Review

What Can DC Polarography Offer Today

Petr Zuman*

Department of Chemistry, Box 5810, Clarkson University, Potsdam, NY 13699-5810, USA

**Corresponding author: Tel. +1-315-268-2340, fax 1-315-268-6610*

** Corresponding author: E-mail: zumanp@clarkson.edu*

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Dedicated to Professor Josef Barthel on the occasion of his 80th birthday

Abstract

Advantages of uses of DC polarography in determination of inorganic and organic species (such as speed of analyzes, highly reproducible and reliable results, good theoretical background, establishment of the degree of conjugation in organic species, possibility to determine the oxidation states in metal ions, lower costs, no use of organic solvents, wide range of heavy metal ions and of reducible organic compounds that can be determined) as well as limitations (lack of qualified supervising personnel, fewer species undergoing oxidations) are stated. Examples of recent successful applications are given: (1) analyses of drugs containing a single physiologically active and electroactive species; (2) investigations in non-homogeneous systems, such as of binding of heavy metal ions, N-nitrosamines and various pesticides in suspensions of lignin; or of formation of slightly soluble small aggregates and well soluble large aggregates in interactions of heavy metal ions with bile salts; (3) determination of equilibrium and rate constants for equilibria occurring in the bulk of the solution and of rate constants of establishment of acid-base and hydration dehydration equilibria that are perturbed by electrolysis in the vicinity of the electrode. It is possible to follow this type of fast reactions, with rate constants in the range between 1×10^4 L mol^{-1} s^{-1} and 1×10^{10} L mol^{-1} s^{-1}.

Keywords: DC polarography, metal ions, drugs, kinetics rate constants

1. Introduction

In the 1950s and 1960s DC polarography (DCP) was according to reports in Analytical Chemistry one of the five most frequently used methods in analysis and in investigations of solution chemistry, of both inorganic and organic species. Since that time it has been replaced in determination of heavy metal ions by techniques based on atomic absorption. In investigations of solution chemistry of organic compounds polarography competes with the more widely used spectrophotometry. In organic analyses now predominates mostly the use of separation technique, such as various forms of chromatography and electrophoresis.

To evaluate the possibilities of current uses of DCP, we shall summarize first the advantages, that polarographic methods offer both in applied and fundamental studies, and then point out the limitation of such applications.

2. Advantages of DC Polarography

The DC polarography (DCP) is a physico-chemical technique, based on recording and measurement of current-voltage curves, obtained with a dropping mercury electrode. The quantities measured most frequently on such current-voltage curves are limiting currents and half-wave potentials. The rate of the reduction or oxidation varies with applied potential. For reductions the rate increases with increasingly negative potentials, for oxidation with increasingly positive potentials.

With the increase of the potential in negative direction, the region of potential is reached, where the rate of the electroreduction becomes faster than the transport of the reducible species to the electrode. As a result, the current due to electron uptakes by the reducible species reaches a limiting value and does not increase with a further increase of the negative potential. Its value remains practically constant until a more negative potential is reached at which another reduction process takes place. The transport controlling the limiting current is, in most instances, diffusion of the electroactive species to the electrode, but can also be limited by a rate of a chemical reaction (including catalyzed processes), which occurs in the vicinity of the electrode surface. Alternatively, the limiting current can be affected by adsorption phenomena. Currents due to reduction processes are denoted cathodic.

The situation of electro-oxidations, manifested by anodic currents, is similar, just the processes and currents involved are a mirror image of cathodic ones. The rate of oxidations, involving a loss of one or more electrons by the oxidized species, increases with increasingly positive potentials. After a potential is reached, when the transport of the species to be oxidized becomes the slow step, the anodic current (flowing in the opposite direction than that observed for reductions) reaches a limiting value.

In most cases, the transport processes observed for oxidations are also diffusion controlled. Nevertheless, in some instances it is the rate of a chemical reaction occurring in the vicinity of the electrode surface that becomes the rate limiting step. Kinetic anodic waves result.

When anodic processes are due to the formation of slightly soluble mercury species, anodic waves are often controlled by adsorption.

Measured limiting currents are often a simple function of concentration of the electroactive species. Measurements of limiting currents can be thus used for determination of the concentration of the reducible or oxidizable species. Such measurements can also allow the following and interpretation of rates of chemical reactions, detect adsorption phenomena and enable to distinguish between some types of adsorption.

The possibility to record limiting currents, together with the fact that each drop of the mercury electrode begins with a fresh, clean, well defined surface results in the main advantage of the use of the dropping mercury electrode (DME) – the possibility to obtain well reproducible results, not only with the same apparatus and in the same laboratories, but between different laboratories using different types of equipment. Due, for example, to the presence of varying concentrations of surface active substances, the peak currents obtained in real life samples in a number of electroanalytical techniques are less well reproducible than limiting currents obtained with DME.

The accuracy of measurements of limiting currents obtained with DME is typically of the order of ±3% in various types of samples. If needed, with greater care, this can be reduced to ±1%. The peak currents obtained for example in linear sweep voltammetry (LSV), cyclic voltammetry (CV) or pulse techniques (like DPP) or square wave techniques (SWP, SWV) in real life samples, like food, natural products, blood or tissues are commonly determined to ±2 to ±5%. Lower reproducibility is frequently observed, when i-E curves are recorded with stationary mercury electrodes (such as hanging drop or pool), and even more frequently when electrodes from other metals and materials are used.

This situation is reflected even more in theoretical studies. This is indicated by the fact that in numerous publications describing the use of CV or LSV curves, only values of peak potentials and/or shapes of the i-E curves are reported rather than the variations in peak currents. Often voltammograms obtained by CV or LSV are discussed only in qualitative terms.

Hence the possibility to obtain and measure limiting currents is the main advantage in the use of DME in DCP. Limiting currents can be obtained also with some other techniques, such a normal pulse polarography or rotating disk electrodes. The limited number of systems studied with these techniques prevents us from comparing their advantages and limitations.

