[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE INSTITUTE OF TECHNOLOGY AND THE DEPARTMENT OF PHYSIOLOGY OF THE MEDICAL SCHOOL OF THE UNIVERSITY OF MINNESOTA]

The Anodic Reaction and Waves of Cysteine at the Dropping Mercury Electrode and at the Platinum Micro Wire Electrode[1]

BY I. M. KOLTHOFF AND CYRUS BARNUM

The oxidation potential of the cystine–cysteine system has been the subject of many investigations. A complete discussion of the literature[2] is given in the thesis of the junior author and is omitted here for the sake of brevity. Cysteine, $HOOC\text{-}CH(NH_2)CH_2\text{-}SH$, which in the following will be referred to as RSH, can be oxidized to cystine by suitable oxidizing agents or by anodic electrolysis at a platinum electrode. Cystine, $HOOC\text{-}CH(NH_2)CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}CH(NH_2)COOH$, designated in the following as RSSR, can be transformed to cysteine by suitable reducing agents or by electrolytic reduction. From potential measurements carried out in the classical way it is apparent that the reaction

$$RSSR + 2H^+ + 2e \longrightarrow 2RSH$$

is not strictly reversible in the thermodynamic sense. When noble metals are used in the measurement of the oxidation potential, it is found that the concentration of cystine does not affect the potential, but that the potential does depend on the hydrogen ion concentration and on the concentration of cysteine according to the equation

$$\pi = \pi_0 + \frac{RT}{F} \ln [H^+] - \frac{RT}{F} \ln [RSH]$$

Barron, Flexner and Michaelis[3] showed that mercury is not an indifferent electrode to cysteine. When cysteine undergoes air oxidation at a mercury surface a mercuric cysteinate is formed

$$Hg + 2RSH \rightleftarrows Hg(SR)_2 + 2H^+ + 2e$$

No investigations on the current–voltage curves (hereafter to be referred to as c. v. curves) of cysteine at the dropping mercury anode or at a platinum micro wire electrode[4] have been reported in the literature. In this paper we deal with the c. v. curves obtained with cysteine. In a subsequent paper the c. v. curves obtained with cystine will be presented and discussed.

Experimental

Materials Used.—Cysteine hydrochloride, C. P., was purchased from Pfanstiehl and contained 0.25% ash. A 0.002 *M* solution at *p*H 7 underwent complete air oxidation in about forty hours, indicating that the product probably contained small amounts of metals catalyzing the oxidation.[5]

Circuit and Apparatus.—A manual apparatus similar to that described by Kolthoff and Lingane[6] was used in this work. All experiments were carried out in a thermostat at 25.0 ± 0.1°. The *m* and *t* values of the dropping mercury capillary used were, respectively, 2.963 mg. of mercury per second and 3.04 seconds when the capillary dipped into 0.1 *M* potassium chloride and no potential was applied to the mercury drop. The drop time of the capillary was determined frequently during the course of each investigation.

Experimental Results

Curve A of Fig. 1 shows the anodic wave obtained at the dropping mercury electrode with a solution of 0.002 *M* cysteine in 0.1 *M* perchloric acid. Hydrochloric acid should not be used in such an experiment as the anodic chloride wave interferes with the cysteine wave at this *p*H. It is seen from Fig. 1 that the anodic reaction begins at a potential of about −0.2 volt with respect to the saturated calomel electrode and that a diffusion current is obtained at a potential of about +0.1 volt. At more positive potentials abnormalities occur. The diffusion current, determined after correction for the residual current, was found to be proportional to the concentration of cysteine as is seen in Table I, and hence this substance can

TABLE I

[RSH]	i_d	i_d/[RSH]
2.5×10^{-4} *M*	−1.01	4.04×10^3
10^{-3} *M*	−4.16	4.16×10^3
2×10^{-3} *M*	−7.97	3.98×10^3

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[1] From a thesis submitted to the Graduate School of the University of Minnesota by Cyrus Barnum in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1940.

