

**POLAROGRAPHIC INVESTIGATION
OF SYSTEMS CONTAINING COBALT(II)
AND CYSTEINE-LIKE COMPOUNDS
WITH SPECIAL REFERENCE TO THEIR ABILITY
TO FACILITATE HYDROGEN EVOLUTION
FROM BUFFERED AQUEOUS SOLUTIONS**

P. MADER

*Department of Biophysics and Plant Physiology,
University of Agriculture, Prague 6*

Dedicated to the 65th anniversary of the late Academician R. Brdička.

Received July 3rd, 1970

The polarographic activity of the cobalt-thiolate system has been reinvestigated. Two new, hitherto unknown types of the catalytic hydrogen currents, in addition to the earlier known Brdička and "SH" currents, have been observed. The acid-base and redox properties of the cobalt-thiolate complexes in various oxidation states are believed to account for the formation of two types of the catalytic hydrogen currents in the studied system. Strong surface interaction between the adsorbed thiol and freshly deposited highly active cobalt atom results in a direct discharge of a hydronium ion on the surface of the thio stabilized metallic cobalt. Nitrogen-free thiols were found to be catalytically active in absence of cobalt; their catalytic activity increases with increasing ionic strength of the solution. Frequently observed adsorption phenomena in the above systems have been explained in terms of the enhanced surface activity of the components after their mixing, anodic reaction of the thiol group with mercury, or slow chemical decomposition of the disulfide into the surface active intermediates. Neither adsorption nor an exhaustion of the proton donor in the vicinity of the electrode surface accounts for the constant value of the Brdička catalytic current at large thiol concentrations. A simple factor enabling the direct comparison of the experimental results obtained with different thiols and in different media has been proposed.

When, in 1933, Prof. Brdička discovered¹ the large polarographic maxima due to the catalyzed hydrogen evolution in cobalt(III) hexammine chloride solution containing human serum albumin, he opened a completely new field in polarography — the polarographic hydrogen catalysis by transition metal complexes. In his subsequent study² Brdička showed that the actual active components in the above system are electrochemically generated divalent cobalt ions and cysteine resp. cystine residua, the latter giving free thiol groups upon their reduction at the mercury electrode. Other thiols were found also to be catalytically active. Divalent nickel in place of cobalt has similar, but much less effect. Cysteine facilitates hydrogen evolution even in cobalt-free solutions; its catalytic activity was ascribed by Brdička to the catalytic action of the SH group⁴.

The pioneering work of Brdička¹⁻¹⁶ was soon followed with numerous investigations which resulted in an extensive and valuable experimental material; it found many applications both in theory and in analytical and clinical practice. Today, the literature on Brdička currents comprises more than thousand papers (for detailed reviews see *e.g.* ref.¹⁷⁻²²).

In spite of such a great effort which has been devoted to the study of the nature of Brdička currents the actual mechanism which leads to the catalyzed evolution of hydrogen is still ill understood. The main reason for this is that the mechanism and the kinetics which determine these currents vary with experimental conditions; this situation makes it sometimes very difficult if not impossible to determine the effect of a single factor on the current. Questions such as the composition of the actual catalytically active species; oxidation state of cobalt in this species; the existence and importance of adsorption for the actual catalytic step; the catalytically active site in the molecule of the catalyst; etc, are still unanswered and there remains much uncertainty and contradiction in the literature concerning their interpretation. Several complicating factors further contribute to this complex situation:

The polarographic behaviour of cystine has been thoroughly studied^{23,24} and is believed to be satisfactorily understood. There remain, however, a few points which need further elucidation. Thus, while the importance of the cystine adsorption at mercury for its reduction in the cystine prewave is today generally accepted, its direct experimental proofs are rather scarce and not much convincing. The polarographic reduction of cystine in three, instead of usually observed two, reduction waves has been observed²⁵. The origin of the third wave is not clear. The peculiar time changes which were observed both in cobalt-free²⁶ and in cobalt-containing²⁷ cystine solutions in alkaline buffers have not been explained, either. Since the vast majority of investigations have used cystine in place of cysteine the secondary properties of the former compound, unrelated to the hydrogen catalysis, could have been easily misinterpreted and attributed to the actual catalyst containing cysteine.

Another source of complications is the *polarographic behavior of divalent cobalt* which is rapidly reduced to the zero state already at potentials much less negative than those at which the Brdička catalytic wave takes place. This makes it difficult to ascribe the proper oxidation state to the cobalt atom in the actual catalytically active species. Sulfur-containing proteins as well as low molecular weight thiols facilitate cobalt(II) reduction and displace its reduction wave to 150–250 mV less negative potentials. This effect has been ignored in all so far proposed quantitative interpretations of the catalytic hydrogen wave.

Finally, *the necessity to use other weakly complexing agents* in large concentrations, makes the study of the Brdička catalytic hydrogen waves more complicated. At pH at which Brdička waves are observed (pH 8–12) divalent cobalt precipitates from the solution in the form of cobalt(II) hydroxide; in order to keep it in a soluble form, weakly complexing agents must be added, which also serve as buffer constituents. Variations of their concentration affect both the pH and the coordination equilibria in the solution and make an already involved system even more complex.

It seemed obvious that any further study of the Brdička catalytic hydrogen currents should be performed only under the most simplified conditions, *i.e.*, with *a*) low molecular weight bi- or tridentate thiolate ligands of the cysteine type which are well soluble in water and which form well water soluble chelates with iron group metal cations; and *b*) divalent cobalt which has less tendency to form polynuclear species with thiolate ligands than nickel(II) ion. Previously reported results with cystine and other disulfides should be reinvestigated with respect to the specific properties of these compounds. The catalysis of cobalt(II) reduction by thiolate compounds should be treated in more detail and taken into consideration in the quantitative interpretation of the catalytic hydrogen mechanism. Aromatic thiols which are much more acidic than their aliphatic analogues should be employed in order to avoid

the necessity to coordinate cobalt with an additional ligand. Finally, the characteristics of the Brdička currents should be studied only under the specified conditions when the results obtained in different media and with different thiols can be compared.

This paper will summarize and evaluate a series of our polarographic investigations on redox, adsorption and catalytic properties of systems containing cobalt(II) and/or cysteine-like compounds, with special reference to their ability to facilitate hydrogen evolution from buffered aqueous solutions. This study has been initiated and then stimulated by the steady interest and help of Prof. R. Brdička.

EXPERIMENTAL

Chemicals

All thiol compounds used are listed in Table I, together with their acid dissociation constants. 0.01 to 0.02M stock solutions in previously deaerated water or ethanol were freshly prepared before each set of experiments.

Three types of buffer systems have been used, *i.e.*, those with the positively charged, uncharged, and negatively charged acid buffer constituent; some experiments in zwitterionic glycinate buffer have also been performed. The pH-values used ranged from about 3 to 12.

In connection with the sensitivity of organic thiols and of cobalt(II)-thiolate mixture toward oxidation by atmospheric oxygen, especially at higher pH, the following procedure was always followed in the preparation of reaction mixtures. An air-free solution of the thiol was added to the previously deaerated buffer solution and upon continued passage of nitrogen, air-free solution of CoCl_2 was added from a microburette. This order of mixing had an additional advantage in that it prevented precipitation of cobalt(II) hydroxide at higher pH and large thiol concentrations, because of complexation of cobalt(II) with the thiol.

Conventional d.c. polarography has been applied to most problems. Supplementary polarographic techniques included a.c. polarography, Kalousek with commutator method, drop-time, differential capacity and instantaneous current *vs.* time measurements, and cyclic voltammetry on a stationary mercury drop. UV irradiation was used in studies of the cystine instability.

All reported currents are average currents. Values of potentials refer to the saturated calomel electrode.

RESULTS AND DISCUSSION

DIFFUSION AND ADSORPTION CURRENTS IN SYSTEMS CONTAINING COBALT(II) AND/OR CYSTEINE-LIKE COMPOUNDS

On the Polarographic Reduction of the Cystine Disulfidic Group

Brdička was the first to reduce cystine polarographically². In spite of the great number of subsequent studies the actual reduction mechanism is still unclear. Particularly puzzling is the formation of the cystine prewave which precedes the main

TABLE I
List of Used Thiols and Their Acid Dissociation Constants in Water

Compounds	pK
Cysteine ^a	pK(COOH) 2.0; pK _A 8.53; pK _B 8.86; pK _C 10.36; pK _D 10.03
Cysteine ethyl ester ^a	pK _A 7.45; pK _B 6.77; pK _C 8.41; pK _D 9.02
Cysteamine	pK ₁ (SH) 8.27; pK ₂ (NH ₃ ⁺) 10.53
Thioglycolic acid	pK ₁ (COOH) 3.55; pK ₂ (SH) 10.68
Thiosalicylic acid	pK ₁ (COOH) ~ 5; pK ₂ (SH) 8.88
3-Mercaptopropionic acid	pK ₁ (COOH) 4.27; pK ₂ (SH) 10.84
2-Mercaptoethanol	pK(SH) 9.72
2-Mercaptoaniline	pK ₁ (NH ₃ ⁺) 2.9; pK ₂ (SH) 5.9
4-Mercaptoaniline ^b	pK(SH) 7.95
Thiophenol	pK(SH) 6.5
Pentachlorothiophenol	pK(SH) 3.5
Reduced glutathione	pK(SH) 8.56
Diethylaminoethanthiol	?
Maleonitrile dithiolate	?

