FIA - ELECTROANALYTICAL TECHNIQUES FOR PHARMACEUTICALS

A. Danet, [1], L. Lahuerta Zamora[2] and J. Martínez Calatayud[3].
[1] Department of Analytical Chemistry. University of Bucarest. Bucarest. Rumania
[2] Colegio Universitario de Farmacia C.E.U. *San Pablo*. Universidad de Valencia. 46100, Moncada, Valencia, Spain
[3] Departamento de Química Analítica. Universidad de Valencia. 46113, Burjassot, Valencia, Spain

Summary

Published articles dealing on pharmaceutical determinations on the basis of a FIA assembly provided with a electrochemical detector are reviewed.

A panoramic view is presented with special emphasis on the analytical figures of the each reported procedure. The characteristics of each detector in flow conditions are also described.

Key-words: Electroanalysis, Flow - Injection, Pharmaceuticals.

Electrochemical methods are very well suited to flow measurements in view of their sensitivity, controllable selectivity, good precision and accuracy, simplicity and easy of signal handling and automation [1 -5].

On the other hand they often suffer from interaction between the sensor and the test liquid leading to poor stability and reproducibility of the active electrode surface [4-6]. To overcome this problem, the experimental conditions must be judiciously selected, which requires knowledge of certain electrochemical principles and experience. This requirements is partly responsible for the fact that electrochemistry is often less popular in analytical laboratories than it deserves.

In our opinion ion selective electrode (ISES) potentiometry together with amperometry, voltammetry and polarography are most important in flow analysis.

Potentiometry

Compared with any other device potentiometry with ISEs is the most economic choice. Potentiometry with

[3] To whom correspondence should be addressed.

ISEs is attractive for FI detection because its simplicity and selectivity. However problems might arise because of the slow response of the sensor, particularly at low analyte concentrations [5].

When flow-through potentiometric detection is used, the electrochemical properties, of the sensors incorporated in the detector cell play the predominant role. This is in contrast with voltammetry under hydrodinamically controlled conditions when the geometry of the detector cell and the hydrodinamic conditions are prevailing [7].

The most important characteristics of the potentiometric sensors which must be studied in order to apply these in flow injection analysis are: measuring range, response time, selectivity, stability and life time.

With the FI technique the linear response range (i.e. the E vs. log a_i plot) as well as the lower limit of detection of the sensors are generally worse than in continuous flow or in batch measurements based on the same measuring cell, instruments, etc. This finding can be attributed partly to the sample dilution, but more important to the dynamic response characteristics of the potentiometric sensors.

The use ISE as detectors in FIA offer the following advantages:

I) ISEs are not affected by the color or turbidity of the sample;

2) ISEs normally do not need the addition of an expensive reagent, since they are in many cases sufficiently selective;

3) Potentiometry offers an extremely small dead volume in the detector (wetted ISE surface);

4) Potentiometry is simple and the instrumentation is not expensive;

5) ISE potentiometry gives information about the state of oxidation or binding, which are essential in speciation analysis;

6) ISE potentiometry can be used over a wide concentration range.

Flow-through arrangements with ISE have been described by Cammann [1]. FIA, with its continuous and controlled flow of certain carrier solutions, is especially beneficial to ISE measurements for the following reasons:

I) The hydrodynamic in front of the sensing electrode surface responsible for the thickness of the Nernst diffusion layer, are controlled and stable. This results in a very reproducible potential generation.

2) In several cases, the selectivity of an ISE can be drastically improved, since the time in which the sample segment passes the sensor surface is too short to establish the full equilibrium potential for the interfering ions;

3) Contamination problems encountered in normal "beaker analysis", in which the salt bridge solution leaks into the sample via the normally used highly diffusible sleeve diaphragm are eliminated since the surface electrode can be positioned downstream.

Amperometry

Another important and well-developed electrochemical technique which can easily be combined with the FIA principle is amperometry. In this technique, the current is measured while the working electrode potential is maintained at a certain fixed potential suitable for an electrochemical oxidation or reduction of the substance to be determined. Since excellent electrochemical detectors [8-10] are commercially available, their superior sensitivity (pg range) can be used to advantage in FIA work. Amperometric detectors are currently the most widely used electrochemical detectors because they provide the best detection limits. However FIA is based on a specific detector, the working conditions for an amperometric detector should be carefully chosen. Often in FIA pharmaceutical analysis the substance to be sensed is the only electroactive (oxidizable or reducible) compound in the sample and the specificity could be not a major problem. If interfering compounds which are electroactive are present in the rather large "potential window" of classical amperometry at a fixed and controlled voltage, modern pulse or alternating current techniques have to be applied in order to narrow this potential window [11-13].

The benefits of applying FIA in amperometry instead of a constant flow-through arrangement are as follows:

I) A clean working electrode surface is absolutely essential for this consumptive technique, in which the analytical signal depends upon the active electrode area and the diffusion layer thickness;

2) If organic compounds are to be detected electrochemically, normal HPLC detectors, working at a constant electrode potential, tend to be covered by polymers produced by the electrochemical reaction of certain compounds at the working electrode surface. If a non-stationary potential is used, together with a rather short contact time of the compound, this problem in the amperometric cells can be eliminated.

