Journal of Pharmaceutical & Biomedical Analysis
Vol. 1, No. 4, pp. 475–490, 1983
Printed in Great Britain

0731–7085/83 $3.00 + 0.00

Application of polarography and voltammetry to drug analysis in industry*

P. M. BERSIER

Ciba-Geigy, AG, Basel, Werk Rosental, R.1055.3.54, CH-4002 Basel, Switzerland

Introduction

Essential to the renewal of interest in polarography and voltammetry in the 1970s were the availability of reliable polarographs offering the pulse mode, with sensitivity down to 10^{-7}–10^{-8} M and higher resolving power; a better understanding of the electrode processes of compounds of pharmaceutical interest (active agents, excipients, additives, antioxidants, etc.); and the application of oxidation waves, making the technique available to a wide range of pharmaceuticals. Davidson [1] has surveyed the use of the voltammetric oxidative mode.

Few modern books [2–4] but several recent review articles [5–10] discuss the electrochemistry (polarography and voltammetry) of drugs. According to Fogg [11] the answer to the question: "Voltammetry, is it worth considering?" is an emphatic "yes" for the determination of drugs in biological materials. Viré and Patriarche [12] comment: ". . . among modern analytical techniques the application of differential pulse polarography in the pharmaceutical sciences is well justified with regard to accuracy and sensitivity, and it is shown to be of use for rapid investigation of complex media . . ." and they conclude: "it is reasonable to think that this technique will be applied more and more in biological and pharmaceutical analysis in the future." The literature of the last few years shows that polarography and voltammetry are particularly applied in pharmaceutical and biological chemistry. The USP XX quotes 16 drugs and formulations assayed by polarography.

Does all this activity mean that polarography and voltammetry are, or will be, used in industry? Osteryoung and Osteryoung [13] note that "The use of electroanalytical methods in industry obviously depends on the availability of individuals knowledgeable in the area, the applicability of methods to solve real problems, and the instrument available."

One of the main objections to polarography and voltammetry advanced by analysts in industry is that both methods are purely academic; polarography is mostly encountered in basic electrochemical courses. Both are more demanding than most other instrumental techniques used in industrial laboratories [13–17, 68], and they are mostly practised in

* Parts of this paper were presented at the 1st International Symposium on Drug Analysis, June 1983, Brussels, Belgium.

highly specialized laboratories. Their scope has not been recognized by many industrial laboratories, or they have not been considered adequate for routine work.

This article formulates answers to such questions as:

(i) Why should an analyst consider polarography or voltammetry instead of techniques such as photometry or chromatography?

(ii) Are polarography and voltammetry used in industrial laboratories?

(iii) If polarography or voltammetry are chosen, which technique should be used?

To illustrate the advantages and limitations of both techniques in the industrial laboratory, examples from the recent literature and practical examples from the author's laboratory are discussed.

Wherever possible, comparisons with other instrumental techniques have been included. All the polarographic and voltammetric curves shown were measured or (in the literature examples) remeasured in the author's laboratory.

Choice of Polarography and Voltammetry

It is reasonable to ask why an analyst should consider polarography or voltammetry, in view of the wide use of spectrometry and chromatography. HPLC in particular offers high resolution of structurally related compounds and sensitivities at least as good as those of polarography and voltammetry. The two following examples illustrate the value of the electrochemical methods.

Polarographic determination of total iodine in thyroid hormones and of thyroxine

The major biological activity of the iodoamino acids produced by the thyroid gland is present in thyroxine (I) and liothyronine (II). 3,3′,5′-Triiodothyronine (III) and 3,5-diiodothyronine (IV) have little or no biological activity and diiodotyrosine (V) and monoiodotyrosine (VI) have no biological activity [18].

Chemical methods [19] are generally based on determining the organically combined iodine after ignition either directly or by oxidation to iodate which is determined by titration, spectrophotometry or polarography.

A differential pulse polarographic method developed by Holak and Shostak [18] for the determination of total iodine in thyroid hormones and thyroid tablets was recently (1982) collaboratively studied by eight laboratories [20]. All the collaborators commented favourably on the method, which is simpler, shorter, more reliable and less cumbersome than its official counterpart. An advantage over the USP method [19] is the reduction of the numbers of solutions and steps required, thereby reducing the errors [20]. The USP also requires separate methods for the analysis of thyroid hormones and thyroid tablets. Figure 1 shows the recovery of thyroxine, determined as iodate by differential pulse polarography using Holaks's procedure [20].

A better indication of thyroid potency would be the determination of thyroxine (I) and liothyronine (II) [18]. Holak and Shostak [18] and Jacobson and Fonahn [23] proposed differential pulse polarographic procedures for the direct determination of both compounds (I, II) in formulations, suitable for content uniformity tests. Cyclic voltammetric experiments and drop time measurements [23] showed that (I) and (II) are strongly adsorbed at the electrode surface, hence the determination was possible without prior separation from surfactants usually present in pharmaceutical formulations (cf. also Ref. 21).

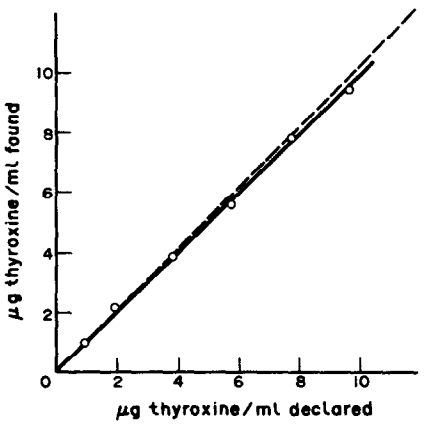


Figure 1
Recovery of thyroxine determined as iodate by differential pulse polarography in model solutions using Holak's procedure [20].

The drawback of the procedures is that thyroxine (I) and liothyronine (II) cannot be determined separately without a prior separation [18].

Voltammetric determination of morphine

Modern electroanalytical procedures for the determination of morphine (see Fig. 2) and of certain morphine derivatives are based on the oxidation of morphine at a platinum or carbon/graphite electrode [28, 29]. With the platinum electrode Proksa and Molnár [28] found the optimal concentration range to be from 9×10^{-6} to 1×10^{-3} M, using linear sweep voltammetry (LSV) at a scan rate of 1 V/min. The advantage lies in the rapid determination of the morphine content of crude morphine and plant material, even if minor alkaloids, like papaverine, codeine and narcotine, are present. The latter compounds, being phenol ethers rather than phenols, are not electrochemically oxidizable [30].

