CLV. THE COLORIMETRIC ESTIMATION OF MINUTE AMOUNTS OF COMPOUNDS OF SILICON, OF PHOSPHORUS AND OF ARSENIC.

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IN the course of some work upon the minor constituents of sea-water and their utilisation by the phytoplankton it was necessary to make use of colorimetric methods and to study any possible interference of one constituent upon the method adopted for the analysis of another. Of the substances investigated the quantities present in sea-water are minute, being for phosphate, as P_2O_5 , 1-40, for arsenic, as As_2O_3 , about 25, and for silicate, as SiO_2 , 40-200 mg. per m.³, viz. per 1000 litres [Atkins, 1923, 1926; Orton, 1924].

The estimation of silicate in presence of arsenite, arsenate and phosphate.

The method of Diénert and Wandenbulcke [1923, 1924] may be used as follows: 2 cc. of 10 % ammonium molybdate and 4 drops of 50 % (by volume) sulphuric acid are added to 50 cc. of solution. A yellow colour results with solutions containing silicate. Permanent standards are obtained by dissolving 36.9 mg. of picric acid in a litre of water and diluting as required. The strength given corresponds to 50 mg. SiO_2 per litre. Thresh and Beale [1925] consider the method as accurate as the gravimetric, but use 40 mg. per litre of picric acid. Working with 100 cc. the writers used 8 drops of acid, but found that a blue tint was apt to develop. The trouble was traced to excess of acid. Taking only 4 drops, with sea-water, the solution is brought to p_H 2.1 and a clear yellow is obtained. The comparisons were made in Hehner tubes, using standards equivalent to 2.0, 0.5, 0.2 or 0.1 mg. SiO_2 per litre; the first is suitable for fresh waters, the others for sea-water. The limit detectable is close to 0.04 mg. per litre. For fresh or estuarine waters allowance has to be made for the tint already present which may be matched approximately against the picric acid standard.

Neither phosphates, arsenates nor arsenites, when present in moderate amount, give any colour with these reagents. Thus a solution of K_2HPO_4 , 0.1916 g. per litre, gave no more tint than that due to 0.2 mg. per litre of SiO_2 and even this may have been derived from the glass of the bottle.

Similarly a solution containing 0.1612 g. per litre of As_2O_5 gave no perceptible tint. The method can accordingly be used for natural waters with no risk of error due to the substances named.

In solutions in which phosphates, arsenites or arsenates are suspected of vitiating the silicate results the latter may be estimated after evaporating down with hydrochloric acid and redissolving in sodium bicarbonate; alternatively the phosphate and arsenate may be removed by ferric chloride and ammonium hydroxide, and the arsenite similarly after oxidation with potassium chlorate and hydrochloric acid.

The estimation of phosphate, in presence of arsenite, arsenate and silicate.

Three colorimetric methods have been used: (a) Denigès [1920, 1921], using, in error, 50 % sulphuric acid by volume, instead of by weight; the modification was satisfactory, so was retained after the error had been pointed out. (b) Bell and Doisy [1920], also with Briggs' modifications [1922, 1924]. These need no description. (c) Pouget and Chouchak [1909, 1911]. All are methods which have been established for phosphate, but have not heretofore been used quantitatively for arsenic compounds, though Denigès, describing his qualitative test for phosphate, mentions a similar reaction—only about half as sensitive—with arsenate. Florentin [1921] also mentions possible interference of arsenate.

(a) *Denigès' method, as used.* To 100 cc. of phosphate solution add 2 cc. of reagent No. 1, followed by one drop of No. 2 and mix. No. 1 contains 100 cc. of 10 % ammonium molybdate with 300 cc. of 50 % (by volume) sulphuric acid. No. 2 is stannous chloride, freshly prepared from 0.1 g. tin, 1 drop 3 % copper sulphate solution and 2 cc. strong hydrochloric acid made up to 10 cc.

The blue colour which develops almost instantaneously is due to the reduction of a phosphomolybdic acid to a blue complex oxide of molybdenum [Vila, 1923; see also Hopkins, 1923]. Under the conditions of the test the excess of ammonium molybdate is not reduced, and so the intensity of the colour is proportional to the phosphate originally present and may be compared with a standard. The method is convenient and delicate, being suitable for sea-water, without concentration, and for soil extracts [Atkins, 1923, 1924, 1925]. Only the minimum amount of stannous chloride should be used. Toluene serves as a preservative for the standard phosphate solutions.

