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ORIGINAL PAPER

Current examples of practical and fundamental applications of DC polarography

Petr Zuman

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Abstract Examples of recent applications—applied, analytical, and fundamental are given. For these types of applications, an understanding of at least the number of electrons and protons transferred and the sequences of electron transfers and chemical reactions is essential. Polarography can be used for the determination of the active component in tablets and injection solutions, where it offers speed and sensitivity of the analytical method. Electroactive species can also be determined in heterogeneous systems without pre-separation. This has been taken advantage of in the investigation of reactions of bile salts with heavy metal ions or of binding of pesticides and other toxins on lignin. Polarography can also be used in the investigation of alkaline cleavage of lignin under mild conditions. Kinetics of this cleavage was used as a tool in proving that humic acids are not natural products. Among fundamental applications belong proofs of limited delocalization in groupings >C=N–N=C<, of diprotonation of hydrazones and oximes at the electrode surface, as well as of formation of imines as intermediates in reductions of hydrazones and oximes. Simultaneous determination of benzaldehyde and its hydrazone enabled providing experimental evidence for formation of carbinolamines as reaction intermediates and interpretation of the pH dependence of the equilibrium constant of this reaction. Differences in hydration of three isomeric phthalaldehydes are discussed as well as the covalent hydration of 1,2,4- and 1,3,5-triazines and pyrimidines. The **ω,ω,ω**-trifluoroacetophenone oxime the C=N bond is covalently hydrated.

Keywords DC polarography · Drug analysis · Binding on and cleavage of lignin · Covalent hydration of C=O and C=N bonds · Diprotonation of hydrazones and oximes · Imines as intermediates · Lack of conjugation in >C=N–N=C<

Introduction

During the 1950s and 1960s, according to reviews in *Analytical Chemistry*, DC polarography (based on recording of current–voltage curves using a dropping mercury electrode) was one of the five most frequently used analytical techniques. With the advance of atomic-absorption-based techniques for the determination of inorganic species and chromatographic and other separation methods for the determination of organic compounds, polarography lost its importance. Related electroanalytical techniques such as differential pulse polarography and voltammetry, square wave polarography and voltammetry, and, in particular, stripping methods enabled the development of trace analysis procedures with extremely high sensitivity. Nevertheless, there are some situations in which even now the classical DC polarography can offer some advantages. The main advantages of this technique, based on recording current–voltage curves using a dropping mercury electrode are the reproducibility of such curves and exhibition of limiting currents, which, over a certain potential range, are practically independent of the applied voltage. Similar shapes of current–voltage curves can be obtained with normal pulse polarography and voltammetry with a rotating disk electrode. Limiting currents in a given solution for a chosen electrode depend only on the concentration of the

Dedicated to Professor Dr. Alan Bond on the occasion of his 60th birthday.

P. Zuman (✉)
Department of Chemistry, Clarkson University,
Potsdam, NY 13699-5810, USA
e-mail: zumanp@clarkson.edu

electroactive species, number of transferred electrons, and diffusion coefficient of the electroactive species. Thus, comparison of limiting currents enables a rapid determination of the number of transferred electrons. Compared to other electroanalytical techniques such as linear sweep voltammetry, cyclic voltammetry, differential pulse and square wave polarography and voltammetry, the limiting current measured in DC polarography does not depend on the rate of the electrode process. This enables the use of polarographic current–voltage curves even in cases of strongly irreversible processes. The limiting currents are also, in general, less affected by the presence of surface-active substances in the investigated solution.

In searching for the best analytical procedure for the determination of a given analyte in a given matrix at lowest cost, polarography can compete with other techniques in some special cases. It can be, for example, the method of choice in analyses of samples containing one or few electroactive species. This condition is sometimes fulfilled, for example, for analyses of pharmaceuticals in tablets and injection solutions or even in blood (but rarely for the determination of these compounds in urine, where structurally related metabolites are usually present) or in some types of determination of some environmentally important constituents in waters, plants, or soils.

The other area in which the applications of polarography offer advantages, are analyses of heterogeneous systems such as suspensions or solutions containing colloidal materials. If the dispersed particles are not hydrophobic, it is often possible to carry out analyses directly in the suspension or in the presence of colloids, without separation.

Polarography can be sometimes applied also as a tool, enabling solution of some basic problems, in inorganic, organic or physical chemistry. Apart of investigations of structure–reactivity relationships, polarography, in some cases, enables investigation of both equilibria and kinetics of chemical reactions. Information about rates and mechanisms of reactions involving both inorganic and organic species can be obtained both for fast reactions taking place in the vicinity of the electrode surface and for slower reactions taking place in the bulk of the solution.

In the following, some examples of the use of DC polarography from our laboratory, dealing with both applied and basic problems, obtained over the past 7 years, are given to demonstrate the usefulness of the dropping mercury electrode (DME).

But first a word of warning: The use of DME involves the use of metallic mercury. Let me stress that this is a metal, the use of which is prohibited in some parts of the world by undeserved hysteria. It is of course necessary—like with many other chemicals—to handle mercury carefully. However, at 25 °C in normally ventilated laboratories, the toxicity of its vapors is negligible. I worked for more than 50 years in laboratories where the mercury in electrodes was daily used. Neither I nor any of my colleagues, nor any of the hundreds of polarographers I met observed any toxic effects, clearly manifested by a shaky handwriting. In the Polarographic Institute of the Czechoslovak Academy of Sciences in Prague in the 1960s, some 60 chemists were working daily with mercury electrodes and some spillage was unavoidable. Monthly inspections by the Institute of Industrial Hygiene measured concentration of mercury vapors in the air in the laboratories. The results were always 100 to 1,000 times lower than the lowest toxic level. Also, quarterly blood tests of practitioners of polarography never indicated an increased level of mercury. Only at temperatures above 80 °C does the vapor pressure of mercury reach the toxic level. Careful precautions have to be taken when drying the mercury.