^a For the determination of microdissociation constants of cysteine and its ethyl ester see⁶⁵; ^b in 40% ethanol.

cystine reduction wave. The kinetic character and weak adsorption properties of this prewave would suggest the participation of mercury. It has been tentatively identified as a cathodic wave, arising from a mercury compound created in the surface layer of the electrode²⁴.

In order to bring more insight into the reduction mechanism of the cystine disulfidic bond, a separate study has been performed²⁷. Studies on instantaneous currents on single drops, effect of temperature, drop-time, reservoir and other studies conclusively confirmed the mixed adsorption-kinetic character of the prewave limiting current. It has been stated in the literature^{28,29} that some disulfides react chemically with metallic mercury forming mercurous and/or mercuric compounds. Hence, we tried the effect of mechanical shaking of the cystine solutions with metallic mercury. In ammonia buffer under anaerobic conditions it indeed resulted in an increase of the cystine prewave, and in a shift of its half-wave potential to less negative values. When the height of the exalted prewave becomes about 200% of the original prewave current it becomes obvious that the exalted wave is, in fact, composed of two separated waves whose half-wave potentials differ a few tens of millivolts. This behavior together with that of cystine at a hanging mercury drop supports Březina's conclusion³⁰ that the species which is being reduced in the cystine prewave

is a particular species which differs from that formed as a product of an anodic depolarization of cysteine with mercury. Experiments performed with mercury cysteinates further support this conclusion²⁷.

Mercury in the form of HgCl_2 greatly increases the prewave current in ammonia medium. The exalted prewave is, in this case, preceded with a large reduction current of free mercury(II) ions. No such current is observed when cystine is treated with metallic mercury. In acetate buffers neither mercury chloride nor metallic mercury has any appreciable effect on the cystine reduction waves. In several instances, a slight decrease of both waves after the treatment with mercury has been observed in acidic media.

Irradiation of dropping mercury electrode with visible and ultraviolet light brings about a large increase of the prewave current similar to that due to mercury. Quartz cell has been used in the latter case. Obviously, the rates of surface reactions on mercury increase in the presence of radiation.

Instability of Cystine in Mildly Alkaline Solutions

Weakly alkaline solutions of cystine are subject to time changes³¹, contrary to the statements in the literature. Upon standing for a few days, a 1 mM solution of cystine in ammonia buffer, pH 9.3, exhibits a new reduction wave (wave C, Fig. 1), in addition to the ordinarily observed two reduction waves of cystine A and B. Another new wave (wave D) appears at still longer times of standing. This wave D increases with time, while the other waves A, B and C gradually diminish and after 7–8 months completely disappear. A detailed study of the cystine instability has revealed³¹ that the new wave C is the reduction wave of the disulfidic anion S_2^{2-} , the latter species being formed in the solution as an unstable intermediate of the cystine degradation. In the presence of air, disulfidic anion is further slowly oxidized to thio-sulphate which, in reaction with the simultaneously formed cysteine, gives the final degradation product, S-sulfocysteine. The latter is polarographically active and

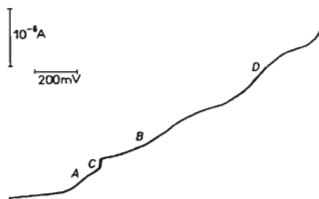


FIG. 1

Effect of Ageing on the Polarographic Behavior of Cystine

1 mM cystine, 0.1M- NH_3 , 0.1M- NH_4Cl , after four months of standing in presence of air and light. From -0.2 V.

gets reduced in the wave D. Strong adsorption phenomena observed sometimes in older cystine solutions are encountered by the adsorption of the strongly surface active disulfidic anion on the mercury surface, and by its inhibition of the electrode process in the prewave A.

S-Sulfocysteine upon reduction gives one molecule of cysteine, compared to two cysteine molecules which are obtained after the polarographic split-off of the cystine disulfidic bond. In the presence of divalent cobalt, S-sulfocysteine gives large Brdička current which is one half in magnitude of that of cystine in the same concentration. The earlier reported time changes in the cobalt-free and cobalt-containing cystine solutions in alkaline buffers can be, thus, explained now in terms of the cystine transformation into the less catalytically active S-sulfocysteine. This process is catalyzed in the presence of Co(II), Ni(II) and particularly Cu(II) ions³². The composition of the final degradation products, however, is independent of the presence of these ions and is determined chiefly by the pH of the solution³¹.

Adsorption of Cystine at Mercury Electrodes

Drop-time curves. Milimolar cystine solutions in unbuffered (KF) as well as in buffered ($\text{NH}_3\text{-NH}_4\text{Cl}$, F; borax-boric acid) solutions exhibit no or only slight changes in surface tension of mercury compared to that in cystine-free solutions. In mildly acidic and alkaline media, cystine is difficultly soluble and its milimolar solutions are almost saturated. On the other hand, in 0.1M-HCl or KOH solutions the cystine solubility is considerably increased and it is possible to work with much higher

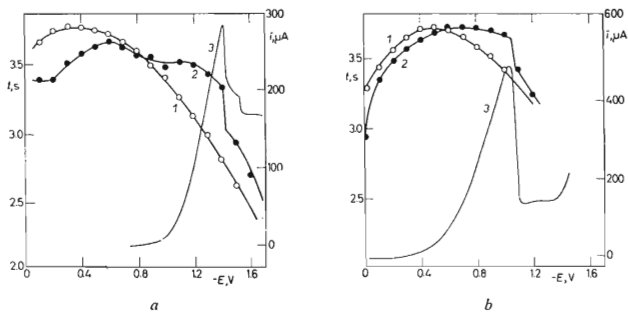


FIG. 2

Drop-Time Curves in (a) 0.1M-HCl; (b) 0.1M-KOH

Conc. of cystine: 1 0; 2 $3.5 \cdot 10^{-2}$ M; 3 D.C. polarogram of 2.

concentrations. Drop-time curves then exhibit a distinct decrease in the surface tension of mercury at potentials less negative than those of the cystine reduction wave. The latter is single and is accompanied with a large maximum of the first kind (see curve 3 in Fig. 2a,b). No maxima on the cystine reduction waves have been described earlier in the literature.

It is of interest to note that we have observed small rounded maximum on the main cystine reduction wave even in its millimolar concentrations. This maximum increases in the presence of certain organic compounds (Fig. 3) and also carborafine. Visual observations shows a slow hydrodynamic movement of the electrolyte around the electrode at the potentials of this maximum.

The surface activity of cystine is greatly increased in the presence of cobalt(II) ions. At higher cobalt concentrations, a pinkish slightly water soluble cobalt(II) cystinate precipitates from the solution.

Cystamine, which is a structural analogue of cystine, more strongly adsorbs on mercury than cystine (Fig. 4). Divalent cobalt further increases the adsorptivity of cystamine (curves 3 and 4 in Fig. 4), and suppresses the large streaming maximum

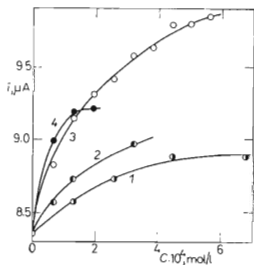


FIG. 3

Exaltation of the Cystine Maximum in the Presence of 1 Aniline; 2 β -Phenylalanine; 3 Glycine; 4 *p*-Aminobenzoic Acid

All solutions 1 mM in cystine, 0.1M in NH_3 , 0.1M in NH_4Cl .

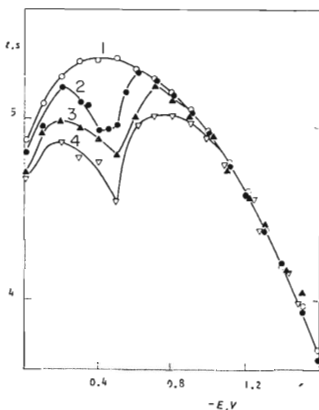


FIG. 4

Drop-Time Curves in 0.06M Borax
Conc. of cystamine: 1 0; 2—4 1 mM. Conc. of CoCl_2 : 1—2 0; 3 4; 4 $6 \cdot 10^{-5}$ M.

which accompanies the cystamine reduction wave (Fig. 5). At higher cobalt concentrations, an adsorption prewave is observed which is followed by an inhibited wave of cystamine. Assuming the reversible course of the reduction in this prewave, an area covered by one molecule of adsorbed cobalt(II) cystaminatate was calculated to be 61 \AA^2 (see³³).

