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REVIEW

Polarography and pharmacy: a centenary of mutual interaction

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Abstract

On the occasion of the centenary of the discovery of polarography, this article studies the mutual interactions between polarography and pharmacy. It highlights the relationship of the discoverer of polarography, Jaroslav Heyrovský (1890–1967), to pharmacy. The article shows how quickly and widely polarography has been applied to pharmacy. The contribution of polarography to the quality control of pharmaceutical substances and dosage forms is discussed, including some interesting solutions to problems with the measurement of substances that are difficult to determine electrochemically. The integration of polarography into pharmacopoeias is also followed. Next, the use of polarography in the determination of drugs and their metabolites in biological samples for pharmacological purposes is discussed. Finally, the application of polarography in the development of new drugs is reviewed. Although classical polarography on a dropping mercury electrode is nowadays seldom used, its contribution to pharmacy is considerable, and, moreover, new voltammetric methods derived from polarography still have a useful and irreplaceable position in pharmacy.

Graphical abstract

Keywords Drugs · Electroanalysis · History of science · Pharmacopoeia · Pharmacy · Pharmacology · Polarography

Introduction

When the Czech scientist Jaroslav Heyrovský (1890–1967) [1, 2] published his discovery of polarography one hundred years ago [3, 4], he certainly had no idea how widespread this new method would become and in how many areas of human endeavor it would eventually be applied [5, 6]. In 1922, Heyrovský laid the foundations for an important area of electroanalytical chemistry which still touches many aspects of human activity, from industry to food processing, pharmaceuticals and medicine, and therefore affects the quality of life of millions of people [7–9]. The importance of polarography is also evidenced by the fact that 37 years after its discovery Heyrovský was awarded the Nobel Prize in Chemistry "for his discovery and development of the polarographic methods of analysis" [10, 11]. This wording shows that the Nobel Committee for Chemistry took particular account of the importance that Heyrovský's discovery had—and still has—for analysis. It should be made clear that only four Nobel Prizes in Chemistry have been awarded for analysis to date.

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One of the important areas of polarography application very soon after its discovery was pharmacy, especially pharmaceutical analysis. Polarography was predestined to do this primarily because, at the time of its discovery, it was the most sensitive analytical method available. Classical polarography is able to determine substances even at a dilution of 1:1,000,000. To give you an idea of the task, it is like accurately locating a single, specific second in a time span of 11.6 days. To this day, one of the daughter methods of polarography, stripping voltammetry, is one of the most sensitive analytical methods available in chemistry for determining trace level [12]. It is a million times more sensitive than polarography, with a detection limit of $1{:}10^{12}$ dilutions. The second great advantage of newly introduced polarography was that only three years after its discovery, Heyrovský and his collaborator, the Japanese chemist Masuzo Shikata (1895–1964), constructed an apparatus for the automatic recording of polarographic curves, which they called a polarograph [13] (Fig. 1). The polarograph became the first ever automatic recording instrument in analytical chemistry [14]. Finally, an important factor in the introduction of polarography into pharmacy was the close connection between chemistry and pharmacy, which was typical of the Faculty of Science of Charles University, where Heyrovský worked and where he made his discovery [15]. At the time of his discovery, pharmacy was taught at the Faculty and several Heyrovský's colleagues were engaged in pharmaceutical analysis. Indeed, Heyrovský himself had a positive and close relationship with pharmacy. During World War I, he served as a pharmacist in the then Austro-Hungarian army in a military hospital in Tábor (a town in the South Bohemian Region) and later in Innsbruck [16] (Fig. 2). Here, he also established a lifelong friendship with the Czech analytical chemist Oldřich Tomíček (1891–1953), who contributed significantly to the development of pharmaceutical analysis [17].

Fig. 1 Jaroslav Heyrovský and his polarograph (1930s)

Of course, polarography has undergone considerable development since its discovery one hundred years ago.

