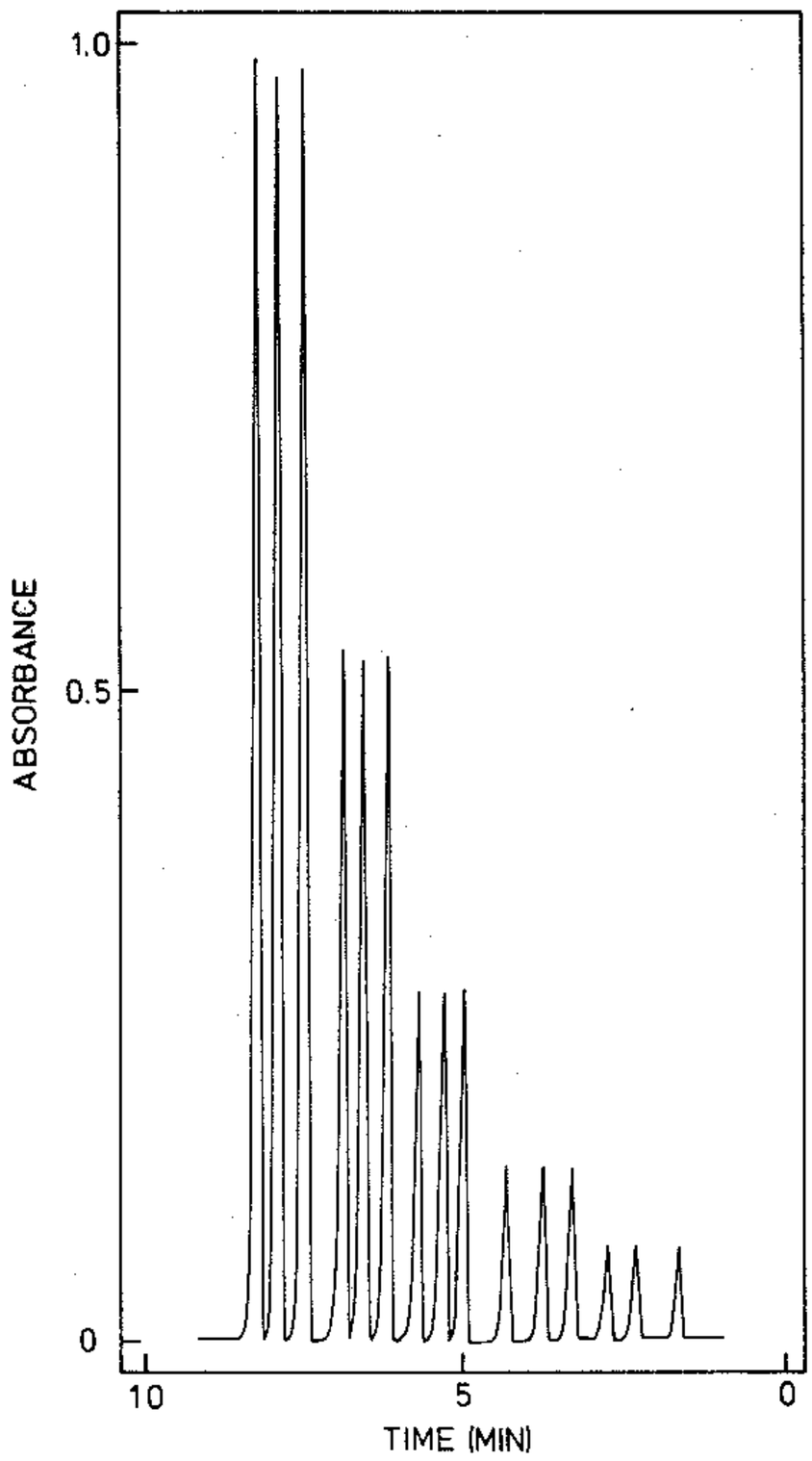

Fig. 3. Flow injection of methyl orange.

