THE EFFECTS OF CERTAIN ANIONS ON THE LINEAR STARCH-TRIIODIDE BLUE COMPLEX

by

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INTRODUCTION AND REVIEW OF LITERATURE

The history of man's use of starch antidates the life of Christ by some 3500 years. Although little was known about the chemical composition of starch until about 1800, the technique of separating starch from many plant sources had developed through the centuries. During this period of time starch was used in pastes, as a size for paper, in textile production and in medicinal and dietary preparations (Walton, 18).

During the late 1700's much was learned about the elementary composition of starch. In 1811, Kirchhoff (3) succeeded in producing glucose from starch which was the first insight into the structure of starch. By 1835 Guerin-Varry (Schoch, 17) recognized that starch was heterogeneous. Efforts to gain more knowledge about the structure of starch in the following century was generally hampered by the following factors: use of impure starch; degradation or retrogradation of the starch when fractionation was attempted; and lack of suitable quantitative criteria for evalutation of the degree of purity of a fraction (Schoch, 17).

About 1940, methods were devised that overcame these difficulties and yielded fractions that gave nearly identical results when subjected to a series of chemical and physical tests (Rundle, et al, 15).

These fractions have been referred to as the "A-fraction" or the linear amylose fraction and the "B-fraction" or the branched amylopectin fraction (Schoch, 17). The amylose appears

to be a linear chain with an average length of 200 - 300 α-D-glucopyranoside units that are connected through the α-1,4 linkage. A characteristic of this fraction is its ability to form a reproducible blue color with iodine (Schoch, 17). It has been postulated that the amylopectin fraction has a tree-like configuation that contains 50 - 70 branches, each being 25 - 30 glucose units in length (Bull, 1). Two characteristics that have been noted of the amylopectin fraction are: the development of a red color with iodine and its function as a protective colloid that surrounds the amylose in the starch granule.

Meyer (8) and also Krishnaswamy and Sreenivasan (4) developed methods for the initial separation of a portion of the amylose fraction from the starch granule by hot water extraction. The initial step was to suspend 1 - 2 percent of starch in water at 60° - 80° C for a number of hours. This causes swelling of the starch granules, which allows the crude amylose to diffuse out of the swollen granule. At about the same time in this country Schoch (16, 17) developed a method for the fractionation of starch that yielded a quantitative separation of the linear starch, or amylose, and the branch chain fraction, or the amylopectin. With this method, purification of the crude amylose was effected by a number of recrystallizations with certain higher molecular weight alcohols. For the initial recrystallization they used Pentasol which was is mixture of various primary amyl alcohols, while the final recrystal-

lizations were effected with n-butyl alcohol.

The use of starch as a reagent to detect traces of free iodine has been useful and interesting to chemists since 1812. In that year Colin and Gaultien de Claubry (2) studied the effects of iodine on a number of organic substances and noted that the most remarkable change was effected when starch and iodine interacted. During the nineteenth century many experiments were carried out under varied conditions in an effort to gain more knowledge about the interaction of starch and iodine. A review of the literature reveals conflicting reports about the combination of iodine with starch but this undoubtedly was due to the variety of conditions that were used for the various experiments.

Because of the extreme sensitivity of starch as an indicator for free iodine it has found wide use in direct and indirect iodimetric methods. The most useful application of the starch-iodine blue reaction was its use as an oxidation-reduction indicator in detecting traces of iodine that were liberated when iodide ion was oxidized to free iodine. It would be well to note that the bright blue color in a solution is due to the interaction of starch and triiodide, I_3^-, ion and not starch and free iodine, I_2. A difficulty that was encountered very early in the use of starch indicator solutions was the deterioration of the starch indicator solutions on standing. Many methods have been suggested to inhibit the deterioration of starch solutions.

Painter (11) devised a method for preparing a very stable starch solution. The method consisted of boiling together equal amounts of sodium carbonate and common rice starch, cooling the resultant solution, and acidifying with hydrochloric acid. Zinc was then added to the acidified solution which was filtered after standing 24 hours.

A stable and sensitive starch indicator solution was prepared by Minovici (9) by disolving starch in boiling water that contains mercuric chloride. Nichols (10) found that the addition of salicyclic acid preserved a starch solution for two months.

The work of Lambert et al (5, 6) produced a starch solution that was reproducible as well as stable for long periods of time. According to these workers, the natural toxicity of the cadmium and the formation of cadmium iodide complexes probably lend stability to the solution. The use of homogeneous linear starch fraction accounts for the fact that reproducible results were obtained with this reagent. The cadmium iodide-linear starch reagent was used throughout this investigation because of these characteristics.

Since the discovery of the starch-triiodide ion blue reaction, the nature of the product formed has aroused a great deal of interest, experimentation, speculation and postulation. The most generally accepted concept of the nature of the product of the starch iodide blue product is that of Rundle et al (12, 13, 14). In their investigations of the nature of the starch iodine

blue product, they used X-ray diffraction, spectrophotometric, potentiometric and optical analysis. By these methods they established that the starch iodide complex was a compound in which the iodine molecules were held, parallel to and inside the helix of the helical amylose chain. The general composition of the complex is one or more turns of the helical amylose chain, which contained six glucose residues, with molecules of iodine inside, parallel to the axis (Fig. 1). They found that the helix with the iodine on the inside yielded a bright blue color and also that the intensity of the color was dependent on the length of the amylose chain or the number of turns in the helix.

Many of the conflicting results of the various investigations of the starch-triiodide ion blue complex are undoubtedly due to the operation of different variables in the various techniques that were employed. The many explanations of the complex could be classified into two main groups (1) the formation of a "compound", (2) solid solution formation or adsorption of iodine.

PREPARATION OF LINEAR STARCH

Background

The method developed in this work for the separation of amylose for use in colorimetric iodimetric studies was undertaken in an effort to obtain a method whereby amylose of high purity could be produced with equipment that is available in most laboratories. The initial separation of the crude amylose is similar to Meyer's and Krishnaswamy and Sreenivasan's methods,

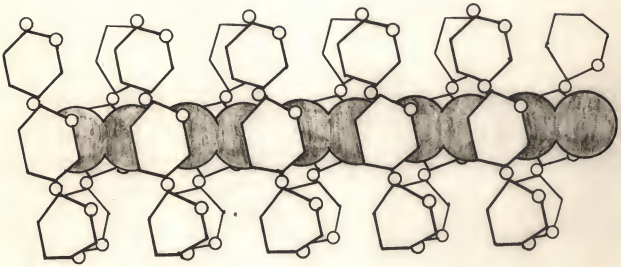


Fig. 1 Iodine Filled Amylose Helix (Rundle et al,15)

while the purification of the crude fraction essentially was an adaption of Schoch's selective precipitation method. The main modification of Schoch's method was the use of n-amyl alcohol as a precipitating agent for all recrystallizations of the amylose. In this method the n-amyl alcohol was found to be superior to n-butyl alcohol for precipitating the linear starch.

Apparatus and Chemicals

Beckman Model DU spectrophotometer.
Stirring motor.
Magnetic Stirrer.
Standard measuring spoon, tablespoon size.
Filter paper, Whatman No. 40 and 41, 11 cm. circles.
Filter paper, Whatman No. 50, 5.5 cm., circles.
Potato starch, Fischer Scientific Company, Catalog No. S-514.
Sodium chloride, reagent grade.
n-Amyl alcohol, Fischer Scientific Company, certified grade.
Methanol, synthetic, purified.
Diatomaceous earth, Johns-Manville Hyflo Super-Cel.
Cadmium iodide, reagent grade.

