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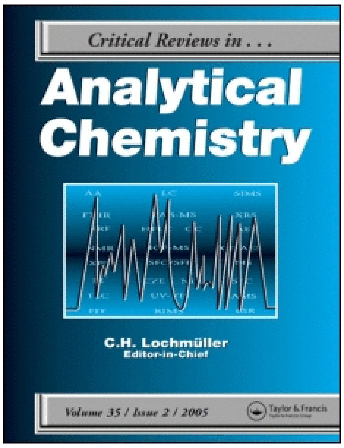

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AUTOMATIC ELECTROCHEMICAL ANALYSIS: PART 2

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V. AUTOMATION IN TITRIMETRIC ANALYSIS

A. Introduction

Titrimetry is the most commonly used procedure among quantitative analytical chemical methods. Automation in titrimetry started with the development of different instrumental end-point indication techniques. Potentiometric, amperometric, and conductimetric end-point indication provides a certain objectivity in following the course of titration and in many cases enables one to perform special analytical tasks, but at the same time the analysis becomes lengthy if the points of titration curves are drawn manually.

Elaboration of titrimetric methods using electroanalytical detection and the principle of titration to a predetermined point, i.e., the titration end-point, accelerated titrimetric analyses.

But at the same time, it is to be noted that from the point of view of reliability of titration results, the application of this idea, i.e., titration to the predetermined end-point, was a retrograde step. Namely, when using this method the indicator electrode is supposed to have a so-called long-term stability, and this cannot be expected for electroanalytical sensors in most cases. The error originated from the change of the indicator electrode characteristics is higher in voltametric measuring techniques than in potentiometric detection.

The second important step in automating titrimetric methods was the development of automatic reagent delivery in mechanisms and systems and their use in the practice of titrimetry. In constructing a titrator, probably one of the hardest realizable tasks is to design a sufficiently precise mechanism for reagent delivery. Therefore, several earlier researchers utilized coulometric reagent dispensers. Due to the rapid progress of the instrumental industry, however, the solution of the above problem became gradually simpler. Complete series of digitally controlled and programmable, highly precise dispensers are commercially available, either separately or as part of the commercially available titrators.

Reagent dispensers presently available are grouped as follows: volumetric and coulometric dispensers. The ways of the reagent addition, i.e., the types of dispensers used, divide titration methods, also, into two parts.

In the beginning, the automation of the coulometric titration procedure developed with a higher speed than that of volumetric titrations. This is due to the fact that the current intensity used for reagent generation can be regulated by relatively simple electronics.

From among the reagent dispensers elaborated for volumetric titration the following are well-known:

- Gravitation dispenser — its operating principle lies in using a magnetic valve to control the flow time of the reagent, where the reagent reservoir is suspended above the titration cell
- Drop-counting reagent dispenser—delivering and measuring the reagent in terms of drops of given reagent volume
- Plunger reagent dispenser
- Reagent dispenser operating with a peristaltic pump

The above reagent dispenser systems connected to the detecting units made possible the construction of "automatic titrators". Prior to the recent electronic revolution, several quite highly automated titrators were available.

B. Hardware-Controlled Titrators

In automatic titrators, frequently reagent dispensing is controlled in a simple way by using detector signal feedback. One of the simplest and most often used methods of hastening the titration is the anticipation principle. It is based on a preset anticipation detector signal level. The reagent is added at a relatively large rate, and when the measured signal reaches the preset level, the reagent addition rate is decreased. An increase of the titration rate is achieved in this way, without considerable loss of precision.

In another technique, the rate of reagent dispensing is controlled by using the first or second derivative of the detector signal.

A large number of titrators that record the whole titration curve are available. One of the most successful realizations is the equipment manufactured by Radiometer, which incorporates a Buret type ABU lb, a Titrator type TTT lc, and a Titrigraph type SBR C. Advantageous applicability is ensured by the fact that the potential from the detector cell, as well as an appropriately chosen linear potential-time function are interconnected to control the reagent dispensing.

The above mentioned first generation of "automatic titrators" will not be comprehensively discussed here. The monography of Phillips[84a] and Svehla[85a] give a detailed survey of them.

Digital computers, making possible a higher level of automation in titrimetry, first played a role in solving problems related to process titration curves.[86–88] They rendered possible the comparison of the precision of various end-point determination methods as well as the determination of the value of various physical-chemical constants. Using mathematical models, the differences between the real equivalence point and experimental end-points obtained by different indication methods could be calculated.

The effect of interfering components can also be calculated and taken into account using computer techniques.

Application of transformation methods (e.g., Gran-transformation[89] elaborated earlier for the evaluation of titration curves) has become significantly simpler with computers.

In everyday titrimetric practice, however, digital computers can only be used in a limited way for the case of manual data acquisition. A considerable step forward in improving automatic titrators was the combining of a digital computer with the titration system itself. Thus, "computerized" titrators have a common feature, namely the data of titration curves are available for digital data processing in a simple way. At the same time, different ways of collecting data pairs of the titration curve are known. Data characteristic of aspects of the signal part of the data pairs can be taken on different bases, e.g., following the signal level stability, the data are taken when the required stability is observed, averaging the detector signal measured in a given interval; or data can be taken at a given time instant after the reagent addition, etc.

Greater differences exist between titrators that control titrations by following the amount of reagent addition to generate the data pairs. Under constant (continuous or quantized) rate of reagent addition the time of dispensing always refers to the actual reagent quantity added. In the case of reagent dispensing controlled by a separate programming unit, the data pairs (signal and reagent volume) have to be appropriately combined.

When using a digital buret, the reagent volume added to the cell is calculated and stored by the computer.

C. Computer-Controlled Titrators

When the whole process of titration is computer controlled through an adequate program, the equipment is considered as the highest degree of automation in titrimetry today.

In this case the following functions of the titrators are usually controlled by a computer:

- The computer "observes" the change in the detector signal level and acquires information on reaching the steady-state within a given precision.
- The computer calculates the amount of reagent to be added in the next step.
- The computer commands the addition of the next reagent amount or refill of the buret.
- The computer stores and monitors the reagent amount added—detector signal data pairs.
- With appropriate calculations the computer searches for the equivalence and end-point, respectively.

Some of the publications dealing with the study of the properties and capability of titration systems developed, while others report on the routine use of titration and evaluation principles and methods known earlier but seldom used because of their tedious feature.

The examples to be mentioned are ranked according to the gradually increasing degree of automation.

In 1974 Kankare et al.[90] described a titrator, including a controlling unit constructed

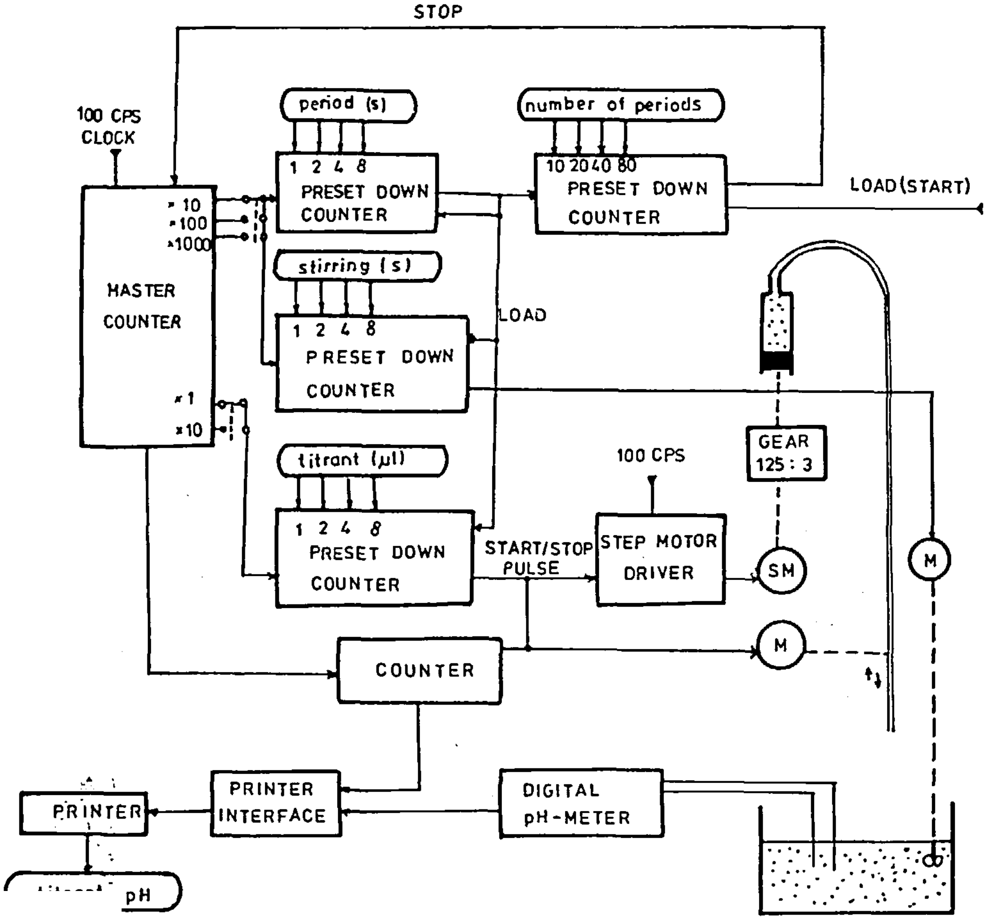

FIGURE 22. Block diagram of digital titrator developed by Kankare et al.[90]

from logic circuits, for the automatic recording of titration curves. The schematic design of the titrator can be seen in Figure 22. Operation of the titrator is controlled by a central master counter connected to a clock unit. Separate digital counter units serve to adjust and control the duration of the measurement, the number of the steps, the duration of reagent mixing in the measuring cell, and for the determination and adjustment of the reagent amount added in each portion by the step motor regulating buret.

In each cycle a motor-driven mechanism lets the tip of the buret dip into the solution mixture to be titrated. The digital pH meter and the unit counting the reagent dispensing steps are joined to a digital printer via an interface. According to a rigid program previously set on the counters, the titrator adds a reagent dose of given volume into the titrating cell. When the previously adjusted waiting and stirring time expire, the serial number of the added reagent dose, as well as the digitalized signal of the pH meter, are printed out. This cycle is repeated as set. After the titration has been completed, the buret is filled manually. The titration data are obtained in digitized form, printed on tape.

The instrument can be combined with a controllable current generator, thus, conveniently serving as a coulometric titrator.

Different types of potentiometric titrators attaining different degrees of automation were elaborated by Hieftje and co-workers.[91] A general functional block diagram of the above titrators is presented in Figure 23.

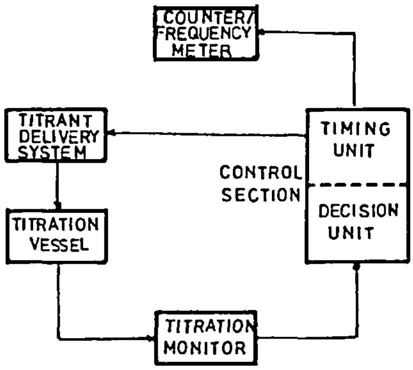

FIGURE 23. Functional block diagram of titrator developed by Hieftje et al.[91]

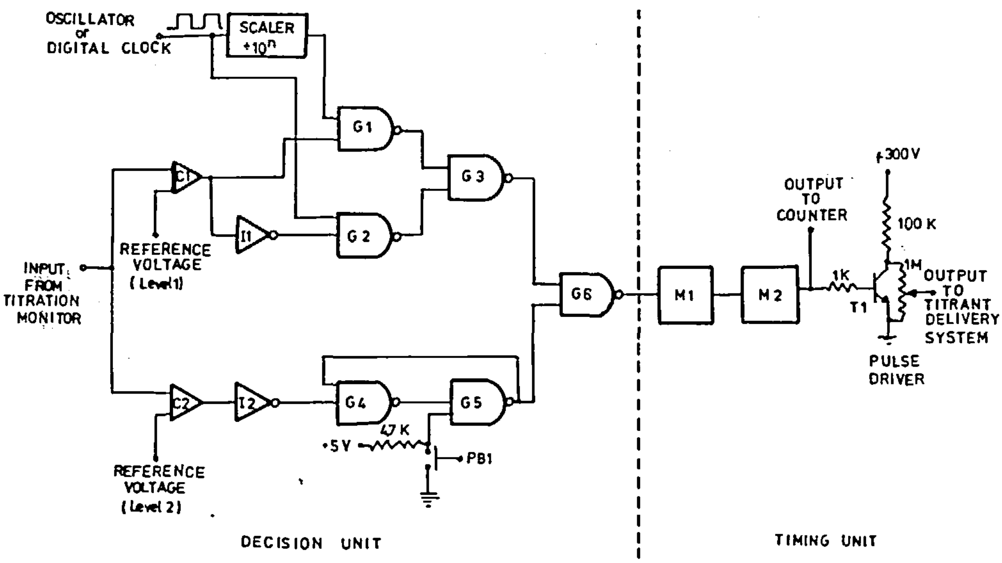

FIGURE 24. Schematic logic circuit diagram of the hardware controller, according to Hieftje et al.[91]

In each case reagent dispensing was done by the dispenser designed earlier by the authors. This unit provides uniform reagent drops with a rate controlled by digital pulses. The standard deviation of the drop size was less than 0.1%. In order to be able to study the properties of the reagent dispensing system, manual titrations with visual indication were also performed.

The progress of the titration is indicated with a titration monitor consisting of the appropriate indicator and reference electrodes, and an electrometer. In the case of the first type, the so-called hardware-controlled titrator, the rate of reagent dispensing is regulated by the output signal of the titration monitor. In addition to this, the logic circuit applied is convenient for indicating the end-point of the titration in that the potential signal given by the titration monitor is compared to a previously chosen reference signal (end-point potential) (Figure 24).

A computer (PDP-12/40)-controlled titrator (Figure 25) renders possible the application of more accurate end-point determination methods. Besides end-point titration

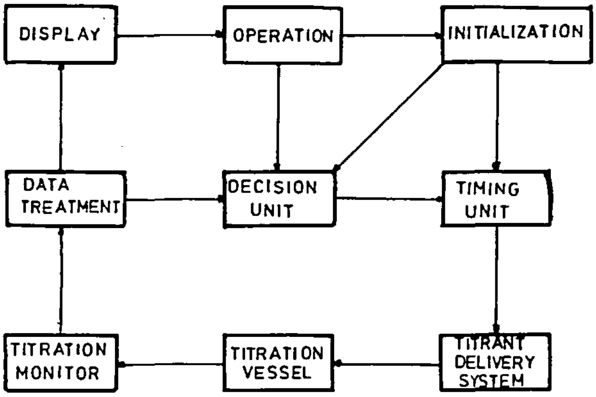

FIGURE 25. Organization of the software for the computer-controlled titrator, according to Hieftje et al.[91]

to a fixed potential and smoothing of titration curves, end-point determinations on the basis of the first and second derivative can be performed. Hieftje et al.[91] found titration curve evaluations based on the second derivative to be the most advantageous.

Another advantage of computer application is the possibility of recording the data of the titration on a magnetic tape, to make possible the subsequent application of various, more complicated evaluation methods.

Pel and Bartscher[92] suggest a digitally controlled titrator for amperometric analyses. The titrator is able to automatically titrate four samples simultaneously. A Metrohm® buret four delivery tubes Type E 412-20 loads reagent charges of 0.1 mℓ into the titration vessels. Prior to reagent delivery, the current intensities from the constant potential cells are measured with the help of a current-to-voltage converter and digital voltmeter. The serial number of the reagent doses and the potential signal value, which is proportional to the cell current, i.e., the data of the titration curve, are punched on the paper tape. The cell current is compared to two different signals with the help of two different comparators. When the value of the cell signal reaches that of the first reference signal, the titration rate falls back to a previously adjusted lower value (anticipation principle). If the reference signal is conveniently chosen, the rate of titrant dispensation can be decreased in the vicinity of the end-point, resulting in better accuracy. When the second reference signal is reached, a counter mechanism counts the reagent dispensing steps decreasing from 9. When 0 is reached the titration is finished. The last measured value is printed and so marked. The titration process can be shortened by indicating the approaching end-point. Recording of points past the end-point can be neglected, since they are not necessary for the evaluation.

The automatic titration system constructed by Bos[93] is a good example for systems using a computer of relatively high performance.

The scheme of the titrator is shown in Figure 26. For the control of the system, consisting of a Mettler® DV 11 automatic buret and appropriate titration cell and pH meter, a PDP-11/10 computer with 16-K core memory takes care.

The characteristics of the system is shown at titration of a mixture of strong and weak acids.

The computer does the following:

- It stores the calibration data of the measuring cell. From these it calculates the standard electrode potential, as well as the slope of the calibration curve of the electrode.

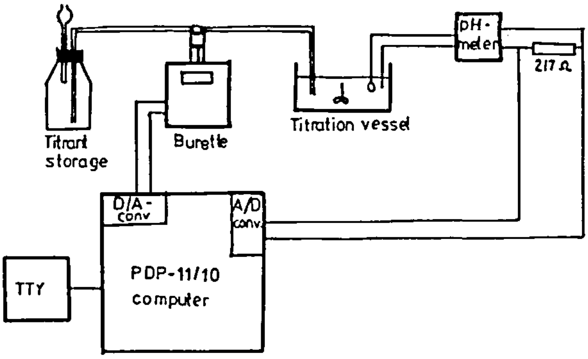

FIGURE 26. Schematic diagram of automatic titration system, according to Bos.[93]

The measured electrode potential data can directly be converted into ion concentration and ion activity data, respectively.

- It controls the titration process. Without any feedback it controls the addition of doses of given, previously determined reagent amounts, and after a certain waiting time the data pair (cell signal-reagent volume) is stored. This working cycle, the delivery of the reagent and data recording, is repeated as many times as needed.
- The collected data are processed off-line by the Wentworth multiparameter curve-fitting method.[94] The three parameters used for curve fitting based on the least-square method are as follows: concentration of the weak and strong acids as well as the dissociation equilibrium constant of the weak acid.

Experimental results proved the analytical applicability both of the technique and titration system. To record a titration curve of 50 points, 4 min are needed. The curve-fitting program operates while recording the next titration curve.

In the microcomputer-controlled potentiometric titration system constructed by Leggett[95] a feedback control is accomplished, i.e., each reagent dose to be added is calculated on the previously measured date.

The microcomputer built in the titrator keeps the following ratio between the volumes of the nth and n + 1 reagent portion to be added:

$$\frac{V_{n+1}}{V_n} = \frac{\Delta E_D}{\Delta E_n} \qquad (1)$$

where V_{n+1} is the reagent volume to be given as n + 1 portion; V_n is the prior reagent volume added; ΔE_n is the signal intensity difference observed as an effect of feeding in reagent volume V_n; ΔE_D is the optimum signal intensity change per portion; it is a constant.

The scheme of the titrator is shown in Figure 27 while the specifications of the units built in are listed in Table 1.

To measure the equilibrated signal level Leggett's device collects a given number of pH or mV data in subsequent 0.2 sec. Then these data are summed up and compared to a sum of data of identical number collected after a given, appropriately chosen time period. If the difference of the two sums is smaller than the previously chosen tolerance value, then the detector signal is accepted as equilibrated by the equipment. In other cases signal recording and observation are continued. If the signal representing the equili-

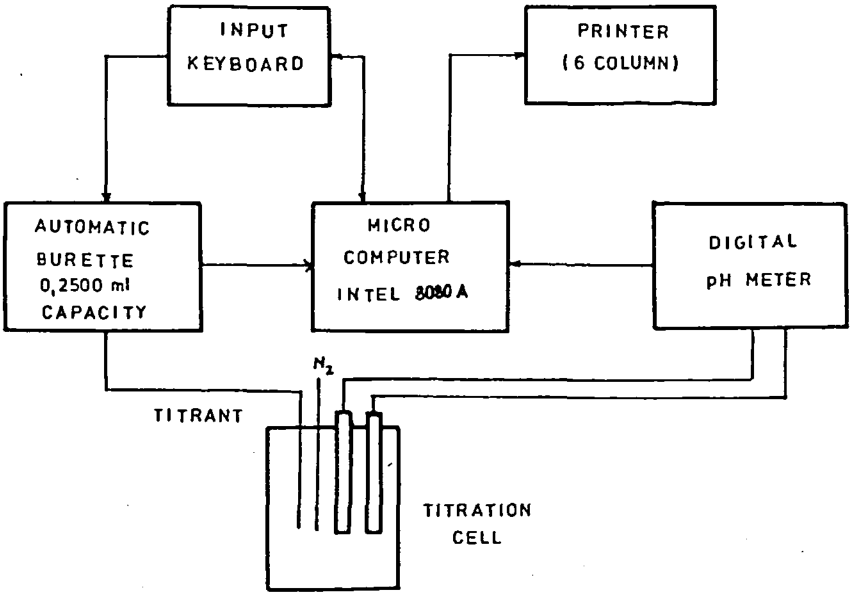

FIGURE 27. Block diagram of the titration system used by Leggett[95] showing the communication lines.

