THE VOLTAMMETRIC APPLICATION OF SILICONE RUBBER BASED GRAPHITE ELECTRODES WITH SPECIAL REGARD TO FLOWING SYSTEMS

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ABSTRACT

The authors report on the silicone rubber based graphite electrode, a sensor applicable in the anodic polarization range. In addition to the voltammetric properties of the electrode, some applications are also presented in which the electrodes, incorporated in flow-through cells, were used for solving some practical problems.

Analysis in flowing systems is not a recent technique. The aim of the first studies of this type was the elucidation of the kinetics of fast reactions. A number of papers appeared in the 1940s in physicochemical journals on kinetic studies and apparatus in which a variety of methods were used for detection.

Later, in addition to problems of theoretical interest, some problems arose in practice which could only be solved by continuous analysis in flowing solution. The monitoring and control of continuous technologies, which are increasingly gaining ground, the better insight into biological processes, and gas and liquid chromatography are all inconceivable without detectors suitable for continuous measurement in fluid medium. The adaptation of various instrumental methods to the analysis of flowing media brings up mainly practical problems. Thus, for example, the continuous filtration of turbid solutions in spectrophotometry, the elimination of streaming potential in potentiometry, prevention of the contamination of the electrode in conductometry, and so on, constitute tasks which are difficult to solve but do not relate to matters of principle. In this respect voltammetry has a special position among the methods of instrumental analysis, since the concentration signal, the current, is controlled by the rate of the mass transport towards the measuring electrode, and thus is sensitive to the relative motion of the electrode and the medium to be analysed. Accordingly, the majority of the studies dealing with the field aim at the elucidation of the parameters of the mass transport due to the relative motion of the electrode and medium.

The general relationship to the description of voltammetric current prevailing under not purely diffusion-controlled conditions has been given by Levich[1] on the basis of theoretical considerations.

Under the conditions used in voltammetric analysis convection and diffusion play a predominant role among mass transport processes in controlling the current. The general equation for describing the convective diffusion has been given by Levich for these conditions as follows:

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} + \frac{\partial^2 c}{\partial z^2}\right) - \left(V_x\frac{\partial c}{\partial x} + V_y\frac{\partial c}{\partial y} + V_z\frac{\partial c}{\partial z}\right) \tag{1}$$

The solution of the above differential equation containing partial differential quotients and variable coefficients is rather involved and is only possible under simple geometric and hydrodynamic conditions. In *Table 1* are presented the equations for the convective diffusion-limiting current in the laminar range, for some electrodes.

In the field of voltammetry carried out under well-defined hydrodynamic conditions—which is called, according to Jordan, hydrodynamic voltammetry—remarkable work has been done by Matsuda, Kimla and Štrafelda, Jordan and co-workers, Marchiano and Arvia, Blaedel and co-workers, and Newman, as well as by Levich. Various authors have used a variety of cells, electrodes of different nature and construction, and different techniques for performing particular analytical tasks. In addition to mercury electrodes, which can be used also in flowing systems, in many cases various solid electrodes (noble metals, graphite, etc.) were used as the measuring electrode. As the reference electrode mostly electrodes of the second kind are applied —in some cases streaming second-kind electrodes to eliminate concentration polarization—as well as redox electrodes and electrodes of the first kind. Besides classical d.c. technique, differential, a.c. square-wave and pulse polarography were used. According to the aim and conditions of the measurement, evaluation can be made on the basis of the complete current (i)–potential (U) curve, or using the voltammetric current recorded at a constant potential chosen within the range of limiting current of the component studied. If the concentration or flowrate of the solution may change during the time of one measurement i–U curves recorded are difficult to evaluate. Measurement of the current at constant potential is of special importance in cases where the signal produced is used for process control. A great advantage of the method is that a simple one-function instrument is sufficient for carrying out tne measurements. Voltammetric measurements in streaming and stirred solutions are not as widely used at present as their potentialities would permit. In some fields, however, hydrodynamic voltammetry is used as a routine technique. Such is, for example, the determination of dissolved oxygen in natural waters, biological fluids, and so on. For this purpose mainly the so-called Clark electrode is used. Furthermore, methods have been developed for the continuous monitoring of other gases (SO_2^2, H_2S^2, CO^3, Cl_2^4). One of the first applications is the continuous measurement of metal ion concentration in various industrial metal salt solutions by polarography (uranium[5, 6], cadmium[7, 8], bismuth[8], copper, zinc, lead[9], etc). Recently, voltammetric detectors have been applied in automatic analysers.

Papers dealing with the application of chromato-polarography as defined

by Kemula[10] constitute a remarkable fraction of the work done using polarography in flowing systems. Polarographic detection following separation by chromatography has been used for the separation and detection of nitroanilines, chlornitrobenzenes, nitrophenols, DDT isomers,

Table 1. Limiting current equations for various types of voltammetric electrodes

Electrode shape	Equation
Rotating disc	$i_L = k\,nFAc_0\,D^{\frac{2}{3}}\,v^{-\frac{1}{6}}\,\omega^{\frac{1}{2}}$
Rotated hemispherical	$i_L = k\,nFAc_0\,D^{\frac{2}{3}}\,v^{-\frac{1}{6}}\,\omega^{\frac{1}{2}}$
Planar	$i_L = k\,nFbh^{\frac{1}{2}}c_0\,D^{\frac{2}{3}}\,v^{-\frac{1}{6}}\,V^{\frac{1}{2}}$
Tubular	$i_L = k\,nFR^{\frac{2}{3}}\,x^{\frac{1}{3}}\,c_0\,D^{\frac{2}{3}}\,V^{\frac{1}{3}}$
Conical	$i_L = k\,nFAL^{-\frac{1}{3}}\,c_0\,D^{\frac{2}{3}}\,v^{-\frac{1}{3}}\,V^{\frac{1}{3}}$
Disc	$i_L = k\,nFAL^{\frac{1}{3}}\,c_0\,D^{\frac{2}{3}}\,v^{-\frac{1}{3}}\,V^{\frac{1}{3}}$
Spherical	$i_L = k\,nFa^{\frac{1}{2}}\,c_0\,D^{\frac{2}{3}}\,V^{\frac{1}{2}}$

i_L is the limiting current; k is a rate constant; n is the number of electrons taking part in the electrochemical reaction; F is the Faraday constant; A is the geometric surface of the indicator electrode; c_0 is the concentration of the electroactive component in the moving solution; D is the diffusion coefficient of the electroactive component; v is the kinematic viscosity of the solution; V is the flowrate; ω is the rotation speed; and b, h, R, x, L, a are the characteristics of the electrodes.