The results obtained on this model system confirmed that:

(a) the output of the instrument closely follows the Lambert–Beer law;

(b) manual injection allows a high precision, which in the case of measurement of the red form of methyl orange gave a regression coefficient of 0.9993 and a standard deviation of 0.05 absorbance unit, corresponding to *ca.* 1% in the concentration of methyl orange;

(c) there is no carry-over between samples even at a rate of 270 samples per hour as the signal still reaches the baseline.

As the change of the yellow colour to red was clearly visible, it was easy to follow the formation of the sample zone, the rate of the acid–base reaction and the transport of the sample within the system. At the point of injection, the carrier solution is pushed aside and the sample zone, during transport, changes colour from the sides towards the middle. Large volumes of sample and or too short a length of tubing, which may not give sufficient time for reaction or enough reagent to equilibrate across the full sample zone, yield a minimum, or valley, at the top of the peak. How-

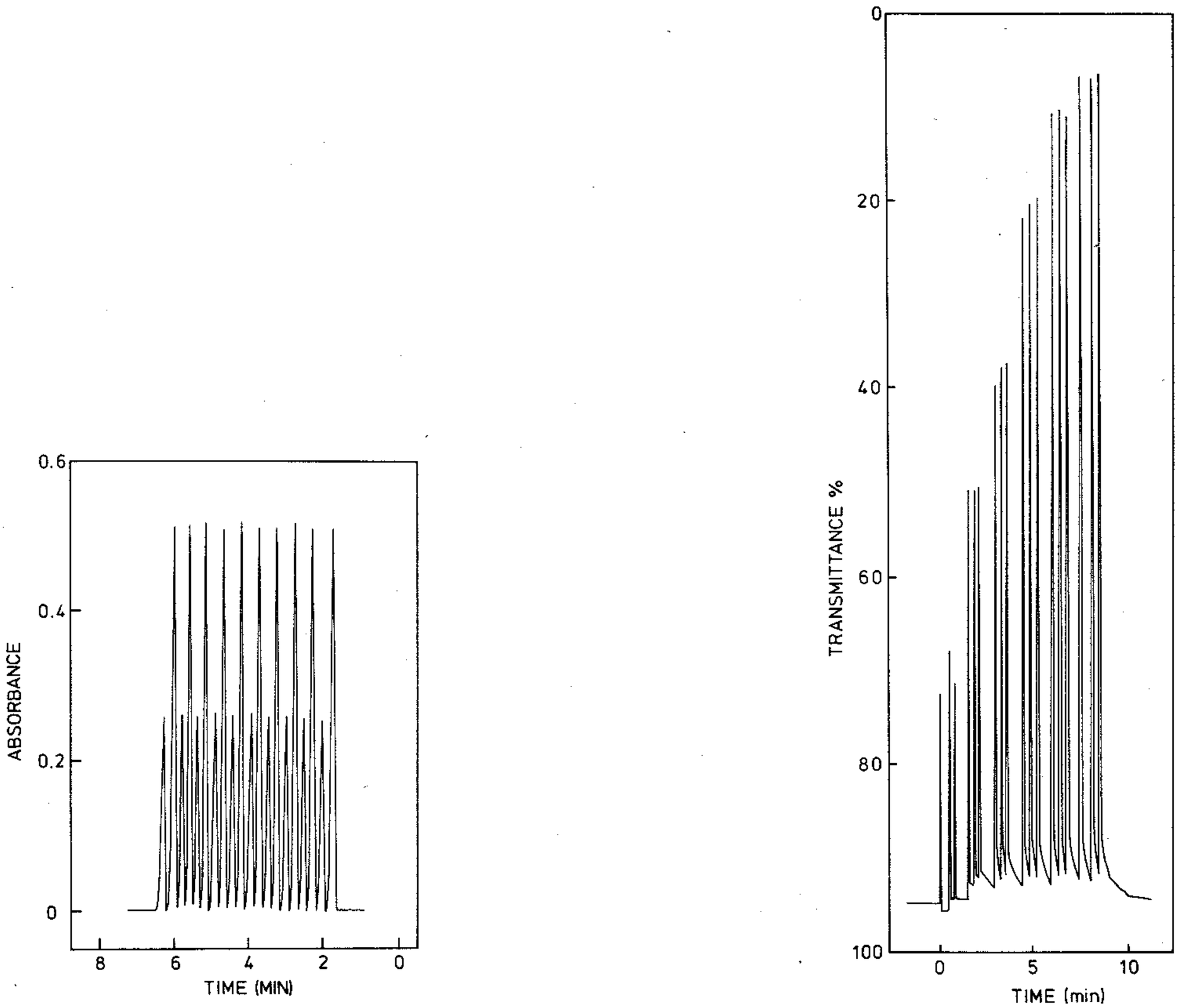

Fig. 4. Flow Injection of methyl orange at a rate of 285 samples per hour (6.25 and $12{,}50 \cdot 10^{-4}$% methyl orange samples).

Fig. 5. Flow Injection Analysis for phosphate by the molybdenum yellow method. Paper speed 0.2 inch min^{-1}. Sampling rate 120 samples h^{-1}. 5.0, 7.5, 10.0, 15.0, 20.0, 25.0 μg P ml^{-1}.

ever, it ought to be pointed out that in the model system, this valley formation occurred only at very extreme conditions.

Spectrophotometric determination of phosphate

The spectrophotometric determination of phosphate was based on the measurement of molybdenum yellow[19] as well as molybdenum blue[20]. This system was chosen because it could serve as a model for simple and fast colorimetric determinations (yellow method), as well as for a more complicated reaction which requires the use of additional reagent downstream and is said[21] to require a considerable reaction time (blue method).

The yellow phosphomolybdate was measured at 362 nm and the manifold was the same as that used in the methyl orange measurement. The carrier stream, pumped at the rate of 18 ml min^{-1}, was 0.005 *M* ammonium molybdate in 0.4 *M* nitric acid. The samples (0.50 ml) contained sodium orthophosphate in amounts of

