Further details of the method and some biological applications will be published elsewhere.

DEPARTMENT OF BIOCHEMISTRY
SCHOOL OF MEDICINE AND DENTISTRY L. A. LIBERMAN
UNIVERSITY OF ROCHESTER ALEJANDRO ZAFFARONI
ROCHESTER 20, N. Y. ELMER STOTZ

RECEIVED JANUARY 30, 1951

PHASE BOUNDARY POTENTIALS OF NICKEL IN FOREIGN ION SOLUTIONS[1]

Sir:

An investigation was started early in 1947 concerning the phase boundary potentials of inert metals in contact with solutions initially free from the common metal ions. The study of such systems, which remain relatively free from common ions, might contribute to a better understanding of the initial processes which induce corrosion of the metal. Our approach aims at very careful control of all experimental factors, since most metals show a greater or lesser tendency to interact with electrolyte solutions.

In a recent publication by El Wakkad and Salem,[2] the behavior of the potentials of mercury in buffer solutions initially free from mercury ions is discussed. An earlier article by Tourky and El Wakkad[3] dealt with an analogous investigation of the potentials of copper in foreign ion solutions.

Since in these laboratories work already has been done on several inert metals,[4] we believe that a preliminary account of our measurements on nickel may be of interest. Stable potentials could be obtained within 5 to 15 hours in a series of potassium hydroxide solutions, and in a series of phosphate buffer solutions covering the entire *p*H range. The stationary potentials, calculated against the standard hydrogen electrode, are plotted as a function of the *p*H of the solutions (Fig. 1).

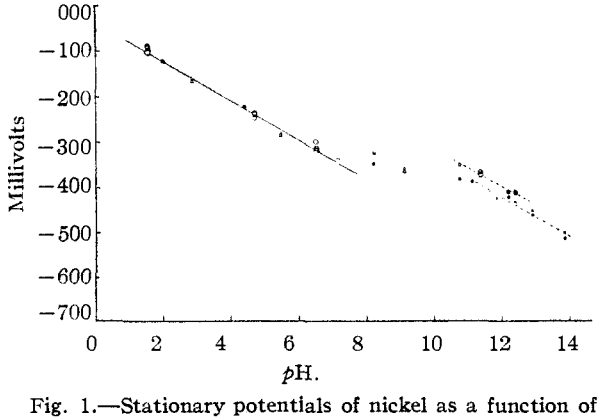

Fig. 1.—Stationary potentials of nickel as a function of *p*H in potassium hydroxide (× runs 4 and 5) and in 0.100 *M* phosphate solutions (O run XI, □ run XII, △ run XIV).

(1) This paper was presented before the General Meeting of the Division of Physical and Inorganic Chemistry of the American Chemical Society, Chicago, Ill., September, 1950.

(2) S. E. S. El Wakkad and T. M. Salem, *J. Phys. and Coll. Chem.*, **54**, 1371 (1950).

(3) A. R. Tourky and S. E. S. El Wakkad, *J. Chem. Soc.*, 740, 749 (1948).

(4) J. J. Singer, Jr., Ph.D. Dissertation, Clark University, 1949; J. H. Rosenbaum, Ph.D. Dissertation, Clark University, 1950.

Since aeration greatly accelerates the corrosion of nickel in acid media, oxygen was excluded from the half cells. Lengths of nickel wire of high purity were thoroughly cleaned and pretreated. The samples were freed from oxides and gases by induction heating first in hydrogen and then in a high vacuum. The solutions were freed from oxygen before making contact with the nickel samples. More details of the apparatus and the procedure will be given in a more extensive article. After each run the solutions were tested for nickel which might have gone into solution. The results were always negative, even in the acid phosphate solutions, unless oxygen had been admitted. The potentials were measured with a Leeds and Northrup K-2 potentiometer, using a Coleman electrometer as null point indicator.

Our graph of the nickel potentials in the phosphate buffers shows analogy with the curves obtained for copper[3] and mercury[2] in contact with a set of buffer solutions. In a more complete article we hope to discuss the interesting aspects both of an experimental and a theoretical nature.

Acknowledgment.—This work was supported by the Office of Naval Research to whom the authors express their appreciation.