Fig. 2 Jaroslav Heyrovský (in the middle with a test tube in his hand) in the military pharmacy in 1915

Today, classical Heyrovský polarography on a dropping mercury electrode is a little-used method and, in addition, the name voltammetry has been adopted for the more recently introduced working electrodes made of other materials (or measurements on a static mercury drop). However, new areas can still be found where polarography/voltammetry can be applied [18]. In particular, advanced digital electronics and the connection of measurement devices to computers allow the use of miniaturized instruments with a broad spectrum of polarographic/voltammetric methods [19]. These applications include countless biosensors, which save lives and protect health through early diagnosis (the most widely used is the glucometer) [20, 21]. Another example is the polarographic/voltammetric study of biomolecules (including DNA), which helps understanding of many processes in living organisms and can detect serious diseases early [22].

The centenary of the discovery of polarography is therefore an appropriate occasion to reflect on the past one hundred years of the interaction between polarography and pharmacy. Naturally, there are several thousand works in this field; so, we will focus mainly on the general principles and highlight the pioneering works.

Polarography in pharmaceutical analysis

For many years, polarography has reigned supreme among analytical methods, mainly due to its high accuracy, the ability to determine very low concentrations of analytes in addition to the high concentration of matrix constituents (often without prior separation), and the relatively low cost of analysis [23]. It was the analysis of compounds used in pharmaceuticals and the possibility of controlling the quality of medical formulations that made polarography an interesting tool for pharmaceutical analysis very soon after its discovery. In fact, Heyrovský himself pointed this out in his article on the application of polarography in pharmacy, which was published in 1934, only 12 years after discovering it [24].

The importance of polarography in pharmaceutical analysis is evidenced by the fact that, only 30 years after its discovery, in 1952, a large Czech monograph by Březina and Zuman "Polarografie v lékařství, biochemii a farmacii" (Polarography in medicine, biochemistry and pharmacy) [25] was published, and soon translated into German [26] and English [27]. Later developments are covered, in particular, in reviews by Zuman [28], Volke [29, 30], Patriarche and Vire [31].

Of course, classical polarography developed widely during the twentieth century and new techniques were invented and introduced, allowing further and further application of the method, and not only in pharmaceutical analysis. Heyrovský himself was, among others, the creator of oscillographic polarography, which he widely recommended for the analysis of organic substances [32]. Polarimetric titrations were another method introduced by Heyrovský, in which polarographic/voltammetric measurements serve as an objective method for indicating the equivalence point in titrations [33]. The broad application of this method for pharmaceutical analysis was reviewed by Kalvoda and Zýka [34].

Polarography reached its highest application in pharmaceutical analysis in the early 1960s. Then, it began to be displaced mainly by separation methods. Admittedly, these methods outperform classical polarography in both sensitivity and selectivity. Thus, polarographic/voltammetric methods are nowadays applied in pharmaceutical analysis mainly as detection techniques after chromatographic separation. The first such detector was introduced by Kemula in 1952 [35].

Inorganic compounds

The first polarographic analyses, which Heyrovský carried out while discovering the principle of polarography itself, were performed in aqueous solutions of inorganic salts. In addition to reducible metal cations, it turned out that some oxygen anions (bromates, nitrates, sulfates) could also be reduced, or by taking advantage of the fact that the corresponding anion forms compounds with mercury, which is the material of the electrode [27]. Gradually, methods of polarographic determination were developed for practically all inorganic compounds that could be converted into a solution. The advantage of polarographic analysis is that several analytes can often be determined at once in a single measurement (assuming that their qualitative characteristics, half-wave potential, differ; Fig. 3). A disadvantage in polarography is when determining an inorganic substance which constitutes the major component of a pharmaceutical substance or a dosage form. In this case there is a need for high dilution to reach the region where polarography provides linear calibration dependencies [27].

