to be 3% per degree. A small maximum was found in the reduction wave but this did not interfere in the dilute solutions used in this study.

Acknowledgments.—The author thanks Mr. Alexander Kaczmarczyk for running the ultraviolet spectra and Dr. R. C. Roark for supplying the anabasine. The author is indebted to the Research Corporation for support which made this work possible.

BEIRUT, LEBANON

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, INSTITUTE OF POLYMER RESEARCH, POLYTECHNIC INSTITUTE OF BROOKLYN]

Ultraviolet and Infrared Spectral Studies of Polyvinylpyrrolidone[1]

BY GERALD OSTER AND E. H. IMMERGUT

RECEIVED JULY 20, 1953

The ultraviolet spectra of polyvinylpyrrolidone (PVP), N-ethylpyrrolidone and N-vinylpyrrolidone are markedly dependent on *p*H at extreme *p*H values. N-Vinylpyrrolidone has a very pronounced absorption maximum at 235 mμ which is present only to a minute extent in the spectrum of the polymer and this is used as a method to follow the conversion of monomer to polymer during polymerization. Iodine exhibits strong interaction with PVP as shown by the alteration in the iodine spectrum at 290 mμ. Infrared spectra of PVP, N-ethylpyrrolidone and N-vinylpyrrolidone have similar absorption bands but the last-named compound also has a strong absorption at 6.13 μ corresponding to the vinyl group. The absorption peak of PVP at 5.96 μ is depressed on addition of KI.

Introduction

One of the most interesting products resulting from Reppe's technique for the handling of acetylene under pressure is polyvinylpyrrolidone (PVP). The importance of this water-soluble polymer lies not only in its usefulness as a blood plasma extender but also in the fact that like serum albumin, PVP binds certain drugs, dyes and toxins. Thus PVP acts as a carrier or vehicle for various substances in the blood stream and has been used as a retardant for drugs and as an eliminant for toxins.[2]

In particular, PVP combines strongly with anionic dyes of the fluorescein family, the binding increasing with increasing number and polarizability of the substituted halogens on the dye molecule.[3a,b] Similar binding properties have been observed for native serum albumin and for other proteins in the denatured state.[4-6] Of great practical importance in preventive medicine is the affinity of molecular iodine for PVP, since the resulting PVP–iodine complex retains the disinfectant properties of the iodine but eliminates its toxicity.

It is the purpose of the present paper to describe the ultraviolet and infrared spectra of PVP and closely related substances in an attempt to explain the peculiar binding properties of PVP.

Experimental

Two PVP samples, both of a number average molecular weight of about 40,000, were kindly supplied by the General Aniline and Film Corporation and by Schenley Laboratories. N-Vinylpyrrolidone (b.p. 96° at 14 mm.) and N-ethylpyrrolidone (b.p. 104° at 20 mm.) were supplied by the General Aniline and Film Corp.

Ultraviolet spectra were determined in a Beckman spectrophotometer. The fine structure illustrated in Fig. 1 was confirmed in a Carey recording spectrometer. The ultraviolet absorption is given in terms of optical densities per cm. path length; fused quartz cells one centimeter in path length were used throughout. Infrared spectra were determined on a Perkin–Elmer double beam spectrometer. Aqueous solutions of PVP were evaporated on silver chloride plates. Since no attempt was made to control the thickness of the films, the transmission data are given in arbitrary units. A sodium chloride liquid cell was employed for N-vinylpyrrolidone and N-ethylpyrrolidone.

