Studies on the Catalytic Double Wave of Cystine by Oscillographic Polarography

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Brdicka[1] has reported that in a conventional polarography cystine and cysteine develop a pronounced single catalytic wave in an ammonia buffer containing divalent cobalt. In the previous paper[2] it was reported that this catalytic wave gives a rounded maximum of good reproducibility by the addition of gelatin and that the maximum wave is split off into two waves by the addition of methyl cellulose (cf. Fig. 1). The form of these double waves resembles that of the catalytic double wave of protein, and the evolution of hydrogen as the electrolytic reductant is facilitated by the catalytic action of the cobalt(II)-cysteine complex* adsorbed on the surface of the dropping mercury electrode (DME). Accordingly, one can expect to clarify the nature of the protein wave from the analogy to the cystine wave.

The present experiment was carried out as a tentative model of the protein wave, and a possible mechanism of the cystine wave is proposed from the consideration of the adsorption equilibria among three surface active substances, i. e., the cobalt(II)-cysteine complex anion, methyl cellulose and gelatin.

Experimental

A Sevcik type oscillographic polarograph[3] constructed in our laboratory[4] was used. Effects of the mercury head and the frequency of the applied triangular sweep on the oscillographic current voltage pattern were observed. The mercury head was changed from 25 to 65 cm., when the life-time of the DME changed from 5.4. to 2.0 sec. under the applied span voltage from -0.28 to -2.16 V. vs. S. C. E. (20 cycles/sec.). The rate of the mercury flow was 0.755 mg./sec. at the mercury head of 25 cm. The frequency applied was 20～60 cycles/sec., when the rate of potential change was 75～225 V./sec.

All the reagents were of analytical grade. The test solutions used are tabulated as follows:

Test solution No.	Constituent
0	0.1 F NH_3-NH_4Cl buffer
1	1×10^{-3} F $CoCl_2+2\times10^{-5}$ F cystine in solution 0
2	0.02% methyl cellulose in solution 1
3	0.01% gelatin in solution 1
4	0.02% methyl cellulose+0.01% gelatin in solution 1
5	0.005～0.08% methyl cellulose in solution 0

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[1] R. Brdicka, *Collection Czechoslov. Chem. Communs.*, **4**, 148 (1933).

[2] M. Shinagawa and H. Nezu, This Bulletin, **33**, 272 (1960).

* This catalytic wave occurs at a more negative potential than the potential of the reduction of cystine to cysteine, and therefore, must be caused by the reduced form, cystein.

[3] A. Sevcik, *Collection. Czechoslov. Chem. Communs.*, **13**, 349 (1948).

[4] M. Shinagawa, H. Imai and S. Chaki, *J. Electrochem. Soc. Japan*, **23**, 132 (1955); H. Imai, This Bulletin, **29**, 276 (1956).

An H-cell with low impedance was used, and the dissolved oxygen in the test solution was not removed. The temperature was 29±0.5°C.

Results and Discussion

Under the experimental conditions described in the preceding section, the cobalt(II)-ammine complex behaves irreversibly at the DME, and the charging current, which flows when the adsorption or desorption of surface active substances occurs, becomes markedly great. The effect of the mercury head on the peak current gives information concerning the difference between the electrolytic current and the charging current and also concerning the irreversibility of an electrode process. One can obtain similar information from the effect of the sweep frequency on the peak current and the peak potential**.

The Oscillographic Current-Voltage Pattern. — The oscillographic current-voltage pattern of solutions 1, 2, 3 and 4 are illustrated in Figs. 2-A, B, C and D, respectively. In Fig. 2-A, two reduction steps represented by i_p1 and i_p2 are noticeable***. i_p1, the peak potential of which is −1.27 V. vs. S. C. E., corresponds to the reduction of the cobalt(II)-ammine complex ion. The peak height of i_p1 is decreased and rounded off by the addition of methyl cellulose and gelatin as is illustrated in Figs. 2-B, C and D, simultaneously the peak potential being shifted toward more negative potentials (E_p=−1.27, −1.31 and −1.36 V. vs. S. C. E. in solutions 2, 3 and 4, respectively.).

