While the very structural features which render detergents protein precipitants may likewise be involved in their denaturing action, the two phenomena may be discerned under certain conditions. Thus, while for anionic detergents precipitation is confined to the acid side of the isoelectric point, denaturation, as revealed by viscosity measurements,[43] occurs to about the same extent in both acid and alkaline regions, with detergents of both the anionic and cationic types. Moreover, while relatively low concentrations of detergents exert a high denaturing action on proteins,[1,5] this effect increases with detergent concentration in the region of detergent excess.[43]

The properties of serum albumin recovered from the precipitated complex approximate more closely those of the protein regenerated from concentrated urea or guanidine hydrochloride solutions[13,44] than they do those of the native material. Since precipitation by SDS leads to changes in properties which may not be wholly reversed even upon subsequent dissociation of the protein-detergent complex, the recovered protein must be denatured according to the general definition proposed for denaturation.[45] However, it is the degree of denaturation that varies with detergent concentration, regardless of whether the combination between protein and detergent leads to precipitation.

While the application of detergents to the isolation of proteins is restricted by the degree of denaturation incurred, nevertheless, their use may be warranted in specific instances, even in the preparation of biologically *active* materials. For example, the serological activity of antibodies[46] or the hormonal activity of insulin[47] have been shown to be rather insensitive to even high degrees of denaturation. Also, under suitable conditions detergents may be employed in the preparation of protein-free filtrates or of regenerated proteins. Since precipitation of a given protein ceases at the isoelectric point, detergents may conceivably be adapted to the separation of protein mixtures under conditions at which the components carry opposite charges, *e. g.*, serum proteins. This aspect of the problem is now being investigated in this Laboratory.

The authors are indebted to the Rockefeller Foundation, to the Lederle Laboratories, Inc., and to the Duke University Research Council for support of this work.

Summary

Investigation of the effects of synthetic detergents on proteins has revealed that anionic detergents precipitate proteins only in the cationic form, precipitation ceasing above the isoelectric point of the protein.

Quantitative study of the system crystalline horse serum albumin-sodium dodecyl sulfate has shown that precipitation is governed by the following factors: protein-detergent weight concentration ratio, *p*H, temperature and ionic strength. At low detergent concentrations the protein may be completely precipitated while in regions of detergent excess redispersion of the precipitate occurs.

Dissociation of the protein-detergent complex by means of barium salts yields a protein which, as indicated by diffusion, viscosity and electrophoresis, is in a regenerated rather than in the native state.

The mechanism of precipitation and the potential application of detergents to the preparation and separation of proteins have been considered.

(43) J. O. Erickson, F. W. Putnam and H. Neurath, unpublished observations; see also ref. 1.

(44) D. G. Sharp, G. R. Cooper, J. O. Erickson and H. Neurath, *J. Biol. Chem.*, **144**, 139 (1942).

(45) H. Neurath, J. P. Greenstein, F. W. Putnam and J. O. Erickson, *Chem. Rev.*, in press.

(46) J. O. Erickson and H. Neurath, *Science*, **98**, 284 (1943).

(47) A. Rothen, B. F. Chow, R. O. Greep and H. B. van Dyke, *Cold Spring Harbor Symposia Quant. Biol.*, **9**, 272 (1941).

DURHAM, N. C. RECEIVED DECEMBER 31, 1943

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF DELAWARE, AND THE AMMONIA DEPARTMENT OF E. I. DU PONT DE NEMOURS AND COMPANY, INC., WILMINGTON, DELAWARE]

Polarographic Examination of Carbonyl Compounds

BY JOHN M. LUPTON AND CECIL C. LYNCH

Although the polarographic reduction of many carbonyl compounds has been reported,[1] it is not possible to obtain reduction waves with saturated, unsubstituted ketones. This fact led us to the study of methods by which carbonyl compounds may be examined polarographically.

In searching for an environment in which carbonyl reaction products would give polarographic waves, several reagents were tested. In acid hydrazine solution,[2] ketones give double waves as shown in Fig. 1. Aldehydes give similar waves. The half-wave potentials vary somewhat with concentration, but for ketones the first wave occurs near -1.1 v. (*vs.* S. C. E.); for aldehydes, near -0.9 v. For both aldehydes and ketones the half-wave potential of the second wave is in the

(1) I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1941.

