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THE COLORIMETRIC ESTIMATION OF SILICON IN TISSUES BY ISAACS' METHOD

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The accumulating evidence of the existence of silico-organic complexes in such material as starch makes a micro method of estimating silicon extremely valuable. Such a method was developed by Isaacs[1] in this Laboratory and was recently published. Almost immediately, Bertrand[2] put forward a very severe criticism of the method and of the results obtained by its use in the estimation of silicon in animal tissues.

The process employed by Isaacs consists of ashing as small a quantity as 0.5 g. of dried tissue by ignition with nitric acid in the presence of boric acid and calcium nitrate. Ignition converts the nitrate to oxide. To reconvert oxide to nitrate the ash is warmed with a few drops of nitric acid. The silica in the ash is dissolved by warming with silicon-free caustic soda. The excess of alkali is neutralized with acetic acid. More acetic acid and distilled water are added and the solution obtained is treated with ammonium molybdate solution and heated for five minutes in a boiling water-bath. The silicomolybdate formed is reduced to a deep blue compound by the addition of sodium sulfite. The color thus produced is compared with the color given by standard silicate solutions. The sensitivity of the method, Isaacs claimed, is very great. In more than one case he was able to estimate as small a quantity of silicon as 0.5 mg. in 100 g. of dried tissue. Since he took only 0.5 g. of tissue for ashing, his colored solution in these cases contained only 0.0025 mg. of silica, or 0.00115 mg. of silicon. It is obvious that in testing such a method, silica present as impurity in reagents even in very small traces would entirely mislead one as to the nature of results.

Bertrand's main criticism of the method was that under the same conditions phosphate would give a phosphomolybdate and that this would also be reduced by the sodium sulfite to give a blue color. Obviously, if this were true, Isaacs' method would be useless for the determination of silicon in tissues, since phosphorus is always present in large quantities. Other points criticized were matters of technique and the stability of the colored compounds produced.

The usefulness of Isaacs' method is established if it can be shown that (*a*) phosphomolybdates do not give a blue reduction product when treated with sodium sulfite in the presence of acetic acid; (*b*) quantitative mixtures of silicate and phosphate do not give a color *more* intense than would be

[1] Isaacs, *Bull. soc. chim. biol.*, **6**, 157 (1924).

[2] Bertrand, *ibid.*, **6**, 656 (1924).

given by solutions having the same concentration of silicate but no phosphate. The present paper contains data which clearly demonstrate these two points.

In all of the experiments performed, we used systems made up as follows, the reagents being added in the order listed. (These mixtures duplicated the actual mixture finally obtained by Isaacs when tissues containing various quantities of phosphate and silicate were ashed.)

(*a*) A measured quantity of a known phosphate solution or, for control, a known solution of sodium silicate. (*b*) 1 cc. of 6% sodium acetate solution. In the process of preparing tissue ash for color determination, Isaacs added 3 cc. of 2% sodium hydroxide solution and later neutralized this with 10% acetic acid. (*c*) 3 cc. of 10% acetic acid or, in special cases noted below, 10% sulfuric acid. (*d*) 1 cc. of a 5% solution of hydrated calcium nitrate, $Ca(NO_3)_2.12H_2O$. This is the amount added by Isaacs in the ashing process. It is, of course, converted to oxide at the red heat employed, but is later converted to nitrate by addition of nitric acid. (*e*) 5 cc. of 10% ammonium molybdate solution. (*f*) Distilled water up to 22 cc.

The mixtures were prepared in Pyrex tubes. They were placed in a boiling water-bath for five minutes, then removed and 3 cc. of saturated sodium sulfite solution was added immediately, as in Isaacs' directions. After an interval of 15 minutes the solutions were compared in a colorimeter.

In testing Isaacs' method, Bertrand used a sodium phosphate solution made from alkali and phosphoric acid, prepared by dissolving metallic sodium and phosphorus pentoxide in distilled water. With this reagent, in the presence of acetic acid, he obtained a molybdate which, on reduction with sodium sulfite, gave a blue color. The color did not, however, appear so rapidly as when the same procedure was applied to a solution of sodium silicate whose content of silica was equivalent to the phosphorus pentoxide content of the phosphate solution. On this evidence, Bertrand suggested that Isaacs' results are really estimates of the amount of the phosphate which is always present in tissues in a concentration far higher than that of silicon.