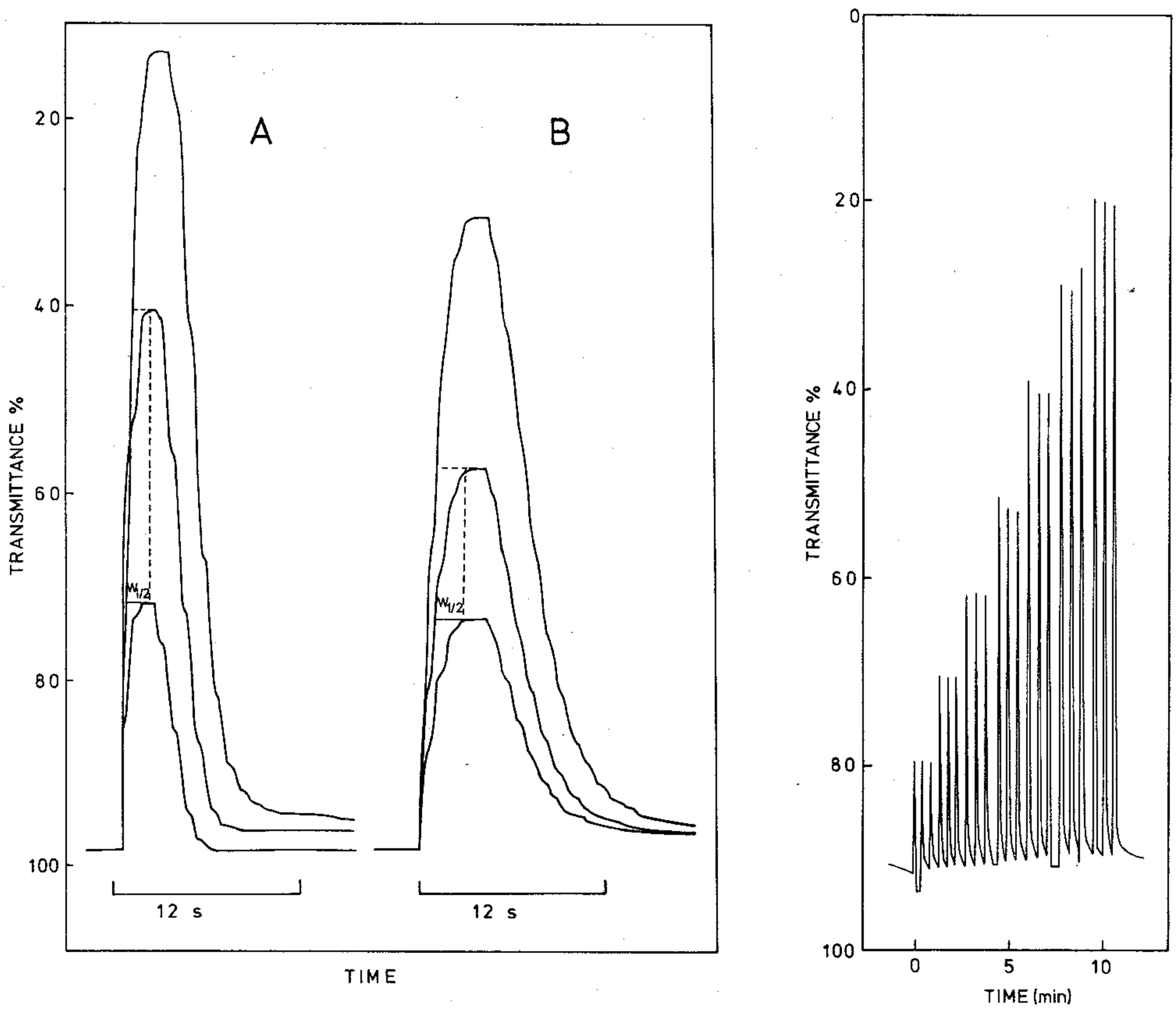

Fig. 6. Flow Injection Analysis for phosphate recorded at high paper speed (10 in. min^{-1}) for evaluation of flow parameters, 5.0, 10.0 and 20.0 μg of P. A. Molybdenum yellow method yielding $W_{\frac{1}{2}}$ of 1.5 s. B. Molybdenum blue method yielding $W_{\frac{1}{2}}$ of 1.9 s.

Fig. 7. Flow Injection Analysis for phosphate by the molybdenum blue method. Paper speed 0.2 in. min^{-1}. Sampling rate 120 samples h^{-1}. 2.5, 5.0, 7.5, 10.0, 15.0, 20.0, 25.0 μg P ml^{-1}.

5.0, 7.5, 10.0, 15.0, 20.0 and 30.0 μg P ml^{-1}. The continuous recording of the transmittance shown in Fig. 5 again demonstrates fair reproducibility and absence of carry-over even at high sampling rates; and when plotted as a calibration curve, $-\log T$ gave a rectilinear response with respect to the phosphate content ($r=0.998$). In order to evaluate the duration of lag phase and half-wash time, the peaks for 5, 10 and 20 μg of phosphorus were recorded at the fastest available (10 in min^{-1}) paper speed (Fig. 6A). The half-wash time estimated from the peak heights, corresponding to a doubled concentration of analyte, was 1.5 s while the lag phase was totally absent. The small undulations on the wave are due to irregularities in the pumping action.