3) If a certain dilution can be tolerated, by mixing the sample in a mixing chamber with carrier solution, the time normally used for de-aeration can be reduced, when the carrier solution is maintained oxygen-free and the injected volume is in the μl range.

Biamperometry

The biamperometric mode is based on the establisment of an difference of potential between two inert identical Pt electrodes. The compound to be monitored should be present in both oxidized states of the redox couple, and the electrodes intermediate electron transference from the oxidized to the reduced specie (no redox reaction are allowed at so small difference of potential). The redox equilibrium should be reversible with a quick kinetic. Almost all of FIA-pharmaceuticals papers are dealing with an indirect procedure based on an auxiliary redox couple like Fe(III)/Fe(II). The pharmaceutical solution

is inserted into a Fe(III) solution stream and the resulting Fe(II) mixed with the Fe(III) excess are led to detector flow-cell were the small electric current intensity produced, reflects Fe(III)/Fe(II) ratio.

Michalowski et al. [14] used this mode to determine phenothiazines (promazine and thioridazine). Samples were injected into a water carrier that was merged with a stream of 2 M HCl containing 0. 18 M Fe(III). The combined stream was passed through a 100 cm coil heated at 60°C on its way to the electrochemical cell, which accommodated two Pt electrodes that were subjected to a potential difference of 150 mV. Calibration graphs were linear over the ranges 40-160 ppm promazine and 40-140 ppm thioridazine, and the detection limits were 0.4 and 0.5 ppm, respectively. The reproducibility, as RSD, was 8%, and the throughput 90 samples/h.

Recently, García Mateo and Kojlo [15] developed a similar method for the determination of epinephrine over the range 0.1-20 ppm, with RSD = 1.5% and a throughput of 153 samples/h. Both this method and the previous one were used to determine active principles in commercially available pharmaceutical preparations.

Voltammetry

In voltammetry, an electrochemical cell containing a microelectrode is subjected to an excitation signal (a potential difference). The signal produces a typical current response on which the technique relies.

The typical voltammetric signal consists of a linear scan where the potential applied to the cell is linearly increased (usually over a range of 2-3 mV) over time. The current produced in the cell is recorded as a function of time - and hence of the applied potential. Pulsed signals are also commonplace in voltammetry. The resulting current is measured at different times during the pulse lifetime, in perfect synchronized with the pulse application. With triangular signals, the potential is cycled between two values; first, it is raised linearly to a maximum and then decreased, also linearly, along a slope of the same value to its original value. It should be noted that continuous-flow potentiodynamic voltammetric variants require the potential scan to be fast enough to allow an entire wave to be executed over a short period during which a given sample concentration (a plug segment of constant concentration in FIA) is held in the detector cavity.

In order to expeditiously obtain reproducible limiting currents (by confining analyte diffusion to a thin liquid layer that does not shift to the solution with time) one must keep the solution or microelectrode in continuous, reproducible motion. This is the principle behind hydrodynamic voltammetry. The operating mode addressed in this review is that where the analyte solution is circulated through a tube onto which the microelectrode is

mounted. This technique is currently being widely used for detecting analytes emerging from an LC column or an FIA manifold. These applications use a thin layer cell. In the cell, the working electrode is usually built into a wall of an insulating block, at a short distance from the auxiliary electrode. The cell volume typically ranges from 0. 1 to 1 ml. In contrast with amperometric detectors, voltammetric detectors offer the advantage of qualitative as well as quantitative information.

In designing and operating a voltammetric detector, three principal problems must be dealt with:

a) The working electrode material must be chosen and the working electrode constructed, so that the accessible potential range is suitable for the given purpose, the residual current and noise are sufficiently low and constant, the surface activity of the electrode is reproducible and the electrode reaction kinetics for the analyte are favourable;

b) The geometry of the cell must allow the effective volume to be as small as possible, in order to suppress analyte zone broadening and distortion;

c) A suitable measuring technique must be selected, so that the measurement is sensitive, reproducible and has the required selectivity.

A discussion on working electrode material, cell geometry and operational parameters for a flow voltammetric determination are well done in the paper of Stulik and Pacakova [6].

Ideally, the electrodes used in the different voltammetric modes should remain stable for an indefinite period of time; also, they should be easy to construct and feature a high signal-to-noise ratio. Such a ratio can be maximized by using materials conducting a minimal amount of residual current and providing high currents in response to the electroactive species. In fact, many solid electrodes (particularly carbon paste electrodes) behave closely to this ideal electrode. Many breakthroughs in this field are of empirical nature; in fact, the materials were found to be appropriate for some purposes and assayed in different applications. Thus, carbon paste was one of the earliest materials used in this context and has proved specially useful for anodic oxidations in aqueous mobile phases. Also, different carbon-based materials have been employed with varying success. Vitreous carbon is probably the most popular material in this context as it allows the construction of electrodes that are easy to assemble and exhibit a reproducible response, however, they feature low signal-to-noise ratios relative to carbon paste electrodes.

The wide variety of materials used as electrodes warrants a separate section in this review.

The simplest and by far the most common measuring technique is d.c. amperometry at a constant potential corresponding to the analyte limiting current. Pulse techniques (square-wave,, normal pulse and differential

pulse voltammetry) have been introduced [16-20] in an attempt to (a) improve sensitivity and selectivity of measurements, (b) suppress signal dependence on solution flow rate and (c) suppress adsorption of substances on the working electrode leading to electrode passivation.