It is very difficult to prepare sodium phosphate absolutely free from silica. Bertrand was mistaken in assuming that by preparing his phosphoric acid from phosphorus pentoxide and his alkali from metallic sodium, he had eliminated all silica. Unless he made and stored these reagents and the distilled water used in vessels of metal free from silica, his precautions were useless. Even then he would in all probability have been forced, as we were, to use phosphorus pentoxide and sodium of which he did not know the previous history and which themselves may have acquired silica by storage in glass.

When glass, the chief source of contaminating silica, is gradually elimi-

nated in the preparation of phosphate and in the carrying out of Isaacs' test, the intensity of color produced on final reduction of the phosphomolybdate is progressively diminished, almost to the vanishing point. A solution of phosphate was prepared by allowing water vapor to act on metallic sodium and phosphorus pentoxide in platinum dishes and titrating the alkali with acid from a glass buret until the mixture was neutral to litmus. The other reagents used were made up in Pyrex vessels and the reaction was carried out in Pyrex tubes. The final reduced solution contained 13.78 mg. of phosphorus in 25 cc. and was of a pale blue-green color, equal to that given by 0.0006 mg. of silicon in 25 cc. When the phosphorus pentoxide was weighed out in glass the addition of phosphoric acid to alkali was made in platinum, the preparation of the other reagents was carried out in nickel, the distilled water collected in nickel and the final reaction performed in platinum, the blue color produced on reduction of a solution containing about 5 mg. of phosphorus per 25 cc. was equal to that given by 0.0005 mg. of silica in 25 cc. Finally, when the phosphorus pentoxide was weighed out in platinum and no glass whatever was used other than that of the colorimeter tubes, a solution containing 4.36 mg. of phosphorus pentoxide per 25 cc. gave an extremely faint color, equal to that produced by 0.00012 mg. of silica in 25 cc. In other words, the color was really too faint to be estimated.

The test was repeated, using diammonium phosphate, $(NH_4)_2HPO_4$, which according to the manufacturer's analysis contained no silica. When employed under the conditions of Isaacs' method, this salt never gave more than the faintest blue coloration on reduction of its molybdate, and the whole of this color could be traced to the other reagents used. We may conclude, therefore, that phosphate free from silicate gives no color in Isaacs' test. It remains to show that a mixture of silicate and phosphate does not give more color in the test than would be produced by the amount of silicate present.

Preliminary experiments were carried out with commercial samples of phosphates containing up to 0.001% of silica. The intensity of the color produced on reduction of the molybdate in the presence of acetic acid was not directly proportional to the amount of phosphate and its impurity, silica. In all cases, with increase in the concentration of the phosphate and so with an increase of the silicate impurity in the final solution, the blue color produced on addition of sulfite increased in intensity up to a maximum, after which it rapidly became less intense and finally failed to appear. These color variations suggest that phosphates may actually retard the reduction of silicomolybdate, to an extent depending upon the concentration of the phosphate present. It is here that the presence of sodium acetate seems important. With all other factors the same, a solution of silicate containing 1 cc. of 6% sodium acetate or its

equivalent, will give in the presence of phosphate a slightly more intense color than a solution not containing the acetate.

The possible retardation of the reduction of silicomolybdate caused by phosphates present in the same system is of importance in the estimation of silicon in tissues. Of all the tissues examined by Isaacs, the brain has under normal conditions the highest phosphorus content. It amounts to about 1.5% of the dry weight of the tissue. This is equivalent to 7.5 mg. of phosphorus in the 0.5 g. of tissue taken by Isaacs for ashing. It was, of course, of interest to find whether this amount of phosphorus, added in the form of a phosphate solution to a solution of silicate, would retard the reduction of the silicomolybdate. A series of mixtures of ammonium phosphate and sodium silicate was made up and examined by Isaacs' method. All contained 0.7 mg. of silicon in the 25 cc. of the final colored solution. The quantity of phosphorus was varied. The results are shown in Table I.

