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Adsorption Effects in Polarography of Berberine

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Adsorption effects in square-wave and d. c. polarography of berberine are investigated in the supporting electrolytes of various pH. Tensametric a. c. and SW polarographic measurements show that both berberine and canadine are strongly adsorbed in the potential range between —0.3 V and —1.7 V *vs.* SCE. The reduction of berberine proceeds from the adsorbed state and requires four electrons. The coefficients $\alpha n_a = 1.32$ and $D = (4.0 \pm 0.5) \cdot 10^{-6}$ cm²/s are evaluated. There are two kind of faradaic SW responses: the first is associated with very low surface coverage while the second appears when the surface is totally covered by the adsorbed berberine. In d. c. polarography a pseudo-prewave and pseudo--main wave appear. If pH > 6 the limiting currents of d. c. polarographic waves are severely depressed.

INTRODUCTION

Berberine (Figure 1A) and canadine (Figure 1B) are protoberberine alkaloids.[1,2] The oxygen from the air oxidizes canadine to berberine (I_2 can be used instead[3]). The reduction of berberine to canadine can be achieved by zinc in acidic solutions.[2] In acidic and slightly basic solutions these alkaloids exist in the forms of berberinium B^+ and canadinium CH^+ ions, respectively. The dissociation constant of berberinium hydroxide is[4,5]: $pK_a = 11.73$ (defined as $K_a = $ conc. $B^+OH^- \cdot$ conc. H^+/conc. B^+) According to Gadamer's theory,[6] the hydrolysis of berberinium ion leads to the formation of its carbinolic form (or pseudo-base B—OH) by the reversible nucleophylic addition of hydroxy group, or alcoholate anion to carbon C8 atom which is positively charged.

Electrochemical properties of berberine have been investigated by several authors.[7-13] In the acidic aqueous media the reduction of berberinium ion is totally irreversible and involves four electrons and four protons.[10] The product is canadinium ion[7] which is polarographically electroinactive and can not be reoxidized at mercury electrode.[11] This type of reduction is characteristic
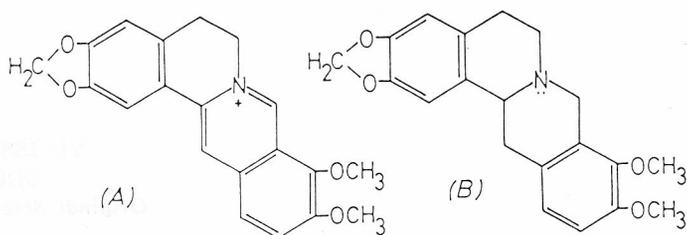
Figure 1. Structural formulas of berberinium ion (A) and of canadine (B).

of many isoquinolines.[14] D. c. polarographic response of 2×10^{-4} mol/dm³ berberinium ions in acidic aqueous media (pH $\leq$ 5) is a single wave with $E_{1/2} = -1.12$ V vs. SCE.[8-12] In neutral media (6 $\leq$ pH $\leq$ 9) the limiting current decreases when pH increases and one, or two very high and irregular maxima appear on the plateau of the wave.[9,10,12] In basic media the response consists of two small waves at -1.0 V and -1.3 V vs. SCE.[9,11,12] These waves have been ascribed to the different tautomeric forms of berberine: either to the open amino-aldehydic[11], or to the carbinolic form.[12] However, spectrophotometric measurements have shown that in aqueous solutions berberine exists only in the form of berberinium ion, even at the highest pH values.[15,16] Carbinolic form should be electroinactive in water solutions because the nucleophylic addition to C8 atom reduces nitrogen atom and the molecule becomes a simple homeocyclic aromatic system which can not be reduced at the potentials more positive than hydrogen evolution potential.[11] Šimanek et al.[12] have supposed that alcoholic adduct can be reduced by the scheme:
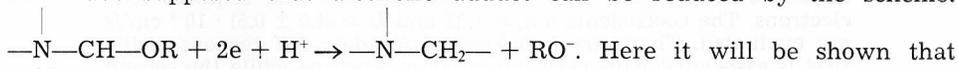
$$-\underset{\cdot\cdot}{N}-CH-OR + 2e + H^+ \rightarrow -\underset{\cdot\cdot}{N}-CH_2- + RO^-.$$ Here it will be shown that

the splitting of berberine polarographic response in two waves may be also ascribed to its adsorption to the DME surface. Although it is known that almost all alkaloids are surface active substances,[17] the investigations of adsorption properties of berberine are rare.[13,18] Pulse polarograms of berberine exhibit maxima which are characteristic consequences of the reactant adsorption to the DME.[13] Berberine adsorbed to the DME inhibits the reduction of Zn^{2+} more strongly than the adsorbed canadine.[18] Adsorption effects in square-wave and d. c. polarography of berberine will be described and analyzed in the present communication. The investigations have been performed at various pH of the supporting electrolyte, ranging from pH 2 to pH 13. The observed effects are significant for a better understanding of the reduction mechanisms of berberine and many other similar alkaloids.