Table 1
INSTRUMENT COMPONENTS OF TITRATION SYSTEM[4,21]

Digital pH meter	Radiometer Model PHM64; accuracy ±0.002 pH; ±0.2 mV, ±0.05% of reading
Automatic buret	Radiometer Model ABU13; autoburette type B230; capacity 0.25 mL; accuracy ±0.25 $\mu L^{+2.0\%}_{-0.5\%}$ electronic counter with BCD output
Digital panel printer	Datel Systems, Inc. Mode. DPP-7
Microcomputer	Intel 8080A microprocessor; intel chips, RAMs and PROMS, SDK-80-B

brated state cannot be reached within a reasonable time — as it frequently happens in nonbuffered media of low ion activity on the curved sections of the calibration curve — the apparatus continues with the titration by feeding in the next reagent portion, simultaneously marking the uncertain values among the printed data pairs. This way the ill-defined sections of the titration curve are separated from the well-defined ones.

An identical level of automation is represented by the digital titrator developed by Christiansen et al. of Radiometer.[96] The titrator is built of the following units produced and marketed by Radiometer:

PRS 10	Alpha printer with built-in microcomputer
pH M 64	pH-Meter
ABU 13	Automatic buret
TTA 60	Titrator assembly
ATS 1	Autopipetting titration station

The intrument differs to some extent from the titration of Leggett mentioned above in the way of calculation of the reagent portion to be added and in the mode of data collection.

The calculation of the actual reagent volume ($V_{n+1,n}$) is carried out by the determination of the local slope of the titration curve with the help of the following empirical equation:

$$V_{n-1,n} = \frac{a}{\Delta_{n,n-1} + b\,(\Delta_{n,n-1} - \Delta_{n-1,n-2})} + c \qquad (2)$$

where $\Delta_{n,n-1}$ means the local slope, i.e.,

$$\Delta E_{n,n-1}/\Delta V_{n,n-1} = \Delta_{n,n-1} \qquad (3)$$

a,b,c are constants. Their actual values depend on the titration reaction and on the volume of the burets.

A rigid real-time program serves for checking the time stability of the detector signal level after each reagent addition. In this the computer takes the mean value of the detector signal intensities from 5 sampling points taken at an interval of 0.5 sec from each other, then compares this value with the next mean and establishes if the steady reading is already reached. It considers the signal in the interval as the average value of 10 measured points after reaching the steady-state. If after 45 sec no steady-state is detected, the mean of 10 signal intensities after this time has elapsed is considered as signal.

Increasing the analysis speed of microcomputer-controlled potentiometric titrators was tried by Yamaguchi and Kusuyama[97] in such a way that the titrator designed by them uses only a few points of the potentiometric titration curve for evaluation. For testing the measuring principle together with the operation of the apparatus, monobasic strong and weak acid sample solutions were titrated.

For the calculation of the titrant volume to be added, the computer roughly estimates the concentration of the sample solution on the basis of the electrode potential or pH value existing in the measuring cell.

For the calculation of the actual reagent volumes added ($V_1 \ldots V_n$), Yamaguchi and Kusuyama suggest the following:

$$V_1 = \frac{1}{2} V_{E_1} \qquad (4)$$

$$V_n = \frac{1}{2} (V_{E_n} - V_{n-1}) \qquad (5)$$

where $V_{E_1} \ldots V_{E_n}$ are the reagent volumes — estimated separately in every case — necessary for attaining the equivalence point. With the use of the titrator constructed and using a linearization technique for the data processing (see later on), one titrimetric measurement could be carried out in 22 sec.

As it was mentioned earlier, one of the most important advantages of the use of digital computers in automation of titration techniques lies in the simplicity of the data evaluation.

The computer plays a role in the improvement of the quality of the titration data (filtering, smoothing of titration curves), on one hand, and on the other hand it makes possible the routine application of different, quite complicated evaluation and calculation procedures.

A great number of publications deal with the off-line and on-line automatic evaluation of titration data, mostly dealing with potentiometric titrations. In the following, a few of them are mentioned as examples.

Koch and co-workers[98] employed an IBM® computer (Type 360/65) to obtain high precision end-point determinations of coulometric acid-base titrations. In this applica-

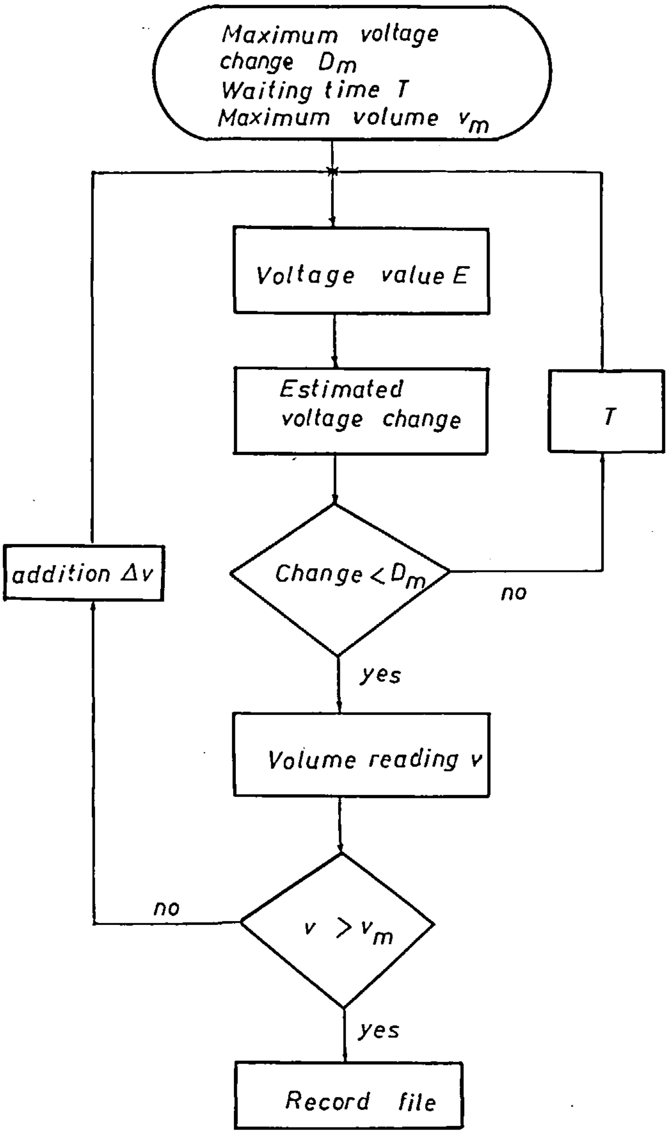

FIGURE 28. Flow chart of the data collection program for computerized potentiometric titrations, according to Nowogrocki et al.[102]

tion the computer served only for the automatic end-point location. The reagent was added with a constant rate via constant current generation according to a rigid program.

In the neighborhood of the equivalence point of the recorded titration curve, 14 data pairs of pH-reagent generation times were fed into the computer's memory, then two third-order equations were fitted to these data pairs by the computer program and the best fitting Q values were calculated.

$$\text{pH} = Q_1 + Q_2\,(\text{time}) + Q_3\,(\text{time})^2 + Q_4\,(\text{time})^3 \qquad (6)$$

$$\text{time} = Q_1 + Q_2\,(\text{pH}) + Q_3\,(\text{pH})^2 + Q_4\,(\text{pH})^3 \qquad (7)$$

The equivalence point is determined by searching for the zero value of the second derivative. The good agreement of data obtained from Equations 6 and 7 proves the applicability of the technique.

Anfält and Jagner[99] compared the most frequently applied evaluation methods by titrating fluoride with thorium nitrate. The theoretical shape of the titration curve was calculated with a computer taking into consideration the equilibria existing in the solution (Program HALTAFALL). The evaluation techniques compared can be divided into three groups:

1. Methods utilizing the sigmoid shape of the potentiometric titration curve
2. Differentiation procedures
3. Techniques based on mathematical models considering the mass balance and equilibria

Methods belonging to the first group (maximum slope, Tubb's method[100] Kohn-Zitko method[101]) have a systematic error of 3 to 4% and accuracy of ±0.3 to ±0.9%.

The systematic error of differentiation methods, especially in evaluation using curves of $\Delta V/\Delta E$ values, is lower compared to the first group.

The lowest systematic error and the highest accuracy characterize the techniques of the third group (titration to previously adjusted potential, Gran-evaluation, multiparameter refinement methods).

The theoretically most correct technique is the multiparameter refinement method, whose application requires a computer of highest capacity.

If the inflection point and the equivalence point don't coincide, or the inflection point is ill-defined, then the location of the equivalence point can be done with different mathematical procedures.

The paper of Nowogrocki et al.[102] deals, first of all, with the automatic evaluation of titration curves having ill-defined inflection points. With the help of a laboratory computer of 16 Kbyte, both the whole titration process and data processing can be performed automatically. In the beginning of the titration process the maximum sample volume which can be added, the waiting time for the equilibration of the potential, and the highest potential change permitted during this time, are entered.

The flow diagram of data acquisition is presented in Figure 28.

As long as the maximum reagent amount to be charged is reached, the titrant is added in equal portions and data reading is continued.

The appropriate data pairs (v, E) are stored on a magnetic tape and processed as follows:

$$v_c = v_o \left\{ H_i^o + \sum_{i=1}^{D} C_i^o (N_i - \bar{n}_{Hi}) - [H] + K_w [H]^{-1} \right\} / \tag{8}$$

$$\left\{ -H_x^o - \sum_{i=1}^{B} C_x^o (N_x - \bar{n}_{Hx}) + [H] - K_w [H]^{-1} \right\} \tag{9}$$

and

$$-\log [H] = E_o + E_{ja} [H] + E_{jb} K_w [H]^{-1} + SE$$

where* H_i^o H_x^o = the concentrations of the strong acid (initial and charged, respectively); C_i^o, C_x^o = the concentrations of the weak acid (initial and charged, respectively); D = the

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*Ionic charges have been omitted for clarity.

initial species; B = the added species; N_i, N_x = number of bonded protons in the initial and added component, respectively; $\bar{n}_{Hi}$ and $\bar{n}_{Hx}$ = acidity constants; K_w = ion product of water; V_c = reagent volume added; V_o = initial reagent volume; E_o = standard potential; S = slope of the calibration curve of the electrode; E_{ja} and E_{jb} = liquid junction coefficients.

The unknown parameters in these equations were determined by a least-square method, by minimizing the sum

$$\sum_{n=1}^{N} W_n (v_n - v_{nc})^2 \qquad (10)$$

where n = index number of reagent addition; N = number of experimental points; W_n = weighting factor correcting the error of potential and volumetric measurement; v_n = experimental value of the added volume for the nth point; v_{nc} = calculated value of the added volume for the nth point.

The above correlations hold for all kinds of acid-base titrations. Unknown parameters can be determined by approximation. The authors demonstrated the applicability of the technique at the determination of the purity of sodium-potassium-tartarate, as well as the composition of difunctional acids. (In the first case the titration curve does not contain an inflection point, while in the second case one single inflection point can be found on the titration curve.)

Yamaguchi and Kusuyama,[97] in their earlier mentioned work, determine the equivalence point on the basis of a few points of a titration curve using Johansson's[86] linearization method:

$$V_e - V = \frac{V_o + V}{C_b}[H] \qquad (11)$$

for strong acids and

$$V_e - V = V[H] + \frac{V_o + V}{C_b}[H]^2 \qquad (12)$$

for weak acids, where V_o = initial volume of sample solution, V = volume of titrant added, C_b = concentration of titrant, and V_e = equivalence volume. The line constructed from the data pairs, with the help of the least-squares method, gives V_e. The authors of this work consider it sufficient to use only 4 points before the equivalence point for evaluation.

There is a certain similarity between the potentiometric multiple standard addition and subtraction method and potentiometric titrations; both techniques can be used with the same hardware. In addition to this, some evaluation methods show similarity in the two measuring techniques. Some devices used in potentiometry can advantageously be employed as titrator, just like the different evaluation methods of potentiometry are of use in the determination of a titration equivalence point. For further c
works of Frazer and Selig.[103-105]

D. Microprocessor-Controlled Titrators

Microprocessors have been used more and more frequently in the last decades, surplanting larger ones for many instruments, and have proved to be capable for the solution of tasks in automatic titrations as well.

Microprocessor-aided instruments can be considered as a new group of digital titrators. A great part of commercial, highly automated digital titrators belong to this

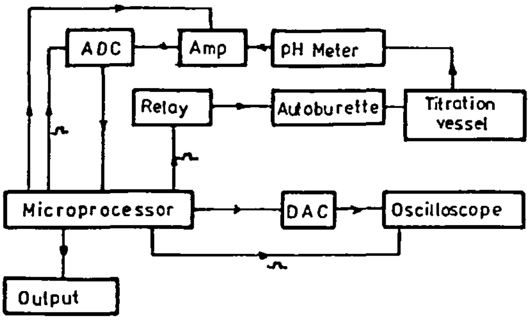


FIGURE 29. Microprocessor-controlled automatic titrator developed by Betteridge et al.[106]

group. One of our examples to be shown is a microprocessor-controlled system constructed in a research laboratory, while the others are commercially available titrators.

Betteridge et al.,[106] in connection with a practical problem, developed a microprocessor-controlled automatic titrator for the determination of the concentration of adipic and boric acid, used in the production of nylon (Figure 29). The instrument is capable of the recording of the total titration curve. During titration, the titrator adds reagent portions in a previously determined number (150) and preset volume (0.1 mℓ) to the cell. The addition of the reagent, which occurs with a Radiometer autoburet (Type ABU 13), is controlled by the microprocessor with a read relay switch, according to the program. The time lapse between the individual reagent dose additions is constant in the course of the titration.

After adding the reagent and waiting for a certain time to elapse (this time can be changed in the program), the cell potential values are entered into the memory of the microcomputer after passing through the pH-meter, amplifier, and AD converter.

The determination of the end-point of the titration is calculated by various methods. The authors found that the Gran-transformation and the application of similar functions does not provide sufficiently accurate results. Best results were obtained by plotting the differences of the neighboring electrode potential values as a function of reagent volume added, and by searching for its maximum. For obtaining accurate results, the curves had to be smoothed. The authors tried three different smoothing techniques (running average of three or five, median smoothing, Hanning smoothing) and found the differential titration curve obtained by the running average of three as the most noiseless.

The hardware applied consisted of the following units: AD converter connected to an amplifier, a relay switch operated by the microprocessor for portioning of the titrant, and a DAC unit for the decoding of data obtained and stored by the microprocessor. These analog signals can be displayed on an oscilloscope or on a recorder.

The microcomputer assembled for this special purpose consists of a microprocessor (Intel 8080), a read-only memory of 1-K words, a read-and-write memory of 2-K words, and a digital input-output unit. The main functions of the software are the following: data collection, derivation and curve smoothing, data display, peak detection, interpolation (determination of the maximum slope of the titration curve by calculation) and display of the results.

By carrying out titrations under different experimental conditions, the authors have chosen the most suitable conditions for the measurement (range of ADC, stirring rate, time interval between an addition of titrant and digitizing voltage). Under optimum conditions, they could determine the concentration of adipic acid with an error of about

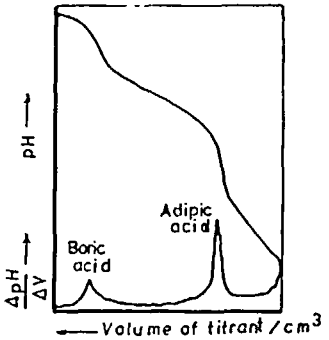


FIGURE 30. Titration curve obtained by titrating a mixture of adipic and boric acids, according to Betteridge et al.[106]

0.2%, and that of boric acid with an error of 0.1%, this latter without the addition of mannite. The time needed for one analysis is 1 to 15 min, depending on the time needed to reach constant potential in an interval. Results obtained by automatic and manual methods agreed excellently. A characteristic titration curve is shown in Figure 30.

Using commercially available units, Martin and Freiser[107] built a low-price potentiometric analysis system. The total cost of the computer hardware with all the input-output cards and interfacing was about $2400 (1979). The computer was a IMSAI 8080 microcomputer kit equipped with 24 K of static memory. A new, high level computer language, CONVERS, was employed in programming, which provided great flexibility. The capability of the analysis system was experimentally studied in both titrimetry and direct potentiometry. The potentiometric titration program worked out used routines similar to that described by Christiansen et al.[96] to calculate the desired titrant increment volume and to locate the end-point.

The highly developed, commercial automatic titrator Methrom® Titroprocessor F636 is controlled by a microprocessor. The preparation of the titration program to be carried out is executed on program cards. The cards are marked with a pencil by the experimenter. When an empty card is used the microprocessor orders the titration to be executed according to the basic program. The instrument can be readily interfaced with an automatic balance or sample handling apparatus. Results of measurements are automatically evaluated. In cases of acid-base titrations it can distinguish between inflection and equivalence points. The instrument provides the titration curve, the reagent volume corresponding to the end-point, calculated concentrations, and conditions of the titration. The titrations are carried out in beaker-type cells containing the pH-sensitive glass electrode.

The Radiometer firm has a tradition of producing automatic titrators. At present the commercial titrators with different grades of automation consist of modules. From these, besides the relatively simple end-point titrator (ETS 822) and the recording potentiometric titrator capable of recording the whole titration curve (RTS 822), a fully automated microcomputer containing a digital titrator (DTS 833) can also be assembled.

The Digital Titrator System DTS 834 also contains a mechanical unit for handling 55 samples. It stores the samples and brings one after the other into the titration vessel. Different types of automatic titrators can be also built of modules made by Mettler®. For example, the automatic titration system SR 10 is constructed of the following units:

- DV 11 Buret drive
- DK 11 Rate and end-point control
- DK 14 Electrode potential amplifier
- DV 103 Command module

VI. AUTOMATION WITH FLOW-THROUGH ANALYSIS CHANNEL

Flow-through analysis has opened an extremely effective field of automation.

For flow-through analysis a new type of mechanization became possible. In discrete analyzers generally the automation of the individual steps of classical analysis is performed, while in flow systems the process of analysis can be essentially different owing to significant simplification of the mechanisms involved. This is mostly due to the simpler possibilities for moving a fluid medium than handling discrete solution volumes. In engineering terms, the flow-through analysis is analogous to continuous operation while the others are batch types.

In devices applying the flow-through principle the steps of analysis are performed in succession in a flow-through channel. For detection flow-through detectors — mostly in a continuous working mode — are used. The sample, together with reagents, pass through the analysis channel via the same route. Individual steps of complicated processes can be carried out in different sections of the flow-through channel. The uniform sample handling, which eliminates the subjective errors in the analysis, often ensures excellent reproducibility. By precise selection of the geometry of the flow channel and flow rate, the time necessary for the elementary processes of analysis can be easily controlled; there is no problem in ensuring identical reaction times. By varying the hydrodynamic parameters, the ratio of the sample and reagents can be increased or decreased.

Flow-through techniques were used earlier, usually in reaction kinetic investigations. The precisely controlled reaction times and the simple methods of ensuring uniform reaction conditions make it possible to determine concentrations not only in fast reactions but also in so-called analytical reactions that provide stable reaction products.

The application of flow-through principle requires a more or less new approach as compared with the classical one. It means that the appropriate analysis time can be achieved by the correct design of the analysis channel and/or by the appropriate selection of the flow rate of the sample and reagent solutions. Moreover, in flow systems not only the sample and reagent concentrations and volumes but also the mass-flow have a definitive effect on the analytical signal. Accordingly, e.g., the extent of chemical reaction between the sample and reagent is realized between the two mass-flows.

The compatibility of the flow-through principle and the properties of the electroanalytical detectors is an important question. Two problems should be studied in this respect: the behavior of the electroanalytical detectors under flow-through conditions, on the one hand, and the conditions needed for their incorporation into flow systems on the other hand.

With respect to the behavior, electroanalytical detectors are especially well-suited for use in flow-through conditions. An important advantage is, for example, that the chemical and mechanical interfering effects on the electrodes during operation, i.e., washing, cleaning, changing of solutions, etc. are significantly smaller in flow systems as compared to classical stationary measuring conditions.

In flowing solutions the compounds formed at the electrode-solution interface and the material getting into the solution from the electrode are physically removed from the volume around the electrodes and, thus, do not have any disturbing effect. Another advantage is that, contrary to classical situations, convection if flow systems advantageously affects the response time of electroanalytical sensors. Under flow-through conditions the detection limit of the electroanalytical detectors is better than in classical systems.

