PARAMETERS AFFECTING THE SHAPE OF CATALYTIC WAVES IN THE SYSTEM Ni(II)–CYSTEINE; QUALITATIVE RESULTS

M. KUIK

Institute of Chemistry,
Copernicus University, Torun, Poland

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

Received June 25th, 1970

During polarographic investigations of ammonia buffer solutions containing Ni(II) and cysteine, in the absence of surface active compounds three peaks were observed in the range of potentials, where waves of the catalysed reduction of hydrogen ions appear (from about −1·5 to −1·9 V *vs.* S.C.E.). The following parameters effect the shape of the catalytic wave: *1.* value of the mole ratio Ni : CyS; *2.* summary concentration ([Ni] + [CyS]) when [Ni] : [CyS] = const; *3.* composition of the buffer solution; *4.* presence of surface active compounds; *5.* height of the mercury column; *6.* drop time (m = const); *7.* temperature of the solution. The results presented show, that a catalytic wave in the form of three peaks is characteristic not only of proteins. In the case of low molecular compounds the effect is probably much more common and might be observed also in other systems after a suitable choice of experimental conditions.

The already well known catalytic double wave of proteins, obtained in the presence of cobalt salts, was discovered by Brdička[1]. Lately Ruttkay-Nědecky and Anderlová[2] reported the appearance of even three catalytic maxima under similar conditions. Regarding low molecular compounds, data concerning catalytic double maxima may be found in relatively not numerous papers[3–16], but only few authors pay attention to this effect.

The present communication deals with three peaks observed during polarographic investigations of ammonia buffer solutions containing Ni(II) and cysteine (no surface active compounds added) within the range of potentials, where waves of the catalysed reduction of hydrogen ions appear (from about −1·5 to −1·9 V *vs* S.C.E.). The aim of the present publication is to present qualitatively the effect of various parameters, examined till now, on the number and shape of the described peaks.

EXPERIMENTAL

Polarograms were recorded by means of the type PO 3k polarograph (Radiometer, Copenhagen, Denmark). The polarographic vessel and characteristics of the capillary according to Smoler[18] used in the experiments, were given in an earlier paper[17]. If not otherwise stated, measurements were performed always at a height of the mercury reservoir $h_{Hg} = 45{\cdot}0$ cm and the vessel thermostated at $20 \pm 0{\cdot}1°C$. The drop time was adjusted by means of a mechanical drop time controller.

Cysteine, pure, a product of the firm Schuchardt (München, GFR) and Bacto Gelatin, produced by Difco Laboratories (Detroit, USA) were used. Other chemicals were of the grade *p.a.*, produced by Polskie Odczynniki Chemiczne (Gliwice, Poland).

Stock solutions of cysteine were prepared and stored according to[17]. The exact concentration of approximately 0·1M-$NiCl_2$ in 0·01M-HCl was determined gravimetrically by means of dimethylglyoxime[19]. In order to obtain an ammonia solution free from pyridine bases, the commercial solution of NH_3 p.a. was additionally purified[20]. The preparation of the remaining solutions and methods of determining their concentration were described in[17].

A methodics developed earlier[17] was applied. The only difference was, that when preparing the solution to be examined in the measuring flask, cysteine was added always last of all. Investigations of the dependence of the catalytic wave on the height of the mercury column were performed from the same solution (it has been stated, that within this period the height of the catalytic wave does not change). Values of h_{Hg} adduced in the figures are corrected against the back pressure. When examining the temperature dependence, for each temperature a fresh solution was prepared.

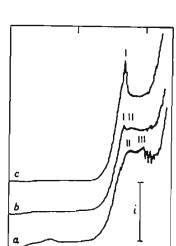

FIG. 1

Denotation of Peaks Occurring on the Catalytic Wave

0·5 mM-$NiCl_2$; 0·1M-NH_4Cl; 0·1M-NH_3; [Cys], mM: *a* 0·25; *b* 0·5; *c* 1·0. The values of *i*, μA: *a* 22·5; *b*, *c* 30.

