

# 行政院國家科學委員會專題研究計畫成果報告

以氣泡取代式無載流自動分析系統偵測海水中矽酸鹽之研究

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## 一、Abstract

An automated bubble flow analysis system is proposed for the determination of silicate in seawater based on the formation of silicomolybdenum yellow reaction. Citric acid, instead of oxalic acid, was used to eliminate interferences from phosphate. The reaction was speeded up with moderate heat at 40 °C. The sensitivity of this system was 2200  $M^{-1}cm^{-1}$  for freshwater and 2150  $M^{-1}cm^{-1}$  for seawater, which covers most naturally occurring Si concentration ranges of up to 500  $\mu M$ . The detection limit was 0.5  $\mu M$  and the precision was less than 1% at 100  $\mu M$  level. A throughput rate of 30 samples per hour can be readily achieved.

Keywords: silicate, sea water

## 二、Introduction

The most widely applied protocols for the determination of silicate in seawater are all based on the silico-molybdenum reactions<sup>(1)</sup>. The major ways have been proposed: the first one is to measure the yellow color at around 400 nm after the formation of a silico-molybdenum yellow complex<sup>(2,3)</sup>,

and the second is to measure the blue color at 810 nm after the yellow color is further reduced to an intense heteropoly blue complex<sup>(4,5)</sup>. The yellow complex, mainly consists of the beta-form silicomolybdenum, is known to be unstable due to the quick transformation to the alpha-form, and the color should be measured promptly to prevent from losing sensitivity. However, since the timing of measuring the yellow color can hardly be controlled to a very precise state by manual procedures, most oceanographers switch their interest to the more stable blue color measurement.

In our experience of using the heteropoly blue approach to measure silicate on the research vessel, we have found that it suffers from several drawbacks: (1) The sensitivity is too great for most estuarine and deep ocean samples. The molar extinction coefficient of the reduced heteropoly color is normally over 20000  $M^{-1}cm^{-1}$ , so at Si concentration more 100  $\mu M$  the reading may go off-scale. (2) The precision of the blue color method is still depending on the stability of the first step yellow color reaction, so the timing is still important. (3) The reaction time

for the last reduction needs more than 5 min to complete at room temperature. As a consequence, the yellow color measurement is put back into the consideration if the timing problems can be solved. In this paper a new concept of automation is proposed which allows the time of reagent additions be controlled at require precision. Elevation of temperature, usually avoided in the manual operation, can be applied in the proposed automated system to shorten the reaction time.

### 三、Experimental

#### *Automated manifold:*

The automated system is presented in Fig. 1. which consists of a Ismatech IPS-8 peristaltic pump, a manifold made of 1 cm i.d. Teflon tubing immersed in a thermostat, a spectrophotometer installed with a 1 cm wide-bore Hellma flow cuvette. The pumping rate ratio of the three pumping tubes was 2.5:0.1:0.1 for the main stream and the two reagents. The actual pumping rates were 4.8 mL/min for the sample and 0.2 mL/min for the reagents. The first reagent (R1) is added right after the up-taking the sample, allow to pass through the pump (~30 s) and react in the thermostat at 40 °C for another 40 s before the second reagent (R2) is added. The mixture is led to the spectrophotometer and the color is measured at 400 nm. The time for a sample to reach the detector was ca. 90 s, and the reading is taken after 120 s when

reaching a stable state.

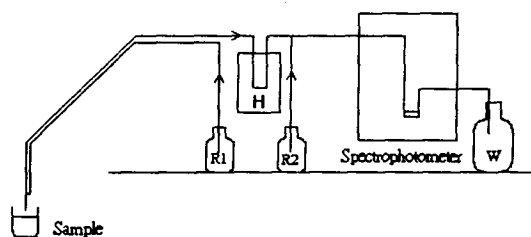


Fig. 1 Systematic layout of the proposed bubble flow analyzer. The pumping rate ratio between the main stream and reagents was 2.5: 1. H: thermostat heater, W: waste

#### *Reagents and standards:*

**Acidic molybdate reagent (R1):** In a plastic beaker, a molybdate solution was prepared by dissolving 32 g of ammonium heptamolybdate in 250 mL distilled water. In another beaker 25 mL of conc. sulphuric acid was poured into distilled water to make up to a final volume of 250 mL. The diluted acid is poured with gentle swirling into the molybdate solution. The molar ratio of  $[H^+]/[Mo]$  in the final mixture was 5.

