COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION of FISHERIES and OCEANOGRAPHY

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REACTIVE SILICATE IN SEA WATER

A Comparison of the Reduced Alpha and Beta Molybdosilicic
Acid Methods of Spectrophotometric Analysis

By B. D. Scott

Marine Laboratory
Cronulla, Sydney
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By B.D. Scott

Abstract

The two commonly used methods for the determination of reactive silicate are compared using samples from vertical profiles in the Tasman Sea. The Grasshoff method which employs the reduced alpha molybdosilicic acid is found to give more variable results than the Mullin and Riley method, which employs the reduced beta acid. The variability in the alpha method is due to turbidity caused by calcium oxalate precipitated from the sea water by the reagents. The relationship between the two methods is shown to be linear, and is expressed by the equation: Alpha = 1.89 + 1.020 Beta, where both results are $m\ mol\ Si\ m^{-3}$. The 40 samples used to obtain this relationship range in reactive silicate content from 0.5 to $84\ m\ mol\ Si\ m^{-3}$, as measured by the beta method. Samples poisoned with 400 mg $HgCl_2\ dm^{-3}$, and stored in polythene bottles at ambient temperatures for eight months showed no significant change in reactive silicate. The use of a range of standard solutions to determine the calibration curve is recommended.

INTRODUCTION

There are two commonly used spectrophotometric methods for the determination of reactive silicate in sea water. Mullin and Riley (1955) described a method which was later modified slightly and pub-lished by Strickland and Parsons (1968) in which reduced beta molybdo-silicic acid is formed at a pH of between 1 and 2. The Grasshoff (1964) method is based on the reduction of alpha molybdosilicic acid at a pH of 3 and 4. These methods will be referred to as the alpha or beta methods in the text below. There are other methods which utilise the yellow colour of the unreduced alpha or beta acids, but these are less sensitive and are less precise than those of the reduced acids, as Sugawara (1969) has shown. The chemistry of the methods has been investigated by Strickland (1952) and the nature of the reactive species has been described by Stumm and Morgan (1970).

Previous comparisons of these two methods at this laboratory (unpublished) show that when coastal waters of about 2 m mol Si m^{-3} are analysed, the alpha method gives results 1 to 3 m mol Si m^{-3} higher than the beta method. The alpha method is also more variable than the beta method. These effects are due to a slight turbidity caused by the precipitation of calcium oxalate· from the sea water with the reagents of the alpha method. This reaction does not occur with the artificial sea water standards which do not contain calcium. Further experiments in which the reagent concentrations and reaction times were changed slightly failed to improve the original alpha method. Liss and Spencer (1969) compared the two methods using low silicate coastal waters from the Menai Straits, and reported that the alpha method gave results 35% higher than the beta method.

Following the tests above, it was decided to discontinue the analysis of sea water samples at this laboratory by the alpha method, and change to the beta method after September 1972. The comparison experiment reported below was undertaken so that the results of the preceding years could be compared with those from the new method.

METHODS

The samples were collected from four oceanic profiles located in the Tasman Sea, 70 km from the coast, at 34°04'S 151°55'E (HMAS *Kimbla* Cruise K7/72, Stations 40, 41 and 42, 10th to 12th September 1972). Ten Nansen bottles were used to collect the samples from each profile, spaced at intervals to a depth of about 1400 metres. The contents of each Nansen bottle were transferred to a 1200 cm^3 polyethylene bottle and 10 cm^3 of 5% mercuric chloride solution added as a preservative. The samples were stored at ambient temperatures (15 to 30°C) until analysis.

The analyses were performed between the 18th and 29th of September 1972 by both methods alternately on the dates shown in Table 1. The 40 samples were measured on four occasions by each method, and in triplicate on each occasion. Blanks and a range of standard solutions were also measured in triplicate on each occasion. To check the sample preservation method, the samples were kept at ambient temperatures until 7th May 1973, then analysed again in triplicate by the beta method.

The alpha method described by Grasshoff (1964) was modified slightly. The standard silicate solution used was the same as that used for the beta method. This is a solution of sodium silicofluoride, containing 5 m mol Si m^{-3}. This stock solution was used to prepare standards of 0, 2, 5, 10, 20, 50, 100 m mol Si m^{-3}. A Spekker absorpt-iometer with Ilford 608 filters was used for measuring the absorbances against distilled water. For concentrations up to 60 m mol Si m^{-3} (absorbance 0.8) 40 mm path length cells were used, and 10 mm cells for higher concentrations. A distilled water blank was used for the

TABLE 1.   Average Net Absorbances of the Standard Silicate Solutions, in artificial sea water.