nitroalcohols, nitropropane and nitrobutane isomers among others by Kemula and co-workers[11]. Chromato-polarography has successfully been used also in the analysis of metals[12] and inorganic anions[13].

THE SILICONE RUBBER BASED GRAPHITE ELECTRODE

Various carbon electrodes can be used to advantage as indicator electrodes in hydrodynamic voltammetry in the positive potential range, since they are more stable and show memory effects less frequently than noble metal electrodes.

My co-workers and I succeeded in developing an electrode from spectrally pure graphite embedded in silicone rubber[14]. The anodic polarization range of the electrode in aqueous solutions is $+1.3$–$+1.4$ V (versus SCE), depending on the medium used. The electrode has the advantages that it is solid and its well-defined measuring surface can be prepared in various sizes, from a few cm² down to a few mm²[15]. The residual current is of the order of one-hundredth μA at $+0.7$ V at the commercially available electrode[16] having a surface area of 0.25 cm².

The response time of the electrode is shorter than the delay of the common recording devices. The standard deviation which is characteristic of the reproducibility of the peak current was found to be 0.5 per cent for components which give a reaction product soluble in the medium used. In Table 2 some compounds are given which have successfully been measured by voltammetry, using a silicone rubber based graphite electrode.

APPLICATION OF SILICONE RUBBER BASED GRAPHITE ELECTRODE IN FLOWING MEDIUM

Measuring cells and methods

For the purposes of continuous measurements silicone rubber based graphite electrodes were usually prepared with a measuring surface of 2–10 mm^2. In most cases an Ag/AgCl electrode of the second kind of streaming solution-phase was used in cell as reference electrode. In most of the measurements it was possible to incorporate the reference electrode into the cell, and, using constant chloride concentration, the streaming solution itself served as the solution of the reference electrode. In some cases a saturated calomel electrode was used as the reference electrode.

Table 2. Half-peak potentials of some compounds at silicone rubber based graphite electrode

Material tested	Supporting electrolyte	$E_{p\frac{1}{2}}$ (V versus SCE)
Hydroquinone	phosphate buffer pH = 7.0	+0.23
p-Phenylene diamine	0.1 M HCl	+0.505
p-Toluidine	0.1 M HCl	+0.83
p-Phenetidine	phosphate buffer pH = 7.0	+0.29
α-Naphthylamine	0.1 M KCl	+0.26
Oxytetracycline	0.1 M KCl	+0.45
Ascorbic acid	B–R buffer pH = 4.0	+0.41
Pyrogallol	B–R buffer pH = 7.0	+0.06
Adrenaline	B–R buffer pH = 3.61	+0.64
Gallic acid	B–R buffer pH = 3.61	+0.40
Isoprenaline	0.1 M KCl	+0.54
α-Methyl-DOPA	0.1 M KCl	+0.60
Chlorpromazine	0.1 M KCl; 0.01 M HCl	+0.80
Diethazine	0.1 M KCl	+0.75
Amidopyrine	0.1 M KCl	+0.45 and +0.7
Uric acid	phosphate buffer pH = 8.3	+0.25
Adenine	acetate buffer pH = 4.8	+1.04
Guanine	acetate buffer pH = 4.8	+0.81
Xanthine	phosphate buffer pH = 8.3	+0.96
Hypoxanthine	phosphate buffer pH = 8.3	+1.12
Adenosine	phosphate buffer pH = 8.3	+1.12
Guanosine	acetate buffer pH = 4.8	+0.80
5'-GMP	acetate buffer pH = 4.8	+0.98
Morphine	4 N H$_2$SO$_4$	+1.0
Ethylmorphine	4 N H$_2$SO$_4$	+1.0
Papaverine	4 N H$_2$SO$_4$	+1.0
Codeine	4 N H$_2$SO$_4$	+1.1

The cell types most often used in our measurements are shown in *Figure 1*. The cells were made in different sizes, according to the particular task to be performed. In the tubular cell shown in *Figure 1(a)* the flow was laminar in the range of flowrates used. In the cell shown in *Figure 1(b)* the direction of the flow is perpendicular to the electrode surface, and the narrow portion of the tube just before the electrode serves to increase the flowrate. Consequently, the flow is turbulent at the electrode surface.
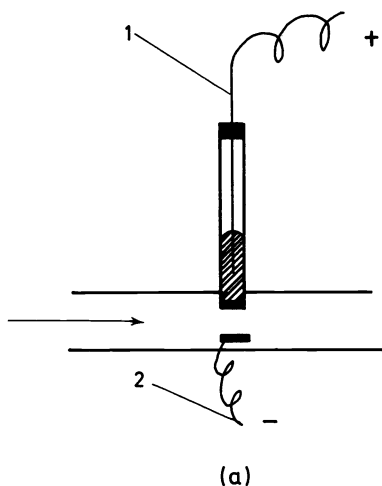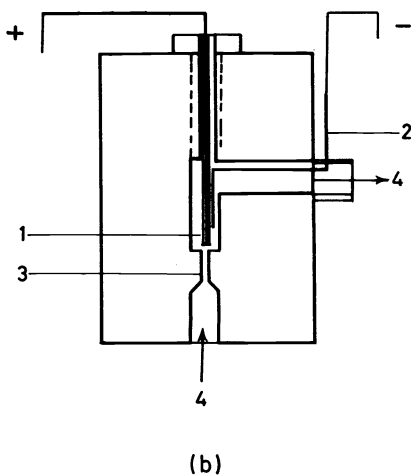
Figure 1. Measuring cells with silicone rubber based graphite electrode. (a) Tubular flow-through cell: 1, indicator electrode; 2, Ag/AgCl reference electrode. (b) Cell constructed for the purposes of chromatographic detection: 1, indicator electrode; 2, Ag/AgCl reference electrode; 3, narrowing; 4, fluid in and out