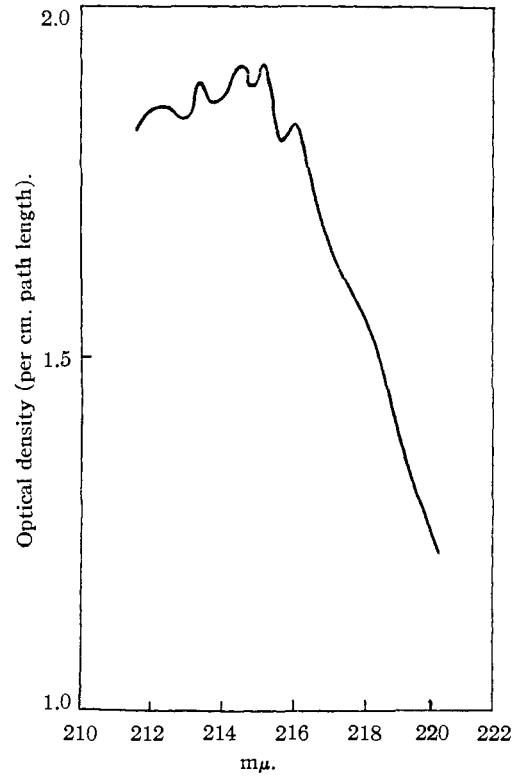


Fig. 1.—Ultraviolet spectrum of PVP in water (0.02%).

Results and Discussion

The fine structure of the ultraviolet spectra of PVP in water at relatively high concentrations (Fig. 1) is entirely reproducible. Very few poly-

(1) Presented at the 121st Meeting of the American Chemical Society, Buffalo, New York, April 19, 1952.

(2) "PVP-Polyvinylpyrrolidone," compiled and published by General Aniline and Film Corp., New York, 1951. This book contains abstracts of all the published work on PVP up to 1950.

(3) (a) G. Oster, *J. Polymer Sci.*, **9**, 553 (1952); (b) G. Oster and A. H. Adelman, data to be published.

(4) I. M. Klotz and J. M. Urquhart, THIS JOURNAL, **71**, 847 (1949).

(5) G. Oster and H. Grimmson, *Arch. Biochem.*, **24**, 119 (1949).

(6) G. Oster, *J. chim. phys.*, **48**, 217 (1951).

mers exhibit fine structure in ultraviolet spectra unless the chain unit is of sufficiently complicated structure. On dilution of the PVP solution with water the spectrum is shifted to shorter wave lengths and below a weight concentration of about 0.002% the maximum has apparently shifted to a wave length below 210 mμ and cannot be recorded on our instrument.

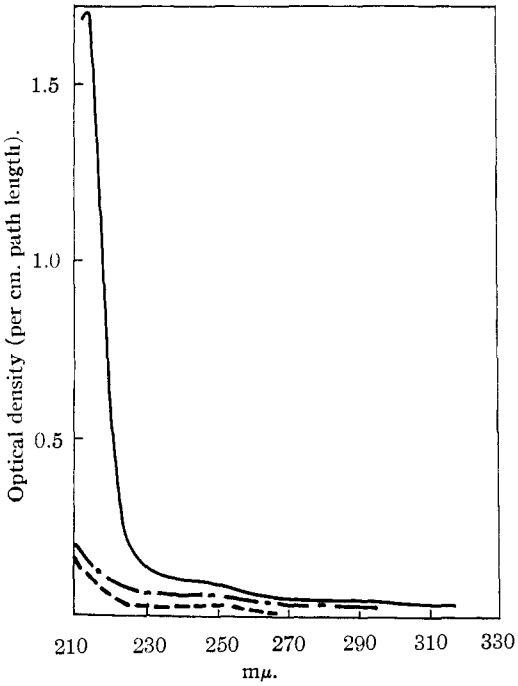
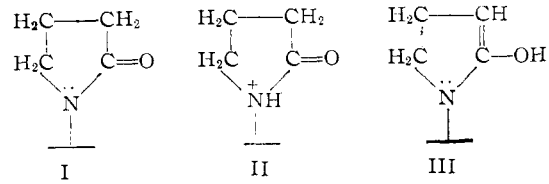


Fig. 2.—Ultraviolet spectrum of PVP (0.001%) at *p*H 0.8 (- - - -); at *p*H 5.0 (—— -); at *p*H 12.0 (——).