In some instances, in particular when investigating adsorption phenomena, the timing of the contact of the electrode (the new drop) with the solution is better defined than that during the immersion of a stationary electrode (such as solid or hanging mercury drop) into the solution. For the latter electrodes the period between immersion and the beginning of measurement is often not well defined.

Another advantage of the use of the DME is the high hydrogen overvoltage of mercury in aqueous solutions. This allows following of electroreductions at potentials which are more negative than those that can be reached when other electrode materials are used as cathodes. Wide range of potentials from +0.4 V to about –3.0 V enables following reductions of a wide variety of both inorganic[1] and of organic[2] species.

Further advantage of polarography is the possibility to apply it not only in investigations in homogeneous solutions, but also in heterogeneous suspensions. In some instances it is possible to determine both inorganic and organic species present in dispersions of inorganic or organic materials or colloidal solutions.

From the theoretical point of view it is of interest that polarography enables examination of equilibria established in chemical reactions, based both on shifts of half-wave potentials and on variations of limiting currents with the composition of the examined solution. The conditions of such applications will be discussed below. The same applies to the use of polarography in a study of fast and slower reactions.

3. Limitations of DC Polarography (DCP)

To be followed by polarography, the investigated species must be electroactive. This means that it can be reduced or oxidized on DME within the available potential range, or alternatively form slightly soluble or complex compounds with mercury ions or that it can affect catalytically some electrode processes.

Great majority of inorganic cations, acids, or anions can be reduced or oxidized at the DME, but the main area of analytical and theoretical applications of DCP in investigation of inorganic species involves the reduction of cations of heavy metals. As opposed to techniques based on atomic absorption, polarography often enables simultaneous determination of several cations present in a mixture. Perhaps even more important is the possibility to distinguish the oxidation states of individual metals. In some cases it is moreover possible to determine metal ions, their complexes and other inorganic species, when they are present in varying oxidation states in a given solution.

Among important reducible inorganic molecules belong O_2, H_2O_2 and SO_2, as well as some acids of elements of the sixth and seventh group of the periodic table. Thus it is, for example, possible to determine periodic and iodic acids in a mixture. Polarography is also a useful tool in investigation of the oxidation state of the central atom and of structures of some complexes. The enumeration above indicates that the limitations of electroactivity among inorganic species are not too serious.

The situation is not principally different concerning the electroactivity of organic compounds. Large number of important organic compounds undergoes reduction at the DME, particularly of those that are physiologically active. These include numerous natural products (particularly when their structures contain double or triple bonds). In some instances a single C–X bond (X = halides, OH, NH_2SH) can be split reductively, but often for such cleavage of the single bond a presence of an activating grouping in a vicinity of the cleaved bond is necessary. In general, a large number of organic compounds, that are chemically reactive, are also electroactive.

The situation is markedly different when considering oxidative processes. Due to an electro-oxidation of metallic mercury at about +0.4 V, it is possible to follow by DCP only the most easily oxidized species. Among inorganic ions, anodic oxidation waves can be only observed in the presence of some metal ions in lower oxidation states, such as Fe(II), Cr(II) or Sn(II).

Similar is the situation, when dealing with the electrooxidation of organic compounds. Anodic oxidation waves are observed in solutions of enediols (such as ascorbic acid), hydroquinones, phenylhydroxylamines as well as some hydrazine derivatives, geminal diol anions in the oxidation of aldehydes, as well as some heterocyclic compounds including reduced forms of some dyes.

The easy oxidation of mercury has, nevertheless, some positive aspects. It is namely possible to observe anodic waves, when mercury ions, generated by oxidation of metallic mercury, can interact with some components of the investigated solution to form slightly soluble mercury compounds or soluble complex species. Representants of inorganic species forming slightly soluble mercury compounds are halides, sulfides and selenides, example of complex forming species are cyanides. Among organic species reacting with mercury ions are thiolates, as well as cyclic and acyclic derivatives of urea and thiourea (for example barbiturates or thiobarbiturates) and some heterocyclic compounds. Organic complex forming species can be represented by derivatives of ethylenediamine tetraacetic acid, that form anodic waves.

But the electrooxidation of most common organic compounds, like phenols, anilines and other amines, takes place at so positive potentials that some solid electrodes – such as various forms of carbon electrodes – have to be used.

Nevertheless, the most serious limitations to the current use of DCP are problems of availability of qualified personnel, particularly of the more highly trained ones. A laboratory technician, an undergraduate or a starting graduate student can master the use of the DME and of the source of applied voltage and the recording device – the polarograph – within one or at most two weeks. The problem is the scarcity of well trained supervising personnel or mentors.

Personnel supervising one of the variants of chromatography or of atomic absorption have to be familiar only with one area of research in order to be able to supervise the laboratory work and to interpret and evaluate results. On the other hand, the personnel supervising the use of electroanalytical methods must be trained not only in electrochemistry, such as in choosing the right electrode, the right supporting electrolyte, the proper voltage range, or direction of the applied voltage, but also in solution chemistry (treatment of equilibria, particularly acid-base, of kinetics of chemical reactions, of the roles of temperature, viscosity, solvation, ionic strength, as well as of the possible effects of cations and anions present in the investigated solution, etc.). Such supervisors or mentors should also understand – at least in principle – structure-reactivity relationships, both for inorganic and organic species, as well as the principles of processes taking place on the boundary between the metallic phase and the solution.

The latter includes in particular the role of adsorption and the types of interactions, which control it, ranging from the roles of cations and anions in the double-layer, to those resulting from van der Waals forces, π–π interactions and chemisorptions: Such interactions can be responsible for a variety of adsorption phenomena, from those resulting in formation of a double layer, to formation of layers that can be penetrated (with different ease) by electroactive species, all the way to formation of rigid, impenetrable films, from formation of isolated islands at low concentrations of adsorbed species to formation of monolayers and multilayers.

A good training in such diverse areas can be achieved only in well functioning research teams, in which an exchange and development of ideas can flourish. Such research groups, which in the past existed at universities and in research institutes worldwide, are currently unfortunately rather rare. This results in the current shortage of well qualified supervising personnel in laboratories in industries and in a limited number of well qualified mentors at schools, who would be interested and able to teach electrochemistry and to direct research in this area.