In some cases, if it was necessary, a separate unit was used for sample or reagent introduction before the measuring cell. The voltammetric current was recorded at constant potential chosen in the limiting current range.

Properties of the measuring system applied

Owing to the simple construction of the cells, the retention volume (the volume of solution passing through the cell during the time elapsed from the appearance of the signal to the point where it reaches 90 per cent of its value) after a sudden change in the concentration is 0.5–3 ml at a flowrate of about 0.1 ml/s, depending on the diameter of the cell and the size of the electrode.

The stability of the cells was checked and found to be very good, as no change was found in the current when a solution containing an electroactive compound giving a soluble product was passed through the system continuously for 8 h. The change in the potential of the Ag/AgCl electrode with streaming solution phase was negligible in the measurements.

Studies on the relationship between voltammetric limiting current and analytical data have shown that a linear relationship exists between the current measured at constant potential and the concentration of the electroactive component within a wide concentration range (about 10^{-6}–10^{-3} M).

The correlation between the voltammetric limiting current and the flowrate can be given, in accordance with theoretical considerations, for cells containing electrodes parallel to the direction of flow, as follows:

$$i_{\mathrm{L}} = knFD^{\frac{2}{3}} v^{-\frac{1}{6}} Lc(a + \sqrt{V}) \tag{2}$$

where i is the voltammetric limiting current; k and a are numerical constants; n is the number of electrons involved; F is Faraday's constant; D is the diffusion coefficient; v is the kinematic viscosity; c is the concentration of the electroactive component in the solution; L is a number depending on the size of the electrode; and V is the flowrate.

SOME APPLICATIONS OF VOLTAMMETRIC MEASUREMENTS IN FLOWING SYSTEMS

Only some methods and devices out of the great number of possible applications of the technique will be given here, which were developed in our Institute. The reason for developing the methods and devices to be reviewed here was our intention to solve some real practical problems.

The methods developed can be classified into two groups: (1) monitoring of processes involving changes in the concentration of electroactive components, and (2) the injection technique (see below).

(1) Monitoring of processes involving changes in the concentration of electroactive components—(a) Dissolution of drugs from pharmaceutical products

A number of organic compounds with therapeutic activity can be determined by voltammetry using the silicone rubber based graphite electrode. In this way the process can be controlled in various phases of the production, and the active ingredient in the final product and dissolution rate thereof can be determined. The latter is a very important parameter of pharmaceutical products and needs to be controlled. This property must be variable over a wide range to encompass products which are assumed to have fast action and those having a long-lasting effect.

The scheme of the apparatus constructed for measuring the rate of dissolution by voltammetric detection is shown in *Figure 2*. The principle of the operation of the apparatus is that sampling from the dissolution vessel (1) is made continuously by a pump (2) ensuring constant volumetric flowrate. This sample is passed through the measuring cell (3) and returns to the dissolution vessel. The current versus time curve recorded during the dissolution of the tablet can be considered as an integrated dissolution curve on the basis of the linear relationship proved to exist between the current measured at constant potential and concentration of the electroactive component in the solution (*Figures 3* and *4*).

An apparatus based on the above principle has been constructed in our Institute which can be operated with both manual and automatic control. The scheme of the apparatus is shown in *Figure 5* and that of the mechanical part in *Figure 6*.
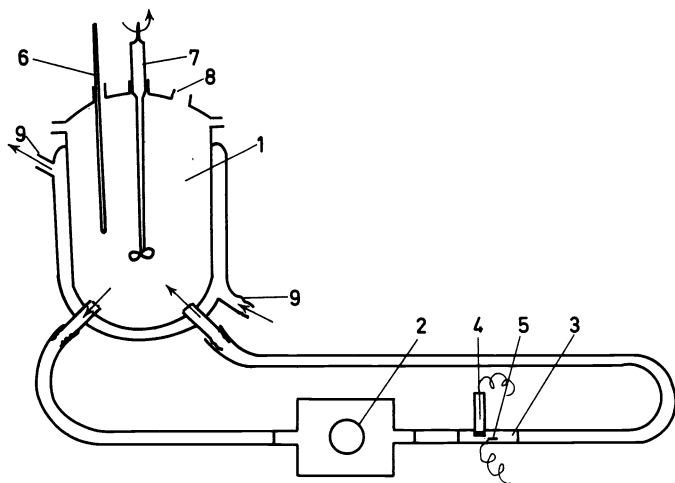
Figure 2. Schematic diagram of the apparatus constructed for measuring dissolution rates: 1, dissolution vessel; 2, pump; 3, measuring cell; 4, indicator electrode; 5, reference electrode; 6, thermometer; 7, stirrer; 8, drug in ; 9, thermostat

(b) Chromato-voltammetric detector cell with silicone rubber based graphite electrode[17]