EXPERIMENTAL

All chemicals were of »analytical grade« purity. Berberine chloride was purified until only one spot in thin-layer chromatography (stationary phase Stahl's silica gel and a mobile phase 15⁰/₀ solution of NH₄OH in methanol) was detected. Mercury for DME was purified chemically and by the double-step distillation under reduced pressure. Doubly distilled water was used.

The supporting electrolytes were 0.1 mol/dm³ solutions of NaCl + HCl (pH 2), citrate buffers (4 $\leq$ pH $\leq$ 7), phosphate buffers (8 $\leq$ pH $\leq$ 12) and NaOH (pH 13). The berberine concentration was changed between 5×10^{-7} and 5×10^{-4} mol/dm³.

The experiments were performed within a conventional electroanalytical cell EA 875—20, with a corresponding universal cap (both Metrohm). The working electrode was DME with a free drop life-time between 6 and 18 seconds (70 cm and 5 cm altitude of mercury reservoir). During the experiments the drop life-time was controlled mechanically. The platinum wire counter electrode and saturated (NaCl) calomel, or Ag/AgCl electrodes as reference completed the three electrode system. The electrolytic bridge between the reference electrode and the solution was filled with 0.1 mol/dm³ NaCl. The solutions were deaerated by extra pure nitrogen 20 minutes prior to the measurements.

For d. c. polarographic, pulse and differential pulse polarographic measurements the polarographic analyzer PAR-174/A (Princeton Applied Research) and the corresponding drop-knocker ASSY-170/47 were used. PAR-174/A was partially rearranged to enable working with drop life-times of 0.1, 0.2, 1.5 and 3 seconds. The potential scan rates did not exceed 2 mV/drop. D. c. polarographic »i—t« curves were recorded similarly to the corresponding d. c. polarogram: the slow potential ramp was applied to the DME with mechanically controlled drop life-time and the response was recorded with x—y recorder HP-7045/A (Hewlett-Packard) using high horizontal resolution (x: 2.5 mV/cm). In this way the current which flowed during the life-time of each particular mercury drop was clearly recorded. For instance, if $t_d = 2$ s, the scan rate was 1 mV/s and the recording of each drop occupied 8 mm of x axis. The current sensitivities of the polarograph and the recorder were the same as those used for the recording of the corresponding polarogram.

A. c. polarographic measurements at a phase angle of 87° (nonfaradaic response) were performed using the PAR-170 electrochemistry system with a hanging mercury drop electrode (Metrohm). The a. c. frequency was 230 s⁻¹ and the amplitude was 10 mV.

The Harwell multi-mode polarograph[19] and x—y recorder HP-7045/A were used for square-wave voltammetric measurements. The working DME was used in a free-dropping mode with the 18 seconds drop life-time. The reference was a (NaCl) saturated Ag/AgCl electrode. Each SW voltammogram was recorder on a single mercury drop. After the fall of the drop was detected by the associated rise in the internal resistence of the cell, the circuit within the instrument oscillated momentarily and started the timing circuits. During a four-seconds delay period the drop was getting charged to the starting potential and then the voltammogram was generated in six seconds. The scan rate was 0.083 V/s. The SW frequency was 225 s⁻¹ and the SW amplitude was 8 mV. The current responses were sampled either at the end of each square-wave half-cycle (mostly faradaic currents), or at its beginning (mostly capacitive currents).

The coulometric electrolysis at a constant potential was performed in a large (1000 ml) home-made cell with the potentiostat-coulometer PAR-273. The working electrode was a mercury pool and Ag/AgCl_sat. was the reference. The counter electrode (the Pt wire was separated from the cathodic compartment by ion--exchanging membranes. The solution was stirred by a propeller. 250 ml of 10⁻⁵ mol/dm³ berberine in 0.1 mol/dm³ NaCl, pH 4, was electrolyzed at —1.250 V vs. Ag/AgCl. The solution was deaerated by extra pure nitrogen 90 minutes prior to the electrolysis.

All current responses were recorded by a HP-7045/A (Hewlett-Packard) x—y recorder. The MA-5723 pH meter (Iskra) and TTT-85 titrator (Radiometer) were used.

RESULTS AND DISCUSSION

Cyclic linear scan voltammetric and double step chronocoulometric measurements have confirmed total irreversibility of berberine reduction in the whole range $2 \leq pH \leq 13$. From the slope of logarithmic analyses of the chronocoulogram of reduction of 10^{-4} mol/dm³ berberine in 0.1 mol/dm³ NaCl, pH 2, $t_d = 2$s, the parameter $\alpha n_a = 1.28$ was evaluated. The coulometric electrolysis at a constant potential ($E = -1.25$ V vs. Ag/AgCl) has confirmed that the reduction of berberine in the acidic medium (pH 4) involves four electrons. Consequently, the transfer coefficient is $\alpha = 0.32$ in the acidic medium.
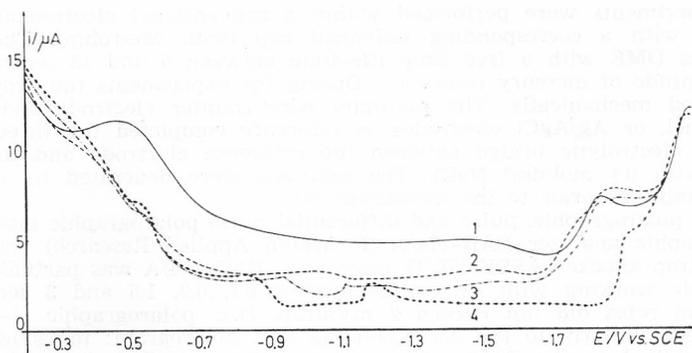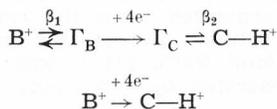
Figure 2. A. c. polarographic responses of berberine at the HMDE; capacitive component. The a. c. frequency 230 s^{-1}, amplitude 10 mV and phase angle 87°. The supporting electrolyte is 0.1 mol/dm³ NaCl, pH 2. Berberine concentrations /mol dm^{-3} = 0 (1), 1×10^{-5} (2), 5×10^{-5} (3) and 1×10^{-4} (4).