(2) The polarographic reduction of the hydrazonium and phenylhydrazonium ions has not been investigated. Since they do not appreciably alter the hydrogen discharge in hydrochloric and sulfuric acid solutions (0.1 *N*) their half-wave potentials must be more negative than -1.5 v.

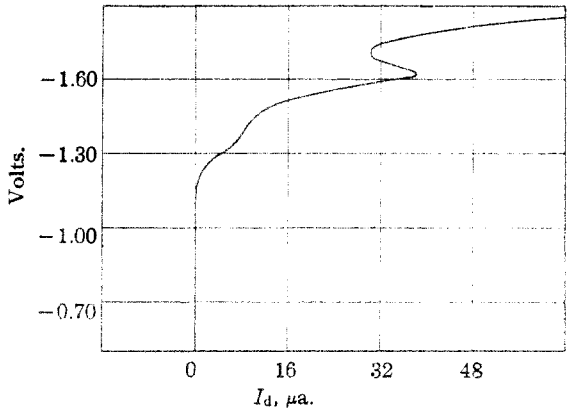

Fig. 1.—The reduction of methyl *n*-amyl ketone (0.006 *M*) in hydrazine solution.

range −1.3 to −1.4 v. The carbonyl compound reacts with the hydrazine solution, forming the hydrazone ($RR'CNNH_2$), the azine ($RR'CNNCRR'$), or a mixture of the two. The constants governing the relative quantities of the hydrazone and the azine produced under these conditions have not been determined here. The data indicate that a state of equilibrium exists in the system.

Acetone and *i*-butyraldehyde give reduction waves in acid phenylhydrazine[2] solution closely similar to those obtained in hydrazine solution, but the difficulty of obtaining and storing pure phenylhydrazine hydrochloride made the hydrazine reagent more satisfactory.

No carbonyl reduction waves were found with solutions of *i*-butyraldehyde and of methyl *i*-butyl ketone in ammonium chloride–hydrochloric acid solutions. Isobutyraldehyde gave no reduction wave in alkaline sodium sulfite solution. In slightly acid and neutral sodium sulfite solution only the bisulfite waves were observed.

Experimental

Work contained in this paper is limited to the investigation of the relationship between diffusion current and concentration in acid hydrazine solution for the following carbonyl compounds: isobutyraldehyde, acetone, methyl *i*-butyl ketone, methyl *n*-amyl ketone.

Apparatus.—The Leeds and Northrup Electrochemograph was used in obtaining all polarograms. Supplementary potential measurements were made with the Leeds and Northrup Universal *p*H Indicator.

The capillary tip was a piece of Pyrex capillary tubing about 0.05 mm. inner diameter, 6 mm. outer diameter, 8 cm. long. The mercury reservoir and regulating device was similar to that described by Furman, Bricker and Whitesell[3] except that connection to the capillary was made by a standard taper 10/30 ground joint. A typical value for the product $m^{2/3}t^{1/6}$ was 1.745 mg.$^{2/3}$ sec.$^{1/6}$, corresponding to a mean chloride diffusion current $i_d' = 5.59\ Ma$, at 0.00100 *M*.

A mercury pool, never less than 3 sq. cm. in area, was used as the anode. The potential of this electrode in the standard hydrazine solution was 0.22 v. *vs.* the saturated calomel electrode. To convert potentials to the saturated calomel electrode base, 0.22 v. must be subtracted from observed values.

A large portion of the polarograms were run using the cell and transfer system shown in Fig. 2. A definite volume of supporting electrolyte solution, usually 25 ml., was added to a weighing buret. A stream of water-saturated nitrogen was passed through the liquid for twenty minutes to remove oxygen. After oxygen removal, the buret was opened briefly for the weighed addition of carbonyl compound, and shaken.