Differential pulse voltammetry

The modest limits of detection afforded by linear sweep voltammetry have been considerably improved by the pulse technique. The two most important pulse techniques are differential pulse and square-wave voltammetry, the former of which is described below.

The differential pulse variant uses two chief types of exciting signals. One is used with analog instruments and is obtained by superimposing a periodic pulse with a linear sweep. The other is usually employed with digital instruments and is a combination of an output pulse with a staircase signal. In both cases, 50 mV pulses are applied for 50 ms and two current intensity measurements are made, one 16.7 ms before the pulse and the other 16.7 ms after it. The intensity difference per pulse, Δi, is recorded as a function of the potential, with which it increases linearly. The result is a peak-shaped differential curve (or one containing as many peaks as pulsed potential sweeps are made under flow conditions), the height of which is directly proportional to the concentration.

One advantage of this technique is that it allows one to obtain individual peaks for substances with half-wave potentials differing by a mere 0.04 or 0.05 V (the difference required for adequate wave resolution in normal sweep voltammetry is about 0.2 V). More important, however, is that the differential pulse technique provides significantly improved limits of detection (typically two or three orders of magnitude lower than those of the linear sweep technique), in the 10^{-7} to 10^{-8} M region. These substantial assets can be ascribed to the increased Faradaic intensity and decreased non-Faradaic current intensity inherent in this technique.

Smotkin *et al.* [21] used differential pulse voltammetry to determine imipramine and desipramine. They employed an antimony-doped tin oxide electrode for this purpose and obtained linear calibration graphs over the range 25-30 ppm for both drugs

Wang *et al.* [22] demonstrated the use of rapid differential pulsed voltammetry for determining paracetamol, dopamine, caffeic acid and chlorpromazine.

Square wave voltammetry

This pulse polarographic mode is highly expeditious and sensitive. The exciting signal is obtained by superimposing the pulse train on the staircase signal. The length of each staircase step and the pulse period are identical and usually about 5 ms. The staircase potential amplitude is typically

10 mV and the pulse magnitude 50 mV. Under these conditions, which correspond to a pulse frequency of 200 Hz, a 1 V sweep takes 0.5 s. Reported limits of detection for square-wave voltammetry are in the range 10^{-7}–10^{-8} M.

Yoshizawa *et al.* [23] implemented this technique in a FIA system in order to determine caffeic acid and vanillic acid. The manifold included an electrochemical detector furnished with two circular vitreous carbon working electrodes and an Ag/AgCl reference electrode. The effect of the flow-rate on the signal was minimized by using an SBSR (single bead string reactor). The detection limits thus achieved were in the nanomolar range.

Yamaguchi *et al.* [24] determined cystine by use of a vibrating Pt electrode coated with Hg. The calibration graph was linear between 1 and 100 pmol cystine in a 20 ml sample.

This voltammetric mode was also used by Scolari and Brown to determine dopamine [25].

Cyclic voltammetry

In cyclic voltammetry, changes in the current intensity are caused by a triangular potential signal. The potential is first varied linearly from its initial value to a greater or smaller one. Then, the sweeping direction is reversed and the potential returned to its original value. The potential range to be used in an experiment is that where the diffusion-controlled oxidation or reduction of one or more analytes takes place. Depending on the sample composition, the direction of the initial sweep can be positive or negative (a sweep to more negative potentials is referred to as a "forward sweep" and one to more positive potentials as a "reverse sweep"). Each cycle lasts from less than 1 ms to 100 s or longer.

Carella *et al.* [26] determined aliphatic alcohols by using this technique and a nickelmodified glassy carbon electrode in conjunction with an Ag/AgCl reference electrode.

Kristensen *et al.* [27] determined dopamine *in vivo* in rat brain by using a carbon fibre microelectrode coated with a Nafion film thinner than 200 nm which was permeable to dopamine and avoided degradation of the voltammetric signal.

Nagels *et al.* [28] developed an effective on-line electrochemical detector providing fast scans that were used to determine various drugs.

Stripping voltammetry

In this mode, the analyte is first deposited on a microelectrode, which is equivalent to an electrochemical preconcentration. After an accurately measured time, the electrolytic process is stopped and deposited analyte is determined by using one of the above-described voltammetric procedures. In this second step, deposited analyte is stripped from the microelectrode, hence the name of the technique. In anodic

stripping methods, the microelectrode behaves as a cathode in the electrodeposition step and as an anode in the stripping step, where the analyte is oxidized to its original form. The opposite process takes place in the cathodic mode. Stripping methods are highly efficient in trace analysis; they typically allow concentrations in the range 10^{-6}-10^{-9} M to be conveniently and expeditiously determined.

Usually, only part of the analyte present is deposited during the electrodeposition stage; therefore, the obtainment of quantitative results relies on the use of an appropriate microelectrode, deposition time and applied potential. The microelectrodes typically employed in stripping methods are constructed from various materials including mercury, gold, silver, platinum and carbon in different forms. The most popular electrode by far in this context is the hanging mercury drop electrode.