Last but not least limiting factor in the use of DCP deals with available instrumentation. The early polarographs allowed recording i-E curves obtained with a DME. The recording system used an adjustable damping, comparable to that of a critically damped galvanometer. The DME was realized by a glass capillary attached at an adjustable height to a reservoir of mercury. The resulting i-E curves have shown oscillations, due to the dropping off of one drop (the electrode) and to the growth of the subsequent one. Observation of the shape of these oscillations can already offer information regarding the electrode process, particularly of some types of adsorption phenomena.

Theoretical treatments of limiting diffusion, kinetics and adsorption currents took into account the increase of the surface of the mercury drop during its life-time. They offered solution for the mean recorded current, corresponding to the center of current oscillations. These old instruments made it possible to vary to some degree the size of the oscillations by damping. It has been repeatedly proved with various instruments that the mean measured current (at the middle of oscillations) remains practically independent of the damping used and size of the observed oscillations.

The principle of recording and current measurement was retained in the early pen-recording instruments, such as the one manufactured by Cambridge Instruments which used a pen linked to a movement of a galvanometer. These were soon followed with electronic instruments, using first lamps, later transistors, such as those manufactured by Radiometer, Sargent, Metrohm, IBM, Amel, or PAR (Princeton Applied Research). All these analog instruments used a pen-recorder and mostly naturally growing mercury drop electrode.

Considerable change took place with the introduction of digital instruments, in which were measured currents, obtained with a mercury electrode either with a constant surface (a hanging mercury drop electrode – HMDE) or with a dropping mercury electrode, the life-time of which was artificially terminated after a chosen time-interval from the beginning of the life-time. The current-voltage curves recorded in this way practically do not show oscillations. The measurements of such limiting currents are easier to handle electronically, it is faster and suitable for analytical applications. But two aspects of this type of recording and measurement of limiting currents should be kept in mind: (1) It has not been demonstrated, how the currents measured in this way agree with those predicted by available theory derived for DME. (2) The information obtainable from the shape of oscillations on i-E curves recorded by analog systems is lost.

The mercury indicator electrodes, recently commercially available, are constructed in such a way that the metallic mercury has no contact with the laboratory air. This leads to a discussion to what can be called “mercurophobia” or “mercury hycteria.” Due to a media reaction to a case of poisioning by organomercurials (chemically different from metallic mercury and considerably more toxic) in Japan by ill informed fanatics and politicians, the use of mercury in laboratories was prohibited in some countries. To a chemist it seems natural that, as with other chemicals used in laboratories (many of them much more toxic than mercury), experiments dealing with metallic mercury must be carried out with care.

This author has worked, or spent considerable part of his life, (more than 60 years) in a laboratory, where metallic mercury was daily used. He never observed marked early symptoms of poisoning of mercury vapors, such as very shaky hand writing. In the 1950s and 1960s in the Polarographic Institute of the Czechoslovak Academy of Arts and Science, where he worked, daily about 70 people either dealt with metallic mercury or worked in rooms where mercury was used. The Institute of Industrial Hygiene tested every month the level of mercury vapors in the air in all laboratories. In all instances the concentration of mercury vapors in all rooms at about 25 °C was two orders of magnitude (100x) lower than the toxic level. The same was true about quarterly tested mercury levels in blood of the chemists working daily with mercury.

A different kind of evidence is based on his meeting during the past more than 50 years with several hundreds of chemists working with polarography and had been in contact with numerous others. Not a single one of them observed or reported any ill-effects due to contact with mercury. On the other hand, he knows of several cases of synthetic organic chemists, who suffered serious health problems, including cancer, resulting from exposure to common organic solvents.

The only case of mercury poisoning, he was aware of, happened ironically in the laboratories of the Institute of Industrial Hygiene in Prague. There a chemist was drying mercury at 105 °C in a hood in a small laboratory. Unfortunately, the hood ill-functioned and instead of removal of the mercury vapors from the hood at this high temperature, it has blown them into the laboratory. The chemist exhibited all the typical symptoms of mercury poisoning, but after treatment with 2,3-dimercaptopropanol the symptoms receded completely during 2–3 weeks and currently at 77 has no health problems.

It is at temperatures higher than about 60 °C that mercury becomes very volatile and toxic. All the reported examples of the horror stories about toxic effects of mercury were based on cases from felt factories in the 1800s, where production was carried out at high temperatures in ill-ventilated workshops.

Last but not least, there is in the Miro Museum in Barcelona (Spain) a fountain, using liquid mercury instead of water in a public accessible site. It seems improbable that its use would be allowed to continue, if any toxic effects were reported.

4. Successful Recent Applications of Polarography

Following discussion of advantages and limitations, it is now possible to point out areas, in which polarography currently is – or could be – successfully applied. It should be stressed that before polarography is applied to a solution of a practical, analytical problem or for solving some basic or theoretical problem, the nature of cathodic, anodic, or catalytic processes involved should be understood, at least in principle. The understanding of processes is essential for an optimal use of the technique. If the nature of processes involved is not understood, it should be investigated before the application to prevent possible errors in interpretation of the results.

4. 1. Pharmaceuticals

A large number of pharmaceuticals can be reduced or oxidized in the available potential range and their waves can be used in their determination. It seems that often the therapeutical activity is paralleled by electrochemical reactivity.

Some drugs can be determined using polarography directly, without separation, in some physiological liquids, for example in blood, lymph and saliva and is some times. Numerous examples of such applications have been reported early[3]. In some cases, nevertheless, simple separation procedures, such as extraction, have to be used. In numerous cases of this type the uses of polarography would result in procedures which are as selective, but faster and less expensive to use than applications of the most widely used chromatographic methods. On the other hand, in analyses of complex mixtures like urine or in the following of metabolic products, the use of separation techniques is definitely preferable.