The spectrum of PVP is sensibly independent of *p*H except at extremes of *p*H (Fig. 2), HCl being used for the low *p*H and NaOH for the high *p*H. A similar spectral dependence on *p*H is exhibited by N-ethylpyrrolidone and hence this behavior must be characteristic of the pyrrolidone ring. Electrophoretic studies[7] show that although PVP at *p*H 7 has almost zero mobility and hence is electrically neutral I, at extreme values of *p*H, PVP is charged. PVP is precipitated by trichloroacetic acid at very low *p*H (below *p*H 1) where the polymer is positively charged II and by $Ba(OH)_2$ at high *p*H where the polymer is negatively charged. At high *p*H values, a proton might conceivably be abstracted from the C atom adjacent to the C=O group. The resulting extra pair of electrons then could shift into the ring, giving the enolic form III with an accompanying shift of the spectrum to longer wave lengths.[8]



A feature of the PVP spectrum at ordinary *p*H values which is not present in the spectrum of N-ethylpyrrolidone is the small maximum at 235 mμ. This maximum is not present in all samples of PVP and is believed to be due to the presence of small amounts of unreacted vinyl groups. This is borne out clearly by the spectrum of N-vinylpyrrolidone (Fig. 3) which shows at neutral and high *p*H values a large peak at 235 mμ.

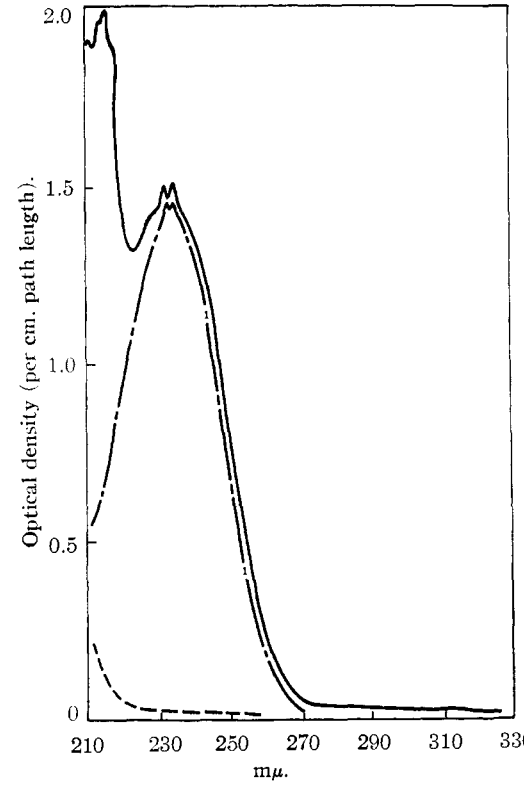


Fig. 3.—Ultraviolet spectrum of N-vinylpyrrolidone at *p*H 0.8 (- - - -); at *p*H 5.0 (—— -); at *p*H 12.0 (——).

The molecular extinction coefficient for the maximum of this peak is 13,500. The disappearance of this peak at low *p*H is probably due to the addition of a proton to the nitrogen of the pyrrolidone ring. This interrupts the cross-conjugation between the vinyl and carbonyl groups, the molecule as a whole becoming positively charged.

We have followed the polymerization of N-vinylpyrrolidone as a function of time by observing the decrease in absorption at 235 mμ. The polymerization was carried out by adding to a 20% aqueous solution of N-vinylpyrrolidone 0.08% by weight of potassium persulfate as polymerization catalyst. From time to time aliquot samples were diluted one thousandfold in water and the absorption was measured. It was found that the weight of polymer produced was close to that calculated from the decrease in absorption.

No appreciable alteration of the spectrum of PVP in water was observed on addition of various anions (other than hydroxyl). Molecular iodine has an absorption in the near ultraviolet which is considerably altered in the presence of PVP (Fig. 4). The alteration in absorption of molecular iodine at 290 mμ as a function of PVP concentration is illustrated

(7) R. A. Sullivan, F. M. Palermiti and R. Annino, Meeting-in-Miniature, New York Section of A. C. S., February 8, 1952, New York.
(8) *Cf.* E. Schauenstein, *Monatsh.*, **80**, 820 (1949).