It has been found in preliminary experiments (see Table 2) that the four non-substituted purine bases can be determined by voltammetry using a silicone rubber based graphite electrode. The flow-through cell operating in the laminar range proved to be not sensitive enough to be used in chromatographic analysis.
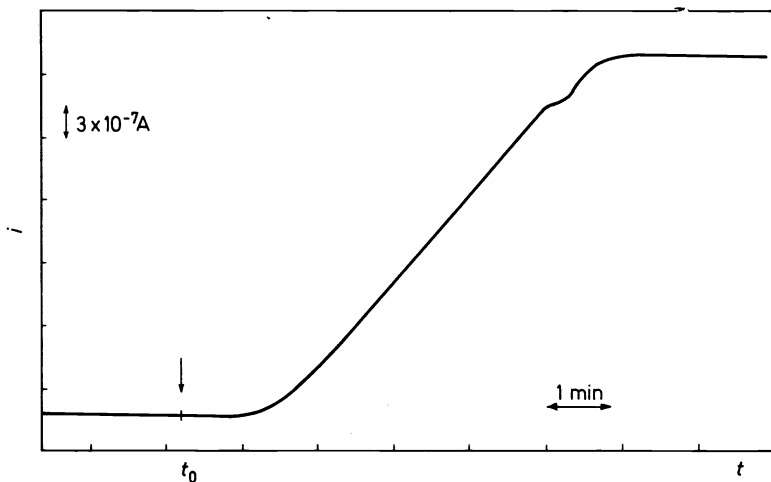
Figure 3. Dissolution curve of a tablet containing amidopyrine (Germicid). Dissolving medium: 10^{-1} M NaCl, 10^{-2} M HCl; $t = 37°C$; potential $= +0.8$ V
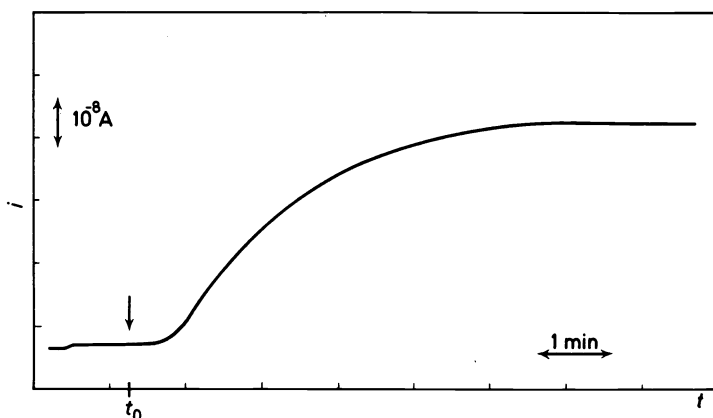
Figure 4. Dissolution curve of a dragée containing promethazine (Pipolphen). Dissolving medium: 10^{-1} M NaCl, 10^{-2} M HCl; $t = 37°C$; potential $= +0.7$ V

The sensitivity of the method could be increased by ensuring turbulent flow at the electrode surface (*Figure 1b*). Investigations concerning the characteristics of the turbulent flow in the cell are in progress. In *Figure 7* is shown the chromatogram obtained in the separation of the four non-substituted purine bases on a 100×0.9 cm Sephadex G-10 column. The 0.1 ml solution poured on the column contained 1.25×10^{-7} mol of each of the four components. The sensitivity of the cell with turbulent flow can
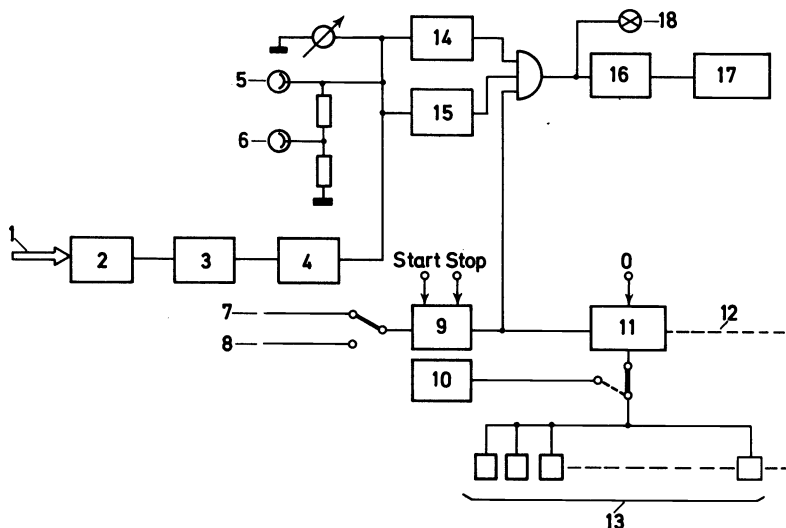
Figure 5. Block diagram of the apparatus for tablet testing: 1, drug introduction; 2, dissolution vessel; 3, measuring cell; 4, amplifier; 5, digital voltmeter; 6, recorder; 7, normal program; 8, fast program; 9, time-base; 10, manual control; 11, program-control; 12, cyclic program; 13, operational controllers; 14, high-level discriminator; 15, low-level discriminator; 16, timer; 17, digital display; 18, level indication
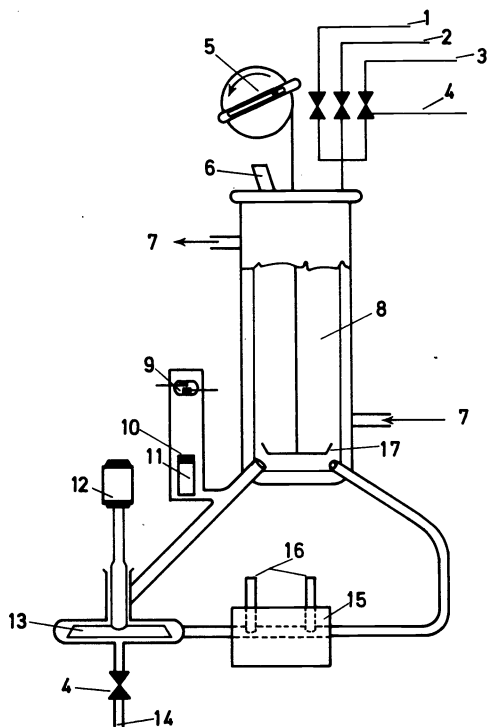
Figure 6. Diagram of the mechanical part of the tablet-testing apparatus: 1, artificial gastric juice; 2, rinsing water; 3, artificial intestinal juice; 4, magnetic valves; 5, mechanism for tray driving; 6, drug introduction; 7, thermostat; 8, dissolution vessel; 9, Reed relay; 10, magnet; 11, glass float; 12, stirring motor; 13, glass centrifugal pump; 14, outlet; 15, measuring cell; 16, electrodes; 17, tray