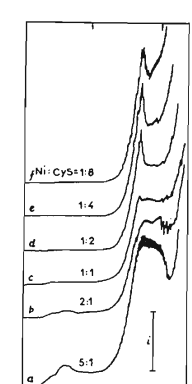

FIG. 2

Effect of the Mole Ratio of Ni: Cys on the Shape of Catalytic Waves

0·5 mM-$NiCl_2$; 0·1M-NH_4Cl; 0·1M-NH_3. The values of *i*, μA: *a* 10·5; *b* 22·5, *c*—*f* 30.

RESULTS

The three peaks mentioned above, which will be further denoted according to the sequence of their appearance as *I*–*III* (Fig. 1), may be obtained from solutions, to which no surface active compounds have been added. Sometimes the peaks transform into waves with a better or worse formed limiting current. Usually, however, only two peaks (or waves) are simultaneously visible.

Irregular dropping of mercury, as a result of an intensive evolution of gaseous electrolysis products, causes deformations of the waves, which make their quantitative evaluation difficult. Such disturbances become considerable for [Ni] > 0·5 mM and depending on conditions, at negative potentials higher than approximately −1·6 V (or even −1·8 V) *vs* S.C.E.

Effect of the Value of the mole Ratio [Ni] : [Cys]

This effect is best illustrated by polarograms obtained under conditions, when all of the peaks may appear (Fig. 2). Fig. 2 shows that the peak *I* becomes distinctly

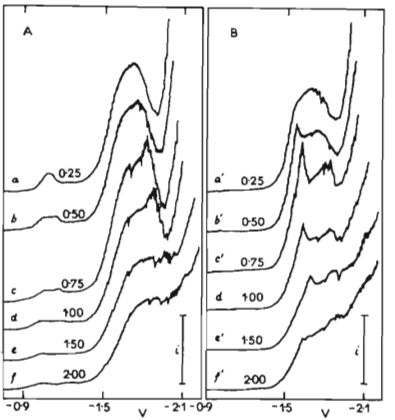

FIG. 3

Effect of the Summary Concentration ([Ni] + [Cys]) when [Ni] : [Cys] = const (A 2 : 1, B 1 : 2), on the Shape of Catalytic Waves

0·1M-NH_4Cl; 0·9M-NH_3. Values of i, μA: *a* 10·5; *b'* 22·5; *a'* 15; *b*, *c'* 30; *c*, *d'* 45; *d*, *e'* 75; *e*, *f'* 105; *f* 150. [$Ni(Cl)_2$], mM, given directly on the curves.

visible only when [Ni] ≤ [Cys], while the peaks *II* and *III* in principle may be distinguished only in solutions, where [Ni] > [Cys]. However, the composition of the buffer solution considerably influences the separation of individual peaks (see p. 1013).

Effect of the Summary Concentration ([Ni] + [Cys]), *when* [Ni] : [Cys] = *Const*

As may be seen from Fig. 3, a change of the summary concentration of both components when their mole ratio was kept constant, to a high degree influences the separation of individual peaks. Under given conditions, at the lowest concentrations (curves *a*, *a'* in Fig. 3) the separation is practically not visible. As the concentration is increased (curves *b* – *d*, *b'* – *d'* in Fig. 3), the separation becomes very distinct and afterwards gets worse (curves e, *f*, e', *f'* in Fig. 3). It is, however, not always so.

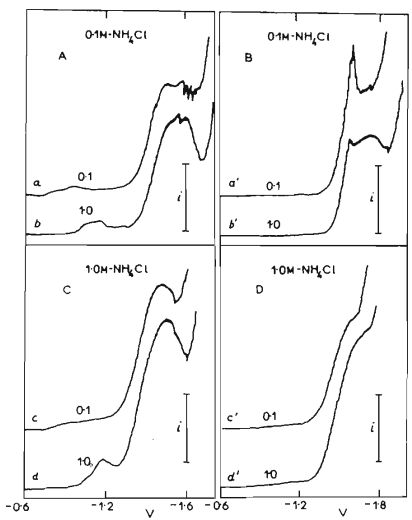

FIG. 4

Effect of the Composition of the Ammonia Buffer Solution on the Shape of Catalytic Waves

0·5 mM-$NiCl_2$; values of *i*, μA: *a*, *b*, *d'* 22·5, *a'*, *b'*, *c'* 30; *c* 15, *d* 10·5. [Ni] : [Cys] = 2 : 1 (A, C), 1 : 2 (B, D). [NH_3], M, given directly on the curves.