Charging current. The anodic charging current of the dropping mercury electrode in ammonia buffer considerably increases in magnitude after adding cystine. Most compounds are known to suppress, rather than increase, the charging current after their adsorption on the surface of the mercury electrode. The anomalous behavior of cystine seems to suggest that the electrode double layer capacity increases after cystine gets adsorbed on the surface of mercury (v.i.).

A.C. polarography. In buffers which permitted to work at potentials near zero (vs S.C.E.), a.c. polarograms showed a positive tensammetric peak corresponding probably to the cystine adsorption. No desorption peak has been observed, however. Also, no decrease of the a.c. current at potentials more negative than those of the positive tensammetric peak has been observed. Instead, a slight increase of this current was sometimes noticed, behavior which is consistent with that of charging current.

Instantaneous current-time curves on single drops. $I-t$ curves at potentials of the cystine prewave are smooth monotonous parabolas whose exponent varies between 0.44 and 0.48. Its value somewhat decreases at elevated temperatures. $I-t$ curves recorded upon applying the voltage at different instants of the drop-life exhibit larger values of the current than when the drop is polarized from the beginning;

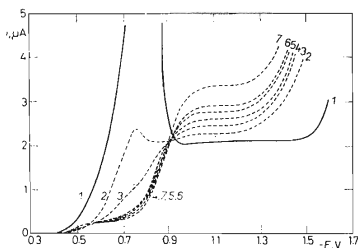


FIG. 5

Reduction of Cystamine in Presence of Cobalt(II)

0.06M Borax, 1 mM cystamine, pH 9.2. Concn. of CoCl_2 : 1 0; 2 4; 3 8; 4 12; 5 16; 6 20; 7 $30 \cdot 10^{-5}$ M.

the exalted current, however, soon falls back to the value obtained when the voltage is applied during the whole drop-life. Evidently, the depolarizer is accumulated in the vicinity of the electrode during the period when the electrode is disconnected from the outer voltage.

$I-t$ curves recorded at potentials of the rising part of the cystamine reduction wave are accompanied with many oscillations indicating that the large maximum on the cystamine reduction wave is due to streaming. Curves at more negative potentials are smooth parabolas with the exponent close to 0.17. In the presence of traces of cobalt(II), the cystamine maximum is almost completely obliterated. $I-t$ curves at larger cobalt concentrations confirm an adsorption character of the cystamine pre-wave (see Fig. 5) and a strong inhibition of the electrode process at more negative potentials (Fig. 6). The validity of the expression³⁴

$$i_c^2 = \text{const} \quad (1)$$

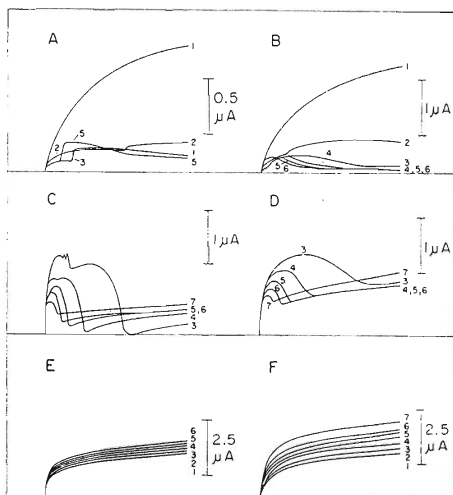


FIG. 6

Current-Time Curves at Different Concentrations of CoCl_2 in 0.06M borax, 1 mM cystamine

Conc. of CoCl_2 : 1 0; 2 4; 3 8; 4 12; 5 16; 6 20; 7 30. 10^{-5} M. Applied potential: A — 0.55; B — 0.60; C — 0.75; D — 0.81; E — 0.95; F — 1.05 V.

(θ denoting the time of full coverage of the electrode surface) indicates that the adsorption equilibrium is established rapidly and that it is diffusion controlled. Under this condition the maximum number of moles of adsorbed species per cm^2 was calculated to be $2.4 \cdot 10^{10} \text{ mol cm}^{-2}$, which corresponds to an area of 69 \AA^2 covered by one molecule of cobalt(II)-cystamine³³.

Suppression of streaming maxima. The large streaming maximum on the reduction wave of oxygen is greatly suppressed in the presence of cystine in small concentrations; it is completely obliterated when the concentration of cystine attains about $2 \cdot 10^{-5} \text{ M}$ (Fig. 7). The effect is the same both in unbuffered (KCl) and in buffered ($\text{NH}_3\text{-NH}_4\text{Cl}$) solutions. Methionine in millimolar concentrations has no appreciable effect on the height of the oxygen maximum.

Competitive adsorption. Surface active substances tylose and gelatin gradually suppress the limiting current of both cystine reduction waves. Half-wave potential of the more negative wave is shifted to more negative potentials. On the other hand, $E_{1/2}$ of the cystine prewave is almost unaffected in the presence of the surfactants. Of the two compounds, tylose is much more effective.

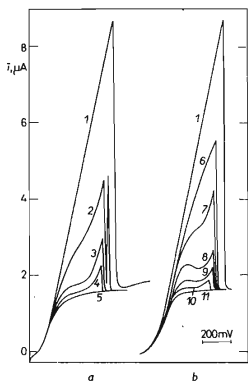


FIG. 7

Suppression of Maximum on the Reduction Wave of Oxygen

a 2 mM KCl; b 2 mM NH_3 , 2 mM NH_4Cl ; saturated with air. Conc. of cystine: 1 0; 2 0.5; 3 1; 4 1.5; 5 2.5; 6 0.25; 7 0.5; 8 0.75; 9 1; 10 1.25; 11 $2 \cdot 10^{-5} \text{ M}$. From -0.2 V .

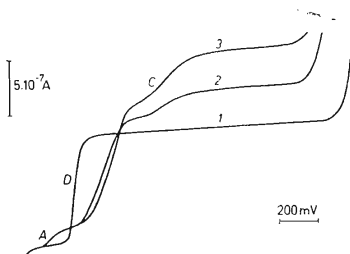


FIG. 8

Formation of the Prewave and Inhibition of the Complexonate Reduction in the Presence of Cystine

0.01M- NH_3 , 0.1M- NH_4Cl , 0.4 mM- CuCl_2 , $3.2 \cdot 10^{-2} \text{ M}$ EDTA. Conc. of cystine: 1 0; 2 1.2; 3 $2,4 \cdot 10^{-4} \text{ M}$. From -0.2 V .

scheme (A), thus being one of the very few known examples of the systems which would produce strongly surface active intermediates in chemical reactions subsequent to their electroreduction, these intermediates being even more strongly adsorbed on the surface of the mercury than the original components.

Adsorption of Cysteine in Alkaline Solutions

The surface activity of cysteine in alkaline medium is restricted to the potentials at which cysteine reacts anodically with mercury giving mercury cysteinate which is strongly adsorbed. With ordinary techniques we could not confirm any adsorption of cysteine at more negative potentials. Above pH 9, cysteine in the solution is predominantly present in the thiolate form and the surface interaction between the sulfur atom and mercury is opposed by the electrostatic repulsion of the cysteinate from the negatively charged electrode. In order to increase the sensitivity of the measurement large concentrations of cysteine have been used. No adsorption of cysteine could be confirmed even under these conditions; a very interesting phenomenon has been, however, observed. Above about 3 mM concentration of cysteine in ammonia buffer of pH 9.3, the single anodic wave of cysteine was found to split into two waves; the height of the (more negative) prewave does not change with further increase

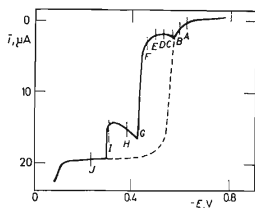


FIG. 9

Anodic Prewaves of Cysteine in Alkaline Medium

0.1M-NH₃, 0.1M-NH₄Cl, pH 9.3. Conc. of cysteine: $1.4 \cdot 10^{-2}$ M.

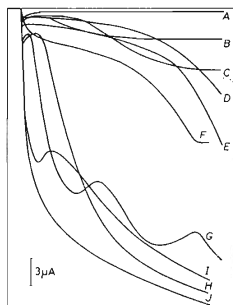


FIG. 10

Current-Time Curves at Potentials of the Splitted Cysteine Anodic Wave

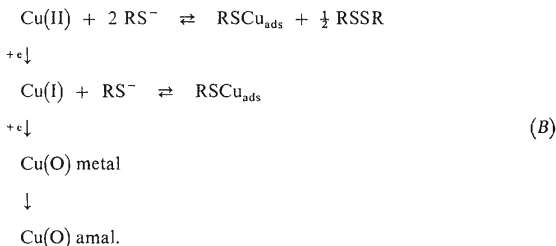
Applied potential: A -0.64; B -0.615; C -0.69; D -0.55; E -0.52; F -0.48; G -0.45; H -0.40; I -0.32; J -0.25 V. For d.c. polarogram see Fig. 9.

in the cysteine concentration. At about 6 mM cysteine, another prewave separates out from the main cysteine anodic wave (Fig. 9), which is again independent of further changes in the cysteine concentration. The sum of all three waves is a linear function of the cysteine concentration up to $1.5 \cdot 10^{-2}$ M, the highest concentration used. The described effect is reversible; diluting the solution, as well as increasing the temperature, pH, or ionic strength, suppress the formation of the prewaves. Higher cysteine concentrations are then necessary for them to separate out. Cooling the solution, decrease of pH and ionic strength, and even the addition of dimethylformamide or dioxan have the opposite effect.