The determination of trace amounts of metals or inorganic substances that may be present in pharmaceutical substances as undesirable contaminants is one of the major applications of polarography. One of the earliest works on this subject is Schwaer's publication [36] from 1933, which describes the use of polarography to determine the purity of commercial pharmaceutical substances. Schwaer determined the bromate impurities in potassium bromide with a detection limit of 2×10^{-4}%, which was unattainable by any other analytical method at that time. He also determined trace amounts of lead or zinc in commercially available ammonium bromide and calcium gluconate (Fig. 4). Similarly, in 1934, Heyrovský analyzed a commercial preparation of citric acid in which he determined 0.003% copper [24].

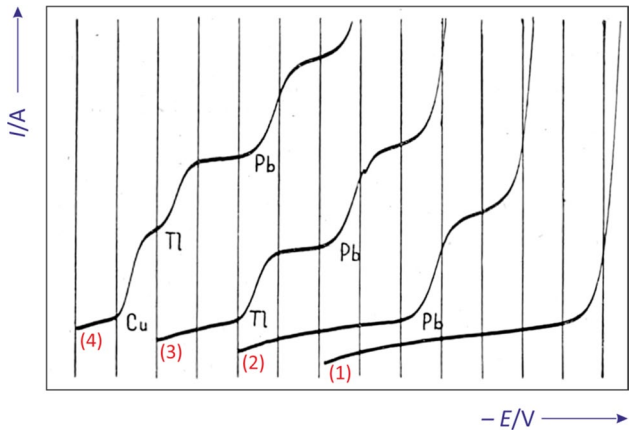

Fig. 3 Polarographic spectrum of (1) pure basic electrolyte, (2) after addition of Pb^{2+}, (3) after addition of Tl^{+}, (4) after addition of Cu^{2+} (measured in acetate buffer pH = 4.0 on dropping mercury electrode). Figure redrawn and adapted from [25]

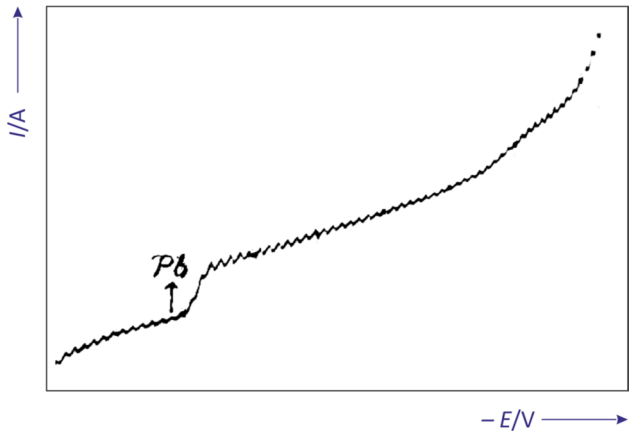

Fig. 4 Polarographic determination of Pb^{2+} in 10% aqueous solution of calcium gluconate. Figure redrawn and adapted from [36]

The first work which focused on the analysis of impurities in dosage forms is that of Bowman et al. [37], who analyzed preparations containing organic drugs (acetylsalicylic acid, sodium citrate) for trace impurities of metals.

Currently, spectral methods, especially AAS and ICP-MS, are preferred for the determination of metals and inorganics in pharmaceutical analysis [38].

Organic compounds

The possibility of polarographic determination of organic substances attracted the attention of Heyrovský and his collaborators from the very beginnings of classical polarography. In 1925, Shikata published the first study on the electrochemical reduction of an organic substance, nitrobenzene, on a dropping mercury electrode [39]. This prompted great interest in the polarography of organic substances, the main aim of which was to determine polarographic activity, design a possible course of electrode transfer in accordance with the structural formula, and, above all, determine whether and in what concentration range the limiting current was proportional to the concentration of the compound being determined. As summarized in Fig. 5, most organic functional groups are electroactive, so the possibilities for the determination of these compounds are very broad.

In the analysis of organic pharmaceutically active substances, polarography is again very useful both as a method to determine the active substance and as a method for determining purity [27]. Its ability to determine low concentrations is a great advantage, since organic drugs are often effective in very small doses. For determining purity, the main advantage is the close relationship between electrochemical activity and the structure of a compound. Therefore, a change in structure (i.e., the presence of another organic substance, often just a derivative of the active substance) is reflected by a change in electrochemical activity (a polarographic wave with a different half-wave potential).