For instance in solutions of 1M-NH_4Cl and 0·91M-NH_3 the best, although incomplete separation of the peaks, takes place just at the lowest concentration of nickel and cysteine[21].

Effect of the Composition of the Buffer Solution

This effect is best illustrated in Fig. 4, which simultaneously takes into account the role of the mole ratio of nickel to cysteine. From presented data, the following conclusions result: *a*) When [Ni] > [Cys], an increase of the ammonia concentration causes a growth of peak *III* (Fig. 4A); an increase of the concentration of NH_4Cl (while keeping the ammonia concentration constant) results in a disappearance of peak *III* (Fig. 4C). *b*) When [Ni] < [Cys], in the presence of 0·1M-NH_4Cl an increase of NH_3 concentration lowers the peak *I*, but simultaneously improves the separation of peaks *I* and *III* (Fig. 4B). An increase of the concentration of NH_4Cl makes worse the separation of the peaks and at the same time changes completely the shape of the

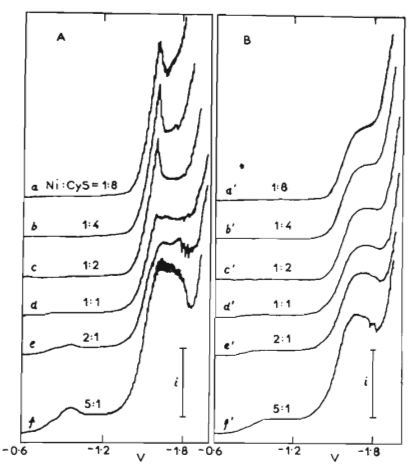

FIG. 5

Effect of Gelatin on the Shape of Catalytic Waves at Different Mole Ratios of Ni : Cys

A Without gelatin, B 0·01% gelatin. 0·5 mM-$NiCl_2$, 0·1M-NH_4Cl, 0·1M-NH_3.; values of *i*, μA: *a*—*d*, *a'*—*d'* 30; e, e' 22·5; *f*, *f'* 10·5.

catalytic wave to a form, resembling normal polarographic waves with a fairly well formed limiting current (Fig. 4D). Conclusions given above on the basis of Fig. 4 were confirmed in a more detailed examination of the effect of the composition of the ammonia buffer solution on catalytic waves in the system studied[22].

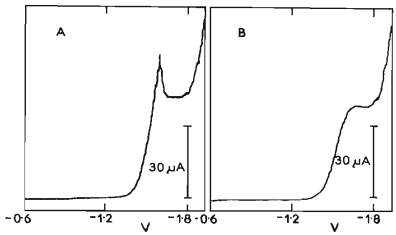

FIG. 6

Effect of Gelatin on the Shape of Catalytic Waves

A Without gelatin, B 0·002% gelatin. 0·5 mM-$NiCl_2$; 0·1M-NH_4Cl; 0·1M-NH_3. Ni : Cys = 1 : 2.

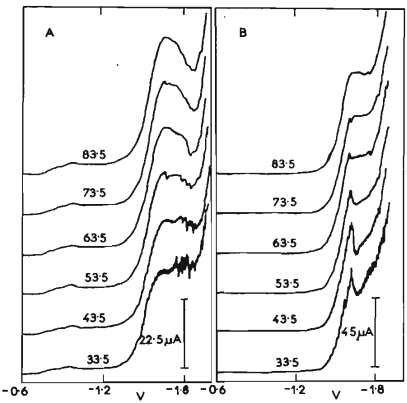

FIG. 7

Effect of the Height of the Mercury Column on the Shape of Catalytic Waves

Ni : Cys: A 2 : 1, B 1 : 8. 0·5 mM-$NiCl_2$; 0·1M-NH_4Cl; 0·1M-NH_3. h_{Hg}, cm, given directly on the curves.

