Amylose-Iodine Complex. III. Potentiometric and Spectrophotometric Studies

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Potentiometric titrations and spectrophotometric measurements have been used as methods of studying the starch-iodine reaction by many authors. Most of these investigations, however, were carried out at room temperature. When the temperature of amylose solution is raised, it may be expected that the amylose molecule enormously increases its flexibility and takes a more random configuration[1]. In parallel with this, it becomes difficult to form amylose-iodine complex with increasing temperature. Hence, it seems interesting to investigate the temperature dependence of the amylose-iodine reaction.

In the present paper, the authors describe the enthalpy change of the amylose-iodine reaction obtained by the potentiometric and/or the spectrophotometric method at wider temperature range than previously reported, and some information concerning the nature of the complex which is deduced from the results obtained.

Experimental

Potentiometric Titrations with Iodine.—The amyloses used for potentiometric titrations were those obtained from potato (white potato), corn and sweet potato starch by hot water extraction[2] at 60°, 69° and 72°C, respectively.

The technique of potentiometric titration was essentially similar to that described by Bates et al.[3]: In our procedure about 0.015 g. of amylose was dispersed in 10 ml. of 0.5 M potassium hydroxide. When dispersal was complete, the mixture was made neutral to methyl orange by the addition of hydrochloric acid, added 10 ml. of 0.5 M potassium iodide, and then filled up to 100 ml. The solution thus prepared was titrated with 0.0005 M iodine in 0.05 M potassium iodide solution. A control solution which contains the same components as that described above but no amylose, was also titrated in the same way.

Titration was carried out in a cell immersed in a thermostat.

For the potential measurements, a Yokogawa high precision potentiometer was used in conjunction with a normal calomel electrode and a bright platinum electrode.

Spectrophotometric Measurements.—The amylose used was obtained from potato starch by a selective precipitation method with *n*-butyl alcohol and isoamyl alcohol[4].

Spectrophotometric titration was carried out as follows: 100 mg. of amylose was dispersed in 100 ml. of 0.5 M potassium hydroxide and diluted to 500 ml. with distilled water after neutralization with hydrochloric acid. Each 25 ml. of the solution was diluted to 100 ml., a requisite amount of 0.1 M potassium iodide being added to make the final solution 0.01 M in iodide after the addition of 1 to 22 ml. of a solution of 0.0005 M iodine in 0.001 M potassium iodide. Although the absorptions of the sample in which 6.5 ml. or less of 0.0005 M iodine solution was contained, were directly measured, those of the sample in which 8.0 ml. or more of the iodine solution was contained, were measured after twofold dilution with water. The absorptions were measured at 15°C at wavelengths of 288, 350 and 640 mμ, respectively.

The change of the absorption with temperature change was measured in the following way: The solution (0.005% amylose, 2.5×10^{-5} M iodine), in which the amount of iodine was nearly half of the amount to saturated amylose, was prepared in the same way as that for the spectrophotometric titrations. This solution was placed in a 250 ml. colored volumetric flask and kept in a thermostat at each given temperature for 20 min. and then aliquots of the solution were used to measure the absorption at wavelengths of 288, 350 and 640 mμ, respectively.

A Shimadzu type QB-50 spectrophotometer with thermo-regulating device was used with 1 cm. thick silica cell throughout this investigation.

Results and Discussion

Potentiometric Titrations.—Titration curves of potato amylose and control solution (0.05 M in potassium iodide) at various temperatures are shown in Fig. 1. It can be seen that the potential of these systems becomes high with increasing temperature. At a given temperature the potential is determined by the concentration of free iodine which is in equilibrium with amylose-iodine complex, since the concentration of iodide ion is kept practically constant.

Referring to the results of Dube[5] and Gilbert et al.[6], it may be considered that the ratio of

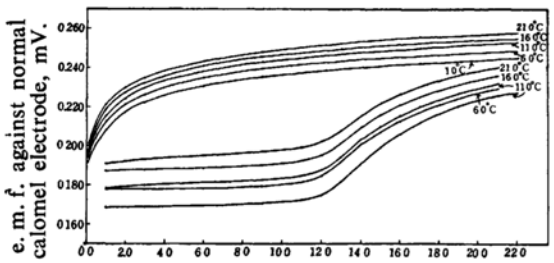
1) S. Ono, T. Kuge and Y. Yoshikawa, presented at the 11th Symposium on Colloid Chemistry held in Sendai, September, 1958.