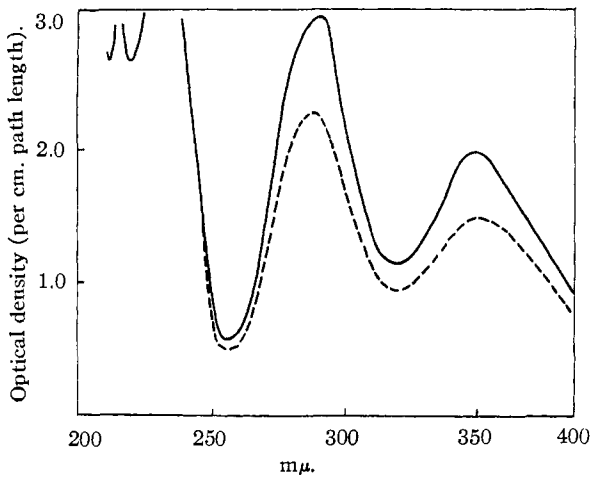


Fig. 4.—Ultraviolet spectrum of iodine (4×10^{-4} *N*) in the absence (dashed) and presence of 0.001% PVP (continuous).

in Fig. 5 and shows that for this concentration of iodine, all the iodine is bound at a concentration of PVP about 0.002%. The results of an experiment

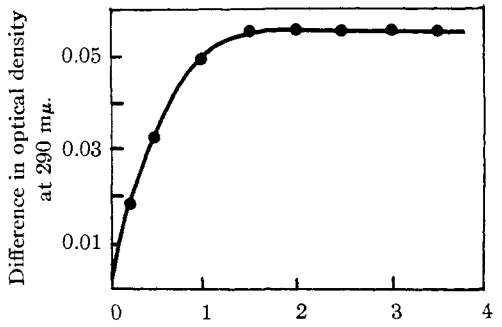


Fig. 5.—Difference of optical densities at 290 mμ of iodine–PVP mixtures and iodine alone as a function of PVP added to iodine.

in which the procedure was reversed are illustrated in Fig. 6. Now varying amounts of iodine are added to a fixed amount of PVP and the difference

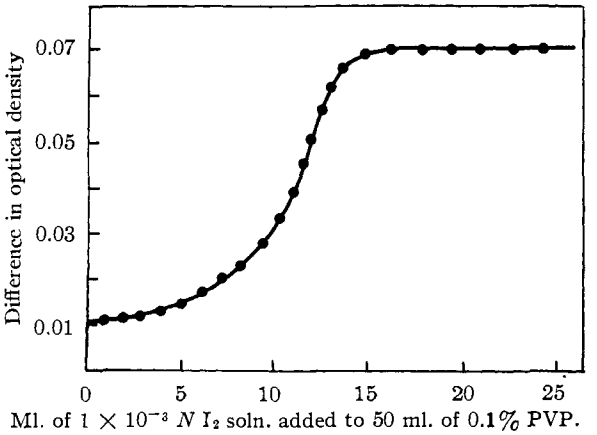


Fig. 6.—Difference of optical densities at 290 mμ of iodine–PVP mixtures and iodine alone as a function of iodine added to PVP.

in absorption at 290 mμ of the solution to that of the same concentration of iodine alone is determined. Figure 6 shows that in order to saturate PVP, 0.033 mole of iodine to one mole of pyrrolidone unit is required. The sigmoidal form of the isotherm suggests that the first few molecules of iodine are bound to PVP with difficulty but further iodine molecules are added more easily until the polymer is saturated. It might be postulated that the initially bound iodine induces a polarizable influence on the succeeding ones. Alternatively, in analogy to the reaction of iodine with amylose[9] and with polyvinyl alcohol,[10] one might suppose that the first few iodines induce a helical form to the polymer and that succeeding iodine atoms enter the interior of the PVP helix.