Current-time curves at different potentials of the anodic waves (Fig. 10) exhibit a complex course at the potentials of both prewaves, while the limiting current of the least negative wave is diffusion-controlled, the $i-t$ curve being a monotonous parabola with an exponent close to 0.17. Diffusion character of this current is further confirmed by its linear increase with the cysteine concentration, and with the square root of the height of mercury in the reservoir. Both adsorption prewaves are virtually independent of both the cysteine concentration and height of mercury. The separation of the prewaves is evidently connected with the completion of an adsorbed layer of mercury cysteinatate on the surface of the mercury drop, and its preventing further electrode reaction between mercury and cysteine until the potential is made positive enough to cause a break down of the film. This conclusion is substantiated by the fact that the value of the surface tension of mercury ceases to decrease just about at such cysteine concentrations at which the first prewave appears on polarogram. Separation of the second prewave requires about double cysteine concentration.

The solubility of the adsorbate increases when the pH, temperature or ionic strength are increased. Addition of the less polar solvent dimethylformamide or dioxan has the opposite effect. The single anodic wave of cysteine then splits into two or three waves even at quite low cysteine concentrations. Thus far, the (one) anodic prewave of cysteine has been described only in acidic and neutral media, and the solubility of the mercury cysteinatate at pH above 8.5 was believed to be sufficient as to prevent formation of a compact film on the surface of the electrode. Our results show that such formation is possible even at pH as high as 11, provided the sufficient concentration of cysteine is used. At still larger cysteine concentrations two separated prewaves are formed, corresponding to the two layers of the adsorbate²⁷.

In the presence of Cu(II)-complexonate, cysteine reacts chemically with divalent copper already in the bulk of the solution. The formed copper(I) cysteinatate strongly adsorbs and hinders the reduction of the complexonate. No adsorption prewave is observed and, instead, the whole complexonate reduction wave is shifted to more negative potentials. Reduced glutathione and diethylaminoethanethiol behave similarly, indicating the generally occurring sequence of reactions³⁵



in which RSH stands for the thiol.

Polarography of Cobalt in Borate Medium

Many experiments of this study have been performed in borate buffers which, in our opinion, have several advantages over the most widely used ammonia buffers. Since the polarography of cobalt in this medium is virtually unknown we have performed a separate study³⁶.

In 0.06M borax, divalent cobalt is reduced in a single irreversible wave whose half-wave potential is -1.25 V. The cobalt solubility and stability in borax were found to be sufficient for our purposes. Both increase on addition of increasing amount of boric acid, but the characteristics of the wave are not affected by the presence of boric acid. Evidence is presented that the aquo-cobalt and not the complex ion is electroreduced at the mercury surface. In the presence of tetrabutylammonium perchlorate the reduction of cobalt(II) occurs at potentials at which the surfactant is desorbed from the surface of mercury. No maxima have been observed on the Co(II) wave in borate medium; such maxima are observed in practically all other buffers and also in unbuffered cobalt(II) solutions³⁷.

KINETIC CURRENTS IN SYSTEMS CONTAINING COBALT(II) AND/OR CYSTEINE-LIKE COMPOUNDS

Catalysis of Cobalt(II) Reduction in Solutions of Cysteine-like Compounds

The considerable overvoltage observed in the polarographic reduction of divalent cobalt is greatly decreased in the presence of cysteine and similar compounds³⁸; the effect results in a shift of the cobalt(II) wave to less negative potentials. In several media, it gives rise to the appearance of a well-developed prewave. We have observed³⁸ particularly distinct prewaves in borax-boric acid buffers (Fig. 11). The considerable dependence of these prewaves on pH and thiol concentration (Fig. 12) as

well as their kinetic character at small thiol concentrations indicate that the species which is being reduced in the prewave is the cobalt(II)-thiolate complex, while the reduction of the thiol-free cobalt(II) occurs only at much more negative potentials. The chemical reaction between the thiol-free cobalt and liberated thiolate ligand

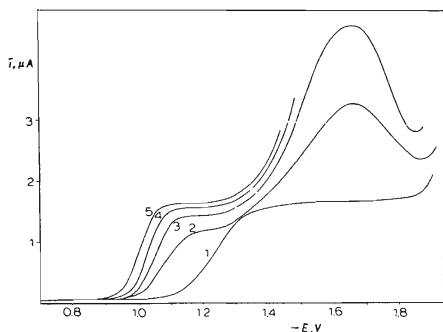


FIG. 11

Cobalt Prewaves in 0.06M Borax, 0.4 mM CoCl₂

Cysteine conc. and $E_{1/2}$: 1 0, -1.25; 2 0.5, -1.08; 3 1.1, -1.05; 4 2.1, -1.03; 5 4.7 · 10⁻⁵M, -1.0 V.

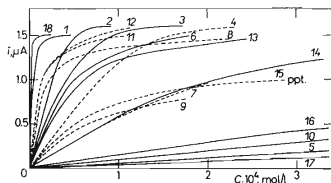


Fig. 12

Plot of Prewave vs Thiol Conc. at Various pH

All solutions 0.4 mM in CoCl₂, 0.06M in borax. Conc. of H₃BO₃ and pH: 1 0, 9.2; 2 0.36, 7.9; 3 0.48, 7.7; 4 0.6, 7.3; 5 satd. with H₃BO₃, 6.0; 6 pH 9.2, 8.2, 7.3; 7 pH 6.0; 8 pH 9.2; 9 pH 8.2; 10 pH 7.3; 11 pH 9.2; 12 pH 8.7, 8.2; 13 pH 7.3; 14 pH 6.0; 15 pH 9.2; 16 pH 8.2; 17 pH 7.3; 18 pH 9.2. Thiol used: 1-5 cysteine; 6-7 cysteine ethyl ester; 8-10 cysteamine; 11-14 thiosalicylic acid; 15-17 2-mercaptoethanol; 18 maleonitrile dithiolate.

and an increased concentration of one of the components due to the adsorption on the electrode surface may further affect the value of the experimentally obtained rate constant and account for its departure from the value obtained in the homogeneous phase. We could not find any exaltation of the Co(II) surface concentration due to the specific adsorption of its complex with cysteine, its ethyl ester, or cysteamine. Such exaltation occurs when thioglycolic, thiosalicylic and 3-mercaptopropionic acid, 2-mercaptoethanol and 2- and 4-mercaptoaniline are used. Prewave of cobalt is then accompanied with a flat maximum which is followed with a flat minimum. The height of the limiting prewave current is limited by the height of the diffusion current of thiol-free cobalt(II). Only at pH which is several units less than the pK of the thiol group two non-streaming maxima have been observed which considerably exceed the diffusion current of cobalt(II). These maxima, which have the characteristics of catalytic hydrogen maxima, have been ascribed to the catalyzed evolution of molecular hydrogen. In several instances, as many as three catalytic hydrogen maxima, including the well-known Brdička catalytic maximum, are simultaneously observed on polarogram (see *e.g.* Fig. 13), indicating that at least three different types of the catalytic mechanism can lead to the catalyzed hydrogen evolution in mixtures containing cobalt(II) and thiols (*v.i.*).

Polarographic Hydrogen Catalysis in Solutions of Divalent Cobalt Salts

Metallic cobalt, as well as other transition metals, is difficultly soluble in mercury and after reduction of its ions at mercury electrodes metal deposits are first formed on the surface of mercury, which are then wetted by mercury to give amalgam and intermetallic compounds inside mercury^{40,41}. Under certain conditions the amalgamation of the deposited metallic cobalt is inhibited⁴²⁻⁴⁸. Hydronium ion then discharges directly at the surface of the deposit as metallic cobalt has much less value of the hydrogen overvoltage than nontransition metals including mercury. The overvoltage at freshly deposited highly active cobalt atoms is still much less than that at ordinarily prepared cobalt surfaces. Discharge of hydronium ion gives rise to the large catalytic hydrogen current. When the amalgamation becomes appreciably fast, the hydronium discharge ceases. The value of the hydrogen overvoltage at transition metal amalgams is equal to that at pure mercury⁴⁹. In very acidic solutions, the liberation of hydrogen accompanies the cobalt(II) reduction even in cases when the metallic deposit is not stabilized^{50,51}.

We have observed a small maximum on the cobalt(II) reduction wave in acetate buffers with a pH as high as 4.6. This maximum is due to hydrogen evolution and not to streaming, as it increases with decreasing pH and ionic strength, and also in the presence of small amounts of the surfactant polyvinylpyrrolidone, the latter increasing the life period of the cobalt metal (Fig. 14).