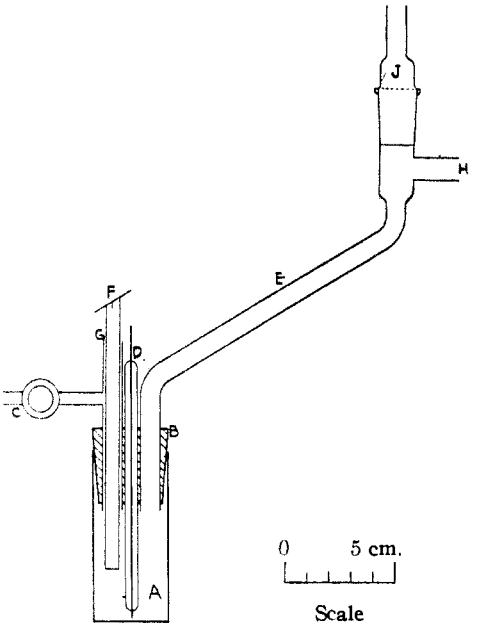

Fig. 2.—Cell and transfer system.

The cell (Fig. 2-A), the bottom covered with a layer of mercury, was fitted tightly over the #5 rubber stopper (B) through which were sealed auxiliary connections: a vent (C) equipped with a stopcock, a glass-covered connection to the mercury pool (D), and a filling tube (E). The dropping mercury electrode (F) entered the cell through the vent to which it must be sealed (G) by a rubber sleeve or by a sealing compound. Nitrogen under a pressure of 15 cm. of water was connected to the filling tube at the hose connection (H) and by the adapter (J).

After assembling the system the vent was opened and the system flushed with nitrogen for about five minutes. The vent was then closed, the ground glass joint opened, and the weighing buret, containing the sample, rapidly inserted between the adapter (J) and the filling tube. The cell and sample were protected from oxygen during this operation by the stream of nitrogen issuing from the filling tube and the adapter. When the buret stopcock was opened the sample solution could flow into the cell.

This system permitted mixing and transfer of solutions without serious absorption of oxygen and without serious loss of volatile carbonyl compounds.

Other runs were made using a large cell (capacity, 25 to 50 ml.) covered with a #10 rubber stopper. The dropping mercury electrode, the connection to the mercury pool, an aeration tube, a vent, and two standard taper 10/30 ground joints, outer members, were sealed into the stopper. A buret, 5 × 0.02 ml. capacity, fitted into one ground joint. The other served as the bearing for a paddle stirrer which could be turned manually through about 60° of angular rotation. In using this cell a definite volume of supporting electrolyte was added and the cell tightly closed except for the vent. Oxygen was removed by bubbling with water-saturated nitrogen. Aeration was then stopped leaving nitrogen at a pressure of about 2 inches of water connected to the vent. A blank was run, then carbonyl solution (in

(3) N. H. Furman, C. E. Bricker and E. B. Whitesell, *Ind. Eng. Chem., Anal. Ed.*, **14**, 333 (1942).

oxygen-free standard supporting electrolyte) was added from the buret, mixed by the stirring paddle with the liquid in the cell, and run. By this means a series of results could be obtained without changing the cell.

The actual cells used corresponded to the above descriptions except that some were provided with water jackets for temperature control. Cells without jackets were controlled by immersion in thermostat water. All polarograms were run at 25.0 ± 0.2°.

Purity and Purification of Materials.—The inorganic reagents were of reagent grade and were used without further purification. Polarographic tests indicated that no significant quantity of impurity was present. The hydrazine sulfate (Baker Analyzed) was assumed to be pure. Sulfuric and hydrochloric acids were analyzed before use. Mercury was of a grade suitable for polarographic work.

Merck Reagent acetone, b. p. 55.5–57.5°, was used without further purification. Other carbonyl compounds were of C. P. or technical grade and were carefully purified in a 3 foot by 0.5 inch rectifying column packed with Fenske rings. Fractions were considered to be pure when they condensed within 0.1° of the accepted boiling point at reflux ratios exceeding 20/1.

Results

Diffusion current values for the four carbonyl compounds were determined over the concentration range 0.0001 to 0.01 M and are summarized in Table I and in Figs. 3 and 4. In this table the data are represented through second degree equations of the type $R = AC + BC^2$. All results were obtained with a standard supporting electrolyte solution having the composition: hydrazine sulfate 0.100 M, sulfuric acid 0.0500 M, hydrochloric acid 0.00100 M. Higher concentrations of hydrazine sulfate were impractical, due to solubility considerations. In this solution the ratio, total electrolyte/carbonyl, falls below the conventional minimum, 50, when the carbonyl concentration exceeds 0.006 M. This would normally lead to coulomb effects. Since, however, diffusion current values are consistent over the concentration range 0.0001 to 0.010 M (i_d/C is a linear function of C) coulomb forces do not appear to be appreciable. Control experiments showed that thirty minutes must be allowed for reaction of the carbonyl compounds with this solution.