The results of a. c. polarographic measurements in 0.1 mol/dm³ NaCl, pH 2, are shown in Figure 2. Curve 1 is the capacitive current of the pure electrolyte. Addition of 10^{-5} mol/dm³ berberine (curve 2) decreases significantly the capacitive current in the wide range between —0.3 V and —1.7 V *vs.* SCE indicating strong adsorption. Berberine is reduced at about —1.1 V *vs.* SCE, but the capacitive currents do not return to the value recorded in the pure electrolyte after this potential. Obviously, the product of berberine reduction is also adsorbed. In the presence of 10^{-4} mol/dm³ berberine, additional depression of the capacity current appears at potentials between —0.9 V and —1.8 V *vs.* SCE. It can be ascribed to the change in surface orientation or structure of the adsorbates. The small peak at about —1.1 V occurs in the range of reduction and can be of faradaic rather than capacitive origin. Very similar results have been obtained in neutral (pH 7) and basic (pH 12) solutions. Those results indicate the general reduction scheme in the acidic medium:

$$B^+ \underset{\beta_1}{\overset{}{\rightleftharpoons}} \Gamma_B \xrightarrow{+4e^-} \Gamma_C \underset{\beta_2}{\overset{}{\rightleftharpoons}} C\text{—}H^+$$

$$B^+ \xrightarrow{+4e^-} C\text{—}H^+$$

Scheme I

The normalized non-faradic capacitive square-wave currents (the sampling at the beginning of SW half-cycles) are shown in Figure 3. They are recorded at —0.7 V *vs.* Ag/AgCl on slowly dropping DME after 4 seconds of its life--time. The SW scan which followed after these initial 4 seconds was not recorded. The current $(i_c)_0$ corresponds to the pure electrolyte, while the currents i_c correspond to different berberine concentrations. The measurements were performed in solutions of different pH. Regardless of pH, three concentration ranges are distinguishable. In conc. $B^+ < 10^{-5}$ mol/dm³, the surface
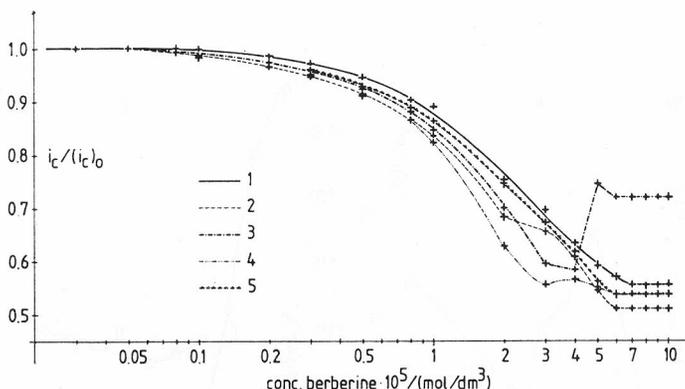
Figure 3. Dependence of the normalized square-wave capacitive currents on berberine concentration and the pH of supporting electrolytes. $(i_c)_0$ recorded in the pure electrolyte. SW frequency 225 s^{-1}, SW amplitude 8 mV, potential —0.700 V vs. Ag/AgCl, accumulation time 4 s, pH = 2 (1), 5 (2), 7 (3), 10 (4) and 13 (5).

coverage is small ($\Theta < 0.5$), but when conc. $B^+ > 6 \times 10^{-5}$ mol/dm^3 (7×10^{-5} for pH 2) the surface is saturated with the adsorbed molecules. In the middle range, the capacity is a more complex function of berberine concentration, especially at pH 7, probably because of some rearrangements of the adsorbed layer under the influence of interactions between adsorbed molecules. The sudden increase of the capacity observed at pH 7, between 4×10^{-5} and 5×10^{-5} mol/dm^3, can be ascribed to the reorientation of the adsorbed berberine molecules from the flat to the perpendicular position. The double-layer capacity of the DME is inversely proportional to the surface concentration and to the area covered by the single adsorbed molecule, as well as to the thickness of the adsorbed monolayer. In its flat position, the berberine molecule covers a significantly larger area than in its perpendicular orientation. Also, the monolayer of perpendicularly oriented molecules is much thicker than the other one. The increase of the surface concentration after the reorientation compensates these two negative effects and the double-layer capacity decreases again. Similar phenomena have been observed with other isoquinolines.[20]