2) K. G. Krishnaswamy and A. Sreenivasan, *J. Biol. Chem.*, **176**, 1253 (1948).

3) F. L. Bates, D. French and R. E. Rundle, *J. Am. Chem. Soc.*, **65**, 142 (1943).

4) E. J. Wilson, T. J. Schoch and C. S. Hadson, ibid., **65**, 1380 (1943).

5) H. A. Dube, Dissertation, Iowa State College, (1947), through "Chemistry and Industry of Starch", edited by R. W. Kerr, Academic Press, Inc., New York, (1950), Chapter XVII, p. 461.



ml. of 0.0005 M iodine solution

Fig. 1. Potentiometric titration curves: 100 ml. of 0.012% potato amylose solution (the lower five curves), 100 ml. of 0.05 M potassium iodide (the upper five curves).

adsorbed iodide ions to adsorbed iodine molecules on amylose is unity in the present experimental conditions (0.05 M in potassium iodide). Therefore, the amylose-iodine reaction can be expressed as

$$\mathrm{Am} + n\mathrm{I}_2 + n\mathrm{I}^- \rightleftarrows \mathrm{Com} \qquad (1)$$

where Am and Com represent amylose and amylose-iodine complex, respectively.

The equilibrium constant K, which is constant for the amylose of a given chain length, is then

$$K = [\mathrm{Com}] / [\mathrm{Am}]\,[\mathrm{I}_2]^n\,[\mathrm{I}^-]^n$$

hence

$$K^{1/n} = [\mathrm{Com}]^{1/n} / [\mathrm{Am}]^{1/n}\,[\mathrm{I}_2]\,[\mathrm{I}^-] \qquad (2)$$

In the amylose-iodine reaction, the activities of the complex and amylose can be regarded as unity, in the manner of a heterogeneous reaction. Eq. 2, therefore, may be written as

$$K^{1/n} = 1/[\mathrm{I}_2]\,[\mathrm{I}^-] \qquad (3)$$

Since the potential of the horizontal part of the titration curve is lower than the lowest potential obtained with the control solution in a given temperature, as can be seen in Fig. 1, direct calculation of free iodine from the potential, which was made by Bates et al.[3], is impossible in the present case. Accordingly, the calculation of $K^{1/n}$ was carried out as follows. The potential difference, E_d, between the potential of the sample at the mid-point of the titration (i. e. characteristic potential[7]) and that of the control solution at the corresponding titration point, can be written as

$$E_d = (\boldsymbol{RT}/2\boldsymbol{F}) \ln ([\mathrm{I}_2]_c / [\mathrm{I}_2]_s) \qquad (4)$$

where $[\mathrm{I}_2]_c$ is the concentration of free iodine in the control solution and $[\mathrm{I}_2]_s$ corresponds to that of the sample, and $\boldsymbol{R}$ and $\boldsymbol{T}$ are gas constant and absolute temperature, respectively, and $\boldsymbol{F}$ is Faraday constant. From Eqs. 3 and 4

$$E_d = (\boldsymbol{RT}/2\boldsymbol{F}) \ln [\mathrm{I}_2]_c\,[\mathrm{I}^-]_s\,K^{1/n} \qquad (5)$$

or

$$E_d = (\boldsymbol{RT}/2\boldsymbol{F}) \ln ([\mathrm{I}_3^-]_c\,K^{1/n}/K_1) \qquad (6)$$

where $[\mathrm{I}^-]_s$ is the concentration of iodide ion in the sample, $[\mathrm{I}_3^-]_c$ is the concentration of triiodide ion in the control solution and K_1 is the equiliblium constant of the following reaction

$$\mathrm{I}_2 + \mathrm{I}^- = \mathrm{I}_3^-$$

Therefore, $K^{1/n}$ can be calculated from E_d, $[\mathrm{I}_3^-]_c$ and K_1 which is given in literature[8], using Eq. 6.

In Table I are listed observed $K^{1/n}$, the enthalpy change of the reaction obtained from the temperature dependence of $K^{1/n}$, characteristic potentials, intrinsic viscosities of amylose in 1 M potassium hydroxide by Ostwald viscometer[9] and the temperature of water extraction of amylose. As the temperature of the water extraction is raised, the mean length of the extracted amylose chain may be expected to become longer. This is inferred from their characteristic potentials[7] and intrinsic viscosities, though the contamination of amylopectin may increase. The enthalpy changes of the reaction with amyloses from different sources agree within experimental error in spite of the difference of the size of molecule among these amyloses. Similar values have also been obtained with amyloses partially degraded by bacterial α-amylase.