The blue phosphomolybdate was measured at 660 nm and as this method involves the reduction of yellow phosphomolybdate, a reducing reagent had to be added downstream. Therefore another mixing coil (length 2.5 m) was added together with a T-piece. Here, the sample, injected into a carrier stream of 0.005 *M* ammonium molybdate–0.4 *M* nitric acid, passed through the first mixing coil (1.2 m) where the yellow phosphomolybdate was formed, and then an aqueous 1% solution of ascorbic acid was added at a rate of 1.5 ml min^{-1} through the T piece into the carrier stream, which further passed through the second (2.5 m) mixing coil into the detector. Surprisingly, the residence time of 6 s during which the reduction occurs in the second mixing coil was sufficient for colour development. Even more interesting was the observation that the substantial increase in the length of the tubing (more than 200%) and the addition of the second reagent downstream, did not create any lag phase and increased the half-wash time only by 0.3 s (Fig. 6B). The continuous record of the transmittance (Fig. 7) shows good reproducibility, and when plotted as a calibration graph gave a rectilinear response of $-\log T$ with respect to phosphate content ($r=0.998$). A study of flow injection analysis for phosphate in plant materials by the blue phosphomolybdate method showed that even with an average analysis rate of 200–250 samples per hour very precise measurements could be achieved (within 1–2%)[22].