Faradaic square-wave responses of berberine are shown in Figures 4—6. Two types of peaks can be recognized. Under the condition of a very low surface coverage, e. g. at 10^{-6} mol/dm^3 berberine, only the first type appears regardless of pH. It is single, simple and almost symmetrical, with $\Delta E_{p/2} = 44$ mV. Its peak current is independent of pH. However, its peak potential and its dependence on berberine concentration are pH dependent. At pH 2, $E_p = -1.135$ V vs. Ag/AgCl and i_p is a linear function of conc. B^+ if conc. $B^+ \leq 10^{-5}$ mol/dm^3. Between 1×10^{-5} and 5×10^{-5} mol/dm^3 berberine, its peak potential shifts strongly in positive direction, its half-peak width increases while the peak current gradually reaches the maximum value. For conc. $B^+ > 5 \times 10^{-5}$ mol/dm^3, the peak current decreases until the peak disappears. At higher pH, these processes occur at the lower berberine concentration, as it can be seen in Figure 4. At pH 13, the first peak reaches the maximum for conc. $B^+ = 10^{-5}$ mol/dm^3 and disappears for conc. $B^+ > 4 \times 10^{-5}$ mol/dm^3.
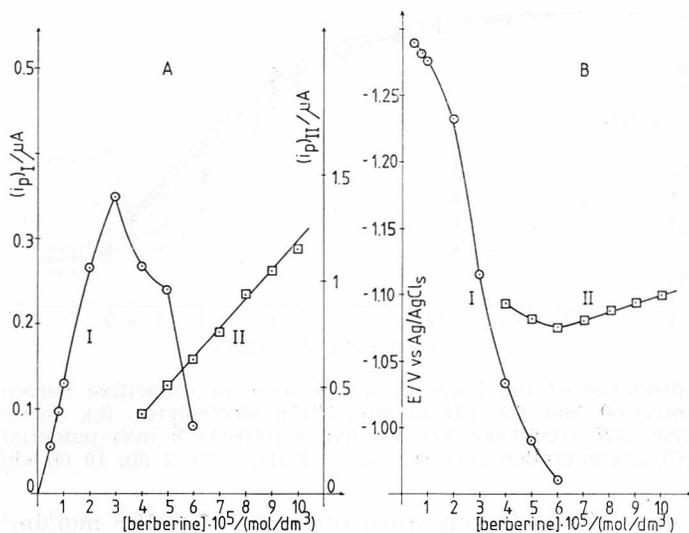
Figure 4. (A) Dependence of maximal faradaic SW peak-currents on berberine
concentration. The left axis- the first peak, the right axis- the second peak. Citrate
buffer pH 5.
(B) Dependence of SW peak potentials on berberine concentration. Citrate buffer
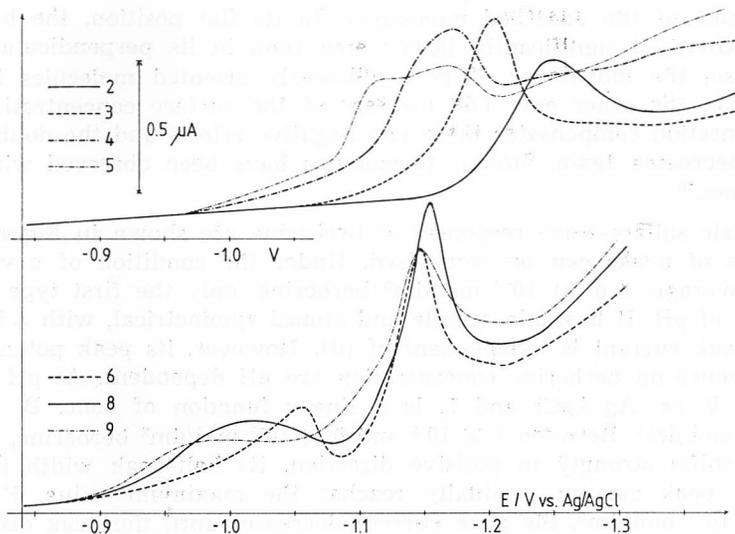pH 5. SW frequency 225 s⁻¹; SW amplitude 8 mV and accumulation time 4 s.