be characterized by stating that it detects as little as 10^{-10} mole of material. The standard deviation, which characterizes the reproducibility, is 3 per cent in the lower range of determination.

(c) *Continuous measurement of the concentration of saturated chlorine water using a gas-diffusion sampling unit*
   In a particular case we had to construct a device for the measurement of
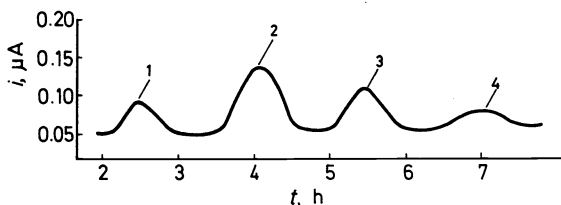

Figure 7. Separation of purines on 100×0.9 cm Sephadex G–10 column. Eluant, 0.05 M NaCl; sample, 1.25×10^{-7} mol of each purine per 0.1 ml; potential, $+0.96$ V; flowrate, (0–6.5 h) 30 ml h^{-1}, (65–9 h) 90 ml h^{-1}. 1, hypoxanthine; 2, xanthine; 3, guanine; 4, adenine

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chlorine in streaming aqueous solution. As shown by our earlier studies[18], chlorine can be determined in acidic chlorine water using silicone rubber based graphite electrode. Under the given conditions, however, the chlorine concentration was too high to be determined by voltammetry and the acid to be added for the measurement to be feasible disturbed the prevailing equilibria, and therefore could cause erratic results.
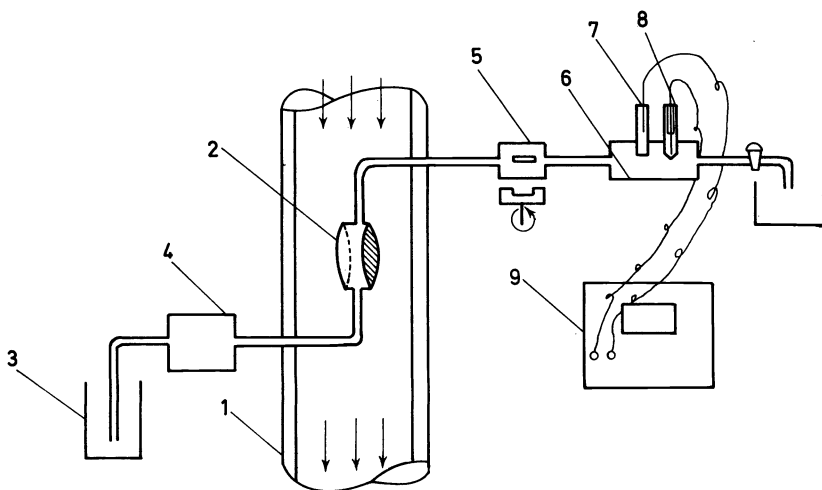

Figure 8. Scheme of a voltammetric analyser working with a gas-diffusion sampling unit: 1, sample introduction; 2, sampling cell; 3, solution reservoir; 4, liquid pump; 5, stirrer; 6, measuring cell; 7, silicone rubber based graphite electrode; 8, saturated calomel electrode; 9, measuring instrument

To overcome this difficulty, a special sampling unit was applied, which was a drum-shaped cell the bases of which were made from a membrane permeable to gases only. The experimental set-up is shown in *Figure 8*. The chlorine water to be analysed flows in a tube (1). The sampling cell (2) is placed within the tube, with the bases of the cylinder parallel to the direction of flow. The 10^{-1} M H_2SO_4 supporting electrolyte is delivered from a reservoir (3) by a pump (4) at a suitable constant volumetric flowrate. The solution within the sampling unit is homogenized by a stirrer (5). The solution containing chlorine in a concentration depending on the chlorine content of the flowing sample solution and on the volumetric flowrate in the measuring system passes through the measuring cell and then goes to waste. Evaluation was made on the basis of the voltammetric current recorded at a constant voltage ($+0.3$ V) applied between the silicone rubber based graphite (7) and saturated calomel electrode (8). The advantage of the system is that the sampling unit makes the method selective, and also that the flowrate of the sample solution has practically no influence on the results.

(2) *Method for the determination of the concentration of single solution samples and of streaming solutions by the injection technique*[19, 20]
    The increase of the demands placed on the analysis of single samples in

the past decades concerns not only the reliability and accuracy of the results but also the amount of data obtained. This latter requirement arising in some fields (clinical analysis, quality control) was very effective in promoting the development of automatic analysers. Modern auto-analysers usually contain a continuously flowing system, which eliminates difficulties encountered in the separate treatment of the samples; that is, the analysis can be performed with a simpler device. In the automatic analysers at present available mostly optical methods are used for detection.
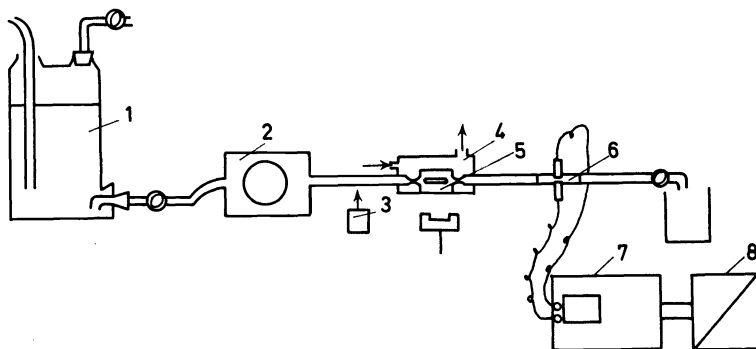