The method is useful at concentrations below 0.0001 M, but the configuration of the waves is such that a highly empirical method for determination of the diffusion current must be used. Because of the large maximum in the second carbonyl wave quantitative measurements of its diffusion current were not attempted. The diffusion current of the first carbonyl wave was determined by locating the inflection point between the two carbonyl waves and by measuring the difference in current between the inflection point and the point at which the extrapolated residual current reaches the potential of the inflection point. The same method was used in evaluating the chloride diffusion current.

Diffusion currents were calibrated by the pilot-ion technique.[1] The carbonyl diffusion current, i_d, was divided by the diffusion current of chloride ion, i'_d, at 0.00100 M, giving the ratio, R. All results were expressed in terms of R but may be converted to absolute values by the tip data above and the corresponding average value for i'_d at 0.00100 M, $i'_d = 5.59$ μa.

$$i'_d/m^{2/3}t^{1/6} = 5.59/1.745 = 3.20$$
$$i_d = i'_dR = 3.20m^{2/3}t^{1/6}R$$

Use of the values for R below with other dropping electrodes and with other polarographs may be made by conversions to absolute values or by the pilot-ion technique.

Two attempts were made to predict total diffusion current for carbonyl mixtures (aldehydes and ketones do not give separate waves) from the

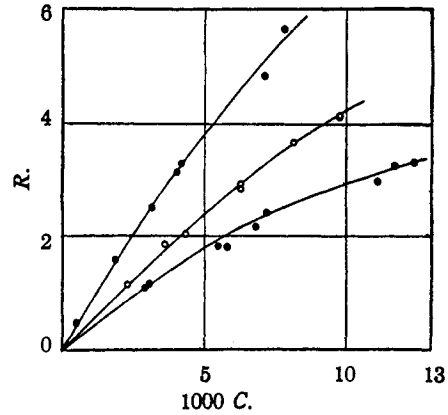

Fig. 3.—Reduction of aldehydes and ketones in hydrazine solution: ●, isobutyraldehyde; ○, acetone; ○, methyl *n*-amyl ketone.

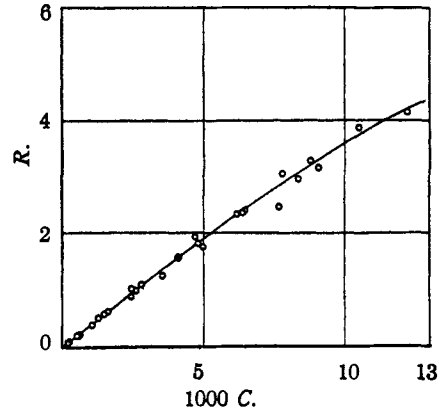

Fig. 4.—Reduction of methyl *i*-butyl ketone in hydrazine solution.

TABLE I

THE REDUCTION OF CARBONYL COMPOUNDS IN STANDARD HYDRAZINE SOLUTION: EMPIRICAL EQUATIONS RELATING R AND C. GENERAL EQUATION: $R = AC + BC^2$

Carbonyl compound	A	B	Mean deviation, %
Isobutyraldehyde	944	−36,300	4.5
Acetone	559	−15,100	2.4
Methyl *i*-butyl ketone	403	−4,960	4.9
Methyl *n*-amyl ketone	416	−12,900	4.4

empirical equations for diffusion current. In each case the experimental diffusion current was much lower than the calculated value.

Discussion.—The chemical nature of the material undergoing reduction at the dropping electrode is not clear. The double wave could result from the presence of two reducible species, but probably represents the stepwise reduction of a single species. The similarity of waves obtained in the hydrazine and in the phenylhydrazine solution (where the azine cannot form) suggests but does not prove that the hydrazone is the major product. In any case, mathematical analysis of the first carbonyl-hydrazine wave shows that the electroreduction is irreversible, a value of 0.62 electron transferred being found.