The binding of anions to PVP is probably due in part to van der Waals forces[2,3] and hence should be manifested by an alteration in the infrared spectra of PVP. This is borne out as shown in Figs. 7A and 7B. On addition of KI (one part in forty) to the polymer, the absorption maximum at 5.96 μ, identified as that due to the carbonyl group, is shifted to 6.00 μ and is decreased relative to the other absorption maxima.

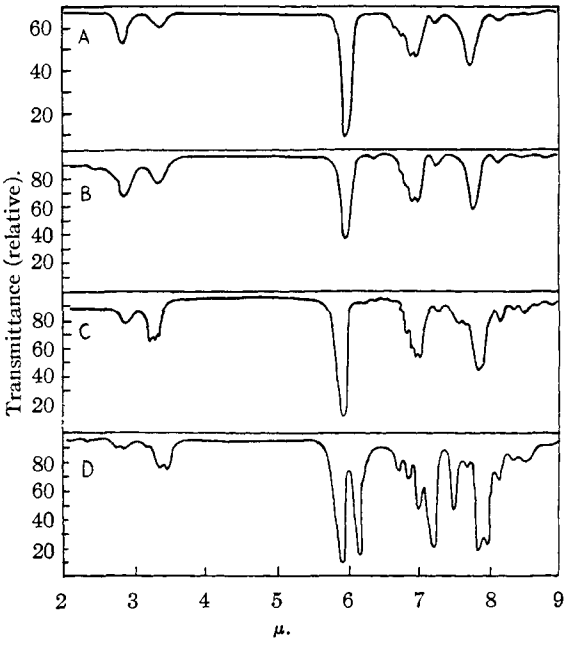


Fig. 7.—Infrared spectrum of: A, PVP; B, PVP plus KI (one part in forty); C, N-ethylpyrrolidone, D, N-vinylpyrrolidone.

The infrared spectrum of PVP bears a resemblance to that of N-ethylpyrrolidone (Fig. 7C) except that in the former the absorption maximum at 2.85 μ, probably due to bound water, is greater than the absorption maxima around 3.4 μ due to CH stretching, whereas with N-ethylpyrrolidone this is reversed. N-Vinylpyrrolidone, on the other hand, (Fig. 7D) shows a strong absorption at 6.13 μ. This absorption peak is due to the pres-

(9) R. E. Rundle and D. French, THIS JOURNAL, **65**, 1707 (1943).

(10) S. E. Sheppard and P. T. Newsome, *J. Chem. Phys.*, **12**, 513 (1944); C. D. West and E. H. Land, "Advances in Colloid Chemistry," Reinhold Publ. Corp., New York, N. Y., 1946.

ence of the vinyl group and appears to a small extent in PVP samples which exhibit an absorption peak in the ultraviolet at 235 mμ.

Acknowledgment.—We should like to express our thanks to Prof. F. R. Eirich for his interest and encouragement in this work. One of us (E. H. I.) was supported by the National Research Council and the Army Surgeon General's Office under contract number DA-49-007-MD III.

BROOKLYN, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

A Study of the Interaction of Dodecyl Sulfate with Bovine Serum Albumin[1,2]

BY M. J. PALLANSCH AND D. R. BRIGGS

RECEIVED AUGUST 3, 1953

The interaction of dodecyl sulfate with bovine serum albumin, in phosphate–sodium chloride buffer of *p*H 6.8 and ionic strength 0.2, was determined at 22° by the dialysis equilibrium method through a range of equilibrium free detergent concentrations of 0–80 × 10^{-5} *M*. The detergent was dissolved in the external buffer solution and allowed to equilibrate with protein in buffer solution inside a cellophane sac. The equilibrium detergent concentration in no case exceeded the critical micelle concentration of the detergent. Each equilibrated system was subjected to electrophoretic analysis at 20° using the external solution as the overlying solution and the protein solution as the underlying solution in the electrophoresis cell. Moles of bound detergent per mole of protein (*r*) covered a range from 0 to 40. At values of $r > 10$, the course of the interaction isotherm deviated greatly from its initial course, reflecting a greater binding capacity of the protein for the detergent than would be expected on the basis of the simple mass action interaction exhibited at *r*-values less than 10. Concomitantly there appeared a second, faster moving, boundary in the electrophoresis patterns. It is pictured that, as the *r*-values exceed 8–10, the protein is caused to undergo a change in physical organization in which form it exhibits a greater binding capacity for the detergent. The over-all binding of the detergent is the summation of the binding by the two forms of the protein and can be adequately described in terms of two simple mass action functions applying, respectively and in degree proportional, to the two forms of protein present in a given equilibrium system. Evidence is presented that the protein molecules are not equally susceptible to the action of initially bound detergent in causing the change in physical form which is accompanied by the change in interaction capacity.