Procedure for Linear Starch Preparation

Two and one-half liters of distilled water and 2.50 grams of sodium chloride were added to a 3-liter beaker and heated to about 55° C. A thin paste containing 50 grams of potato starch

was then added to the mechanically stirred warm salt water. Stirring was continued and the temperature of the mixture was elevated to the 57° - 60° C. range and maintained for two hours. The stirring rate was moderate, being just fast enough to keep all the starch granules in suspension. Control of the temperature of the mixture was easily accomplished with an ordinary laboratory burner. After two hours at 57° - 60° C., the mixture was set aside for 5 to 7 hours. During this time the mixture cooled and the insoluble portion of the starch settled to the bottom of the beaker. The supernatant liquid containing the crude linear starch fraction was then carefully decanted. A tablespoon of diatomaceous earth per liter of the crude extract was then added while stirring the solution. It was then filtered through a previously prepared mat of diatomaceous earth in a Buchner funnel. This mat was prepared by pouring a slurry of three tablespoons of diatomaceous earth in about 300 ml. of distilled water onto a 11 cm. circle of No. 40 or 41 Whatman filter paper in a No. 3 Buchner funnel. The resulting mat was then washed three or four times with about 200 ml. of distilled water.

The filtrate was then diluted to 2.5 liters, with distilled water, 2.5 grams of sodium chloride was added and this solution was then heated to 80° - 90° C. The hot solution was then poured into a beaker containing enough n-amyl alcohol to saturate the solution and form a 1/2 - 3/4 inch layer on top of the solution. To insure rapid saturation of the solution

with the n-amyl alcohol, the mixture was poured back and forth from one beaker to another three or four times. This mixture was then set aside in a beaker covered with a watch glass and allowed to cool to room temperature. Usually the n-amyl alcohol-starch complex began to precipitate within one hour and maximum precipitation was attained in four to six hours. After maximum precipitation was attained, most of the liquid above the precipitate (about 2 liters) was decanted. The excess n-amyl alcohol was separated and saved for recovery by redistillation.

The remainder containing the precipitate was then diluted with distilled water to 2.5 liters, 2.5 grams of sodium chloride was added, and the resultant mixture was heated to 80° - 90° C., with subsequent redissolving of the precipitate. The saturation of the hot solution with n-amyl alcohol was carried out as in the first precipitation. The beaker was covered with a watch glass and set aside to cool. Maximum precipitation was again attained in four to six hours.

The liquid above the precipitate was decanted and the crude n-amyl alcohol was recovered for purification. The solution containing the precipitate was diluted to 2.5 liters, 2.5 grams of sodium chloride added and this mixture was heated to 80° - 90° C. as before. The hot solution was saturated with an excess of n-amyl alcohol and set aside to cool and to allow the linear starch-alcohol complex to precipitate.

As soon as the precipitate settled sufficiently, the excess

liquid was decanted. While stirring the starch-alcohol slurry, methanol was added until the volume increased about threefold. This mixture was set aside, and in approximately 30 minutes, the starch-alcohol complex had settled until most of the liquid portion was easily decanted. The starch-alcohol complex was then filtered through a 5.5 cm. No. 50 Whatman filter paper in a No. 1 Buchner funnel.

The moist residue was then broken into small clumps. These were added to about 200 ml. of n-amyl alcohol that was being vigorously stirred by a magnetic stirrer. The stirring was continued for three hours with occasional stops to break up lumps if they occurred. The alcohol was then decanted and replaced with about 400 ml. of n-amyl alcohol and stirred for another six hours. Most of the alcohol was then decanted and the linear starch was collected on a 5.5 cm. circle No. 50 Whatman filter paper in a No. 1 Buchner funnel. A firm pad of linear starch resulted which was then broken into small clumps. Drying of the linear starch was completed by either; (1) placing the clumps of starch in a medium-fritted borosilicate Buchner funnel and drawing air of low relative humidity through the funnel with an aspirator for eight or ten hours, or (2) air drying at low relative humidities for about a week. This product was readily soluble in boiling water and had a faint odor of n-amyl alcohol.

The blue complex of this linear fraction and of Schoch's linear starch "A-fraction" with triiodide ion, I_3^-, yielded

absorption curves of similar shape, and with maxima, minima and inflection points at the same wavelengths. Recently a new and six-month-old refiltered cadmium iodide reagent were used to test a solution containing a trace amount of triiodide, I_3^-. These two reagents yielded identical absorption curves even though they were prepared at different times from different batches of linear starch that was prepared by this method. This attests to the ability of this method for preparation of a reproducible starch fraction for volumetric iodimetry.

Preparation of Cadmium Iodide-Linear Starch Reagent (Lambert et al 5)

To about 200 ml. of distilled water, 5.50 grams of reagent grade cadmium iodide was added. The resulting solution was gently boiled at approximately constant volume for 15 minutes. Linear starch, 1.25 grams, was slowly added as gentle boiling was continued. This solution was then cooled, filtered through No. 40 Whatman filter paper and then diluted to 500 ml. with boiled distilled water.

EFFECTS OF CERTAIN ANIONS ON THE LINEAR STARCH-TRIIODIDE BLUE COMPLEX

Background

The use of the cadmium iodide-linear starch reagent in iodimetric methods has been reported in the literature (5, 6 and 7). Since this reagent finds uses under varied conditions, this study was undertaken in an effort to find out the effects of certain anions on the linear starch-iodine blue color.

After some preliminary work, it was decided to control the concentration of free triiodide ion released in the standard by using fixed amounts of the cadmium iodide-linear starch reagent, 0.2 N hydrochloric acid, potassium iodide solution and potassium iodate solution. The idea was to regulate the amount of iodide that was oxidized to free iodine by the addition of a fixed amount of iodate ion to a solution containing the acid and the excess iodide.

Apparatus, Chemicals and Reagents

Beckman Model DU spectrophotometer.

Beckman Model H2 pH meter.

Cadmium iodide linear starch reagent.

0.2 N hydrochloric acid.

Potassium acetate, chemically pure grade.

Potassium biiodate, G. F. Smith, 99.95%

Potassium bromide, chemically pure grade.

Potassium bromate, reagent grade.

Potassium chlorate, reagent grade.

Potassium chromate, chemically pure grade.

Potassium iodate, reagent grade

Potassium nitrate, reagent grade.

Potassium oxalate, reagent grade.

Potassium periodate, chemically pure grade.

Potassium persulfate, reagent grade.

Potassium monobasic phosphate, chemically pure grade.

Potassium acid phthalate, Baker and Adamson, meets A.C.S. requirements.

Potassium sulfate, reagent grade.

Experimental Procedure

In the early stages, some qualitative work was done to gain some insight into the effects of some anions on a system using the cadmium iodide-linear starch reagent to test for excess iodine or triiodide ion. Even though the concentrations used were too great to be of use in this semiquantitative investigation, it was observed that the most reproducible results were obtained if the iodide ion was mixed with the interfering anion and diluted to a convenient volume, with the final additions of the reagents necessary to release free iodine followed by the starch indicator.

In the presence of an excess of iodide ion in an acid media, enough iodate to oxidize 5 p.p.m. of iodide ion to free iodine, caused a suitable intensity of the blue starch iodine complex for this study. To accomplish this experimentally, a solution containing 13.2 p.p.m. of iodate as potassium iodate was prepared. This concentration of iodate was such that the 5 ml. of it oxidized 5 p.p.m. of iodide in 50 ml. of solution.

