indicates that the 9.1-μ band of tetrahydropyran is of type A'' and is to be associated with the A_{1u} band of cyclohexane and the A_u band of *p*-dioxane. Although this is not the assignment accepted by Burket and Badger[2] it is not in serious disagreement with their work.

Although it is not expected that a vibration of this type will necessarily give rise to prominent infrared bands in the hydrocarbon series, we can attempt to choose from previous assignments for these molecules those vibrations which correspond to analogs of the antisymmetric stretching vibrations of the ethers. For cyclopropane this is the degenerate ring deformation at 11.5 μ. For cyclobutane Wilson[7] has assigned 10.6 μ to the B_{1g} vibration. The A_{1u} band of cyclohexane has been given the value 9.7 μ by Beckett, Pitzer and Spitzer.[8] Assignments for cyclopentane are less definite. The most satisfactory correlation is provided by choosing the mean of the two bands at 9.7 and 10.5 μ interpreted by Miller and Inskeep[9] as arising from the splitting of the E_2' ring deformation due to lack of complete planarity of the ring.

The regularities in the series of strong infrared bands of the cyclic ethers and the assigned antisymmetric vibrations of the corresponding hydrocarbons are shown, together with the Raman frequencies in Fig. 3. Although the trends are not so parallel as in the case of the totally symmetric vibrations, it seems clear that in both the ether and hydrocarbon series we are dealing with a similar type of vibration, *i.e.*, one in which we can picture the ring as undergoing a deformation consisting approximately of stretching and compression of alternate bonds.

The trend of the infrared bands of the cyclic ethers leading to that for the open chain ethers suggests also that the B_1 skeletal stretching mode for propane should, in view of the cyclic hydrocarbon series, be assigned a value of about 9.5 μ unless a rather large interaction exists in this molecule. Such interactions have been suggested by McMurry and Thornton[10] but assignment of this vibration to a band at 9.5 μ has been made by Gates[11] while values of about 10.8 μ have been suggested by Wu and Barker[12] and by Pitzer.[13]

As with the Raman bands it appears that the trend of frequencies of the infrared bands to greater frequencies with increasing ring size is a reduced mass effect rather than an immediate consequence of bond strain in the smaller rings. We will present shortly a more detailed account of the spectra of trimethylene oxide and trimethyleneimine and we hope then to be able to determine reasonably reliable force constants for these molecules.

Acknowledgment.—The authors wish to express their appreciation to Mr. Morris Knepp and Miss Rosalind Guy for obtaining some of the spectra presented here.

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Some Factors Which Influence the Iodine Affinity of Amylose as Shown by Potentiometric Titration[1]

BY EUGENE F. PASCHALL AND JOSEPH F. FOSTER

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The effects of certain experimental variables on the iodine titration behavior of several amylose preparations are investigated. Results obtained clearly do not represent an equilibrium state but reflect the previous history of the amylose preparations. Of particular importance are the time of aging of the alkaline solution prior to neutralization, and the time of aging and *p*H of the solution following neutralization. The affinity of amylose for iodine is enhanced by pretreatment with iodine. An attempt is made to interpret the results in terms of the state of aggregation of the amylose molecules.

The potentiometric titration method for study of the iodine-binding properties of starch and its fractions as introduced by Bates, *et al.*,[2] has proved of outstanding value for the estimation of amylose content. It has been shown that there is some correlation between binding affinity of amylose preparations and molecular weight.[2,3] Furthermore, since the reaction appears to be an all-or-none phenomenon,[3,4] amylose molecules tending to saturate successively with iodine, the intriguing possibility exists that if the titration could be carried out under equilibrium conditions it should be possible to determine molecular weight distributions by this technique. As a preliminary step in this direction a study has been made of some factors which influence the titration behavior of amylose. It is shown that equilibrium does not prevail under the condition customarily employed and that secondary effects are sufficient to mask completely relatively minor differences resulting from variation in molecular weight distribution.

(1) Journal Paper No. J-2143 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 817. Supported in part by a grant from the Corn Industries Research Foundation. Taken from a thesis presented by E. F. Paschall in partial fulfillment of the requirements for the degree Doctor of Philosophy, Iowa State College, 1951.
(2) F. L. Bates, D. French and R. E. Rundle, THIS JOURNAL, **65**, 142 (1943).
(3) J. F. Foster, Ph.D. Thesis, Iowa State College, 1943.
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Materials and Methods.—Of the three samples used, the amylose sub-fractions designated by the code number P 5/6 A(9a) and C146 A(1b) were supplied by Dr. T. J. Schoch. These samples had been sub-fractionated with *n*-octyl alcohol after a primary separation from starch had been accomplished with Pentasol.[5]