The capacity for and intensity of the interactions of a protein with small molecular weight substances of known chemical structure, together with any recognizable effects upon the protein molecule which occur as a consequence of such interactions, must reflect important features of the physicochemical structure of the protein. Through the description and interpretation of these interaction properties, a clearer understanding of the complex molecular configuration of the protein itself may eventually be attained.

The interactions of proteins with anionic detergents, particularly with the alkyl and alkyl-aryl sulfates and sulfonates, have received considerable study.[3-7] At low concentrations, dodecyl sulfate has been observed to enhance the resistance of serum albumin in solution to the denaturation effects of heat[3] while at higher concentrations such detergents promote denaturation to such a degree as to allow fiber formation from native corpuscular proteins.[4]

Putnam and Neurath, from studies of the precipitating action of dodecyl sulfate on proteins at *p*H values acid to their isoelectric points[5] and from electrophoresis studies of the complex formed in a phosphate–sodium chloride buffer of *p*H 6.8, ionic strength 0.2,[6] concluded that the interaction involved was of the nature of a stoichiometric binding which occurs to an extent determined by the number of available ionized cationic groups on the protein molecule. With horse serum albumin (P) they reported the formation of two distinct complexes, PD_n and PD_{2n}, where *n* was approximately 55. As increasing proportions of detergents were mixed with the protein, the stepwise formation of PD_n from P occurred in increasing degree, followed by a second stepwise conversion of PD_n to PD_{2n}. From their interpretation of the process it would appear that, at all stages, practically all of the detergent is bound in the complexes and that, starting with the native protein, the individual protein molecules react with *n* molecules of detergent or not at all. When sufficient detergent is present to convert all of the native protein to the PD_n complex an all or none process is repeated to form the PD_{2n} complex. Electrophoresis patterns, obtained with the protein–detergent–buffer as underlying solution and with buffer alone as the overlying solution in the cell, showed peaks for P, PD_n and PD_{2n} and no intermediates, emphasizing the stepwise and apparent all or none nature of the process. When an individual protein molecule is capable of combining with a large number of detergent molecules, it would appear from mass action considerations that such an all or none type of reaction is improbable. However, the sequential and stepwise conversion of the complex into distinct electrophoretic entities of uniform mobility requires explanation.

Karush and Sonenberg[7] used differential dialysis techniques to follow the binding of dodecyl sulfate by bovine serum albumin in 0.025 *M* phosphate buffer, *p*H 6.1. Their studies were restricted to fairly low mixing ratios of detergent and protein where the moles of detergent bound per mole of protein did not exceed approximately 10. The bind-

(1) Paper #3036. Scientific Journal Series, Minnesota Agricultural Experiment Station. A part of a thesis to be submitted by M. J. Pallansch to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Ph.D. degree.

(2) This study was supported in part by a research grant from the National Institutes of Health, Public Health Services.

(3) E. L. Duggan and J. M. Luck, *J. Biol. Chem.*, **172**, 205 (1948).

(4) H. P. Lundgren, THIS JOURNAL, **63**, 2854 (1941); *Textile Res. J.*, **15**, 335 (1945).

(5) F. W. Putnam and H. Neurath, THIS JOURNAL, **66**, 692 (1944).

(6) F. W. Putnam and H. Neurath, *J. Biol. Chem.*, **159**, 1952 (1945).

(7) F. Karush and M. Sonenberg, THIS JOURNAL, **71**, 1369 (1949).