The excess of iodide ion was supplied to the system by the cadmium iodide-linear starch reagent and an aliquot of potassium iodide solution that would amount to 5 p.p.m. of iodide ion in

the solution examined. In actual practice, a solution of 200 p.p.m. iodide ion as potassium iodide was prepared and 1.25 ml. of this solution was added to a sample with a final volume of 50 ml. About 450 p.p.m. of iodide was supplied by cadmium iodide to a sample with a final volume of 50 ml. and to which had been added 3.0 ml. of cadmium iodide-linear starch reagent.

From previous work with this reagent, it was possible to predetermine a concentration of acid that would be in excess of the requirements for the production of free iodine and consequently produce an intense blue starch-triiodide ion complex with an excess of the reagent. This amount of acid was calculated to be 4.0 ml. of 0.2 N hydrochloric acid. To six different samples containing 10 p.p.m. of iodide ion, 5.0 ml. of 13.2 p.p.m. iodate ion solution, 4.0 ml. of 0.2 N hydrochloric acid and enough water to make the total volume 50 ml. after the cadmium iodide-linear starch reagent was added in aliquots of 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 ml. respectively. These samples were allowed to stand about thirty minutes for color development and then their intensities were determined at 600 mμ with a Beckman Model DU spectrophotometer. The results indicated that 3.0 and 4.0 ml. of the starch reagent produced a slightly increased absorbance. Thus, 3.0 ml. of the starch reagent were considered sufficient for the conditions that were employed in this investigation.

Another group of six samples were prepared similar to those

used to determine the proper amount of starch indicator solution. This time the amount of 0.2 N hydrochloric acid added was varied as follows: 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 ml. to a 50 ml. sample, and the amount of starch indicator added was 3.0 ml. in each instance. Samples containing three or more milliliters of 0.2 N hydrochloric acid required essentially the same amount of time for color development. Thus, the use of 3.0 ml. of the 0.2 N hydrochloric acid was considered satisfactory for this investigation.

For the studies involving color development and adsorption curves the order of addition of the various components was the same. First, 1.25 ml. of 200 p.p.m. iodide was added; second, the interfering anion was added from a 500 p.p.m. or 5000 p.p.m. solution of the anion; third, 5.0 ml. of the 13.2 p.p.m. iodate solution was added; fourth, the solution was diluted to 44.0 ml. with distilled water; fifth, 3.0 ml. of 0.2 N hydrochloric acid was added and sixth, 3.0 ml. of the cadmium iodide-starch reagent was added.

The color development curves were obtained at 600 mμ with a Beckman Model DU spectrophotometer. The starting point for elapsed time was considered to be when the starch reagent was added. Each sample was run concurrently with a control, containing no interfering anion, that had an absorbance of 0.725.

For the absorption curves the samples were prepared and allowed to develop to their maximum absorbancies. The data for their curves were then obtained from 400 - 700 mμ in 10 mμ

increments with a Beckman Model DU spectrophotometer. These data were always taken concurrently with a control containing no interfering anions.

The pH of the solutions containing acetate and several other ions at equilibrium were determined with a Beckman Model H pH meter. The pH of the controls were in the range from 2.7 - 2.8.

Discussion of the Effects of Certain Anions on the Linear Starch-Triiodide Blue Complex

When the elementary conditions of the system were fixed, they naturally excluded the study of certain anions. The use of hydrochloric acid to produce a fairly constant hydrogen ion concentration excluded the study of the effect of chloride ion on the starch-triiodide blue complex in this system. The acidity also eliminated the possibility of studying the effects of carbonate and cyanide ions on the starch-triiodide blue complex in this system. Even though some anions were excluded, it was possible to obtain twelve potassium salts and study some of their effects on the starch-triiodide blue complex. The anions studied were: acetate, bromide, bromate, chlorate, chromate, nitrate, oxalate, periodate, persulfate, monobasic phosphate acid phthalate and sulfate. Because of the pronounced changes noted with the acetate ion, it was investigated more fully than the other anions that were studied.

To minimize the possibility of other ion effects, only salts with a common cation were used. Potassium salts were chosen because of their availability, purity and other desirable

properties. The other cations introduced into the system were cadmium and hydrogen, but the amounts introduced were always nearly the same, so their effects were essentially constant.

After the decision to use iodate as the oxidizing agent to release the free iodine to give the triiodide ion, I_3^-, the problem of another ion resulting from reduction of the oxidizing agent was eliminated because the iodate was reduced to iodine.

A cursory study of the color development effects caused by hydrochloric acid led to the conclusion that the use of from 2.5 ml. - 5.0 ml. of 0.2 N hydrochloric acid in a total volume of 50.0 ml. effected a rapid equilibrium which in about 15 minutes produced a constant intensity of the starch-iodine blue complex at maximum absorption.

A study to determine the proper amount of cadmium iodide-linear starch to be used gave some interesting information. An insufficient amount of the reagent produced a green color instead of a blue color, while an excess of the reagent produced a blue color. For the concentrations of triiodide, I_3^-, encountered in this investigation, 3.0 ml. of the reagent was sufficient to produce a rapid reproducible blue color of a constant intensity.

The possibility of using the same relative concentrations of the various anions when studying their effects on the starch-iodine blue complex was eliminated very early. The reason was that relatively high concentrations of some anions had little or no effect, while minute amounts of other anions caused

profound effects on the color development time and the absorption curves involved. Two factors limited the concentration of interfering ion used: (1) for oxidizing anions the limiting factor was the lowest concentration of an anion that caused maximum absorption; (2) the concentration of an anion that would cause precipitation of the linear starch-triiodide blue complex.

The procedure followed was to determine the oxidizing effect of the anions. Then with the oxidants, two usable concentrations were chosen for further investigation. A usable concentration was one that did not cause a maximum absorption at 600 mμ that was too intense for accurate determination.

For anions that were not oxidants, the concentration of the interfering anion was increased until precipitation of the linear starch-triiodide blue complex occurred. Then two concentrations of the anion lower than that necessary for precipitation and such that they would not precipitate the blue starch-triiodide complex in less than 24 hours were studied.

Color Development. For anions, oxidizing and otherwise, that showed marked differences between their behavior and the behavior of the control, a most striking effect was noted in their color development.

The effect of the periodate ion was of particular interest because it was so different from the other oxidizing anions. For the concentrations of this ion tested, the intensity of the starch iodine blue complex was essentially constant for

from one to eight hours (Plate I).

A contrast to the action of periodate ions was noted when compared to similar low concentrations of bromate ions. When low concentrations of bromate ions were added to the linear starch-triiodide system, about seventeen hours were required to attain a constant maximum absorption for the blue complex. The effect of the chlorate ion on the starch-triiodide complex maximum absorption is similar to the effect of bromate, but much less intense (Plate I).