In addition to the observations already made on the model system with methyl orange, which were confirmed by these phosphate determinations, it was learned that:

(a) the half-wash time is less than 2 s and the lag phase is negligible;

(b) neither the addition of a second reagent downstream nor additional tube length (of 2.5 m) significantly alters these parameters.

These results give reasonable grounds to suppose that the proposed system will find wide practical applications, as there are many spectrophotometric determinations which are fast and simple enough to be performed in similar manner, *i.e.*, by injecting the sample into a buffer and adding the second reagent downstream.

Potentiometric determination of ammonia

The potentiometric determination of ammonia was investigated as a model system with an ion-selective electrode as a detector. The carrier stream of 0.05 *M* sodium hydroxide was pumped at a rate of 10 ml min^{-1} through tubing with a total length of 1.5 m, two thirds of it made into a mixing coil. The aqueous samples of ammonium chloride (1.0, 5.0, and $10.0 \cdot 10^{-3}$ *M*) were injected in the usual way and were converted quantitatively into ammonia gas, which when trans-

ported into the flow cell, diffused across the air gap and caused an increase of pH at the gas-sensitive surface of the air-gap electrode[18]. The signal of this electrode, which is a special glass electrode in conjunction with a reference electrode, was continuously monitored by a pH meter and recorded. The recorded signal consisted of a series of peaks, each of which corresponded to one sample injection (Fig. 8). While the increase in pH was due to an increase in the ammonia partial pressure in the flow chamber, the fall part of the curve was caused by its decrease when the chamber was washed by the carrier stream and by the air which was entering the chamber by a side vent (see flow-cell). In this case, the digital readout at the top of a peak (readable to 0.001 pH unit) was used to obtain better precision. The resulting calibration curve had a regression coefficient of 0.99996 and a standard deviation of 0.0038 pH units, corresponding to a standard deviation of 0.9% in the concentration of ammonia.