For the polarographic determination of the active organic molecule in pharmaceutical dosage forms, it is advantageous that the organic substance is often soluble in a non-aqueous solvent, so that it can be easily separated from the interferents or the matrix [29, 30]. It is also very advantageous that, unlike UV/Vis spectrometric methods, polarography is not affected by the coloration or even turbidity of the solution. Moreover, a small volume of the order of 1 cm^3 to a few mm^3 is sufficient.

Plant alkaloids were one of the first groups of organic pharmaceutically active substances where polarography was used as a control method. The first work on quinine polarography was published in 1925 by Podroužek [40]. A detailed study of the polarography of isoquinoline alkaloids was published by Pech in 1934 [41]. The first work on the determination of alkaloids in dosage forms was published by Reimers in 1939, who determined strychnine in tablets [42]. Polarography was also successfully applied to the determination of the quality of essential oils, the first work on this subject being published by Bitter in 1950 [43]. Polarography can also be used for determination of active substance in plant drugs. For example, Šantavý determined santonin in *Flores cinae*, the unexpanded flower-heads of *Artemisia maritima*, as early as 1947 [44].

The analysis of the famous drug that saved millions of people, penicillin [45] also relates back to the early days of polarographic analysis of organic pharmaceuticals. Direct determination of this substance on a dropping mercury electrode is not possible, but as Page found in 1946 [46], penicillin can be easily converted to penicillamine. Penicillamine is identical to dimethylcysteine which can be easily determined by the Brdička reaction (see below). For this extremely

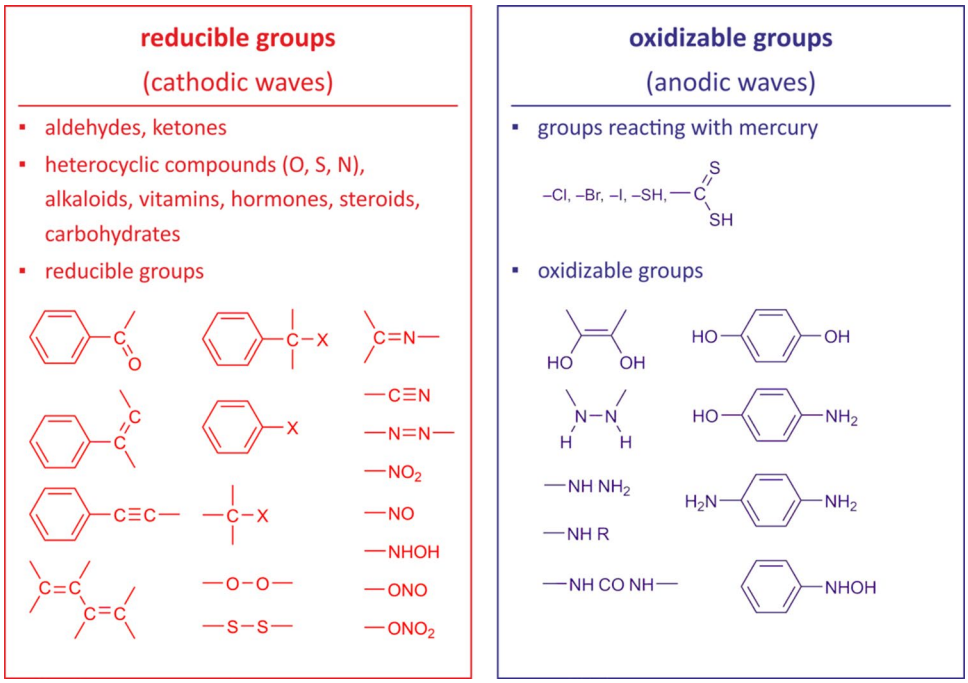

Fig. 5 Examples of polarographically and voltammetrically active reducible and oxidizable functional groups in organic compounds

important drug, Trifonov [47] proposed in the same year a measurement based on the bacteriostatic action of penicillin. The decrease in the oxygen concentration in a nutrient solution with a culture of *Staphylococcus* is measured over a period of time. After the addition of the penicillin sample, the decrease in the oxygen concentration is again measured over the same time period. This subsequent decrease is smaller due to the bacteriostatic effect of penicillin.