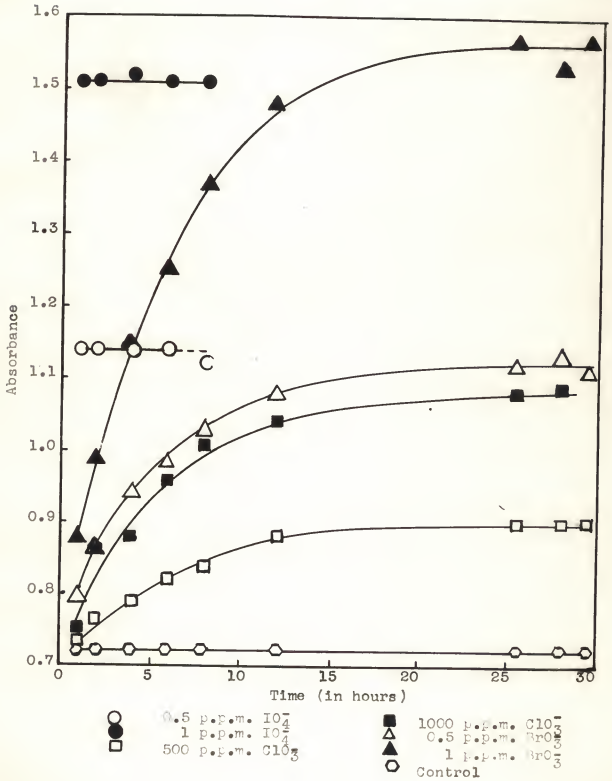
The other common oxidizing anions tested required 24 to 30 hours to attain a constant value for the maximum absorption of the blue starch iodine complex. Among those were chromate and persulfate (Plate II).

The color development times for the oxidants studied apparently were related to the establishment of equilibria for each concentration of the anions studied. Extinction coefficients per unit valence change were calculated from the maximum optical density obtained and from the concentrations that were studied. These are reported in Table 1; the optical density values were obtained from Plates I and II. This extinction coefficient was an index of whether the theoretical concentration of the linear starch-triiodide blue ion was attained. Thus, the extinction coefficient, when based on a maximum optical density, afforded a means of determining whether the oxidizing anion was used only for the production of iodine, I_2, and subsequently triiodide ion I_3^-. Most of the calculated values for the extinction

EXPLANATION OF PLATE I

Color development curves for the linear starch-triiodide blue ion without an interfering anion, and with 0.5 and 1 p.p.m. IO_4^-; 500 and 1000 p.p.m. ClO_3^-; 0.5 and 1 p.p.m. BrO_3^-.

PLATE I


1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
Absorbance
0
5
10
15
20
25
30
Time (in hours)
C
0.5 p.p.m. IO_4^-
1 p.p.m. IO_4^-
500 p.p.m. ClO_3^-
1000 p.p.m. ClO_3^-
0.5 p.p.m. BrO_3^-
1 p.p.m. BrO_3^-
Control

EXPLANATION OF PLATE II

Color development curves for the linear starch-triiodide blue ion without an interfering anion, and with 1 and 2 p.p.m. $CrO_4^{=}$; 5 and 10 p.p.m. $S_2O_8^{=}$.

PLATE II

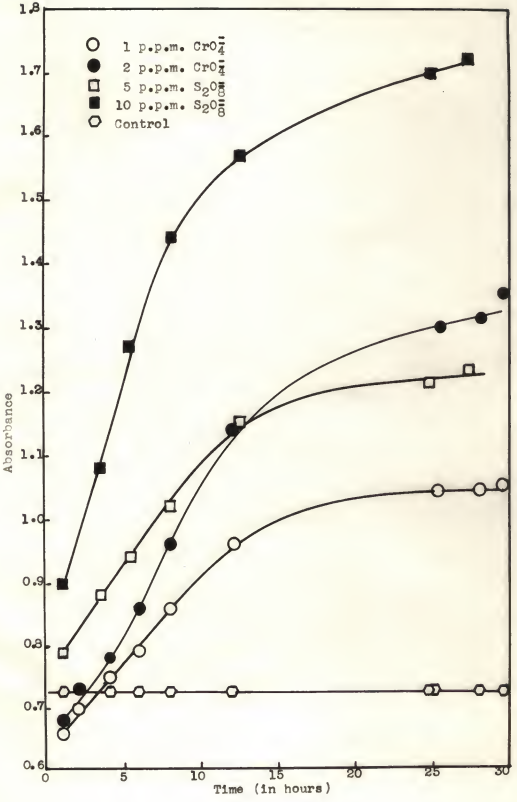

1 p.p.m. $CrO_4^{=}$
2 p.p.m. $CrO_4^{=}$
5 p.p.m. $S_2O_3^{=}$
10 p.p.m. $S_2O_3^{=}$
Control
Absorbance
Time (in hours)
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0
5
10
15
20
25
30

Table 1. Calculated values of extinction coefficients per unit valence change with values necessary for their calculation.

Anion	p.p.m.	Electron Change When Reduced	Formal Concentration	Optical Density D_{obs}	Extinction Coefficient per Unit Valence Change (ϵ Calc.)
IO_4^-	0.5	8	3.8×10^{-6}	1.14	37,000
	1	8	7.6×10^{-6}	1.52	24,900
ClO_3^-	500	6	8.8×10^{-3}	0.905	16.7
	1000	6	1.8×10^{-2}	1.08	10.2
BrO_3^-	0.5	6	5.1×10^{-6}	1.12	36,700
	1	6	1.0×10^{-5}	1.56	25,500
$CrO_4^=$	1	3	1.4×10^{-5}	1.05	24,400
	2	3	2.9×10^{-5}	1.33	15,400
$S_2O_8^=$	5	2	3.8×10^{-5}	1.22	15,800
	10	2	7.7×10^{-5}	1.72	11,150

coefficient in this study are of the same magnitude as the value of 17,000 per equivalent of oxidizing agent that was determined by Lambert (6). So, with the exception of the chlorate ion, it appears that the oxidizing anions in this system oxidized only iodide. Chlorate may oxidize some other ion or it may form a product that interfers with the development of the linear starch-triiodide blue ion complex.

A number of anions that usually are not classified as oxidants showed relatively small constant interferences in the color development curves at specified concentrations for a period of time from two hours to eight hours after preparation. The anions comprizing this group were the following: monobasic phosphate, nitrate, bromide, sulfate and acid pthalate (Plate III, Figs. 1, 2 and 3; and Plate IV, Figs. 1 and 2).

The acetate and oxalate ions caused definite lowering of the constant maximum intensity of their respective color development curves. Both of these curves were also dependent on the concentration of the anions that would cause precipitation of the color complex. Two and three thousand parts per million of the oxalate caused a moderate lowering of the absorption from that of the control that contained no interfering anion. The intensity of maximum absorption for oxalate was unchanged for from two hours to twelve hours after preparation of the starch-triiodide blue ion complex. The initial study of acetate with 500 - 1000 p.p.m. indicated a behavior similar to oxalate, but further investigation revealed that the higher concentration of acetate required a long time to reach maximum intensity. This led to a more intensive study of the effect of acetate ion on the system used.

Data from samples containing varying concentrations of acetate ion and hydrochloric acid were obtained and plotted (Plates V, VI, VII, VIII and IX). A perusal of these graphs indicated some connection between length of color development

EXPLANATION OF PLATE III

Fig. 1. Color development curves for the linear starch-triiodide blue ion without an interfering anion and with 2000 and 3000 p.p.m. Br^-.

Fig. 2. Color development curves for the linear starch-triiodide blue ion without an interfering anion and with 500 and 1000 p.p.m. NO_3^-.

Fig. 3. Color development curves for the linear starch-triiodide blue ion without an interfering anion and with 200 and 500 p.p.m. $H_2PO_4^-$.