Figure 9. Measuring set-up for the injection technique: 1, solution reservoir; 2, peristaltic pump; 3, injection unit; 4, thermostat; 5, stirrer; 6, detector cell; 7, measuring instrument; 8, integrator unit

We have developed a method using the cell mentioned earlier, which contains silicone rubber based graphite electrode and works in the laminar range, which may later serve as the basis of an automatic analyser. The principle of the injection technique—a device for the application of which is shown in *Figure 9*—is that a small volume of such a solution is injected fairly rapidly through the wall of a tube containing a solution streaming at a constant volumetric flowrate, which causes a remarkable change in the parameter of the streaming solution indicated by the detector cell. A small flow-through mixing unit between the place of injection and the detector cell serves for delivering into the detector cell a solution homogeneous in the direction perpendicular to that of the stream. The signal—which is in a well-defined correlation with the concentration, although the nature of the correlation depends on the mode of detection, on the rate of possible chemical reactions—produced by the detector cell after injection of a small dose changes along a maximum-type curve with time (*Figure 10*).

The shape of the curve can be explained by considering that the signal characteristic of the flowing solution changes as the dose reaches the detector, and falls back to its original value with the passage of the material. Accordingly, a well-defined area (T) is determined by the signal versus time curve and the baseline for a given sample, which can be used for estimation.

The injection technique has been combined with various methods of detection (photometric, potentiometric and voltammetric) in our Institute.

On this occasion only the results of measurements obtained using a voltammetric detector cell containing silicon-rubber based graphite electrode will be presented.

According to theoretical considerations, if an electroactive solution is injected into a stream of the supporting electrolyte, the electrochemical reaction is fast, and the stirring unit shown in *Figure 9* together with a detector cell is used for which equation 2 is valid in the given range of concentration and flowrate, the area (*T*) mentioned above can be given as follows:

$$T = (KM/V)(a + V^{\frac{1}{2}}) \tag{3}$$

where M is the amount of material injected, V is the flowrate of the solution, a is a constant, and K is a coefficient given by $K = knFD^{\frac{2}{3}} v^{-\frac{1}{2}}L$.
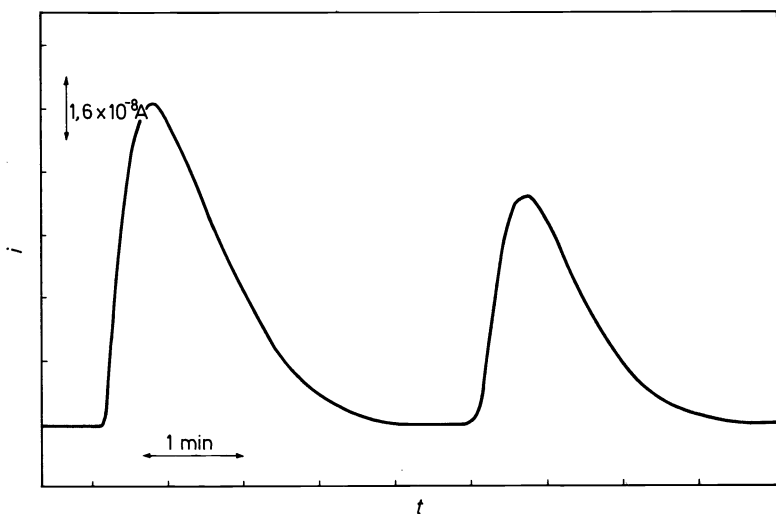

Figure 10. Recordings after injection

The physical significance of T in this case is the amount of charge passing through the cell during the time of analysis. The validity of the above equation was supported by the linear calibration graphs obtained (*Figure 11*) and the agreement of T values calculated and measured at different flowrates of the supporting electrolyte (*Figure 12*).

It can be concluded that small volumes of electroactive solution samples can be analysed by the technique described.

The electrochemical activity of the solution passing through the detector cell may be changed not only by an electroactive sample injected but also by a chemical reaction taking place between the injected and the flowing solution. In view of this the field of application of the injection technique may be widened remarkably, as, by appropriate choice of the streaming supporting electrolyte and sample injected nearly all tasks can be performed which can be done by amperometric titration.