The Brdička reaction, just mentioned, was an important contribution to the determination of organic substances, especially proteins. It was made by Heyrovský's student Rudolf Brdička (1906–1970) [48]. In 1933, while studying the polarographic reduction of cobalt ions in an ammonia buffer, Brdička discovered a pronounced catalytic hydrogen evolution reaction caused by proteins and other substances containing – SH and – S – S – groups in these solutions [49]. Brdička's discovery soon proved to be useful in medical diagnosis, especially of cancer. It then entered the world literature as the "Brdička reaction", and is still a phenomenon studied today [50, 51].

In addition to medical diagnostics, the Brdička reaction can also be used for the polarographic determination of substances containing the functional groups – SH and – S – S –, that is, mainly proteins. A nice example is the determination of the effectiveness of insulin, which Zadina attempted to study polarographically in 1937 [52]. He compared the height of the polarographic wave measured in Brdička solution (0.1 M NH_3, 0.1 M NH_4Cl, and 2 mM $CoCl_2$) for different commercially available insulins. However, further work was required to find the correct polarographic method for determination of the effectiveness of insulin [27].

If the substance itself is not electrochemically active, it can be functionalized, i.e., an electroactive group can be introduced into the structure of the substance to enable the determination. One of the first such determinations was that of the famous antisyphilitic, salvarsan, proposed by Brdička in 1933 [53, 54]. The determination is based on the easy chemical oxidizability of salvarsan to polarographically active products. Another famous procedure was the polarographic determination of morphine after reaction with nitric acid in a hydrochloric acid environment, reaching a detection limit of 1 μg cm^{-3} [55]. An interesting method of functionalization is the reaction of a polarographically inactive substance with picryl fluoride, which greatly increases the sensitivity of determination because 12 electrons are consumed in the electrochemical reaction of the reaction product (according to the Ilkovic equation, the current flowing through the dropping mercury electrode is directly proportional to the number of electrons exchanged) [56]. The introduction of new electrode materials that allowed the extension of voltammetric determinations into oxidation regions meant that in many cases direct determinations could be made without the use of the above functionalization. For example, in the case of morphine, this was determined by direct oxidation on a platinum electrode [57].

Polarography in pharmacopoeias

If polarography was such a success in the pharmaceutical analysis of individual substances and dosage forms, it was of course only a step towards its introduction as an official

method in the pharmacopoeias. Unfortunately, this effort was partly hampered by the events of World War II. The first pharmacopoeia in which polarography appeared as an analytical method was the of Heyrovský's native country pharmacopoeia, the *Czechoslovak Pharmacopoeia, 2nd edition,* of 1954 [58]. The general section on polarographic analysis in this pharmacopoeia was written by Jaroslav Heyrovský and his student Jiří Volke (1926–2011). The pharmacopoeia prescribed polarographic determination for ascorbic acid, chlorotetracycline, chloramphenicol, nicotinamide, and of an insulin zinc suspension. However, with the decline in the popularity of polarography, this method is gradually disappearing from pharmacopoeias (except for polarimetric titration). Therefore, polarography is no longer encountered in the current *European Pharmacopoeia*. On the other hand, *The United States Pharmacopeia,* in which polarography has appeared since the 16th edition of 1960 [59], still prescribes polarography as one of the pharmacopoeial control methods.

The main reason for the retreat of polarography from the pharmacopoeias is, of course, the predominant use of separation methods (especially HPLC) in current pharmaceutical analysis. This is a pity, because in polarographic analysis of dosage forms such as tablets, but also ointments, the determination is very simple [30]. It is sufficient to extract the dosage form with a suitable solvent (e.g., acetonitrile, methanol, or water). The extract obtained is simply mixed with a basic electrolyte, and a polarographic measurement is performed.