Schwartz and Benjamin [30] carried out differential pulse voltammetric (d.p.v.) determinations of morphine in poppy straw concentrates at a stationary glassy carbon electrode and obtained results similar to those obtained by HPLC [32] and gas chromatography [31].

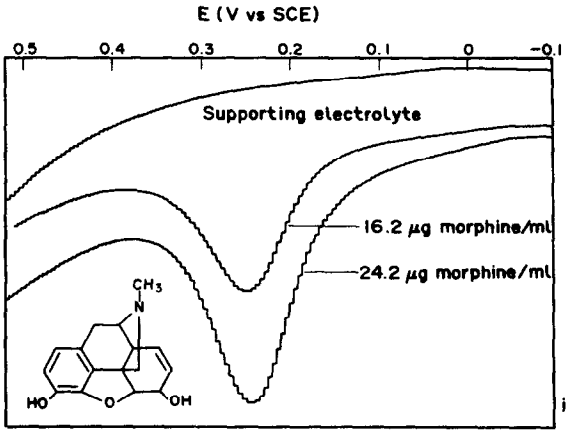


Figure 2
Anodic differential pulse voltammograms of morphine, measured at a stationary glassy carbon electrode. Supporting electrolyte — carbonate buffer (pH 10)/methanol; pulse amplitude = 50 MV; scan rate 10 mV s^{-1}; synchronization 0.5s; sensitivity = 10 µA.

The method is rapid (<5 min) and involves no sample preparation other than dissolving and transferring to the supporting electrolyte. Linear calibration graphs were obtained over a range of 10–40 ppm. Gas chromatography [31] involves extraction and derivatization steps, with an increase in working time and the possibility of sample loss. HPLC [32] requires minimal sample preparation and profiles all the major alkaloids in a single run, but is slower than the d.p.v. method. For the polarographic determination of morphine and its derivatives indirect methods have been proposed, based on nitrosation (formation of 2-nitro-morphine [33]) and *N*-oxide [34].

Recently, liquid chromatography has been used to separate thyroid hormones (e.g. Ref. 24). Separation of 3,3',5-triiodothyronine, 3,3',5'-triiodothyronine and thyroxine by reverse-phase HPLC and amperometric detection was proposed by Hepler *et al.* [25]. Tatwawadi *et al.* [26] described the combined oxidation and hydrolysis of thyroxine and 3,5-diiodothyronine at a stationary carbon paste electrode. Peterson *et al.* [36] also determined morphine by HPLC with electrochemical detection, and oxidative dimerization to fluorescent pseudomorphine at a transparent gold micromesh electrode enabled McLeod and West [37] to assay morphine selectively in papaveretum with a detection limit of 5×10^{-7} M.

Sensitivity, rapidity, selectivity and specificity

Sensitivity. The useful ranges of modern polarographic and voltammetric techniques, and the drug concentration ranges most commonly encountered in formulations, forensic analysis and pharmacological studies, are summarized in Fig. 3. A distinction must be

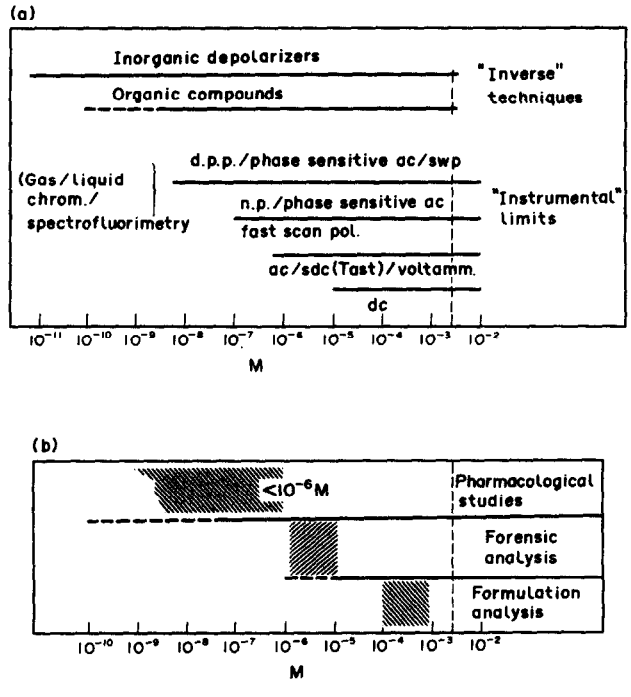


Figure 3
(a) Ranges of practical use of polarographic and voltammetric techniques (see also [112, 113]) and (b) concentration ranges in drug analysis (see also [114]).

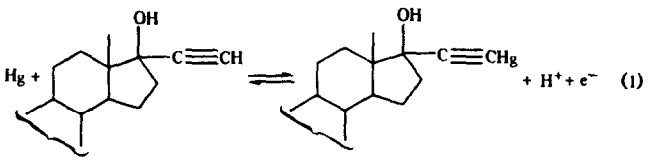
made between the sensitivities provided by the different available techniques (Fig. 3, 'instrumental' limits), and the increased sensitivity provided by the stripping or 'inverse' voltammetric techniques developed in the last few years (Table 1).

Table 1
Lower detection limits of organic compounds by inverse voltammetry

Compound		Reference	
Uracil, cytosine, thymine	5×10^{-8} M	Palecek (1980)	[35a]
Adenine	2×10^{-9}	Palecek (1980)	[35b]
Mercapto-pyridine-*N*-oxide	8×10^{-10}	Csejka (1975)	[27]
Thioamide drugs in plasma, urine	2×10^{-8}	Davidson (1980)	[106]
Ethinyloestradiol	5×10^{-9}	Bond (1982)	[43]
Penicillins	10^{-10}	Forsman (1983)	[107]
Pesticides containing thiourea	1 ng/ml	Osteryoung (1980)	[108]
Vitamin K_1	10 ng/ml	Hart (1981)	[109]

The practical limitation of detection is given by the magnitude and slope of the background signal. Processes contributing to the background in polarography are the charging current of the electrical double layer (the limits of detection of electrochemical techniques depend primarily on their ability to achieve a favourable faradaic-to-charging current ratio [15, 38]), faradaic reactions of impurities, and oxidation and reduction of the electrode surface, the last factor often being predominant with solid electrodes. With detection limits down to 10^{-6}–10^{-8} M, pulse methods and phase sensitive ac polarography are well within the required range for formulations and forensic work. For pharmacological studies separations and preconcentrations are often necessary. In view of the large number of electrochemically reactive compounds, HPLC with electrometric detection [39, 40] has been widely adopted in drug metabolism but is also used in quality control.