Polarographic Hydrogen Catalysis in Solutions of Cysteine-Like Compounds

The catalytic effect of cysteine on the polarographic reduction of hydronium ions was first studied by Brdička⁴. He ascribed the catalytic activity of this compound

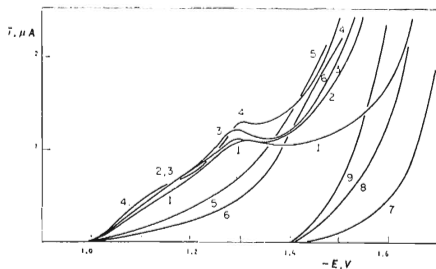


FIG. 14

Effect of Polyvinylpyrrolidone on Reduction of Cobalt(II)

0.1M-NaAc, 0.1M-HAc, 0.4 mM-CoCl₂, pH 4.55. Conc. of PVP: 1 0; 2 0.5; 3 1; 4 5; 5 8; 6 11 . 10⁻³%. Curves 7, 8 and in the absence of Co(II): 7 0; 8 1; 9 11 . 10⁻³% PVP.

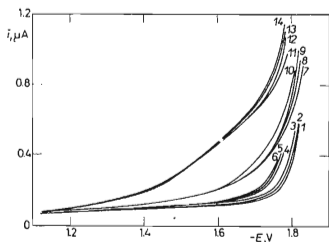


FIG. 15

Catalytic Hydrogen Activity of Thioglycolic Acid and Its Variation with Acid Buffer Constituent Concentration and Ionic Strength

All solutions 0.01M in Na₂BA; ionic strength maintained constant with NaClO₄. Conc. of NaHBA: 1 0.02; 2 0.03; 3 0.06; 4 0.01; 5 0.05; 6 0.06; 7 0.005; 8 0.02; 9 0.03; 10 0.04; 11 0.005; 12 0.02; 13 0.06; 14 0.08M. Conc. of TGA: 1-6 0; 7-14 1mM. Ionic strength: 1-3, 7-10 0.1; 4-6, 11-14 0.3M. H₂BA is *p*-hydroxybenzoic acid.

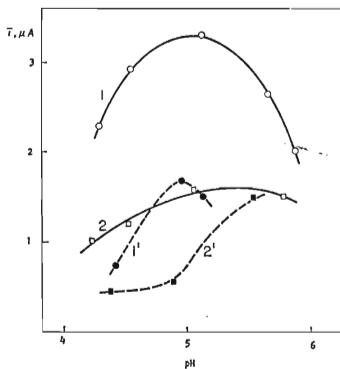


FIG. 16

Plot of Currents at Maximum *A* vs. pH

0.4 mM-CoCl₂, 1 . 10⁻⁵M MAN, acetate buffer. Solid lines: Conc. of NaAc 0.1M, conc. of HAC was changed. Dashed lines: Conc. of HAC 0.01M, conc. of NaAc was changed. Ionic strength was kept constant with NaClO₄: 1, 1' 0.1; 2, 2' 0.3M.

to the SH group, from which hydrogen is believed to be reduced more easily than when in the form of a hydronium ion. Later, v. Stackelberg and coworkers⁵² performed a systematic study of 230 model compounds and came to the conclusion that the catalytic activity of organic thiols is solely due to the action of the nitrogen-containing groups; the latter were present in the molecule of all active catalysts. According to v. Stackelberg⁵², nitrogen-free thiol compounds are entirely catalytically inactive. We have investigated a number of thiols in various buffer systems²⁷ and found that, according to their catalytic activity, they can be divided into three main groups. By far the most active were cysteamine, cysteine ethyl ester, and 2- and 4-mercaptoanilines, *i.e.* compounds which contain an amino group in addition to the SH group. Fully protonated forms of these compounds are monovalent cations. Catalytic hydrogen current of these substances decreases with increasing the ionic strength of the solution.

Cysteine exhibits an intermediate catalytic activity. Its fully protonated form under our experimental conditions is a zwitterion. The catalytic current of cysteine is much less sensitive to the changes of an ionic strength than the first group.

Finally, compounds such as thioglycolic, thiosalicylic and 3-mercaptopropionic acids are almost catalytically inactive. Small catalytic hydrogen currents are observed only when very large concentrations of these substances are used. Their catalytic activity, however, greatly increases upon increasing the ionic strength (Fig. 15), the effect of which is opposite to the behavior of all organic catalysts studied so far. The fully protonated forms of these compounds under the experimental conditions of our study are univalent anions. Experiments with the last group conclusively showed that the SH group does participate in the catalytic mechanism, and confirmed the original Brdička's interpretation. At a given pH, the catalytic activity of all used thiols was found to be independent of the concentration and charge of acid buffer constituent. This situation makes it possible to a certain extent to work under the corresponding states, even when different thiols and buffer systems are used. As is well known, low molecular weight thiols do not give rise to the limiting current but cause only a shift of the hydronium discharge potentials to less negative values. When working at the same thiol concentrations, current densities, ionic strength and at pH which is the same number of units less than the pK of the respective thiol group, we can compare quantitatively the catalytic activity of various thiols under otherwise different conditions.

The chemical reaction which leads to the reformation of the active catalyst after its reduction at the electrode surface involves the reaction of the thiolate with hydronium ion, rather than with the acid constituent of the buffer. Ionic strength of the solution affects the rate of this reformation, its influence depending on the charge of the thiolate ion. The observed increase of the catalytic activity of thiols with decreasing negative charge of the catalyst is connected with the electrostatic interaction between the catalyst and the mercury cathode; this interaction has a direct influence

on the surface concentration of the catalyst, and on its orientation in the vicinity of the electrode surface.

All thiols studied were found to be catalytically active only at pH which was of the order of, or less than, the pK of the respective thiol group. Their activity greatly increases with decreasing the pH of the solution.

Polarographic Hydrogen Catalysis in Solutions Containing Cobalt(II) and Cysteine-Like Compounds

A-currents. These currents are generally observed at the potentials of the cobalt(II) prewave (see Fig. 13). They are particularly well developed with aromatic thiols such as thiophenol, pentachlorothiophenol, 2- and 4-mercaptoaniline. The solubility of the first two compounds, and of their cobalt(II) salts, in water is very low. Hence, most of our experimental work on *A*-currents has been performed with mercaptoanilines^{5,3}. 2-Mercaptoaniline (MAN) gives large *A*-current in the pH-region of about 4–7 (Fig. 16). It is the highest at the pH which is close to 5, *i.e.*, somewhat less than the pK of the SH group of MAN (Table I). From the considerable dependence of the current on the concentration of acid buffer constituent it may be concluded that the protonation reaction which leads to the reformation of the active catalyst involves the acid constituent of the buffer. Ionic strength greatly affects the *A*-current (Fig. 16). Surfactants, even in small concentrations, suppress the current *A* (Fig. 17A).

Current-time curves (Fig. 21A), effect of temperature and height of reservoir confirm the pronounced adsorption-kinetic character of current *A*. Drop-time curves exhibit a decrease of the surface tension at potentials just preceding the maximum *A* after adding cobalt to the solution of MAN in acetate buffer. Stationary polarograms on a hanging mercury drop exhibit a large maximum at potentials of the maximum *A*; at larger current densities, bubbles of molecular hydrogen are visible on the surface of the stationary electrode.

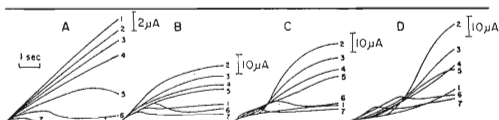


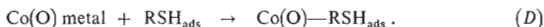
FIG. 17

Current-Time Curves in Presence of PVP in Solution of 0.1M NaAc, 0.1M HAC, 0.4 mM $CoCl_2$ and $1 \cdot 10^{-4}M$ MAN, pH 4.55

Conc. of PVP: 1 0; 2 0.5; 3 1; 4 2; 5 5; 6 8; 7 $11 \cdot 10^{-3}\%$. Applied potential: A -1.0; B -1.25; C -1.30; D -1.35 V.

The *A*-currents are always composed of the two currents, the cobalt(II) prewave-current and the catalytic hydrogen current. It is not possible to evaluate the quantitative representation of these currents from the experimentally obtained data. Hence, the interpretation of the *A*-currents must always consider the possible dual effect of some of the factors on these two types of currents.

B-currents. The *B*-currents occur only at potentials where the cobalt(II) reduction in the absence of thiol approximately attains its limiting value (Fig. 13). They are again observed only at pH of the order of, or less than, the pK of the thiol group, *i.e.*, when at least part or all thiol is present in an undissociated form. The uncharged or positively charged mercaptoamine is much more surface active at the negatively charged interface than its thiolate form. Due to the great chemical affinity of organic thiols toward metallic cobalt the adsorbed mercaptan may be expected to react chemically with highly active cobalt atom which is freshly deposited into its neighbourhood, thus preventing its amalgamation:



Symbol — denotes the chemisorption bond between cobalt and thiol. Proton can be reduced on the surface of such stabilized cobalt atoms. The electrolytic evolution of hydrogen from the protonated form of the adsorbed thiol can also take place. Protonation of the thiolate ion in reaction with hydronium ions accounts for the reformation of an undissociated active form of the thiol catalyst.