The sample designated simply as corn amylose was prepared from a 2 to 3% aqueous starch slurry which had been autoclaved for two to three hours under 15 to 18 pounds pressure. This amylose was recrystallized twice with Pentasol. The cut 2 sub-fractionated sample was prepared from the Pentasol fractionated corn amylose by crystallization from a 15% pyridine solution at 25°. The amylopectin impurity had been previously removed by recrystallizing the amylose several times from 15% aqueous pyridine maintained at 45°. This procedure will be reported in greater detail in another communication.[6]

For potentiometric iodine titration a sample of slightly greater than 40 mg. was dried in a vacuum oven for four hours at 50° and weighed. To each 40 mg. of sample, 5 ml. of 1.000 *N* KOH was added and the solution stirred frequently until complete dispersion of the amylose was effected. This was usually accomplished in less than 30 minutes. The alkaline solution was neutralized to a *p*H value of 6–6.5 with 0.500 *N* HCl. A 15-ml. aliquot, which contained 40 mg. of amylose, was transferred to a 100-ml. volumetric flask. Ten ml. of 0.500 *N* KI was added and the volume adjusted to 100 ml. with distilled water. The amylose solution was titrated with 0.00157 *N* iodine solution which was 0.05 *N* with respect to both potassium iodide and chloride. Thus, the salt concentration was essentially constant throughout the titration.

A three-necked 250-ml. flask, flattened on the bottom to accommodate a magnet sealed in glass, was used as a container for the solution to be titrated. This flask was immersed in a cylindrical jar filled with water which was maintained at 25 ± 0.03° by pumping water from a thermoregulated water-bath through a copper tube coiled to fit the inside of the jar. The cylindrical jar was placed upon a magnetic stirrer. This arrangement also permitted the titrations to be carried out under nitrogen if desired.

A commercial calomel electrode was used as reference electrode, the potassium chloride concentration being maintained at 248 g. per liter. A centimeter square of bright platinum served as the inert electrode. Each electrode was fitted into a neck of the three-necked flask by means of rubber stoppers. The third neck of the flask was used as an inlet for the iodine solution.

All forward titrations were carried out by measuring the potential three minutes after the addition of an iodine increment. A Leeds and Northrup Type K potentiometer and Leeds and Northrup galvanometer were used in measuring the potential.

The above procedure permitted duplication of results to within 0.5 millivolt throughout the entire titration. The importance of temperature control in this regard cannot be over-emphasized.

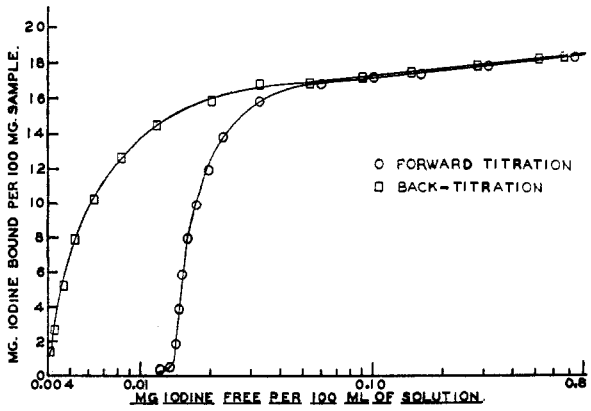

Fig. 1.—Comparison of forward and back-titration curves for corn amylose C146A(1b) showing non-equilibrium character of the titration.

(5) S. Lansky, M. Kooi and T. J. Schoch, THIS JOURNAL, **71**, 4066 (1949).

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Experimental Results

1. **Back Titration of the Amylose–Iodine Complex.**—In a conventional forward potentiometric iodine titration a continuous decrease in free iodine concentration is observed as shown by the potential drop after the addition of an increment of iodine. A constant free iodine concentration is not reached even after several hours. This was shown to be true by observing the behavior with time when iodine was added to a stoichiometric excess of amylose and the system kept under nitrogen for several hours with constant stirring. These results indicate that equilibrium conditions do not exist under the conditions of the conventional iodine titration.

A back-titration of the amylose–iodine complex using standard sodium thiosulfate was performed on the corn amylose preparation C146A(1b.). The results of a forward and back titration are shown in Fig. 1. The very large increase in the iodine binding affinity is of particular interest. The marked hysteresis confirms the impression that equilibrium conditions do not prevail, at least in the forward titration. The shift or decrease in free iodine concentration as a function of time is greatly reduced or entirely eliminated during the back titration, possibly indicating substantial equilibrium between iodine and amylose.