The extraordinary sensitivity of stripping techniques with detection limits of 10^{-8}–10^{-10} M arises from the preconcentration of traces of material onto a microelectrode before the process is reversed and the substance is stripped from the electrode surface (Table 1). The trace determination of ethinyloestradiol is a good example. Polarographic methods for the determination of ethinyloestradiol have so far relied exclusively on prior derivatization [42, 43, 48, 49] (other methods for its determination are exemplified in Refs. 50 and 51). Bond, Heritage and Briggs outlined [43] the stripping determination of this compound at the static mercury drop electrode (STME [44]). Using plating times of 3 min and a stripping scan of 100 mV/sec detection limits of *ca.* 5×10^{-9} M were obtained in pure solution: similar detection limits would be required for biological media. According to Bond *et al.* [43] the most plausible explanation is a one-electron oxidation/reduction transfer:



(1)

The reaction of Ag^+ [42, 45, 46] and Cu^+ [43] with terminal acetylenic groups is well known and forms the basis of the titrimetric determination of ethinyl steroids [42, 45].

Belal Saied and Issa Nevine [47] determined ethinyloestradiol by a colorimetric method involving the formation of adducts with $HgCl_2$, i.e. (R–C = CH.$HgCl_2$). The mechanism (I) is thus consistent with insoluble compound formation, pH dependence and the lack of response at non-mercury electrodes [43].

Rapidity. Because of rising costs and the need to analyse larger numbers of samples, speed has become an important factor in the industrial laboratory. The main disadvantage of differential pulse polarography is the relatively long time required for each measurement, as each pulse is imposed only once during the drop life, and slow potential scan rates (1–5 mV/sec) are required.

Increasing effort is nowadays being expended on fast scan methodology, including normal pulse, differential pulse and square-wave modes [52, 53]. In practice, however, the time advantage of such techniques can be misleading. The additional few minutes required for conventional d.p.p. may be considered negligible in relation to the time required for the analysis as a whole. In the absence of pretreatment procedures a read-out can be obtained in 5–10 min with an error of 2–3%, which is well within the limits of most official methods. For example, flumethasone is a synthetic corticosteroid incorporated in various formulations. Figure 4 shows differential pulse (curve 1) and sampled dc (curve 2) polarograms of flumethasone. The direct polarographic determination of flumethasone is definetely simpler and just as precise as the time consuming tetrazolium blue method of the USP [54] (blue tetrazolium [42, 55, 56], phenylhydrazine [42, 57], isoniazid [58]) and UV methods for corticosteroids are subject to interferences from several sources [59, 60]. The reproducibility of eight replicates was 0.6% (r.s.d.); the recovery of seven samples was 99.7%, with a relative standard deviation of 0.6%.

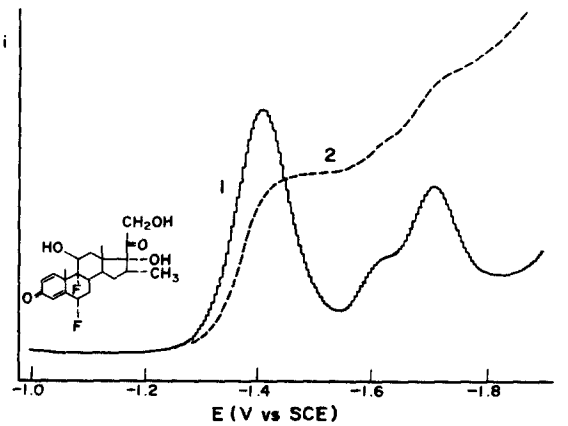


Figure 4
Differential pulse (1) and sampled dc (SDC) (2) polarogram of flumethasone in 0.04 M TEAH/95% methanol supporting electrolyte. D.p.p.: pulse amplitude = 10 mV; scan rate 5 mV s^{-1}; t = 1s.

The automation of polarography and voltammetry has been described [3, 61]. Alexander and Shah [62, 63] remark that the lack of reliable automated equipment has certainly delayed the widespread application of polarographic methods in clinical chemistry. An automated differential pulse polarographic method for protein determination using short controlled drop times with Brdicka's reagent (hexaminocobalt (III)

chloride) is comparable in sensitivity to other automated methods. Sampling rates up to 120 per hour with a precision of 1% (r.s.d.) and carryover of <3% are possible [62].

The introduction of content uniformity tests in pharmaceutical quality control increases the work load up to ten-fold [64], making automation almost a necessity. Such tests lend themselves to automation, as all samples are very similar in composition. A spectrophotometric and polarographic routine serial analyzer described by Feher *et al.* [64] combines flow and discreet analysis, but most other automated systems so far proposed are based on flow injection analysis.

Selectivity and specificity. A serious shortcoming of electrochemical techniques is their poor selectivity. Over-optimistic presentations of modern polarographic instruments often claim that their selectivity and specificity characteristics permit unequivocal identification of components of complex systems, even by an untrained user. Smyth [114] puts 'specificity' in the correct perspective: "Although polarography can be used to provide information on the functional groups present in the drug and, in some instances, the molecular environment of the functional group, it has so far not been possible to use the technique to establish the identity of the compound as can be done with infrared spectroscopy and with mass spectrometry". Dusinsky and Faith [115, 116] propose oscillopolarography with constant current amplitude for the rapid identification of medicaments and venoms, based on tabulated Q values, and hope that the results (nearly 200 medicaments were examined) will assist quick identification in forensic analysis, toxicology, etc. The utility of pattern recognition (which has been used with success in the determination of biological activity, and in spectral interpretation) was evaluated for qualitative electroanalysis by Perone *et al.* [105] using a database which contained specific information on both adsorption and solution–analyte interactions.

The selectivity is best exploited using those polarographic and voltammetric techniques which produce a peak response, e.g. ac, cathode ray (LSV) and differential pulse polarography, and derivative techniques (Fig. 4). Species with peak potential values that differ by at least 40–60 mV can be resolved providing the peaks are sharp and well defined. Thus many analytical problems which never could have been resolved by dc techniques become tractable [65]. The limitations and advantages of polarography are demonstrated by two examples.

(i) Limitations due to poor selectivity: nitrate esters. The advantages of polarographic methods for nitrate ester analysis are that they can be used in samples with high salt content, and that a linear calibration curve is obtained over a large concentration range [66].