The differential pulse anodic mode is the most frequently used in the stripping step. The results usually take the form of sharp peaks that are specially suitable for analysing mixtures. One other way of obtaining sharp peaks is by using a mercury film electrode. Because the average length of the diffusion pathway from the film to the solution interface is much shorter than in a dropping electrode, the analyte is stripped much more rapidly and voltammetric peaks are much more narrow and tall as a result; this, in turn, leads to increased sensitivity and enhanced mixture resolution. However, the hanging mercury drop electrode appears to produce more reproducible results, especially at high analyte concentrations.

Adsorptive stripping voltammetry

In this voltammetric mode, a microelectrode (usually a hanging mercury drop electrode) is brought into contact with the analyte solution for a preset time. The analyte is deposited onto the electrode by physisorption rather than by an electrolytic mechanism. After an adequate amount of analyte is deposited, the analyte is determined in the linear sweep or pulse mode. In aqueous solutions, many compounds of clinical and pharmaceutical interest tend to be strongly adsorbed on the mercury surface, particularly if this is maintained at a potential about –0.4 V *vs* SCE, where mercury possesses a zero charge. The limits of detection thus achieved can be as low as 10^{-10}–10^{-11} M.

Kopanica and Stara [29] determined Cyadox by using this technique. The analyte was inserted into the system and adsorbed from the sample plug onto a hanging mercury drop electrode. After the whole plug was passed, the electrode was subjected to differential pulsed stripping up to -l.O V at a scan rate of 20 mV/s and a pulse amplitude of -50 mV, with a pulse duration and pulse interval of 0. 1 s. The calibration graph was linear from 3 to 150 ng/ml. The method was applied to the

determination of the drug in porcine plasma, with a detection limit of 10 ng/ml.

Bouzid and Pacakova [30] used the same method to determine 5-fluorouracil at concentrations between 0.1 and 1.5 mM.

Wang and Freiha [31] used a new electrochemical detection FIA assembly based on the accumulation of an analyte on the surface of a carbon paste electrode by selective adsorption.

The preconcentration can be accomplished by spontaneous adsorption of the analyte or covalent bonding to the surface (via specific functional groups introduced for this purpose). Because the species retained on the surface preserve their electrochemical properties, they can be quantified by using conventional voltammetric procedures. This preconcentration step considerably improves sensitivity and selectivity, and facilitates application to complex samples. The selectivity can be raised by transferring the electrode from the complex sample to a blank electrolyte solution prior to measurement in FIA, this involves preconcentration while the sample is being circulated and measuring while the carrier is, between samples. The sample is injected into a 0. 1 M phosphate buffer carrier and, after a 5 s delay, preconcentration is started by applying a potential of +0.3 V for 1 min, which deposits the analyte on a carbon paste working electrode (the reference electrode is Ag/AgCl and the auxiliary electrode Pt). Adsorbed analyte is then quantified by applying an anodic pulse potential ramp of +0.9 V for 1 min. The method was applied to the direct determination of chlorpromazine in urine (following 1:3 dilution) in the presence of excess (1:10) amounts of species with a similar redox potential. The throughput was 24 injections/h and the detection limit a few nanograms.

The application of pulse measurements to solid electrodes always produces S/N ratios poorer than in d.c. measurements, primarily because the redox reactions of the surface functional groups cause residual currents that decay slowly and cannot be eliminated by current sampling. However sometimes the selectivity can be improved. The results are better when pulse methods are applied to mercury electrodes.

It can be concluded that d.c. amperometry is generally preferable for flow measurements. Application, of pulse techniques might sometimes be useful for enhancing the measuring selectivity and minimizing the dependence of the signal on the flow rate.

The reason for which voltammetric and polarographic detectors are applied in practical analysis are their sensitivity and/or selectivity for certain groups of substances. Selectivity is especially important in analysis of clinical and biological samples that contain many substances that interfere in e.g. UV

photometric detection but not in electrochemical measurement.

The extremely high sensitivity of voltammetric detectors to catecholamines and their metabolites is well known [16]. Another group of substances that can be electrochemically detected with a high sensitivity are carcinogenic aromatic amines [32].

Many techniques have been studied and applied to pretreatment of solid electrodes in order to maintain a constant activity of the electrode surface and possibly also to improve the sensitivity and/or selectivity of measurement. These techniques are necessarily rather empirical and involve mechanical, chemical, electrochemical and physical procedures [33].

A voltammetric detector with a fibre microelectrode has been described which exhibited greatly reduced charging currents permitting scan rates of up to 1 Vs^{-1} [34]. However this detector is limited to use with open tubular chromatographic systems.

An alternative to direct voltammetric detection is so named voltammetric/amperometric detection, which is based on a dual-electrode thin-layer transducer in a series configuration [34]. The potential of the upstream electrode is scanned while the downstream electrode is maintained at a constant potential. The downstream electrode is used to monitor the redox reaction occurring at the upstream electrode without the charging current associated with scanning the potential. Therefore, voltammetric information can be obtained at an electrode operated in an amperometric mode, and the detection limits are similar to those attainable with direct amperometric detection.

The series configuration of the dual-electrode detector used in an amperometric mode can greatly increase selectivity because only chemically reversible redox couples are detected [35].

Microelectrode array has received much attention for its various attractive features. A multichannel electrochemical detection system using an individually addressable potentiostated microelectrode array placed parallel to flowing streams was reported [36,37]. This system provides three-dimensional results (i.e., current, potential and time) without potential scanning. The 16 channel detection was carried out by collecting current response at each microband electrode to show the three-dimensional results. Compared to rapid -scanning voltammetry using a single electrode this system is possible to hasten voltammetric detection. Unlike potential scanning methods, this type of detector does not suffer from the undesired effect of charging current, since the potentials for the individual electrodes were held at constant value.