Applied problems

For analyses of samples containing a single or a few electroactive components, application of polarography results in procedures, which are sometimes faster and enable higher sensitivity than those using other techniques. The possibility to determine them by means of polarography has been demonstrated for numerous inorganic species, especially those containing heavy metal ions, as well as for more than 30,000 organic compounds.

For application of any analytical method, it is essential to understand the nature of both physical and chemical processes involved, not only to find optimum conditions for analysis, but also to limit the hazards due to the presence of other components of the analyzed sample. Thus, for any practical application of polarography, the nature of the electrode process involved should be understood. This requires the understanding of the structure of the analyte: in inorganic species, the oxidation state of the element, the role of a ligand and of the structure of the inorganic ion or molecule or the substitution inert complexes. For organic compounds, the nature of the electroactive group that undergoes changes during the electrolysis is of primary importance. The number of electrons transferred in the given electrode process should be known, as well as the sequence of electron transfers and chemical reactions involved in the electrode process. Only if this information is available is it possible to understand and predict interferences due to the presence of other components of the analyzed sample.

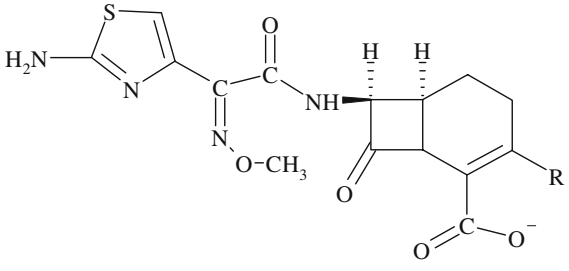
Whereas in analyses of samples containing complex mixtures of species, as encountered, for example, in the

detection and identification of metabolites, separation techniques rightfully predominate, in the analyses of samples containing one or few electroactive species polarography can offer analytical procedures, which are sufficiently accurate and sensitive and are faster and cheaper than some others. Such types of applications are often found in the analyses of drugs and pharmaceutical preparation such as tablets or injection liquids. Alternatively, successful applications may be found among environmental analyses, where often the presence of inorganic and organic components does not interfere with the determination of a single or few electroactive species.

Examples of analyses of pharmaceuticals

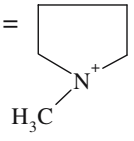
In the following, only the nature of the electrode process used in the given analysis will be pointed out. For description of procedures, their limits and validation, the reader may consult the cited references.

Cephalosporins



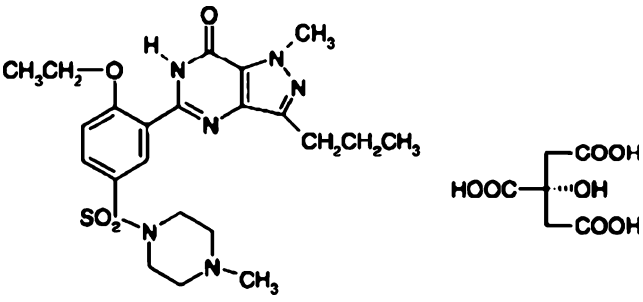
Ia = CH_3

Ib =



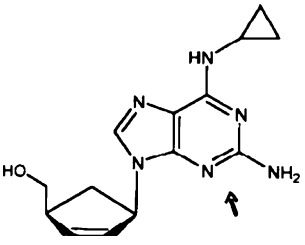
Cephalosporins represent a group of antibiotics structurally related to penicillins. Their use does not show some of the limitations encountered in the applications of penicillins. Their molecules contain several electroactive groups [1]. In two investigated cephalosporins, cefetamet [2, 3] (Ia) and cefepime (Ib) is present an oximino (>C=NOR) grouping that undergoes at pH <8 polarographic reduction in an overall four-electron process [4, 5]. The variation of the waves of cefepime with time was used for investigation of its alkaline degradation [5].

Viagra (II)



Sildenafil citrate (II) is a drug used under the name Viagra that controls erectile dysfunction. The compound undergoes electrooxidation involving the piperazine ring [6]. The resulting anodic currents enable rapid determination in preparations with high accuracy.

Abacavir (III)



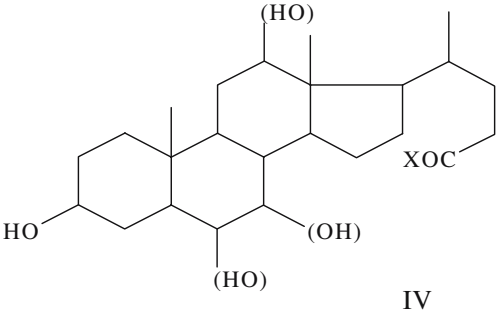
Abacavir (III) is an inhibitor of HIV, which undergoes in acidic media a four-electron reduction of the pyrimidine ring. This reduction process resembles the processes that take place in the reduction of adenine and adenosine-5-phosphate (Özkan et al., unpublished results). The determination of this compound in pharmaceutical preparations is quick and accurate, and the procedure was validated.