Lastly, in flow-through systems the reference electrode can be placed generally downstream, thus eliminating the contaminating effect of salt bridge electrolyte and ensuring a stable continuously renewing sample-reference electrolyte interface. The latter is especially important in potentiometric measurements.

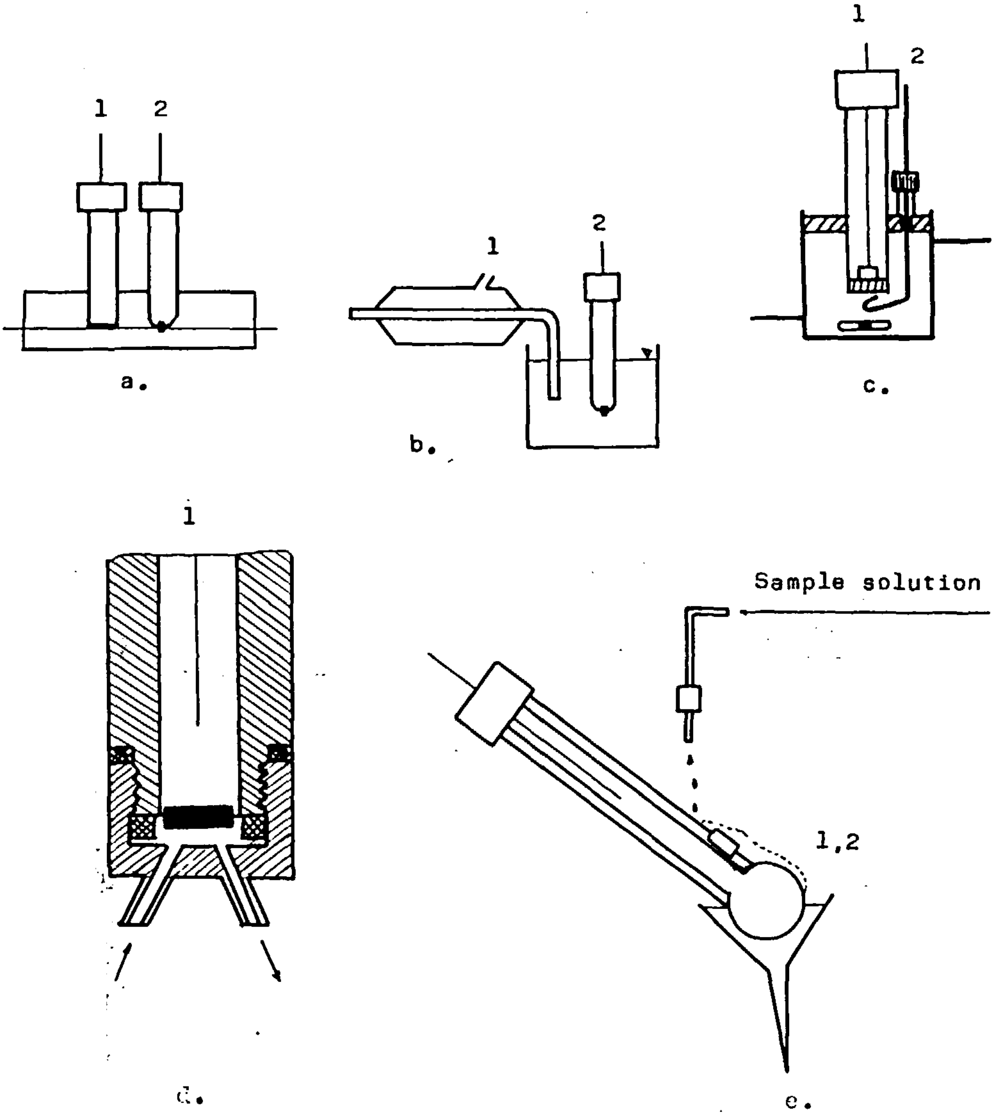


FIGURE 31. Electroanalytical methods adaptable to continuous flow analysis, according to Snyder et al.[111]

Besides considering the advantages of the analysis in flow-through systems with electroanalytical detectors, some new aspects must also be considered. Namely, the signals of electroanalytical detectors placed in flowing solutions (e.g., voltametric detectors) can depend on the flow rate of the solution. This drawback is not very serious in analytical systems, due to the usual constant flow conditions needed in most cases. The solution carrying pulsating pumps generally used in flow-through instruments generate oscillating signals. Several methods are known for decreasing or eliminating this phenomenon. Automated data processing computerized procedures can be used for filtering out this periodic noise. Besides, electroanalytical detectors that have relatively large surface area, noisy signals may be obtained due to local differences in the concentration at the electrode surface.

For possible design and several placements of flow-through detectors, the reader is referred to Figure 31.[108]

Automation of flow-through methods will be surveyed below in the following order:

1. The automatic proportional analytical systems (CFA) based on air segmentation will be discussed first.
2. Thereafter, the so-called flow-injection technique (FIA) will be treated.
3. Some special automated techniques including the standard addition and subtraction ones, and devices for measuring concentrations will be dealt with in a separate paragraph.
4. A further part will be devoted to the more-or-less automated titrations in flow-through systems.

A. Continuous Analyzers with Air Segmentation

The most important step in advancing the flow-through principle in mechanized chemical analysis was made by Skeggs.[109] The development of different types of AutoAnalyzer systems by Technicon® was based on his concept. The continuous flow analysis (CFA) has the following properties in addition to the general features characterizing the flow-through systems:

1. The measurements are generally based on chemical reactions; different reagents are mixed with the sample in an appropriate ratio, and most often the concentration of a colored product formed in the reaction is followed. For the evaluation of the analytical concentration the extent of the chemical reaction is not necessarily considered.
2. The determination of concentrations is based on a steady-state detector signal — that means that by the selection of the sample and reagent streams, the time of reaction, and the dispersion, a condition is produced where the detector signal is constant for a certain time. An important advantage of the evaluation process is that the signal intensity vs. time has a plateau, that is, the signal in the plateau region is an average value.
3. In the analysis channel the flowing solution is segmented by air or gas bubbles. By suitable timing of the bubbles — which is the invention of Skeggs — several serious problems can be overcome: the separation of subsequent samples is more complete; a higher specific speed of analysis in ensured; the carry-over is minimized; the mixing efficiency of the sample and reagents streams becomes satisfactorily intensive; and stable flow conditions are maintained.
4. The AutoAnalyzer systems most often used in analytical practice are built of modules. In all of the systems suitable for solving different analytical tasks, there is a sampler for the storage and moving of the samples and a peristaltic pump. The design of the analytical unit depends on the type of chemical reaction applied for the determination. Figure 32 shows a general scheme.

Usually visible photometers or colorimeters are used as detectors. For the display of the data a recorder and printer are connected to the detector in the simplest case.

In the 20 or more years ensuing since the first AutoAnalyzer appeared, the design of the instrument has significantly changed. Today the third generation of this type (SMAC) is on the market.

For the complete automation (mechanization) of the different analytical processes, units for sample preparation were developed (see also earlier) and connected to the analytical unit. Such are, e.g., the SolidPrep unit for the preparation of solid samples (grinding, dissolving), the continuous filtering unit, and the digestion unit.

Considerable efforts have been made for the mechanization and automation of the

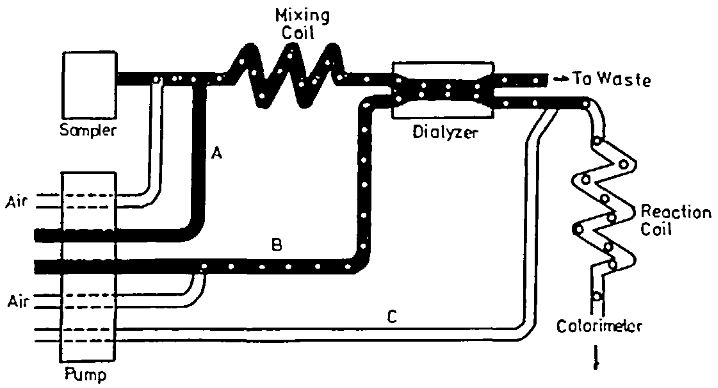


FIGURE 32. Potential analytical applications of the injection technique, according to Pungor et al.[136] (M) Sample solution; (R) reagent; (P) product; (A) component detected; (J) component injected; (S) component flowing. Sample concentrations increase from left to right.

separation of the components to be measured from the interfering ones (e.g., filtering, flash distillation, solvent extraction, ion exchange, gas diffusion, and dialysis).

It should be noted that a great number of authors deal with Technicon® AutoAnalyzer systems (or very similar to them) with different detectors, e.g., polarographic or potentiometric, but commercially available instruments are still mainly equipped with photometric detectors (colorimeter, UV-colorimeter, or fluorometer).

A number of papers deal with the theory and practice of continuous flow analysis. Results achieved by different authors by means of Technicon® analyzers or their modified variants are published in the Proceedings of Technicon Congresses, in addition to those appearing in analytical journals. Besides, in 1975 a bibliography was published, mainly on the practical applications of CFA.[110]

Concerning the theory of CFA, the reader is first referred to the paper of Snyder et al.,[111] but the publication of Thiers et al.[112] should also be consulted. Snyder and co-workers discuss the continuous flow analyses performed with Technicon® AutoAnalyzers and compare it with other analyzer systems in a paper of 1976. They point also to new trends in the development of CFA.

In the formation of a reproducible signal at the detector, which is in an unambiguous function with the concentration of the sample, the following factors have decisive roles: the dispersity of the sample, mixing, and flow stability. The effect of these factors was investigated in detail recently, especially since the beginning of general use of the flow-injection technique.[111]

When discussing the role of the dispersity of the sample, besides the regularities in the flow tube, the effect of the segmentating air bubbles has to be considered as well. Dispersity depends mostly on the following parameters: flow rate of the liquid, inner diameter of the flow tube, rate of segmentation, viscosity of the flowing liquid, and time.

Good mixing of the sample and reagents should be ensured in all solution segments; this is accomplished by a mixing coil, which also ensures a long enough reaction time for the chemical reaction serving as the basis of analysis. Studies showed that in the efficiency of mixing, the properties of the flowing liquid and the geometry of the flow tube (inner diameter, diameter of the coil, length of the segments) had the most important role.

The problem with flow stability is mainly due to the pulsation of the flowing liquid produced by relatively inexpensive peristaltic pumps. Segmentation by air has a significant role in decreasing the fluctuation and achieving a stable flow; according to the original idea of Skeggs an appropriate air-bar opens the way for the pressurized gas in the

Table 2
ELECTROANALYTICAL METHODS ADAPTABLE TO CONTINUOUS FLOW ANALYSIS[111]

Analyte	Enzyme or substrate	Measurement method[a]	Species monitored
Glucose	Glucose oxidase	A	$O_2, H_2O_2(I^-$ via ISE)
Urea	Urease	P	NH_4^+, HCO_3^-, $NH_{3(g)}$, $CO_{2(g)}$
Creatinine	Creatininase	P	NH_4^+,$NH_{3(g)}$
Uric acid	Uricase	A (P)	$O_2, H_2O_2(I^-$ via ISE)
Cholesterol	(Cholesterol oxidase) (cholesterol hydrolase)	A (P)	$O_2, H_2O_2(I^-$ via ISE)
Lactatic acid	Lactic dehydrogenase	A	$NAD^+/NADH$
Inorganic phosphorus	Alkaline phosphatase	A	O_2, H_2O_2
Lactate dehydrogenase	Lactic acid	A	$NAD^+/NADH$
Glutamic-pyruvic transaminase	Lactic dehydrogenase	A	$NAD^+/NADH$
Creatine phosphokinase	Hexakinase, glucose-6-phosphate dehydrogenase	A (P)	O_2,$H_2O_2(I^-)NADP^+/NADPH$
Cholineesterase	Acetylcholine	P	H^+
Alkaline phosphatase	Phenyl phosphate polyphenoloxidase	A	O_2,
Proteins		P	Ag^+
Lipase	Triglyceride	P (P)	H^+

[a] A = amperometric methods with Pt. glassy carbon electrodes as transducers. P = potentiometric methods with ISEs as transducers.

tube only, when, in accordance with the properties of the peristaltic pump, the flow momentarily stops. The stable, continuous flow is also aided by the elastic medium represented by the air-bubble.

Concerning the application of electroanalytical detectors in segmented liquid flow systems, it has already been mentioned that they are not widespread in practice yet. Snyder[111] published a table (Table 2) about the adaptable electroanalytical methods.

Lectures at different conferences and papers in literature gives accounts of various experiments and successful applications. Several of them will be discussed here, as examples.

Among electroanalytical detectors in analyzers applying segmented liquid flow, voltametric (polarographic) detection is especially rare. The scarce use of polarographic detectors in automatic analyzers is explained by the difficulties in constructing a flow-through polarographic cell with suitable properties. It is expected that the volume of the cell be small, but, at the same time, this cannot be decreased at will because of the deformation of the mercury drop. The problems of adequate electrode configuration and detector cell design is dealt with in detail by Lund and Opheim.[113]

In polarographic detector cells often mercury electrodes with a large surface area are applied as reference or auxiliary electrodes. According to literature data and also to our own experiments, the use of noble mental auxiliary and reference electrodes is more advantageous, mainly from a hydrodynamic point of view.

In polarographic analyses the removal of oxygen is an important step, which is generally realized by bubbling an inert gas through the analate. This would be a complicated task in a flow-through system. In most cases the problem is overcome in that liquid-segmentation is carried out with nitrogen instead of air. For this purpose, Lento and Cullen[114-115] built a mixing coil into the system in which the saturation of the sample solutions with nitrogen is carried out. Silvestri[116] mixes the samples with large amounts of oxygen-free background electrolyte.

In the device built by Lund and Opheim[113] three samples are simultaneously deoxygenated by bubbling inert gas through them in the sample holder. Considering, however, that the sampler sucks in air between the sampling cycles, the usual measuring system had to be modified in such a way that the pump did not work between the individual samplings. This makes the apparatus more complicated, and, in addition, disadvantageous from the point of view of achieving uniform steady liquid flow. Despite this fact the authors found that by stopping the pump temporarily, the scatter of the data decreased.

The above authors also studied the problem of analysis speed. This is limited by the time needed for reaching the steady state, and the carry-over effect. Lento[114] found that the condition of obtaining a steady-state signal is fulfilled at a speed of 20 samples per hour. According to Alexander and Shah,[117] the steady-state signal is approached to within 98% at a speed of 60 samples per hour in a differential pulse operation mode, the extent of carry-over being negligible. Lund and Opheim[113] found results reliable and well evaluable at a speed of 30 samples per hour.

The effect of the liquid flow-rate on the measured signal at voltammetric techniques is a well-established fact. The constancy of the flow-rate is usually ensured upon using automatic analyzers. There are several points in choosing the suitable flow-rate. The signal intensity increases with increasing flow-rate, but at the same time, the reagent consumption can be very high at high flow rates. The increase of the signal with increasing flow-rate can be limited by the fact that the electrode process on which the voltametric determination is based — or more precisely, the electron transfer process — is not fast enough.

Several works are known in the literature, where the authors report on the use of voltametric electrodes other than dropping mercury electrodes in automated systems. Fleet and West[119] studied the applicability of mercury drop, mercury-coated platinum, and graphite electrodes in solving a special analytical task (determination of Ca and Mg based on their complex-forming reactions with EDTA and EGTA). They found the hanging mercury drop electrode to be the most suitable for this purpose.

McDonald and Duke[120] studied the applicability of platinum electrodes in the Technicon® AutoAnalyzer system.

In addition to the classical D.C. polarographic working mode, A.C. and D.P.P. methods have also been used. The application of these measuring techniques can be motivated by the intention to achieve higher selectivity and sensitivity. It should be mentioned, however, that hydrodynamic voltametry (i.e., voltametry carried out under convective diffusion conditions) at constant electrode potentials — hydrodynamic amperometry — (which is almost exclusively applied in automated systems) is one of the most sensitive methods by itself, thus, the application of a highly sensitive measuring technique is not necessarily needed.[121-122]

After the above discussion of results achieved by voltametric detection, several examples will be mentioned for the coupling of proportional analyzers applying bubble-segmented liquid flow and potentiometric detectors. The degree of automation in the examples to be shown is different.

Oliver and co-workers[123] determined fluoride-ion concentrations in gases and plant samples. Analysis of the latter was performed after a distillation step as is shown in Figure 33 while the analysis of gas samples was carried out in a system as is seen in Figure 34. Combined fluoride-ion selective electrodes and pH-sensitive glass electrodes were used for detection. Concentration data were obtained on the basis of calibration curves measured with standard solutions.

Porter and Sawyer[124] applied the CFA method with a redox-detector for determining

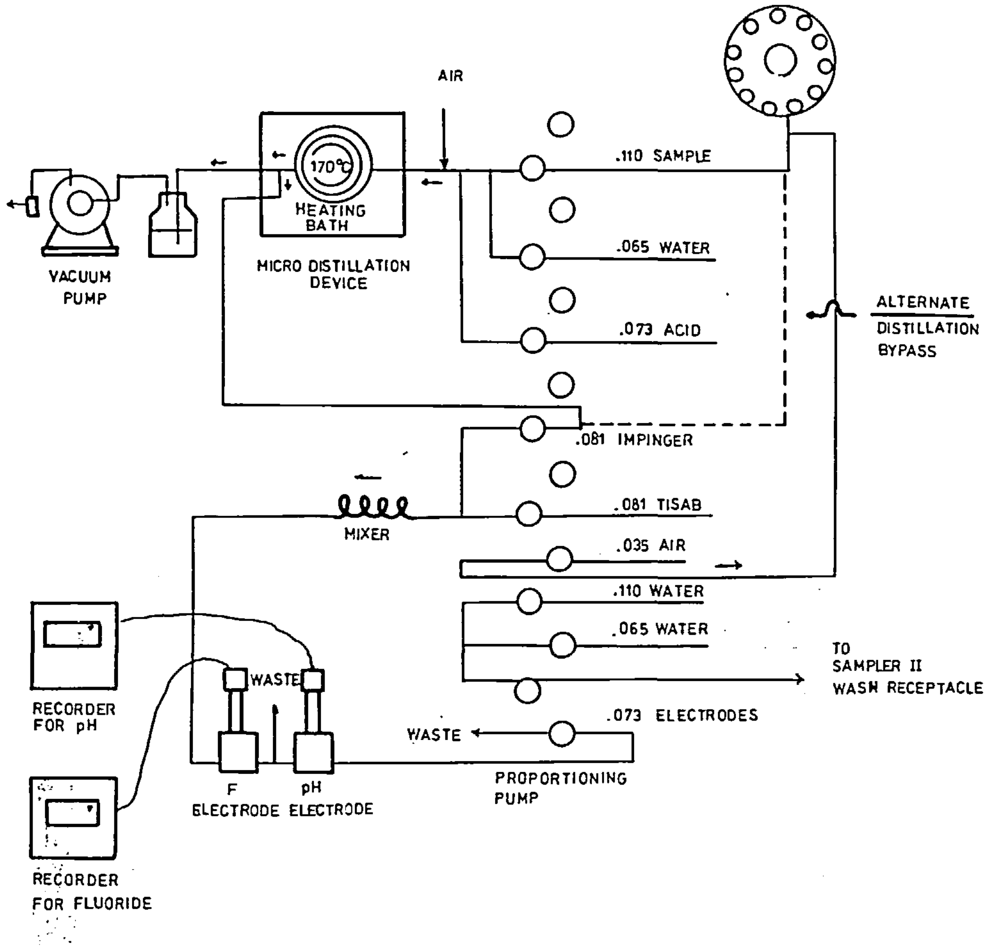


FIGURE 33. A survey on the application of the injection technique with potentiometric detection, according to Pungor et al.[138]

the sugar content of different foods. A hexacyanoferrate (III) solution was the reagent, and the change in the redox-potential accompanying the formation of hexacyanoferrate (II) was detected by a platinum and a calomel electrode (Figure 35). The flow-diagram of the system used is shown in Figure 36.

Upon measuring the sugar content of different samples (marmalade, invert sugar, glucose syrup, etc.) before and after hydrolysis, results obtained were in agreement with those of manual analysis.

When investigating the properties of different gas-sensing probes (ammonia, sulfur dioxide, and nitrogen oxide), Bailey and Riley[125] found that the response time of the above electrodes allows their application in CFA. The electrodes, supplied with a flow-through cap, have been built into the flow channel, as shown in Figure 37.

Between every two samples, electrode conditioning solutions were used for washing, e.g., a solution containing NH_4^+ ions in a lower concentration than that of the samples when measuring ammonia, and diluted $K_2S_2O_5$ solution in the determination of SO_2. An analysis rate of 60 samples per hour could be achieved when determining ammonia, while 30 sulfur dioxide samples were analyzed per hour.

