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ERRORS IN THE VALUES FOR NITRATE AND SILICATE CONCENTRATIONS IN SEAWATER, AS LISTED IN THE CSIRO AND WORLD DATA BANKS

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Abstract

Values of nitrate in deep seawater samples determined by a manual strychnidine method are higher than either those determined by an automated cadmium reduction method or those determined by the strychnidine method with standard additions to each sample. The error increases with increasing concentration. Two possible causes were investigated: incorrect use of the strychnidine standard curve; and interference due to a matrix effect caused by concentrations of other constitutents that increase with depth in oceanic waters. The former was found to be the cause. High concentrations of nitrate determined by the two different techniques should not, ideally, be compared to assess long-term changes in the composition of deep water masses. However, if such comparisons are unavoidable, an approximate correction factor can be subtracted from the strychnidine data.

Concentrations of silicate in seawater that were determined by the manual method were lower by a constant amount than those determined by the automated method. An incorrect reagent blank value had been subtracted from each sample absorbance. The true value can be twice as large as the listed value for the low concentrations found in surface water, but for deep waters (high concentrations) the relative error is small. Some of the incorrect silicate data could be corrected for studies of long-term changes in the composition of surface water masses if analytical data is accessed and the incorrect blank concentration replaced by a suggested realistic value. For all cruises, silicate data should be discarded if nitrate values in deep water were depleted, because silicates are too high in such examples.

INTRODUCTION

From 1947-84, CSIRO Marine Laboratories determined nitrate, silicate and phosphate in seawater by manual colorimetric methods (Rochford, 1947; Major et al., 1972). After 1976, some nutrients were determined by automated methods (Airey and Sandars, 1984); for intercalibration purposes, some nutrients were determined by both methods (see Table 3). During 1984, all manual methods were replaced by automated methods.

This report describes the differences in results of nitrate and silicate determinations in samples analysed by manual and automated methods. Phosphate concentrations measured by both techniques were the same within the accuracy of the new techniques (Airey and Sandars 1984).

DISCUSSION OF NITRATE METHODS

Strychnidine is a multi-ringed complex that gives an intense red colour with nitrate if conditions of pH and chloride ion concentrations are correct. Because of the intensity of the coloured complex, samples are diluted. The standard curves used (Fig. 1) are not linear. Algorithms have been changed several times to accommodate gross changes in the shape of the curve. Absorbances vary from day to day due to ageing of the reagent and changing operational procedures, or grossly due to batches of reagent being prepared by different manufacturers. Dal Pont (pers. comm.) suggests nitrate has a catalytic action, and that below 50 µg/l insufficient nitrate is present for total colour development at low concentrations, which results in a curved calibration graph. Nitrate concentrations determined by this method are now listed in CSIRO Marine Laboratories computer centre and in the World Data Centre.

The strychnidine method has been superseded because it cannot be used at sea; reagent blank values are high; there is large variability in replicate analyses (for example, 5 replicates for a 200 µg/l standard often have a

standard deviation of 60 µg/l; samples were not analysed routinely in replicate); noxious fumes are emitted from the reagents; reaction times are lengthy; bubbles form in the viscous solutions and interfere randomly during the spectrophotometric readings; such bubbles are not manually eliminated from modern suction curvettes; colour development differs if the temperature varies because of inconsistencies in the speed of combining reagents; and results for the same concentration differ if the geometry of the reaction flasks are not identical e.g. the neck width.

The automated cadmium reduction method is that recommended worldwide for nitrate in seawater (Strickland and Parsons, 1972). Details of the autoanalyser manifold and operational procedures is given elsewhere (Airey and Sandars, in press).

Oceanic Water

Nitrate concentrations in water sampled at several stations during Sprightly Cruise 12 in 1978 were determined by both the automated cadmium reduction method and the strychnidine method, and then compared (see Fig. 2). The 1:1 line is the curve the data should have taken if the results were comparable. The curve shows that at high concentrations either the strychnidine method gives results with a positive bias or the automated method gives a negative bias. The deviation from the 1:1 line increases as the nitrate concentrations increase with depth. At low values of nitrate in samples, in standards, or in spiked surface seawater up to 100 µg/l, the comparisons between pairs of results are tolerable, apart from the large scatter in replicate strychnidine values about the mean value. On rare occasions, comparisons of samples > 400 µg/l have also been tolerable, but one cannot determine when and why this happens.