The situation is different, when the content of the drug is to be determined in matrices, in which the drug is the only electroactive species. Such situation is often faced in analyses of tablets, solutions like eye drops or those used for injections, but also in analyses of some creams and ointments. In such cases the solution to be analyzed should contain the electroactive species at concentrations varying between 1×10^{-5} and 1×10^{-3} M. In such solutions DC polarography is the preferable technique of choice. The method is sufficiently sensitive and measurements of limiting currents are less affected than those of peak currents, used in other electroanalytical procedures, by the presence of electroinactive components of the sample. Presence of components in the sample, like that of starches, of some polymers or long-chain saturated compounds, has little effect on limiting currents, but can affect peak currents, as obtained in differential pulse or square wave variants.

To use of chromatographic methods for analyses for such simple matrices resembles using a canon on a fly. To use polarographic methods for analyses of such simple matrices yields results often much faster, with a better accuracy and without using organic solvents. But, in addition to the lack of trained supervising personnel, mentioned above, there is another, administrative hurdle preventing a wider use of polarographic methods in the determination of drugs. Pharmaceutical companies will use, whenever possible, officially approved methods of analysis. In the past, some polarographic analytical procedures were listed in numerous Pharmacopoeias. It should be a goal of electroanalytical chemists around the world to have them listed again. The lower costs, faster results, and the possibility for quickly detecting mishandlings by technicians, are powerful arguments.

4. 2. Analyses of Heterogeneous Systems

Two successful recent applications of the following of processes taking place in non-homogeneous systems will be discussed here: The interactions of heavy metal ions with bile salts and the investigations in suspensions of lignin.

The interaction of bile salts with heavy metal ions was of interest, because such heavy metal ions are present in bile. In the investigations of interactions of heavy metal ions, such as Cu^{2+}, Cd^{2+}, Zn^{2+} and $Fe^{2+,}$ the concentration of the metal ion was kept constant and the concentration of the bile salt was gradually increased. In such interactions with bile salts, the pH was kept at pH > 7, to assure a complete dissociation of the carboxyl group, in investigations of taurine conjugates of bile salts it was kept at pH > 2, to assure a complete dissociation of the $–SO_3H$ group.

Results of those studies[4–11] can be summarized as follows: At low concentrations of bile salts, where they are present as monomeric anions, their interaction with Me^{2+} is negligible and the solution remains homogeneous. At a certain concentration of bile salt, that depends on its structure and the nature of the metal ions, the investigated solution becomes cloudy. In this concentration range of the bile salt it forms aggregates, containing 2–4 molecules. The Me^{2+} ions interact with these aggregates to form a slightly soluble species. Its formation is accompanied by a decrease in the limiting current of the metal ion. With further increase in bile salt concentration, more of the slightly soluble aggregate is formed and the limiting current of the metal ion decreases. This decrease continues, until a saturation of the solution is reached and the solution becomes saturated by the aggregate containing the metal ion. Over a certain range of the bile salt concentration, the limiting current remains independent of the bile salt concentration. Above a concentration of the bile salt, which is close to the reported critical micelle concentration of the given bile salt, the limiting current of the reduction of the metal ion gradually increases (Fig.1).. This is attributed to a formation of aggregates consisting of a larger number of bile salt molecules. The presence of further free carboxylate groups assists the higher solubility of the larger aggregates.

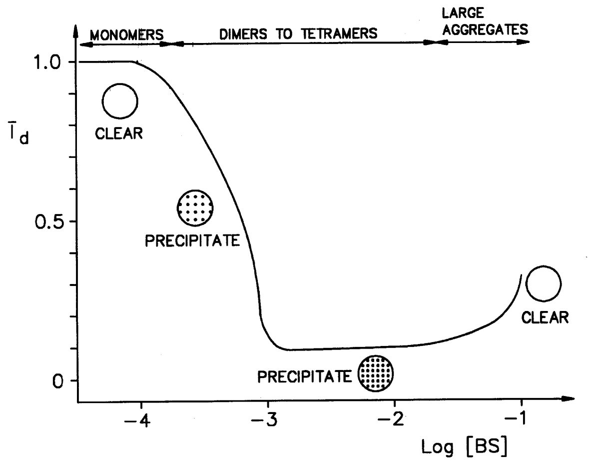

Figure 1. Reactions of bile salts (BS) with dications of heavy metal ions. Schematics of the dependence of the limiting current of the reduction of the metal(II) ion "i" as "i/id"(where id is the diffusion current of the current of the reduction of Cu(II) ions in the absence of BS) on the concentration of BS.

Changes in the side chain of C-17 on the steroid ring, such as replacement of the COO- group by the SO3- group, strongly affect the reactivity towards Me^{2+} ions. Whereas all four investigated trihydroxy bile salts have shown a similar behavior, indicating similar structures of the aggregates, no such similarity was observed for the six dihydroxy bile salts investigated. It is assumed that the stacking of bile salt molecules in small aggregates is different for individual dihydroxy isomers.

Two types of investigations have been carried out in suspensions of lignin. Lignin (Fig. 2) is a natural polymer present in plants, particularly in trees and shrubs, but evening grasses and in straw. It contributes to their mechanical strength of trunks, branches and stems. In industry, it is an unwanted side-product in the manufacture of paper and of some cellulosic polymers. It promises to become a green material for detoxication and a renewable source of organics for chemical industry.