The general design of an apparatus suitable for the determination of enzyme activity, consisting of the parts of the Technicon® Analyzer-type air segmented flow, is shown

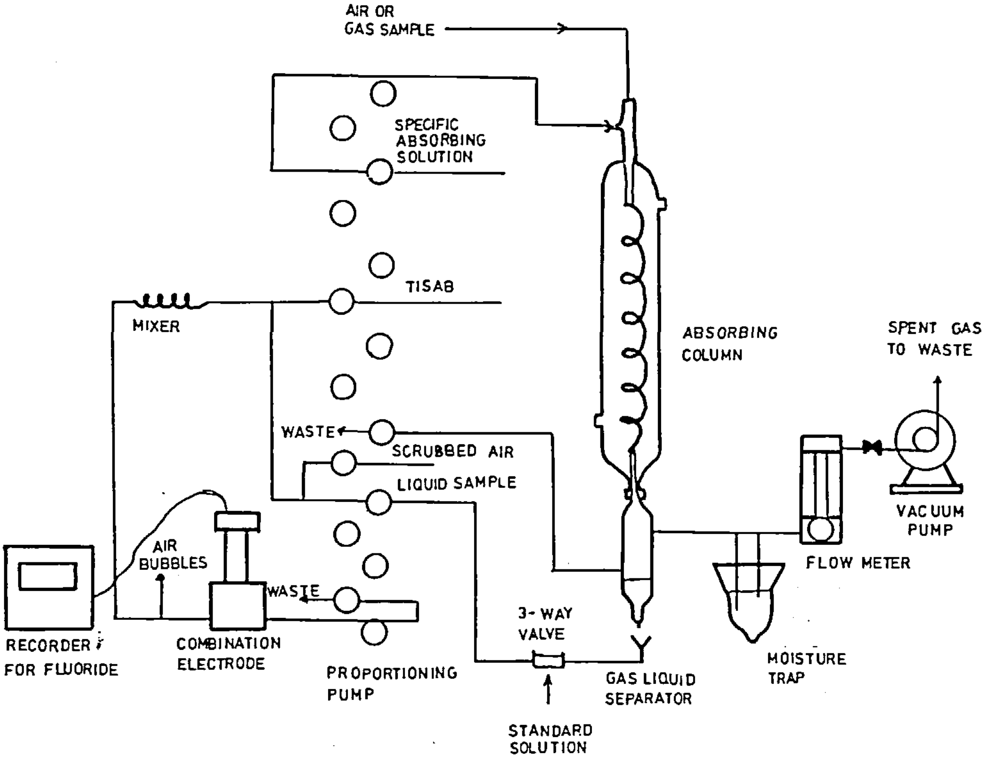


FIGURE 34. Examples for the application of the injection technique with voltammetric detection, according to Pungor et al.[132]

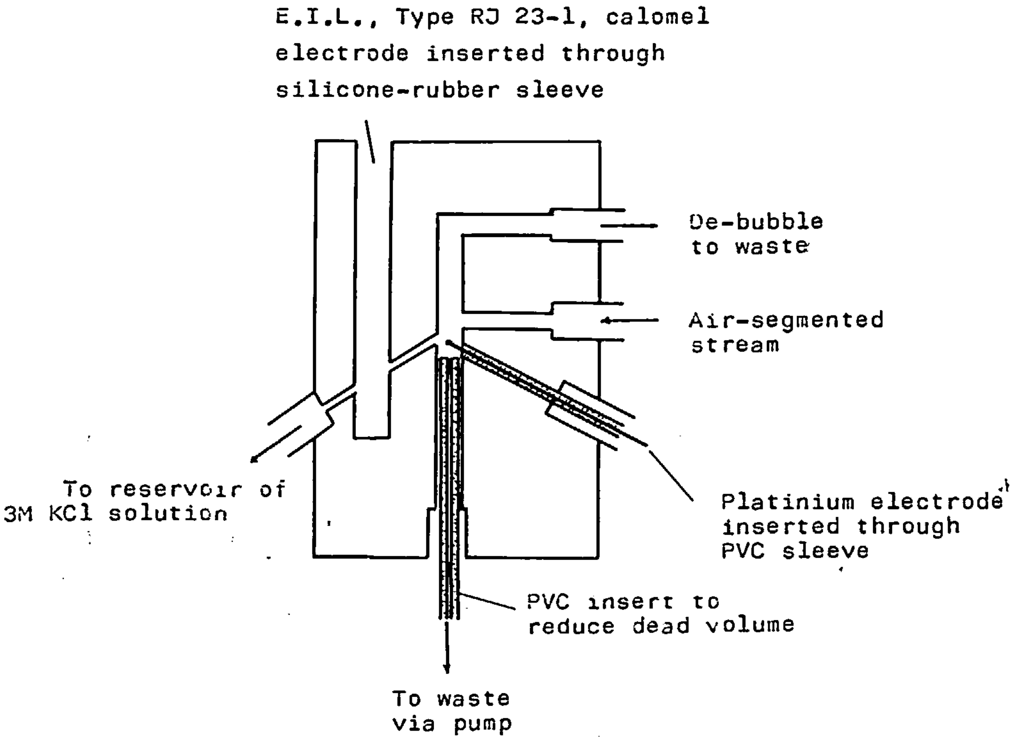


FIGURE 35. Flow cell, redox assembly used for sugar determinations by Porter and Sawyer.[124]

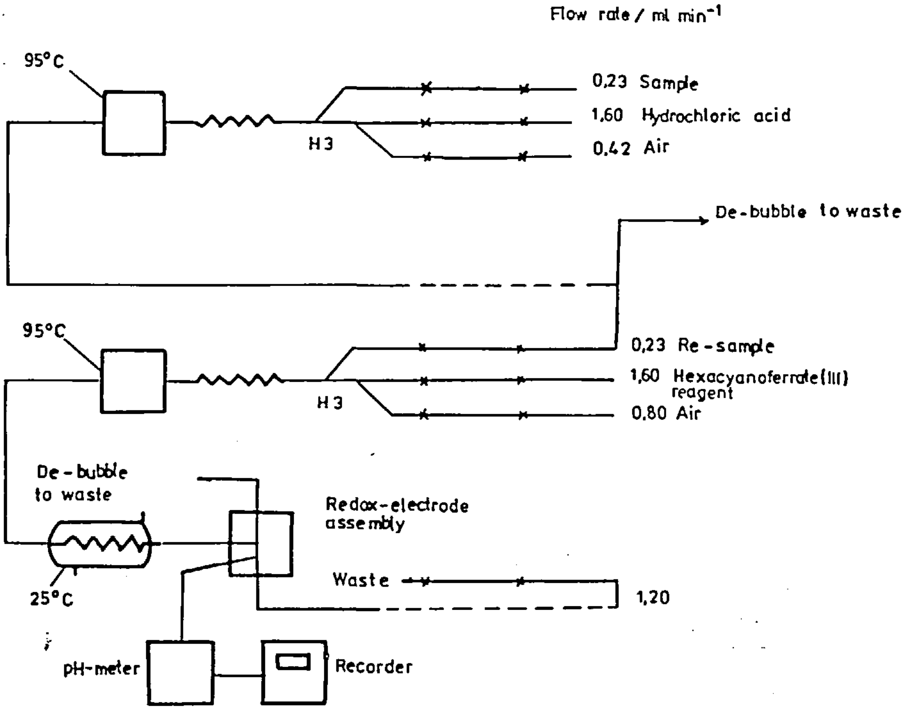


FIGURE 36. Manifold for sugar determinations, according to Porter and Sawyer.[124]

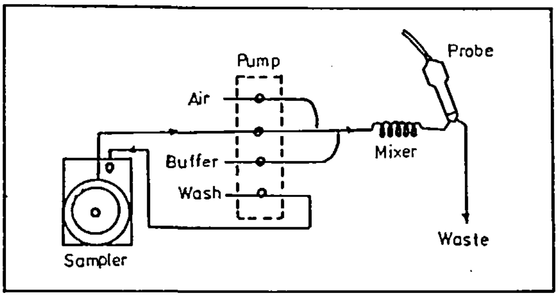


FIGURE 37. Diagram of flow system containing ammonia sensing probe according to Bailey and Riley.[125]

in Figure 38. The solution of the substrate prepared with a suitable buffer after passing a thermostating water bath first meets the segmentating air bubbles and next with the enzyme solution originating from the sampler or the washing solution. The unified liquid-flow goes into the reaction zone, thermostated by a water bath, which ensures identical reaction extent. The reaction zone ends where the quenching reagent streams into the system. After this part a debubbler is built in and the solution leaving the debubbler goes into the flow-through measuring cell containing the ion-selective electrode as detector.

The enzyme activity is measured via a reaction with the substrate in the reaction zone, giving rise to a product detectable by the ion-selective electrode, or removal of one, depending on the activity of the enzyme.

Rechnitz and Llenado[126] studied the efficiency of three different variants of the

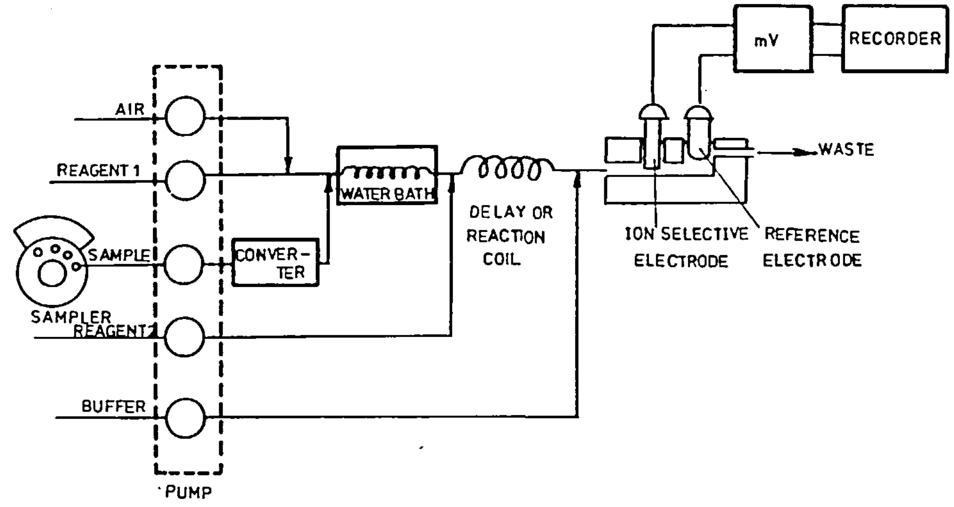


FIGURE 38. Universal flow-diagram for enzyme-activity measurement.[163]

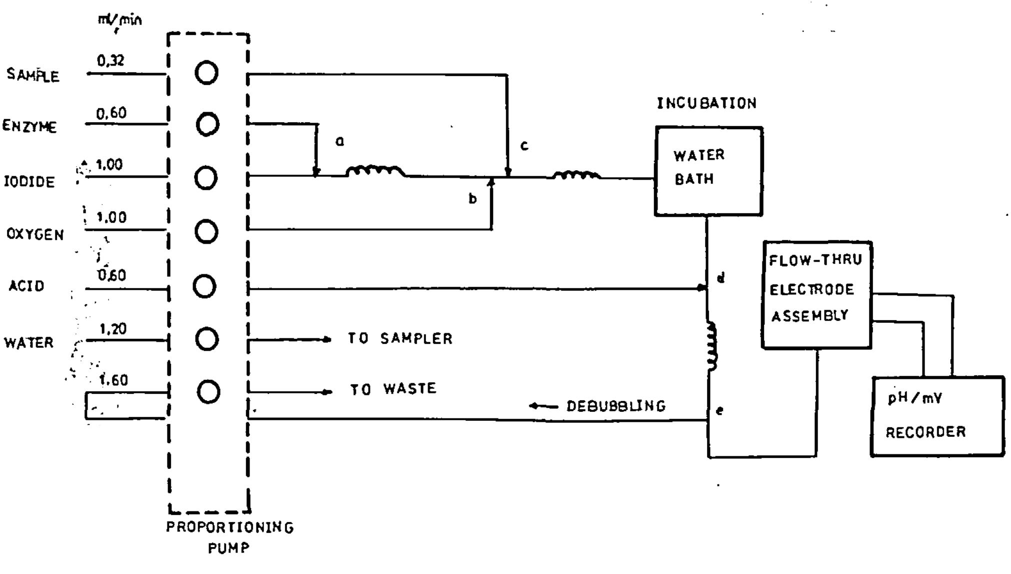


FIGURE 39. Flow diagram of the system used for glucose determination by Llenado and Rechnitz.[127]

apparatus shown in Figure 39. The following reaction has been used by the above authors for determining the activity of the β-glucosidase (E.C. 3.2. 1.21) enzyme:

$$\text{Amygdaline} + H_2O \xrightarrow[\text{(E.C.3.2.1.21)}]{\beta\text{-glucosidase}} \text{benzaldehyde} + 2\,\text{glucose} + HCN$$

Detection was by a CN^--selective electrode.

Similarly, the measurement of the activity of rhodenase (E.C.2.8.1.1.) has been carried out by using a cyanide ion-selective electrode. The chemical reaction utilized is the following:

$$S_2O_3^{2-} + CN^- \xrightarrow[\text{(E.C.2.8.1.1.)}]{\text{rhodenase}} SCN^- + SO_3^{2-}$$

The activity of the glucose oxidase enzyme has been determined by applying a two-step reaction:

$$\beta\text{-D-glucose} + H_2O + O_2 \xrightarrow[\text{oxidase}]{\text{glucose}} \text{D-gluconic acid} + H_2O_2 \quad \text{(E.C.1.1.3.4)}$$

$$H_2O_2 + 2H^+ + 2I^- \xrightarrow{\text{Mo(VI)}} 2H_2O + I_2$$

In this case, the potentiometric detector follows the change in the activity of the iodide ions formed in the second step.

Sufficient accuracy has been achieved at an analysis speed as high as 70 samples per hour.

Nevertheless, in connection with the enzyme activity measurements with CEA, it has to be noted that those analytical methods, which detect only one single point of the concentration vs. time curve, are not especially accurate.

Instruments based on reaction rate measurements applying the flow analysis channel principle are relatively disadvantageous due to the above reason, when compared to centrifugal analyzers or automated batch analyzers.

In another work[127] Llenado and Rechnitz report on an automatic determination of glucose on the basis of the above-mentioned two steps. The concentration of glucose is also determined through the measurement of the iodide ions by means of a flow-through iodide ion-selective electrode.

In the system shown in Figure 39, a fixed time is ensured for the enzymatic reaction in which a steady state is reached.

Sekerka and Lechner[128] elaborated and successfully applied a flow-through system with several electrodes in a batch-type automatic potentiometric apparatus for the analysis of potassium, sodium, and ammonium ions. The indicator electrodes are Orion Na^+ and K^+ ion selective, and NH_3 gas electrodes built into a plexiglass flow-through cell. The detector cells are coupled to the millivoltmeter with a BCD output through a unit containing a three-channel switcher and an amplifier. The mV meter is connected to a Wang®-60 minicomputer (Figure 40).

The pumping of the samples coming from the turntable sampler (Fischer-type 9-315-50) is ensured by a Manostat-type multichannel pump. The analysis process is controlled by the central control module. The computer calculates the concentration of the three ions in the sample on the basis of a calibration curve recorded previously. The sample solution is distributed into three different analysis channels where they are unified with the flows of the auxiliary solutions necessary for the potentiometric measurement. Air segmentation and a debubbler are also incorporated. As the mode of pumping interferes with the measurement of the cell potential, the pump is stopped for the time when data are collected. The computer only served to store the data and calculate the concentrations from the signal (cell potential).

Scott[129] discusses the determination of the Na^+, K^+, Cl^-, and CO_2 content of blood serums with ion-selective electrodes. His measurements have been carried out in a Technicon® Stat/Lyte Analyzer controlled by a microcomputer. This analyzer can be used in both batch and flow-through operation mode.

A simplified flow diagram of the apparatus for determining Na^+ and K^+ ion concentrations is shown in Figure 41. The following electrodes have been applied.

- Flow-through capillary glass electrode for determining Na^+

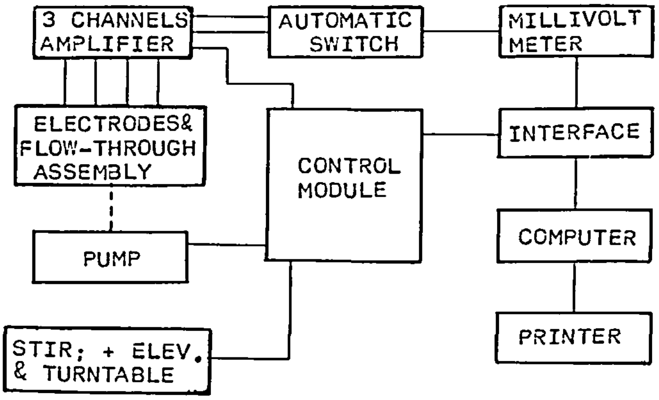


FIGURE 40. Block diagram of instrumentation for automated determination of ammonia, sodium, and potassium, according to Sekerka and Lechner.[128]

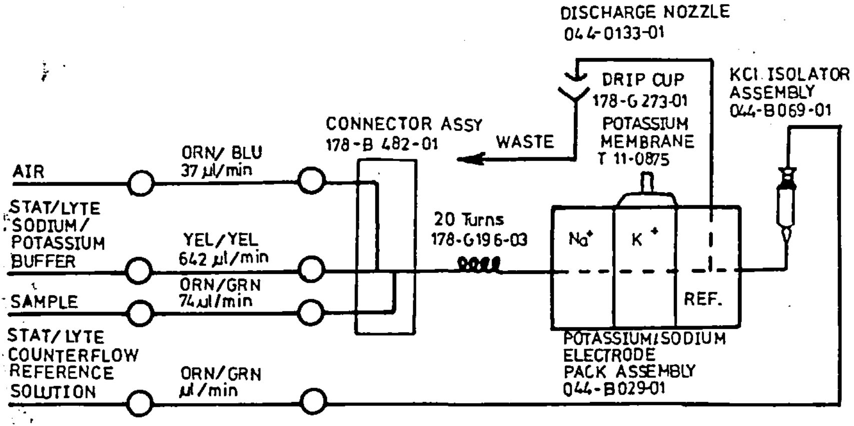


FIGURE 41. Simplified flow diagram of the sodium and potassium determination, according to Scott.[129]

- Valinomycin-PVC membrane electrode for determining K^+
- Ag/AgCl disk electrode for determining Cl^-
- pH Sensitive glass electrode for determining CO_2

Fully automatic calibrations and analyses are carriedout according to a strict program. In the automatic working mode, every half hour a two-point calibration takes place, while the checking of the computer occurs every 2 hr. In order to achieve maximum accuracy, in addition to these, a single-point calibration is also made before every measurement. The speed of analysis is 36 samples per hour. The microcomputer displays the results, together with all the necessary data characterizing the sample.

If the system does not work at its optimal conditions, this fact is indicated, and self-monitored routines can also be carried out.

B. Flow Injection Systems

Another well-defined field of application of the flow-through principle is related to the so-called flow-injection technique. Its recent popularity is shown by the fact that

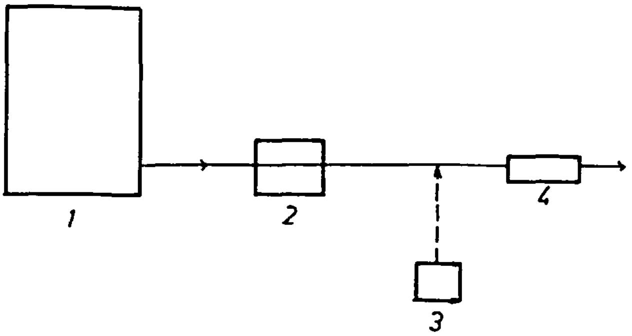


FIGURE 42. General pattern of flow-injection devices. (1) Carrier solution, (2) pump, (3) injector, (4) detector.

almost any noteworthy general analytical journal contains at least one publication on the flow-injection technique. The flow-injection principle proved to be a very advantageous one in solving various analytical problems using different detection techniques, sample preparation, and separation units.