**Citric acid (R2):** The solution was prepared by dissolving 105 g of trisodium citrate into 500 mL of distilled water.

**Silicate standards:** A stock Si standard solution of 1 g/L was prepared from Merck Titrisol ampoule. Aliquot of 14 mL of this stock was diluted to 100 mL with distilled water to give a concentration of 5000  $\mu$ M. Working standards were diluted from this solution as required.

#### *Procedure:*

After starting the automated system and checking the settings of

spectrophotometer, thermostat, peristaltic pump, and flows in the manifold, distilled water was loaded through the inlet tube. When liquid filled up all manifold and the readings become stable, the instrument was set zero as the blank. The water sample was introduced and the reading was taken 120 s later. Other samples were loaded sequentially.

#### 四、Results and discussion

##### *The bubble-flow design*

The proposed system is distinguished from the traditional flow injection analyzer (FIA) by its carrier-free design and its direct sample loading. The advantages are: (1) the sample section does not receive dilution effect from the carrier so the sensitivity is higher than that of FIA, (2) air bubbles are generated inside the tubings between two sample sections, thus gives minimal carry-over, (3) no injector is need as the sample is directly taken up into the manifold, and (4) the system can be used for freshwater to seawater without worrying the change of light refractory index.

##### *Color formation kinetics*

The time control is essential to the formation of the beta-form silicomolybdic acid. The kinetics study shows that the reaction rate is affected by: (1) acidity (2)  $[H^+]:[Mo]$  molar ratio, (3) temperature (4) salt matrix. A series of

color formation curves was obtained at various conditions. The curve was an exponential type, and a useful parameter:  $t(90\%)$  was used to evaluate the reaction rate. In definition the  $t(90\%)$  is the time required for the color to reach 90% of its maximum value.

In the first set of test the sample was treated with various concentrations of molybdate and acidity at fix temperature (25 °C). The results are shown in Table 1. It shows that the  $t(90\%)$  tends to be shorter at low  $[H^+]:[Mo]$  ratio ( $<5$ ), and becomes very low at  $[H^+]:[Mo]>10$ . Small difference was found between the freshwater set and the seawater set. Another consideration is the easiness of preparing the mixed reagent. Since at ratio less than 3 the mixed reagent has a tendency of precipitation, the ratio value 4 and 5 becomes a pair of thresholds for the optimization of the reaction.

##### *Temperature effect*

Table 2 shows the effect of temperature to the  $t(90\%)$  at a fixed  $[H^+]:[Mo]$  ratio at 4. In general high temperature did have strong promotion to the reaction rate. The  $t(90\%)$  was ca. 80s for freshwater at 20 °C, and shortened to less than 16s at 40 °C, and less than 11s at 50 °C. The trend also fit for seawater samples. However, most protocols did not take the advantage of high temperature due to the stability of the color.

### *Fading of color*

After the formation of the beta-form silicomolybdic acid, the color is gradually faded due to the transformation of the beta-form to a less color-intense alpha-form. The fading rate is related to all the factors that might affect the formation rate. For example, the color faded rapidly at elevated temperatures, higher salinities, and lower [H<sup>+</sup>]:[Mo] ratios. Table 3 shows the results. At 20 °C, the color fading rate was -2% /hr for freshwater and -4%/hr for seawater. At 30 °C, that rates became -4%/hr and -12%, and at 40 °C, -12%/hr and -27%/hr respectively. In manual operation the complete reaction requires 5-10 min to develop at room temperature, and the measurement needs to be carried out immediately to avoid the fading effect. At elevated temperatures, e.g. 40 °C, the  $t(90\%)$  is as short as 15 s, so the measurement can only be performed on an automated continuous flow system. With the proposed system the time span for the first reaction was ca. 60 s (30s at room temperature and 30 s at ambient temperature), which allowed a more than 98% of completeness.