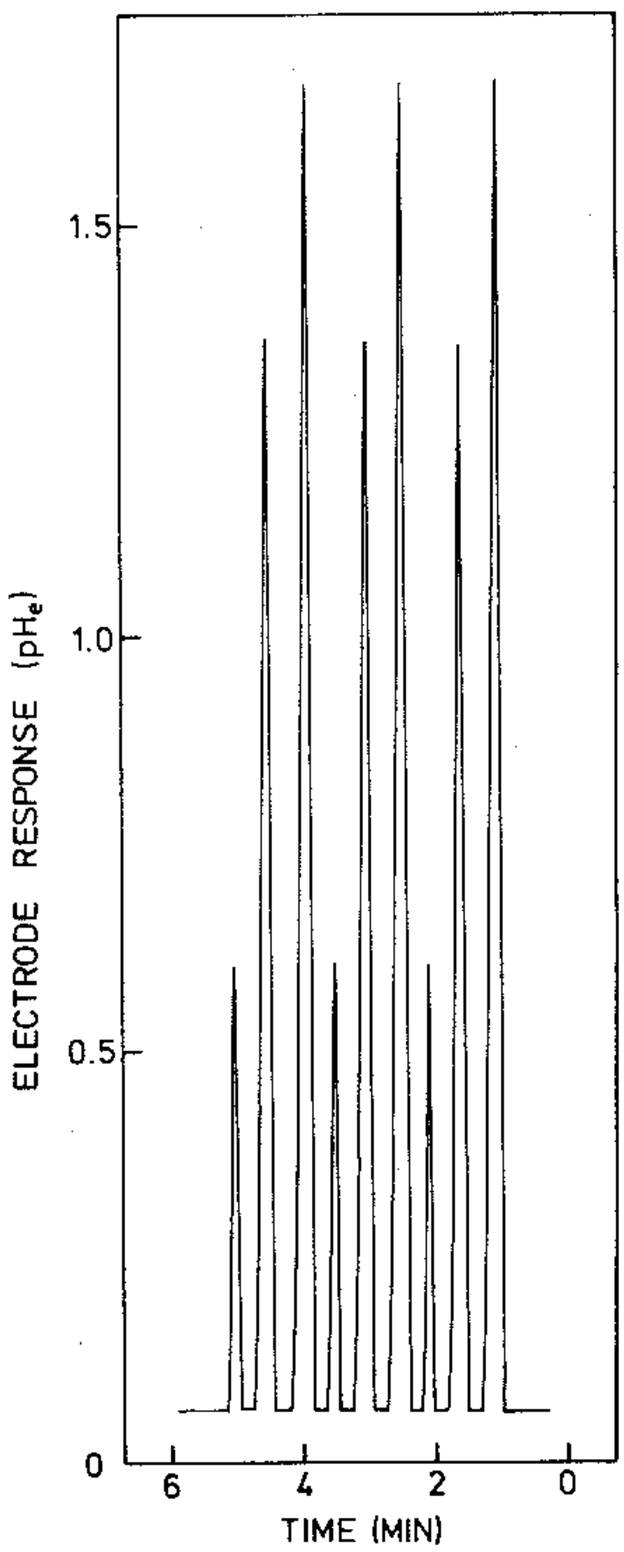

Fig. 8. Flow Injection Analysis for ammonia with the air-gap electrode as sensor ($1.0 \cdot 10^{-3}$, $5 \cdot 10^{-3}$ and $1 \cdot 10^{-2}$ *M* NH_4Cl).

These results confirm that a linear response with a Nernstian slope can be obtained in a Flow Injection Analyser furnished with a potentiometric sensor, and that the reproducibility of the determination can be as good as that obtained by a spectrophotometric measurement. The low sampling rate of *ca.* 60 samples per hour was due to a large dead volume of the flow cell (2.3 ml of solution and *ca.* 4 ml of air constituting the air gap), and to a slower pumping rate.

CONCLUSIONS

From the above results, it is evident that instant discrete sampling by injection into a carrier stream allows continuous analysis to be performed in a new, much simplified way. Because of the absence of lag phase, at a half-wash time of 2 s and a sample: wash ratio of 1:1.5, 360 samples per hour can be theoretically analysed (at 75% peaking). With manual injection, rates in the vicinity of 280 analyses per hour were actually achieved. The analyser is extremely simple to construct, and with a little practice even manual injection gives results with a standard deviation better than 1%. For larger numbers of analyses, however, the contruction of an automatic injector (similar to that used in stop-flow analysers[23]) must be undertaken to utilize the productivity of the system in full.

In contrast to the AutoAnalyser, where the sample and wash solutions are alternately aspirated, pumped, air-segmented and supplied with reagents, and thus form a continuously moving stream of air and solutions slugs, Flow Injection Analysis is based on the formation of well defined sample zones in a rapidly moving carrier stream of reagent. Because of the absence of air segmentation, the carrier stream of reagent flows more regularly as it is not compressible. Consequently, except for detector warm-up, the system is ready for instant operation immediately after the pump has been switched on. Thus, even a small number of samples can be conveniently assayed in a very short span of time in "computer-like" fashion with a good economy of reagent consumption. The new concept, and absence of air segmentation, allows the use of a simple manifold with fewer pumping tubes and easier programming.

The present stage of development leaves, however, a number of open questions. It is not yet known if the sample can be split for multiple analysis and it is doubtful that dialysis can be performed on the injected sample without distorting the flow parameters. The relationships between sampling rates, sample volumes and sensitivity as well as the influence of tube lengths and tube material wettability, has recently been investigated[22]. Obviously, over a certain length of tubing, the turbulent flow will become less effective than air segmentation, and will result in decreased peak height and loss of sensitivity. Thus it can be visualized that the continuous flow analysis will be eventually performed on:

(a) sample injected into a turbulently flowing stream,
(b) sample injected into an air-segmented stream, or
(c) sample aspirated and pumped into an air-segmented stream.