For comparison the Duboscq and Kober colorimeters were used, also Hehner tubes mounted on a nesslerising stand, with a sheet of cardboard in front of the tubes to screen them from extraneous light. On the opal plate of the stand a white card, with ruled lines, was arranged so as to form half of the field of each tube; the device is a considerable aid to accurate working. The triple field constituted by the Hehner tubes and the white plate, coupled with the greater length of the liquid columns, render work with these tubes

as accurate as with the more costly colorimeter and preferable for very dilute solutions.

Table I. *Estimation of arsenate, as As_2O_5 , using Denigès' reagents and phosphate standards. All with Kober colorimeter, save first with Hehner tubes.*

Substance tested	Treatment	Taken mg. per 100 cc.	Found mg. per 100 cc.	Difference %
As_2O_5 (B.D.H.)	—	0.00806	0.0072	- 11
"	—	0.00806	0.0079	- 2
As_4O_6 (B.D.H.)	Evaporated, with 10 cc. pure re-distilled nitric acid, to dryness on sand-bath	0.00675	0.0058	- 14
"	As before but with "Arsenic test" nitric acid (B.D.H.)	0.00675	0.0068	+ 1
"	Oxidation with 0.15 cc. of $N/10^3$ iodine solution, with bicarbonate	0.0067	0.0077	+ 14
"	Oxidation with 0.15 cc. of $N/10^3$ iodine solution, with bicarbonate	0.0070	0.0065	- 8

Table I shows the results obtained with solutions of arsenate, or of arsenite oxidised to arsenate. The amounts taken were very minute, so that the agreement between them and the quantities found is fairly satisfactory. It should be added that the arsenate solutions were compared with standard phosphate for convenience and because arsenic oxide is hygroscopic. The agreement between the amounts taken and found shows that this is legitimate, as might be expected from the fact that the "molybdenum blue" compound is the same whether derived from the reduction of phosphomolybdate or arsenomolybdate.

Arsenious oxide solutions were tested without oxidising, and though a blue colour was obtained, the results for As_4O_6 present were very low, showing only a partial reaction. The presence of arsenic pentoxide was suspected and Table II shows that the pure arsenite solutions do not give the Denigès reaction. The solutions, made up with bicarbonate, were all faintly alkaline and their reducing properties indicate that they have a tendency to spontaneous oxidation.

Though the foregoing conclusion appeared well established, it was thought that the presence of arsenate in the dilute arsenite solutions might be confirmed by titration with iodine before and after reduction with sulphurous acid. Such attempts were unsuccessful as even $N/10^5$ iodine is quite colourless.

Table II. *Estimation of arsenious acid, as As_4O_6 , using Denigès' reagents and phosphate standard. All with Kober colorimeter, save first with Hehner tubes.*

Substance tested	Taken mg.	Found mg.	Difference %
As_4O_6 Kahlbaum, 99.98 % (for analysis)	0.0049	0.0011	- 77
"	0.0195	0.0057	- 71
"	0.0195	0.0056	- 71
As_4O_6 Kahlbaum resublimed	0.0070	0.0005	- 93
As_4O_6 Kahlbaum resublimed, solution 14 days later	0.0070	0.0010	- 86
As_4O_6 Kahlbaum resublimed	0.0700	0.0044	- 94
"	0.0700	0.0045	- 94
As_4O_6 (B.D.H.)	0.0675	0.0015	- 98

In Table I results obtained by oxidising arsenious acid with nitric acid have been shown, but this requires care. It was accomplished by evaporating the solution to dryness in a beaker on a sand-bath. It is necessary to remove the beaker immediately dryness has been reached, otherwise low results are obtained. Care must be taken to avoid "sputtering," but the use of a conical flask is inadmissible, as to remove all acid too high a temperature has to be used. In oxidising with iodine, adding bicarbonate, a large excess of the reagents must be avoided. Blank determinations were made upon all the reagents used for the purposes of this research.