DEPARTMENT OF CHEMISTRY D. MACGILLAVRY
CLARK UNIVERSITY J. J. SINGER, JR.
WORCESTER 3, MASSACHUSETTS J. H. ROSENBAUM

RECEIVED JANUARY 29, 1951

AN INTERMEDIATE IN THE CONVERSION OF FIBRINOGEN TO FIBRIN[1]

Sir:

When bovine fibrinogen and thrombin react in the presence of 0.4 *M* hexamethylene glycol (at *p*H 6.3, ionic strength 0.45), no clot is formed. However, the fibrinogen, whose sedimentation constant is about 9 *S*, appears to be gradually replaced by a new molecular species with a sedimentation constant of 25 *S*, which is evidently an intermediate polymerization product.[2] (All sedimentation constants given here are extrapolated to zero protein concentration.) We have now found very similar behavior with urea instead of glycol as the inhibitor. At *p*H 6.3 in 1.0 *M* urea, or at *p*H 7.5 in 2.35 *M* urea (ionic strength 0.15), sedimentation diagrams of a fibrinogen–thrombin system show, after 24 hours, two peaks; the sedimentation constant of one corresponds to that of unaltered fibrinogen, and the other is about 25 *S*.

Urea, unlike hexamethylene glycol, can in concentrated solution dissolve fibrin clots prepared in the absence of calcium and an unidentified serum factor.[3] A solution of fibrin in 3.5 *M* urea at *p*H 7.5, ionic strength 0.15, shows a single component in the ultracentrifuge with a sedimentation constant of 8 to 9 *S*; and its intrinsic viscosity is the same as that of fibrinogen, so that the fragments appear to

(1) This is paper 4 of a series on "The Formation of Fibrin and the Coagulation of Blood" from the University of Wisconsin, supported in part by research grants from the National Institutes of Health, Public Health Service. Grateful acknowledgment is made also of a grant from Eli Lilly and Company.

(2) S. Shulman and J. D. Ferry, *J. Phys. Coll. Chem.*, **55**, 135 (1951).

(3) E. Mihályi, *Acta Chem. Scand.*, **4**, 344 (1950); L. Lóránd, *Nature*, **166**, 694 (1950).

be very similar to fibrinogen molecules in size and shape. But when the urea concentration is reduced to 2.35 *M*, two peaks appear in the sedimentation diagram, with constants of 9 and 25 *S*.

The presence of the fast peak, both in partly polymerized fibrinogen inhibited by glycol or urea, and in depolymerized fibrin in urea, is always accompanied by a high viscosity which depends markedly on the rate of shear. The reduced specific viscosity falls rapidly with dilution of the protein, however, approaching that characteristic of the original fibrinogen, indicating dissociation of long linear aggregates.

The behavior of the intermediate represented by the fast peak is thus the same whether it is formed from fibrinogen by the action of thrombin or from urea-depolymerized fibrin by decreasing the concentration of urea.[4]

We are much indebted to Professor J. W. Williams for use of the Svedberg oil turbine ultracentrifuge.

(4) In general agreement with the experience of Mihályi,[5] there was no evidence, under the conditions of our experiments, of the denaturation which is observed[5] at somewhat higher urea concentrations or temperatures (or lower *p*H). Fibrinogen in 2.35 *M* urea had the same intrinsic viscosity as in the absence of urea; and the solubility of fibrinogen was not impaired by contact for 18 hours with 3.5 *M* urea, at *p*H 7.5, room temperature, and subsequent removal of the urea by dialysis. These criteria are of course not applicable to fibrin, but the susceptibilities of fibrinogen and fibrin to denaturation should be similar. Also, the viscosity of a fibrin solution in 3.5 *M* urea at *p*H 6.3 showed no change with time for two days, indicating that no progressive changes were taking place.

(5) E. Mihályi, *Acta Chem. Scand.*, **4**, 317 (1950).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN
SIDNEY SHULMAN
PAUL EHRLICH
JOHN D. FERRY

RECEIVED FEBRUARY 12, 1951

DIETHYL CHLOROARSENITE AS A REAGENT FOR THE PREPARATION OF PEPTIDES

Sir:

In the course of an investigation in these Laboratories of methods of peptide synthesis, new reagents for forming the peptide linkage at either the amino or carboxylic function of an aminoacid or peptide chain have been found. The use of diethyl chlorophosphite has been reported recently.[1] Similarly, diethyl chloroarsenite[2] reacts readily with aminoacid esters and with N-substituted aminoacids to give highly reactive amides and anhydrides, respectively. The new reagent has advantages over the phosphite analog in being stable and readily prepared. Comparable yields are obtained with either reagent.