The second step (E_p=ca. −1.7 V. vs. S. C. E.)

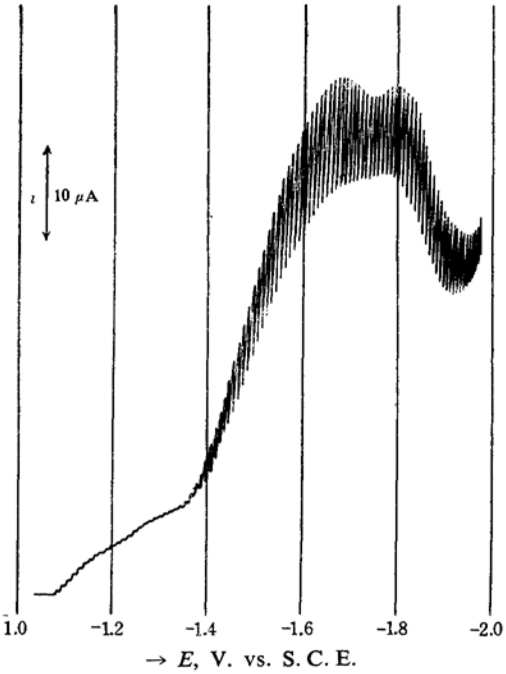
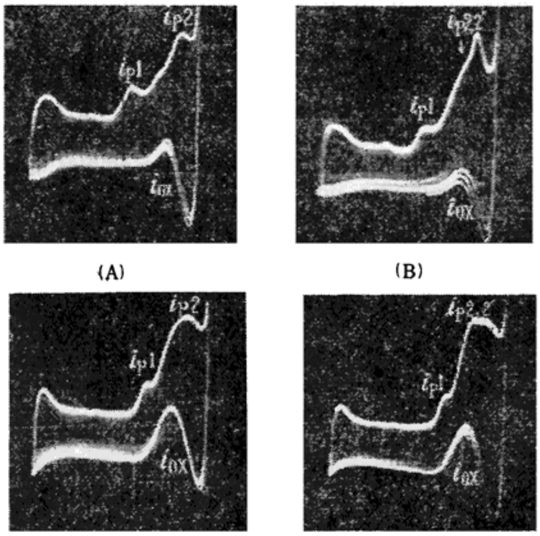


Fig. 1. The catalytic double wave of cystine by a conventional polarograph. Solution: 1×10^{-3} F $CoCl_2$, 2×10^{-5} F cystine, 0.002% methyl cellulose in 0.1 F NH_3-NH_4Cl buffer. m=2.46 mg./sec., t=3.5 sec.



(A) (B)

(C) (D)

Fig. 2. The oscillographic current-voltage pattern of 1×10^{-3} F $CoCl_2$ and 2×10^{-5} F cystine in 0.1 F ammonia buffer (A). (B): +0.02% methyl cellulose, (C): +0.01% gelatine, (D): +0.02% methyl cellulose and 0.01% gelatin, Sweep frequency=20 cycles/sec., v=75 V./sec., h=25 cm., t=4.9 sec.

** These points of view are summarized in detail as follows in order to help the subsequent considerations.

(1) The peak of the oscillographic pattern is rounded off and the peak potential (E_p) is shifted toward more negative potentials in the cathodic branch as the electrode process becomes irreversible[5].

(2) No reoxidation peak can be observed in the anodic branch unless the DME forms the amalgam with the reductant.

(3) The maximal peak current (i. e. the peak current just before the electrode mercury droplet falls) of a reversible electrode process ($i_{p,r}$) is independent of the mercury head, while that of an irreversible one ($i_{p,ir}$) varies with the mercury head[6].

(4) $i_{p,r}$ is proportional to the square root of the rate of the potential change (v)[6], while $i_{p,ir}$ deviates from this proportionality[7].

(5) The maximal charging current is independent of the mercury head, and it is linearly proportional to v.

(6) The peak potential of the charging current, which flows at the adsorption or the desorption potential of surface active substances, will be shifted by the change in the concentration of the adsorbed substance.