Since the results obtained at temperatures higher than those described above are confused owing to the vaporization of iodine during the procedure, the analysis of the results is not made as those obtained at a lower temperature. However, it seems worth while to mention that the end-point of titration shifted to higher concentration of iodine and became obscure with increasing temperature, from which the occurrence of a complex having a short polyiodine-chain (also suggested in the following spectrophotometric studies) at a higher temperature might be supposed.

Spectrophotometric Studies.—Fig. 2 shows the results obtained from spectrophotometric titrations at 15°C. At the beginning of the titration, the curve for 640 mμ has a small lag and thereafter increases linearly with the amount of iodine added until the vicinity of end-point.

6) G. A. Gilbert and J. V. R. Marriott, *Trans. Faraday Soc.*, 44, 84 (1948).

7) J. F. Foster, Dissertation, Iowa State College, Ames, Iowa (1943), through "Chemistry and Industry of Starch", edited by R. W. Kerr, Academic Press, Inc., New York, (1950), Chapter XVII, p. 460.

8) M. Davies and E. Gwynne, *J. Am. Chem. Soc.*, 74, 2748 (1952).

9) Viscometric studies were carried out in a way similar to that described by S. Lansky et al., *J. Am. Chem. Soc.*, 71, 4066 (1949).

TABLE I. SOME CONSTANTS OBTAINED WITH AMYLOSES FROM DIFFERENT SOURCES

Amylose	Temp. of water extraction	Equilibrium constant $K^{1/n}$ at 16°C	Enthalpy change kcal./mol. iodine	Charact. potential v.s. N. C. E. at 16°C	Intrinsic viscosity
Potato	60°C	2.2×10^{9}	−15.5	0.1890	0.80
Corn	69°C	2.4×10^{9}	−15.8	0.1878	1.20
Sweet potato	72°C	3.0×10^{9}	−15.3	0.1856	2.15

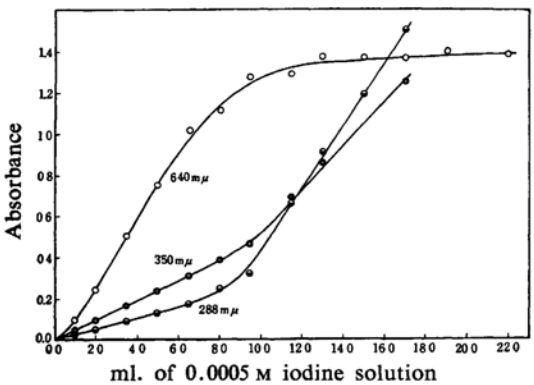


Fig. 2. Spectrophotometric titration curves: 100 ml. of 0.005% potato amylose solution.

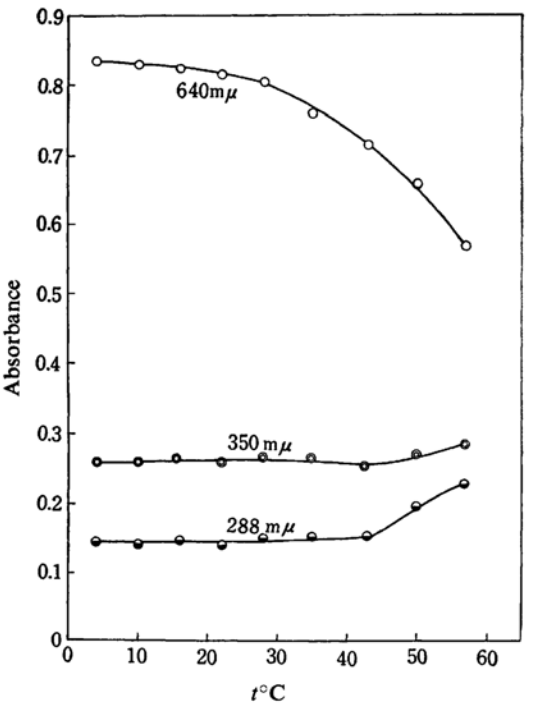


Fig. 3. Temperature dependences of absorptions at wavelengths of 640, 350 and 288 mμ.