Alpha Method

Analysis date	20.ix.72	23.ix.72	26.ix.72	29.ix.72	Cell path length
m mol Si m^{-3}					
2	0.035	0.030	0.037	0.035	40 mm
5	0.076	0.074	0.085	0.082	"
10	0.136	0.145	0.155	0.153	"
20	0.263	0.274	0.293	0.292	"
50	0.660	0.655	0.670	0.714	"
50	0.164	0.173	0.194	0.195	10 mm
100	0.324	0.348	0.370	0.367	"

Beta Method

Analysis date	18.ix.72	21.ix.72	24.ix.72	27.ix.72	7.v.73
m mol Si m^{-3}					
5	0.059	0.059	0.058	0.058	0.059
10	0.115	0.117	0.115	0.115	0.116
25	0.284	0.287	0.283	0.282	0.282
50	0.557	0.562	0.552	0.552	0.554
75	0.822	0.831	0.814	0.821	0.814
100	1.085	1.095	1.070	1.086	1.059
150	1.582	1.613	1.561	1.590	1.529
200	2.067	–	–	–	–

TABLE 2. Reactive Silicate Content of the Sea Water Samples. (m mol Si m^{-3}). The average of twelve analyses are reported together with the standard deviation, for each sample and for each method.

Sample No.	ALPHA METHOD		BETA METHOD	
	Average	Std. Dev.	Average	Std. Dev.
1	2.53	0.18	1.66	0.07
2	6.34	0.75	4.31	0.14
3	7.18	0.79	5.26	0.18
4	10.29	0.57	8.28	0.25
5	11.60	0.57	8.96	0.18
6	15.00	0.94	12.05	0.16
7	26.93	0.68	24.26	0.14
8	48.32	2.42	44.40	0.40
9	71.92	3.54	69.58	0.51
10	89.16	6.34	83.39	0.35
11	4.32	0.85	2.68	0.12
12	4.70	1.06	2.80	0.09
13	6.09	0.88	4.18	0.06
14	8.72	0.76	6.72	0.09
15	11.15	0.77	8.74	0.20
16	13.32	0.66	10.80	0.15
17	25.65	0.83	22.55	0.32
18	41.53	1.48	39.53	0.29
19	64.25	4.55	60.64	0.45
20	82.63	3.42	80.92	0.44
21	4.54	0.48	3.10	0.06
22	6.00	0.71	4.33	0.05
23	6.26	0.55	4.59	0.08
24	7.67	0.57	5.82	0.06
25	9.87	0.64	7.70	0.28
26	11.09	1.08	8.51	0.20
27	19.22	0.58	16.48	0.16
28	33.12	1.13	30.58	0.23
29	56.37	2.20	54.62	0.26
30	78.02	5.12	74.92	0.31
31	2.21	0.85	0.52	0.05
32	4.78	0.75	3.30	0.05
33	7.09	0.71	5.11	0.09
34	8.96	0.59	7.16	0.14
35	9.56	0.80	7.43	0.07
36	10.82	0.48	8.64	0.06
37	19.06	0.86	16.42	0.17
38	35.00	2.03	32.04	0.18
39	54.78	2.14	52.93	0.26
40	79.64	5.78	75.43	0.73

correction of the sample absorbances, and an artificial sea water blank for the correction of the standards. The reducing agent was added one hour after the molybdate, and the absorbances read after leaving the colour to develop overnight. Since some of the material which causes the turbidity precipitates to the bottom of the reaction vessel, care was taken to draw off the supernatant solution without disturbing it.

The beta method described by Strickland and Parsons (1968) was also modified. The analyses were carried out in polyethylene beakers of 75 cm^3 capacity. The standards prepared were equivalent to 0, 5, 10, 25, 50, 75, 100, 150, m mol Si m^{-3}. The reducing agent was added 15 ± 2 minutes after the mixing of sample and molybdate, and the absorbances were read after 3 ± 0.25 hours. The absorbances were read on a Varian spectrophotometer, model 635, against distilled water, at 810 mm, using a 10 mm path length flow cell. For absorbances of less than 0.1 the scale expansion was used.