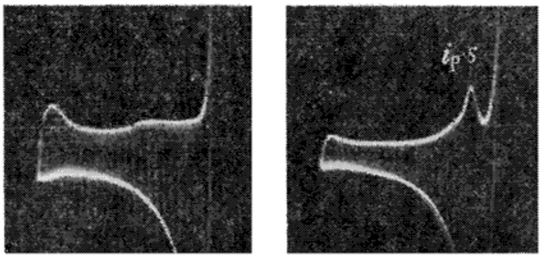
5) P. Delahay, *J. Am. Chem. Soc.*, **75**, 1190 (1953); H. Matsuda and Y. Ayabe, *Z. Elektrochem.*, **59**, 494 (1955).

6) P. Delahay, *J. Phys. Colloid Chem.*, **54**, 630 (1950); H. Imai, *J. Electrochem. Soc. Japan*, **23**, 530 (1955).

7) P. Delahay and G. Perkins, *J. Phys. Colloid Chem.*, **55**, 586, 1146 (1951).

*** i_p2 is accompanied by a barely perceptible peak at −1.5 V. vs. S. C. E. This peak corresponds to an irregular wave observed at the same potential by a conventional polarograph. The nature of this wave is unknown at this stage.

represented by i_p2 in Fig. 2 is the catalytic current. This catalytic current is accompanied by a sharp peak at −1.72 V. vs. S. C. E. in the presence of methyl cellulose. Since a similar sharp peak is developed at −1.7 V. vs. S. C. E. by the desorption of methyl cellulose in solution 5 (cf. Fig. 3), the above-mentioned sharp peak of solution 2 can be assigned to the charging current change caused by the desorption of methyl cellulose. By the addition of gelatin, the catalytic current is rounded off (E_p=1.72 V. vs. S. C. E.) as is illustrated in Fig. 2–C, and this wave is split off into two waves by the addition of methyl cellulose (Fig. 2–D), the potential of the first and the second peak being −1.62 and −1.73 V. vs. S. C. E., respectively. Undoubtedly, these double peaks are different in nature from the catalytic double waves developed by a conventional polarograph (Fig. 1), because the charging current becomes predominant in an oscillographic method. It may safely be assumed, however, from the coincidence of the peak potential of i_p2' with the desorption potential of methyl cellulose that the desorption of methyl cellulose is closely connected with the occurrence of i_p2' and presumably with that of the second maximum wave in the conventional polarography.



(A) (B)

Fig. 3. The oscillographic current-voltage pattern of 0.1 F ammonia buffer (A) and 0.06% methyl cellulose in 0.1 F ammonia buffer (B). Sweep frequency=20 cycles/sec., v=75V ./sec., h=45 cm., t=2.4$_5$ sec.

In the anodic branch, the current change represented by i_{ox} is noteworthy. The pattern of i_{ox} is pronounced by the addition of gelatin, and in this case it can be seen from Figs. 2–C and 2–D that this current is obviously cathodic (the reduction current). A cathodic current of a similar character has been observed in the solution containing indium(III) and chloride[8], corresponding to the well-known minimum wave in the conventional polarography. It is supposed that this minimum wave is caused by the electrostatic repulsion of $InCl_6^{3-}$ from the negatively charged electrode. By analogy, it can be assumed that the cobalt(II)-cysteine complex anion behaves likewise. When the electrode potential is shifted toward less negative values in the anodic branch after the repulsion of the complex anion in the cathodic branch, the complex anion will be readsorbed on the electrode surface and contributes to the catalytic reduction of the hydrogen ion. Thus, the mechanism of i_{ox} leads to the idea of the repulsion of the cobalt(II)-cysteine complex anion at the potential more negative than −1.63 V. vs. S. C. E.