TABLE I

THE INFLUENCE OF PHOSPHATE ON THE REDUCTION OF SILICOMOLYBDATES

P in 25 cc. of final colored solution, mg.	Result of reduction of silicomolybdate
None	Immediate deep blue color
9.3	Color much less intense than control
6.2	Color very slightly less intense than control
3.1	Immediate deep blue color, equal in intensity to control

To find how the concentration of acetic acid would influence the action of the phosphate, solutions were prepared containing 0.7 mg. of silicon and 7.75 mg. of phosphorus in 25 cc. of the final colored solution. The amount of acid was varied as in Table II.

TABLE II

THE INFLUENCE OF ACETIC ACID ON THE RETARDING OF REDUCTION OF SILICOMOLYBDATES IN THE PRESENCE OF PHOSPHATE

10% acetic acid added, cc.	Result of reduction
3[a]	Immediate deep blue color
2, 3, 4	Color not fully developed in 24 hours
5	After 2 hours color was 9/10 as intense as control
6, 7	Immediate deep blue color equal to control

[a] No phosphorus present.

The color given by the phosphate-silicate mixture was never more intense than that of the control, even when 10 cc. of the 10% acetic acid was used.

The mechanism of the retarding action of phosphates is not clear. Using indicators, it was found that a mixture made up as above, with the omis-

sion of silicate or phosphate, has a P_H value of about 3.6 at room temperature. By addition of 3 cc. of saturated sodium sulfite solution this is changed to 5.7. This latter value is, of course, approximate, since the acid solution of sulfite rapidly decolorizes the indicator. The presence of ammonium phosphate, containing up to 7 mg. of phosphorus in the mixture, before adding sulfite, raises the P_H value slightly, but the final P_H, after addition of sulfite, is the same as that of mixtures not containing the phosphate. The action of the phosphate, therefore, must be ascribed to something other than a change in acidity. It may be due to removal of molybdate necessary for the formation of silicomolybdate, even though ammonium phosphate retards when present in a concentration as low as 0.01 *M*, when 5 cc. of 10% ammonium molybdate in 25 cc. represents a molybdate concentration of 0.1 *M*. It may be that phosphate and silicate combine to give a complex which either does not form a molybdate or which forms a molybdate not reduced at the P_H of the medium. This complex formation might be prevented in a more acid medium than that used by Isaacs, for we have shown that when the reaction contains 7 instead of 3 cc. of 10% acetic acid its P_H is about 5.2 and the silicomolybdate is completely reduced.

It appears from these results that Isaacs' values for silicon in tissue containing much phosphate are probably too low, rather than too high, as Bertrand implies. There is a factor, however, which may possibly neutralize this error, due to phosphate. At the red heat employed in the process of ashing the tissue the phosphate will be converted into pyrophosphate. Although the ash is moistened and warmed with a few drops of nitric acid before the test solution is made up, it does not necessarily follow that all of the pyrophosphate is converted by the acid into orthophosphate. Pyrophosphate does not give a yellow color with ammonium molybdate, even in the presence of more acetic acid than Isaacs used, and addition of pyrophosphate to the test solution in quantities up to the equivalent of 12 mg. of phosphorus does not retard, to the slightest degree, the production of a blue color on reduction. The fact that pyrophosphate is much less soluble than orthophosphate increases the possibility that only a fraction of the phosphate in the tissue will appear in the silicate mixture in a form capable of disturbing the silicon determination. Consequently, Isaacs' results are very likely correct.

Bertrand states that the color of the blue solution increases in intensity in course of time. This is true, but the fact does not invalidate Isaacs' method. If standard and test solution are prepared at the same time, even after standing for 27 hours, the ratio of color intensity of the two solutions is not greatly different from the color ratio of the freshly prepared solutions. With the standard at 10 mm. on the colorimeter scale in both cases, an unknown silicate solution matched the standard at 22.2 mm.

when freshly prepared and at 22.5 mm. 27 hours later. The calculated amounts of silicon in the unknown, on the basis of 0.7 mg. of silicon in the standard, were 0.315 and 0.311 mg., respectively. The difference corresponds to an error of 1.2%, which is within the limits of error of a colorimetric method. Isaacs omitted to say that standard and unknown should be made at the same time, but this statement was hardly necessary in view of the general practice in colorimetric work.