This behavior is not due to the salts formed during the back titration with thiosulfate. An experiment was performed in which a slight excess of iodine neutralized with an equivalent amount of thiosulfate was added at the beginning of a normal titration. This titration curve was identical to the one obtained when iodine neutralized with thiosulfate was not added.

2. **Effect of Amylose Concentration.**—To minimize aggregation effects it would appear desirable to titrate at the lowest possible amylose concentration. Also, for interpretation of results of later sections, particularly in cases where a part of the amylose had been removed by retrogradation, it was essential to ascertain the effect of amylose concentration. Figure 2 summarizes results at four concentrations covering a range of almost threefold. The shape of the curves is uninfluenced by concentration and the shift in binding affinity, while definite, is relatively minor. This result is in accord with the idea that the binding is an all-or-none reaction possessing a high order in iodine.

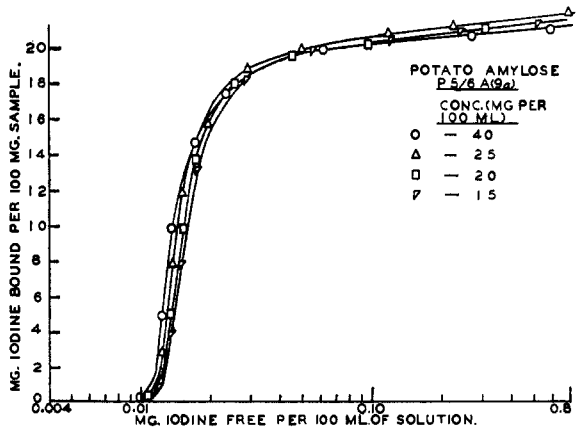

Fig. 2.—Effect of amylose concentration on the titration behavior of potato amylose.

3. **Effect of Regeneration and Iodine Pretreatment.**—Freshly prepared amyloses exhibit a slightly higher binding affinity than amyloses which have been allowed to age for several months in the dry condition. The iodine binding affinity of these aged samples can be greatly increased by regenerating the amylose from neutral solution with aqueous ethyl alcohol. The binding effect is still greater if the neutral solutions of the aged amylose samples are pretreated with an excess of iodine and, after reduction of the iodine, the amylose is again regenerated with 50% aqueous ethyl alcohol. This behavior is shown in Fig. 3 for a Pentasol

fractionated corn amylose sample that had been prepared several months in advance.

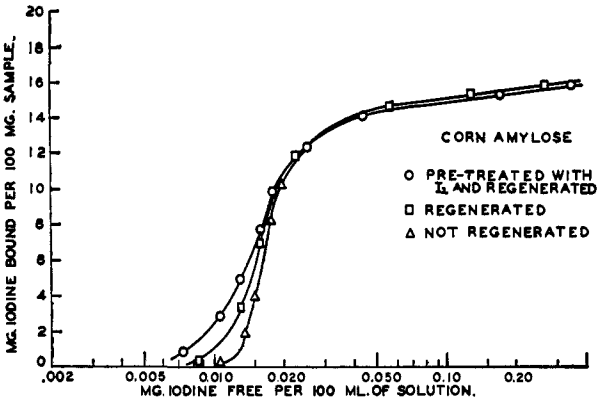

Fig. 3.—Effect of regeneration (with ethanol) and iodine pretreatment on the iodine binding behavior of corn amylose.

The neutral amylose solutions were prepared by dispersing the amylose in N KOH and neutralizing with HCl to a pH of 6.3. Regeneration was accomplished by recrystallizing the amylose several times from a 50% aqueous ethyl alcohol solution. After each crystallization the complex was centrifuged and dispersed in water. After three crystallizations most of the potassium chloride had been eliminated. Residual water was removed from the alcohol complex by washing five times with absolute ethyl alcohol. This regenerated amylose was finally dried in a vacuum oven for four hours at 50°. For iodine pretreatment, a slight excess of iodine dispersed in an aqueous potassium iodide solution was added over a period of 30 minutes with constant stirring. The iodine complex was then destroyed by reducing the iodine with an equivalent amount of sodium thiosulfate. The amylose was subsequently regenerated with ethyl alcohol in the manner described above and iodine titrations run.

The result of the preceding experiment served to show that either iodine or ethyl alcohol (or probably any complexing agent used prior to or for regeneration) influences the subsequent behavior of amylose toward iodine, this behavior being manifest in the reduction of the free iodine concentration necessary for complex formation.