Pugh [67] proposed a dc polarographic assay for glyceryl trinitrate using methyl nitrate (BDH, Poole, UK) as internal standard. This gave results in good agreement with the BP method [67]. Lannigan *et al.* [69] proposed a differential pulse polarographic method for a nitroglycerine single tablet assay. Minimization of sample preparation and of the effects of tablet excipients were emphasized. Nitroglycerine on lactose substrate or in single sublingual capsules can be determined directly by d.p.p., but not always by chromatographic methods. The determination of nitroglycerine as an active compound or in tablets is thus simpler, faster and just as precise as official methods. Recent reports have indicated that intravenous nitroglycerine solutions lose potency during preparation or on storage due to degradation or absorption on to a plastic matrix [70–73]; contact with rubber and plastic surfaces should thus be minimized [74]. The breakdown occurs [70] by a stepwise loss of nitrate groups.

The relevant peak potentials are: nitroglycerine, −990, −1020 mV; 1,2-dinitrogly-

cerine, −965, −1090 mV; 1,3-dinitroglycerine, −1040 mV; 2-nitroglycerine, −1030 mV; 1-nitroglycerine, −995 mV (vs SCE. in 0.01 M TMNCl/0.01 NH_4OH/NH_4Cl/80% isopropanol [117]).

These figures show that the selectivity of d.p.p. is not sufficient for the simultaneous determination of nitroglycerine and the various degradation products, though all with the exception of glycerine are polarographically active. Baaske *et al.* [70] were the first to achieve the direct determination of nitroglycerine in intravenous solutions in the presence of breakdown products, using a rapid HPLC method.

(ii) Advantages of poor selectivity. In some cases lack of selectivity can be useful, e.g. when knowledge of individual metabolites is not essential.

Direct d.p.p. is used in the author's laboratory to determine the sum of different nitro-drugs and their biological nitro-metabolites in the blood and urine (faeces) of test animals in resorption and toxicity studies. Reasonably good agreement between the polarographic data and C^{14} values was observed (Fig. 5). Differences arise after 48 h because polarography determines only the nitro moiety, while the C^{14} technique covers the whole molecule. The main non-nitro metabolites are voltammetrically active amines exhibiting anodic waves, so the total should be close to the C^{14} value over the entire time period.

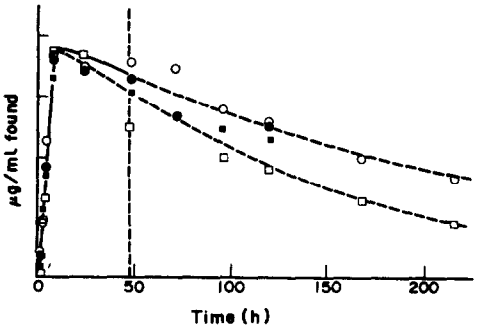


Figure 5
Resorption studies of NO_2-CGP-X. Differential pulse polarographic determination of total NO_2-CGP-X + NO_2-metabolites in dog blood. ■ ● C^{14} method, □ d.p.p., ○ 5 mg kg^{-1}, 20 mg kg^{-1} □.

Use of Polarography and Voltammetry in Industry

Birch, in a recent lecture [75], cast doubt on the general application of polarography and voltammetry in industry. In practice both techniques are used, although the sum of publications on electroanalytical drug analysis has decreased, whereas applications of HPLC combined with electrometric detection are on the increase. The polarographic determination by LSV or d.p.p. of traces of the equatorial β-epimer in the axial α-form of the fluorenone (Fig. 6), an intermediate in steroid production, is an example. Less than 0.3% of the β-fluorenone can be determined directly by differential pulse polarography in the α-product. The peak currents are proportional to concentration over the useful range 0.3–3% β-fluorenone. The relative standard deviation is 1.7% for d.p.p. and 3.7% for LSV. The determination is made via at least three standard additions: the use of conventional calibration curves is fraught with danger in view of matrix problems, especially in the study of formulations [78] or of biological samples.

The different steric position does not necessarily mean a great shift in the reduction potentials of the axial and equatorial form, as was found in the case of α- and β-methyl-steroids with a ΔE_p of ~40 mV compared to the ~230 mV for the fluorenone couple (see also [87–90, 92]). The applications of polarographic and voltammetric methods in structure analysis have been discussed by Zuman [22, 41, 110] or Nürnberg [91].

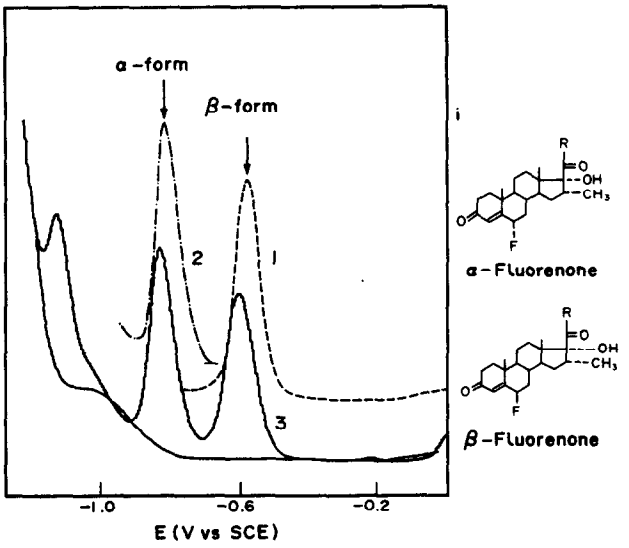
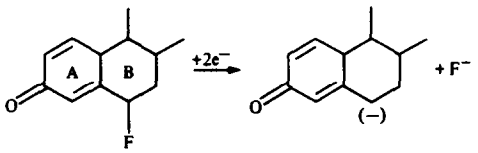


Figure 6
Differential pulse polarograms of β-(axial) and α-(equatorial) fluorenone in Britton Robinson buffer, pH 1.9/30% DMF: pulse amplitude 50 mV; scan rate 5 mV s^{-1}; t = 1 sec. (1) axial form, (2) equatorial form (3) (1:1) mixture.

According to Heftmann and Hunter [79] the resolution of epimeric corticoic drugs by reverse-phase HPLC deserves special mention. Görög *et al.* [80], Wickby *et al.* [81], Juenge and Brower [82] and Roth *et al.* [83] reported the HPLC determination of different epimeric ketone steroids. As little as 0.5% α-ethynodiol diacetate (EDDA) could be determined in the β-form with acceptable precision [80]. Juenge and Flinn [84] described a TLC system sensitive enough to identify and allow quantitation of the 16-methyl α- and β-epimers of 9-fluoro-11β-hydroxy-16-methylandrosta-1,4-diene-3,17-dione. The applications of polarographic and voltammetric methods in structure analysis have been discussed by many authors [87–91].