Accordingly, an injection technique can be used, beside the analysis of electrochemically active samples, in the following cases:
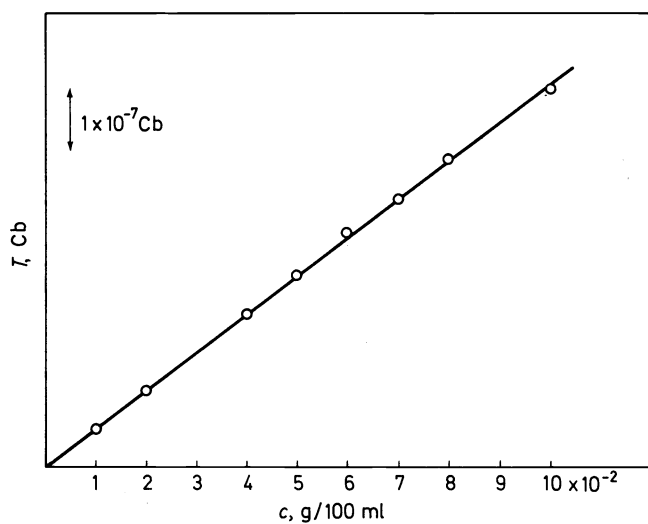
Figure 11. Calibration curve obtained for chlorpromazine using the injection technique. Supporting electrolyte, 10^{-1} M KCl, 10^{-2} M HCl; potential $= +0.7$ V; $V = 7.5$ ml/min; amount injected, 100 μl
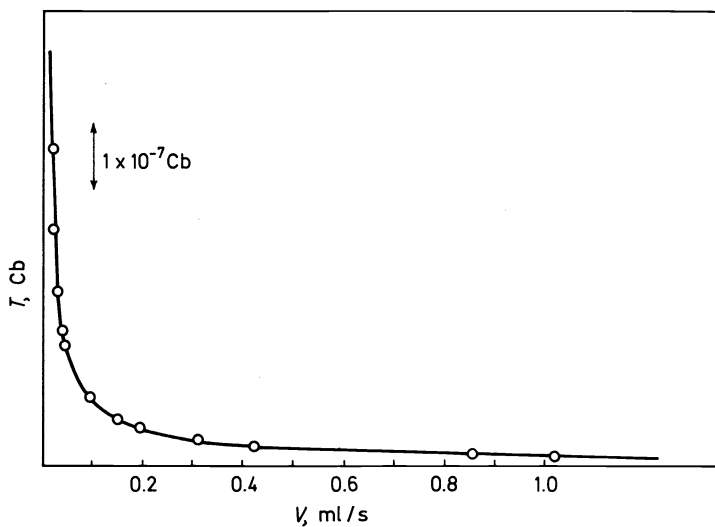

Figure 12. Comparison of calculated and measured T-values. Supporting electrolyte, 10^{-1} M KCl, 10^{-2} M HCl; test solution, 10^{-2} M chlorpromazine; amount injected, 100 μl; potential $= +0.7$ V. — Calculated from equation 3; ○ measured

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(a) *Analysis of electrochemically inactive samples*—In this case the electro-chemically inactive sample can be injected into two types of reagent solutions: in the first case, the sample is injected into a supporting electrolyte containing an electroactive component which reacts with the sample to be analysed and the product of reaction is electrochemically inactive. Thus the signal characteristic to the reagent solution first decreases, and then increases to reach the original level again, that is, a so-called negative peak is obtained.

In the second case the sample is injected into a supporting electrolyte containing a reagent which is not electrochemically active but produces an electrochemically active compound in a reaction with the sample.

In this case a positive peak is obtained the area of which increases with increasing concentration of the sample to be analysed.

(b) *Analysis of flowing electroinactive solutions*—In this case a reagent solution is injected into a steam of the solution to be analysed, which also contains supporting electrolyte. In this way a chemical reaction is caused to occur which results in a change in the electrochemical activity indicated by the detector cell. The reagent may be an electroactive component which gives an electroinactive product in the reaction with the sample.

The peak area obtained will correspond to the total amount of reagent injected if the streaming solution does not contain the component to be analysed. The concentration of the component can be determined from the reduction in peak area.

The reagent itself may be electroinactive, if the product of its reaction with the sample is electroactive. In this case a positive peak is obtained the area of which increases with increasing concentration of the component to be determined.

Whether a compound is electroactive or electroinactive obviously depends on the conditions used.

Whether the sample is chosen to flow or to be injected, depends in certain cases on the task to be performed; sometimes the sample concentrations determine which mode is to be used.

Our experience gained in connection with the injection technique can be summarized as follows:

(i) it is expedient to choose the constant electrode potential used in the measurements within the limiting current range of the voltammetric curve of the electroactive component.

(ii) the relationship between the flowrate and the signal obtained is given by equation 3 for determinations not involving chemical reactions and for those in which the chemical reaction is instantaneous. In the case of slow reactions the flowrate has an influence on the signal by influencing the time of reaction as well.

(iii) one of the points to be considered in choosing the concentration of the reagent solution is that the electroactive reagent or the electroactive product of chemical reaction should give a signal proportional to the concentration in the entire concentration range occurring in practice. Another point is that the reagent should be in excess (taking also the dilution into consideration).

(iv) the medium used may affect the electrochemical reaction; thus for

example affecting the potential to be used, and also the rate of the chemical reaction.

(v) calibration of the indicator electrode can be done by injection of a standard solution of known concentration in the case of analysis of solution samples, or by passing the supporting electrolyte or a standard solution through the system if the concentration of flowing solutions is to be determined.

Using the injection technique, a method has been developed for the analysis of injection solutions containing various derivatives of phenothiazine as the active ingredient. Our technique is particularly useful in this case, as the analysis of rather concentrated solutions (2.5 per cent) can be carried out without preliminary dilution. In the determination of electroinactive compounds, complexation, oxidation or reduction and precipitation reactions have been used. Only two examples of these applications will be noted here.

Small concentrations of phosphate have been successfully determined. A reagent solution containing Fe^{3+} ions was added to the sample solution adjusted to a suitable pH and the voltammetric reduction limiting current of Fe^{3+} was recorded. A linear relationship was found to exist between the decrease in the charge passed through on the addition of a single dose and phosphate concentration[21].

The heavy metal ions in electroactive samples could sometimes be successfully determined by injecting them into a stream of solution containing ascorbic acid. In this case a linear correlation was found to exist between the area of the negative peak and the amount of metal ion present in the solution injected.