All furnished evidence points to the above interpretation. *B*-currents were found only to rise with decreasing pH, being, at the same time, independent of the concentration of acid buffer constituent (Fig. 18). They increase upon decreasing the temperature, and also in the presence of surfactants in small concentrations (Fig. 17 B, C, D). At larger coverages of the electrode by the added surfactant the *B*-current decreases again. Increase in an ionic strength or charge of an indifferent cation greatly suppresses current *B* (Fig. 18, 19). A hydrogen overvoltage at the stationary cobalt wire electrode is much less than that at mercury, and is further decreased in the presence of the thiols.

Brdička catalytic hydrogen currents. For the description and interpretation of the earlier results we refer to the numerous reviews which have appeared in the last decade¹⁷⁻²². Despite such an extensive study which was devoted to these currents the actual catalytic mechanism leading to the Brdička currents is still unknown. The Brdička currents are known to increase with increasing thiol concentration. The shape of this concentration curve at a given cobalt concentration which resembles a Langmuir adsorption isotherm has led several authors to the conclusion that a cobalt(II)-thiolate complex is adsorbed on the surface of the mercury electrode. Only recently it has been shown^{54,55} that for low molecular weight thiols this as-

sumption is consistent neither with theory nor with experimental facts and that the resemblance with Langmuir adsorption isotherm is fortuitous. Recent studies⁵⁶⁻⁵⁸ have revealed that the characteristics of the Brdička currents vary with experimental conditions, in particular, with the ratio of concentrations of the actual active components cobalt(II) and thiol. We have studied the concentration dependences in more detail and found that, when the Brdička current approaches its limit, it becomes entirely diffusion controlled. On the other hand, when current becomes less than about 20% of its limiting value, it is entirely kinetic in nature. Between about 20 to 100%, Brdička currents exhibit mixed diffusion-kinetic character. Variation of the cobalt concentration affects the course of the concentration dependence (Fig. 20); even then the characteristics of the Brdička currents are determined only by the ratio of current to its limiting value. The same situation occurs when thiols are replaced with sulfur-containing proteins.

The above situation makes it possible for the first time to compare the Brdička currents recorded with different thiols and in different media. Many unsuccessful attempts have been made earlier to find a simple factor which would provide for such correlation. Müller's "protein index"⁵⁹ can serve as an example. The extreme variability of the published results, and conclusions as well, can now be explained

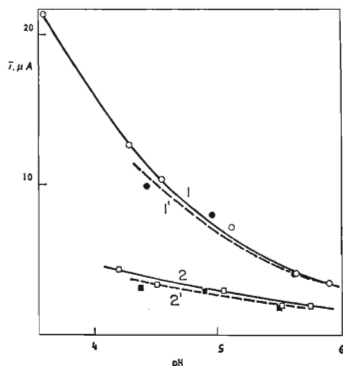


FIG. 18

Plot of Currents at Maximum B vs pH

0.4 mM- CoCl_2 , $1 \cdot 10^{-5}$ M MAN, acetate buffer. Solid lines: Conc. of NaAc 0.1M, conc. of HAc was changed. Dashed lines: Conc. of HAc 0.01M, conc. of NaAc was changed. Ionic strength was kept constant with NaClO_4 : 1, 1' 0.1; 2, 2' 0.3M.

in the light of the non-equivalent experimental conditions which had been used in these studies. Many investigations on the Brdička currents have been performed only at one concentration of cobalt and/or thiol. The non-corresponding conditions must have necessarily led to such diversity of results, and interpretations based on them.

The concentration dependences in Fig. 20 explain also the variability in the reported dependences of Brdička current on the cobalt concentration. As follows from Fig. 20, three different types of such dependences can be generally observed, *i.e.*, linear, broken or parabolic with the exponent less than one. When the concentration ratio of thiol to cobalt is always greater than a certain value, L_r , the current *vs* cobalt concentration curve is linear. When $[RSH]/[Co(II)] < L_r$, the dependence is parabolic. Finally, when the value of this ratio is initially higher than, but during adding cobalt, it falls below, the value of L_r , the resulting dependence is broken, being initially linear and then parabolic. The fact that the diffusion-controlled Brdička current always linearly increases with increasing cobalt concentration makes it clear that the earlier proposed theory which assumed the exhaustion of the proton donor in the vicinity of the electrode as a limiting factor for the height of Brdička current

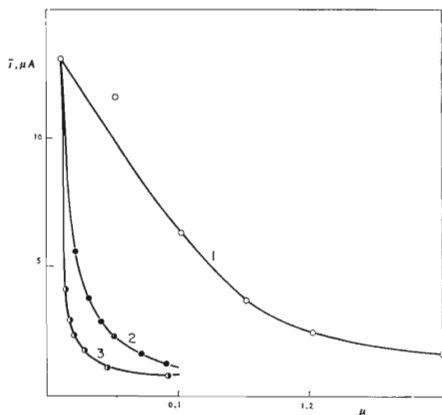


FIG. 19

Plot of Currents at Maximum B vs Ionic Strength

Solution of 0.01M-NaAc, 0.01M-HAc, 0.4 mM-CoCl₂, $1 \cdot 10^{-5}$ M MAN. 1 NaClO₄; 2 CaCl₂; 3 LaCl₃.

was incorrect. It has been confirmed by numerous investigations that the Brdička current exceeds many times the diffusion current of cobalt(II); obviously the protonation reaction takes place but, under the diffusion control of the Brdička current, it is not rate-determining.

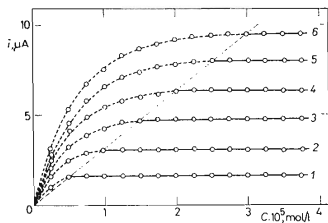


FIG. 20

Dependence of Brdička Current on Cysteine Concentration

0.18M tris, 0.02M tris-HCl, pH 9.05. Conc. of CoCl_2 : 1 1; 2 2; 3 3; 4 4; 5 5; 6 6. 10^{-5}M .

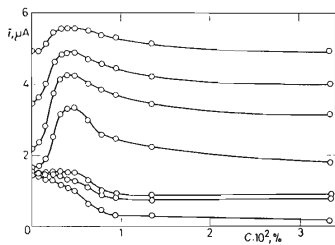


FIG. 21

Variation of Current with PVA Concentration

0.06M borax, 0.4 mM- CoCl_2 , $1 \cdot 10^{-5}\text{M}$ cysteine. Values of potential: 1 -1.1; 2 -1.2; 3 -1.215; 4 -1.3; 5 -1.4; 6 -1.5; 7 -1.6 V.

The characteristics of the diffusion- and kinetically controlled Brdička currents have been described in detail elsewhere⁶⁰. Here, we will mention just one peculiar effect which is, in our opinion, of a great value for understanding the catalytic activity of Brdička mixtures. Addition of the surfactants polyvinylalcohol (PVA), polyvinylpyrrolidone, Triton X-114, tetrabutylammonium perchlorate and gelatin to the cobalt-thiolate system brings about an increase of the Brdička current at potentials at which the surfactant is adsorbed (Fig. 21). In many instances, the well developed double maximum, similar in shape to that of proteins, is observed. Since proteins are surface active substances, their adsorption may greatly contribute to the formation of the Brdička proteinic double-wave. The much more pronounced adsorption character of the less negative of the two proteinic maxima^{61,62} supports this conclusion and explains why the use of polypeptidic or proteinic catalysts has been intentionally avoided throughout this study. The high surface activity of these compounds would only bring an additional complicating factor to an already very involved system.

Polarographic Hydrogen Catalysis in Cobalt(III)-Thiolate Mixtures

Replacement of divalent cobalt with cobalt(III) has no apparent effect on the height and shape of the cobalt(II) prewave, nor on currents *A* and *B*. When in the form of the cobalt(III) thiolate, trivalent cobalt gives also exactly the same Brdička current as the divalent cobalt in the same concentration. Only in instances when cobalt(III) hexammine chloride is used the Brdička current of low molecular weight thiols becomes considerably less than that with Co(II). It, however, greatly increases in height in the presence of surface active substances⁶⁰.

With rare exceptions⁶³ trivalent cobalt used in Brdička studies was exclusively in the form of the hexammine complex. This complex is much more stable than the corresponding complex with the thiolate ion. At potentials of the cobalt(II) prewave, all Co(III) $(\text{NH}_3)_6^{3+}$ is reduced to the Co(II) aquo amino complex which, in reaction with thiolate, gives the Co(II)-thiolate complex. At potentials of the maximum *B*, Co(III) is reduced rapidly to Co(O) which is then stabilized by the adsorbed thiol. This rapid reduction almost prevents formation of the Co(II)-thiolate complex, and no, or only very small, Brdička catalytic current is observed. In the presence of the surfactant, the rate of the Co(III) to Co(II) reduction becomes slower, and more thiolate complex with divalent cobalt can be formed. This account for an observed increase in the height of the Brdička catalytic hydrogen wave.