Analyses of heterogeneous systems

In suspensions of small solid particles or solutions of colloids—provided that particles are not hydrophobic and adsorbed at the electrode surface—it is possible to determine polarographically inorganic ions or organic compounds. The determination, based on measurements of limiting currents, can be carried out directly, recording the current–voltage curve using the dropping mercury electrode immersed directly into a suspension of such particles. In numerous instances, the presence of small solid or colloidal particles does not interfere with the electroreduction or electrooxidation of the dissolved electroactive species. Separations of either the remaining dissolved electroactive species, or oppositely of the slightly soluble materials that

have to be carried out when either spectrophotometry or chromatography were used, are not necessary in applications of polarographic analyses. It is possible to carry out the electrolysis using the dropping mercury electrode immersed directly into the investigated suspension.

Formation of aggregates of bile salts



IV

X= ONa
NH_2CH_2COOH
$NH_2(CH_2)_2SO_3Na$

In the gall bladder and other parts of the digestive system, bile salts (IV) exist in the presence of some heavy metal ions such as Cu^{2+} and Fe^{3+}. In aqueous solutions of bile salts containing only sodium ions, which are the predominant counter-ion in biological fluids, self-aggregation of bile salts takes place. Such aggregations result from hydrophobic–hydrophobic interactions involving the steroidal skeleton of bile salts, as well as from hydrogen bond formation between the OH groups of stacked bile salt anions.

Our investigations [7–16] dealt with interactions between bile salts and ions of heavy metals such as Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, and Fe^{2+}. They were carried out at a constant initial concentration of the heavy metal ion and at varying concentrations of the bile salt. On a typical plot of the dependence of limiting currents of heavy metal ions on concentration of added bile salt (Fig. 1), it is possible to distinguish four regions: At low concentrations of bile salts (region I), both currents and half-wave potentials of metal ions remain independent of bile salt anions concentration.

This indicates negligible interaction between bile salt anions and metal ions, while the solution remains homogeneous. This reflects the low stability of carboxylate complexes. At concentrations of bile salts corresponding to region II (Fig. 1), the limiting current and, hence, the concentration of free metal (II) ions decrease with increasing concentration of anions of bile salts. Simultaneously, the solution becomes turbid, and the half-wave potentials of the reduction of the metal ion are shifted to more negative values. In this region are formed small aggregates binding metal ions, containing two to four molecules of bile salt per metal ion. In region III (Fig. 1), an equilibrium is established between the small aggregates of bile salts and metal ions. Finally, in region IV larger aggregates of bile salts are formed. Due to an increased number of hydrophilic OH and COOH groups at the surfaces of these aggregates, the solubility of these large aggregates is increased, binding of Me^{2+} ions on aggregates is decreased and this results in an increase of limiting currents of free metal ions.

Properties of aggregates of all trihydroxy bile salts were similar. For example, the sequence of stabilities of adducts of Me^{2+} ions to individual bile salts were similar for various Me(II) ions. This indicates that the composition and properties of Me(II) aggregates are similar for all trihydroxy bile salts studied. On the other hand, no such regularity was observed for the seven dihydroxy bile salts investigated. This shows that the structures and properties of aggregates formed by individual bile salts differ from one dihydroxy bile salt to another.

Properties of lignin suspensions

Lignin is—after carbohydrates—the second most frequently encountered organic polymer on the surface of the Earth. Wood, straw, and similar parts of plants contain between 10 and 30% of lignin. Natural lignin is a complex, water-insoluble three-dimensional polymer with a molecular weight of more than 100,000. It consists predominantly of 3-phenylpropane subunits, which are more or less randomly linked. By the random linkage of the 3-phenylpropane units substituted with hydroxy, alkoxy, or carboxy groups, lignin differs from most naturally occurring polymers (like proteins, celluloses, gums, etc.), which contain repeating structural units.

Use of lignin as detoxicant In the presence of a toxin, such as some pesticides, nitrosamines or heavy metal ions such as Cu^{2+}, Cd^{2+}, Pb^{2+}, UO_2^{2+}, and Zn^{2+} in a suspension of lignin, it is possible (after the equilibrium is established) to determine the concentration of the unbound toxin using DC or differential pulse polarography. This enables—for the given toxin and the particular lignin sample—construction of an adsorption isotherm. Between 30 and 60% of a given pesticide is bound to lignin. Considerable fraction (40–60%) of this binding is irreversible over a period of several days. This binding ability indicates that lignins are promising detoxicants. Knowledge of binding of a given pesticide should allow estimating what fractions of the pesticide become biounavailable. Results of the degree of binding allow an estimate of the pesticide to be needed for a successful application.

Removal of toxins from water streams should be possible by passage through a lignin-filled barrier. Lignins are considerably cheaper than other commonly used

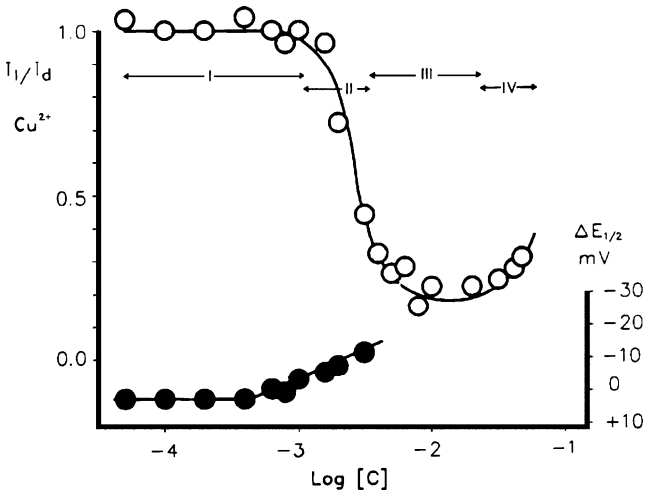
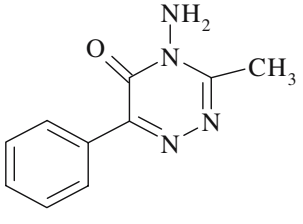