Figure 5. Forms of faradaic SW responses of X ×10⁻⁵ mol/dm³ berberine in citrate
buffer, pH 7. The numbers in the legend are the values of X. Parameters of the
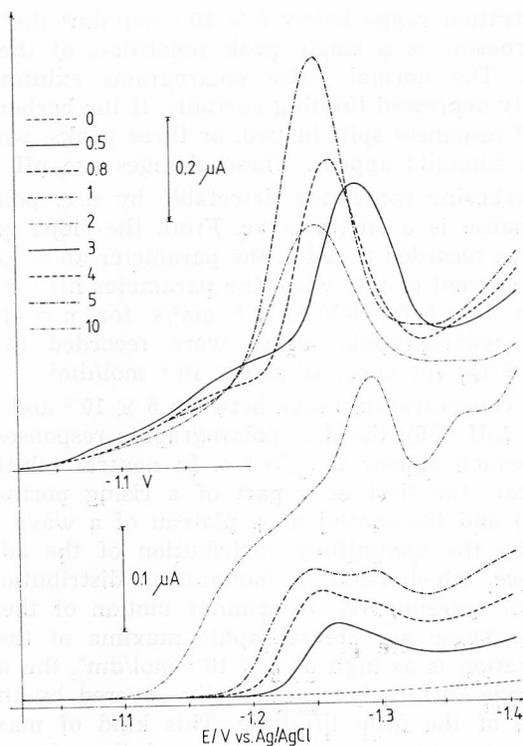excitation signal as in Figure 4.

Figure 6. Forms of faradaic SW responses of $n \times 10^{-5}$ mol/dm^3 berberine in 0.1 mol/dm^3 NaOH, pH 13. The numbers in the legend are the values of n. Parameters of the excitation signal as in Figure 4.

The second SW peak appears in the concentration range between 5×10^{-5} and 1×10^{-4} mol/dm^3 (if pH $\leq$ 10), but if pH = 13 it can be noted even for conc. B$^+$ = 10^{-5} mol/dm^3. Its peak current is linearly proportional to conc. B$^+$ if pH $\leq$ 6, but if pH $\geq$ 7 it increases nonlinearly with increasing conc. B$^+$ until some maximum and then decreases with the further increase of conc. B$^+$. At pH 7 it reaches the maximum for conc. B$^+$ = 8×10^{-5} mol/dm^3, while at pH 13 for 6×10^{-5} mol/dm^3.

The first type of SW responses corresponds to the simple reduction from the adsorbed state: $\Gamma_B + 4e \rightarrow \Gamma_C$ under the conditions of a small surface coverage. This basic reaction occurs in all solutions, regardless of pH. The energy required for this charge transfer depends on the interactions between the adsorbed molecules and decreases if Θ increases. This observation indicates the existence of (probably electrostatic) repulsion between the adsorbed berberinium ions and maybe the attraction between the adsorbed molecules of canadine.[21] The second type of SW peaks appears when the electrode surface is almost, or totally, saturated. It appears as a single peak indicating the single charge transfer in the whole range $2 \leq$ pH ≤ 13.

In the concentration range below 5×10^{-5} mol/dm³ the differential pulse polarogram of berberine is a single peak regardless of the pH of solutions (for $2 \leq$ pH ≤ 13). The normal pulse polarograms exhibit well developed maxima and heavily depressed limiting currents. If the berberine concentration is higher, the DPP responses split in two, or three peaks, while complex NPP maxima with two summits appear. These changes are pH dependent.

The lowest berberine contration detectable by d. c. polarography is 10^{-5} mol/dm³. The response is a single wave. From the slope of the logarithmic analysis of the wave recorded at pH 4, the parameter $\alpha n = 1.32$ was evaluated. From the limiting current of this wave the parameter $nD^{1/2} = (8.0 \pm 0.5) \times 10^{-3}$ cm/s$^{1/2}$ (wherefrom $D = (4.0 \pm 0.5) \times 10^{-6}$ cm²/s for n = 4) was calculated. Such simple d. c. polarographic waves were recorded in all investigated solutions ($2 \leq$ pH ≤ 13) for conc. $B^+ \leq 5 \times 10^{-5}$ mol/dm³.

In the berberine concentration range between 5×10^{-5} and 2×10^{-4} mol/dm³, in acidic solutions (pH < 6), the d. c. polarographic responses are complicated by high maxima which appear if $t_d > 1$ s. In neutral solutions ($6 \leq$ pH ≤ 8) two maxima appear: the first as a part of a rising portion of a wave (as in acidic solutions) and the second on a plateau of a wave. The maxima are probably caused by the nonuniform distribution of the adsorbed molecules at the DME surface, which leads to nonuniform distribution of the charges at the surface and, consequently, to laminar motion of the mercury on the surface of a drop. These are polarographic maxima of the third kind.[22] If berberine concentration is as high as 5×10^{-4} mol/dm³, the maxima disappear because the electrode surface becomes totally covered by the adsorbed layer in the early stage of the drop life-time. This kind of maxima appear also as a consequence of sudden rearrangements of the adsorbed layer, or the formation of a new adsorbate.[22] The second maximum observed in neutral solutions can be ascribed to such phenomena.