[2] M. Dixon and J. H. Quastel, *J. Chem. Soc.*, **123**, 2943 (1923); E. C. Kendall and F. F. Nord, *J. Biol. Chem.*, **69**, 295 (1926); M. Dixon and H. E. Tunnicliffe, *Biochem. J.*, **21**, 844 (1927); E. C. Kendall and D. F. Loewen, *ibid.*, **22**, 649, 669 (1928); M. Dixon, *Proc. Roy. Soc.* (London), **101B**, 57 (1927); D. C. Harrison and J. H. Quastel, *Biochem. J.*, **22**, 683 (1928); L. Michaelis and L. B. Flexner, *J. Biol. Chem.*, **79**, 689 (1928); J. W. Williams and E. M. Drissen, *ibid.*, **87**, 441 (1930); E. K. Fischer, *ibid.*, **89**, 753 (1930); J. C. Ghosh, S. N. Raychadhuri and S. C. Ganguli, *J. Indian Chem. Soc.*, **9**, 43 (1932); J. C. Ghosh and S. C. Ganguli, *Biochem. J.*, **28**, 381 (1934); D. E. Green, *ibid.*, **27**, 678 (1933); J. S. Fruton and H. T. Clarke, *J. Biol. Chem.*, **106**, 667 (1934); H. Borsook, E. L. Ellis and H. M. Huffman, *ibid.*, **117**, 281 (1937).

[3] E. S. G. Barron, L. B. Flexner and L. Michaelis, *J. Biol. Chem.*, **81**, 743 (1929).

[4] H. A. Laitinen and I. M. Kolthoff, THIS JOURNAL, **61**, 3344 (1939).

[5] S. Sakuma, *Biochem. Z.*, **142**, 68 (1923); D. C. Harrison, *Biochem. J.*, **18**, 1009 (1924).

[6] I. M. Kolthoff and J. J. Lingane, *Chem. Rev.*, **24**, 1 (1939).

be determined polarographically in a medium of 0.1 *M* perchloric acid.

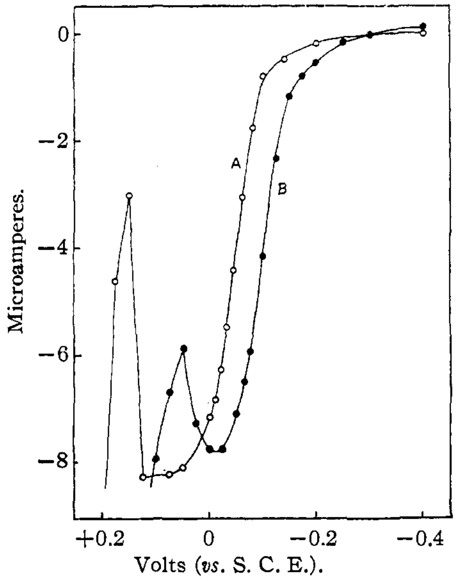

Fig. 1.—Curve A, 0.002 *M* cysteine at *p*H 1.0; Curve B, 0.002 *M* cysteine at *p*H 2.0.

By application of the Ilkovic equation[7] it can be shown that the anodic reaction of cysteine involves one electron per molecule ($n = 1$). The Ilkovic equation states that

$$i_d = 605nD^{1/2}Cm^{2/3}t^{1/6}$$

when the current is expressed in microamperes, the concentration, C, in millimoles per liter, and the product $m^{2/3}t^{1/6}$ in the units mg.$^{2/3}$sec.$^{-1/2}$. From this equation the diffusion coefficient of cysteine was calculated and found to be 3.10×10^{-5} cm.^{2}/sec. in 0.1 *M* perchloric acid.

Current–voltage curves were also determined for cysteine at other *p*H values. Curve B of Fig. 1 shows the c. v. curve obtained with 0.002 *M* cysteine in a Clark and Lubs buffer of a *p*H of 2. It is seen that an abnormality occurs just before the wave reaches its diffusion current. As the *p*H increases above 2, the waves become even more abnormal as is seen from Fig. 2 which depicts the c. v. curves of cysteine in an acetate buffer at *p*H 4.73 and in a phosphate buffer at *p*H 6.0. The *p*H of the buffers was measured with a quinhydrone electrode. From Fig. 2 it may be seen that as the *p*H increased the waves started at more negative potentials but that the waves levelled off to give a constant current (false diffusion current) of about 1 microampere despite the fact that the expected diffusion current was about 8 microamperes. The current did finally increase at more positive potentials but no diffusion current region was established. This abnormal behavior is attributed to the formation of a film of mercurous cysteinate (*vide infra*) around the drop which prevents further interaction between the cysteine and the mercury. Other cases have been found in this Laboratory where film formation causes great irregularities in the shape of the c. v. curves. In agreement with the above explanation it was found that the small value of the constant current (1 microampere) was independent of the concentration of cysteine presumably because the leveling off of the current occurs at a value at which a film of certain thickness is formed. Only when the concentration of cysteine was reduced to 0.00025 *M* (see Fig. 2) or less was a true diffusion current obtained since the latter was smaller than the current at which film interference occurred.