4. **Effect of Aging at Various pH Values.**—The iodine binding affinity of amylose is to some extent dependent upon the pH value to which an alkaline amylose solution is adjusted just prior to potentiometric iodine titration. This dependence is particularly evident at low pH values. In Fig. 4 are presented the results of three titrations in which the alkaline solutions were adjusted to pH values of 3.2, 6.2 and 9.0. The sample used was the subfractionated potato amylose P 5/6 A(9a). At a pH value of 3.2 a significant decrease in the iodine binding affinity occurred.

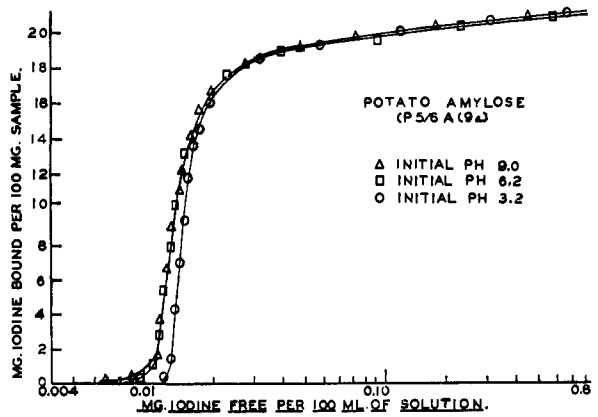

Fig. 4.—Comparison of titration curves carried out at differing initial pH values. The pH in all cases approaches 6 during the course of the titration as a consequence of the buffering action of the iodine solution.

The pH effects, however, are more strongly pronounced when amylose solutions are allowed to age at various values. The results of several iodine titrations are presented in Fig. 5 in which pH value and time of standing are variables. The above octyl alcohol sub-fractionated amylose was again used. This amylose was found to give nearly constant results for potentiometric iodine titrations carried out over a period of six or eight months.

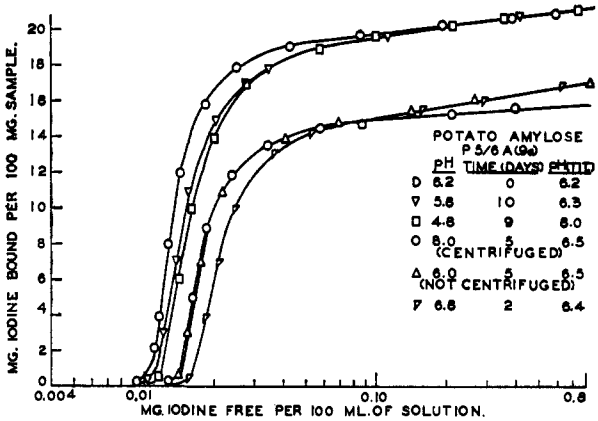

Fig. 5.—Effect of aging in solution at various pH values on the titration behavior of potato amylose.

It was found that the tendency of the amylose to retrograde was much more pronounced at pH values of between 6 and 7 than at lower values of from 4 to 5.5; however, at pH values of 8 to 9, the tendency toward retrogradation was intermediate. Potentiometric iodine titrations were carried out in most cases when retrogradation was visibly evident. At low pH values titrations were performed after 9 to 10 days even though a visible turbidity did not develop. The pH values of all the samples were readjusted to values ranging from 6 to 6.5 before titrating. The results presented in Fig. 5 indicate that solutions which stood at a pH below 6 showed a progressive decrease in binding affinity as the pH was lowered. However, the percentage of iodine bound did not change appreciably from the control value obtained by titrating amylose immediately after neutralizing to a pH of 6.2.

The solution which had been allowed to stand at pH 6.8 for 2 days showed a greatly reduced binding affinity with the percentage of iodine bound being reduced by about one-third. The reduction in the capacity for iodine may be attributed to a loss of soluble amylose, but the increase in free iodine concentration necessary for complex formation cannot be attributed to this in view of Fig. 2.

The sample which stood at a pH value of 8 for 5 days and developed some turbidity was titrated before and after centrifuging (at 20,000 g) in order to observe the possible effect of retrograded particles on the binding affinity. The two curves are almost identical except at high free iodine concentrations at which the retrograded amylose apparently binds some iodine.

5. **Effect of Aging in 1 N KOH.**—Corn amylose was aged in 1 N KOH with and without nitrogen for various time intervals. A reduction in the iodine binding affinities was observed in both cases with perhaps a greater reduction occurring with samples aged under air.

For aging in the presence of air, the required concentration of Pentasol fractionated corn amylose necessary for an iodine titration was dispersed in 1 N KOH and kept in a stoppered buret to prevent evaporation. At various time intervals the required aliquot was withdrawn, neutralized to a pH value of 6.3 and titrated with iodine. Figure 6 shows the results of several titrations performed during 13 days in KOH. A gradual decrease in the iodine binding affinity with time occurs. It is perhaps significant that the binding affinity of a sample after aging 13 days in 1 N KOH is to a large extent restored by regeneration to the value obtained after one hour in KOH. Thus, regeneration at least partially counteracts the influence of the long KOH treatment in air.