The absorptions of ultraviolet region (at 288, 350 mμ) appear to have no such lag and their sharp increases due to free triiodide ion are seen after the end-point. Using the slopes of the initial linear parts of the titration curves, the molecular extinction coefficients of iodine in the complex were estimated to be 36300, 10500 and 5230 at wavelengths of 640, 350 and 288 mμ, respectively. The latter two values are in good agreement with that calculated from Mould's report[10] by the present authors.

The temperature dependences of absorptions are shown in Fig. 3, in which it can be seen that the absorption at 640 mμ decreases in a regular fashion with increasing temperature, while the absorption of the ultraviolet region (at 288 and 350 mμ) remains almost unchanged and has rather a tendency to increase at temperature higher than 40°C.

Free triiodide ion in an aqueous solution exhibits two ultraviolet absorption bands whose maxima are at 288 and 353 mμ[11], respectively. Since the concentration of free iodine molecules which are in equilibrium with the complexes may increase with increasing temperature and consequently the concentration of free triiodide ions may increase, the above described temperature dependence of absorption in the ultraviolet region may be principally ascribed to the increase of the free triiodide ions.

The absorption at 640 mμ may be considered to be due to the complex alone, since free iodine concentration is low enough that its contribution to the absorption can be neglected. Assuming that the absorption of the ultraviolet region is determined by the concentrations of the complex and free triiodide ion, as a first approximation, the following equations can be derived.

$$E_{640}=C_c\varepsilon_{c640} \tag{7}$$

$$E_{288}=C_c\varepsilon_{c288}+C_i\varepsilon_{i288} \tag{8}$$

$$E_{350}=C_c\varepsilon_{c350}+C_i\varepsilon_{i350} \tag{8'}$$

where E represents the absorbance observed, C_c and C_i are the concentrations of the complex and free triiodide ion, respectively, ε_c and ε_i are the molecular extinction coefficients of the complex and free triiodide ion, respectively, and the numerical suffixes represent their corresponding wavelengths in mμ. The concentrations of the complex, for the sake of convenience, are expressed as moles of iodine bound.

10) D. L. Mould, *Biochem. J.*, **58**, 593 (1954).
11) A. D. Awtrey and R. E. Connick, *J. Am. Chem. Soc.*, **73**, 1842 (1951).

TABLE II. TEMPERATURE DEPENDENCE OF SOME PROPERTIES OF POTATO AMYLOSE

Temp. t°C	Concn. of complex C_c mol./l.	Concentration of free triiodide ion C_{1288} mol./l.	C_{1350} mol./l.	C_{1c} mol./l.	Concn. of short polyiodine C_u mol./l.	Equilibrium constant $K_s^{1/n}$	$K^{1/n}$ a)	Enthalpy change kcal./mol. iodine
1.0	—	—	—	—	—	—	9.2×10^{9}	
4.0	2.29×10^{-5}	5.8×10^{-7}	6.2×10^{-7}	5.6×10^{-7}	1.5×10^{-7}	2.4×10^{9}	—	
6.0	—	—	—	—	—	—	4.8	
10.0	2.28	5.6	6.9	4.9	5.2	2.3	—	
11.0	—	—	—	—	—	—	4.2	
16.0	2.27	6.1	9.2	4.6	1.1×10^{-6}	2.1	2.2	−15.5[a]
21.0	—	—	—	—	—	—	1.3	
22.0	2.24	5.3	9.2	3.4	1.4	2.4	—	
28.0	2.22	8.1	1.31×10^{-6}	5.6	1.8	1.3	—	
35.0	2.09	1.06×10^{-6}	1.81	6.9	2.8	9.1×10^{5}	—	
43.0	1.97	1.24	1.88	9.2	2.4	6.0	—	
50.0	1.81	2.51	3.12	2.21×10^{-6}	2.3	2.2	—	−20.8
57.0	1.57	3.65	4.62	3.17	3.6	1.4	—	

a) This value was obtained by potentiometric titration.