Automatic zero pipettes were used for dispensing the samples, and syringe dispensers for the reagents, in both methods. This results in better precision in measuring the volumes, and better mixing of the reactants. The timing of the reagent additions, particularly the reducing agent, was carefully controlled since earlier experiments had shown that this improved precision in both the alpha and beta methods. Both these methods incorporating some of the modifications listed above, have been reported by Major *et al* (1972). In order to determine the nature of the precipitate obtained in the alpha method, a composite sample of about five litres was used. After developing the colour overnight the supernatant liquid was decanted, and the remaining liquid with the precipitate filtered through a millipore membrane. The filter cake was washed several times with distilled water then dried under vacuum. The dried material was then examined in a Philips PW1540 X-ray spectrometer to determine the elements present.

RESULTS

The absorbances of the standard silicate solution were corrected by subtracting the average reagent blank, and the three net absorbances for each concentration were averaged. These averaged standards are shown in Table 1. A cubic curve, constrained through the origin, was fitted to each set of standards by the least squares method. The average reagent blank was subtracted from each sample absorbance, then the standard curve used to determine the silicate content. The average of the twelve results obtained by each method are shown in Table 2, together with the standard deviations. The analyses which were performed eight months later are shown in Table 3. The means of all the samples analysed on each day, by each method are shown in Table 4.

The data from which these tables were compiled are available for reference at these laboratories. None of the data were rejected.

The X-ray spectrogram of the precipitate obtained by the alpha method showed the components to be Ca > Hg > P > Mo. Further chemical tests and microscopic comparisons showed the precipitate to be calcium oxalate. The minor elements present would be adsorbed on this precipitate.

6

TABLE 3. Reactive Silicate Content of the Sea Water Samples.
(m mol Si m^{-3}). The individual results from the
beta method analyses on 7th May 1973.

Sample No.	Individual Results		
1	1.69	1.78	1.69
2	4.32	4.32	4.32
3	5.25	5.42	5.42
4	8.12	8.20	8.20
5	9.14	9.05	9.14
6	11.90	11.98	11.81
7	24.16	24.16	24.34
8	44.86	44.77	44.77
9	70.18	70.00	70.00
10	84.58	84.48	84.58
11	2.80	2.80	2.71
12	2.88	2.88	2.97
13	4.15	4.24	4.24
14	6.58	6.67	6.67
15	8.72	8.63	8.63
16	10.69	10.69	10.86
17	22.19	22.37	22.19
18	39.28	39.38	39.38
19	60.73	60.54	60.73
20	81.30	81.39	81.39
21	3.39	3.47	3.39
22	4.41	4.41	4.41
23	4.66	4.74	4.66
24	5.59	5.68	5.68
25	7.78	7.61	7.69
26	8.63	8.46	8.46
27	16.35	16.35	16.26
28	30.35	30.35	30.35
29	54.54	54.54	54.54
30	75.28	75.28	75.56
31	0.85	0.85	0.93
32	3.30	3.39	3.39
33	5.00	5.00	5.00
34	7.01	7.18	7.09
35	7.35	7.26	7.26
36	8.46	8.46	8.55
37	16.43	16.26	16.26
38	31.68	31.68	31.68
39	52.91	53.00	53.00
40	76.02	76.20	76.02

DISCUSSION

Before the alpha and beta silicate analysis methods were compared, the possible effect of the delay between sampling and analysis was considered. The results obtained by the beta method both before and after the eight month delay were compared (Table 2 and 3). The later results were examined and 77 of the total of 120 (64%) were found to lie within one standard deviation of the averages of the previous results. The number of these results to either side of the previous averages was about equal. The means of all the beta method results for September 1972 and May 1973 are 23.03 and 23.10 m mol Si m^{-3} respectively. It is therefore improbable that the reactive silicate content would have changed by a measurable amount over the eleven day period of the initial series of analyses. However the sample means for each analysis batch (Table 4) do show a slight trend for each method. An analysis of covariance showed a small but significant change in the analysis results between days, but when the absorbances were used, without using the standards to calculate the silicate concentrations, then there appeared to be no difference between days. Thus the trend in the analyses from day to day is due to some slight change in the standards for which no explanation has been found. Since these changes were small they were disregarded for the purpose of this study and the averages of the twelve results obtained by each method in the initial series were compared.

TABLE 4. Reactive Silicate Content of the Sea Water Samples.

(m mol Si m^{-3}). The mean of all the samples

analysed by each method, each day.