The splitting of the d. c. polarographic response of 5×10^{-4} mol/dm³ berberine in two waves is shown in Figure 7. Only one wave (i_1) appears if $t_d < 1$ s. Its limiting current does not depend on the drop life-time. The second wave (i_2) appears for drop life-times longer than 1 s. The broken lines on curve 5 represent the local maxima on »$i - t$« curves. The variations of currents during each single drop (»$i - t$« curves, $t_d = 2$ s) are displayed in Figure 8. The development of the first wave (i_1) is presented by sequence »a«. The faradaic reaction proceeds on the free electrode surface until it is totally covered by the adsorbed canadine which inhibits the reduction process. The continuation of the reduction on the covered electrode surface is much slower and can be seen as the increase of the current after minimum. The left side of sequence »b« represents the limiting current of the first wave. The right side of this sequence exhibits the development of the second wave (i_2) which obviously appears when the overpotential is high enough to increase the rate of the reduction on the covered surface to a considerable extent. Sequence »c« exhibits the limiting current of the second wave. According to the nomenclature of Mairanovskii[23] the first wave (i_1) is a pseudo-prewave and the second (i_2) is a pseudo-main wave. Both waves correspond to the same totally irreversible redox reaction complicated by the reactant and product adsorption (Scheme I). The dependence of d. c. polarographic limiting

currents on berberine concentrations is linear, but two slopes were observed: for conc. $B^+ \leq 8 \times 10^{-5}$ mol/dm^3 $\partial i_{lim}/\partial$ conc. $B^+ = 5.6$ A cm^3/mol, while for $8 \times 10^{-5} \leq$ conc. $B^+/dm^{-3} \leq 5 \times 10^{-4}$ $\partial i_{lim}/\partial$ conc. $B^+ = 3$ A cm^3/mol. The results were obtained at pH 4, with $t_d = 1$ s.
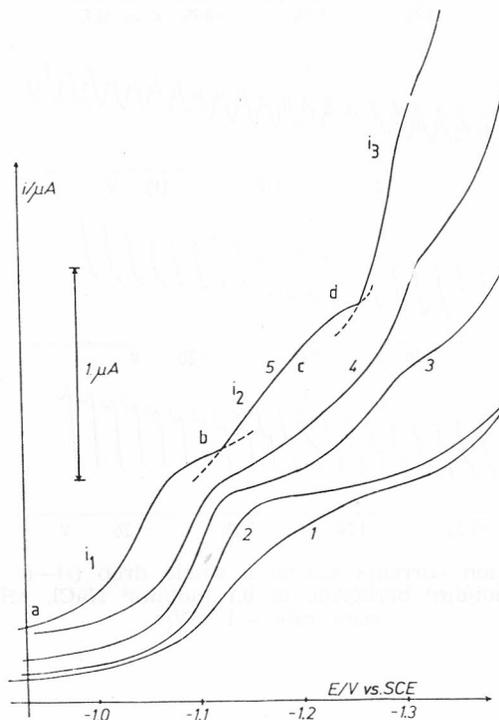

Figure 7. D. c. polarographic waves of 5×10^{-4} mol/dm^3 berberine in 0.1 mol/dm^3 NaCl, pH 2.2; $t_d/s = 0.1$ (1), 0.2 (2), 0.5 (3), 1 (4) and 2 (5). i_1, i_2 and i_3 — the first, second and third waves; a, b, c and d — parts of the wave investigated by »i—t« curves.

It is well known that the product of berberine reduction catalyzes the reduction of hydrogen.[8-10,17] The catalytic wave exists for pH $<$ 10. Sequence »d« in Figure 8. corresponds to the development of the third wave (i_3) in Figure 7, curves 3—5. The current of the reduction on the free electrode surface increases and forms the third wave. The current can be increased only if additional electrons are exchanged. This maens that reduction of some other electroactive species develops. The most probable reaction is the reduction of hydrogen ions to the atoms adsorbed to the surface film of canadine. Further increase of the potential initiates masive evolution of hydrogen. The third wave exists in neutral (see Figure 9), but not in basic solution (see Figure 10).

D. c. polarograms of 5×10^{-4} mol/dm^3 berberine in 0.1 mol/dm^3 citrate buffer, pH 7.1, are shown in Figure 9. The responses consist of one, two, or three waves (i_1, i_2 and i_3). The first wave at —0.95 V vs. SCE reaches

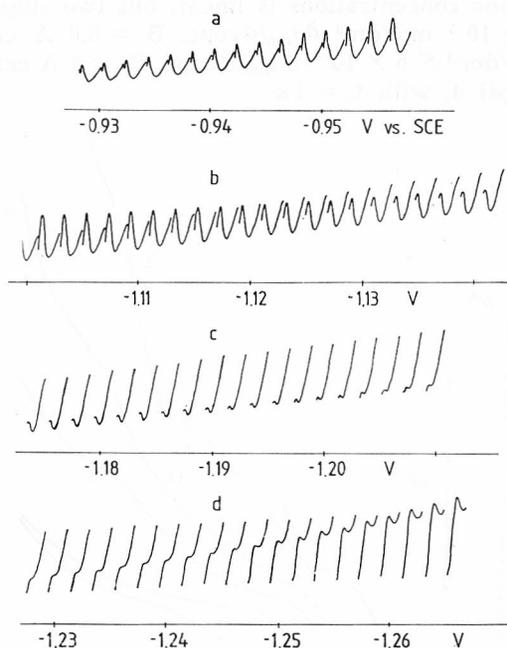Figure 8. Depolarization currents during a single drop (»i—t« curves) at various potentials. 5×10^{-4} mol/dm³ berberine in 0.1 mol/dm³ NaCl, pH 2.2; $t_d = 2$ s; the scan rate —1 mV/s.
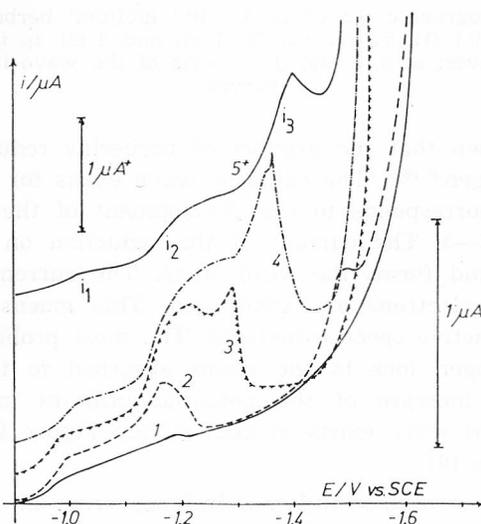