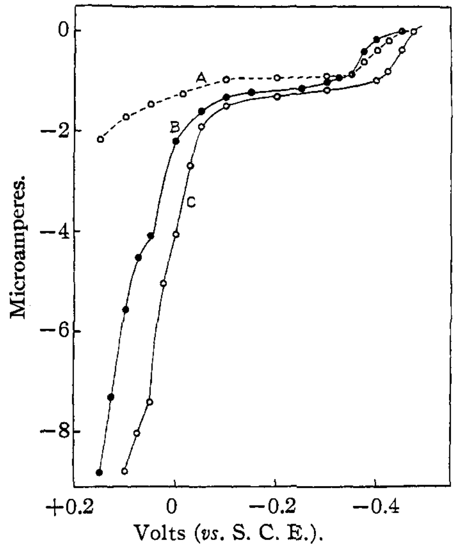

Fig. 2.—Curve A, 0.00025 *M* cysteine at *p*H 6.0; Curve B, 0.002 *M* cysteine at *p*H 4.73; Curve C, 0.002 *M* cysteine at *p*H 6.0.

The film formed during electrolysis is apparently soluble in strongly acid medium (*p*H 1) and also in alkaline medium. At a *p*H of 9.2 (borax) a discontinuity was still found in the c. v. curve (see Fig. 3), but the diffusion current was well defined, while in 0.1 *N* sodium hydroxide a normal wave was obtained until the diffusion current was reached. Slight irregularities were found, however, when the potential became more positive than −0.4 volt in 0.1 *N* sodium hydroxide.

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(7) D. Ilkovic, *Collection Czechoslov. Chem. Commun.*, **6**, 498 (1934).

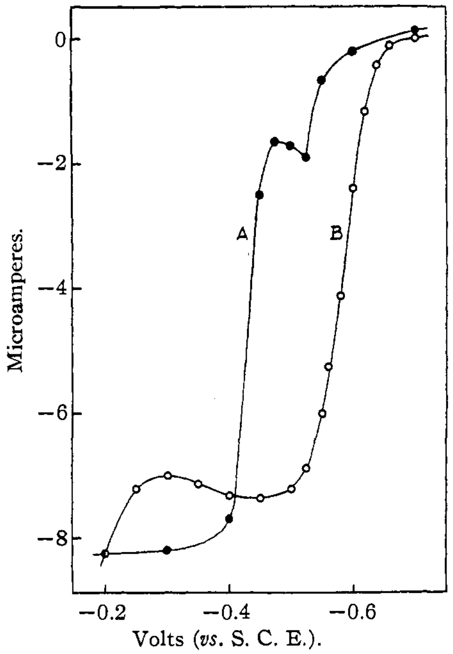

Fig. 3.—Curve A, 0.002 *M* cysteine at *p*H 9.2; Curve B, 0.002 *M* cysteine in 0.1 *N* sodium hydroxide.

Current–Voltage Curves Obtained at a Platinum Micro Wire Electrode.—The same technique was used as given by Laitinen and Kolthoff.[4] Measurements were recorded after the current had reached a constant value (about five minutes after the application of a particular e. m. f.). A typical c. v. curve at a *p*H of 2 is given in Fig. 4 and for comparison a c. v. curve obtained at the same *p*H with the dropping mercury electrode is included. The c. v. curve obtained with the platinum electrode showed an indication of reaching a diffusion current but abnormalities occurred when the potential was made more positive than +0.7 volt. The striking difference between the curves obtained with the platinum and the dropping mercury electrodes is that the wave at the platinum electrode started at a potential about 0.6 volt more positive than did the wave at the dropping mercury electrode. Apparently, the reaction occurring at the platinum electrode involves an oxidation of cysteine to cystine while the reaction occurring at the mercury electrode involves the formation of a mercurous cysteinate as will be shown in the next section.