The new technique of stopped flow injection coulometric titration combines the advantages of FIA and coulometric titrations [38,39]. An injected sample is

carried into a 0.7 ml mixing chamber containing a magnetic stirring bar. The flow is stopped at a predetermined time, arresting a fixed portion of the sample in the chamber, creating a gradient dilution. The sample is then titrated by electrochemical generation of the titrant as a coiled platinum electrode, at the bottom of the cell, until an end point is recorded by a potentiometric, amperometric, conductometric or photometric method. The system must be calibrated for a given flow stop time. The range of sample concentrations that can be titrated is a function of the stop time and the general current selected. The entire system is under computer control. It should be a valuable tool for the automatic assay of many pharmaceuticals using only a few microliters of sample solution.

ELECTRODES

Carbon paste electrodes

This type of electrode is made by mixing graphite or powdered carbon with an organic liquid immiscible with the solution where the electrode is to be immersed. It is frequently used for anodic oxidation on account of its low residual current and the wide range of positive potentials it affords. The earliest electrode of this type used in LC was employed to determine catecholamines by anodic oxidation [40]. The limits of detection thus achieved were much lower than those afforded by existing methods.

Carbon paste electrodes can be used for months with no surface renewal; its favourable features are a result of the purity of the materials used to make the paste. One composition that gives good results in aqueous carriers is 25% nujol by weight. Because the optimal composition of the paste depends on the intended use of the electrode, a wide variety of mixtures and proportions have been studied; some authors have even assayed using solid materials to bind the carbon particles. The limits of detection of these electrodes are fairly good as the chief result of their low residual current. The organic liquid fills the voids between particles, thus keeping particles together and reducing the residual current. In fact, the residual current has been shown to be inversely proportional to the amount of organic liquid used; however, an increased amount of liquid has also been found to hinder heterogeneous electron transfer. A high transfer rate is desirable in order to ensure well-defined voltammetric waves and facilitate selection of the applied potential. However, different preparations of the same paste have been found to give disparate results so the optimal component ratio continues to be determined empirically.

Notwithstanding the favourable limits of detection, this type of electrode has some disadvantages. One is the above-mentioned variability in the preparation of the carbon paste. One other is that it is only suitable for anodic oxidations (the oxygen in the paste would

be reduced at a potential below 0.00 V *vs* SCE and produce a residual current). Finally, it tends to be dissolved in organic carriers.

Villar *et al.* [41] determined mitoxanthrone in pharmaceutical preparations by oxidation at a carbon paste electrode at +0.9 V, using Ag/AgCl as the reference electrode. The sample was injected into a 0.1 M $HClO_4$ carrier at pH 1.2 at a flow-rate of 4 ml/min. The linear range thus achieved was 0.05-10 mM.

Vitreous carbon electrodes

Vitreous carbon is obtained by heating a phenol–formaldehyde resin in an inert atmosphere. It is gas-impermeable but gives rise to higher residual currents than carbon paste [42]. However, vitreous carbon electrodes can be used at both negative and positive potentials over a 2 V range in aqueous solutions. The response has been shown to remain fairly stable over time. Also, should the electrode be fouled, it can be regenerated electrochemically without the need to disassemble the cell [43,44]. This type of electrode can be constructed in a variety of shapes (tubular, thin layer) and dimensions.

Sánchez Pérez *et al.* [45] reported a method for the amperometric determination of vitamin D_3 and one of its metabolites, 25-OH D_3, by using a vitreous carbon working electrode, an Ag/AgCl reference electrode and an Au auxiliary electrode. Samples were dissolved in 70:30 V/V methanol/water and injected into a carrier consisting of 0.075 M $LiClO_4$ dissolved in 60:40 V/V methanol/water. The vitreous carbon electrode was polished with alumina and polarized at + 1.40 V for 30 min in the carrier stream. The potential used to detect the vitamin and its metabolite was +1.05V. The method was applied to the determination of both analytes in commercially available pharmaceutical formulations. The detection limits achieved were 1.2×10^{-7} M for the vitamin and 1.8×10^{-7} M for its metabolite. The method offers substantial advantages over existing alternatives in terms of throughput (35 samples/h) and simplicity (it requires no prior chromatographic injection, even though the electrode surface must be renewed every 60 injections).

Cox and Dabek [46] used the same electrode and treatment to determine methionine. The electrode catalyses the oxidation of the analyte at +0.92 V *vs* Ag/AgCl. A sample volume of 7.5 ml was injected into the same carrier as in the previous method and at an identical flow-rate. The resulting linear calibration range was 0.6-180 mM and the sensitivity remained unchanged after three weeks of daily use.

Shah and Stewart [47] reported a determination for propantheline bromide in tablets involving the injection of 20 ml samples dissolved in an acetonitrile/0.05 M NaH_2PO_4 carrier at pH 6.2 that was circulated at a flow-rate of 1 ml/min. The potential applied to the vitreous carbon

electrode was +1.4 V *vs* Ag/AgCl. The linear calibration range was 2-16 ppm.