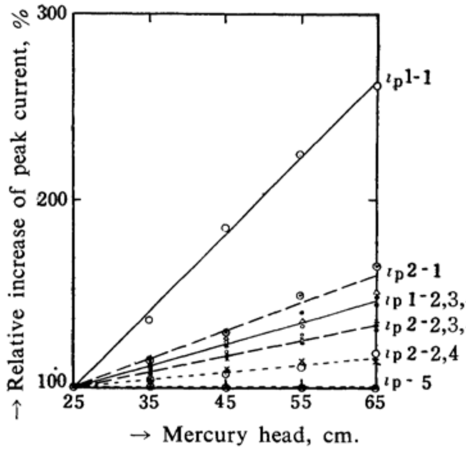


Fig. 4. The relative variation of the peak current with the mercury head. The symbols of the peak current are given in Fig. 2, and the second figure of the symbol represents the solution number given in the section of the experimental. Sweep frequency=20 cycles/sec., v=75 V./sec.

The Variation of the Peak Current with the Mercury Head.—The peak current is plotted against the mercury head in Fig. 4, where the peak current is represented by the same symbols as those used in Fig. 2, and the second figure in the symbols represents the solution number given in the section of Experimental. In Fig. 4 three groups are classified according to their inclination. The first group involves i_p1–1 and i_p2–1 which have the steepest inclination among i_p1's and i_p2's, respectively. When the recovery of the diffusion layer in each cycle of the voltage sweep imperfect owing to the imcomplete reoxidation of the reductant (Note that i_p1 and i_p2 give no reoxidation peak in Fig. 2.), the repetition of the reduction process during the life-time of the DME results in the depletion of the oxidant at the vicinity of the electrode surface except for the supply due to the duffusion from the bulk of solution. Under

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[8] M. Shinagawa, H. Imai and H. Sunahara, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), 77, 1482 (1956).

this condition, the thickness of the diffusion layer will be markedly decreased by the rapid rate of the DME growth. Moreover, the fresh surface of the DME is increased by the rapid rate of the DME growth. Since the rate of the DME growth increases as the mercury head is increased, it is easy to see why these currents increase as the mercury head is increased.

The second groups involves i_p1–2, 3, 4 and i_p2-2, 3, 4, which show a moderate change with the mercury head. In this group, the effect of the drop growth seems to be retarded by the presence of the surface active substances.

The third group involves i_p–5. This desorption peak current of methyl cellulose is practically independent of the mercury head.

The peak heights of i_p2'–2 and i_p2'–4 were measured from the height of the charging current without paying any attention to the superposition of the preceding peak current, i_p2, which is markedly affected by the mercury head. Keeping this fact in mind, one can assign the slight inclination of i_p2' to the superposition of the intrinsic current of i_p2' on the decreasing current of i_p2. Accordingly, it is assumed that i_p2' is practically independent of the mercury head and will be tacitly controlled by the desorption of methyl cellulose. This assumption is best realized from Fig. 5 which illustrates the relative change of i_p2–4 and i_p2'–4 with the mercury head.

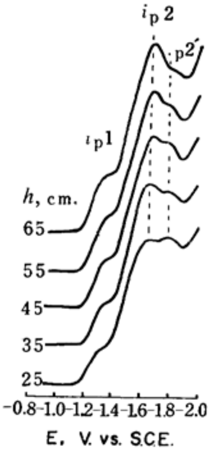

Fig. 5. The variation of the oscillographic current-voltage pattern of 1×10^{-3} F $CoCl_2$ and 1×10^{-5} F cystine in 0.1 F ammonia buffer containing 0.02% methyl cellulose and 0.01% gelatin with the mercury head (*h*, cm.). Sweep frequency=20 cycles/sec., v=75 V./sec.

The Effect of the Sweep Frequency. — The effect of the sweep frequency on the cathodic peak current is illustrated in Fig. 6. Any peak, except for the desorption peak of methyl cellulose, becomes more rounded and the peak potentional is shifted toward more negative potentials as the sweep frequency is increased. This result indicates that all of these electrode processes are irreversible. It must be pointed out here that the electrode process of i_p2'–4 is irreversible in nature, though it relates to the desorption of methyl cellulose as is described in the preceding section.