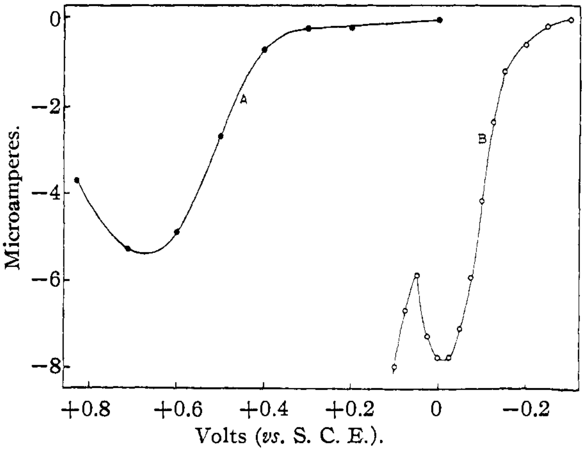

Fig. 4.—0.002 *M* cysteine at *p*H 2.0: Curve A, platinum micro wire electrode; Curve B, dropping mercury electrode.

Discussion

From a mathematical analysis of the wave at the dropping mercury electrode it should be possible to get an indication of the mechanism of the anodic wave of cysteine. If cysteine were oxidized to cystine and the reaction were reversible

$$2\mathrm{RSH} \rightleftarrows \mathrm{RSSR} + 2\mathrm{H}^+ + 2e \qquad (1)$$

the potential, π, of the electrode at each point on the wave should be given by

$$\pi = \text{const.} + \frac{RT}{F}\ln [\mathrm{H}^+]_0 + \frac{RT}{F}\ln \frac{[\mathrm{RSSR}]_0}{[\mathrm{RSH}]_0^2} \qquad (2)$$

The subscript zero denotes concentrations at the surface of the mercury drop (concentrations are written here instead of activities). Since all measurements have been carried out in well-buffered solutions, $[\mathrm{H}^+]_0$ is equal to the hydrogen ion concentration in the bulk of the solution at all points on the wave. It is easily shown[8] that at each point on a wave

$$i = k([\mathrm{RSH}] - [\mathrm{RSH}]_0) \qquad (3)$$

in which [RSH] is the concentration of cysteine in the bulk of the solution. When the diffusion current i_d is reached

$$i_d = k[\mathrm{RSH}] \qquad (4)$$

From equations (3) and (4) it follows that

$$[\mathrm{RSH}] = (i_d - i)/k \qquad (5)$$

The concentration of the oxidation product at the electrode should be proportional to the current

$$[\mathrm{RSSR}]_0 = k'i \qquad (6)$$

Hence, if equation (1) represents the mechanism, the wave should be determined by

$$\pi = \text{const.} + \frac{RT}{F}\ln [\mathrm{H}^+] + \frac{RT}{2F}\ln \frac{i}{(i_d - i)^2} \qquad (7)$$

or at a constant *p*H

$$\pi = \text{const.} - \frac{RT}{2F}\ln \frac{(i_d - i)^2}{i} \qquad (7a)$$

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[8] J. Heyrovsky and D. Ilkovic, *Collection Czechoslov. Chem. Commun.*, **7**, 198 (1935).

It also is possible that the oxidation of cysteine to cystine occurs with the intermediate formation of a free radical, RS. If the first reaction were reversible

$$\mathrm{RSH} \rightleftarrows \mathrm{RS} + \mathrm{H}^+ + \mathrm{e} \qquad (8)$$

$$2\mathrm{RS} \longrightarrow \mathrm{RSSR} \qquad (9)$$

we would derive the following equation of the wave

$$\pi = \text{const.} + \frac{RT}{F}\ln\,[\mathrm{H}^+] - \frac{RT}{F}\ln\frac{(i_\mathrm{d} - i)}{i} \qquad (10)$$

In the introduction it has been mentioned that Barron, Flexner and Michaelis[3] found that upon interaction of cysteine with various forms of mercury a mercuric cysteinate, $\mathrm{Hg(SR)_2}$, is formed.

$$\mathrm{Hg} \rightleftarrows \mathrm{Hg}^{++} + 2\mathrm{e} \qquad (11)$$

$$\mathrm{Hg}^{++} + 2\mathrm{RSH} \longrightarrow \mathrm{Hg(SR)_2} + 2\mathrm{H}^+ \qquad (12)$$

Without going through the various steps, it may be said that the equation of the wave corresponding to such a reaction is similar to expression (7).