Belal [48] developed a similar system based on a methanol/aqueous phosphate buffer at pH 7 for the determination of phenazopyridine hydrochloride dissolved in the carrier solution. The applied potential was +0.950 V *vs* Ag/AgCl. Sample injections of 50 ml provided a linear range of 1-80 ppm and 0.2 ng as the detection limit. The method proved highly selective.

The previous author used the same system to determine sodium warfarin in tablets and injectables [49]. The applied potential was +1.05 V. The linear calibration range thus achieved was 1-40 ppm, the detection limit 5 ppb and the throughput 200 injections/h.

Mazzo *et al.* [50] determined butylated hydroxyanisol by use of a vitreous carbon electrode at +0.60 V *vs* Ag/AgCl. The sample was injected into a carrier consisting of 25 x 10^{-3} M NaH_2PO_4 (pH 4, H_3PO_4), acetonitrile and methanol in a 33:55:12 proportion that was circulated at a flow-rate of 1 ml/min. The samples, injected in portions of 4 ml, were dissolved in the carrier solution.

Ruiz *et al.* [51] determined bromazepam in capsules by reduction at a vitreous carbon electrode. They used a 10:90 V/V HAc/NaAcO (pH 4.6)-methanol carrier and obtained a linear range from 6 to 32 ppm.

González *et al.* [52] determined nicarpidin and a piperazine derivative over the ranges 0.2-1.6 and 0.3-50 ppm, respectively, by oxidation at a vitreous carbon electrode.

Pinilla *et al.* [53] developed a method for quantifying theophylline in serum. Serum samples (25 ml) were incubated at room temperature for 30 min, using 200 ml of alkaline phosphatase-theophylline (I) conjugate from the EZ-BEAD kit and a bead coated with anti-I antibodies. The bead was then washed and added to 1 ml of 4-aminophenylphosphate solution. After 15 min, 20 ml of the solution was withdrawn and the 4-aminophenol formed was determined by flow injection analysis using 2-(ethylamino)ethanol as the mobile phase (at a flow-rate of 1 ml/min) and amperometric detection with a vitreous carbon electrode at +0.1 V *vs* Ag/AgCl. The calibration graph was linear up to 100 mg/l . The between-assay coefficient of variation was 3.9-7.0%.

Tang *et al.* [54] developed an electrochemical enzyme immunoassay for phenytoin in serum by flow injection analysis with a redox coupling agent. Assays were performed according to the EMIT instructions (Syva Co., Palo Alto, CA), except that the reaction mixture was not transferred to a flow-cell. At intervals of 1 and 5 min after the enzymelabelled phenytoin (reagent B) was added, a 50 ml portion of the reaction mixture and phosphate buffer at pH 7 (250 ml) were supplied to 300 ml of 0.42 mM 2,6-dichlorophenol indophenol (I). The rate of NADH production was measured at 5 min by injecting the solution into a flow-

injection amperometric assembly from Bioanalytical Systems (West Lafayette, IN) fitted with a vitreous carbon working electrode and an Ag/AgCl reference electrode for detection of reduced I. The results compared well with those provided by fluorescence polarization immunoassay.

Other solid carbon forms

Pyrolyte is an isotropic pyrolytic form of carbon. Its electrochemical properties are similar to those of carbon paste, yet it can be used at more negative potentials than this. Like vitreous carbon, it can be molded into planar shapes, which facilitates the construction of very thin flow-through amperometric cells.

Reticulated vitreous carbon possesses a large surface that resembles a sponge. Because of the manufacturing procedure used, it has a shiny appearance and exhibits lower residual currents than other forms of porous carbon. This material has also been used with its pores filled with epoxy resin [55]; the resulting surface resembles carbon paste as it consists of carbon islands surrounded by an insulating material.

Carbon fibres are microscopic strands formed by pyrolysis of pitch or polyacrylonitrile. They have been used to form batches of disks 10 mm in diameter each [56]. The detection limits they provide are slightly better than those of vitreous carbon at positive potentials because the background signal for the electrode array is less markedly dependent on oscillations in the carrier flow-rate.

Belal and Anderson [57] developed a flow injection method for the determination of three N-substituted phenothiazine drugs by using amperometric detection at a carbon-fibre electrode array. The rod electrode was constructed by embedding a multi-strand carbon-fibre yarn in 43:7 Epon 828 resin (Miller Stephenson, Chicago, IL)/m-phenylenediamine. The active end of the array was sanded and polished. The detector cell was of the three-electrode, fully developed wall-jet type, and the mobile phased consists of 1:1 methanol/phosphate buffer at pH 7 containing 2% NaAcO, and circulated at 1.0 ml/min. Samples of perphenazine (I), fluorpromazine hydrochloride (II) and fluphenazine hydrochloride (III) in the previous mobile phase were injected into the electrolyte stream, the current being measured at + I. 1 V *vs* Ag/AgCl/3.5 M KCl. The current vs concentration graph was linear over the range 1-50 mg/ml for each drug. The reproducibility was quite good: the coefficient of variation (peak height) was 0.8%, 0.95% and 1.2% for 50 ml injections of solutions containing 20 mg/ml I, II and III, respectively. The detection limit was 5 ng/ml for the three analytes. Sampling frequencies as high as 200 h^{-1} were achieved. The method was used to determine I, II and III in dosage forms, with good accuracy and precision.