Assuming that these molecular extinction coefficients are independent of temperature, the concentration of the free triiodide ion at each temperature may be found, using Eqs. 7 and 8 or 8′ [12]. The values obtained are shown in Table II, in which C_{1288} and C_{1350} are the concentrations of free triiodide ion estimated by using the absorbance observed at wavelengths of 288 and 350 mμ, respectively. It can be seen that C_{1288} and C_{1350} do not agree with each other and that the former is smaller than the latter at any temperatures. This seems to suggest that Eq. 8 or 8′ would not be an adequate expression for the observed absorbance. A more favorable equation than Eq. 8 or 8′ is seemed to be written as

$$E=C_c\varepsilon_c+C_1\varepsilon_1+C_u\varepsilon_u+C_a\varepsilon_a \qquad (9)$$

where C_u represents the concentration of the polyiodine of short length and is also expressed as moles of single iodine molecule, ε_u is the molecular extinction coefficient of iodine in the polyiodine, C_a and ε_a represent the concentration and molecular extinction coefficient of the triiodide ion loosely adsorbed on the surface of amylose, respectively. It would appear that little attention has been given to the ultraviolet absorption of the amylose-iodine complex and an investigation of the ultraviolet absorption has been inconclusive[10,13]. However, the fact that the titration curve at 640 mμ has a small lag may reasonably suggest the existence of polyiodine of short length which exhibits no appreciable absorption at 640 mμ but ultraviolet absorption similar to that of the complex. It may be assumed that the absorption spectrum of the triiodide ion is unchanged whether in the adsorbed state described above or in an aqueous solution.

The use of Eq. 8 or 8′ instead of Eq. 9 would cause an error ΔC_1 being always positive, in the estimation of the concentration of the free triiodide ion. The error can be expressed as follows.

$$\Delta C_1=C_u\varepsilon_u/\varepsilon_1+C_a\varepsilon_a/\varepsilon_1 \qquad (10)$$

Since, ε_u may be roughly assumed to be not appreciably different from ε_c and ε_a may be considered as equal to ε_1, the ratio of ε_u to ε_1 depends on wavelength while that of ε_a to ε_1 seems to be unity, independent of wavelength. The smaller the ratio of ε_u to ε_1, the smaller the error becomes. Therefore, C_{1288} should be smaller than C_{1350}, and the experimental results given in Table II satisfy this requirement.

The true concentration of free triiodide ion, C_{1t}, would be written as

$$C_{1t}=C_{1288}-C_u\varepsilon_{u288}/\varepsilon_{1288}-C_a\varepsilon_{a288}/\varepsilon_{1288} \qquad (11)$$

or

$$C_{1t}=C_{1350}-C_u\varepsilon_{u350}/\varepsilon_{1350}-C_a\varepsilon_{a350}/\varepsilon_{1350} \qquad (11')$$

Therefore, C_u can be obtained by subtraction of Eq. 11 from Eq. 11′. C_u obtained thus is shown in Table II, and the increase of C_u with increasing temperature is seen. C_{1c} listed in Table II is the corrected concentration of free triiodide ion in respect to C_u. It should be noted that C_{1c} is not free from the error due to C_a. C_{1c} was used to calculate $K^{1/n}$, and the

12) ε_{1288} and ε_{1350} used in the calculation were 40000 and 26000, respectively, which were obtained from our experiments and have no essential difference from the values in reference 11.

13) Y. Tanizaki, T. Kobayashi and N. Andô, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **80**, 445 (1959).

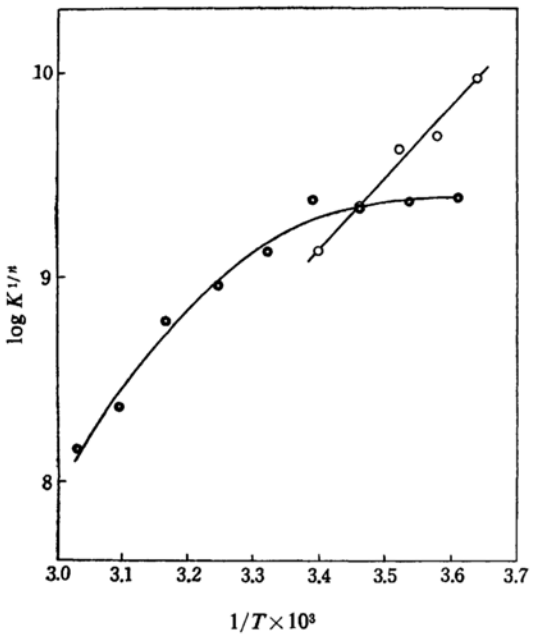


Fig. 4. $\log K^{1/n}\sim 1/T$ plot for potato amylose: —◎—◎—, $\log K_s^{1/n}$, obtained from spectrophotometric method; —○—○—, $\log K^{1/n}$, obtained from potentiometric titration.