The first reduction peak at −560 mV for the axial form and −790 mV (vs SCE) for the equatorial form (Fig. 6) are attributed to carbon–fluorine fission involving the transfer of two electrons. De Boer and van Oort [85, 86] formulated the first reduction wave of fluoprednisolone in the following manner



The conjugate system of C-4 and the double bond make the reduction possible. This was proved by monitoring the fluorine concentration with a fluorine-selective electrode during potentiostatic reduction at the peak potential of the first wave. The resulting fluoride concentration equalled the original fluoprednisolone concentration. No fluorine is set free with the fluorine at C-9, as it is too isolated to be reduced in the available potential range.

The differential pulse polarographic assay proved to be very useful for the rapid assessment of the quality of commercial products. Figure 7 shows two products, one exhibiting a large additional reduction wave at −780 mV (vs SCE).

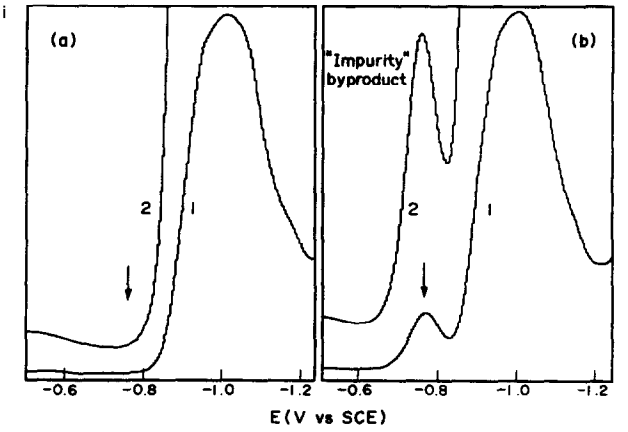


Figure 7
Quality control of active agents (steroids). Comparison of differential pulse polarograms of two products (1, sensitivity 5 μA; 2, sensitivity 1 μA).

In general polarography is more suitable for the determination of low concentrations of a given substance in a solution or a mixture, rather than for a quantitative assay (titre determination) of a pure substance.

The author's laboratory uses sampled dc polarography for the quantitative determination of α-fluorenone raw products. Relative standard deviations of 0.7–1.3% for α-fluorenone contents from 58 to 99% were found.

In 1981 Görög [50] surveyed the state of the art in the assay of pharmaceutical dosage forms containing steroids. The electrochemistry of steroids is well documented [42, 92, 93]. The last 3–5 years have seen a strong interest in the polarographic behaviour of pure substances and the polarographic assay of formulated dosage forms [50, 76, 85, 86, 94].

Position and aim of polarography and voltammetry in the industrial laboratory

For many years the author's laboratory has studied the application, adaptation and development of quantitative polarographic, voltammetric and amperometric procedures for inorganic depolarizers and organic compounds, as 'main' and 'secondary' methods.

'Main' methods are procedures for routine work, for example the direct determination of formaldehyde at below 5 ppm in different starch samples [111].

'Secondary' methods are procedures used as an alternative to other techniques. It is becoming increasingly desirable or even advisable to have two totally different assays for many materials. Differential pulse polarography is currently tested for the determination of the iodide contents of homogeneous gelatin thin films employed as calibration standards [95] for the X-ray-fluorescence analysis of iodide and organically bound iodine in biological matrices. Figure 8 shows the iodide content (measured as iodate) as a function of the film weight. The reproducibility of eight replicates showed a relative standard deviation of ±5.8%. 'Secondary' procedures are also used for checking

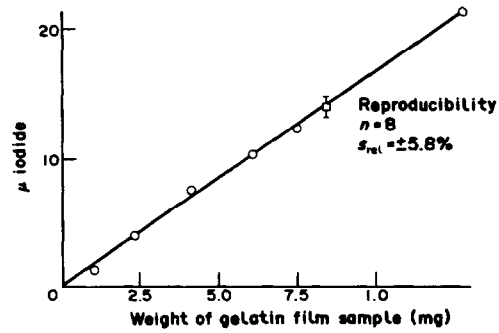


Figure 8
Determination of iodide as iodate as a function of the weight of homogeneous gelatin thin films.

established analytical methods such as chromatographic, spectrometric or microbiological procedures in organic and X-ray fluorescence and atomic absorption spectroscopy in the inorganic field.

Figure 9 shows the comparison of ac-polarographic data and microbiological results, measured in two series each of 20 single dragées. Both methods showed the same variation of the folic acid content of a single dragée (66.6/73–155/163 μg folic acid). The average value of 106 μg found by the two methods agreed with the value supplied by the manufacturer, the assay being performed with a sample of 20 finely ground tablets. For the ac-polarographic determination a single dragée was weighed and dissolved by sonication and the suspension polarographed.

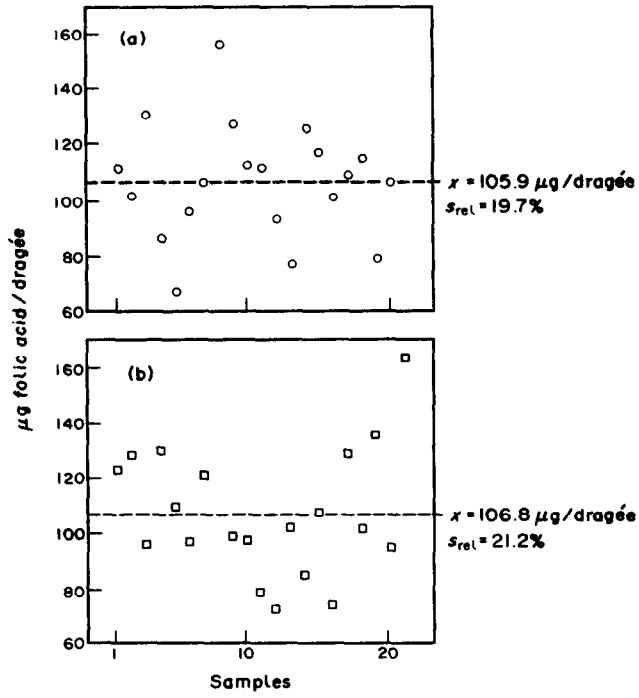


Figure 9
Comparison of folic acid contents of two sets of 20 single multivitamin dragées determined by ac polarography (a) and by a microbiological method (b).

An advantage of polarographic and voltammetric methods in the analysis of formulations is that excipients often do not interfere to such an extent as they do in other methods. As mentioned earlier, the determination of drugs can be performed by differential pulse polarography without any previous separation, provided that the drug is more strongly adsorbed on the electrode than the surfactant present in the formulation (injection, tablet, dragée, oily solution, ointment, cream, etc.). To cope with the real problems confronting the analyst in his day-to-day work, a good knowledge of the literature is indispensable. Whenever possible published procedures are used for a particular problem, and not much of the work is original.