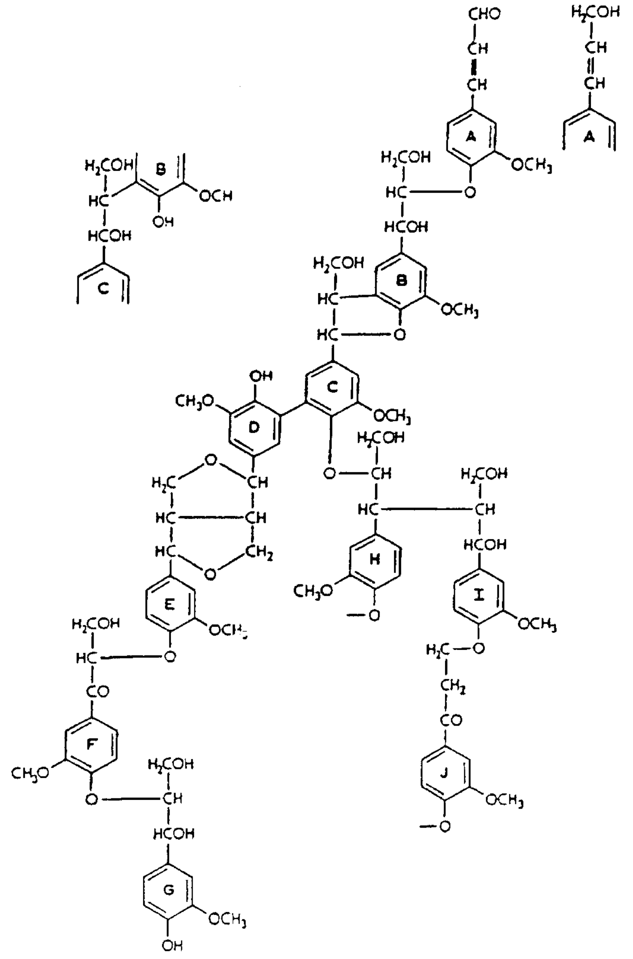

Figure 2. A cartoon showing possible components and linkages in natural lignin. As opposed to polymers like cellulosics or proteins, in the polymeric lignin there is no repetition of structural units.

To demonstrate its usefulness as a detoxicant, its interactions with potentially toxic materials have been studied. The approach was opposite than in the investigation of the binding of metal ions on bile salts: A chosen amount of powdered lignin was dispersed in a buffer of chosen pH and composition. To this ink-like suspension, the studied species was gradually added and the concentration of the remaining free species determined, after placing the DME into the suspension. Resulting adsorption isotherm indicated the degree of interaction of the electroactive species with lignin.[12] In this way was studied the binding of some heavy metal ions,[13–15] nitrosamines,[16] bile salts[17] and various pesticides.[18–20]

To follow depolymerization of lignin under mild conditions (25 °C, pH 9–12) a suspension of lignin in a chosen buffer was prepared and polarographic i-E curves were recorded as a function of time. Increases of waves of 3,4-substituted benzaldehydes and of α,β-unsaturated carbonyl compounds were followed. In this way, it was possible to follow homogeneous kinetics in a heterogeneous suspension (Fig. 3). The dependence of the rate of cleavage on pH indicated that the initial step in the alkaline cleavage of lignin under used mild conditions, is a dissociation of a group with pK_a about 11 (probably of a phenolic OH group) rather than a nucleophilic addition of OH^- ions to the double bonds in lignin.[21,22] Most recently, a comparison of the difference in kinetics of clea-

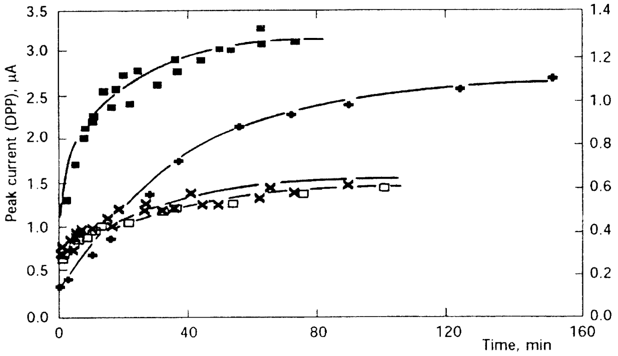

Figure 3. Alkaline cleavage of some lignins: Dependence of currents of reductions of substituted benzaldehydes on time during hydrolyses of three rotwood lignins (full and open squares and x, left hand current scale) and of a kraft lignin (+, right hand current scale). Borate buffer pH 9.

vage of lignin and of humic acids proved that humic acids are a man-made rather than natural product.[23]

4. 3. Basic Studies

Polarography proved to be useful for determination of oxidation states of metals, both in the ionic form and in complexes. Both the equilibria of labile complexes and properties of substitution inert complexes as well as characteristics of metalorganic compounds may be investigated may be investigated by DC polarography (DCP). Structure-reactivity relationships for such species can be established.

In investigations of organic compounds, polarography enables distinguishing the degree of the role of conjugation, of effects of ring size, of substituent effects in Linear Free Energy Relationships[24] and other structure-reactivity relationships. Recently, the comparison of polarographic half-wave potentials indicated limited conjugation in compounds containing the >C=N–N=C< grouping, as opposed to the extensive conjugation for those containing groupings –N=C–C=N–.[25,26]

4. 3. 1. Rapidly Established Equilibria

Polarography is also a useful tool in investigations of solution chemistry, in particular of equilibria and kinetics. Thus for rapidly established equilibria, both acid-base and of formation of complexes, involving reducible heavy metal ions and a variety of ligands, the equilibrium constants can be determined based on shifts of potentials, using graphs in which these measured quantities are plotted as a function of pH or of a logarithm of ligand concentration. In some instances this enables determination of values of equilibrium constants of reactions involving organic compounds that would be difficult to obtain by other techniques. Similarly, from the shifts of half-wave potentials of the reduction of metal ions, it is possible to obtain information about stability constants of formation of some labile complexes. Polarographic investigations also enabled detection of some unusual species formed in acid-base equilibria, for example of the diprotonated forms of hydrazones[27,28] and oximes.[29,30]

Whereas DC polarography is well suited for investigation of chemical equilibria preceding the electron transfer (systems denoted as CE) and for identification of relatively stable intermediates in consecutive electroreductions, for investigation of faster chemical reactions following the electron transfer (EC systems) and for studies of properties of short-lived intermediates, cyclic voltammetry is the technique of choice.