CONCLUSIONS

In the first part of our study we attempted to elucidate the nature of processes leading to various diffusion and/or adsorption currents in Brdička mixtures, and their possible connection with the catalytic hydrogen mechanism. It has been observed that the surface activity of the individual components is greatly increased after their mixing. An increased hydrophobic character of cobalt(II)-disulfides, complex formation between the divalent cobalt and specifically adsorbed thiolate ligands, anodic reaction of the SH group with mercury, or chemical decomposition of cystine into the surface active intermediates account for the frequently observed pronounced adsorption phenomena in the above systems. The surface activity of cobalt-thiolates varies with changes in the concentration ratio of cobalt (II) to thiol; the electroneutral species are usually less water soluble and more surface active than their charged analogues. Preference is given to the thiols, their disulfidic forms being much more surface active and less chemically stable in mildly alkaline aqueous solutions.

The hydrogen catalysis was the main objective of this study. Two new, hitherto undescribed types of the catalytic hydrogen currents in Brdička mixtures have been observed (currents *A* and *B*), together with the earlier known Brdička catalytic hydrogen current and the catalytic hydrogen current due to the SH group. All these currents are observed only at pH of the order of, or less than, the pK of the respective thiol, group. They, further, occur only at potentials at which polarographic reduction

of divalent cobalt in some or all present forms takes place. The protonation of the thiolate sulfur, and its relation to the cobalt(II) to cobalt(O) reduction, thus, must be considered in the catalytic hydrogen mechanism.

Current *B* is indeed due to the catalytic action of the freshly deposited and thiol-stabilized metallic cobalt on the surface of the mercury. Both current *A* and the Brdička current, however, exhibit much more complex behavior. On the contrary to the currents of all other known hydrogen catalysts, these two currents decrease with decreasing pH after the latter becomes 1–2 units less than the $pK(\text{SH})$. This very unusual behavior strongly suggests that the formation of a complex between divalent cobalt and the thiolate ligand is an essential part in the catalytic mechanism. Since this complex is rapidly reduced to the zero state already at potentials of the cobalt(II) prewave, the oxidation state of a cobalt atom in the catalytically active species becomes questionable.

Considering all possible chemical, redox, and acid-base properties of the cobalt(II)-thiolate complexes, and of their primary reduction products, five hypothetical mechanisms leading to the catalyzed hydrogen evolution can be proposed: *a*) Coordinated thiolate ligands are in a rapid dynamic equilibrium with cobalt-free thiols in the solution. Exchange of many ligands is known to proceed *via* the stage in which the coordinated ligand is protonated. Hydrogen from such species may get reduced more easily than when in the form of a hydronium ion. *b*) The basicity of the free and coordinated thiolate sulfur be comparable; a thermodynamic acid-base equilibrium involving protonated coordinated ligand is established. The latter species gets reduced to give hydrogen and the original complex. *c*) The cobalt(II) complex with thiolate is rapidly reduced but the metal in the lower oxidation state remains coordinated for a certain period of time. Resulting species is a powerful reductant and reacts with hydronium ion giving molecular hydrogen and divalent cobalt complex which is again reduced. *d*) The sulfur atom in an unstable low-valent cobalt complex is basic enough to get protonated at the used pH; hydrogen is then reduced from the protonated form of the catalyst. *e*) The low-valent cobalt atom in the thiolate complex is a potential Lewis base in that it contains non-bonding electron pairs. Proton can add directly to the metal to form another type of the protonated complex species. This species may be either reduced directly to Co(O) complex and molecular hydrogen, or undergo an electronic transformation to give a coordinated hydronium species, in which cobalt attains back its formal two-valent oxidation state. This species is unstable in water and reacts with hydronium ions to give H_2 and cobalt(II)-thiolate complex, the latter being reduced back to the zero-valent oxidation state.

Mechanisms *a*), *b*), *c*) and *e*) can hardly account for the appearance of the Brdička current. The rates of the homogeneous chemical reactions are virtually independent of the electrode potential and the catalytic hydrogen current due to these processes should appear at potentials preceding or immediately following those of the cobalt(II) prewave. The extremely high rate of electroreduction of the Co(II)-thiolate complex

at the potentials of the Brdička wave would completely prevent formation of the protonated species in mechanisms *a*) and *b*), while the chemical reactions in mechanism *c*) and *e*) should immediately follow the low-valent cobalt thiolate complex formation at the surface of the electrode. The mechanism *b*) is highly improbable in any case, as the basicity of the thiolate sulfur is only low and is further decreased after combining with the divalent cobalt cation.

From the viewpoint of the chemical stability all mechanisms *c*), *d*) and *e*) are acceptable. Thiolate ligands are potential π -acid type ligands in that they stabilize transition metals in unusually low oxidation states by forming π -bonding which supplements the σ -bonding arising from the lone pair donation. Nothing is known, however, about the redox or acid-base properties of these compounds. The existence of a π -bond would suggest an increase of electron density on the sulfur atom, and its increased tendency to get protonated. All furnished evidence points to this conclusion and to the mechanism *d*) as the most probable sequence of reactions leading to the formation of the Brdička currents⁶⁰. More difficult is the explanation of current *A*. No definite conclusion can be made at this point, our contemporary knowledge of chemistry of low-valent cobalt-thiolates being inadequate to provide any exact interpretation of these currents. It is felt that further studies using NMR and other techniques for detection and investigation of the short-lived intermediates of the electrochemical and chemical reactions is the only means how to get further insight into the mechanism and kinetics of this peculiar kind of the hydrogen catalysis.

CONFRONTATION WITH OTHER THEORIES

All theories proposed so far deal only with the Brdička currents, the other two currents *A* and *B* being unknown.

Brdička² ascribes the catalytic activity in the above mixtures to the freshly generated zerovalent cobalt complex with thiolate, whose sulfur atom is basic enough to get protonated. All evidence furnished in this study supports this interpretation of the Brdička catalytic hydrogen wave.

Mairanovskii⁶⁶ believes that it is a divalent cobalt-thiolate complex which is highly catalytically active and prior its electroreduction it catalyzes the hydrogen evolution. Besides the unexplained rather high basicity of the coordinated thiolate sulfur, this mechanism (*b*) would suit much better to the current *A*, then to the Brdička catalytic hydrogen current.

Calusaru⁶⁷ has proposed a very interesting mechanism which is largely based on his results with another catalytically active system cobalt(II)-nitrohydroxylaminat (v.i.). He assumes that the 1 : 2 and 1 : 3 Co(II)-thiolate complexes are reduced only with a large overvoltage and, being at the same highly catalytically active, they reduce the hydrogen overvoltage even when in very low concentrations. While this interpretation accounts well for the behavior of the cobalt-nitrohydroxylaminat system⁶⁸, it seems inappropriate to the Brdička type of hydrogen catalysis. Our studies with the cobalt(II) prewave convincingly showed³⁸ that the 1 : 2 and 1 : 3 cobalt-thiolate complexes are reduced to the zero oxidation state at potentials much preceding the Brdička catalytic hydrogen wave. Large Brdička currents are observed also when traces of thiol compared to the cobalt(II) concentration are used, and when practically no bis or tris-complexes are formed in the solution. The peculiar high basicity of the sulfur atom in the cobalt(II) complex remains also to be explained.

Other existing theories are more or less derived from the above three main concepts of the Brdička type of hydrogen catalysis.

OTHER TRANSITION METAL-LIGAND HYDROGEN CATALYSTS

For almost thirty years, the cobalt (nickel)-thiolate system was the only known example of the hydrogen catalyst of the transition metal ligand type. Recently, however, a great number of many other systems with the catalytic hydrogen activity has been found, *i.e.*, Cr(II)-dipyridyl^{69,70}; Fe(II)-dipyridyl⁶⁹; Co(II)-dimethylglyoxime^{71,83}, 8-hydroxyquinoline⁷², dipyridyl⁶⁹, nitrohydroxylamine⁸⁴, cyanide⁷³, oxyquinoline⁷⁴, uric acid^{75,76}, histidine and derivatives^{76,77}, carboxyalkylphosphines⁷⁸, glycerine aldehyde and dihydroxyacetone⁷⁹; Ni(II)-8-hydroxyquinoline⁷², dipyridyl⁶⁹, pyridine⁸⁰, and ethylene diamine⁸¹; Pd(II)-dipyridyl⁷²; and Ru(III)-dithiooxamide⁸². It is of interest to note that all metals in these mixtures are transition metals which are difficultly soluble in mercury and under suitable conditions they cover mercury forming transition metal electrodes at which hydronium ion is discharged much more easily than at mercury. Furthermore, all ligands in the catalytically active mixtures are N, P, O or S-donor ligands which are catalytically active also in the absence of metal cations and which are also potential π -acid type ligands able to stabilize transition metals in unusually low oxidation states. Finally, many ligands of the above group have strong chemical tendency toward transition metals and are known to be strongly adsorbed on their surfaces. Since they are also often adsorbed on mercury strong surface interaction between the freshly deposited highly active transition metal atom and the adsorbed ligand may be expected.