The exact degree of acidity at which phosphomolybdates, on treatment with sodium sulfite begin to give a blue color, has not been determined. Using the usual procedure, we compared the color produced in systems containing 15.5 mg. of phosphorus (as diammonium phosphate) and 1.4 mg. of silicon (as sodium silicate). Two tubes of each salt solution were prepared, one containing 0.5 cc. of 10% sulfuric acid in place of the 3 cc. of 10% acetic acid usually employed, the other containing 0.25 cc. of the sulfuric acid. In both cases the acidity of the mixture was much higher than would be the case if 3 cc. of acetic acid were used. Both phosphate and silicate mixtures gave a yellow color on heating with ammonium molybdate, but on adding sulfite the phosphate systems gave merely a white precipitate, while the silicate systems were at once reduced to a deep blue color.

We believe that the above results prove that Isaacs' method for the determination of silicon in tissues is reliable and, since it is far more sensitive than the gravimetric method and gives consistent results with much smaller amounts of tissues, we regard his figures as the most accurate yet obtained. The method might be slightly improved, perhaps, in estimating silicon in the presence of large amounts of phosphorus by adding up to 7 cc. of 10% acetic acid in making up the test solution. This still keeps the acidity far below that required for the reduction of phosphomolybdate.

Finally, the fact that both silicon and phosphorus can be estimated by the color produced on reduction of their molybdates would suggest that the results for the phosphorus content of tissues obtained by this method are actually estimations of phosphorus plus silicon. We have experimental evidence that some procedures for estimation of phosphorus by reduction of the phosphomolybdate can be used equally well for the estimation of silicon. This question is worthy of a separate investigation. We are undertaking further research on this point and on the whole mechanism of the reduction of molybdates.

Summary

1. In confirmation of Isaacs' work, we have found that silicomolybdates are reduced by sodium sulfite in the presence of a much lower concentration of hydrogen ions than is necessary for reduction of phosphomolybdates.

2. The reduction of silicomolybdates, in mixtures of silicomolybdates

and phosphates, is retarded or inhibited by phosphates if the latter are present in sufficient concentration. Within the limits of phosphate concentration found in the ash from animal tissue, the retarding action of phosphate can be removed by slightly increasing the acidity of the system before addition of the reducing agent.

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THE VELOCITY OF IONIC REACTIONS

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1. Introduction

In previous communications[2] it has been shown that neither the classical expression for the velocity of chemical reactions, which for a bimolecular reaction has the form

$$h = k_1 \cdot c_A \cdot c_B \qquad (1)$$

nor the expression given by the "activity theory"

$$h = k_2 \cdot a_A \cdot a_B \qquad (2)$$

is compatible with existing evidence based upon the study of ionic reactions. In Equations 1 and 2, c and a indicate concentration and activity, respectively. On the basis of theoretical considerations, the following expression was derived

$$h = k \cdot c_A \cdot c_B \cdot f_A \cdot f_B / f_X \qquad (3)$$

in which f_A, f_B, and f_X denote the activity coefficients of A, B and X, respectively. X is a complex ion formed by the collision of A and B, the reaction components. For the details of this theory, the reader is referred to the original papers.[2]

The applicability of Equation 3 to the calculation of reaction velocities depends upon the fact that the activity coefficients of ions in dilute solution are largely governed by their electric charges and only to a smaller extent by their individual properties. Since the charge of X is the algebraic sum of the charges of A and B, the charge of each substance entering is known, and a numerical calculation is possible.

Despite the large amount of evidence which has already accumulated in favor of the new theory, it is highly desirable to test its scope by new experiments, particularly in the region of very dilute salt solutions where the behavior of activity coefficients is least influenced by specific effects. The present investigation has been carried out from this point of view.

[1] Bruce Howard Memorial Fellow from the University of California.

[2] (a) Brönsted, *Z. physik. Chem.*, **102**, 169 (1922); (b) **115**, 337 (1925). (c) Brönsted and Delbanco, *Z. anorg. Chem.*, **144**, 248 (1925).