Both the amides, $(C_2H_5O)_2AsNHCH(R)COOR'$, and the anhydrides, $R'NHCH(R)COOAs(OC_2H_5)_2$, are non-distillable oils which are conveniently prepared and reacted without isolation. The reactions are accomplished in an inert solvent in the presence of an equivalent of triethylamine as the acid acceptor. After removal of the precipitated triethylamine hydrochloride, the solution of the intermediate diethylarsenite amide or anhydride is refluxed one hour with an equivalent of a second N-substituted aminoacid or aminoacid ester, respectively. The by-product, presumably diethyl arsenite in both cases, is precipitated quantitatively as arsenic trioxide by addition of water.

The N-substituted peptide ester prepared by either of these procedures is obtained crystalline by first extracting the reaction solution successively with dilute sodium bicarbonate and dilute hydrochloric acid and then concentrating in an air stream. One crystallization from ethanol–water or ethyl acetate–petroleum ether generally has given pure products.

Prepared by the intermediate amide method were carbobenzoxyglycine anilide[3] (79%), m.p. 144–145°; carbobenzoxyglycine morpholide[4] (70%), m.p. 144–145°; ethyl carbobenzoxyglycyl-DL-phenylalanate[5] (59%), m.p. 91–92°; ethyl phthalyl-DL-alanyl-DL-valinate[4] (71%), m.p. 121–123°; and ethyl carbobenzoxyglycyl-L-tyrosinate (74%), m.p. 125–126° (a mixed m.p. with an authentic sample[6] was not depressed).

Prepared by the anhydride method were carbobenzoxyglycine anilide[3] (63%), m.p. 146–147°; ethyl carbobenzoxyglycyl-DL-phenylalanate[5] (52%), m.p. 92–93°; ethyl carbobenzoxy-DL-alanyl-DL-phenylalanate[4] (60%), m.p. 104–106°; ethyl carbobenzoxy-L-leucyl-DL-phenylalanate[4] (74%), m.p. *ca.* 90° $[\alpha]^{24}D$ −9.2° (*c* = 5, 95% ethanol) and ethyl carbobenzoxyglycylglycyl-DL-phenylalanate monohydrate[4] (30%), m.p. 80–82° (from ethyl phenylalanate and the diethyl arsenite anhydride from carbobenzoxyglycylglycine).

(3) Wieland and Sehring, *Ann.*, **569**, 122 (1950).
(4) Carbon, hydrogen and nitrogen analyses were satisfactory.
(5) Neurath, *et al.*, *J. Biol. Chem.*, **170**, 222 (1947).
(6) Bergmann and Fruton, *ibid.*, **118**, 412 (1937).

CHEMOTHERAPY DIVISION
STAMFORD RESEARCH LABORATORIES
AMERICAN CYANAMID COMPANY
STAMFORD, CONNECTICUT
JAMES R. VAUGHAN, JR.

RECEIVED FEBRUARY 7, 1951

CRYSTALLINE XYLOBIOSE AND XYLOTRIOSE

Sir:

Charcoal chromatography of partially hydrolyzed xylan permits the separation and isolation of a considerable amount of crystalline xylobiose and xylotriose. This is the first isolation of crystalline di- and trisaccharides composed only of pentose sugar units.

In one instance a 2% solution of xylan in 42% hydrochloric acid was hydrolyzed at 0° until the reaction was 66% complete as indicated by reducing value and by optical rotation. The hydrolyzate was neutralized with sodium bicarbonate and chromatographically separated on charcoal columns following the method of Whistler and Durso.[1] After washing the column with water, xylobiose was removed with 5% ethanol. The sirupy concentrate from this extraction was dissolved in a small amount of warm water and hot methanol added. On cooling, crystallization occurred. The yield was 4.8% of the xylan used, m.p. 186–187°; $[\alpha]D^{25}$ −32.0→−25.5 (1 hour) (*c*, 1 in water).

(1) Anderson, Welcher and Young, THIS JOURNAL, **73**, 501 (1951).
(2) McKenzie and Wood, *J. Chem. Soc.*, **117**, 406 (1920).

(1) Roy L. Whistler and Donald F. Durso, THIS JOURNAL, **72**, 677 (1950).