Further tasks of the polarographic laboratory include the elucidation of mechanisms of electrode reactions in connection with electrosynthetic work; and the determination of physico-chemical, thermodynamic and kinetic parameters in the real conditions needed in industrial processes or for basic biological and pharmaceutical research. A practical example is the electrochemical characterization of nitroheterocyclic drugs such as nitroimidazoles in connection with biological reactions [96–99].

Choice of Techniques

If polarography and voltammetry are considered, which should be used? The literature shows that more and more studies are being carried out using different methods.

Patriarche *et al.* [100] and Sengün [101] recently described the assay of clozapine, cliothiapine and loxapine. The electrochemical characteristics of loxapine were determined using dc, ac, d.p.p. and cyclic voltammetry [100]. Loxapine can easily be determined by dc and d.p.p. in 0.1 N H_2SO_4/10% ethanol. The method has been successfully applied to the direct assay of loxapine succinate in tablets. Excipients (calcium phosphate, magnesium stearate, stearic acid, methylcellulose) do not interefere [100]. Sengün [101] applied dc or linear sweep voltammetry (LSV) to the content uniformity test of cloxapine in injection solutions, in acetate buffer, pH 4.6 — 10% isopropanol. Dc and LSV yielded comparable results.

In the author's laboratory differential pulse polarography has been used for the determination of bibenzo (b,f) azepines (Fig. 10). The nitro and keto compounds showed

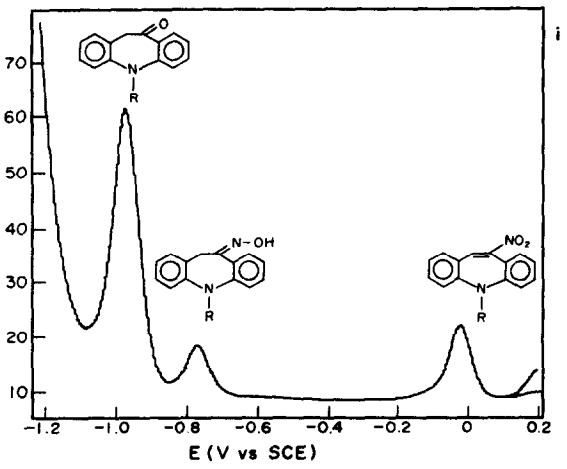


Figure 10
Differential pulse polarogram of different (b,f) azepin compounds. Supporting electrolyte 2 M H_2SO_4/50% ethanol. ΔE = 25 mV, scan 1 mV/s, t = 1 sec, E = 4 × 10^{-8} A mm^{-1}.

a linear dependence of peak height on concentration from 2 to 150 ppm. For crude keto products similar results to those obtained by HPLC were found.

To solve industrial problems all the various polarographic and voltammetric techniques including advanced techniques such as square wave, alternate drop differential pulse or reverse pulse polarography and voltammetry must be considered. Wang and Dewald [102] demonstrated the utility of reverse pulse voltammetry for the automatic assay of chlorpromazine in injections (29–174 ng for 83 µl injection) using a wall-jet detector. At a flow rate of 10 ml/min injection rates of 180 samples per h were obtainable.

Basically two types of instruments are of interest for the industrial laboratory: a simple, very reliable instrument for day-to-day routine work which provides dc, sampled dc, pulse modes and if possible ac or square-wave modes; and a large instrument which provides great flexibility of available techniques and a large choice in the selection of instrumental parameters.

The use of different modes is illustrated by the following example. In a multivitamin dragée, 25 substances out of the 37 formulated were amenable to polarographic or voltammetric determination. The vitamin K_3 contents, found by UV assay to be significantly lower than the values indicated by the manufacturer, were corroborated by d.p.p.

Besides the requested vitamin K_3, vitamins C, B_2, folic acid and nicotinamide could be monitored simultaneously by proper choice of the instrumental mode and the medium. Merely by changing the mode from d.p.p. to ac the waves of K_3 ($E_p = -65$ mV) and of folic acid ($E_p = -550$ mV vs SCE) can be made to appear. The behaviour of the folic acid is worthy of note. The ac-wave is about 30 times higher than the d.p.p. wave. Jacobson and Bjørson [103] attributed these findings to adsorption processes. They proposed a simple, rapid ac-polarographic method for the determination of folic acid in tablets. The method does not involve time-consuming separation of iron salts and insoluble constituents. The ac technique thus seems the best for quantitative assays of folic acid, although cathodic [77] and anodic [104] dc, and d.p.p. procedures have been described.

Conclusions

This article has shown the diverse ways in which polarography and voltammetry can be used and it is clear that industrial laboratories in the pharmaceutical field could usefully consider the benefits these techniques can offer. They are not general-purpose methods: they have various disadvantages and a critical assessment of their applicability must be made in each case. Both techniques can be rapid and sensitive for the measurement of drugs in pure solutions, bulk materials, dosage forms and biological fluids, so they can be considered as alternatives to chromatographic and photometric methods. The relationship between polarography and voltammetry and other instrumental methods in drug analysis is thus complementary rather than competitive.

Acknowledgements: The author thanks P. H. Wenzel and J.-P. Worch for technical assistance.

References

[1] I. E. Davidson, in *Polarography of Molecules of Biological Significance* (W. F. Smyth, Ed.), p. 127. Academic Press, London (1979).

[2] W. F. Smyth (Ed.), *Polarography of Molecules of Biological Significance*. Academic Press, London (1979).