Figure 9. D. c. polarograms of 5×10^{-4} mol/dm³ berberine in 0.1 mol/dm³ citrate buffer, pH 7.1; t_d/s = 0.1 (1), 0.2 (2), 0.5 (3), 1 (4) and 2 (5). i_1, i_2 and i_3 — three waves.
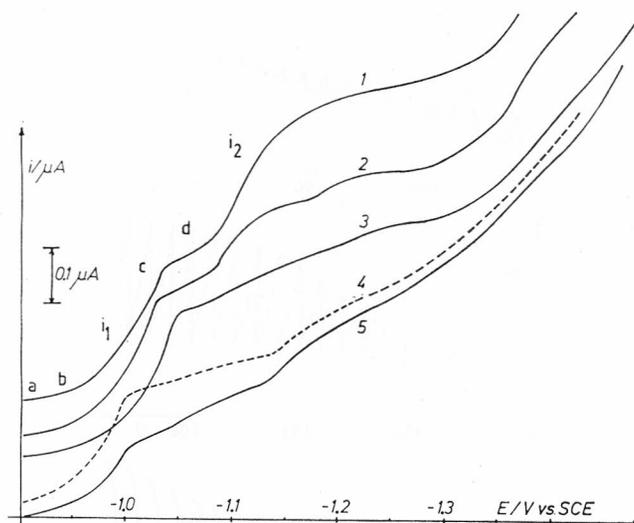
Figure 10. D. c. polarograms of 5×10^{-4} mol/dm^3 berberine in 0.1 mol/dm^3 NaOH, pH 13; $t_d/s = 2$ (1), 1 (2), 0.5 (3), 0.2 (4) and 0.1 (5); a, b, c and d — parts of the wave investigated by »$i—t$« curves.

the maximum value at $t_d = 0.2$ s and does not increase any further. At longer drop times waves i_2 at — 1.18 V and i_3 at —1.35 V increase with increasing the drop-time. The shape of these two waves is affected by adsorption phenomena, resulting in a decrease of the limiting current. The minima on limiting currents occur gradually at more negative potentials with increased drop-time: from —1.2 V ($t_d = 0.2$ s) to —1.4 V for $t_d = 2$ s. The analysis of »$i — t$« curves has shown that the first, small wave (i_1) corresponds to the reduction on the free surface of the DME. The adsorbed product inhibits reduction. The second wave (i_2) arises from the reduction of berberine on the surface totally covered by the adsorbed product. Waves i_1 and i_2 are pseudo-prewave and pseudo-main wave, respectively. In the potential range where the third wave (i_3) appears, the current on the free DME surface suddenly increases significantly. A similar phenomenon has been observed in acidic media (Figures 7 and 8). By analogy, the third wave in Figure 9 can be ascribed to the catalytic reduction of hydrogen. On the plateau of the third wave the reduction becomes almost totally supressed, which could be due to the formation of the condensed film of the adsorbed canadine. Similar decreasing of the limiting current has been observed in polarography of $Co(NH_3)_6^{3+}$ and $In(NO_3)_3$ in KCNS medium,[24] of some platinum complexes[25] and of several inorganic anions.[26] The existence of a certain deactivation mechanism in neutral solutions is also indicated by faradaic SW measurements. The second SW peak sharply diminishses if conc. $B^+ > 8 \times 10^{-5}$ mol/dm^3.