Finally, the reaction of cysteine at the mercury drop may lead to the formation of mercurous cysteinate. If the latter has the composition $\mathrm{Hg_2(SR)_2}$, the equation of the wave again would be the same as given by equation (7). On the other hand, if the composition would correspond to HgSR, equation (10) should fit the analysis of the polarographic wave. In the experimental part of this paper it has been shown that a normal anodic wave was obtained only at a *p*H of 1 (see Fig. 1). Hence, only this wave lends itself to accurate analysis. If equation (7) describes the wave, then a plot of π against log $(i_\mathrm{d} - i)^2/i$ should yield a straight line with a slope of 0.059/2. On the other hand, if equation (10) determines the wave, the plot of π against log $(i_\mathrm{d} - i)/i$ should yield a straight line with a slope of 0.059. From Fig. 5 it is evident that only the analysis corresponding to equation (10) yields a straight line and that this line has a slope equal to 0.056, which is in good agreement with the calculated value of 0.059. Furthermore, it was found that the half-wave potential, that is, the potential at which $i = i_\mathrm{d}/2$, remained constant when the concentration of cysteine varied from 0.00025 to 0.002 *M*. This constancy of the half-wave potential is a characteristic of equation (10) but not of equation (7). Also the wave obtained with cysteine in 0.1 *N* sodium hydroxide (see Fig. 3), which closely approximates a normal polarographic wave, analyzes to give a straight line for the plot of π against log $(i_\mathrm{d} - i)/i$ but in this case the slope was found equal to 0.049 instead of 0.059.

These analyses indicate that the anodic reaction of cysteine at the dropping mercury electrode occurs either according to equations (8) and (9) or by the formation of mercurous cysteinate of the composition HgSR. The fact that oxidation at the platinum electrode occurs at much more positive potentials than does the anodic reaction at the dropping mercury electrode makes it probable that HgSR is formed at the mercury electrode. The interference caused by film formation also points to this conclusion. To obtain more conclusive evidence of the product of the anodic reaction, a solution of cysteine was electrolyzed at

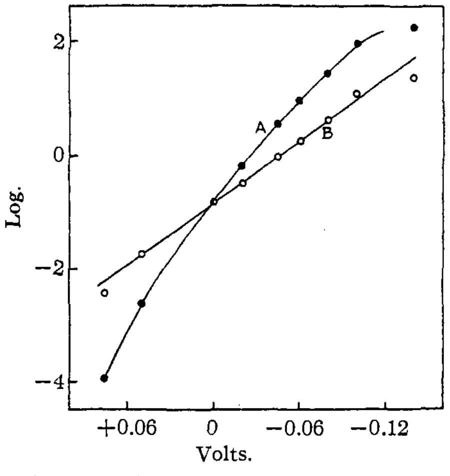

Fig. 5.—Analyses of wave of 0.002 *M* cysteine at *p*H 1.0: A, plot of π *vs.* log $(i_\mathrm{d} - i)^2/i$; B, plot of π *vs.* log $(i_\mathrm{d} - i)/i$.

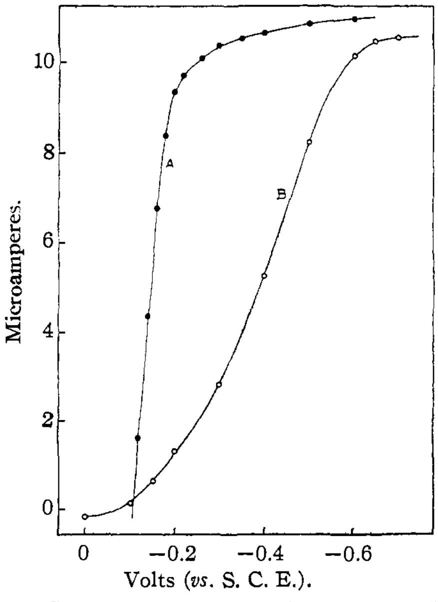

Fig. 6.—Curve A, cysteine solution after anodic electrolysis at a mercury electrode at *p*H 1.0; Curve B, 0.0015 *M* cystine at *p*H 1.0.