PLATE III

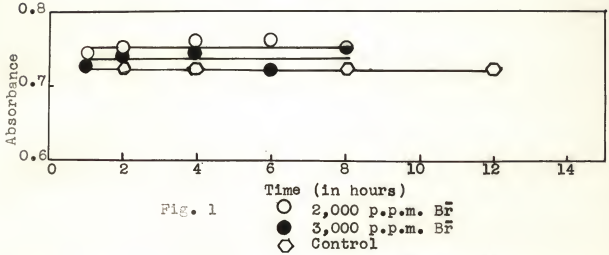


Fig. 1

○ 2,000 p.p.m. Br^-
● 3,000 p.p.m. Br^-
⬡ Control

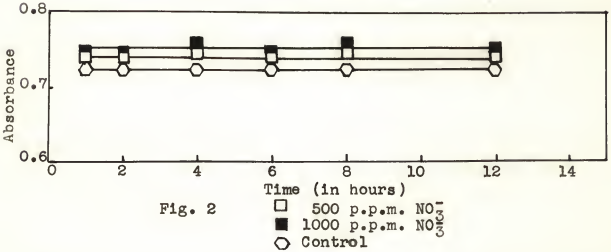


Fig. 2

□ 500 p.p.m. NO_3^-
■ 1000 p.p.m. NO_3^-
⬡ Control

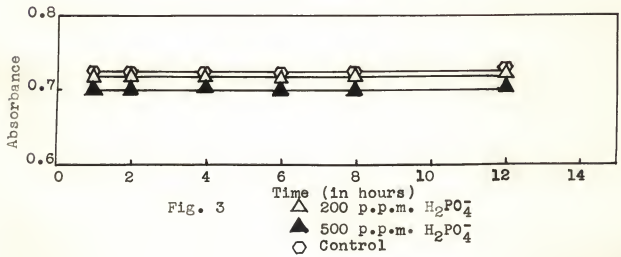


Fig. 3

△ 200 p.p.m. $H_2PO_4^-$
▲ 500 p.p.m. $H_2PO_4^-$
⬡ Control

EXPLANATION OF PLATE IV

Fig. 1. Color development curves for the linear starch-triiodide blue ion without an interfering anion and with 1000 and 2000 p.p.m. acid phthalate.

Fig. 2. Color development curves for the linear starch-triiodide blue ion without an interfering anion and with 2000 and 2500 p.p.m. $SO_4^{=}$.

PLATE IV

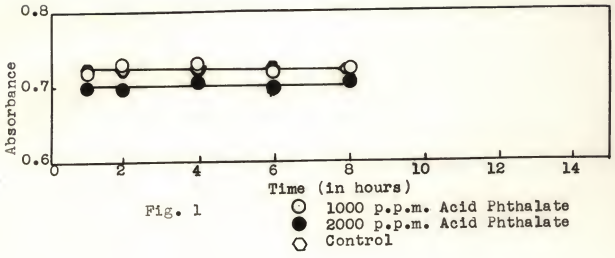


Fig. 1

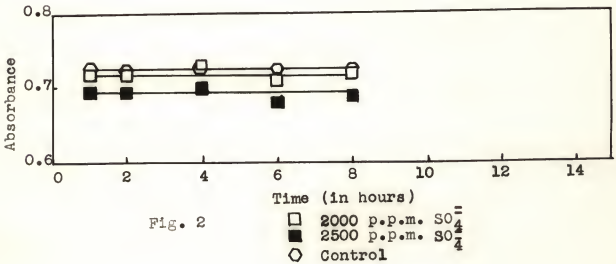
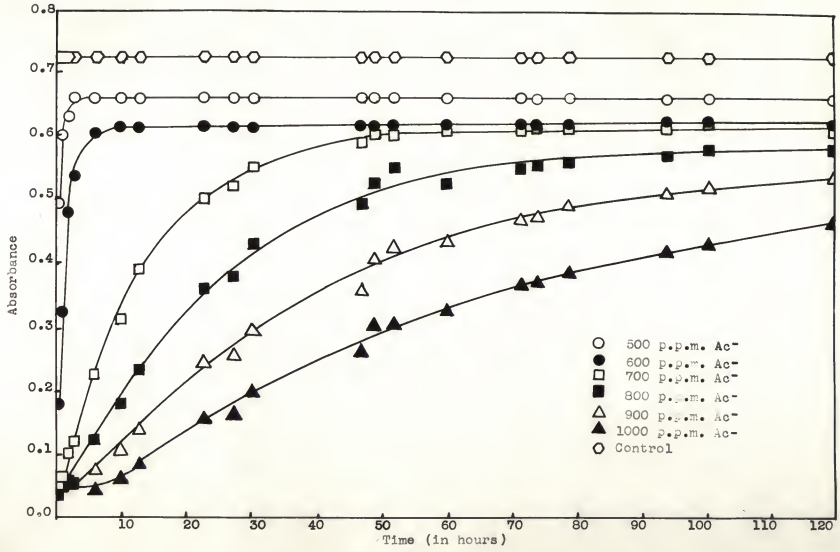


Fig. 2

EXPLANATION OF PLATE V

Color development curves for the linear starch-triiodide blue ion without an interfering anion, and with 500, 600, 700, 800, 900 and 1000 p.p.m. Ac^-.

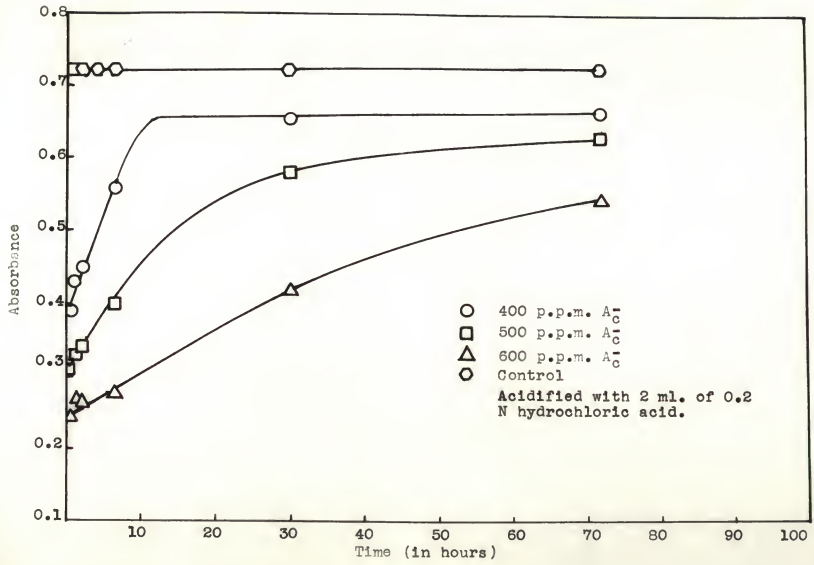
PLATE V


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Absorbance
10
20
30
40
50
60
70
80
90
100
110
120
Time (in hours)
500 p.p.m. Ac-
600 p.p.m. Ac-
700 p.p.m. Ac-
800 p.p.m. Ac-
900 p.p.m. Ac-
1000 p.p.m. Ac-
Control

EXPLANATION OF PLATE VI

Color development curves for the linear starch-triiodide blue ion without an interfering anion, and with 400, 500 and 600 p.p.m. Ac^-, when the samples were acidified with 2 ml. of 0.2 N hydrochloric acid.