4. 3. 2. Slowly Established Equilibria

At the other extreme, when investigated equilibria are established slowly, as compared to the rate of the electrode process, the equilibrium constants can be determined based on measurements of limiting currents. The rates of dissociation of OH, SH or NH_3^+ type acids are too fast to be followed in this way, but some equilibria involving cleavage of the C–H bond are established relatively slowly and can be investigated based on the dependence of the limiting current of the conjugate acid on pH. This approach was possible in establishing the acid-base properties of 3-thianaphthenone,[31] ethyl benzoylacetate,[32] ethyl benzoylbenzoates,[33] ω-cyanoacetophenone[34] and of 1-phenyl-1,3-butanedione.[35]

Another group of processes, where the equilibria are not completely shifted in the favor of the products and are established relatively slowly, are some nucleophilic additions to carbonyl groups. Thus the formation of a separate wave of an imine at more positive potentials than that of the reduction of the carbonyl group, made it possible to determine equilibrium constants of additions of amines to aldehydes and ketones.[36–39] The intermediate of such reactions is a carbinolamine, the presence of which was demonstrated and properties followed in the reactions of benzaldehyde[40] and terephthalaldehyde[41] with hydrazine.

Analogous additions of water to aldehydes and some ketones are usually too fast for such a treatment. Exceptions are reactions of orthophthaladehyde and naphthalenedicarboxaldehyde with water, where the slow step is the consecutive formation of the cyclic hemiacetal[42,43] (Fig. 4). Slowly established equilibria between some carbonyl compounds and water has been also observed in compounds, where there is a strong resonance effect of the interaction between the aldehydic group and an electronegative substituent on the benzene ring, as is the case of the second formyl group affecting the reactivity of terephthalaldehyde[44] or the pyridine nitrogen in pyridinecarboxaldehydes.[45] In aliphatic carbonyl compounds the hydration can be facilitated by adjacent strongly electronegative groups, such as carboxyl[46] or a trifluoromethyl group.[30,47,48] On the other hand equilibria in additions of

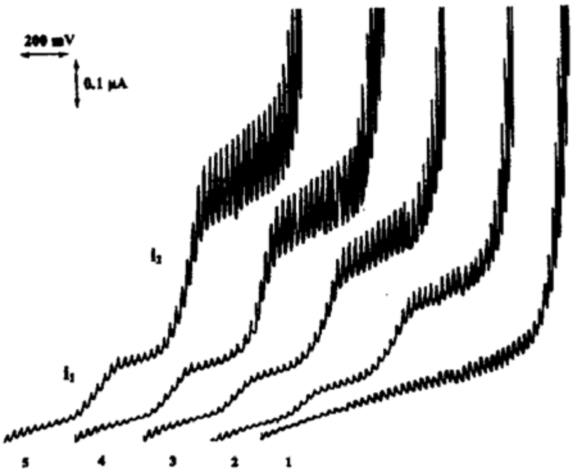

Figure 4. Two reduction polarographic waves of orthophthalaldehyde (OPA) used in the determination of equilibrium constants between the unhydrated (Ia), acyclic monohydrated (Ib) and cyclic hemiacetal (Ic) forms of OPA. First, most positive, wave is proportional to the concentration of the unhydrated form Ia. The second, more negative wave is a simple function of [Ib]. Curves were recorded in a phosphate buffer pH 5.8 at varying [OPA]: (1) 0.0; (2) 0.04; (3) 0.08; (4) 0.12 and (5) 0.18 mM.

SH^-, CN^- or SO_3H^- ions to a carbonyl group of the orthophthalaldehyde are too rapidly established to be followed by polarography.

4. 3. 3. Kinetics of Fast Reactions

Finally, there are some equilibria which are established neither extremely rapidly nor extremely slowly. The rates of their establishments are comparable with the rate of the electrode process. In these cases, where the rate of the establishment of a chemical equilibrium, that takes place before the electron transfer, is comparable with the rate of the transfer of the electron, the rate of the re-establishment of the chemical equilibrium perturbed by electrolysis controls the limiting current. When the equilibrium is perturbed by removal of the electroactive component by reduction or oxidation, the re-establishment of such equilibrium takes place. The rate constant of this establishment of the equilibria can be obtained, provided that the value of the equilibrium constant is known, or obtained by another technique, such as potentiometry or spectrophotometry. For such reactions taking place within the reaction layer in the vicinity of the dropping mercury electrode (a layer which is much narrower than the diffusion layer) it is possible to obtain rate constants of the order of 10^4 to 10^{10} L mol^{-1} s^{-1}. In those cases, where the same reaction was followed by relaxation techniques, good agreement was found. The condition must be fulfilled that the reaction studied electrochemically takes place as a homogeneous process in the solution in the vicinity of the DME.

The currents controlled by the rate of a chemical reaction are called kinetic and are observed particularly for compounds undergoing chemical reactions belonging to two large groups: some acid-base equilibria and some hydration-dehydration processes.

Investigations of acid-base equilibria by means of DC polarography are possible, because the reductions of conjugate acids invariably take place at potentials more positive than those of the conjugate base. On i-E curves of such reducible acids, obtained at varying pH-values, it is possible to observe waves of the acid form decreasing with increasing pH and the waves of the corresponding base increasing at more negative potentials with the total limiting current remaining constant. When the wave of the acid form becomes smaller than about 25% of the diffusion limiting current, it becomes controlled purely by the rate of protonation of the base and a kinetic current results. The value of the pH, at which the reduction wave of the acid reaches 50% of the diffusion current, is denoted pK', which is larger than pK_a of the acid obtained for example by spectrophotometry. From values of pK' and pK_a it is possible to calculate the rate constant of the reaction: base + H^+ = acid. In this way the rate constants of protonations of anions of some α-ketoacids[46] or maleic and fumaric acids[49] for example, have been determined.