In many respects, the characteristics of the catalytic hydrogen currents in the above systems resemble those observed in Brdička mixtures. Thus, *e.g.*, the exalted catalytic hydrogen maximum of the freshly deposited metallic ruthenium on mercury in the presence of dithiooxamide strongly resembles the *B*-current of the thiol-stabilized metallic cobalt. The less negative maximum which is observed only in the simultaneous presence of both ruthenium(III) and dithiooxamide is probably due to the catalytic action of the Ru-dithiooxamide complex. Except the system cobalt(II)-nitrohydroxylamine, however, the experimental material on these currents is very scarce and no detailed conclusions can be made on the catalytic hydrogen mechanism leading to the catalyzed evolution of hydrogen from these systems.

REFERENCES

1. Brdička R.: This Journal 5, 112 (1933).
2. Brdička R.: Ref. 1, p. 148.
3. Brdička R.: Ref. 1, p. 238.
4. Brdička R.: This Journal 8, 366 (1936).
5. Brdička R.: This Journal 9, 76 (1937).
6. Brdička R.: This Journal 11, 614 (1939).
7. Brdička R.: Research 1, 25 (1947).
8. Brdička R.: Biochem. Z. 272, 104 (1934).
9. Brdička R.: J. Chim. Phys. 35, 89 (1938).
10. Brdička R.: Biol. listy 22, 39 (1937).
11. Brdička R.: Nature 139, 330 (1937).
12. Brdička R.: Acta Unio Intern. contra Cancrum 3, 13 (1938).
13. Brdička R.: Acta Radiol. Cancerol. Bohemiae Moraviae 2, 7 (1937).
14. Brdička R.: Nature 139, 1020 (1937).
15. Brdička R.: Klin. Wochschr. 18, 305 (1939).
16. Brdička R.: Klumpar J.: Čas. čes. lékár. 17, 234 (1937).

17. Brdička R.: Z. Physik. Chem. (Sonderheft) 1958, 165.
18. Brdička R.; Březina M., Kalous V.: Talanta 12, 1149 (1965).
19. Müller O. H. in the book: *Methods of Biochemical Analysis* (D. Glick, Ed.), Vol. 11, p. 329. Interscience, New York 1963.
20. Mairanovskii S. G.: J. Electroanal. Chem. 6, 77 (1963).
21. Calusaru A.: J. Electroanal. Chem. 15, 269 (1967).
22. Březina M., Zuman P.: *Polarography in Medicine, Biochemistry and Pharmacy*. Interscience, New York 1958.
23. Kalousek M., Grubner O., Tockstein A.: This Journal 19, 1111 (1954).
24. Nygård B.: *Thesis*. Acta Univ. Upsaliensis 104, 1 (1967).
25. Zieliński M.: *Thesis*. Technische Hochschule für Chemie, Leuna-Merseburg 1966.
26. Kuik M., Basiński A.: Roczniki Chem. 39, 1327 (1965).
27. Mader P., Volke J., Kúta J.: unpublished results.
28. Konek V.: Ber. 53, 1666 (1920).
29. Lecher H. Z.: Ber. 48, 1425 (1915).
30. Březina M.: This Journal 24, 3509 (1959).
31. Mader P., Volke J., Kúta J.: This Journal 35, 552 (1970).
32. Michlík J., Blažej A.: *Sborník prác chemickéj fakulty SVŠT*, p. 157. Bratislava 1965.
33. Mader P., Kolthoff I. M.: Anal. Chem. 41, 932 (1969).
34. Koryta J.: This Journal 18, 206 (1953).
35. Mader P., Kúta J., Volke J.: This Journal 33, 3625 (1968).
36. Mader P., Kolthoff I. M.: J. Polarog. Soc. 14, 42 (1968).
37. Mairanovskii S. G.: J. Electroanal. Chem. 12, 547 (1966).
38. Kolthoff I. M., Mader P., Khalafalla S. E.: J. Electroanal. Chem. 18, 315 (1968).
39. Davies G., Kustin K., Pasternak R. F.: Trans. Faraday Soc. 64, 1006 (1968).
40. Kemula W., Galus Z.: Bull. Acad. Polon. Sci., Ser. Sci. Chim. Geol. Geograph. 7, 729 (1959).
41. Hovsepian B. K., Shain I.: J. Electroanal. Chem. 12, 397 (1966).
42. Babkin G. N.: Izv. Vyš. Učeb. Zaved. Chim. Chim. Technol. 7, 90 (1964).
43. Shirai H.: Nippon Kagaku Zasshi 85, 322 (1964).
44. Babkin G. N., Sambetov Š. S.: Izv. Vyš. Učeb. Zaved. Chim. Chim. Technol. 7, 95 (1964).
45. Ivanov V. F., Ioffa Z. A.: J. Phys. Chem. (USSR) 38, 563 (1961).
46. Ivanov V. F., Ioffa Z. A.: Dokl. Akad. Nauk SSSR 140, 1368 (1961).
47. Mindowitz J.: Electrochim. Acta 5, 202 (1961).
48. Babkin G. N., Kazakisheva M. S., Grašina M. Z.: J. Phys. Chem. (USSR) 39, 73 (1965).
49. Butler J. N., Makrides A. C.: Trans. Faraday Soc. 60, 938 (1964).
50. Reibel I. M.: Trudy Kišinevsk. Selskochoz. Inst. 1962, 35; Chem. Abstr. 59, 9594c.
51. Reibel I. M.: Teorija i Prakt. Polarograph. Analiza, Akad. Nauk Moldavsk. SSR, Materialy Pervogo Vses. Sovesch. 1962, 323; Chem. Abstr. 59, 6032 (1962).
52. Stackelberg M. v., Hans W., Jensch W.: Z. Elektrochem. 62, 839 (1958).
53. Kolthoff I. M., Mader P.: Anal. Chem. 41, 924 (1969).
54. Klumpar J.: This Journal 13, 11 (1948).
55. Kuik M.: Roczniki Chem. 42, 143 (1968).
56. Březina M., Gultjaj V.: This Journal 28, 181 (1963).
57. Kútová M., Březina M.: This Journal, 31, 743 (1966).
58. Basiński A., Ceynova J., Gadek Z.: Roczniki Chem. 40, 101 (1966).
59. Müller O. H.: Arch. Biochem. 15, 39 (1947).
60. Kolthoff I. M., Mader P.: Anal. Chem., 42, 1762 (1970).
61. Kútová M., Březina M.: Abhandl. Deut. Akad. Wiss. Berlin, Elektrochem. Meth. Prinz. Molekular-Biologie, p. 463. Symposium Jena, May 1965. Akademie Verlag, Berlin 1966.

62. Berg H.: Ref. 61, p. 479.
63. Ito M.: *Mic Med. J.* 14, 95 (1964).
64. Grubner O.: *This Journal* 19, 444 (1954).
65. Benesch R. E., Benesch R.: *J. Am. Chem. Soc.* 77, 5877 (1965).
66. Mairanovskii S. G.: *Catalytic and Kinetic Waves in Polarography*. Plenum Press, New York 1968.
67. Calusaru A.: *Compt. Rend., Ser. C.* 262, 676 (1966).
68. Calusaru A.: *Compt. Rend., Ser. C* 262, 4 (1966).
69. Toropova F. V., Elizarova G. L.: *Ž. Anal. Chim.* 18, 4 (1963).
70. Vlček A. A.: *Nature* 189, 393 (1961).
71. Fini G., Todeschini F.: *Ricerca Sci.* 38, 787 (1968).
72. Toropova V. F., Elizarova G. L.: *Ž. Anal. Chim.* 19, 174 (1964).
73. Hanzljk J.: *Thesis*. Czechoslovak Academy of Sciences, Prague 1967.
74. Ivanov I. D.: *Ž. Fiz. Chim.* 34, 2518 (1960).
75. Büchner M., Brahmer Ch., Pietsch A., Piasny W.: *Naturwiss.* 43, 423 (1956).
76. Březina M.: *Advances in Polarography*, p. 933. Pergamon Press, London 1960.
77. Gudbjarnason S.: *Biochem. Biophys. Acta* 177, 303 (1969).
78. Issleib K., Matschiner H., Naumann S.: *J. Electroanal. Chem.* 16, 563 (1968).
79. Lamprecht W., Gudbjarnason S., Katzelmeier H.: *Z. Anal. Chem.* 181, 201 (1961).
80. Říha J.: *Electrochim. Acta* 6, 75 (1962).
81. Mark H. B. jr, Schwartz H. G. jr: *J. Electroanal. Chem.* 6, 443 (1963).
82. Hojman J.: Private communication.
83. Stromberg A. G., Zelienskaja A. I.: *Ž. Obšč. Chim.* 15, 303 (1945).
84. Calusaru A., Kúta J.: *This Journal* 31, 814 (1966).