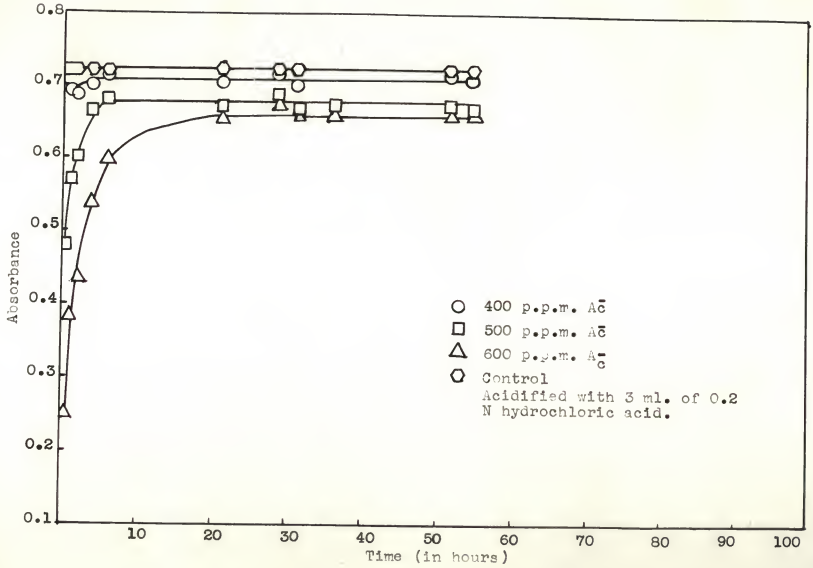
PLATE VI


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Absorbance
10
20
30
40
50
60
70
80
90
100
Time (in hours)
400 p.p.m. Ac
500 p.p.m. Ac
600 p.p.m. Ac
Control
Acidified with 2 ml. of 0.2 N hydrochloric acid.

EXPLANATION OF PLATE VII

Color development curves for the linear starch-triiodide blue ion without an interfering anion, and with 400, 500 and 600 p.p.m. Ac^-, when the samples were acidified with 3 ml. of 0.2 N hydrochloric acid.

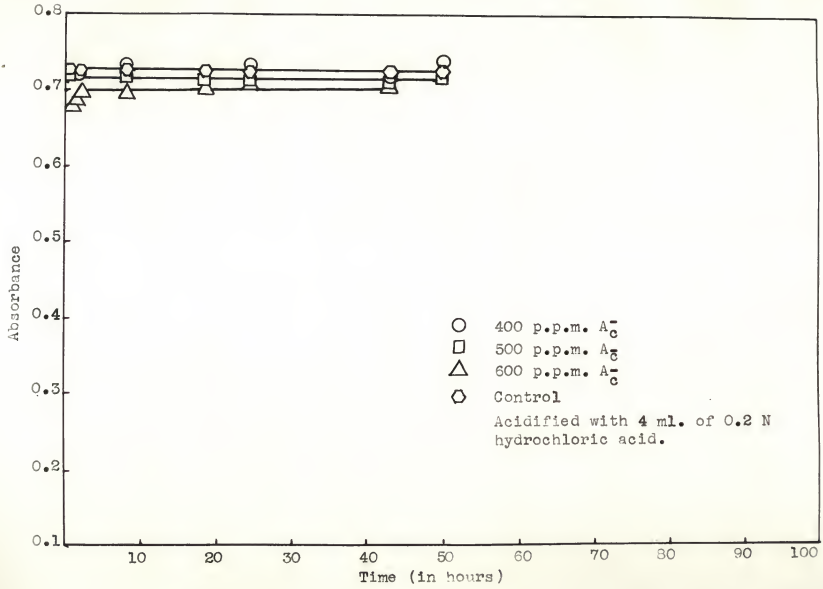
PLATE VII


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Absorbance
10
20
30
40
50
60
70
80
90
100
Time (in hours)
400 p.p.m. Ac̄
500 p.p.m. Ac̄
600 p.p.m. Ac̄
Control
Acidified with 3 ml. of 0.2 N hydrochloric acid.

EXPLANATION OF PLATE VIII

Color development curves for the linear starch-triiodide blue ion without an interfering anion, and with 400, 500, and 600 p.p.m. Ac^-, when the samples were acidified with 4 ml. of 0.2 N hydrochloric acid.

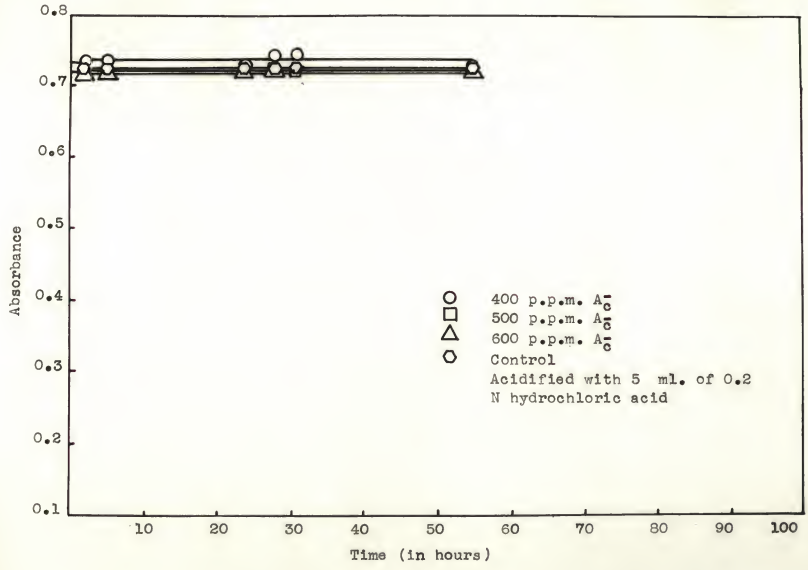
PLATE VIII


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Absorbance
10
20
30
40
50
60
70
80
90
100
Time (in hours)
400 p.p.m. A_c^-
500 p.p.m. A_c^-
600 p.p.m. A_c^-
Control
Acidified with 4 ml. of 0.2 N hydrochloric acid.

EXPLANATION OF PLATE IX

Color development curves for the linear starch-triiodide blue ion without an interfering anion, and with 400, 500 and 600 p.p.m. Ac^-, when the samples were acidified with 5 ml. of 0.2 N hydrochloric acid.

PLATE IX


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Absorbance
10
20
30
40
50
60
70
80
90
100
Time (in hours)
400 p.p.m. Ag
500 p.p.m. Ag
600 p.p.m. Ag
Control
Acidified with 5 ml. of 0.2 N hydrochloric acid

time and the acetate and acid concentration. The concentration of acetate ion in the various samples was readily calculated since the concentration of acetate ion added was known and the respective hydrogen ion concentrations were readily obtained from pH measurements. Correlation of free acetate ion concentration (Table 2 and Plate X) with color development time (Plates V, VI, VII, VIII and IX) indicated that in the main, as the concentration of free acetate ion increased, the maximum absorption decreased and the length of color development time increased.

Rundle et al (12) found that the "hole" in the center of the linear starch helix was 6 Å in diameter. When a molecular scale model of acetate ion was constructed and measured, it was found to vary from a minimum of 4 Å to a maximum of 6 Å in size. The maximum absorption decreased with higher acetate ion concentration. From these facts it is believed that acetate ions may have associated with iodine molecules in solution to yield a iodine-acetate ion, I_2Ac^- complex which could then enter the linear-starch helix without developing a characteristic blue color formed by the triiodide ion. A competition for iodine molecules may have developed between acetate and iodide ion to produce the color development effect that was observed. When equilibrium was attained there may have been a constant ratio of triiodide ions to iodine acetate ions.