value obtained is given in Table II expressed as $K_s^{1/n}$. In Fig. 4, $\log K_s^{1/n}$ is plotted against reciprocal absolute temperature, and it can be seen that the slope of the curve becomes steeper with increasing temperature. Comparing $K_s^{1/n}$ with $K^{1/n}$ obtained by the potentiometric titrations, it can be found that they have similar values at about 16°C, but the slopes as above described, from which the enthalpy change of the reaction would be calculated, are quite different from each other in the neighborhood of the temperature. Such difference may be reasonably explained in terms of the error in the spectrophotometric estimation of free triiodide ion concentration at low temperature region. Considering the data obtained by the potentiometric titrations, it can be seen that free iodine concentration in equilibrium with the complex should decrease with decreasing temperature. However, C_{1c} rather increases with decreasing temperature in the temperature region lower than 22°C. This fact suggests that the amount of the triiodide ion adsorbed on the surface of amylose may be comparable with that of free triiodide ion in the temperature region below 22°C, and a serious error is caused in the estimation of free triiodide ion concentration. It may also be supposed that the lower the temperature, the more the contribution of C_a to C_{1c} becomes. Therefore, it can be inferred that $\log K_s^{1/n}\sim 1/T$ plot should be steeper than that seen in Fig. 4, especially in the lower temperature region. Consequently, the equilibrium constant obtained spectrophotometrically should be larger than that obtained potentiometrically. This result may not be unreasonable, since $K^{1/n}$ is considered as a function of chain length of amylose, being larger with increasing chain length of amylose[9,14], and amylose used in the spectrophotometric study is thought to be longer than that used in potentiometric titration. The selective precipitation method of amylose seems to give amylose with longer chain length than that obtained by the hot water extraction.

For the reason described above, the spectrophotometric method may not be applicable for the estimation of free iodine concentration at room temperature, at which potentiometric titrations are adequate. However, with increasing temperature, when the concentration of free iodine in equilibrium with the amylose-iodine complex increases, the concentration of the free iodine appears to be reasonably estimated by the spectrophotometric method, since, at the higher temperature, C_a can be neglected without a serious error, compared with the free iodine concentration. Even when the vaporization of iodine takes place, free iodine concentration may remain approximately constant as far as amylose-iodine complex exists in solution. Therefore, the present results obtained at a relatively high temperature region may be available for the discussion of the temperature dependence of the amylose-iodine reaction.

From the inspection of the slope of $\log K_s^{1/n}\sim 1/T$ plot in Fig. 4, the enthalpy change of the reaction appears to increase with increasing temperature. The enthalpy change obtained at 50°C is -20.8 kcal. Comparing this value with that obtained potentiometrically at 16°C, -15.5 kcal., it seems certain that the enthalpy change increases as the temperature is raised. This may also be checked with the fact that all the enthalpy changes of the reaction hitherto obtained at different temperatures below 35°C by other authors[5,6,15,16] are -11 to -19 kcal.

At the temperature at which the color of the complex disappears, the free energy change of the reaction should be nearly zero so that the entropy change should have a large value almost corresponding to the enthalpy change at the temperature. This seems to indicate that the amylose, which is free from iodine, would come to take more random configurations

14) Also seen in Table I.

15) Presented at the 12th Symposium on Structural Chemistry held in Fukuoka, November, 1957, by H. Azumi et. al.

16) J. Holló and J. Szejtli, *Periodica Polytechnica*, **1**, 223 (1957).

with increasing temperature through the rupture of both inter- and intramolecular hydrogen bonding. This thought is also supported by the results obtained from the studies in viscosity and optical rotatory power[1].

Summary

The amylose-iodine reaction has been studied in a wider temperature range than hitherto reported by both potentiometric and spectrophotometric methods.

The enthalpy change of the reaction obtained potentiometrically is −15.5 kcal. at 16°C, and appears to be independent of the chain length and the kind of parent starch of amylose.

The change of the absorptions of the amylose-iodine complex with temperature has been pursued at wavelengths of 650, 288 and 350 mμ, respectively.

The triiodide ion adsorbed on the surface of amylose causes an error in the spectrophotometric estimation of free iodine concentration. At the higher temperature, however, this error may be neglected, and the results obtained seem to be reliable.

The enthalpy change appears to increase with increasing temperature and −20.8 kcal. has been obtained at 50°C.

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