The last approach, represented by the AutoAnalyser design, will clearly remain useful for the more involved types of multiple analyses. The first possibility investigated and described here, may very well replace the AutoAnalyser method in numerous analyses involving fast reactions and the addition of one or two reagents downstream. Work is under way to test and adapt various analytical methods for this purpose. Injection of very small samples into the air-segmented stream remains an intriguing possibility.

The authors wish to express their thanks to Dr. J. W. B. Stewart, UNDP Expert, for discussion and reading of the manuscript; to the Centro de Energia Nuclear na Agricultura, Piracicaba, São Paulo, Brasil, for support in experiments

with phosphate analysis during J.R.'s leave of absence on U.N. Development Project BRA/71/556; to Polymetron A/S, Switzerland for a loan of the peristaltic pump; and to the Danish Natural Science Research Council for a partial financial support.

SUMMARY

The concept of a new continuous flow analyser system is described. Based on instant discrete sampling by injection into a carrier stream, the system allows continuous flow analysis to be performed in a fast, much simplified way. As the continuous flowing stream is characterized by a turbulent rather than a laminar flow, the discrete instant sampling creates geometrically well-defined segments of sample solution within the flowing stream. Because of the absence of lag phase, an unprecedented sampling rate for continuous flow analysis of well over 200 samples per hour can be achieved; and even manual injection of the samples allows a very high degree of accuracy and precision to be obtained ($\leqslant \pm 1\%$). Uses of the system in various analytical procedures are described and discussed. A potentiometric sensor (the air-gap electrode used in a flow-through unit) and a spectrophotometric arrangement with a flow-through cell have been used as detector units.

REFERENCES

1 J. T. van Gemert, *Talanta*, 20 (1973) 1045.
2 R. T. Knock, *Analytical Instruments, Stanford Research Institute, Report No. 447*, October 1971.
3 N. G. Anderson, *Anal. Biochem.*, 28 (1968) 207.
4 H. W. Malmstadt, E. A. Cordos and C. L. Delaney, *Anal. Chem.*, 44 (1972) 26A.
5 P. M. Beckwith and S. R. Crouch, *Anal. Chem.*, 44 (1972) 221.
6 L. T. Skeggs, *Amer. J. Clin. Pathol.*, 28 (1957) 311.
7 R. E. Thiers, R. R. Cole and W. J. Kirsch, *Clin. Chem.*, 13 (1967) 451.
8 W. H. C. Walker, C. A. Penncock and G. K. McGowan, *Clin. Chim. Acta*, 27 (1970) 1576.
9 H. S. Strickler, P. J. Stanchak and J. J. Maydak, *Anal. Chem.*, 42 (1970) 1576.
10 W. H. C. Walker, *Clin. Chim. Acta*, 32 (1971) 305.
11 R. E. Thiers, J. Bryan and K. Oglesby, *Clin. Chem.*, 12 (1966) 120.
12 R. L. Habig, B. W. Schlein, L. Walters and R. E. Thiers, *Clin. Chem.*, 15 (1963) 1047.
13 E. H. Hansen and J. Růžička, *Danish Pat. Appl. No. 4846/74.*
14 R. E. Thiers, A. H. Reed and K. Delander, *Clin. Chem.*, 17 (1971) 42.
15 R. D. Begg, *Anal. Chem.*, 44 (1972) 631.
16 A. L. Chaney, *Technicon Symp.*, 1 (1967) 115.
17 J. R. Gerke and A. Ferrari, *Technicon Symp.*, 1 (1967) 531.
18 E. H. Hansen and J. Růžička, *Anal. Chim. Acta*, 72 (1974) 353.
19 J. F. Kennedy and D. A. Weetman, *Anal. Chim. Acta*, 55 (1971) 448.
20 S. R. Dickman and R. H. Bray, *Ind. Eng. Chem., Anal. Ed.*, 12 (1940) 665.
21 D. F. Boltz and M. G. Mellon, *Ind. Eng. Chem. Anal. Ed.*, 19 (1947) 873.
22 J. Růžička and J. W. B. Stewart, *Anal. Chim. Acta*, in press.
23 H. W. Malmstadt and H. L. Pardue, *Anal. Chem.*, 34 (1962) 299.