[3] W. F. Smyth (Ed.), *Electroanalysis in Hygiene, Environmental, Clinical and Pharmaceutical Chemistry*. Elsevier, Amsterdam (1980).
[4] S. P. Miskidjian and L. P. Kravchenyk, *Polarografiya Lekarstvennich Preparatov*. Kiev (1976).
[5] H. Siegerman, *Electroanal. Chem.* **11**, 291 (1979).
[6] G. J. Patriarche, M. Chateau-Gosselin, J. C. Vandenbalck and P. Zuman, *Electroanal. Chem.* **11**, 141–289 (1979).
[7] G. J. Patriarche, *Labo-Pharma-Probl. Techn.* **26**, 689 (1978).
[8] G. J. Patriarche, *Labo-Pharma-Probl. Tech.* **27**, 953 (1979).
[9] G. J. Patriarche, J.-C. Viré, C. A. Mairesse-Ducarmois, J. L. Vandenbalck and G. D. Christian, *Bioelectrochem. Bioenerg.* **6**, 147 (1979).
[10] J. V. Geil und F. Kintschel, Die polarographische und voltammetrische Bestimmung von Wirkstoffen in pharmazeutischen Zubereitungen. *Metrohm Monographien*, März 1982.
[11] A. G. Fogg, *Anal. Proc.* 387 (1981).
[12] J.-C. Viré and G. J. Patriarche, *Pharm. Int.* **2**, 119 (1981).
[13] R. A. Osteryoung and J. Osteryoung, *Phil. Trans. R. Soc. Lond. A* **302**, 315 (1981).
[14] A. J. Bard and L. R. Faulkner, *Electrochemical Methods, Fundamentals and Applications*. John Wiley, New York (1980).
[15] A. M. Bond, *Modern Polarographic Methods in Analytical Chemistry*. Marcel Dekker, New York (1980).
[16] B. Ya. Kaplan, *Impulsnaya Polarografiya*, Ser. Metodi Analiticheskoi Khimiya, M. Khimiya (1978).
[17] Z. Galus, *Fundamentals of Electrochemical Analysis*. Ellis Horwood, Chichester (1976).
[18] W. Holak and D. Shostak, *J. Pharm. Sci.* **68**, 338 (1979).
[19] USP, XX, p. 800–801.
[20] W. Holak, *J. Assoc. Off. Anal. Chem.* **65**, 1059 (1982).
[21] E. Jacobson, in *Electroanalysis in Hygiene, Environmental, Clinical and Pharmaceutical Chemistry* (W. F. Smyth, Ed.), p. 227. Elsevier, Amsterdam (1980).
[22] P. Zuman, *Z. phys. Chem. (Leipzig)* **3**, 161–171 (1963) (through [110]).
[23] E. Jacobson and W. Fonahn, *Anal. Chim. Acta.* **119**, 33 (1980).
[24] B. R. Hepler, S. G. Weber and W. C. Purdy, *Anal. Chim. Acta* **138**, 221 (1982).
[25] B. R. Hepler, S. G. Weber and W. C. Purdy, *Anal. Chim. Acta* **113**, 269 (1980).
[26] S. V. Tatwawadi, S. Piekarski, M. D. Hawley and R. N. Adams, *Chem. Listy* **61**, 624 (1967); *Chem. Abstr.* **67**, 17764h (1967).
[27] D. A. Csejka, S. T. Nakos and E. W. DuBord, *Anal. Chem.* **47**, 322 (1975).
[28] B. Proksa and L. Molnár, *Anal. Chim. Acta* **97**, 149 (1978).
[29] P. Surmann, *Arch. Pharm. (Weinheim)* **312**, 734 (1979).
[30] R. S. Schwartz and C. R. Benjamin, *Anal. Chim. Acta* **141**, 365 (1982).
[31] D. Furmanec, *J. Chromatogr.* **89**, 76 (1974).
[32] B. C. Pettitt and C. E. Damon, *J. Chromatogr.* **242**, 189 (1982).
[33] H. Lund, *Acta Chem. Scand.* **12**, 1444 (1958).
[34] H. Hoffmann, *Arch. Pharm.* **305**, 254 (1972).
[35a] E. Palecek and F. Jelen, *Coll. Czech. Chem. Commun.* **45**, 3472 (1980).
[35b] E. Palecek, *Analyt. Biochem.* **108**, 129 (1980).
[36] R. G. Peterson, B. H. Rumack, J. B. Jr. Sullivan and A. Makowski, *J. Chromatogr.* **188**, 420 (1980).
[37] C. W. McLeod and T. S. West, *Analyst* **107**, 1 (1982).
[38] H. Blutstein and A. M. Bond, *Anal. Chem.* **48**, 248 (1976).
[39] W. F. Smyth, D. Dadgar and M. R. Smyth, *Trends Anal. Chem.* **1**, 215 (1982).
[40] K. Stulik and V. Pacakova, *J. Chromatogr.* **192**, 135 (1980).
[41] P. Zuman, *Substitution Effects in Organic Polarography*. Plenum, New York (1967).
[42] S. Görög and G. Szasz, *Analysis of Steroid Hormone Drugs*. Elsevier, Amsterdam (1978).
[43] A. M. Bond, I. D. Heritage and M. H. Briggs, *Anal. Chim. Acta* **138**, 35 (1982).
[44] W. M. Peterson, *Am. Lab.* **11**, 69 (1979).
[45] S. Görög, *Acta Chim. Hung.* **47**, 1 (1966).
[46] USP XIX, 1975, p. 191.
[47] Belal Saied and Issa Nevine, *Farmazie* **37**, 297 (1982).
[48] W. P. van Bennekom, H. J. E. M. Reeuwijk and J. B. Schute, *Anal. Chim. Acta* **74**, 387 (1975).
[49] S. Görög, A. Lauko and Zs. Sziklay, *Analyst* **104**, 196 (1979).
[50] S. Görög, *Fresenius Z. Anal. Chem.* **309**, 97 (1981).
[51] K. R. Bagon and E. W. Hammond, *Analyst* **103**, 156 (1978).
[52] V. Gajda and K. Horak, *Anal. Chim. Acta* **134**, 219 (1982).
[53] J. A. Turner and R. A. Osteryoung, *Anal. Chem.* **50**, 1496 (1978).
[54] USP XX, p. 326.
[55] USP, XIX, 1975, p. 622. National Formulary, XIV, 1975, p. 976.
[56] S. Görög and P. Horvath, *Analyst* **103**, 346 (1978).