Effect of Surface Active Compounds

The observed peaks are very sensitive to the presence of surface active compounds in the solution. As may be seen from Fig. 5, gelatin suppresses practically all subtleties of the wave, especially for [Ni] ≥ [Cys] (compare curves $c-f$ and $c'-f'$ in Fig. 5). When [Ni] > [Cys] the wave keeps admittedly the shape of a rounded peak, the peaks *II* and *III* however practically disappear. Although under certain conditions, even in the presence of 0·01% gelatin, from the shape of the wave one might conclude of its heterogeneity, the outlines of the described peaks *II* and *III* are very weak[23].

Peak *I* is particularly sensitive to the presence of surface active compounds. It is completely suppressed already in the presence of 0·002% gelatin (Fig. 6).

Effect of the Height of the Mercury Column

As may be seen from Fig. 7, separation of the peaks is markedly influenced also by the height of the mercury column. Best separation is obtained at the lowest outflow of mercury. An increase of the height of the mercury column leads under given conditions to the formation of a single wave. Sometimes, however, the shape of the wave changes completely (Fig. 8).

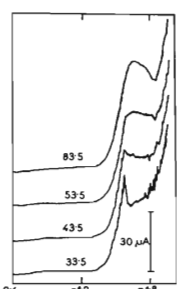

FIG. 8

Effect of the Height of the Mercury Column on the Shape of Catalytic Waves

0·5 mM-$NiCl_2$; 0·1M-NH_4Cl; 0·1M-NH_3. Ni : Cys = 1 : 1. h_{Hg}, cm, given directly on the curves.

Effect of the Drop Time (m = const)

Fig. 9 shows, that for both values of the mole ratio of Ni : Cys, a shortening of the drop time results always in a disappearance of the "fine structure" of the catalytic wave. For a mole ratio Ni : Cys = 2 : 1, as the drop time is shortened, a gradual

disappearance of peak *III* takes place, whereas at Ni : Cys = 1 : 2, both peak *I* as well as the outline of peak *III* disappear (Fig. 9).

Effect of the Temperature

An increase of the temperature of the solution causes a rise of all observed peaks (Fig. 10). When Ni : Cys = 2 : 1, as the temperature is increased, the separation of the peaks gets considerably worse as a result of a shift of peak *III* towards more positive potentials. The position of peak *I* and *II* is almost independent of temperature.

DISCUSSION

One of the most important statements of the present work is, that a catalytic wave in the shape of two or even three peaks is characteristic not only of proteins. The described effect could be formally assumed to confirm the thesis of Ruttkay-Nedecky and Anderlova[2], that cysteinyl residues are responsible for the appearance of three catalytic maxima in the case of proteins. However, due to difficulties in determining the character of separate peaks, such a conclusion cannot be temporarily accepted, and the results of Calusaru[14], too, induce to a certain caution.

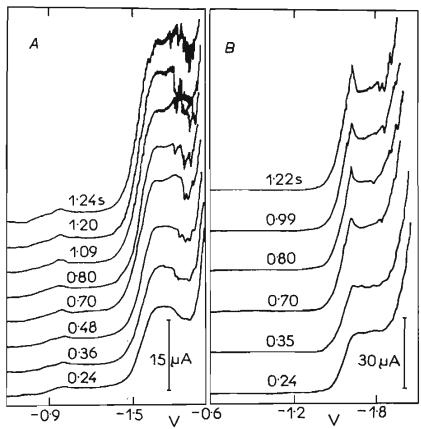

FIG. 9

Effect of the Drop Time on the Shape of Catalytic Waves

Ni : Cys A 2 : 1, B 1 : 2. 0·5 mM-$NiCl_2$; 0·1M-NH_4Cl; 0·12M-NH_3. *t*, s, given directly on the curves.

From data presented it becomes clear, why a finding of the complex shape of the catalytic wave in the system Ni(II)–cysteine has not been successful earlier. The presence of gelatin makes the formation of all peaks practically impossible, although even in this system under certain conditions a catalytic double wave may be observed[24]. It seems that the role of the above described parameters in the splitting of the catalytic wave may differ, depending on the kind of compounds and specific properties of the system studied.