APPLICATION OF THE SILICONE RUBBER BASED GRAPHITE ELECTRODE TO *IN VIVO* MEASUREMENTS[22]

As very small concentrations can be measured using the method and cell developed, and the cell has a fairly high stability and can be prepared in very small sizes, the method could be used in *in vivo* measurements. The purpose of our measurements of this type was not to obtain information about the absorption, metabolism and clearing out of drugs (studies of this type are important for those working in the field of pharmaco-kinetics) but to gain experience concerning the applicability of the system to *in vivo* measurements. It has been checked in *in vitro* preliminary experiments that blood, due to its sufficiently high and constant salt content, may serve as the supporting electrolyte. As a reference electrode, the Ag/AgCl electrode can be used, the solution phase of which is the blood sample itself. In *in vivo* measurements a tubular flow-through cell with a diameter of 1–2 mm was used. The tube was introduced into the artery and vein in the rear limb of a narcotized test animal (cat) in a way that the measuring cell connected the two ends of the vein previously cut. Thus the cell did not cause any change in the blood stream, and as the analysis was essentially carried out in a closed circulatory system, it did not cause any loss of blood either. Simultaneously with voltammetric measurements, other parameters characteristic of a living organism were also measured by ordinary methods. The results of our measurements can be summarized as follows:

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(i) Since the voltammetric current measured is very sensitive to changes in the flowrate, very small amounts of compounds which influence the flowrate, e.g. sympathomimetics, can be measured.
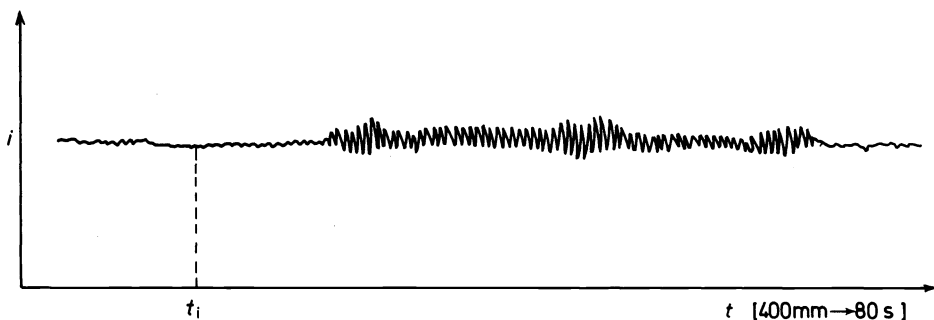

Figure 13. Effect of noradrenaline on the voltammetric current recorded in vein. Drug amount, 1 μg/kg; potential = +0.9 V

As shown in *Figure 13*, the amplitude of the oscillation of the current due to the heart beating, increases to 3 to 5-fold on introducing 1 μg/kg of noradrenaline.

(ii) The distribution of the electroactive drug (e.g. ascorbic acid, amidopyrine, promethazine) introduced by intravenous injection is indicated by both cells, that incorporated in the artery and that in the vein. The current versus time curves provide valuable information concerning the conditions prevailing in a living organism.
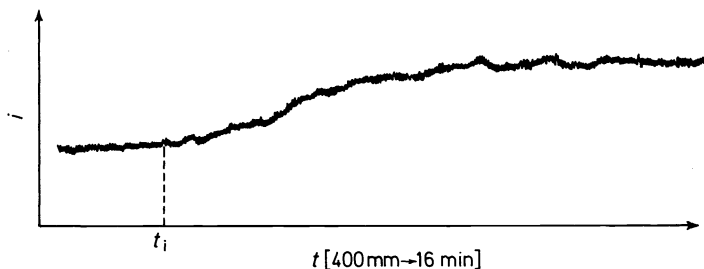

Figure 14. Current–time curves recorded in vein after the administration of amidopyrine. Drug amount, 15 mg/kg; potential = +0.8 V; t_i = time of the injection

In *Figures 14* and *15* are shown the voltammetric current versus time curves obtained by the detectors in the vein and artery, respectively.

The curves give information about the streaming of drug through the circulatory system, distribution in the body and evacuation from it.

There is a linear correlation between the amount of solution added by intravenous injection and the integral with respect to time of the current intensity rises recorded using the detector in the artery (*Figure 16*).
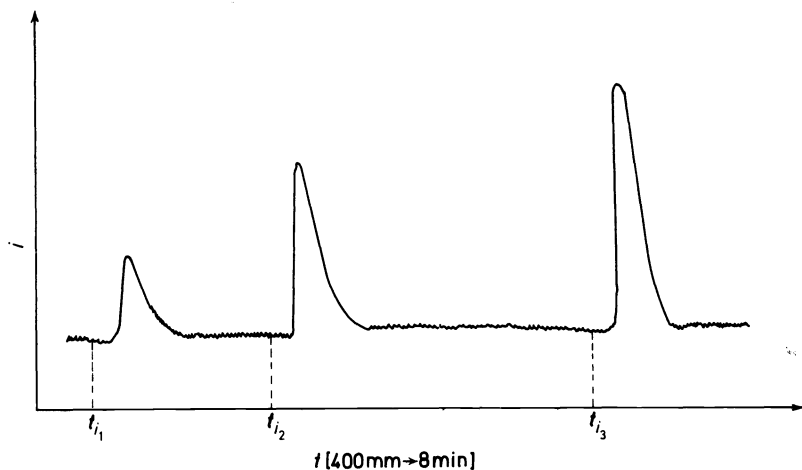
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t [400 mm → 8 min]

Figure 15. Current–time curves recorded in artery after the administration of amidopyrine. Drug amounts, 0.4; 0.8 and 1.2 mg/kg, potential = +0.8 V, t_i = time of injection
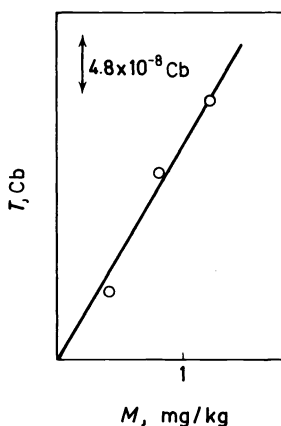

M, mg/kg

Figure 16. Relationship between peak area and amount of drug injected. Potential = +0.8 V

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