In basic media, d. c. polarograms of 5×10^{-4} mol/dm^3 berberine consist of two waves, as shown in Figure 10: the first (i_1) with $E_{1/2} \approx$ —1.0 V and the second (i_2) with $E_{1/2} \approx$ —1.15 V vs. SCE. The limiting current of the first
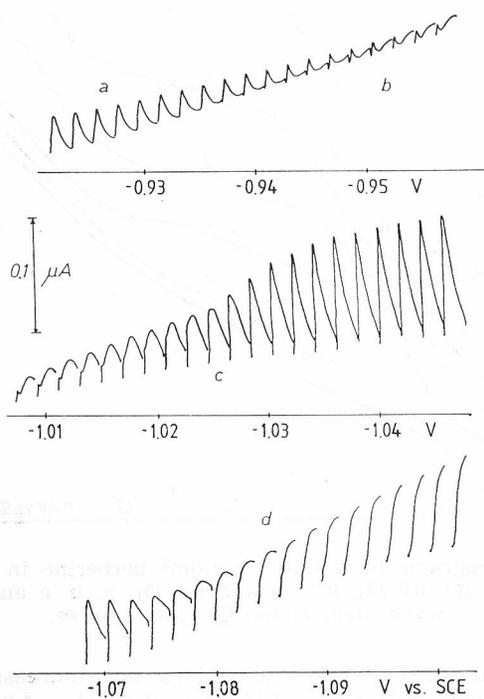
Figure 11. Depolarization currents during a single drop (»i—t« curves) at various potentials. 5×10^{-4} mol/dm³ berberine in 0.1 mol/dm³ NaOH, pH 13; $t_d = 2$ s; the scan rate —1 mV/s.
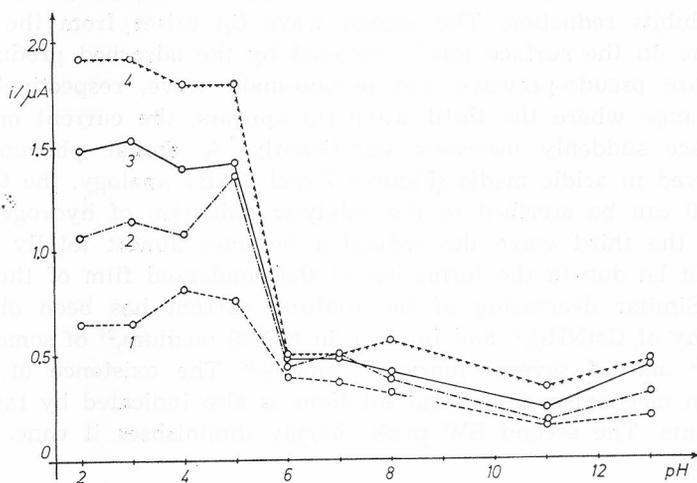

Figure 12. Dependence of limiting currents of d. c. polarograms of 5×10^{-4} mol/dm³ berberine on pH of supporting electrolytes, $t_d/s = 0.2$ (1), 0.5 (2), 1 (3) and 2 (4).

wave increases proportionally to the drop life-time if $t_d \leq 0.5$ s, but then stagnates. The second wave (i_2) exists even at the lowest drop life-times, but increases proportionally to t_d only if $t_d > 0.5$ s. The corresponding »$i - t$« curves can be seen in Figure 11. They are rather unusual. The residual current (sequence »a«) is under the influence of the berberine adsorption. However, the first wave (i_1) does not develop on a free surface, as in acidc media, but on a surface which is totally covered by the adsorbed berberine (sequence »b«). The product of this reduction inhibits the further reduction. Gradually, the inhibition by the product becomes dominant (the left side of sequence »c«), while the initial influence of berberine disappears because it is all reduced. The limiting current of the first wave (the right side of sequence »c«) is defined by the strong inhibition of the reduction by the adsorbed product. The second wave (i_2) develops when the inhibition by the product disappears (sequence »d«). Tensametric measurements did not indicate desorption of canadine in this potential range. It is hard to guess what causes the second wave.

The dependence of limiting currents of d. c. polarograms of 5×10^{-4} mol/dm^3 berberine on the pH of solutions is given by Figure 12. This relationship is in agreement with the results reported by Šantavy.[9] In neutral solutions limiting currents are significantly depressed and the waves are highly irregular. In basic media the responses are depressed and consist of two small waves. All these observations can be ascribed to the adsorption of berberine and canadine to the DME surface. In the whole range between pH 2 and pH 13, the reduction of berberine is the same and requires four electrons. This is proved by the measurements at very low berberine concentrations when the reduction proceeds only from the adsorbed state. Interactions between the adsorbed molecules of reactant and product may significantly influence the results of polarographic measurements in the higher concentration range. These influences are pH dependent. In d. c. polarography two kind of waves appear: the pseudo-prewave which is caused by the reduction of the adsorbed berberine to the adsorbed canadine and the pseudo-main wave which is caused by the reduction of the dissolved berberine on the DME surface modified by the adsorbed canadine. The latter appears only for a very high berberine concentration.

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SAŽETAK

Utjecaj adsorpcije na polarografiju berberina

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Istražen je utjecaj adsorpcije na d. c. i kvadratno-valne (square-wave) polarograme berberina u elektrolitnim otopinama raznih pH. Tenzametrijska mjerenja upućuju na snažnu adsorpciju berberina i kanadina u području potencijala između —0,3 V i —1,7 V prema ZKE. Adsorbirani berberin reducira se primajući četiri elektrona. Određeni su koeficijenti $\alpha n_a = 1,32$ i $D = (4,0 \pm 0,5) \cdot 10^{-6}$ cm^2/s. Postoje dvije vrste faradajskih SW odziva: prvi nastaje kod niskih pokrivenosti površine, a drugi pri potpunoj pokrivenosti. D. c. polarogrami mogu se sastojati od pseudo--predvala i pseudo-glavnog vala. Ako je pH > 6, granične struje d. c. polarograma jako su snižene.