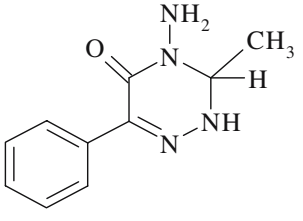
Fig. 1 Dependence of the relative limiting current (i/i_d) (open circles, left-hand scale) and half-wave potentials (full points, right-hand scale) in solutions containing 0.1 mM Cu(2+) ions and 0.15 M sodium nitrate on concentration of sodium cholate at 25 °C. The approximate limits of ranges I–IV are indicated

adsorbents. In polluted areas, which are either wet or are sprayed first with water, a dispersion of a layer of lignin should enable removal of toxins. Removal of this layer, for example, by raking, should result in detoxification [17–25]. Possible residue of lignin after its application would not be harmful, as lignin would undergo natural cleavage over a period of months, as it happens in the formation of manure. Adjustment of pH in the wet areas would allow to control the selectivity of this detoxicant.

Alkaline cleavage of lignin During the investigation of the pH dependence of the binding of toxins on lignin (Use of lignin as detoxicant), it was observed that suspensions of lignin undergo cleavage already at pH greater than about 8 even at 25 °C. This is contrary to industrial practices in which 100 °C and higher and high pressure at pH >13 are used for the cleavage of lignin [26]. The rate of the cleavage of lignin under mild conditions follows first-order kinetics. Dependence of rate constants of this cleavage on pH indicates base catalysis. It was possible to exclude addition of hydroxide ions to α,β-unsaturated carbonyl groupings as responsible for this catalysis. The pH dependence of rate



Va



Vb

constants of the cleavage is thus attributed to an acid-base equilibrium with pK_a between 11 and 12, established before the rate-determining step. This equilibrium can correspond either to a dissociation of a phenolic OH group or to an addition of hydroxide ion to an aromatic aldehydic group.

Use of lignin as a raw material of the future After exhaustion of oil and coal as raw materials for organic syntheses, carbohydrates might be used as raw materials for aliphatics and some heterocyclics, whereas lignin seems to be a promising renewable source for aromatics. Strongly alkaline media and high temperatures are used currently in total cleavage of lignins in paper and cellulosics industries. Resulting mixtures of low m.w.compounds and small polymers is not suitable as a source for industrial organic syntheses. Mild conditions in procedures described in *Alkaline cleavage of lignin* yield at room temperatures simple mixtures of aromatic aldehydes substituted by OH and OCH_3 groups. Such mixtures seem to be promising raw materials for syntheses of aromatic compounds.

Are humic acids natural products? Investigation of kinetics of the alkaline cleavage of lignin allows obtaining of further information in another area. The pH dependences of rate constants of cleavage of soil organic matter and the organic components of deposits from rivers were compared with the cleavage of lignin and humic acids. It has been proven [27] that the kinetics as well as the pH dependence of rate constants in suspensions containing soil organic material and lignin were very similar. On the other hand, the kinetics of cleavage of suspensions of humic acids and its pH dependence are completely different. Based on this comparison, it was concluded that humic acids are man-made polymers rather than a natural product. These polymers are formed during the preparation of humic acids. In commonly used procedures, the soil containing the organic material is dispersed in a strongly alkaline medium where it undergoes cleavage. The solubilized fraction is acidified. In this process, a new slightly soluble polymer is formed called “humic acid”. We proposed that lignin and some of its decomposition products, which are part of the soil organic material, are cleaved in the strongly alkaline (pH >13) medium used. Fragments formed in alkaline solutions repolymerize after acidification. The new polymer formed in this way, the humic acid, differs in its composition, reactivity, and properties from those of the natural product—lignin.

Solving of basic problems

Experimental evidence obtained by polarography in some instances indicated the presence of unexpected phenomena, like the extent of delocalization, formation of unexpected

species such as unusually protonated compounds, less common properties or reactions such as hydration and reactive intermediates of both reactions taking place in the vicinity of the electrode surface and those occurring in the bulk of the solution. Observations made, based on polarographic current-voltage curves, led in some instances to extensive studies by other techniques.

Limited delocalization in the grouping >C=N–N=C<

When investigating the mechanism of the reduction of the herbicide metamitron [30], which is 4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4H)-one (Va), it has been proven that the reduction of the 1,6-C=N double bond takes place at potentials by about 0.6 V more positive than that of the 2,3-N=C bond. When the reduction of metamitron (Va) was compared with a chemically prepared 2,3-dihydroderivative (Vb), it has been observed that the reduction potential of the 1,6-C=N bond in the parent compound Va and in the dihydroderivative Vb are identical between pH 2 and 10. This indicates that the energy needed for the reduction of the 1,6-bond C=N remains unchanged; whether in position 2 or 3, a double bond >C=N– or a single bond >CH–NH– is present. Equal potential of the 1,6-C=N– bond in the presence of either 2,3-N=C< or 2,3-NH–CH< indicates absence of influence of the bond 2,3-N=C< on the reduction of the bond 1,6-C=N–. This is interpreted as due to a limited conjugation or delocalization in the system >C=N–N=C<, and this makes the grouping >C=N–N=C< a delocalization stopper [28, 29].