Beginning in the second half of the 1970s, Ruzicka and co-workers have been very active in popularizing and further developing this technique. This activity is well characterized by the fact that even the invention of the flow-injection principle is attributed to them, regardless of the fact that the invention and some successful application of this technique have been reported far before Ruzicka.[130-133,149,194-198]

The basic idea of the injection-measuring technique is shown in Figure 42; from reservoir (1), a solution flows into the analysis channel with a constant rate, which is ensured by a pump (2) or by a constant hydrodynamic pressure. In the flow-injection technique no bubble segmentation is applied. A suitable injector unit (3) injects a small dose of the solution into the flowing liquid at a given point of the analysis channel. This volume is then transported by the continuous stream. While traveling, a deformation occurs in the initial — injection- and flow condition-dependent — C_{inj} vs. time profile, i.e., dispersion takes place. Beside dispersion other processes, e.g., a chemical reaction between the flowing solution and the components in the injected solution, can also occur. A flow-through detector (4) indicates the peak-shaped signal-time profile, which depends on the detector characteristics, the geometry of the analysis channel, and the concentration of the analate. Evaluation of the results is carried out by using calibration curves obtained with standard solutions, on the basis of the following functions: area under the peaks vs. injected amount, area under the peaks vs. concentration, or peak heights vs. the above parameters.

Thus, this method uses a dynamic signal vs. a time transient function and not a steady-state signal.

Because the signal obtained is first of all characteristic for the quantitative composition of the injected solution, this method is especially suitable for the serial analysis of samples with small volumes. At the same time, under certain conditions, the signal is also applicable for the analysis of continuously flowing solution. Accordingly, different monitors can be used employing the flow-injection technique.

In connection with this method, several summarizing studies have been published.[134-137] They deal with the general basic problems of the method, solutions of these problems elaborated by different schools, and the instrumentation used by the different teams working in this field. In flow-injection analytical (FIA) procedures described to date, absorption photometric detection has usually beed used; thus, the FIA instruments developed for serial analysis are built in accordance with this. In order to diminish

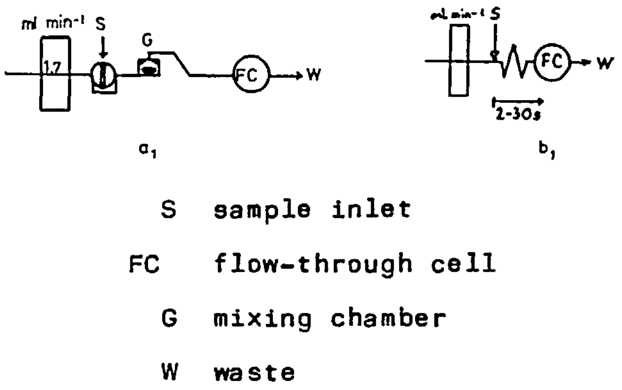


FIGURE 43. Flow-injection system without mixing chamber (a) and with mixing chamber (b), according to Ruzicka and Hansen.[137]

dispersion, the dispersion region of the analysis channel usually consists of a thin tube 0.5 to 1 mm in diameter, and the injector also incorporates a loop with the same diameter temporarily inserted in the flow. Local inhomogeneities perpendicular to the flow direction are more or less smoothed by the use of an integrated type, e.g., photometric detector. When using electroanalytical detectors, several conditions have to be fulfilled for their appropriate functioning. For this and for other reasons, it is sometimes advisable to have proper mixing conditions in the flow, which can very simply be realized with a mechanically stirred mixing chamber.

It may not be superfluous to discuss the advantages and disadvantages of the use of a mixing chamber, especially because several attempts have already been made to work without them.

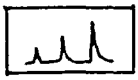
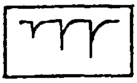
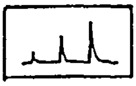
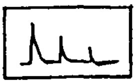
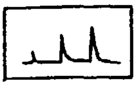
In Figure 43a a flow-injection system is shown containing a mixing chamber, and in Figure 43b the same system without it. The mixing chamber, which contains a magnetic stirrer, produces a relatively high dispersion depending on the volume of the chamber, which is disadvantageous from the point of view of analysis speed and sensitivity. This effect, a little exaggerated, shown in Figure 44, originated from the work of Ruzicka.[137] At the same time, the mechanically propelled mixing unit determines the dispersion and the concentration vs. time profile in systems provided with an appropriate mixing chamber. In the case of samples with different viscosities, the small differences in the dispersity in the channel are leveled by the stirrer; thus, at suitable stirring rates the dispersion conditions in the mixer are independent of the viscosity of the solution entering the mixer. Similarly, the effects of differences in specific density, temperature, detergent content, etc. of the samples on the concentration profile can be remarkably reduced.

The higher dispersion, which is a consequence of the use of mixing chambers, results in only small changes in the concentration of the flowing solution during the analysis. Consequently, the conditions for the detection can easily be ensured. The carrier stream, containing the buffer, ionic-strength adjusting buffer, background electrolyte, complexing, masking, protective colloid-forming, etc. agents are able to produce constant conditions suitable for the operation of electroanalytical detectors.

The concentration vs. time profile can easily be brought into the region of measurable concentrations by changing the volume of the mixing chamber. By diluting the sample, the interfering effect of foreign components can be eliminated, or the segregation of insoluble components can be prevented.

Another aspect is that the application of a mixing chamber increases the contact time between the sample and the detector. Thus, electrodes with longer response time (enzyme

Table 3
POTENTIAL ANALYTICAL APPLICATIONS OF THE INJECTION TECHNIQUE[131]

Direct sample injection $M_A^J + R_I^S \rightarrow M_A$	
Indirect sample injection $M_I^J + R_A^S \rightarrow R_A^S - P$	
$M_I^J + R_I^S \rightarrow P_A$	
$M_I^S + R_A^J \rightarrow R_A^S - P$	
$M_I^S + R_I^J \rightarrow P_A$	

Note: M = sample solution, R = reagent, P = product, A = component detected, J = component injected, S = component streaming. Sample concentrations increase from left to right.

electrodes, membrane-coated gas electrodes) can also be advantageously used in the flow-injection analysis systems.

Readers who are interested in comparing experimental results obtained by applying systems with and without a mixing chamber are referred to Reference 136.

There are cases when a chemical reaction occurs between the injected and the flowing solution, which results in a variation in the concentration of the detected component. For flow-injection systems using electroanalytical detection, a variety of measuring methods have been worked out, depending on whether a component of the injected solution, that of the carrying solution, or the product of the reaction, is detected. As a result, a decrease or increase in the transient signal can take place. A survey is given in Table 3 of the possible methods and the resulting signal-time curves.

Tables 4 and 5 give a survey of application of flow-injection measuring techniques applying potentiometric and voltametric detectors, respectively, for different analytical purposes,[138] while Betteridge[135] summarizes the use of both electroanalytical and other detectors in the flow-injection technique.

The development and use of the injection technique itself can be regarded as an important step towards the automation of chemical analysis. Moreover, serious efforts have been made for the automation of the flow-injection technique in a more rigorous sense.

Several results have been achieved regarding the development of injector systems for special tasks and of designing automatic injector units. In this respect important improvements have been made on the injectors known in chromatographic practice. Experiments are in progress for developing units applicable for sample preparations preceding the flow-injection analysis.

Encouraging results have been obtained in the automation of data processing and evaluation. In connection with that, new types of integrators, devices for searching the curve maxima, microprocessors, and microcomputers have been designed and coupled to the measuring systems.

Table 4
A SURVEY ON THE APPLICATION OF INJECTION TECHNIQUE WITH POTENTIOMETRIC DETECTION[138]

Streaming solution	Solution injected	Component determined in	The aim of the determination	Detector
KI + KNO_3	I^-	Injected one	I^- in pharmaceuticals	I^-
KI + KNO_3	I^- + Cl^-	Injected one	I^- in Cl^-	I^-
KI + KNO_3	CN^-	Injected one	CN^- samples	CN^-
KCl + KNO_3	Chlorpromazine HCl, diethazine HCl, melipramine HCl	Injected one	Cl^- content in pharmaceuticals	Cl^-
KBr + KNO_3	Gastrixone, methylhomatropinium bromide	Injected one	Br^- in pharmaceuticals	Br^-
TRIS pH = 7.0	Urea	Injected one	Urea samples	Urea electrode
TRIS + urea	Urease enzyme	Injected one	Urease enzyme activity	NH_4^+
KI + KNO_3	CN^-	Injected one	CN^- samples	CN^-
Cl^- + KNO_3	Cl^-	Streaming one	Cl^- content of drinking water	Cl^-
CN^- + KNO_3	CN^-	Streaming one	CN^- content of sewages	CN^-
CN^- + KNO_3	Ag^+	Streaming one	Cn^- content of sewages	CN^-, Ag^+
I^- + buffer	Glucose	Streaming one	Glucose samples	Glucose enzyme electrode (26)
CN^- + KNO_3	Ag^+	Streaming one	CN^- content of sewages	CN^-, Ag^+
Thio-cholinesterase	Colinesterase enzyme	Injected one	Cholinesterase enzyme activity	S^{2-}
S^{2-} + NaOH	Ag^+	Streaming one	S^{2-} content in samples	S^{2-}
S^{2-} + NaOH	Hg^{2+}	Streaming one	S^{2-} content in samples	S^{2-}
TRIS pH = 7.0	Urea	Injected one	Urea samples	Urea electrode
Water solutions	Acid or base solution	Streaming one	Buffer capacity	pH (glass)

Table 5
EXAMPLES FOR THE APPLICATION OF INJECTION TECHNIQUE WITH VOLTAMMETRIC DETECTION[132]

Material	Supporting electrolyte	Applied potential/V
$K_4[Fe(CN)_6]$	10^{-1} M KCl	+0·6
Hydroquinone	10^{-1} M KCl	+0·7
Variamine blue	10^{-1} M KCl; 10^{-2} M HCl	
Ascorbic acid	10^{-1} M KCl; 10^{-2} M HCl	+0·8
Isoprenaline	10^{-1} M KCl	+0·8
Chlorpromazine	10^{-1} M KCl; 10^{-2} M HCl	+0·7
Diethazine	10^{-1} M KCl; 10^{-2} M HCl	+0·7
Amidopyrine	10^{-1} M KCl	+1·0
Morphine	4 N H_2SO_4	+1·0
Ethylmorphine	4 N H_2SO_4	+1·0
Papaverine	4 N H_2SO_4	+1·0

Among the wide range of publications that have appeared on flow-injection analysis, only those will be treated in the following which are closely related to the automation of the technique and system in a broader sense. Thus, injectors, sample preparation techniques, and measuring devices will be discussed.

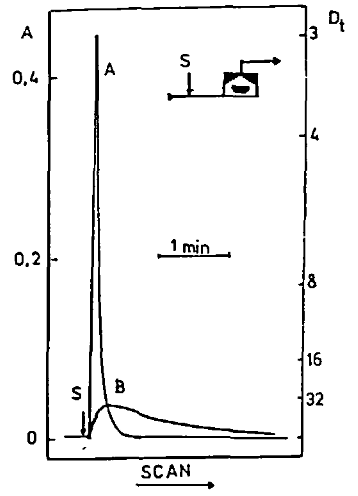


FIGURE 44. Detector signal-time transients obtained for low (A) and high (B) dispersion, according to Ruzicka and Hansen.[137]

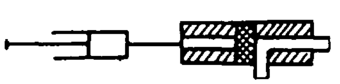


FIGURE 45. Injection port.

An important unit of the flow-injection system is the *injector*. The first type used in FIA was the septum, making manual injection possible (Figure 45). This was soon followed by the rotating valve type injector, the movement of which was realized by manual, magnetic, and pneumatic force. Frequently, a dosing unit known from liquid chromatography is applied for injection. An injector designed in our laboratory is shown in Figure 46. This injector consists of two stators made of hard PVC and of a Teflon® rotor. For moving the rotor, electromagnets with end microswitches (M_1 M_2) are applied. The carrier solution flows into the channel formed by fitting together holes of the three parts. There are three holes of the same diameter in the rotor. One of them is blind.

A sample channel is formed by connecting the exit joinings (A, B) of the blind hole. The volume of the sample channel in the rotor is a given value. This volume can be changed by varying the diameter and length of the tube connecting the A and B pipe stubs.

In the "injection" position the given volume of the sample streams into the flow of the carrier solution. In position "load" the sample can be loaded into the sample channel through a bypass sample loop or by a syringe.

The design of injectors used in different instruments is, in most cases, similar.

The higher demand of reagents is often mentioned as a disadvantage of analytical methods using flow methods. In order to get rid of this disadvantage, a new method was proposed called "merging zone's principle". The idea is that small volumes of both the reagent and the sample are injected into the analysis channel in such a way that they accurately meet and mix with each other. This method is attributed to Bergamin et al.[139] and to Mindegaard.[140] It is demonstrated in the summarizing work of Ruzicka and Hansen[137] (Figure 47).

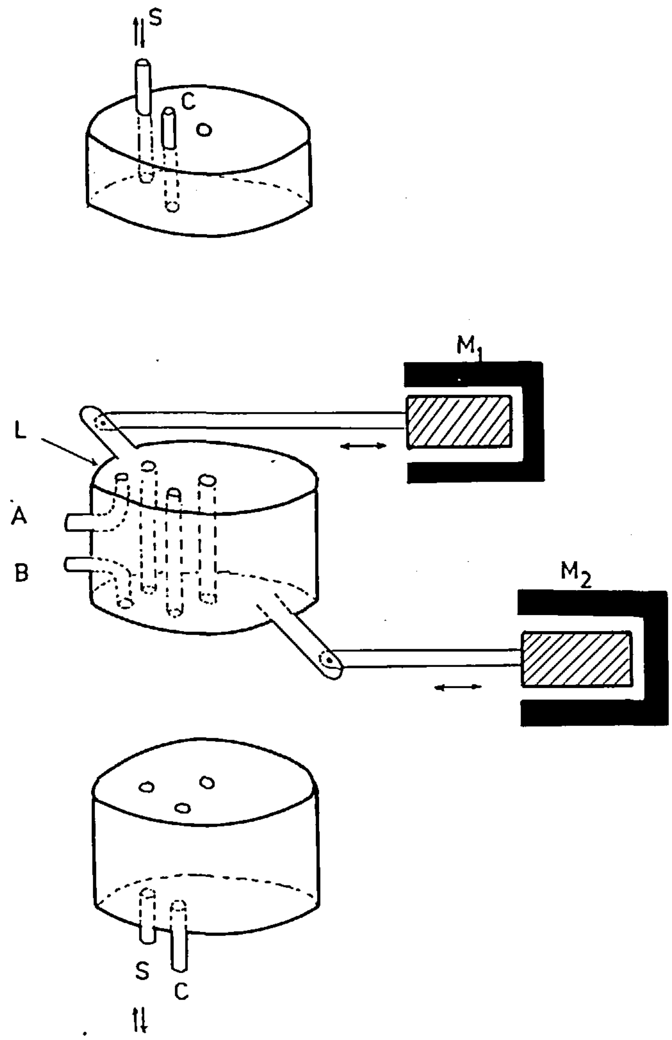


FIGURE 46. Rotary valve-type injector with sample loop and electromagnetic drive (details in text).

The basic idea of the injector developed for parallel double injection is shown in Figure 48.

In the development of the flow-injection technique the sample preparation unit is built into the functional system of the apparatus. However, it is to be noted that in this version of the flow-injection technique other problems appear than in the case of the proportional analyzers utilizing the steady-state signal.

The different preparation units placed after the injector may affect the dispersion produced in the flow tube. This, in turn, can lead to an increase of the experimental error and uncertainty, and in addition to that, the analysis speed can be significantly reduced.

The injector operates as follows: In the loading position (Figure 48A) the sample (S) is aspirated to fill the sample loop (L_s), which exactly defines the sample volume; the excess of sample goes to waste (W). Simultaneously, the reagent (R) is pumped to fill the reagent loop (L_R); its excess, slightly diluted by the reagent carrier stream (C_R), is accumulated in the reagent recovery vessel (D). In this position, the sample and carrier streams (C_S and C_R) are pumped to merge at point A, then going to the manifold (M).

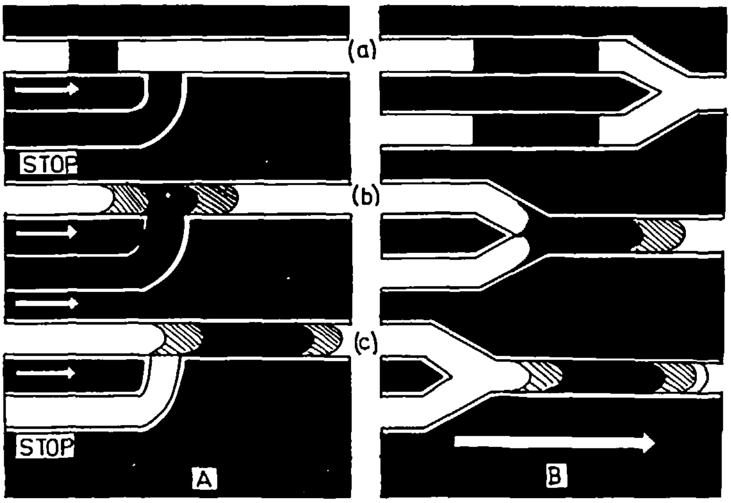


FIGURE 47. The "merging zone principle", according to Ruzicka and Hansen.[137] (A) Merging zone system with intermittent pumping. (a) the sample zone is injected into line I and propelled forward (by pump I) by an inert carrier solution, while pump II controlling the reagent solution is stopped. When the sample zone has reached the merging point (b), pump II is activated, delivering reagent, and pump I is stopped. After addition of reagent, pump I is reactivated and pump II stopped. (B) Synchronous merging of two zones in a symmetrical system with continuous pumping: (a) equal sample and reagent volumes are injected, (b) merge with identical velocities after passing through equal length of tubing, (c) continue downstream while being mixed and dispersed into the carrier stream.

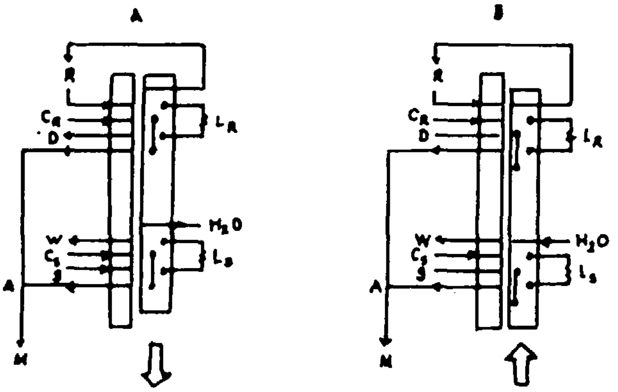


FIGURE 48. Injector developed for parallel double injection, according to Bergamin et al.[139] (Details in text.)

In the injection position (Figure 48B) the selected volumes of sample and reagent are pushed away by the carrier streams, merging at point A simultaneously, and then going to the manifold. The sample aspiration tube aspirates water and the pumped reagent is directed back to the reagent reservoir. However, in numerous cases the coupling of a properly functioning, continuous sample preparation unit and the injection system has been successful.

Bergamin et al.[141] use an automatic sample preparation unit consisting of an ion-exchange column for concentrating the sample in a flow system incorporating photo-

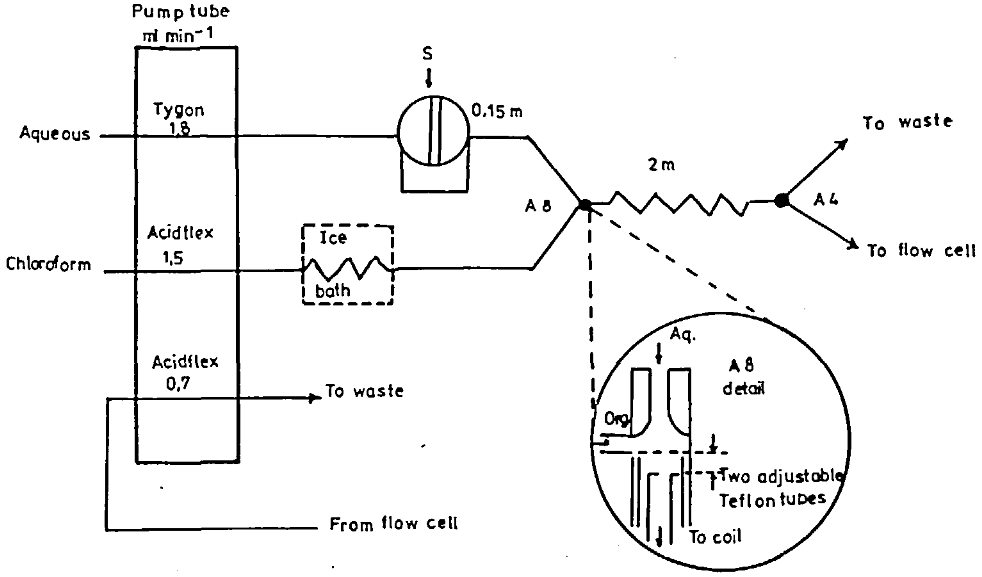


FIGURE 49. Manifold with extraction unit for the determination of codeine, according to Karlberg et al.[143]

metric NH_4^+-detection. The ion-exchange column in the sample-line retards the NH_4^+-ions, while at the other end-point of the injector an eluent solution streams through the column, thus eluting the NH_4^+-ions. The eluted NH_4^+-ions meet with injected Nessler-reagent solution when entering the analysis channel, and after a suitable delay the mixture reaches the photometric detector. For keeping the time of both ion exchange and elution constant a homemade electronic timing unit has been used. The application of ion-exchange columns for sample preparation can be useful also in systems with electroanalytical detectors.