The relatively low diffusion currents obtained at high concentrations and with carbonyl mixtures suggest that equilibrium exists in the system. At very low carbonyl concentrations the hydrazine–carbonyl concentration ratio will approach infinity and the equilibrium, if it exists, will be displaced toward complete reaction. Under these conditions the term in C^2 will become negligible and the current will be determined principally by A and C. Thus the constant A is related to the diffusion coefficient and the irreversibility factors, while B is related to the equilibrium constant of the reaction or reactions.

The mean deviation of all values for R is about 4% and is about three times the mean deviation of values for the chloride diffusion current. The increased deviation is due to the carbonyl wave, possibly because the system has not been adequately controlled. It is more probable, however, that the increased deviation is due to difficulties in locating precisely the inflection point between the carbonyl waves and to the possibility that the recorded wave, resulting from a dynamic instrument, does not accurately or consistently represent the rapidly changing electrode processes in the region of an inflection point.

Summary

It has been shown that aldehydes and ketones can be analyzed polarographically in acid hydrazine solution. The relationship between diffusion current and concentration has been determined for one aldehyde and three ketones over the range 0.0001 M to 0.01 M, and has been expressed by second degree equations. The precision of the method varies between 2 and 5%.

NEWARK, DELAWARE RECEIVED JANUARY 3, 1944

[COMMUNICATION NO. 963 FROM THE KODAK RESEARCH LABORATORIES]

Oxidation Processes. XVII.[1] The Autoxidation of Ascorbic Acid in the Presence of Copper

BY A. WEISSBERGER AND J. E. LUVALLE

Introduction

In the preceding paper of this series we reported on the autoxidation of *l*-ascorbic acid at *p*H from 4.7 to 9.2, in the presence of sufficient cyanide and thiocyanate to suppress the catalytic action of metals. Under these conditions, both the monovalent and the divalent ion of the acid participate in the reaction. With oxygen of atmospheric pressure, the divalent ion reacts 10^5 times faster than the monovalent ion. If the partial pressure of the oxygen is reduced to 1/5, the reaction rate of the divalent ion sinks in proportion to the oxygen concentration, but not the reaction rate of the monovalent ion. The latter ion undergoes a slow process before it reacts with the oxygen, probably forming a reactive radical in the interaction with dehydroascorbic acid. In the absence of cyanide and thiocyanate and in the presence of copper, the reaction rate of the monovalent ion is proportional to the oxygen concentration.[1] This shows that the metal-catalyzed autoxidation of the monovalent ion is more rapid than the slow process just mentioned. The question arises whether the effect of the metal on the reaction of the monovalent ion explains fully the metal catalysis in the autoxidation of ascorbic acid, or whether the autoxidations of unionized ascorbic acid and divalent ion are likewise catalyzed by copper. This problem is treated in the present paper.

(1) Part XVI, Weissberger, LuValle and Thomas, THIS JOURNAL, **65**, 1934 (1943). (On p. 1936, right column, line 15, it should read 0.001 *m* mole instead of 0.001 mole.)

Materials and Methods

***l*-Ascorbic Acid.**—Eastman Kodak Co. *l*-ascorbic acid was used.

Water was redistilled in an all-Pyrex still.

Oxygen in cylinders (Linde) was used.

Sodium *p*-Phenolsulfonate.—Eastman Kodak Co. sodium *p*-phenolsulfonate dihydrate was recrystallized several times from distilled water, which for the last two recrystallizations had been redistilled as stated above.

All other chemicals were Baker Analytical or General Chemical Company Reagent Grades.

Apparatus and techniques were those described in the preceding papers of this series, using the two-chamber reaction vessel.[2] The temperature was 20.03 ± 0.02°. Each volume read was corrected to a barometric pressure of 760 mm. The reaction mixtures had a volume of 50 ml. The buffers, present in a concentration of 0.20 molar, were monopotassium phosphate, potassium hydrophthalate, sodium acetate, dipotassium phosphate and sodium *p*-phenolsulfonate. They were adjusted to the proper *p*H with nitric acid or potassium hydroxide.

(2) Weissberger, Mainz and Strasser, *Ber.*, **62**, 1942 (1929).