Illinois Academy of Science Transactions, Vol. 49, 1956

POLAROGRAPHY OF SULFHYDRYL-DISULFIDE SYSTEMS

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The polarograph has been applied with success to the determination of cysteine, cystine or thioglycolic acid by Brdička (1933a, b). By this method, the sulfhydryl and disulfide contents of protein hydrolysates were also ascertained and these agreed with values obtained by other procedures (Brdička, 1934, 1938). Although it was not possible to distinguish between sulfhydryl and disulfide catalytic waves, Brdička resolved this problem by the use of potassium iodoacetate. The latter, upon reaction with an aliquot sample of protein hydrolysate over a period of one hour, did not affect the disulfide content but abolished the sulfhydryl contribution to the total wave, according to the following:

$$RSH + ICH_2COOH \rightarrow RSCH_2COOH + HI$$

In the present work, the polarographic determination of the sulfhydryl and disulfide contents of thioglycolate - dithioglycolate mixtures was attempted in the light of the satisfactory results with protein hydrolysates. The study also included a comparison of the sulfhydryl catalytic waves of α- and β-mercaptopropionic acids.

EXPERIMENTAL

Thioglycolic acid was twice distilled under vacuum just prior to use (b.p. 105-108° C. at 20 mm.; collected in water). Dithioglycolic acid, after two recrystallizations from water, melted at 107-108° C. The rectified α-mercaptopropionic acid boiled at 91-93° C. (10 mm.) while the β-mercaptopropionic acid melted at 18.6° C. Cysteine hydrochloride and cystine, both of high purity, originated from Nutritional Biochemicals Corporation. In all instances, mixtures of thioglycolic and dithioglycolic acids were freshly prepared.

Waves were recorded with a Sargent Model XXI polarograph at 25° ± 2° C. The rate of mercury flow at pH 8.45 was 3.23 mg./sec. with a capillary-drop time of 2.65 seconds. Correction of $E\frac{1}{2}$ readings of the cell to S.C.E. was made with a Beckman Model G pH meter employing a platinum anode.

Except for some modifications, the procedure of Brdička was employed. Determinations were carried out in 0.1 N NH_3 - 0.1 N NH_4Cl solution, previously freed of oxygen by bubbling nitrogen through the cell for a period of ten minutes prior to the addition of cobaltous chloride (final concentration = 0.002 N).

The aqueous solution containing sulfhydryl, disulfide or a mixture of these in amounts of 0.01 - 0.001 N was diluted as follows as based on

TABLE 1.—**Polarographic Data with Thioglycolate and Dithioglycolate.**

Composition in cell, eq. x 10^6		
Sulfhydryl + disulfide	Disulfide	Sulfhydryl determined, eq. x 10^6/1./μa.
8.72[1]	0.0	0.66
8.40[1]	2.58[2]	0.64
8.57[1]	5.48[2]	0.56
8.50	2.09[2]	0.58
31.1	0.0	0.86

[1] K^+ concentration = 0.2 N.

[2] Reaction in presence of iodoacetate proceeded with complete exclusion of catalytic wave.

a total volume of 2.06 ml.: 1) 0.10 ml. test solution, 0.50 ml. 1 N KOH and 1.46 ml. water; and 2) 0.10 ml. test mixture, 0.66 ml. 1 N KOH and 1.30 ml. 0.1 N iodoacetic acid. The latter solution, which contained additional KOH in amount required for the formation of the iodoacetate, was allowed to stand for a period of 1-2 hrs. at 25° C. Both mixtures were then diluted in the following manner upon introduction into the cell: 0.10 ml. Solution (1) or (2), 9.80 ml. 0.1 N NH_3 - 0.1 N NH_4Cl and 0.10 ml. 0.2 N cobaltous chloride.

In several experiments, the potassium ion concentration and pH were varied so as to study their effect on the sensitivity and reproducibility of the catalytic waves, as well as to ascertain the conditions wherein iodoacetate reacts solely with thioglycolate. Alteration of the pH of the ammonia solutions was affected by the addition of concentrated hydrochloric acid.

RESULTS

Representative polarographic data for thioglycolate, dithioglycolate and mixtures of these are presented in Table 1. The effect of varying pH is shown in Table 2 which also includes results with α-and β-mercaptopropionic acids, cystine, and cysteine.

DISCUSSION

It will be observed that catalytic waves for thioglycolate, dithioglycolate or their mixtures were well-defined in the pH range of 2.1 - 9.4. As would be expected, the disulfide yielded twice the wave increment on a molar basis as that obtained with thioglycolic acid. The reaction of iodoacetate with the sulfhydryl-derivative was complete at a pH of 7.5 or higher but only partial at pH 6.7. The wave was poorly defined in buffered solutions of pH greater than 9.5. The present method failed in the analysis of sulfhydryl and disulfide mixtures since reaction with iodoacetate was also noted in the presence of dithioglycolate to the total elimination of the wave. This could not be obviated by variations in potassium ion concentration or pH.

The procedure was checked by repetition of Brdička's experiments

TABLE 2.—Effect of pH on the Catalytic Waves of Thioglycolate-Dithioglycolate Solutions; Results with Meracaptopropionic Acids, Cysteine and Cystine.

Experiment	Composition in Cell, eq. x 10^6		pH	Sulfhydryl determined, eq. x 10^6/1./μa.
	Sulfhydryl	Disulfide		
Thioglycolic acid (II-18)	65.5	....	6.1	7.55
Thioglycolic acid + iodoacetate (II-18A)	65.5	....	6.7[1]	22.2
Thioglycolic acid (II-5)	193.0	....	7.5	0.99
Dithioglycolic acid (II-10)	.....	50.9	7.15	2.54
Dithioglycolic acid (I-12)	.....	6.3	9.8[2]	....
Dithioglycolic acid (I-13)	.....	60.0	8.15	1.54
α-Mercaptopropionic acid (II-15)	127.2	....	8.2	3.72
β-Mercaptopropionic acid (II-4)	91.6	....	8.6	55.9
Cysteine (II-7)	36.7	....	7.65	0.85
Cystine (II-8)	....	42.6[3]	8.45	1.20

[1] At this pH, only part of iodoacetate reacted.
[2] Catalytic wave was poorly defined at this pH.
[3] Disulfide determined: 38.4 x 10^{-6} eq.

with the amino acids, cystine and cysteine; only the latter reacted with iodoacetate and in good agreement with theory. A definite difference is thus apparent between the cysteine-cystine and thioglycolate-dithioglycolate systems. This variation in behavior undoubtedly resides in the dipolar nature of the amino acids and in the differences in stability of the disulfides in basic solution. The half-wave potentials were found to be —1.70 volt vs. S.C.E. at pH 8.2 for thioglycolate-dithioglycolate and —1.58 volt for cystine-cysteine.

A comparison of the catalytic waves obtained with the mercaptopropionic acids indicated a greater wave height for the *a*-isomer (Table 2).

SUMMARY

Although the total sulfhydryl and disulfide content of thioglycolate-dithioglycolate solutions can be determined polarographically, the individual components of such mixtures did not lend themselves to analysis by these criteria and with the aid of iodoacetic acid. In direct contrast, however, determinations could be carried out with cystine and cysteine, corroborating earlier published findings. The height of the catalytic wave obtained with *a*-mercaptopropionic acid exceeded that of the β-derivative.

ACKNOWLEDGMENT

This study was aided by a grant from The Toni Division of the Gillette Company, Chicago, Illinois.

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