Continuous extractors as sample preparation units have been applied in flow-injection systems first by Karlberg and Thelander.[142-143] The block scheme of the instrument is shown in Figure 49.

After passing the injector, the aqueous carrier solution meets with a flow of a cooled solution of chloroform. The suitably designed merging zone ensures an appropriate ratio of the flows of the organic and inorganic phases in each interchanging segment. Passing through a pipe coil, the aqueous and chloroform phases are separated by a separator. The chloroform phase, having higher density, passes through the detector. From the injected solution the compounds that are to be determined, caffeine[142] and codein picrate,[143] get into the chloroform phase. Thus, different pharmaceutical analysis problems could be successfully solved. Bergamin et al.[144] also mention the application of a similar extraction unit in flow-injection analysis.

Hansen and Ruzicka[145] report on the use of continuous dialyzer in flow-injection analysis. The so-called gas diffusion separation unit was also tested.[146] This consists of two liquid channels separated by a membrane permeable only for gases. Thus, the liberated gas content of the sample solution (e.g., CO_2, NH_3) gets to the analysis line separated from the interfering components.

The so-called distillation unit similar to the separation system based on gas diffusion is also developed and used in the flow-injection technique.

The continuous film distiller described recently in the literature can be considered as an experimental unit, and it proved to be applicable combined with both potentiometric and photometric detector for the determination of NH_3.[147]

Nagy and Pungor[148] have used enzyme electrodes for the determination of different amino acids in flow-injection systems. The reaction layer containing an immobilized enzyme catalyst can be considered as a sample preparation unit which causes only very low dispersion by itself.

The application of specific column reactors containing enzyme catalyst as a sample preparation unit in the flow-injection analysis offers an easy and effective shortcut.

Bergmeyer and Hagen[149] describe a device for measuring glucose by the injection technique. An important element of the apparatus is the flow-through analytical reactor column containing immobilized glucose oxidase enzyme. In the analysis channel a phosphate buffer saturated with oxygen flows with a constant rate. The injection of the sample portions containing glucose is done into the stream before it reaches the reactor. The oxygen concentration of the flowing solution decreases in a manner related to the extent of the reaction between glucose and oxygen which is catalyzed by the enzyme, i.e., the decrease is dependent on the glucose concentration. The applied oxygen electrode in the detector system continuously provides a signal that is linearly proportional to the oxygen concentration. The glucose concentration of the sample solutions can be evaluated on the basis of the negative oxygen peaks. The analysis speed of the apparatus can be as high as 60 samples per hour.

The possibilities of using the flow-injection technique for the simultaneous analysis of more than one component of a sample has been pointed out by Stewart et al.[150] In their technique, elaborated for the determination of phosphor and nitrogen in plant samples, the analysis channel is divided after injection into two lines. Different reagent solutions were fed into the two channels.

A promising new technique is the combination of the stopped-flow and flow-injection methods.[137] Among other advantages, it offers the possibility of the elaboration of analytical methods based on reaction rate measurements. An automatic timer is needed for the application of this method. These studies are only at the beginning of their development; just the surveying of their applicability is going on now.

For the purpose of flow-injection analysis, devices have been made at different levels of automation. Some of them are already commercially available.

Ruzicka and Hansen[151] applied a simple apparatus in order to determine the sodium, potassium, and nitrate content in soil and blood samples. In connection with this problem, they tried to apply a potentiometric detector cell. For the evaluation of the data they used the value of the peak potential, which was registered by a homemade electronic data digitizer as well as using an analog X-t recorder. The same device was applied also for the determination of the area under the injected peaks, i.e., for integration.

The simplest realization of the flow-injection technique with an electroanalytical detector is commercialized by Metrohm®. A flow-through cell containing the plug-in voltametric glassy carbon electrode is connected to a voltametric measuring unit, and a recorder is suitable for the flow-injection determination of some compounds used as medicines.

Pungor et al.[152] developed an automatic flow-injection system applying voltametric detection. The flow chart of the apparatus is shown in Figure 50. The functions of the different units are the following:

1. The multichannel peristaltic pump ensures the flow of the carrier solution, the introduction of the sample, and the delivery of the washing liquid into the injector.
2. The automatic sample changer is capable of storing and changing 200 samples or standard solutions, with a washing cycle between the samples. The sample changer is controlled by the electronic control unit.
3. The analytical unit contains the flow-through analytical channel, together with the accessories necessary to ensure the automatic or manual performance of the measuring principle based on injection. The analytical unit comprises the following main

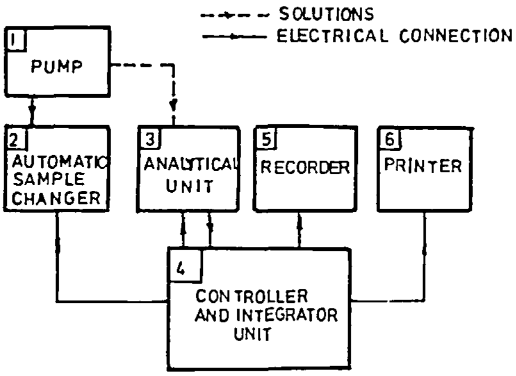


FIGURE 50. Block diagram of injection system developed by Pungor et al.[152]

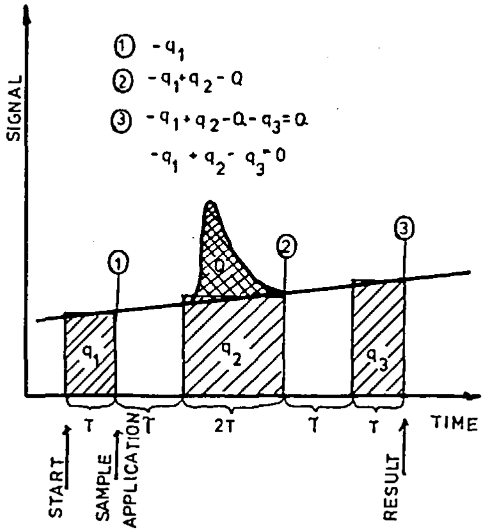


FIGURE 51. Principle of integration with baseline correction (T, 2T: periods of integration; τ: intermission; q_1, q_2, q_3, Q: areas).

parts: voltammetric plexiglass measuring cell, sampling valve for injector valve, sampling head for manual injection, mixing cell and magnetic stirrer, magnetic valve, and electric parts.

4. The control and integrating unit carries out three main tasks:
   - (a) It ensures the electrical conditions for the voltammetric measurement (polarizing voltage, current-voltage transformation, and diffusion current compensation) are met
   - (b) It carries out signal processing and determines the area of the current-time peaks by baseline-corrected integration
   - (c) It controls, synchronized with the phases of signal processing, sample changing, sample aspiration, administration of the sample, the individual phases of integration, and printing of the results.

The principle of the baseline-corrected integration is illustrated in Figure 51. The total measuring cycle is divided into five periods; the first (q_1) and the last (q_3) periods

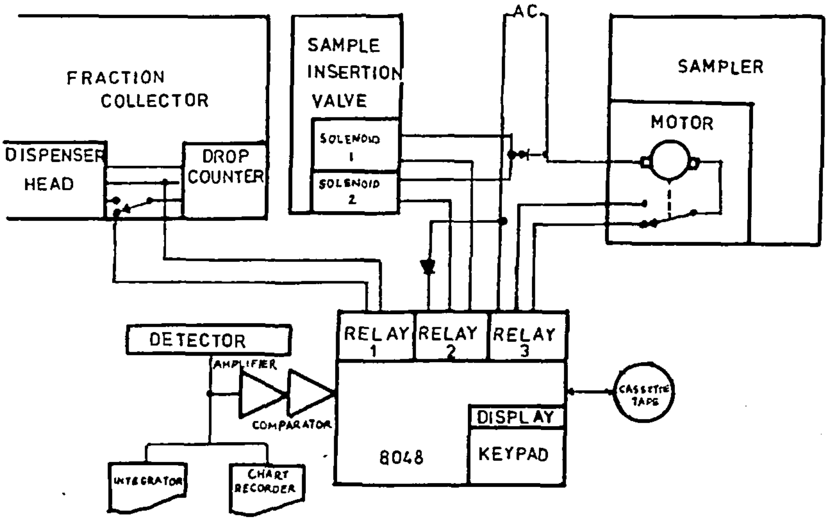


FIGURE 52. Schematic diagram of the automated multiple flow injection system developed by Stewart et al.[153]

ensure the determination of the baseline component of the medium period (q_2) on the basis of the trapezoid-correction principle. The components of the subtotal and total results appearing at the end of the integration periods can be seen in Figure 51. It is characteristic for the operation of the integrator that only deviations from the actual baseline are presented as results, independently of the position of the baseline and of the relative indication of signal and baseline. The system makes possible signals produced in both direct and indirect determinations to be processed. Adjustment of the integrator can be carried out by the determination of the periods T and τ on the basis of experimental data. The result of integration can be read from the display on the integrator unit. In automatic mode it can be seen which phase of the analysis is going on at a given time. In manual mode the display controls the introduction of the sample.

5. The recorder, which can be connected to the apparatus, records the current peaks produced in the measuring cell, vs. time.
6. At the end of the integration cycle, the printer prints out the integrated signal value which is proportional to the concentration of the sample.

The automatic analyzer can be operated in three different working modes: manual, automatic, or monitor. Based on the work of Ruzicka and Hansen, a new flow-injection analysis apparatus with a photometric detector was developed and offered by BIFOK. This instrument is designed for the fast serial analysis of samples with small volumes, when coupled with sample changer and recorder. One of the main advantages of this device is the high analysis speed in direct sample analysis.

Stewart et al.[153] developed a system with a high level of automation for the realization of the automated multiple flow-injection analysis controlled by a microprocessor. The schematic diagram of the apparatus is shown in Figure 52.

The basis of the control system is an IMSAI-8048 control computer which controls the Technicon® sample changer, the magnetic valves, and through an appropriate interface sends a stepping signal to the fraction collector. It also participates in the processing of the signals originating from the colorimetric or fluorescent detector.

The injection system elaborated is used in three different working modes: classical

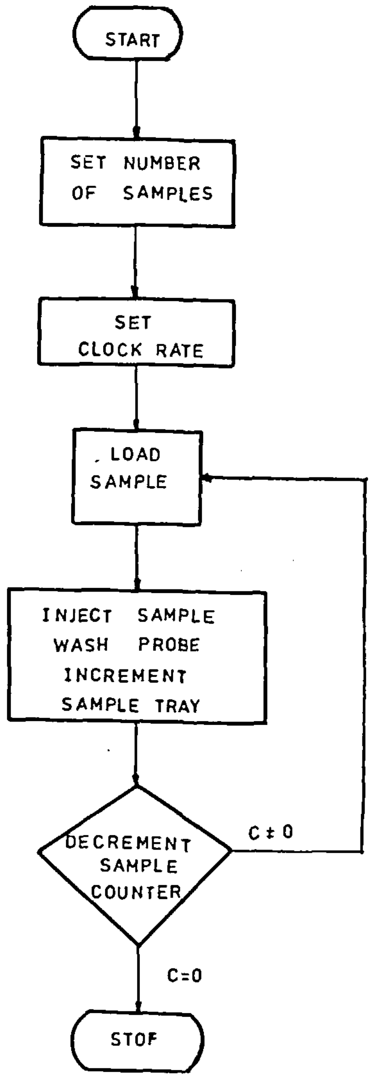


FIGURE 53. A typical automated multiple flow injection analysis program according to Stewart et al.[153]

serial analysis mode, dilution mode, and titration mode. In the first two working modes dispersity is small or medium, and in the third it is higher.

Automatic data control and processing is especially important in the first two cases. The flow diagram of a program for the first, classical flow-injection analysis is shown in Figure 53.

The authors emphasize the simplicity of programming such a system as the main advantage and, in addition, that the precise control and simple evaluation of the results in the multiple flow-injection analysis can be readily solved.

Landis[154] uses a PDP 11/40 computer for data collection and processing in a flow-injection measuring system applying photometric detection. The corticosteroid content of pharmaceuticals has been successfully determined by this apparatus.

Slanina et al.[155] designed a computer-controlled flow-injection system with spectrophotometric and potentiometric detection using ion-selective electrodes.[156] The

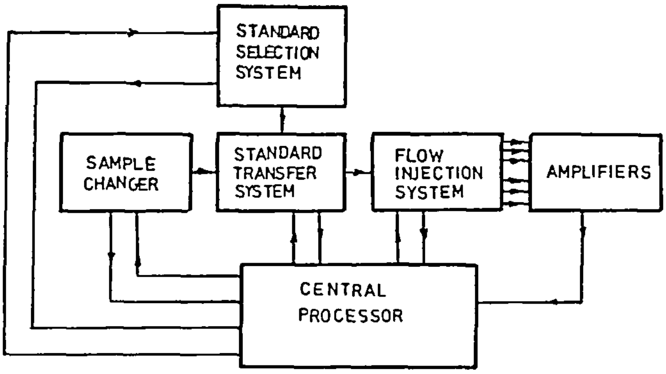


FIGURE 54. General outline of the apparatus developed by Slanina et al.[156]

general setup of the instrument, which is connected to a PDP 11-03 computer with 32 K memory, is shown in Figure 54. The aim of the studies with ion-selective electrodes was the determination of the fluoride content in rain water. The main problem has been that in the concentration region needed for the determination, the response of the fluoride electrode does not follow the Nernst rule, nor is it linear, and in addition to that, the response time of the electrode proved to be strongly dependent on concentration. For improving the measuring conditions, fluoride-containing solution was added to the flowing solution (TISAB), thus shifting the concentration region to be measured towards higher concentrations, hereby improving the response-time properties of the electrode.

In the computer-controlled case, problems originating from the non-Nernstian and nonlinear properties of the electrode do not represent a serious problem (curve fitting being simple). Thus, it is easier to achieve more accurate results.

Analysis in the computer-controlled working mode is performed as follows. Every standard is analyzed twice. If the results differ to a greater extent as previously given, the analysis is repeated. The calibration curve is constructed from the results of the determination of six standards and the carrier solution. Before starting the analysis, calibration is repeated and compared to the results of the first calibration. The system is newly calibrated with a standard after every ten samples. From the results, the conclusion can be drawn that reliable analytical data can be only obtained by frequent calibration and active control of the system.

Pungor et al. developed an automatic instrument for following the dissolution of drug content from pharmaceuticals, based on the flow-injection analysis principle.[157] The apparatus is capable of injection of sample doses from three dissolution vessels and two standard vessels into the flowing voltammetric background solution according to a given program. This is possible through the application of a special five-injector block and the connecting sampling loop system. The control unit governs the time program of the valves ensuring suitable conditions for the dissolution and for the injectors. The flow chart of the solution circuits is shown in Figure 55.

In this work voltammetric and UV-spectrophotometric detectors have been tested, as well as the application of microcomputer-aided data evaluation.[152]

Watson and Keyes[160] describe a commercial device for determining blood urea nitrogen in blood on the basis of flow-injection principle, and they report on their experiences about the operation of the apparatus. In the device (Owens Illinois Inc., Kimble Division, BUN) small volumes of samples are injected into the buffer solution through an analytical reactor containing immobilized urease enzyme. The ammonium ions formed upon hydrolysis are passed through a flow-through ammonia-gas electrode

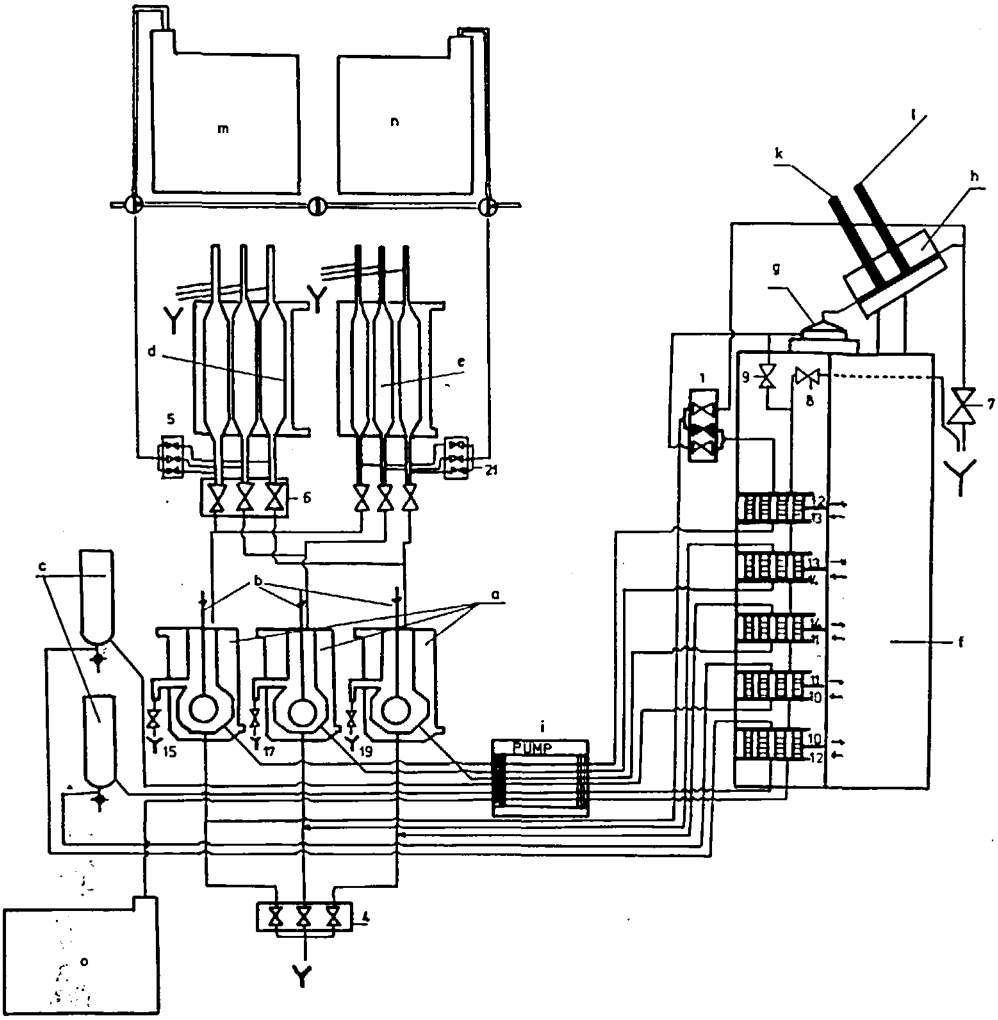


FIGURE 55. Flow diagram of the solution network in the apparatus constructed by Pungor et al.[151] for drug dissolution tests. (a) Dissolution vessels, (b) rotating baskets, (c) vessels for standard solutions, (d) pipettes for sampling the acidic dissolution media (gastric media), (e) pipettes for sampling the dissolution media of higher pH (intestinic media), (f) injector system, (g) mixing chamber, (h) voltammetric measuring cell, (j) peristaltic pump, (k) graphite working electrode, (l) calomel reference electrode, (m) artificial gastric juice, (n) artificial intestine juice, (o) carrier solution, (1 to 21) magnetic valves.

for detection. The Kimble BUN Analyzer provides the automatically evaluated results in a digital form given in concentration units.

An instrument for the determination of phosphatidyl choline in blood serum working on the injection principle is described by Karube et al.[161] The instrument detects amperometrically the hydrogen peroxide formed in the enzyme-catalyzed reaction. In the apparatus, analytical column reactors containing immobilized phospholipase D and choline oxidase enzymes are used.

Watson et al.[162] report on an automatic apparatus applying the injection technique for measuring glucose. The instrument includes an analytical reactor column containing immobilized glucose oxidase enzyme. Its working is based on the catalytic oxidation of glucose by oxygen, thus providing hydrogen peroxide which is detected voltammetrically. From the peaks generated by one sample dose in the flow-through voltammetric measuring cell, the instrument automatically determines the intensity of the peak current corrected for residual current and displays the glucose concentration of the sample if standardization was adequate. On the basis of the signals of built-in thermistors, an automatic temperature correction is also carried out. At the end of the measuring cycle, i.e., when the change in the current decreases below a certain value (approximately the residual current), the device indicates that the next injection can be performed. The accuracy of the measurement was found to be satisfactory for clinical analysis, being about ±5%.