In the other group of compounds, in which the current is limited by the rate of an antecedent chemical reaction, the investigation of the covalent hydration equilibria was restricted to reductions of organic compounds that bear a carbonyl or imino group, which is reducible within the protential range available. Such studies are made possible by the fact that the resulting hydrated form, the geminal diol or carbinolamine, is not reducible. The limiting reduction current is governed by the rate of dehydration. If the reduction current is controlled by this rate (and represents another example of a kinetic current), it is possible by using the value of equilibrium constant of dehydration $K_d = [>COO]/[>C(OH)_2]$ to obtain the rate constants of dehydration k_d. The rate of dehydration is often acid, sometimes acid-base catalyzed. By using the values of k_d at individual pH-values and the value of K_d, it is possible to obtain the rate constants for base or acid-base catalyzed dehydration. Recent investigation of reactions of orthophthalaldehyde[43] has shown that whereas the hydration process is general acid/base catalyzed, the rate of the dehydration is only specific catalyzed. This indicates, that whereas the rate of hydration is controlled by the rate of addition of water to the carbonyl group, the rate of dehydration in this case is controlled by the rate of the ring opening.

The investigation of the rates of chemical reactions accompanying electro-oxidation is made possible by the fact that the conjugate base of the oxidized species undergoes oxidation at more positive potential (that is easier) than the corresponding acid form. Examples are oxidations of benzaldehydes[50] and some heterocyclic aldehydes.[51] In these processes the electroactive form is a geminal diol anion, a Lewis base formed by nucleophilic addition of hydroxide ions to the carbonyl group of the aldehyde or alternatively a Brønsted base, resulting from dissociation of the hydroxy group in the hydrated form of the corresponding acid, the geminal diol. The limiting current of the anodic wave decreases with decreasing pH. The value of pH where the current = $i_d/2$ is denoted pK'. From these values and the values of $K = [>C(OH)/O^-]/[<CHO][OH^-]$ it is possible to calculate the rate constant of the addition of OH^- to the unhydrated form of the aldehyde.

4. 3. 4. Bulk Reactions

Polarography can also be used as an analytical tool in the study of kinetics of reactions, where $\tau_{1/2} > 0.5$ min, often as a complementary method to spectrophotometry.

For reactions with half-lifes $\tau_{1/2}$ between about 0.5 min and 5.0 min it is possible to record the current continuosly as a function of time at a constant potential, corresponding usually to the limiting current (Fig. 5). For reactions with $\tau_{1/2} > 5$ min the segments of the i-E curve, where changes of waves occur with time, are recorded after chosen time intervals (Fig. 6). On such curves the limited currents at individual times are plotted as a function of time or as a function of a log t. Such approach is possible when dealing with simple zero, first or second order reactions. For the treatment of higher order or more complex reactions solutions can be found in treatises on reaction kinetics. The rate constants obtained from these plots at varying compositions of the reaction mixture can be used in interpretation of the mechanism involved. Some examples of such successful applications of polarography are listed below.

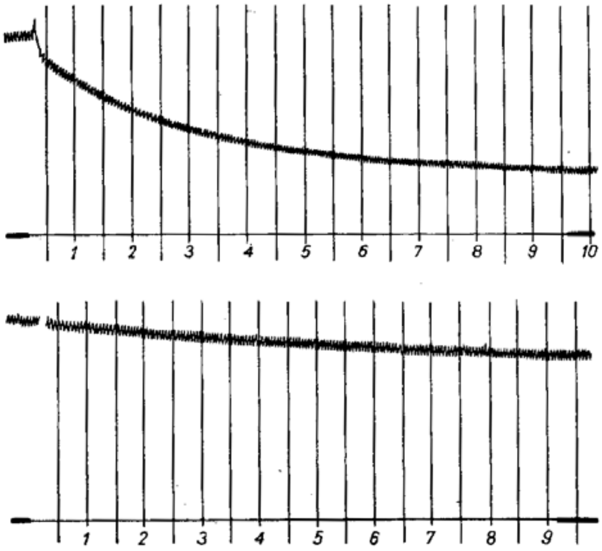

Figure 5. Continuous record of the time dependence of the limiting current of periodic acid in an acetate buffer pH 4.7. Top curve in a reaction with threo-dihydrobenzoin, lower curve with the erythro-dihydrobenzoin.

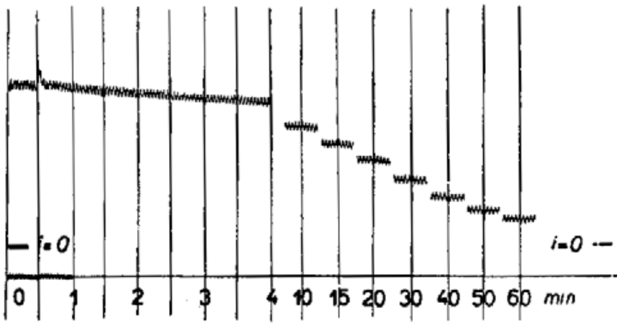

Figure 6. Recording of the limiting current at selected time intervals for the reaction of erythro-2.3-butanediol with periodic acid at pH 5.6.

4. 3. 4. 1. Hydrolyses

Polarography is well suited for determination of thiols based on their anodic waves, corresponding to oxidation of mercury electrodes, by following reactions between mercury ions and thiolate ions. Changes of limiting currents of observed anodic waves with time are then used in kinetic studies. The measurement of anodic currents of thiolates, formed in the course of hydrolysis of isothioureas[52] (Fig. 7) and dithiocarbamates,[53,54] enabled investigation of structural effects on these reactions.

Following the changes of waves at various pH-values, measurement of polarographic currents enabled identification of the reactive form[55] and study of the kinetics[56] of the non-enzymatic hydrolysis of the side chain in pyridoxal-5-phosphate. Kinetics of hydrolyses of various heterocyclic compounds, such as 1,3,5-triazines,[57] carbimazole,[58] hexazinone[59] and dihydrometamitrone[60] were also followed.