### *Masking agent*

Since phosphate can also react with molybdate to cause interference, a masking agent is applied. In literature at least four organic acids have been used namely, oxalic acid, tartaric acid, citric acid, and manitol acid. All of them can

eliminate phosphomolybdic acid effectively. Among those, citric acid showed to be the strongest because the amount of citric required was 1/2 compared with oxalic acid and 1/3 with tartaric acid. It did however, cause fading of the silicomolybdate color.

### *Sensitivity and precision*

A calibration test was made on measuring spiked distilled water and seawater. The results are shown in Table 4. In distilled water the molar extinction coefficient was ca. 2200 M<sup>-1</sup> cm<sup>-1</sup>, while in seawater medium the coefficient was about 2.5% less, to be ca. 2150 M<sup>-1</sup> cm<sup>-1</sup>. The precision was satisfactory, to be less than 0.5 % in the tested range. These test were run at a throughput rate of ca. 30 samples per hour.

## **五、Conclusion**

The proposed automated flow system provide a novel way of automation in the silicate measurement. The carrier-free and injector-free designs allowed the determination to have the highest sensitivity and the least interference. It is especially of practical value when performing on board a research vessel.

## **六、References**

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Table 1 Formation kinetics of silicomolybdic acid (presented as t(90%) in seconds) at various acidity and molybdate concentrations (temperature 25 oC)

Acidity (mM)	[Mo] concentration (mM)				
	10	20	30	40	50
t(90%) (sec) in freshwater medium					
40	106	62	42	32	29
80	175	62	49	46	41
120	>300	109	61	54	43
160	>1000	200	101	71	58
t(90%) (sec) in seahwater					
40	135	80	50	40	42
80	150	80	50	45	40
120	>200	95	60	50	45
160	>1000	130	75	55	50

Table 2 Effect of temperature on the formation kinetics for the silicomolybdic acid (Si concentration range from 50 -150 uM)

[Si] (uM)	Temperature (oC)					
	25	30	35	40	45	50
t(90%) (sec) in fresh water						
50	75	35	21	16	14	11
100	80	36	22	16	13	9
150	80	36	-	15	13	10
t(90%) (sec) in seahwater						
50	65	29	-	15	13	12
100	65	28	20	15	14	11
150	70	28	-	16	14	-

Table 3 Fading of the yellow silicomolybdc acid at elevated temperatures

[H+] (mM)	[Mo] (mM)	[H+]:[Mo] ratio	Temperatrue (oC)		
			20	30	40
Fading rate (%/hr) in freshwater					
40	50	0.8	-3	-8	-24
40	20	2	-2	-5	-13
80	40	2	-2	-5	-13
120	50	2.4	-2	-5	-14
120	30	4	-2	-4	-12
120	30	4	-2	-4	-11
Fading rate (%/hr) in seawater					
40	50	0.8	-6	-12	-38
40	20	2	-4	-10	-38
80	40	2	-4	-9	-29
120	50	2.4	-4	-8	-30
120	30	4	-4	-8	-27
120	30	4	-4	-8	-27

Table 4 Calibration of silicate with the proposed automated system

Sample	Conc. spiked (uM)	Abs(400 nm, 1 cm)	RSD% (n=7)	e(M/cm)
Dist. Watr	0	0.000		
	100	0.201	0.000	2178
	200	0.399	0.001	2161
	500	1.005	0.002	2178
Seawater	0	0.037	0.000	
	100	0.235	0.001	2145
	200	0.435	0.002	2156
	500	1.018	0.001	2126