[57] R. H. Silber and C. C. Porter, *J. Biol. Chem.* **210**, 923 (1954).
[58] E. J. Umberger, *Anal. Chem.* **27**, 768 (1955).
[59] P. A. Williams and E. R. Biel, *J. Pharm. Sci.* **70**, 530 (1981).
[60] J. B. Landis, *Anal. Chim. Acta* **114**, 155 (1980).
[61] W. F. Smyth, J. S. Burmicz and A. I. Ivaska, *Analyst* **107**, 1019 (1982).
[62] P. W. Alexander and M. H. Shah, *Talanta* **26**, 97 (1979).
[63] P. W. Alexander and M. H. Shah, *Anal. Chem.* **51**, 2139 (1979).
[64] Zs. Feher, G. Horvai, G. Nagy, Z. Niegreisz, K. Toth and E. Pungor, *Anal. Chim. Acta* **145**, 41 (1983).
[65] J. B. Flato, *Anal. Chem.* **44**, 75A (172).
[66] J. Asplund, *Int. Symp. on Electroanalysis in Biomedical, Environmental and Industrial Sciences*, Cardiff, 5–8 April (1983).
[67] W. J. Pugh, *J. Pharm. Pharmacol.* **31**, 421 (1979).
[68] J. Osteryoung, *J. Chem. Educ.* **60**, 296 (1983).
[69] N. A. Lannigan, J. R. Johnson and R. H. Pryce-Jones, *J. Pharm. Pharmacol.* **33**, 9 P (1981).
[70] D. M. Baaske, J. E. Carter and A. H. Amann, *J. Pharm. Sci.* **68**, 481 (1979).
[71] H. L. Fung, *Am. J. Hosp. Pharm.* **35**, 528 (1978).
[72] A. H. Amann and D. M. Baaske, *J. Pharm. Sci.* **71**, 473 (1982).
[73] A. W. Malik, A. H. Amann, D. M. Baaske and R. G. Stoll, *J. Pharm. Sci.* **70**, 798 (1981).
[74] A. H. Scheife, J. A. Grisafe and L. Shargel, *J. Pharm. Sci.* **71**, 55 (1982).
[75] B. J. Birch, Int. Symp. on Electroanalysis in Biomedical, Environmental and Industrial Sciences, Cardiff, 5–8 April (1983).
[76] J. C. Schaar and D. E. Smith, *Anal. Chem.* **54**, 1589 (1982).
[77] L. Rozanski, *Analyst* **103**, 950 (1978).
[78] A. M. Bond, I. D. Heritage and M. H. Briggs, *Anal. Chim. Acta* **127**, 135 (1981).
[79] E. Heftmann and I. R. Hunter, *J. Chromatogr.* **165**, 283 (1979).
[80] S. Görög and B. Herenyi, *J. Chromatogr.* **152**, 240 (1978).
[81] A. Wikby, L. Nilsson and G. Hällsås, *J. Chromatogr.* **157**, 51 (1978).
[82] E. C. Juenge and J. F. Brower, *J. Pharm. Sci.* **68**, 551 (1979).
[83] G. Roth, A. Wikby, L. Nilsson and A. Thalen, *J. Pharm. Sci.* **69**, 766 (1980).
[84] E. C. Juenge and P. E. Flinn, *J. Pharm. Sci.* **70**, 415 (1981).
[85] H. S. DeBoer, W. J. van Oort and P.Zuman, *Anal. Chim. Acta* **120**, 31 (1980).
[86] H. S. DeBoer, Thesis, Utrecht (1980).
[87] V. Delaroff, M. Bolla and M. Legrand, *Bull. Soc. Chim. France* **1961**, 1912.
[88] O. Hrdý, *Collect. Czech. Chem. Commun.* **27**, 2447 (1962).
[89] A. I. Cohen, *Anal. Chem.* **35**, 128 (1963).
[90] P. Kabasakalian and J. McGlotten, *Anal. Chem.* **31**, 1091 (1959); **34**, 1440 (1962).
[91] H. W. Nürnberg, *Z. Anal. Chem.* **273**, 432 (1975).
[92] P. Zuman, *Talanta* **12**, 1337 (1965).
[93] K. Ponsold und H. Kasch, *Z. Chemie* **22**, 157 (1982).
[94] H. S. DeBoer, J. Den Hartigh, H. H. J. L. Ploegmakers and W. J. van Oort, *Anal. Chim. Acta* **102**, 141 (1978).
[95] J. Pavel and U. Frey, *Int. J. Environ. Anal. Chem.* **13**, 89 (1983).
[96] D. I. Edwards, in *Progress in Medical Chemistry* Vol. 18 (G. P. Ellis and G. B. West, Eds.), p. 87. Elsevier, North Holland Biomedical Press, Amsterdam (1981).
[97] A. Breccia, in *Nitroimidazoles, Chemistry, Pharmacology and Clinical Application* (A. Breccia, B. Cavalleri and G. E. Adams, Eds.), p. 35. Plenum Press, New York (1982).
[98] D. I. Edwards, R. J. Knox, D. A. Rowley, I. M. Skolimowski and R. C. Knight, in *Nitroimidazoles, Chemistry, Pharmacology and Clinical Application* (A. Breccia, B. Cavalleri and G. E. Adams, Eds.), p. 105. Plenum Press, New York (1982).
[99] R. C. Knight, I. Skolimowski and D. I. Edwards, *Biochem. Pharmacol.* **27**, 2089 (1978).
[100] J.-C. Viré, J.-M. Kauffmann and G. J. Patriarche, *Anal. Lett.* **15**, (B16), 1331 (1982).
[101] F. I. Sengün, *Chim. Acta Turc.* **8**, 155 (1980) (*Chem. Abstr.* **94**, 180735v).
[102] J. Wang and H. D. Dewald, *Talanta* **29**, 901 (1982).
[103] E. Jacobson and M. Wiese Bjørnsen, *Anal. Chim. Acta* **96**, 345 (1978).
[104] J. Ballantine and A. D. Woolfson, *J. Pharm. Pharmacol.* **32**, 353 (1980).
[105] W. A. Byers, B. S. Freiser and S. P. Perone, *Anal. Chem.* **55**, 620 (1983).
[106] I. E. Davidson and W. F. Smyth, *Anal. Chem.* **49**, 1195 (1977).
[107] U. Forsman, *Anal. Chim. Acta* **146**, 71 (1983).
[108] J. Osteryoung, J. W. Whittaker and M. R. Smyth, in *Electroanalysis in Hygiene, Environmental, Clinical and Pharmaceutical Chemistry* (W. F. Smyth, Ed.), p. 413. Elsevier, Amsterdam (1980).
[109] J. P. Hart and A. Catterall, *Anal. Chim. Acta* **128**, 245 (1981).
[110] P. Zuman, *Topics in Organic Polarography*. Plenum, New York (1970).
[111] P. M. Bersier, unpublished results.

[112] J. A. F. de Silva and M. F. Brooks, Voltammetric methods, in *Drug Fate and Metabolism* (E. R. Garrett and J. L. Hirtz, Eds.), p. 1. Marcel Dekker, New York (1978).
[113] H. W. Nürnberg, Proc. Symp. Uppsala, Sweden, 22–27 Sept. 1977, in *Structure and Dynamics in Chemistry*, p. 270. Uppsala (1978).
[114] W. F. Smyth, *Proc. Analyt. Div. Chem. Soc.* 187 (1975).
[115] G. Dusinsky and L. Faith, *Pharmazie* **22**, 475 (1967).
[116] L. Faith and G. Dusinsky, *Identification of Medicaments and Venoms by Oscillopolarography*. Vydavetel'stvo Osveta (1974).
[117] K. Thoma and R. Groning, *Deut. Apoth. Zig.* **115**, 1456 (1975).

[*Received 8 June 1983*]