Oxidation by means of hydrogen peroxide was also attempted, the latter being then decomposed by boiling, but on adding the Denigès reagents a greenish-yellow developed, due to the ammonium molybdate alone; the colour can be destroyed by stannous chloride, but a turbidity remains. Blanks carried out with hydrogen peroxide showed that it was necessary to heat to dryness and then quite strongly before a residue could be obtained which gave no colour with ammonium molybdate solution. The hydrogen peroxide, stated to be free from barium salts, probably contained traces of barium peroxide; phosphate, also said to be absent, was present to about one part per million. The heating of the residue is not desirable in the presence of arsenic compounds. Electrolytically prepared peroxide gave no better results.

Silicate up to 8 mg. SiO_2 per litre gives no reaction with the Denigès reagents and so does not lead to an over-estimate for phosphate. Higher concentrations were not examined.

(b) *Bell and Doisy's method* for determining phosphate was tested for the estimation of arsenate. A trial was first made with two phosphate solutions of known strength, assuming one to be the standard. Taking 0.500 mg. in 50 cc., 0.517 mg. was found; difference + 3.4 %. Taking 0.0201 mg. of arsenic oxide, the amount found was 0.0208; difference + 3.5 %. The reaction is therefore applicable to the estimation of arsenates also.

Using solutions of arsenious oxide varying shades of green and blue were obtained. These it was impossible to match against the phosphate standards. It should be possible to oxidise to arsenate and then to estimate, but this was not tried.

(c) *Pouget and Chouchak's method*. The phosphate solution is acidified with nitric acid and to it is added sodium molybdate similarly acidified; these are followed by a filtered neutral solution of strychnine sulphate. After energetic shaking the yellow opalescence which appears is compared with a similarly treated standard solution. The method is less sensitive than is that of Denigès. Matthews [1916, 1917] found the reaction suitable for work with sea-water, but the process is lengthy as the phosphate has to be precipitated with ferric chloride from 500 cc.

Pouget and Chouchak state that arsenic acid gives no precipitate with strychnine molybdate under the conditions of the determination, but its presence is disturbing when the weight of arsenic attains two or three times

that of phosphorus. Their method does not involve warming the solution. Matthews carried out the estimation in presence of arsenious acid, without oxidising, and found that it did not interfere with the accuracy of the analysis.

Certain experiments carried out by Matthews after oxidation suggested to us the presence of arsenious compounds, rather than phosphorus in organic combination. Accordingly qualitative tests were performed upon solutions of arsenite, arsenate and phosphate, blanks being carried out simultaneously upon the reagents.

The results of these and other qualitative tests are shown in paragraphs 6-8 of the Summary.

SUMMARY.

1. The reagents used by Diénert and Wandenbulcke for estimating silicate give no colour with any moderate quantities of phosphate, arsenate or arsenite.

2. The amounts of phosphate and arsenate ordinarily present in natural waters do not suffice to interfere with the method of Diénert and Wandenbulcke for silicate.

3. The method of Denigès for phosphate may be employed:

(a) for estimation down to 0.0002-0.0004 mg. of P_2O_5 in 100 cc.;

(b) for estimation of equivalent quantities of arsenate, as As_2O_5 , but with arsenite it gives a faint colour only, which may be attributed to the presence of arsenate.

4. The amounts of silicate ordinarily found in natural waters do not interfere with the accuracy of Denigès' method for estimating the phosphate.

5. Bell and Doisy's method does not appear to be as convenient or as delicate as that of Denigès for the estimation of phosphate. It can be used for arsenate also, but solutions of arsenious acid give very irregular results.

6. The reaction of Pouget and Chouchak for phosphates is not given by arsenites, save very faintly on warming, due apparently to arsenate present. It is only given very faintly in the cold with arsenates, but on heating turbidity and a yellow colour develop. With phosphate a strong colour and a marked turbidity appear in the cold immediately, save with very dilute solutions, and become more intense on heating.

7. The Denigès reaction is not given by glycerophosphate, save in so far as free phosphate is present; triphenyl phosphate does not give the reaction, nor is it readily hydrolysed on boiling the aqueous solution as is glycerophosphate; tricresyl phosphate does not give the reaction even after prolonged boiling with water.

8. The Denigès reaction is not given by vanadates, by tungstates or by phosphotungstates. With the latter however a purple tint is slowly produced, but this is not a sensitive reaction.

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