The effect of the existence of so many parameters of catalytic waves is, that:

a) the particular peaks are often not sufficiently well separated and their height cannot be measured. This makes impossible a detailed examination of the effect of only one parameter on the heights of separate peaks. *b*) The investigation of parameters basic for the determination of the character of currents, does not as arule give typical dependences, suitable for a univocal interpretation. The possibility of establishing a scheme of the electrode process is thereby considerably complicated.

Adduced data allow to believe, that the demonstrated effect of individual parameters on catalytic waves is of a general significance and also in the case of other

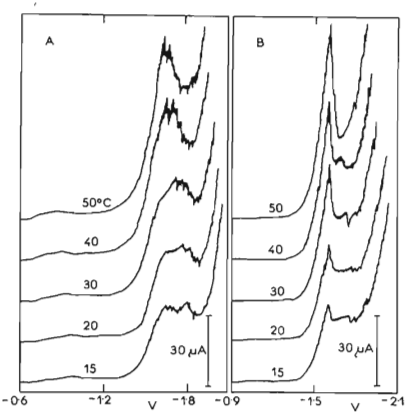

FIG. 10

Effect of Temperature on the Shape of Catalytic Waves

0·5 mM-$NiCl_2$; 0·1M-NH_4Cl; 0·1M-NH_3.Ni: Cys A 2 : 1, B 1 : 2. Temperature, °C, given directly on the curves.

low molecular compounds, a suitable choice of conditions should enable a splitting of catalytic waves in their components. A similar conclusion concerning proteins was put forward by Ruttkay-Nedecky and Anderlova[2]. One might also assume, that in cases, when the examination of parameters determining the character of the wave does not give typical results, the existence of two not separated peaks may be expected.

REFERENCES

1. Brdička R.: This Journal *5*, 112 (1933).
2. Ruttkay-Nedecky R., Anderlova A.: Nature *213*, 564 (1967).
3. Brdička R., Biochem. Z.: *272*, 104 (1934).
4. Brdička R.: This Journal *9*, 76 (1937).
5. Paleček E.: Pharmazie *11*, 551 (1956).
6. Březina M., Zuman P.: *Die Polarographie in der Medizin, Biochemie und Pharmazie.* Leipzig 1956.
7. v. Stackelberg M., Hans W., Jensch W.: Z. Elektrochem. *62*, 839 (1958).
8. Zuman P., Kuik M.: This Journal *24*, 3861 (1958).
9. Lamprecht W., Gudbjarnason S., Katzlmeier H.: Z. Physiol. Chem. *322*, 52 (1960).
10. Březina M.: *Advances in Polarography*, Vol. 3, p. 933. London 1960.
11. Březina M.: *I. Jenaer Symposium 1962*, p. 198. Berlin 1964.
12. Shinagawa M., Nezu H.: Bull. Chem. Soc. Japan *33*, 272 (1960).
13. Shinagawa M., Imai H., Nezu H.: Bull. Chem. Soc. Japan *34*, 445 (1961).
14. Calusaru A.: Compt. Rend., Ser. *C 266*, 956 (1968).
15. Calusaru A.: Rev. Chim. Minerale *5*, 387 (1968).
16. Calusaru A.: Naturwiss. *53*, 475 (1966).
17. Kuik M., Basiński A.: Roczniki Chem. *39*, 1327 (1965).
18. Smoler I.: This Journal *19*, 238 (1954).
19. Struszyński M.: *Analiza Ilosciowa i Techniczna*, Vol. 2. Warszawa 1952.
20. Kariakin Yu. W., Angelov I. I.: *Čistje Chimičeskje Reaktivy*, p. 31. Moscow 1955.
21. Kuik M.: Unpublished results.
22. Kuik M., Masłowski A., Rakowska A.: Unpublished results.
23. Kuik M.: *Thesis*. The Copernicus University, Toruń 1962.
24. Kuik M., Krassowski K.: Unpublished results.