Such attribution is further supported by a comparison of substituent effects. Whereas variation of substituents on C-1 results in expected shifts of potentials of the 1,6-C=N bond corresponding to linear free energy relationships [31], variation of substituent on C-3 has practically no effect on the reduction of the 1,6-C=N bond. That confirms that the grouping C=N–N=C is a conjugation stopper.

This deduction based on polarographic data may be questioned based on limited understanding of solute–solvent interactions involved and the role of antecedent protonation. To indicate that limited delocalization is an inherent property of the >C=N–N=C< grouping, crystallographic properties of a group of some 35 compounds bearing this grouping were compared (Ludvik, unpublished results). The average value of bond lengths of the C=N bonds in the >C=N–N=C< grouping indicate that the C=N bond is practically a pure double bond, whereas the lengths of the N–N bond corresponds to that of a single bond.

Diprotonation of hydrazones and oximes

It has been understood in reports in earlier literature references that a proton transfer precedes the transfer of the first electron in four-electron reductions of hydrazones and oximes. Nevertheless, no attempt has been made to estimate the number of protons involved. The evidence that the reducible species is formed by a transfer of two protons before the first electron uptake is presented in this paper first for the reductions of hydrazones, then for the reductions of oximes [32].

Reduction of hydrazones

The proofs for the reduction of diprotonated forms of hydrazones [33] derived from benzaldehyde, acetophenone, benzophenone, and fluorenone were based on comparisons (a) of shapes of plots of i=f(pH); (b) of slopes of plots of $E_{1/2}$=f(pH); (c) with reduction of **ω**,**ω**,**ω**-trialkylhydrazonium ions.

(a) Plots of i=f(pH) have different shapes for processes, in which the first electron uptake is preceded by a transfer of one or two hydrogen ions. The plot of i=f(pH) for a transfer of one proton is less steep, with a decrease from 90% i_d to 10% i_d in about two pH units. The analogous decrease in limiting current i for processes, where two protons are transferred before the first electron transfer, is steeper and the decrease from 90% i_d to 10% i_d occurs with increasing pH within a single pH unit. For investigated hydrazones, the plots of i=f(pH) were steep, corresponding to a reduction of a diprotonated species.

(b) Even for irreversible processes, the plots of $E_{1/2}$=f(pH) have slopes between 30 and 60 mV/pH for processes involving a transfer of one proton and slopes between 60 and 120 mV/pH for those involving a transfer of two protons before the first electron uptake. At least over a part of the plot of $E_{1/2}$=f(pH) hydrazones manifested plots with slopes between 70 and 110 mV/pH. That also supports the transfer of two protons before electron uptake.

(c) Finally, it has been confirmed that the half-wave potentials of reductions of trialkylhydrazonium derivatives with structures $PhC(R) = NNR_3^+$ depend on pH between pH 5 and 11. This indicates a transfer of a proton before the first electron uptake. This can be only interpreted as due to a protonation of the azomethine nitrogen. Resulting protonated species, $PhC(R) = NH^+NR_3^+$ bears two positive charges on adjacent N atoms. The species reduced thus strongly resembles the reducible form of above hydrazones. The reduction of the diprotonated species is initiated by a cleavage of the N–N bond. This cleavage is facilitated by a pre-formation of NH_3 or NR_3 as good leaving group as well as a positive charge on the azomethine nitrogen.

Reduction of oximes

The reduction of a variety of oximes [34] (such as benzaldehyde, acetophenone, and benzophenone oximes

that can be substituted both on the aromatic ring and in the side chain) takes place also in a four-electron step, initiated by the cleavage of the N–O bond. This reduction can also occur in the diprotonated form of the oxime, $ArC(R) = NH^+OH_2^+$. Similar to hydrazones, the transfer of two protons before that of the first electron has been proven by (a) the shape of plots of $i=f(pH)$; (b) the slopes of the dependence of $E_{1/2}$ on pH, and (c) based on a comparison with electroreductions of nitrones (*N*-alkyl oximes). The evidence obtained from dependences (a) and (b) is similar to that described for hydrazones. (c) For nitrones, it has been reported in the past [35] that their limiting currents decrease with increasing pH and that their half-wave potentials are shifted with increasing pH to more negative values. These authors correctly attributed the observed phenomena to a transfer of a proton before that of the first electron, but it escaped them that this evidence indicated reduction of a species with two positive charges on adjacent heteroatoms, $Arc(R)=NH^+–OH_2^+$. Hence, evidence reported above under (a)–(c) confirms that the initial cleavage of the N–O bond in oximes takes place in the diprotonated form, $>C(R)=NH^+OH_2^+$.

Similar to hydrazones, the cleavage of the N–O bond in oximes is facilitated by the presence of H_2O as a good leaving group and by the positive charge on the azomethine nitrogen. Additional information is available for oximes, based on the shape of *i–E* curves, their dependence on pH and on the concentration of the oxime. This experimental evidence indicates that at pH higher than about 5, the unprotonated form of the oxime is strongly adsorbed at the surface of the mercury-dropping electrode. At this adsorbed layer, the diprotonation takes place in a heterogeneous process, which can be termed catalytic.

At a pH lower than about 5 the, situation is different. This is indicated by the plots of $E_{1/2}=f(pH)$ in this pH range. These plots show several linear segments: The segment above pH 5 has a large slope (70–120 mV/pH), the segment at a pH of about 5 to about 2 or 0.0 has a smaller slope (30–60 mV/pH), and finally the slope in the most acidic region is zero, as the species predominating in the bulk of the solution is reduced. The pH at the intersection of the linear segments of the plot at pH 5 to about 2 with the pH-independent segment at lower pH values corresponds to the pK_a of the dissociation of the monoprotonated form of the oxime. The values of pK_a obtained from polarographic measurements are in good agreement with the values of pK_a obtained for the dissociation of the monoprotonated form of oximes, obtained from the pH dependence of UV spectra of the given oxime.