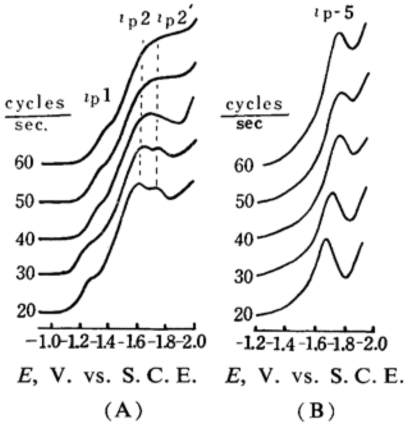

Fig. 6. The variation of the oscillographic current-voltage pattern of 1×10^{-3} F $CoCl_2$ and 1×10^{-5} F cystine in 0.1 F ammonia buffer containing 0.02% methyl cellulose and 0.01% gelatin (A) and the desorption charging current of 0.06% methyl cellulose (B) with the sweep frequency. h=45 cm.

Conclusion

By a conventional polarograph, cystine develops the pronounced double maximum in an ammonia buffer containing divalent cobalt, gelatin and methyl cellulose. This double wave is of interest because of the resemblance to the protein wave in the wave form and in the catalytic behavior. In the following description, it is explained why the cystine wave is developed in the form of the double maximum.

In this experiment, it was confirmed that cobalt(II)-cysteine complex, which acts as a catalyser for the depolarization of the hydrogen

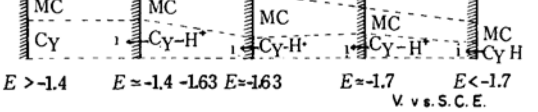

Fig. 7. The schematic representation of the adsorbed quantities of gelatin (G), methyl cellulose (MC) and the cobalt(II)-cysteine complex (Cy) on the unit surface area of the DME at each electrode potential. In this figure, i←Cy–H⁺ means that Cy behaves as a catalysis of the hyrogen ion depolarization.

ion, desorbs from the electrode surface at about −1.63 V. vs. S. C. E. When the electrode potential becomes more negative than −1.7 V. vs. S. C. E., methyl cellulose also desorbs from the electrode surface. Accordingly, the quantity of the surface active substances adsorbed on the unit surface area of the DME at each electrode potential can be schematically expressed as is illustrated in Fig. 7.

In Fig. 7, G, MC and Cy represent the adsorbed quantity of gelatin, methyl cellulose and cobalt(II)-cysteine complex anion, respectively, on the unit surface area of the DME. The quantity of G, MC and Cy depends on each adsorbed concentration, which is in equilibrium with each concentration of the bluk of the solution and on the mutual equilibria among G, MC and Cy on the electrode surface. When the electrode potential is less negative than −1.6 V. vs. S. C. E., all of the surface active substances are adsorbed on the electrode surface as is illustrated in Fig. 7-A. As the electrode potential becomes more negative than −1.5 V. vs. S. C. E., the catalytic current gradually increases (Fig. 7-B). The cobalt(II)-cysteine complex anion, however, begins to desorb at −1.63 V. vs. S. C. E. and results in the decrease of the catalytic current (Fig. 7-C). Thus, the first maximum wave will be developed by the conventional polarograph. It is noteworthy, here, that the quantity of G and MC will be increased, corresponding to the desorption of Cy-complex. This is because the surface area to be occupied by G and MC will increase when Cy-complex desorbs.

At the potential of −1.7 V. vs. S. C. E. methyl cellulose begins to desorb from the electrode surface (Fig. 7-D). In this case, the quantity of G and Cy-complex will be increased, though the increase of Cy-complex is limited in the potential region where the desorption of methyl cellulose appreciably proceeds. Accordingly, the catalytic current will temporarily increase near the potential of −1.7 V. vs. S.C.E. (Fig. 7-E). Thus, the second maximum wave will be developed.

In the above-mentioned conclusion, the present authors proposed a possible mechanism of the cystine double wave from the consideration of the adsorption equilibria among gelatin, methyl cellulose and cobalt(II)-cystein complex anion. The criticism of this idea and its application to the protein wave is left for future.

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