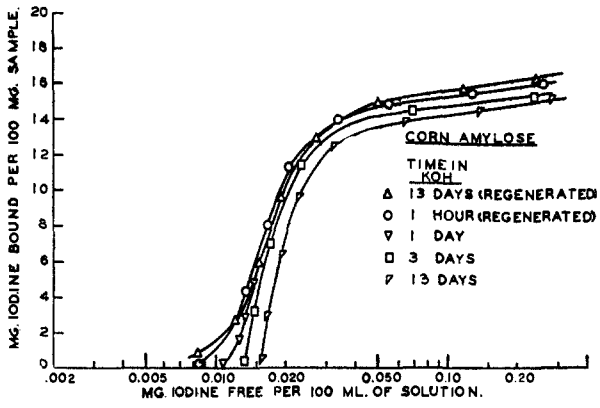

Fig. 6.—Effect of aging in *N* KOH on the titration behavior of corn amylose.

To exclude the possibility of air oxidation, similar experiments were performed on the Pentasol fractionated corn amylose and on the cut 2 sample standing in alkali under a nitrogen atmosphere. The titration data for the corn amylose after various time intervals in KOH are shown in Fig. 7. Prior to amylose dispersion as much dissolved air as possible was eliminated from the KOH solution by first heating to boiling and then bubbling nitrogen through the cooled solution for 30 minutes. A separatory funnel fitted with a two-holed rubber stopper served as a container for the sample. The amylose samples not only were kept under nitrogen but were dispersed by bubbling nitrogen through the KOH solution as the amylose was added. The solutions were stoppered under nitrogen at a positive pressure. The results of potentiometric iodine titrations performed on the two amyloses show a gradual decrease in iodine binding affinity but possibly not to the extent shown by the amylose stored under air.

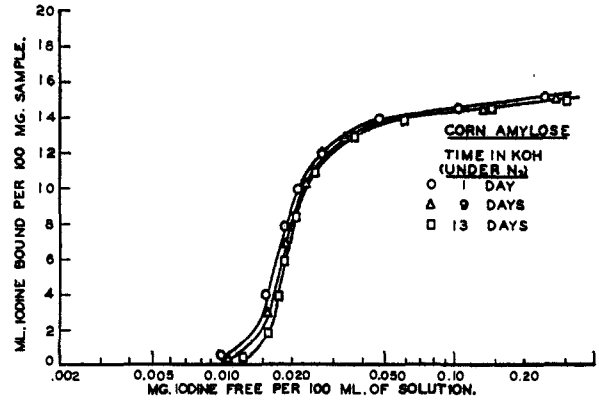

Fig. 7.—Effect of aging, in *N* KOH under nitrogen, on the titration behavior of corn amylose.

Discussion

These results show that the iodine titration as usually carried out does not yield results at all representative of an equilibrium state. Whether the curves obtained by the back-titration method reflect an equilibrium situation cannot yet be stated but because of the much smaller drift in potential with time it seems probable that they represent at least a closer approach to equilibrium. Further study of this procedure is planned.

The slow drift in binding affinity with time of aging in alkali is probably a consequence of the disaggregation which has been demonstrated under such conditions.[7] One puzzling feature is the fact that this effect appears to be at least partially reversible in so far as the iodine binding behavior is concerned whereas the disaggregation has not been found to be reversible.[7]

The behavior on aging in neutralized solution is more complex. The reduction in binding capacity is without much question due to the formation of retrograded aggregates and consequent reduction in the amount of amylose available for titration. That small retrograded particles can bind some iodine at high iodine levels is indicated by comparative titrations on uncentrifuged and centrifuged solutions after aging. On the other hand, the decrease in the binding affinity is probably not due to retrogradation but rather to the breakdown of the unique aggregates which have been suggested.[7] This effect is examined in greater detail in another communication.[6]

The variations in the iodine titration curves produced by such factors as time of standing of neutralized solution, *p*H of solution and previous history of sample (for sample pretreatment, regeneration, etc.) are comparable to, or even greater than, differences found on samples thought to differ rather markedly in molecular weight.[2] The conclusion that without marked refinements in technique the method cannot be used for studies of molecular weight or molecular weight distribution seems unavoidable.

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(7) E. F. Paschall and J. F. Foster, *J. Polymer Sci.*, **9**, 73, 85 (1952).