4. 3. 4. 2. Nucleophilic Additions

The nucleophilic additions to α,β-unsaturated carbonyl compounds take place on the β-carbon rather than on the carbonyl carbon. This has been observed, for example, for the addition of hydroxide ion to chalcone,[61] where

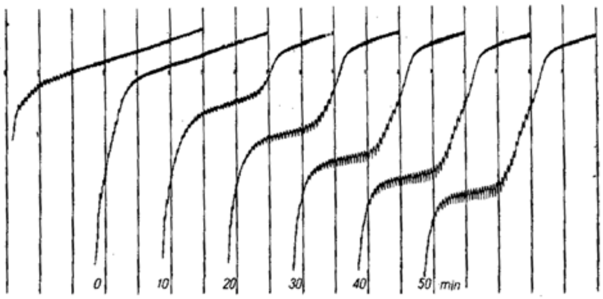

Figure 7. Following of a slower reaction by recording whole i-E curves after selected time intervals. Changes of anodic waves of benzylmercaptan were recorded in the course of an alkaline hydrolysis of S-benzylthiourea. Anodic waves of resulting benzylmercaptan were recorded in 10 min intervals.

this addition is followed by a cleavage of the C–C bond. In this case, polarography enables simultaneous following of concentration of the starting material (chalcone), the intermediate adduct and that of the product (benzaldehyde). Similar simultaneous determinations of starting material and products in situ were possible in other additions to activated double bonds in investigations of additions of hydroxide ions[62] and amines[63] to phenyl vinyl sulfone. Other examples of additions of hydroxide ions are those to 1,2-diketones[64] and to the pyridine ring in 4-position in 1-methylpyridinium carboxaldehyde,[65] which results in a formation of a pseudobase.

Addition of hydroxide ions to nitrosobenzenes[55,67] resembles that to carbonyl compounds and shows similar effects of substituents on the benzene ring.

Addition of hydroxylamine to pyridoxal[68] depends on the degree of protonation of the pyridine ring, on the dissociation of the hydroxy group in position 3 on this ring, and in the side-chain in position 5.

Dependence of addition of hydrazine to aromatic aldehydes on pH offered information about acid-base equilibria accompanying formation and dehydration of the carbinolamine intermediate. This was possible to follow, when the sum of concentrations of the starting benzaldehyde and that of the hydrazone formed were compared with the initial total, analytical concentration of the benzaldehyde. The difference between the sum of concentrations of the starting material and the product and the initial concentration of benzaldehyde was equal to concentration of the carbinolamine.[40] Based on dependence of the concentration of carbinolamine on pH, it was possible to offer an interpretation of the mechanism involved.

In the reaction of orthophthalaldehyde with ammonia,[39] polarography enabled following the rate of formation and subsequent reactions of a carbinolamine. This was made possible by following time changes in the decrease in concentration of the starting dialdehyde, the changes in concentrations of the monoimine formed in a side reaction and by following the increase in concentration of the product, a reducible isoindole derivative.[39] The investigation indicated that the carbinolamine first undergoes dehydration to an imine, but in the further stages the ring formation of 1,3-dihydroxy isoindole takes place, which is dehydrated to yield the reducible isoindole derivative. As in established equilibria the isoindole derivative predominates, the imine is gradually converted back to the carbinolamine and that into the isoindole derivative in a sequence of equilibria. After sufficient time and at a sufficiently high concentration of ammonia, only the starting dicarboxaldehyde and the isoindole derivative are present in an equilibrium.

4. 3. 4. 3. Other Reactions

Among other investigated reactions using the time-dependence of limiting currents, the study of the elimination of Mannich bases[69–71] was particularly informative. This was made possible by the choice of β-morpholinopropiophenone as the substrate. The elimination of the Mannich base takes place only in solutions, in which the unprotonated form predominates. With Mannich bases derived from other amines, the pK_a of its protonated form is usually higher than 9. In their elimination the resulting α,β-unsaturated ketone undergoes further addition of hydroxide ions in a retroaldolisation reaction. The morpholino derivative has a $pK_a = 6.6$. This enables an investigation of the elimination step, which is not complicated by the consecutive additions of OH^- ions to the α,β-unsaturated ketone, formed by elimination.

In investigation of oxidation of thiols it was possible to follow both the decrease in concentration of the investigated thiol and the increase in concentration of resulting disulfide, which is reducible.[72]

In oxidations of 1,2-diols by periodate[73–75] the concentration of the reagent is followed. Hence instead of the commonly used excess of reagent and (pseudo) first order reaction conditions, it was necessary to work at equal concentrations of the diol and periodate. These studies enabled elucidation of the mechanism of such oxidations.

5. Conclusions

Even when polarography does not find the wide range of applications as analytical method, as was the case in 1950s and 1960s, there are some areas, where it fulfills the conditions to be the method of choice for solution of a given problem. Two examples of successful current practical applications are given: (1) Analyses of pharmaceuticals containing a single components, which is both physiologically and electrochemically active, yielding a reduction or oxidation wave. (2) The possibility to determine an electroactive species in heterogeneous suspensions. – Polarography is also a useful technique in physical organic chemistry, and can be used for investigation of equilibria and reaction kinetics. In some cases polarography can offer quantitative information about equilibrium and rate constants, where use of other techniques would be difficult or impossible.

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Povzetek

DC polarografija je metoda, ki se široko uporablja pri določanju tako anorganskih kot tudi organskih ionov v različnih medijih. Članek obravnava njene prednosti (hitrost analitskega postopka, zelo ponovljivi in zanesljivi rezultati, dobro teoretično ozadje, ni potrebna uporaba organskih topil, uporabna za številne ione težkih kovin ter organskih spojin) kot tudi slabosti (pomanjkanje usposobljenih strokovnjakov za nadzor, delci, ki se ne oksidirajo). Navedeni so nekateri novejši primeri uspešne uporabe DC polarografije: (1) analiza zdravilnih učinkovin, ki vsebujejo vsaj eno fiziološko aktiven in elektroaktiven species; (2) proučevanje nehomogenih sistemov npr. vezanje ionov težkih kovin, N-nitrozaminov in različnih pesticidov v suspenzijah lignina ali tvorbe slabo topnih majhnih agregatov in dobro topnih večjih agregatov pri interakcijah ionov težkih kovin z žolčnimi solmi; (3) določanje konstante ravnotežja ter konstante reakcijske hitrosti za različne ravnotežne procese (v raztopini, v bližini elektrod).