+0.1 volt (to the saturated calomel electrode) in the absence of oxygen at a large, quiet mercury electrode for seven and one-half hours. When hydrogen sulfide was passed through this solution after electrolysis, a black precipitate formed, showing the presence of soluble mercury in the solution. Also a portion of this electrolyzed solution was analyzed at the dropping mercury electrode and a cathodic wave was obtained which was not at all similar to the cathodic wave given by cystine (see Fig. 6), but corresponded to the reversible reduction of mercury from a complex compound. From the evidence obtained we feel justified in concluding that the product of the anodic reaction of cysteine at the dropping mercury electrode is a mercurous cysteinate of the composition HgSR.

This is the first case in the literature indicating that dissolved mercurous mercury can exist as Hg^+ instead of as Hg_2^{++}. In agreement with the literature we conclude from polarographic investigations carried out in this Laboratory with mercurous nitrate solutions that at mercurous ion concentrations greater than 10^{-5} *M* practically all of the mercury is present as Hg_2^{++}. However, it should be realized that with decreasing total mercurous ion concentration the equilibrium $Hg_2^{++} \rightleftarrows 2Hg^+$ is greatly shifted to the right. The mercurous ion concentration of the mercurous cysteinate can be calculated to be of the order of 10^{-20} *M* and at this small concentration practically all of the mercurous mercury may be present as Hg^+.

Summary

1. Cysteine can be determined with the dropping mercury electrode in a medium of 0.1 *M* perchloric acid. The diffusion current was found proportional to the concentration of cysteine. The diffusion coefficient of cysteine was calculated to be 3.10×10^{-5} cm.²/sec. at 25°. The half-wave potential in the above medium ($pH = 1$) was found to be −0.05 volt (to the saturated calomel electrode) and independent of the cysteine concentration.
2. At *p*H values greater than 2 the anodic waves were quite irregular. Over a wide range of potentials a small constant current was obtained which was smaller than the diffusion current. This abnormality is attributed to the formation of a film of HgSR.
3. The anodic waves of cysteine at the dropping mercury electrode do not correspond to the formation of cystine but rather to the formation of a mercurous compound, HgSR. The anodic reaction is given by the equation

$$RSH + Hg \rightleftarrows HgSR + H^+ + e$$

At a platinum electrode the anodic waves of cysteine occur at a potential about 0.6 volt more positive than at the mercury electrode and correspond to the formation of cystine.

4. Evidence is obtained that at mercurous ion concentrations of the order of 10^{-20} *M* practically all of the mercurous mercury is present in solution as Hg^+ and not as Hg_2^{++}.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DEPAUW UNIVERSITY]

The Composition of the Fatty Oil from *Carya Cordifornia* Nuts

BY J. L. RIEBSOMER, ROBERT LARSON AND LAWRENCE BISHMAN

Some studies have been made on the physical properties of oils from *Carya amarya* and *Carya ovata* nuts[1] but no work has been reported on *Carya cordifornia*. The quantity of *Carya cordifornia* oil available is relatively small, which accounts for the lack of interest. The nuts from these trees are from two to four centimeters in diameter. The shell is hard and in color quite similar to the English walnut. The meat from these nuts has a bitter taste and even animals refrain from eating them. This bitter material is not extracted with the oil.

Experimental

The nuts used for this analysis were collected in central Indiana in November, 1938. The nuts were ground (shell included), dried and extracted with petroleum ether (b. p. 30–60°). The moisture content of the ground nuts was 4.1%, and the petroleum ether extract which was a reddish brown oil was 27% calculated on the basis of the dried nuts.

The physical and chemical characteristics of the ex-

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(1) Peterson and Bailey, *J. Ind. Eng. Chem.*, **5**, 739 (1913).