These observations can be interpreted as due to a change in the mechanism of the electroreduction at a pH of about 5: at pH 5–10, the diprotonated form, which is generated at the electrode surface in a heterogeneous process, is reduced. At pH 2–5, the monoprotonated form is generated in a homogeneous layer (reaction layer) in the vicinity of the electrode and reduced. At pH lower than about 2, the monoprotonated form, which predominates in the bulk of the solution, is reduced. As no additional energy is needed to convert the predominant species into the electroactive form, the $E_{1/2}$ in this pH range becomes independent of pH.

With some oximes there is a more direct proof of the reduction in di- and monoprotonated forms. For these oximes, for example, those bearing a cyano group either in the aromatic ring or in the side chain, the decrease of the wave observed at pH < 7 is accompanied by an increase of another wave at more negative potentials. With a further increase in pH, even this wave decreases [36]. This behavior shows that three different forms of oxime are present. The reduction of anion $ArC(R)=NO^-$ in the second wave can be excluded based on comparison of the pH range in which the decrease of current with increasing pH is observed with the pK_a of the dissociation $ArC(R)=NOH \rightleftharpoons ArC(R)=NO^- + H^+$ determined spectrophotometrically. Hence, the three reducible species must be $ArC(R)=NH^+OH_2^+$; $[ArC(R)=NOH]H^+$ and ArC(R)=NOH.

Formation of imines as intermediates in the reduction of hydrazones and oximes

Formation of imines by a cleavage of the N–N bond in hydrazones or N–O bond in oximes has been anticipated, but not proved. Presented experimental evidence for imine formation was in the reduction of hydrazones based on comparison with some stable imines for that of oximes on their behavior in acidic solutions.

Imines as intermediates in the reductions of hydrazones

In the reduction of hydrazones, it was assumed that imines are formed as reactive intermediates, following the pattern (1),(2)

$$> C = NNH_2 + 2e + 2H^+ \rightarrow > C = NH + NH_3 \quad (1)$$

$$> C = NH + 2e + 2H^+ \rightarrow > CH - NH_2 \quad (2)$$

As most hydrazones are reduced up to a pH of about 7 in a single, four-electron step, no direct experimental evidence for the imine formation was available. Moreover, a comparison of the reductions of imines with those of hydrazones was limited by the lack of stability of most of the imines, which readily undergo hydrolysis. The proof was made possible by the exceptional behavior of benzo-

phenone and fluorenone hydrazones and imines [33]. These imines are stable within 30 min at pH between 5 and 10 and are reduced in two one-electron steps, following the pattern (3)–(5):

$$> C = NNH_2 + 2e + 2H^+ \rightarrow > C = NNH + +NH_3 \quad E_1 \quad (3)$$

$$> C = NH + e + H^+ \rightarrow > C - NH_2 \quad E_2 \quad (4)$$

$$> C - NH_2 + e + H^+ \rightarrow > CH - NH_2 \quad E_3 \quad (5)$$

Moreover, potential E_2 is so close to potential E_1 that these two reduction steps overlap in a single wave. Thus, reduction of those hydrazones takes place in one three-electron step [(3)+(4)] and one one-electron step (5). Most direct experimental evidence of the above scheme is the identical potential of the one-electron step (5) obtained as the second wave of the hydrazone with that of the second wave of the imine. Additionally, potential E_2 of the first one-electron wave of the imine (4) is close to the potential of the first wave of the hydrazone (3).

Imine intermediates in the reduction of oximes

To prove the presence of the imine intermediates in the course of reduction of oximes, the approach used in proving Imines as intermediates in the reductions of hydrazones for the analogous problem in the reduction of hydrazones cannot be applied. The reductions of imines of benzophenone and fluorenone take, namely, the place at potentials more positive than those of corresponding oximes. This results in a single four-electron step.

In a different approach used, attention was paid to the separation of a four-electron reduction into two two-electron steps, observed in the reduction of some oximes in acidic media [37]. These separations were observed for benzaldehyde and acetaphenone oximes, particularly in the presence of electron donating and weak electron withdrawing (up to *p*-Cl) substituents in para-position. The more negative two-electron wave corresponds to the reduction of imine (2) formed in the first two-electron step (1).

The separation of the two waves at a sufficiently high acidity is attributed to a difference between the pK_a of the oxime and pK_a of the corresponding imine: the pK_a of the protonated form of the imine is always higher than the pK_a of the monoprotonated form of the corresponding oxime. This difference is reflected by the fact that below a certain pH value, the half-wave potential of the reduction of the protonated form of the imine becomes pH-independent. That happens at pH <pK_a, as under these conditions the same species, which predominates in the bulk of the solution, is also reduced. The reduction of the protonated form of the oxime in this pH range (up to pK_a of the protonated form of the oxime) is still shifted to more positive values with decreasing pH. This shift of the half-wave potential of the oxime in the pH range where the half-wave potential of the imine remains constant results in the separation of the two two-electron waves, which represent the proof of formation of the imine as an intermediate.