Metal electrodes

Metal electrodes have been used to a lesser extent than carbon electrodes as they exhibit poorer detection limits. This is the chief result of the high residual currents that arise from surface oxidation when positive potentials are applied.

Utley [58] reported a flow injection method for the determination of cyanide, a toxic ion arising from the decomposition of the salts of the anticholinergic pralidoxine. Hydrogen cyanide diffusing from the carrier was determined amperometrically by using a working Ag/AgCl electrode and a calomel electrode. The method is highly sensitive and selective.

Luo *et al.* [59] developed an analytical system for the amperometric determination of amino acids by use of a Cu electrode at +0.55V vs Ag/AgCl. The detection limit thus achieved ranged from 1 to 50 ppm, depending on the particular amino acid.

Koprowski *et al.* [60] reported a method for the amperometric quantitation of penicillins by using a gold electrode. The reaction was observed in a strongly acidic solution; however, the best compromise between stability and sensitivity was obtained with an acetic/acetate buffer. The detection limit thus obtained for benzylpenicillin was 0.4 mM.

Metal oxide electrodes

Hui and Huber [61] reported a method for the determination of amines and aminoacids based on a nickel oxide electrode used at +0.49 V vs calomel (with Pt as the auxilliar electrode). Sample injections of 25 ml (at a concentration of 10^{-3} M) provided detection limits in the region of 0.2 mg .

The same authors subsequently reported a similar system for the determination of caffeine and other organic compounds [62].

Smotkin *et al.* [63] used a tin oxide electrode doped with Sb and an applied potential of +0.8 V against Ag/AgCl for the determination of imipramine and desipramine in the differential pulse and cyclic modes. The carrier consisted of 50 mM Tris and 150 mM NaCl at pH 7.4, and was circulated at l. 17 ml/min. Calibration graphs were linear between 25 and 300 ppb for both drugs.

Mercury electrodes

In addition to the well known mercury drop electrode, other polarographic electrodes use the same material. For example, the mercury film electrode is obtained by electrolytic deposition of the metal onto a disk electrode.

Wang and Dewald [64] used a vitreous carbon electrode coated with a mercury film to determine Cu and Zn in pharmaceutical preparations. Following adsorption from the injected plug at -0.8 V, both metals were quantified by anodic stripping

voltainmetry. Their mutual interference was overcome by using a system comprising two working electrodes at selective deposition potentials.

The hanging drop mercury electrode consists of a very thin capillary tube connected to a mercury pool. The metal is forced out of the capillary by a piston that is displaced with a micrometer screw. This last allows to obtain drops with surface areas that are reproducible to within ± 5%. The applications of these electrodes are describes in the "Adsorptive stripping" section.

Chemically modified electrodes

Chemically modified electrodes (CMES) represent an effective means for enhancing the power of amperometric flow detectors [65]. Most of the materials used for constructing electrodes can be modified chemically to incorporate substances that catalyse electrochemical processes, alter redox potentials or coat the electrode surface in order to make it selectively permeable to a given substance. An promising avenue is to tailor the surface of such detectors to achieve a catalytic response. Several surface-bound redox indicators have been useful for enhancing the electrontransfer kinetics for important analytes. For example Baldwin´s group has demonstrated the utility of surface-bound cobalt phthalocyanine for enhancing the detection of numerous biological compounds [66,67].

In the paper [68] is described a new catalytic CME based on a mixture of cobalt(II, III) oxides which exhibits a powerful catalytic activity toward several analytically important compounds which suffer from a sluggish redox potential. When used for amperometric detection of flowing streams this type of electrode greatly improved the detection.

Systems involving enzymes are very popular in electroanalytical applications on account of their extreme selectivity for major analytes. One rather commonplace electrode in this context is the Clark oxygen sensor. By immobilizing an enzyme such as glucose oxidase or tyrosinase on the polypropylene membrane of the sensor (constructed in a thin-layer design of V = 7 ml), glucose and pyrocatechol were determined by FI; the response for both was stronger and faster than that obtained by using a reactor containing the enzyme immobilized on Sepharose 4B. Turk *et al.* [69] determined tricyclic drugs (amitriptyline, nortriptyline and proptriptyline) by cyclic voltammetry using "polymer electrodes" that were constructed by plating conducting layers onto reticulated vitreous carbon supports from 1 mM or 10 mM carbazone or thiophene at + I. 1 V and +1.5 V, respectively, against SCE. An electrochemical response was observed for the tricyclic drugs -even for protriptyline, which was previously reported to be inactive.

Gao *et al.* [70] determined ascorbic acid used polypyrrole-dodecylsulphate

filmcoated electrodes. The low oxidation potential involved rendered the proposed electrodes suitable for the FIA determination of the analyte.

Wang *et al.* [71] developed an effective enzyme methodology for avoiding the interference of paracetamol with the amperometric detection of glucose. The strategy involves the *in situ* destruction of the interference by use of the enzyme tyrosinase; this is incorporated onto the carbon paste electrode at the time of preparation from ground mushrooms, which are rich in the enzyme (the reference electrode was Ag/AgCl and the auxiliary electrode Pt). Measurements are made at +0.5 V for the working electrode and a flow-rate of 1 ml/min. The calibration curves thus obtained have a near-zero intercept (as in the absence of paracetamol); by contrast, those provided by an unmodified carbon paste electrode have a slope of 360 mV. The calibration curve is linear up to l. 1 x 10^{-2} M glucose.