Polarography and pharmacology

Monitoring the fate of a drug in a living organism is essential for truly rational use of pharmacy, designing appropriate dosages, and minimizing the possible negative effects of a drug on an organism. While pharmaceutical analysis is concerned with the determination of a substance in vitro, in pharmacology the interest is focused on the determination of a substance in vivo or ex vivo if a sample is taken from a living organism. This means determination in much more complex matrices than in the case of dosage forms and with a much higher content of possible interferents. Nevertheless, polarography was successfully applied very soon after its discovery to the determination of drugs and their metabolites in fluids or tissues from humans or animals [60, 61]. Later developments are summarized by Březina and Volke [62].

The first work of this kind may be considered to be that of Goodwin and Page [63], who in 1943 polarographically studied the excretion in urine of antimony administered as drugs for tropical diseases (including speciation to trivalent and pentavalent antimony). Similarly, Teppermann in 1947 studied the detoxifying effects of 2,3-dimercaptopropanol (British anti-Lewisite [64]) in rabbits injected with an intravenous solution of cadmium chloride [65]. In this context, mention may also be made of the monitoring of metal poisoning, which had already been carried out by Heyrovský's student and collaborator Shikata. In 1927, he and his collaborators proposed the determination of lead in cerebrospinal fluid, because poisonings with this metal were quite common in Japan at that time [66].

Although most pharmacological analyses are nowadays performed by separation techniques in conjunction mainly with mass detection, electroanalytical detection can sometimes be valuable in these measurements (e.g., the analysis of neurotransmitters for which mass detection is unsuitable [67]). In certain cases, it is an obvious advantage that some drugs can be determined using polarography/voltammetry directly, without separation, in bodily liquids such us blood, lymph, and saliva. In numerous cases of this type, the use of polarography would result in procedures which are as selective, but faster and less expensive, than the most widely used chromatographic methods.

Polarography and development of drugs

In addition to the quantitative and qualitative aspects of polarography in pharmaceutical analysis and pharmacology studies, knowledge of the electrode processes may also help to elucidate some mechanisms of the action of drugs in vivo. Most biochemical reactions occurring in a living organism are redox reactions, i.e., reactions based on electron transfer. These reactions play a fundamental role in the interaction between the organism and the drug. Therefore, in some cases, the therapeutic activity is paralleled by electrochemical reactivity.

The relationship between structure and electrochemical activity was discovered by Heyrovský, who in 1934 defined the conjugation rule [68]: "The polarographic reduction will be easier the higher the number of conjugated bonds in an organic molecule." Four years later, Shikata and Tachi established another empirical rule [69]: "The half-wave potential will be the more positive, the more electronegative the substituent is." The quantitative relationships between structure and half-wave potential were then studied in detail by Zuman [70]. But it was not until the 1980s that the relationships between the electrochemical behavior of substances, as determined by polarographic/voltammetric measurements, and biological activity began to be studied. This issue is addressed in detail in our previous review [71].

Conclusions

Polarography, discovered by Jaroslav Heyrovský one hundred years ago, has found a wide range of applications in many fields of human activity. One of these fields is pharmacy, where polarography has been applied both as a

method for quality control of drugs and dosage forms and as an analytical technique for pharmacological studies. Although separation or spectrometric analytical methods dominate the solution of common analytical problems today, there are areas where polarographic/voltammetric techniques are still indispensable. They continue to be applied as a detection technique in flowing fluids or as an objective method of indicating the end of titration. Polarography/voltammetry has also been widely applied in the study of chemical and electrochemical reaction mechanisms, and as one of the QSAR tools in the development of new drugs [72].

Although classical polarography on a dropping mercury electrode is nowadays a relatively rarely used method, Heyrovský's discoveries and ideas remain vital and have a firm and important place in scientific research and practical, everyday chemistry even after a century.

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