Several attempts have been made for comparing the possibilities and efficiency of the two most frequently used flow-through techniques, namely the continuous flow analysis (CFA) and the flow-injection technique (FIA). Different opinions were expressed on the properties of the two systems, inducing scientific arguments, which contributed to the development of both methods. In the following our opinion will be summarized.

In cases where automation of the analysis requires only the connection between the detector and the sample (direct sample analysis) or where the basis of the determination is a fast chemical reaction, the injection technique is certainly to be preferred. Its simplicity and speed in sample handling can be very profitable. It can be nicely utilized in research laboratories and in different fields of chemical analysis. In other cases, however, where the task of the analysis is the determination of the composition of more complex sample or if the chemical reaction, which the analysis is based on, is a relatively slow one, the technique based on measuring the steady-state signal, which is well established and proven, is undoubtedly more favorable.

Preparation units suitable for continuously handling small sample volumes are more easily fitted to CFA due to the fact that their effect on the dispersion does not significantly influence the analysis speed.

C. Miscellaneous Flow-Through Systems Using Direct Measuring Techniques

In addition to the above two most frequently applied techniques, numerous other types of flow-through analytical methods were developed with different levels of automation, which cannot be listed among the above. A few of them should also be mentioned in this review article. For further examples, the reader is referred to the summarizing work of Pungor et al.[122,163]

Erdmann and Taylor[164] designed an automatic analyzer for measuring the specific conductivity and pH of natural waters. With the system built of Technicon® AAII modules (sampler, pump, ISE module, flow-through electrodes, recorder, printer) and of the CD-314 flow-through conductivity cell by Radiometer, measurements could be carried out in the pH range of 4 to 9 and in the conductivity range of 1 to 15,000 μmho cm^{-1} with an accuracy better than 1%. The wide range of specific conductance of waters necessitated the design of such a system, in which the change of the measuring range is also automated.

Carlson[165] developed a flow-through instrument with conductometric detection for the determination of ammonia after Kjeldahl digestion in biological samples (Figure 56). Accordingly, the samples are mixed with sodium hydroxide and the liberated ammonia is allowed to diffuse through silicon rubber hollow fibers into distilled water, which has passed through the conductivity cell.

Upon studying the properties of this measuring system Carlson found that, though the surface area available for gas diffusion is relatively large, no equilibrium is reached between the absorbing distilled water and the sample, and, thus, the process is controlled by a steady-state gas transport. As both the diffusion coefficient and the specific conductivity of the ammonia are temperature dependent, temperature stabilization has been applied to within ±0.01°C. Among interfering components, volatile amines are worst.

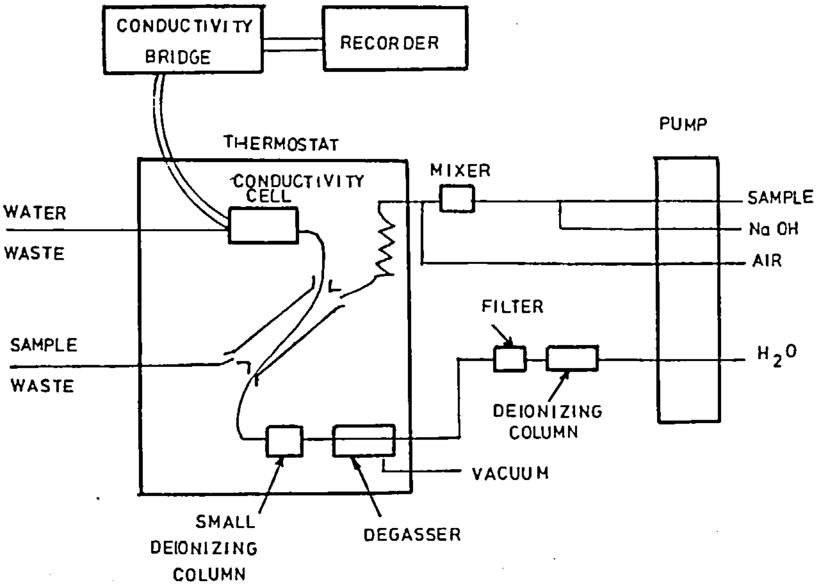


FIGURE 56. Block diagram of the instrument developed by Carlson for the determination of ammonia and dissolved carbon dioxide.

When comparing the analytical data with those obtained by classical methods, good agreement has been found; a mean relative standard deviation of 0.66% has been obtained for the analysis of 39 samples. The system is capable of measuring the total carbon dioxide content.

The Leeds and Northrup Co. offers a family of flow-through analyzers other than the Technicon®-type one. The Enzymax analyzers apply dialyzing membrane sampling. The sample solution coming from the dialyzer is diluted with a buffer solution and introduced into the reactor column containing the immobilized enzyme catalyst. The solution flow leaving the column goes into the amperometric detector cell which has a constant electrode potential. The detector measures the amount of hydrogen peroxide formed in the enzyme-catalyzed reaction.

In the two-channel variant of the apparatus the sample solution is split and directed into two reactors placed parallel and filled with different catalysts, and then into the two identical detector cells. On the basis of the detector signals the sample concentration is calculated and displayed. By using appropriate enzymes, the instrument can be used for determining the concentration of glucose, lactose, and sucrose, respectively.

Papariello et al.[166] report on the development and study of an analyzer designed for following the fermentation process of penicillin. In the flow-through analysis channel of this instrument a pH-sensitive glass electrode is used as the detector. The ferment liquor — cleaned by a dialysis membrane, if necessary — passes through the analytical column containing the immobilized β-lactamase enzyme, and then it is introduced into the detector cell. The penicillin concentration is determined on the basis of the rupture of the lactam ring due to the catalytic effect of the enzyme, resulting in a change of the pH. By following this pH variation, the penicillin concentration can be determined. This device, together with an automatic sampler, is a very useful one; one complete analysis can be made in 10 min.

In analyses performed in flowing systems standard addition or subtraction methods are seldom used. Considering electroanalytical detections, the standard addition method

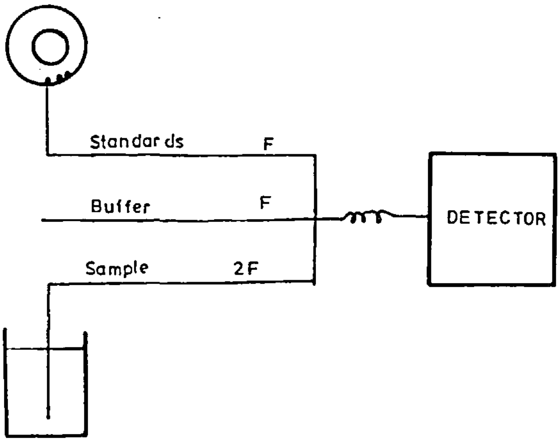


FIGURE 57. Flow chart of computer program used by Fleet and Ho[167] for the evaluation of standard addition measurements.

provides significant advantages when employed in combination with potentiometric sensors.

By single or multiple standard addition technique the concentration of the unknown, the slope, and the standard potential of the detector electrode can be easily calculated with the help of a suitable computer.

In the following paragraphs some results obtained with the use of standard addition methods and potentiometric detection will be surveyed.

Fleet and Ho[160] tried to work out a fully automated method based on the multiple standard addition technique, with the use of the Technicon® AutoAnalyzer modules. From time to time, standard solution portions are mixed to the continuously flowing sample solution and the concentration is calculated from the measured potentials with the help of the Gran-evaluation procedure (Figure 57).

If the flow-rate of the sample solution is 2F mℓ/min, a buffer with a flow-rate of F mℓ/min flows to the sample, and a standard solution with a flow rate of F mℓ/min is mixed, the electrode potential (E_i) can be expressed as follows:

$$E_i = \text{const} + S\cdot\log\frac{2FC_o + FC_i}{4F} \qquad (13)$$

where C_o is the unknown concentration and C_i is the concentration of the standard. When plotting $10^{E_i/S}$ as a function of C_i, the intercept on the volume axis (C_A) gives the unknown concentration

$$C_A = -2C_o, \qquad (14)$$

The computer program used performs data storage, calculation of $Y = 10^{E_i/S}$ values, the line fitting with the least-squares method, and the determination of the intercept. In addition, the calculation of the unknown concentration is also carried out.

The authors employed the flow system for the analysis of cyanide samples with concentrations between 0.001 and 0.006 *M*, and also performed the analysis of samples ten times more dilute than the ones mentioned above.

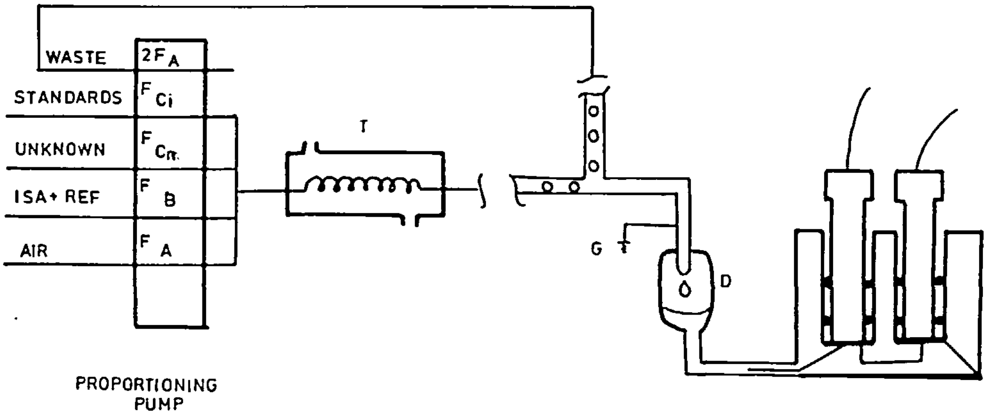


FIGURE 58. Schematic diagram of flow system used by Perone et al.[168] (T) Thermostated mixing coil, (C) glass capillary, (B) cell block.

Perone and co-workers[169] worked out a computer-controlled determination of the concentration of flowing solutions by using ion-selective electrodes with the standard addition method. The measuring system developed on this principle makes possible the use of five different ion-selective electrodes. The aim of the authors was to solve instrumentation problems, on the one hand, and to write a computer program most adequate for handling and processing the measured data on the other hand. They considered the effect of noise, the drift of the electrode and electronics, the response time of the electrode and the electronics, and the accuracy of the data measured. On this basis they worked out a suitable data selecting and processing algorithm.

The scheme of the full arrangement is shown in Figure 58. The analytical unit of this system consists of a Technicon® pump, a mixing coil, flow-through potentiometric detector cell, and Orion-type chloride and fluoride ion-selective electrodes. Data processing occurred on-line, according to a rigorous nonlinear iterative least-squares method.[170]

The basic equation valid for the fluoride ion-selective electrodes applied in this special system is the following:

$$E_i = K - S \log \left[\frac{F_{C_n} C_n + F_{C_i} C_i}{F_{C_n} + F_{C_i} + F_B} \right] \tag{15}$$

where E_i is the potential measured after the addition of the standard; C_i is the concentration of the standard; C_n is the unknown concentration; K includes the standard potential of the electrode and the junction potential; S is the slope of the electrode calibration curve; and F_{C_n}, F_{C_i}, F_B are the flow rates of the unknown, standard, and buffer solutions, respectively.

The nonlinear least-squares method was selected for the evaluation as it was observed that in Gran-type evaluation the results were strongly influenced by the slope of the electrode calibration curve. Considering that in this case the calculation includes the determination of this slope itself, also, it can be better adjusted to the character of the standard addition method carried out with ion-selective electrodes.

According to the above method, the initial estimated C_n, S, and K values are corrected on the basis of the $C_i - E_i$ data pairs, and each C_i value will be compared with its value obtained earlier. If the difference between the following C_i values is larger than a given one, then the correction procedure is repeated.

The effect of the properties of the measuring system on the accuracy and reliability of the results has been studied by generated data. Thus, the effect of the resolution of the digitalizing and data collecting system (ADC), the drift of the K value, the slope of the electrode characteristics, the systematic error of the potential measurement (too short waiting time for achieving the steady potential value) and the effect of the change in the flow-rates have also been studied.

Solutions containing fluoride ions were analyzed according to all the following three programs: (1) operator-controlled data collection and calculation; (2) computer-controlled data collection after previously set time lapses; and (3) selection of data-collection regions based on real-time computer identification of successive voltage plateaus in the potentiometer output during a series of standard additions.

In automatic data collection the noise generated by mixing of the sample and the standard caused some problems.

D. Flow-Through Titrimetry

The advantages of the flow-through analysis channel principle and those of titrations can be amalgamated by combining the two methods.

The realization of titrations in flow systems raises special problems. Firstly, it has to be considered that in flow systems the reaction time can be varied by the proper selection of the flow rate and the geometry of the flow system. The second problem is connected to the realization of the programmed sample or reagent addition necessary to carry out complete titrations, or the control of the sample or reagent flow to ensure a fix titration degree in flowing solutions.

Titrations in flow systems can be accomplished in two different ways. One of them uses a complete titration curve, i.e., the sample or reagent mass flow is constant, while the other is varied according to a predetermined rigid program, and the detector signal vs. reagent mass flow-based titration curve is obtained. According to the other method, however, the detector signal is compared with a predetermined reference signal, the difference signal regulates the reagent or the sample flow through a feedback control circuit with an appropriate transfer function, and the other mass flow is kept constant. The feedback controls flow rates so that a fixed ratio between the sample and reagent mass flows, i.e., a chemical equivalence between the sample and reagent material, is achieved.

Instruments called continuous titrators are of the latter type. Their operation presumes long-term stability of the detectors, thus, mostly potentiometric detectors can be advantageously used. When applying detectors with a linear concentration response, the change of the activity of the detector can cause significant errors. This can be overcome by the frequent checking of the detector signal level in the range corresponding to the end-point of the titration.

A simple variant of continuous titrators is described by Blaedel and Laessig.[171-172] In their continuous titrator a titrant of given concentration with a variable flow-rate is mixed with the sample solution that flows at a constant rate. The flow-rate of the titrant is controlled by a servo-controller system in such a way that the potential value measured in the flowing solution after mixing the sample and the reagent should be equal to a preset constant value. Thus, the flow rate of the reagent is linearly proportional to the concentration of the sample. The scheme of the apparatus is seen in Figure 59.

Griepink et al.[172] reported in several papers on their work for the development of continuous titrators. Their continuous titrator equipped with electroanalytical detectors was used for monitoring the titration chloride ion with silver nitrate titrant. The scheme of the apparatus is shown in Figure 60.

Sample solution (1a) and the buffer (1b) needed for pH control is pumped with a peristaltic pump (2) into the tube-shaped titration vessel (3). The titrant is also introduced

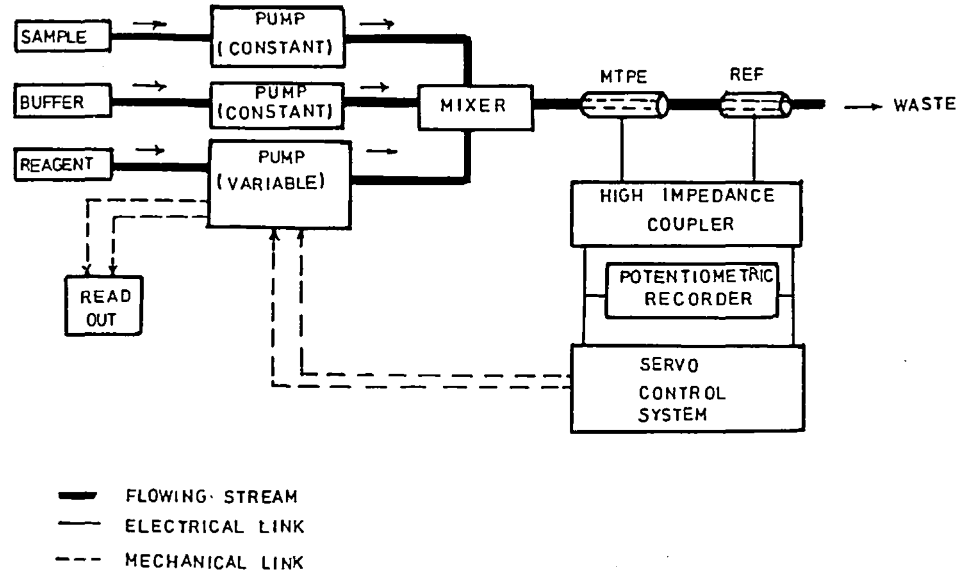


FIGURE 59. Continuous titrator developed by Blaedel and Laessig[171] (MTPE) Mercury coated tubular platinum electrode.

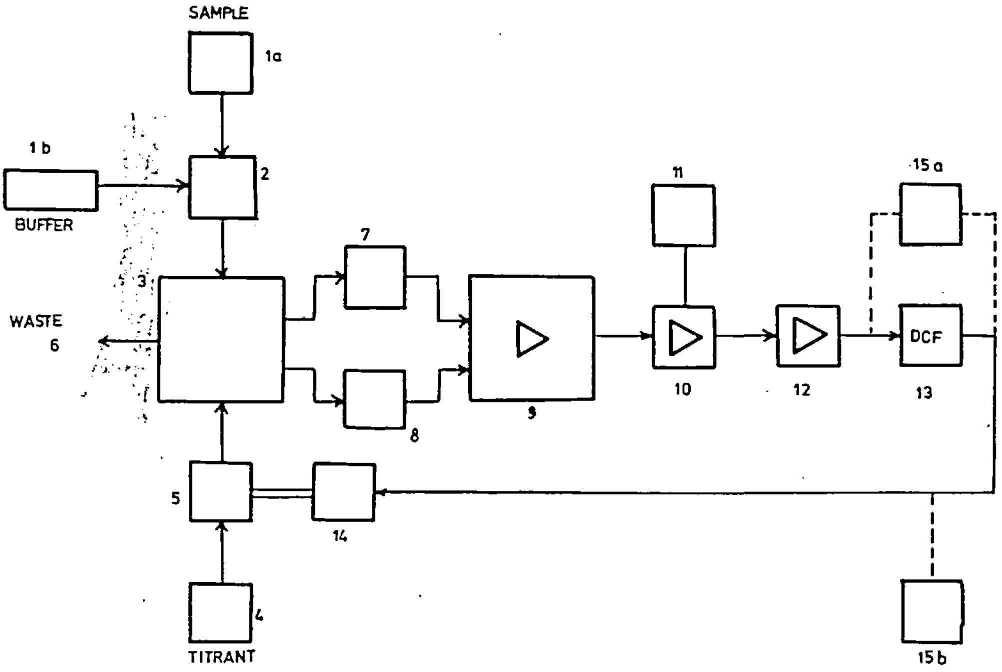


FIGURE 60. Continuous-titrator developed by Osch and Criepink[172] (see text for details).

by a peristaltic pump equipped with a stepping motor (5) into the flow-through titration vessel incorporating the indicator and reference electrodes (7, 8). A brush is rotating at a high speed for cleaning the electrodes. The cell potential goes to the comparator (10) through an impedance transformer with a high input resistance (9). The comparator subtracts the preset end-point value from the cell potential (11), and the signal thus generated goes to a PID controller (12) and goes to a DC/F converter, the impulses of which control the flow rate of the reagent solution via the stepping motor. Results are displayed in the voltmeter (15a) or the frequency meter (16b).

By correctly setting the P, I, and D controller values, a good automatic measuring system is developed which works for chloride in the concentration range of 10 to 100 ppm and has a response time of 78 sec. The short-time stability (1 day) is better than 0.1% and the long-time stability can be kept better than 1% (14 days).

Titrations using complete titration curves can be grouped on different bases, thus, on the type of the chemical reaction utilized, on the mass-flow program used for the titration, etc. In our opinion, for titrations under flow-through conditions, the most useful classification is based on the variable that is controlled; that can be the titrant or sample flow or the time allowed for chemical reaction.

In the following paragraphs several titration techniques will be presented according to the above classification. It has to be noted, however, that methods based on the evaluation of the titration curve as applied in flow-through systems are not well-elaborated or widely used.

In the gradient titration technique, described first by Eichler[173] and investigated in more detail by Fleet and Ho,[174] the concentration of the sample, the flow rates of sample, and reagent are constant, and the concentration of the reagent increases linearly. Thus, the detector signal-time function is actually a titration curve corresponding to the linear mass flow program.

Equality of the mass flows of sample and reagent, i.e., the end-point of the titration, is indicated by a potentiometric cell incorporating an ion-selective electrode. The concentration of the sample solution was found to be proportional to the time elapsed between the beginning of the reagent addition and the end-point.