Absorption Curves. The effects of the various anions on the absorption curve of the fully developed linear starch-

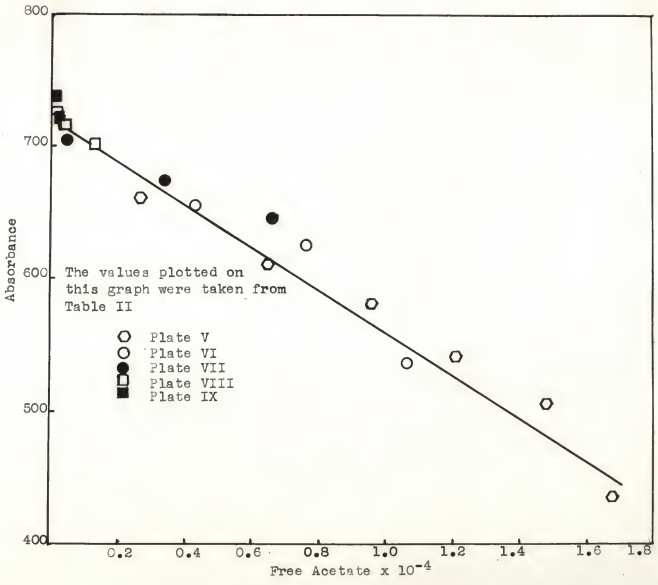
Table 2. Calculated values of free acetate ion in solution, with experimental values necessary for their calculation.

Plate	p.p.m. Acetate Added	pH	Absorption Maximum	Concentration of Free Acetate Ion in Solution
V	500	4.15	.660	0.27×10^{-4}
	600	4.6	.610	0.64×10^{-4}
	700	4.8	.580	0.96×10^{-4}
	800	4.9	.540	1.21×10^{-4}
	900	5.0	.510	1.48×10^{-4}
	1000	5.1	.440	1.78×10^{-4}
VI	400	4.6	.655	$.43 \times 10^{-4}$
	500	4.9	.625	$.75 \times 10^{-4}$
	600	5.1	.535	1.07×10^{-4}
VII	400	3.4	.705	$.046 \times 10^{-4}$
	500	4.3	.675	$.34 \times 10^{-4}$
	600	4.6	.660	$.65 \times 10^{-4}$
VIII	400	3.0	.725	$.018 \times 10^{-4}$
	500	3.3	.715	$.045 \times 10^{-4}$
	600	3.7	.700	$.13 \times 10^{-4}$
IX	400	2.9	.735	$.015 \times 10^{-4}$
	500	3.0	.720	$.023 \times 10^{-4}$
	600	3.1	.715	$.035 \times 10^{-4}$

EXPLANATION OF PLATE X

A plot of acetate ion versus absorbance from the values in Table 2. This illustrates the general relation of decreasing absorbance with increase of acetate ion.

PLATE X


800
700
600
500
400
Absorbance
The values plotted on this graph were taken from Table II
Plate V
Plate VI
Plate VII
Plate VIII
Plate IX
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
Free Acetate x 10^{-4}

triiodide blue ion complex in the presence of an interfering anion was to cause either an increase, a decrease or no effective change in the intensity of absorption. If a large change was noted in the absorption curve maximum, the whole curve would be moved up or down on the absorption axis. If a small change was noted in the maximum, the only observable change would be near the maximum. Examples of the large changes are Plates XI and XII. An example of a minor change is Plate XIII. The curves show that these anions do not cause these curves to shift their maxima, minima or inflection points, but the change is entirely one of intensity of the absorption curve.

SUMMARY

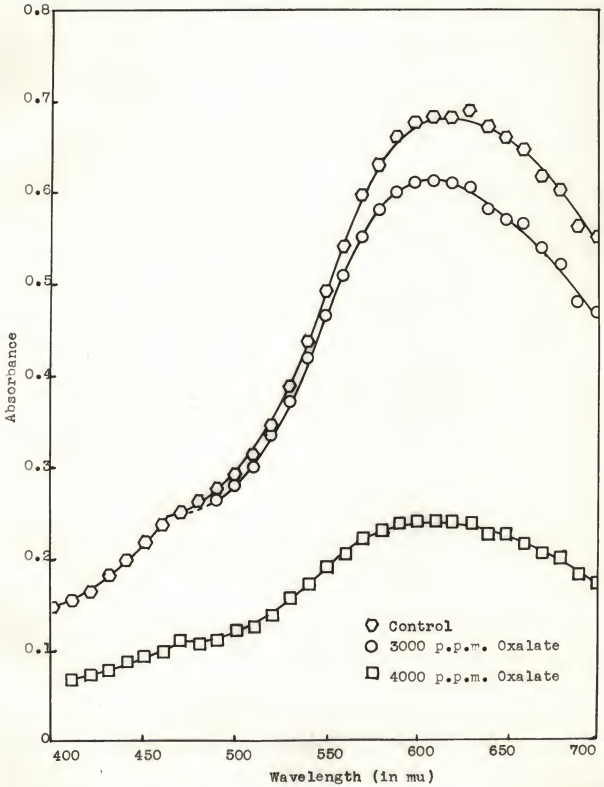
The method described for the extraction and purification of linear starch fraction produces a product that is useful and reproducible. When incorporated in the cadmium iodide-linear starch reagent, this linear starch gives reproducible results when used in quantitative colorimetric iodimetry. The linear starch-triiodide blue complex is stable when fully developed, provided an excess of an ion that would precipitate the complex is not present.

Certain concentrations of some of the anions studied in this system caused definite interferences in the color development time of the linear starch-triiodide blue complex, while other anions have little effect on the color development

EXPLANATION OF PLATE XI

Absorption curves for the linear starch-triiodide blue ion without an interfering anion, and with 3000 and 4000 p.p.m. oxalate.

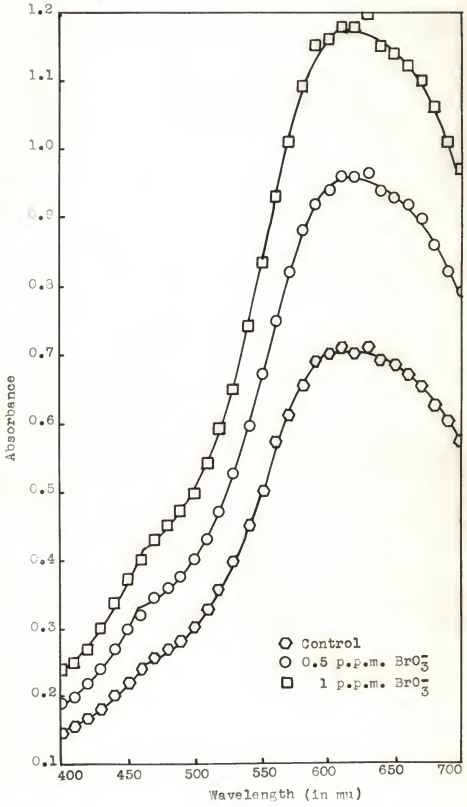
PLATE XI


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Absorbance
400
450
500
550
600
650
700
Wavelength (in mu)
Control
3000 p.p.m. Oxalate
4000 p.p.m. Oxalate

EXPLANATION OF PLATE XII

Absorption curves for the linear starch-triiodide blue ion without an interferanion, and with 0.5 and 1.0 p.p.m. BrO_3^-.