Alpha Method

Analysis Date	20.ix.72	23.ix.72	26.ix.72	29.ix.72	
Mean Silicate	25.79	25.75	25.73	24.31	

Beta Method

Analysis Date	18.ix.72	21.ix.72	24.ix.72	27.ix.72	7.v.73
Mean Silicate	22.88	22.97	23.06	23.24	23.10

A preliminary examination of these data indicated a linear relationship. Since the alpha method was known to be more variable, the beta method was regressed as a linear function of the alpha method. The regression equation obtained was:

Alpha = 1.89 + 1.020 beta (m mol Si m^{-3})

The standard error of the regression coefficient was ± 0.005, and the correlation coefficient was 0.996 (n=40). This relation shows that the two methods are measuring the same observable quantity, but there is a systematic difference of 1.89 m mol Si m^{-3}, due to the turbidity of the alpha solutions. The slight difference between the regression coefficient of 1.02 and unity may be due to a slightly increased yield of calcium oxalate from the deeper waters where increased buffering effect would produce a better final pH for the precipitation.

The standard deviations of the samples in Table 2 appeared to be related to the reactive silicate content. In the case of the alpha method, the standard deviation was in the region of ± 0.70 m mol Si m^{-3}, on the Baltic Sea water. This apparent difference in precision may be partly due to the fact that the results reported here are from four sets of triplicates analysed on different days, whereas those of Grasshoff probably were determined on the same day.

The relationship of the results of the beta method to their standard deviations was also examined. The deviation rose linearly from ± 0.09 at low silicate contents to ± 0.06 at 90 m mol Si m^{-3}. This error is about half that quoted by Strickland and Parsons (1968). This increase in precision probably is due to the greater precision in measuring the sample and reagent volumes, and the timing of the reagent additions. The timing of the reagent additions, and salt effects, as shown by Fanning and Pilson (1973) to be an important factor in the precision of the beta method.

The greater error in the alpha method cannot be attributed to instrumental error, since the variation in the replicates obtained for the artificial sea water standards was about the same as that observed for the beta method samples or standards. Thus the greater errors experienced in the alpha method are due to a variable amount of turbidity caused by the calcium oxalate. This turbidity can only be removed by either filtration, which would make the method tedious, or by decreasing the pH to between 1 and 2 where the method would become that used for the reduced beta molybdosilicic acid.

The precision of an analytical method is usually defined as the range each side of any experimental result in which the true result will lie. For most work the 95% confidence limit, which is equal to about two standard deviations, is adequate. The detection limit is defined in a similar manner, being the precision at, or near, the zero or blank level. For the alpha method the detection limit is 1.40 m mol Si m^{-3}, and for the beta method 0.18 m mol Si m^{-3}.

The accuracy of either method may be impaired by the method used for the standardisation and calculation. Grasshoff (1964) states that the relationship of silicate to absorbance is "exactly linear" and that one standard at 20 m mol Si m^{-3} is sufficient. If this method were applied to the absorbances obtained by the alpha method (Table 1) then an error of up to + 0.8 would occur at the 5.0 m mol Si m^{-3} level, and -2.0 at the 50.0 m mol Si m^{-3} level.

Strickland and Parsons (1968) also recommend the use of one standard of 100 m mol Si m^{-3} and assume the silicate to absorbance ratio is constant. This procedure when applied to the beta method absorbances in Table 1 gives an error of up to + 1.00 at the 25 m mol Si m^{-3} level and -9.00 at the 200 m mol Si m^{-3} level. The alpha method standardisation curve is close to linear to silicate concentrations of less than 20 m mol Si m^{-3}, and one standard of 10 m mol Si m^{-3} may be sufficient, when analysing samples within the 0 to 20 range.

CONCLUSIONS

1. The method employing the reduced beta molybdosilicic acid is preferred to that using the reduced alpha molybdosilicic acid. The alpha method is to be avoided because of the turbidity caused by the precipitation of calcium oxalate from sea water samples. The beta method is also faster and is easier to convert to an automated analysis method.

2. The relationship of the two methods is expressed by:

$$\text{Alpha} = 1.89 + 1.020 \text{ Beta (m mol Si m}^{-3})$$

3. The addition of mercuric chloride (400 mg dm^{-3}) to the sea water samples stored in polyethlene bottles is an effective preservative for up to at least eight months.

4. A range of at least four standards should be used to cover the probable range of silicate concentrations in the samples. A curve should be fitted to these standards in order to eliminate significant errors in the calculated reactive silicate content of the samples.

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