Chen *et al.* [72] reported an electrode array consisting of four carbon paste electrodes doped with different metal oxides that act as catalysts for various analytes (Cu_2O, RuO, NiO and CoO). Each modifier exhibits a differential electrocatalytic behaviour towards each analyte. By coupling the unique response of the sensor array to various statistical regression techniques, one can determine individual carbohydrates or aminoacids in different mixtures by amperometry. For mixtures of two and three components, partial least-squares (PLS) calibration provides an average error of 2.3%. The potential sequentially applied to the electrodes of the sensor array is +0.45 V *vs* Ag/AgCl (with Pt as the auxiliary electrode), and the flow-rate 1.5 ml/min.

Hoogvliet *et al.* [73] used a similar device consisting of four electrodes (carbon paste, C paste modified with cobalt phthalocyanine, Hg-Ag amalgam and Au) for the amperometric determination of glutathione. Each electrode responded in a differential manner to the analyte (the response of the unmodified C paste electrode was virtually zero whereas that of the amalgam electrode was the highest). Because each electrode responds differently to a given substance, the information it provides is much wealthier than that obtained from a similar detector using a single electrode (two substances can give a similar response at two electrodes but not at all four).

Wang and Naser [74] used a thin layer carbon paste electrode (with Ag/AgCl as the reference electrode) for the amperometric determination of paracetamol and dopamine following removal of interferents by use of bioreactors accommodating various immobilized plant tissues containing appropriate enzymes. The reactor containing papaya avoided the interference of proteins with the determination of paracetamol; that containing zucchini overcame the interference of ascorbic acid with the quantitation of dopamine; and that

accommodating potato prevented phenols from interfering with the determination of paracetamol and dopamine.

Previously [75], these authors had developed a thin layer carbon paste electrode modified with plant tissue from ground courgette. The presence of ascorbic acid oxidase in the tissue suppressed the interference of ascorbic acid with the determination of norepinephrine and dopamine. The ensuing method is much more economical than that using the pure enzyme.

Cox and Gray [76] developed a method for the determination of insulin by use of a modified electrode. Prior to surface modification, the vitreous carbon electrode was polished successively with 1, 0.3 and 0.05 M alumina on a metallographic polishing cloth with deionized water as the lubricant. The electrode was then thoroughly rinsed and sonicated prior to assembly in a flow-cell that was filled with a plating solution containing 2 mM $RuCl_3$, 2 mM $K_4Ru(CN)_6$ and 0.5 M KCl (pH 2, HCl). The vitreous carbon indicator was cycled between 500 and 1100 mV *vs* Ag/AgCl (50 mV/s, 50 cycles). The cell was subsequently filled at open circuit with the carrier solution and stored until needed. Measurements were made at +0.96 V vs Ag/AgCl, using 0.2 M K_2SO_4 (pH 2) at 1.0 ml/min as carrier and injections of 7.5 ml. The linear calibration range was 8.2-204 ng and the detection limit 5 ng.

Schmidt and Emons [77] determined phospholipids indirectly by the inhibitory effect of phospholipids adsorbed on a vitreous carbon electrode on the electrode reactions of quinol. The detection limit thus achieved was 1.0 mg.

Zhon and Wang [78] reported a modified vitreous carbon electrode for the amperometric determination of promethazine. The electrode was polished with 0.5 μm alumina particles, ultrasonicated in bidistilled water, rinsed with ethanol and allowed to dry in the air. Then, it was coated with a 1 % Nafion solution in order to cover the active disk and its surroundings. The film thickness was estimated from the volume and density of the Nafion solution, and the active area of the electrode. Naflon is a perfluorinated, sulphonated ion-exchange polymer selective to cations and highly permselective to hydrophobic organic actions.

Wang, Naser and Ojzsojz [79] developed one other selective method by using a similar vitreous carbon electrode that was coated with Naflon first and with cellulose acetate then. The electrode was used for the amperometric determination of cationic neurotransmitters (adrenaline, noradrenalin and dopamine) in the presence of large amounts of uric acid and ascorbic acid.

Wang *et al.* [80] also developed an electrode containing the enzyme theophylline oxidase for the determination of theophylline. The drug

was oxidized against the acceptor ferricytochrome C, which subsequently reacted with ferricyanide ion. This in turn was oxidized anodically at +0.4 V against the Ag/AgCl electrode. Ferricyanide was supplied in the carrier, which was circulated at a rate of 2.0 ml/min. The electrode was constructed by polishing a Pt disk that was subsequently coated with a mixture of the enzyme and cofactor with l% Nafion. This coat was protected by another of polymeric Nafion. In this way, detection limits of 2 x 10^{-6} M and a throughput of 180 samples/h were achieved.

The best signal to noise ratios are obtained on carbon paste electrodes. This is due to the fact that these electrodes actually behave as arrays of microelectrodes and has been shown [81,82] that microelectrodes have many advantages [5].

The main drawbacks of classical carbon paste electrodes are poor mechanical stability, difficulty in making the electrode surface even and smooth and diluent bleeding. These problems can be overcome by using composite electrodes, in which the electrode material particles are dispersed in a suitable porous polymer [16].

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