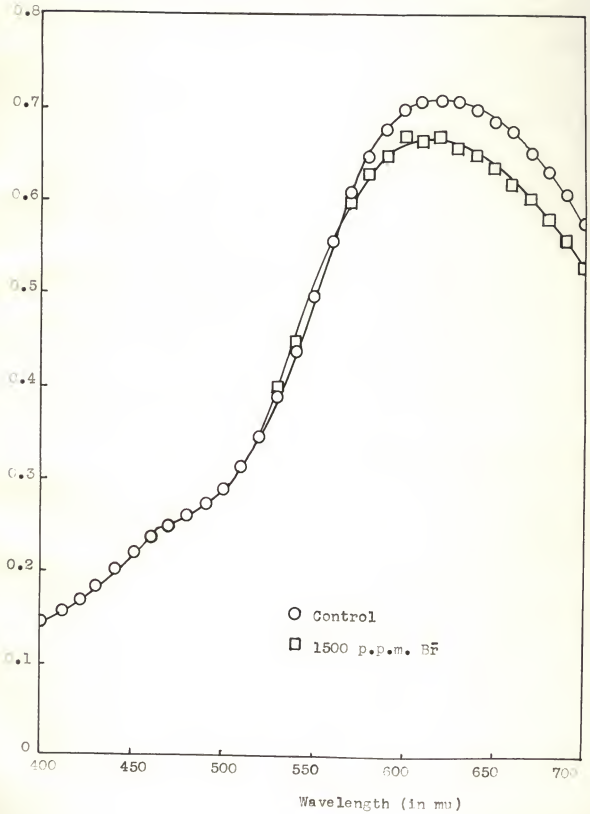
PLATE XII


1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Absorbance
400
450
500
550
600
650
700
Wavelength (in mu)
Control
0.5 p.p.m. BrO_3^-
1 p.p.m. BrO_3^-

EXPLANATION OF PLATE XIII

Absorption curves for the linear starch-triiodide blue ion without an interfering anion and with 1500 p.p.m. Br^-.

PLATE XIII


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
450
500
550
600
650
700
Control
1500 p.p.m. BF^-
Wavelength (in mu)

time of the complex.

Bromide, nitrate, monobasic phosphate, sulfate, oxalate and acid phthalate ions caused essentially no change in the normal color development time for the linear starch-triiodide blue complex in this system. All of these anions except oxalate ion, did cause minor changes in the final absorption maximum. For 2000 and 3000 p.p.m. of oxalate ion definite decreases in the final absorption maximum for the linear starch-triiodide blue complex were observed. Also, the respective absorption maxima were not as intense for the oxalate ion.

Acetate ion caused a very definite interference with the color development for the linear starch-triiodide blue complex. For 800, 900 and 1000 p.p.m. of acetate ion the color development time was in excess of three days. The final maximum absorption apparently was dependent on the acetate ion concentration. An increase in the acetate ion concentration was also accompanied by a decrease in absorbance of the fully developed linear starch-triiodide blue complex. It is possible that the iodine-acetate ion was formed that displaced the triiodide ion from the starch helix and caused a corresponding increase in color development time and a decrease in maximum absorbance.

The effect of periodate on the system did not extend the normal color development time appreciably. The effect of periodate was similar to the addition of more iodate, and

caused the maximum absorption to be more intense.

The color development effects of most of the oxidizing anions studied produced color development curves that required fifteen to thirty hours to attain maximum absorbance. Included in this group of anions were bromate, chlorate, chromate and persulfate. The extinction coefficients per unit valence change for these anions were calculated and compared to a known value for the linear starch-triiodide blue ion. The reasonable correlation between the known value and those calculated indicated that the interference encountered was mainly due to oxidation of iodide to iodine, but chlorate did not follow this pattern.

The effects of the various anions on the absorption curves of the fully developed starch iodine blue complex in the presence of an interfering anion was to cause either an increase, a decrease or no effective change in the intensity of absorption. If a large change was noted in the maximum, the whole curve would be shifted up or down on the absorption axis. If a small change was noted in the maximum, the only observable change would be near the maximum. The absorption curves indicated that the anions studied did not cause the curves significantly to shift the wavelength of their maxima, minima or inflection points, but to change the intensity of the respective absorptions.

ACKNOWLEDGMENT

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THE EFFECTS OF CERTAIN ANIONS ON THE LINEAR STARCH-TRIIODIDE BLUE COMPLEX

by

STANLEY COPELAND RHOADS

B. S., Abilene Christian College, 1951

AN ABSTRACT OF A THESIS

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MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1958

The cadmium iodide-linear starch reagent has been used in quantitative colorimetric iodimetry for detecting trace amounts of free iodine, I_2, as the triiodide ion, I_3^-.

The purpose of this work was twofold: (1) to develop a simplified method for isolating a reproducible linear starch fraction; (2) to study the effects of certain anions on the linear starch-triiodide blue color.

The crude linear starch fraction was separated from potato starch by leaching in a very dilute solution of sodium chloride for two hours at 57° - 60° C. Purification was effected by twice precipitating the linear starch from a very dilute solution of sodium chloride at 80° - 90° C with n-amyl alcohol. The final linear starch slurry was precipitated by methanol and the floculant precipitate suspended in two changes of n-amyl alcohol. The final starch n-amyl alcohol complex was air dried.

The method described for the extraction and purification of linear starch fraction yields a product that is stable and reproducible. When used to prepare the cadmium iodide-linear starch reagent, reproducible results were obtained in quantitative colorimetric iodimetry.

When studying the effects of certain anions on the linear starch-triiodide blue complex, the concentration of free triiodide ion released in the standard was controlled by using fixed amounts of cadmium iodide-linear starch reagent, 0.2 N hydrochloric acid, potassium iodide solution and potassium iodate solution. The theoretical amount of iodide that oxidized to

free iodine was regulated by the addition of a fixed amount of iodate ion to a solution containing the acid and the excess iodide ion. Appropriate concentrations of the anions under study were added to their respective samples before the reagents were added, and their effects were studied spectrophotometrically. Data obtained in this way were plotted as color development and absorption curves.

Bromide, nitrate, monobasic phosphate, sulfate, oxalate and acid phthalate ions caused essentially no change in the normal color development time for the linear starch-triiodide blue complex in this system. All of these anions except oxalate ion, caused minor changes in the final absorption maximum. For 2000 and 3000 p.p.m. of oxalate ion, definite decreases in the final absorption maxima of the linear starch-triiodide blue complex were observed.

Acetate ion caused a very definite interference with the color development time for the linear starch-triiodide blue complex. For 800, 900 and 1000 p.p.m. of acetate the color development time was in excess of three days. The final maximum absorption apparently was related to the acetate ion concentration. An increase in the acetate ion concentration was also accompanied by a decrease in absorbance of the fully developed linear starch-triiodide blue complex. The proposed iodine acetate ion that was formed is thought to displace some triiodide ion from the starch helix and cause a corresponding increase in color development time and a decrease in maximum absorbance.

The effect of periodate ion on the system did not extend the normal color development time appreciably. The effect of periodate was similar to the addition of more iodate, so the only effect was to cause an increase in maximum absorption.

The color development effects of most of the oxidants studied extended to fifteen to thirty hours, the time necessary to attain maximum absorbance. Included in this group of anions were bromate, chlorate, chromate and persulfate.

The extinction coefficients per unit equivalent of oxidant were calculated and compared to a known value for the linear starch-triiodide blue ion. The reasonable correlation between the known value and those calculated indicated that the interference encountered was due mainly to oxidation of iodide to iodine, but chlorate did not follow this pattern.

The effects of the various anions on the absorption curve of the fully developed starch-triiodide ion blue complex in the presence of an interfering anion was to cause either an increase, a decrease or no effective change in the intensity of absorption. If a large change was noted in the maximum, the whole curve would be shifted up or down on the absorbance axis. If a small change was noted in the maximum, the only observable change would be near the maximum. These studies show that these anions do not cause the curves to shift their maxima, minima or inflection points, but that the change is one of intensity of absorption.