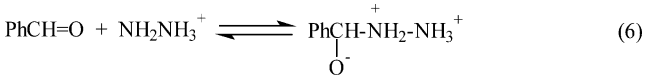
Carbinolamines as intermediate in the formation of benzaldehyde hydrazone

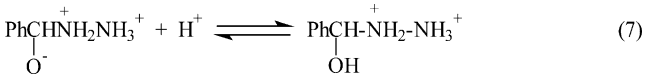
Whereas previous two examples demonstrated the possibility to elucidate the nature of electrochemical and chemical processes taking place in the vicinity of the electrode by means of polarography, the following example shows the possibility of using polarography for following chemical reactions that take place in the bulk of the solution.

Investigations of mechanisms of formation of azomethine compounds such as imines, hydrazones, semicarbazones, and oximes are examples often quoted in physical organic chemistry. The reaction is considered to take place in two principal steps: (a) the condensation of the amine with a carbonyl compound and (b) the dehydration of a carbinolamine, formed as an intermediate [37]. This conclusion was based mostly on investigation of kinetics and the dependence of rate constants on substituent effects. It has been concluded that in acidic media, addition of amine is the rate-determining step. At higher pH values, the slow step is considered to be the dehydration of the intermediate, a carbinolamine. No information was provided concerning the position of the two equilibria involved in such sequence, nor about their dependence on pH. The main limitation of past studies was their restriction to sole use measurements of absorbance in the UV–visible range. The overlapping of absorption bands of the carbonyl compound, the carbinolamine, and the resulting azomethine compound, prevented to obtain reliable values of equilibrium concentrations of individual components.

Polarography enables, for the reaction of benzaldehyde with hydrazine, a simultaneous determination of benzaldehyde, the starting material, and the hydrazone, the final product. From the difference between the initial concentration of benzaldehyde and the sum of equilibrium concentrations of benzaldehyde and hydrazone, it is possible to calculate the equilibrium concentration of the carbinolamine. Kastening et al. [38] used a similar approach in an investigation of hydrolysis of benzylideneaniline, in a publication hardly ever quoted.

Restricting our investigation to pH <7, where hydrazine is predominately present as $NH_2NH_3^+$, which reacts with benzaldehyde, it was possible to obtain values of the overall equilibrium constant $K=[PhCH=NNH_2]/[PhCHO][NH_2NH_3^+]$ as a function of pH and equilibrium constants of formation and dehydration of the carbinolamine. The observed data are in accordance with the following Schemes (6, 7, 8, 9, 10, 11):


(6)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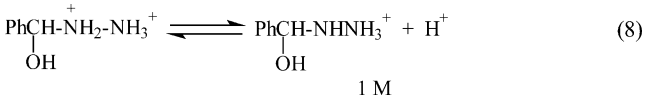
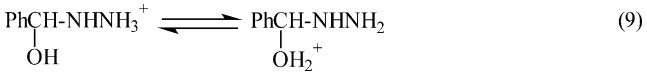
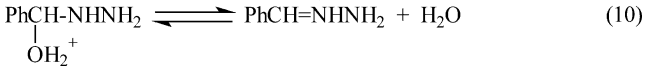

(8)


(9)


(10)


(11)

Kinetics of the formation of the carbinolamine and of its dehydration is currently under investigation.

Covalent hydration

In aqueous solution, organic compounds bearing a carbonyl group or an azomethine bond can covalently add water.

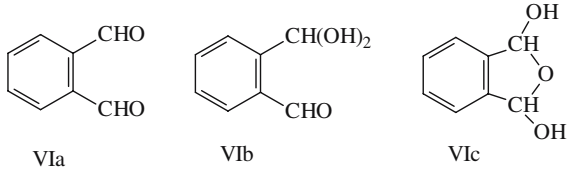
Covalent hydration of the carbonyl group

Covalent hydration of carbonyl compounds in aqueous solutions has been extensively studied, mainly in the aliphatic series [39–41]. The degree of such hydration is larger for aldehydes than for ketones and increases when the carbonyl group is adjacent to a carbonyl, carboxy, or carbalkoxy group or when the adjacent carbon bears one or more halogens. The hydration is also affected by the nature of a ring to which an aldehydic group is attached. Generally, low hydration is observed for benzene-, furane-, thiophene- or pyrrolecarboxaldehydes, whereas considerable hydration takes place for pyridine- or imidazolecarboxaldehydes. Electron-withdrawing substituents on aromatic rings increase hydration, particularly if such substituents are located in para- or ortho-position relative to the formyl group. Thus, when the majority of substituted benzaldehydes are in aqueous solutions less than 5% hydrated, *o*- or *p*-mononitrobenzaldehydes are about 8–10% hydrated and the hydration increases with increasing number of nitro groups [42].

The importance of mutual position of two electroactive centers on a benzene ring was demonstrated by comparison of the behavior of the three isomeric benzenedicarboxaldehydes (phthalaldehydes) [41]. The meta-derivative, the 1,3-benzenedicarboxaldehyde [43], in which there is no resonance interaction between the two formyl groups, behaves like most of the other substituted benzaldehydes and is present in aqueous solution in less than 5% in the hydrated form. On the other hand, the para-derivative, the 1,4-benzenedicarboxaldehyde or terephthalaldehyde [44, 45], is in aqueous solution hydrated in about 20%. The effect of the second formyl group on the reduction of the first one is much stronger than would be expected based on comparison of Hammett substituent constants σ_{p-x}. Comparison of $\sigma_{p\text{-}NO2}$=0.81 with $\sigma_{p\text{-}CHO}$= 0.45 would indicate a much stronger hydration of *p*-nitrobenzaldehyde when compared to terephthalaldehyde. Experimental evidence indicates that, oppositely, terephthalaldehyde is almost twice as strongly hydrated than *p*-nitrobenzaldehyde (which is 8–10% hydrated). This indicates an exceptionally strong resonance interaction between the two formyl groups in terephthalaldehyde. Such resonance interaction would be characterized by a substituent constant $\sigma_{p\text{-}CHO}$=0.92.