In the gradient titration, the authors used Technicon® AutoAnalyzer modules; to produce a concentration gradient a method used in liquid chromatography is utilized. Accordingly, a solvent of a given volume (V_{B_0}) and concentration (C_{B_0}) is placed into a vessel equipped with a stirrer, reagent with a concentration of C_A flows in with a constant flow rate (F_A), while the titrant stream leaves the vessel with a constant flow rate (F_B). The concentration of the titrant (C_B) can be described by the following equation:

$$C_B = C_A + (C_{B_0} - C_A)\left[\frac{(F_A - F_B)t + V_B}{V_{B_0}}\right] \exp \frac{F_A}{F_B - F_A} \qquad (16)$$

A linear concentration profile is produced if the condition $F_B = 2\ F_A$ is fulfilled. Thus,

$$C_B = C_A + (C_{B_0} - C_A)/V_{B_0} \cdot (V_{B_0} - F_A \cdot t) \qquad (17)$$

This interesting technique was proved to be applicable for the mercurimetric determination of solutions containing sulfide ions. However, it can only be easily used if the gradient can be produced automatically. A drawback of the technique is related to the uncertainity of the determination of the starting point of the titration needed for the evaluation.

A linear titrant-time titration program is applied also in the triangle-programmed titration technique developed by Pungor et al.[175-176] At this variation of flow titrations the flow rates of the sample V and titrant solutions are constant and the titrant concentration is programmed. At the first part of the program, the concentration is increasing ($C_R = n.t$) for the time $t = \tau$.

It is supposed that in the flow stream the following chemical reaction takes place:

$$aS + bR \rightarrow dP_1 + gP_2 \qquad (18)$$

With time, infinitesimal solution segments with a greater and greater degree of titration are formed after the confluence of the two solutions. The titrant concentration-time program is so constructed that at time $t = \tau$ the sample solution is overtitrated. At time τ the titrant concentration has its maximum value and then it decreases continuously with the same slope *n*, and $C_R = (2\tau - t)\ n$ for the course of one triangle-programmed titration. Accordingly, the mass flows of the titrant and the sample twice achieve chemical equivalence: at times $t < \tau$ and $t > \tau$ correspondingly, two titration curves are obtained being the mirror images of each other when plotting the detector signal vs. time function. The equivalence points are well-defined, and usually can be located with high accuracy. The evaluation is based on the time interval elapsed between the equivalence points (Q). The concentration of the sample solution (C_S) can be determined according to the following equation:

$$C_S = / \, 2\tau - Q \, / \, \frac{bn}{aV} \qquad (19)$$

Thus, the method eliminates errors owing to the uncertainties in the determination of the starting point of the titration process.

To illustrate the basic idea for sample solutions of different concentrations, detector signal vs. time titration curves are shown in Figure 61, with potentiometric and amperometric detection. The latter approach measures the excess amount of the reagent.

The instrument developed for the titrimetric analysis of flowing solutions is seen in Figure 62. In order to match the apparatus to the mechanical simplicity of devices applying the flow-through analysis channel principle, current-programmed coulometric reagent generation is used for obtaining the appropriate titrant concentration-time titration program.

The electrolytic titrant-generating procedure (according to the triangular current vs. time program) takes place in a two-compartment generator cell. The half cells are separated with a dialysis membrane in order to prevent mixing of the reaction products of titrant generation. The type of the generator electrodes (1) or the generator cell depends on the nature of the titrant required for titration. The other half-cell incorporates the auxiliary electrode (2) required for the electrolysis. In the latter half-cell, an auxiliary solution of given composition is streamed, while in the generator half-cell a solution of proper composition used for the titrant generation is streamed (outer titrant generation).

The triangle-shaped current vs. time programs are produced by a potentiostat and a function generator. The sample solution (11), the titrant generating solution, as well as the auxiliary solution are conveyed by a peristaltic pump. A drip-vessel (7) ensures the instantaneous mixing of the sample and the reagent, and it electrically insulates the generator and the detection system (10). After passing through the drip vessel, the mixture of the sample and the reagent solution enter the detector cell.

So far the following reagents were prepared electrically in our work:

- Silver ions with the anodic oxidation of silver metal
- Mercury ions with the anodic oxidation of mercury
- Bromine with the anodic oxidation of bromide ions
- Iodine with the anodic oxidation of iodide ions
- Hydroxide as well as hydrogen ions with the electrolysis of the solution of an appropriate salt

With this set-up, measurements with flow-through potentiometric cells containing different ion-selective electrodes, as well as amperometric detectors with a platinum electrode, have been accomplished.

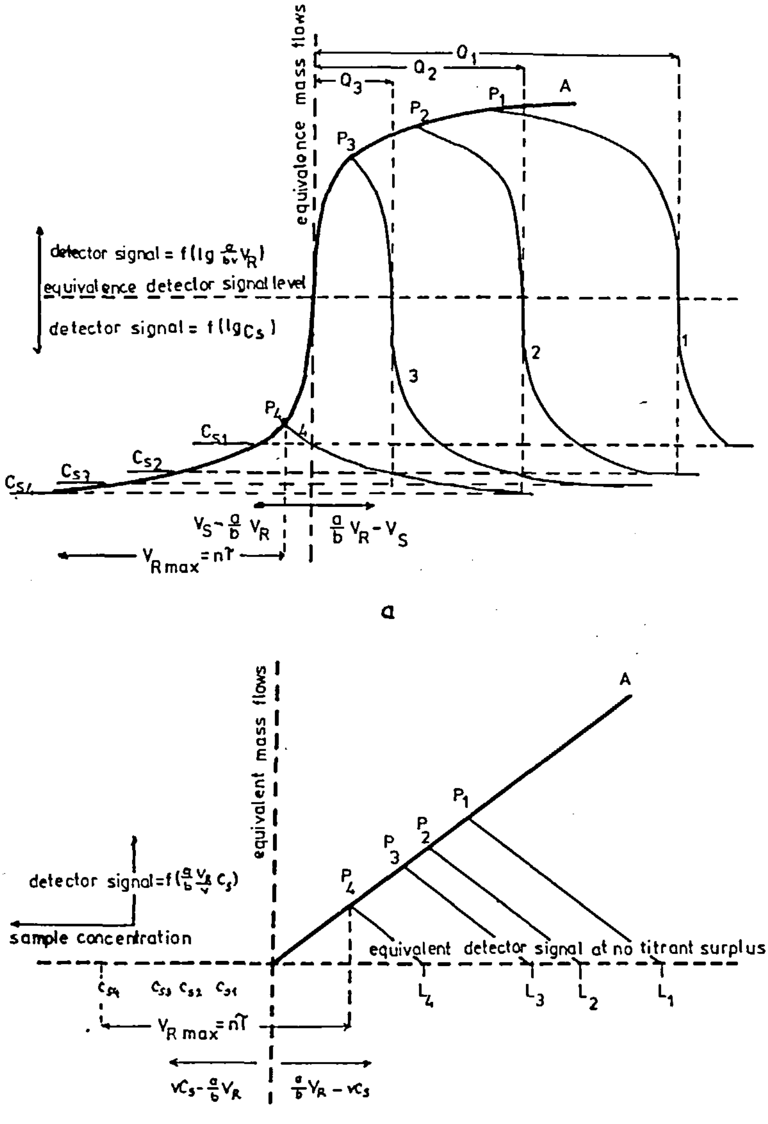


FIGURE 61. Triangle programmed titration curves obtained using potentiometric (a) and voltammetric (b) detection.

To the measuring instrument units necessary for the automatic evaluation coupled to the detector were fitted. After a titration the evaluation is performed by a minicomputer of 8 Kbyte on the basis of the detector signal-time data stored in its memory. The first step of this evaluation is the search for the two end-points of the titration, which was done by calculating the intersection of straight lines fitted to different points of the titration curve, when a detector with linear response function is used. The computer program for potentiometric titrations determines the equivalence points by calculating the second derivatives. The curve is smoothed, and from the value of Q and known values of the necessary parameters fed in previously, the computer calculates and prints the concentration of the sample.

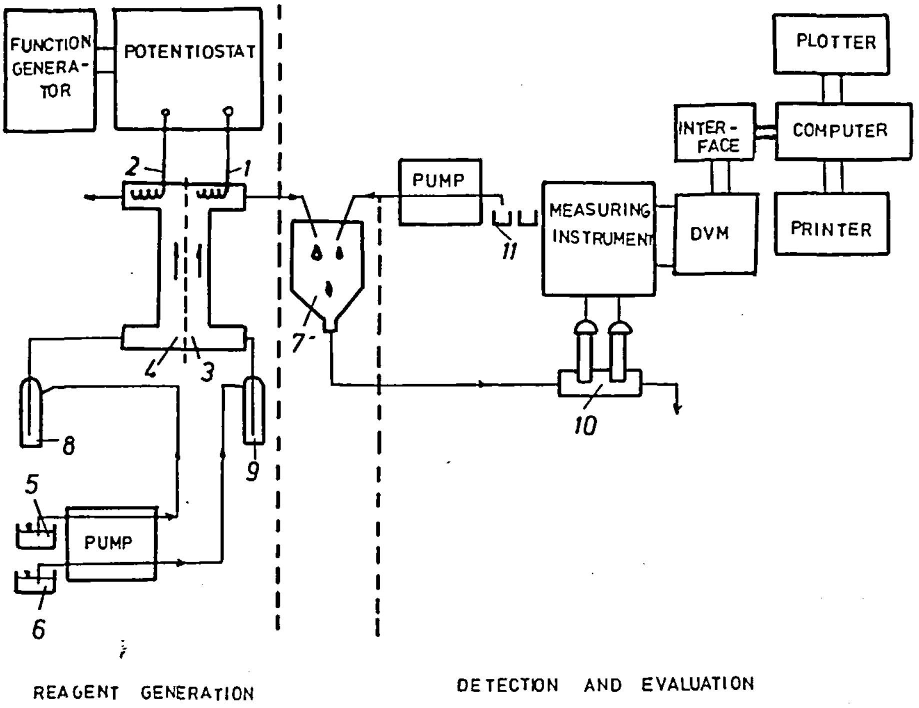


FIGURE 62. Experimental set-up used for triangle programmed titrations with electrically generated titrants (1) Generator electrode, (2) auxiliary electrode, (3) generator half cell, (4) auxiliary half cell, (5) auxiliary solution, (6) titrant-generating solution, (7) drip vessel, (8, 9) buffer vessels, (10) detector cell, (11) samples.

The triangle-programmed titration principle proved to be applicable for various analytical problems. The instrument developed for this purpose excels in disturbance-free operation and easy handling. Several publications on the successful application of the measuring technique have been issued.[177-182]

Abicht[183] developed a so-called controlled dynamic titrator based on the idea of Ashworth.[184] The scheme of the titrator is shown in Figure 63. The solvent used as a carrier and the titrant of a given concentration are fed into the small volume mixing cell (40 to 70 $\mu\ell$) with a constant flow-rate, while the sample is pumped with a linearly increasing rate. From the flow tube, which ensures adequate time for the titration reaction to occur, the mixture goes into the detector. The authors applied photometric, conductimetric, and potentiometric detection. For pumping the sample a buret was used, in which a piston was moved with a linearly increasing speed by a motor. The whole titration process is controlled by a motor-driven programmer. A typical potentiometric titration curve is seen in Figure 64.

Weak and strong acids (hydrochloric acid, potassium hydrogen phthalate, acetic acid, citric acid, oxalic acid, tartaric acid, maleic acid) were titrated with sodium hydroxide by the application of photometric and potentiometric detection. Coulometrically generated reagents were also used, e.g., for the iodometric determination of ascorbic acid and thiosulfate. Titrations were performed generally in a concentration range of 10^{-2} to 10^{-3} M with a relative error of about 1%. Reproducibility of the results was better than 1%.

Pauschmann[185] described a continuous automatic titrator, in which the sample (together with the color-indicator previously added to it) and the titrant streamed in two lines connected by a thin channel. As the two solutions flow parallelly, the reagent driven

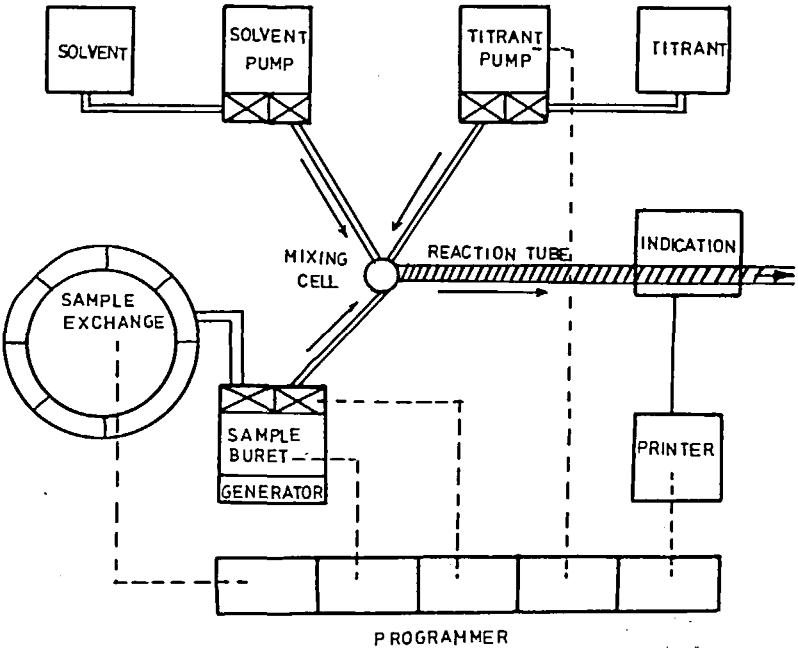


FIGURE 63. Schematic diagram of controlled dynamic titrator developed by Abicht.[183]

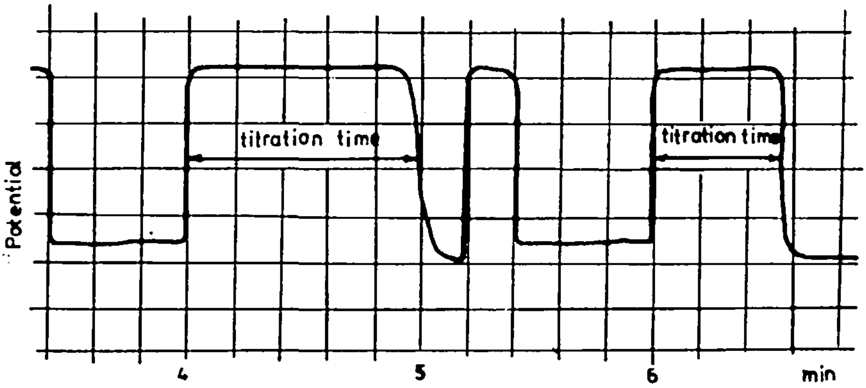


FIGURE 64. Titration curves obtained with the controlled dynamic titrator, according to Abicht.[183]

by a constant gradient diffuses into the sample solution. The whole tube system is built into a transparent plastic block. In one segment of the sample solution, the ratio of the reagent increases as the solution advances in the tube. The color indicator marks the segment in which the sample and reagent amounts are in chemical equivalence. The further in the tube the equivalence segment is, the higher is the sample concentration. In this way the distance of the equivalence segment in the tube is a measure of the concentration. By certain modifications of the instrument other indication methods can also be applied (e.g., potentiometry, conductometry, etc.).

Measurements carried out with reagent mixtures giving linear potential response at

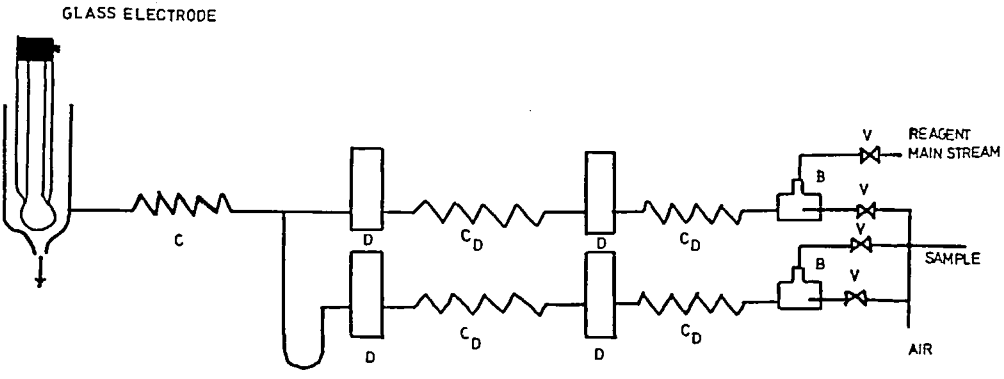


FIGURE 65. Schematic diagram of apparatus developed for measurement of basicity of water by Pungor et al.[191]

potentiometric detectors (single-point titration)[186-190] are suitable for application in flowing systems. Automated monitors can be developed taking advantage of this principle. In order to illustrate this possibility, the method and automatic apparatus developed by Pungor et al. for the determination of the basicity of Danube water can be mentioned.[191] The schematic drawing of the apparatus is shown in Figure 65. The operation of the apparatus is based on the following process: to the given volume of the reagent solution flowing with a constant rate, a given volume of the sample solution is added by means of a suitable volume quantation unit (air injector [B] and bubble sensing detector [D]) at given intervals. A pH-sensitive glass electrode placed after the confluence observes the pH difference when pure regent flow is switched to the mixed reagent-sample flow.

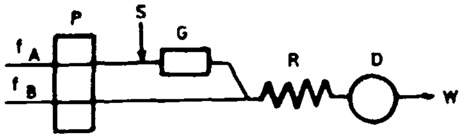
Another possibility for the application of the single-point titration method in flowing systems was pointed out by Åström.[192] He combined the flow-injection technique with the principle of the single-point titration. He determined acid and base concentrations by injection of the sample solution into a solution of the reagent of linear potential response, continuously flowing. He used a potentiometric cell with a glass electrode in a buffered medium as the detector. In this way the advantages of the use of a glass electrode in a buffered medium ensuring optimal operation of the sensor and the quickness, simplicity, and the possibility for automation of the flow-injection technique are realized.

The feature of the flow-injection technique for which evaluation of results occurs on the basis of a well-defined concentration vs. time transient signal offers a possibility for determinations based on chemical reactions. These methods can be called — with some exaggeration — flow-injection titrations.

In the system used most often for this purpose, the concentration and flow rate of the reagent are kept constant. A certain added amount of the sample meets the flow of reagent in a part of the analysis channel (constant volume mixing chamber). As the two solutions are mixed, a special concentration profile is formed in the analysis channel. That means that solution segments containing the sample material will differ in the degree of titration. The profile itself is a function of the dispersion properties of the system and the concentration of the sample. Keeping the first circumstances constant, the profile — detectable with an appropriate detector cell — is characteristic for the concentration of the sample.

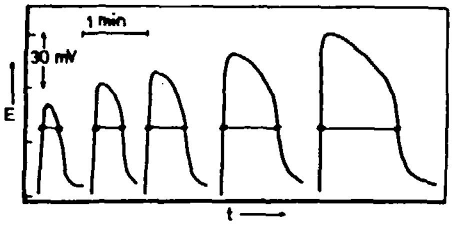
On this principle flow-injection titrations were carried out by Horvai[193] and Ruzicka.[194]

By applying this technique, the question arises how the concentration of the sample can be calculated from the detector signal vs. time function.



a;

S	sample inlet
$f_A : f_B$	flow rate of diluent and reagent,resp.
P	pump
G	gradient chamber
R	delay coil
D	detector
W	waste



b;

FIGURE 66. Measuring set-up for carrying out flow injection titrations, according to Ruzicka et al. (b) Potentiometric titration of calcium with EDTA, according to Ruzicka et al.

A consequent method with the titration technique would be where the signals were transformed into a classical titration curve. However, until now the different evaluation methods used necessitate previous calibration. For the illustration of this method the work of Ruzicka et al. is quoted. The titrating apparatus is shown in Figure 66. The instrument can work with various detectors, e.g., for determining calcium, titration with EDTA was applied by using a potentiometric detector containing a calcium ion-selective electrode.

A series of titration curves obtained in a determination of calcium is seen in Figure 66a. The determination of the reagent mass flow equivalent to the mass flow of the sample is carried out — as it is seen in the figure — on the basis of the time interval between the points of maximum detector signal changes.

VII. CONCLUSION

It is striking that in the last 20 years the different electroanalytical techniques are more and more flourishing. It is true for the development of new sensors and techniques as well as their use in different fields of chemical analysis. Looking at the electroanalytical methods in respect of automation, it can be seen that their feature is especially attractive from this aspect.

The present state of the automation of electrochemical analysis can be characterized by the appearance of automated instruments and analytical systems, the increasing use of earlier developed, but hardly used, methods, and the introduction of novel analytical techniques.

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