It was assumed that the 1,2-benzenedicarboxaldehyde or orthophthalaldehyde (VI) exists in aqueous solutions completely as a cyclic hemiacetal (VIc). Appearance of two polarographic reduction waves [41, 45] indicated the presence of three forms (Via, Vib, VIc). The first two forms (VIa and VIb) are electroactive in a reduction process in which a transfer of two electrons yields an alcohol, whereas form VIc is not electroactive.

Concentration of form VIc can be obtained when subtracting from initial concentration the sum of concentrations of forms VIa and VIb. In equilibrium, the form VIa is present in about 10%, form VIb in about 5%, and form VIc is about 85%. The presence of three forms in solutions of orthophthalaldehyde was supported by NMR spectra. Kinetics of conversion of individual forms, its acid and base catalysis, role of concentration of acetonitrile in the reaction medium, and reaction kinetics of the addition of various nucleophiles to orthophthalaldehyde is currently under investigation (Salem and Kulla, unpublished results). The investigation of orthophthalaldehyde and its reactions with nucleophiles is of importance in view of the fact that



VIa VIb VIc

this aldehyde (in the presence of a nucleophile) is widely used in the determination of amino acids and that the mechanism of reactions involved remains unknown [41]. All three isomeric phthalaldehydes at a pH higher than about 10 add hydroxide ions. Resulting geminal diol anion ($-CH(OH)O^-$) can be anodically oxidized.

Covalent hydration of the azomethine group

Addition of water to the azomethine (C=N) bond in acyclic molecules usually results in a formation of an unstable adduct, which readily undergoes hydrolysis. Nevertheless, considerable evidence has been accumulated for covalent addition of water to C=N bonds present in heterocyclic compounds [39].

Investigation of the electroreduction of herbicides metamitron [30] and metribuzin [46], which are both 1,2,4-triazine derivatives with two azomethine (C=N) bonds in positions 1,6- and 2,3-, revealed covalent hydration of both C=N bonds, stronger for the 1,6- than for the 2,3- C=N double bond. This was concluded based on a decrease of the limiting current. This decrease occurs within a pH range where the rate of dehydration is neither acid- nor base-catalyzed.

Similarly, based on a decrease of the limiting current between pH 2.5 and 4.5, it was possible to prove covalent hydration of the azomethine bond in position 2,3- in hexazinone. This is a herbicide, which is chemically a derivative of 1,3,5-triazine [47].

For 2-aminopyrimidines substituted on the amino group, covalent hydration was manifested by the decrease in limiting current between pH 2 and 5. In this case, addition similar to that of water was observed for several aliphatic alcohols (Privman and Zuman, unpublished results).

Role of an adjacent CF_3 group on hydration

A very strong increase in hydration was reported in literature for molecules, which bear a CF_3 group adjacent to a carbonyl group. Thus, the carbonyl groups in compounds like fluoral or **ω,ω,ω**-trifluoroacetophenone are very strongly hydrated [40]. A similar effect was observed for **ω,ω,ω**-trifluoroacetophenone oxime [37]. The limiting current of this compound is between pH 2 and 8 lower than 5% of the diffusion controlled value. As this small current is kinetically controlled by the rate of dehydration, it can be estimated that the C=N bond in this oxime is about 99% hydrated. Increase in the limiting current at pH <2 is due to an acid-catalyzed dehydration. We believe that this experimental evidence is a proof of a strong hydration of the C=N bond due to the inductive effect of the CF_3 group.

Conclusions

DC polarography can be currently used both for practical and analytical applications and for solving some fundamental problems of chemistry of organic compounds. For both types of applications, it is essential to understand, at least in principle, the nature of electrochemical and chemical processes involved.

Among promising areas of analytical applications of polarography included the analyses of drugs mentioned earlier in this paper, which foundd their applications in laboratories that cannot afford expensive instrumentation, used by large pharmaceutical companies. Another area where polarography offers advantages is in the analyses of heterogeneous systems. In such applications, direct determination of electroactive species is possible in the presence of solid and colloidal particles, without time-consuming separation. Such types of applications enabled investigations of reactions of bile salts with heavy metal ions, of binding of various toxins on lignin, as well as following the alkaline cleavage of lignin under mild conditions. It made possible a demonstration that lignins can undergo alkaline cleavage even under mild conditions—at pH 8–12 at room temperature. Investigation of kinetics of such cleavage offered experimental evidence that “humic acids” are a man-made polymer rather than a natural product.

DC polarography proved to be useful for following the extent of conjugation or delocalization and indicated limited extent of such process in the grouping >C=N–N=C<. Polarography is an underestimated tool in investigations of both fast chemical reactions taking place before the first electron uptake in the vicinity of the electrode and of kinetics and equilibria of slower reactions taking place in the bulk of the solution. Among the investigated problems belonging to the first group belong some acid-base reactions such as diprotonation of hydrazones and oximes and establishment of hydration–dehydration equilibria. To the latter group belong investigations of covalent addition of water to 1,4- and 1,2-benzenedicarboxaldehydes, to C=N bonds in 1,2,4- and 1,3,5-triazines and pyrimidines, and in compounds bearing the C=N group in oximes adjacent to a CF_3 group.

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