Standard additions

To determine which method was incorrect, water from 500 m was spiked with nitrate standards and determined by both methods. The strychnidine method gave high values. Figure 3 shows the measured increase in nitrate levels versus the spike added. The results for the cadmium reduction method are on the 1:1 line. Further confirmatory tests using standard addition eliminated the possibility that the cadmium reduction method was giving low values (Fig. 4a).

To determine whether a deep seawater matrix effect was causing the high values, the standard addition technique was then used to validate the strychnidine method (Fig. 4b). When absorbance was plotted aginst the spike value, the concentration determined could be down to a half of the expected value. This varied for each test; a result, we believe, of the graph of the

absorbance and nitrate concentration being curved (see below). However, when each nitrate value to be plotted was determined from the strychnidine standard curve and then plotted against the spike value, the standard addition value was lower and more consistent with the autoanalyser values. The difference in strychnidine values determined by the routine strychnidine method and those determined by the autoanalyser are shown in Fig. 5a; values determined by the routine strychnidine method and the modified standard addition method are compared in Fig. 5b. From the standard addition experiments, it was not possible to support the hypothesis that a matrix effect was causing the error in the strychnidine method. We could only state that strychnidine results were too high and the cadmium reduction results were correct.

Standard curves

The cause of the errors was found to be the method used to construct and use the strychnidine standard curve. The instruction manual (Major et al., 1976) states that, after the reaction with strychnidine, samples and standards with absorbances greater than one unit must be diluted with 50/50 concentrated sulphuric acid and artificial seawater. Since about 1977, each curve was constructed only for concentrations up to 200 µg/l. To save time, samples with higher concentrations were diluted with artificial seawater before analysis. Unfortunately, high standards were not treated in the same way.

Originally samples, blanks and samples were analysed in triplicate because of the variability in replicates, and the same procedure was followed for all analyses. The results of analysis by the autoanalyser method and the strychnidine method as the latter was performed in 1976 (without replicates, but with standards and samples correctly diluted after the strychnidine reaction) are shown in Fig. 5c. The horizontal bars show some standard variations we have measured in 5-10 replicate standards. As this was the only intercomparison until 1978, after which the anomaly between the two methods existed in every intercomparison, we suggest data that was analysed between 1977 and 1984 should be corrected if it is to be compared with data derived outside this period.

The absorbances of samples diluted with artificial seawater, even up to 200 µg/l were always found to be higher than expected. We cannot explain why this was so (nor do we wish to investigate the matter further as the method is not now being used in CSIRO laboratories). The error became sizeable when the incorrect concentrations were multiplied by dilution factors of 2, 5, or 10. Figure 6 shows schematically that part of the problem, besides the high absorbances, arose from the assumption that linear dilution could be used for nonlinear standard curves. Different depths were diluted by different analysts. Undiluted samples lay in the range 0-150 or 200 µg/l; times one dilution lay in the range 150-200 to 200-250 µg/l; and times five dilution lay in the range 200-250 µg/l for the rest of the samples. So in some areas, the error may be times two or times five, depending on which dilution was selected. This may account for some of the scatter in Fig. 5a.

Another reason for the scatter in Fig. 5a is that errors were not consistent from day to day, due to variability in the determinations and the

standard curves. We also found that if replicate samples, with appropriate standards, were left at different temperatures during the diffusive mixing period, the results of replicates differed (see Table 1). For more than ten years the diffusion reaction has been allowed to take place with the boxes at room temperatures. Originally, samples were cooled for two hours in a refrigerated room. Reagents at the same temperature were added and the diffusive reaction allowed to take place in the same room. This explains why at some times of the year the error was not as great as in other periods.

Correction procedure

Data determined by different methods must be compared with caution. We suggest that Figure 5a be used to make rough adjustments to data if data determined by the two methods are to be compared. The points below the line are due to random analytical variability at low concentrations. Also, in surface waters where there are plankton blooms, nitrate levels increase to concentrations of 4-6 µg/l. Such concentrations of nitrite are determined quantitatively by the cadmium reduction method, but are not determined by the strychnidine method until much higher levels are present (unpublished data). Where nitrite data were measured by the autoanalyser method, they have been subtracted to give nitrate-only values. To use Figure 5a, select the strychnidine data to be corrected, read off from the envelope an approximate correction value, and subtract it.

DISCUSSION OF SILICATE METHOD

Intercomparisons between manual and automated silicate determinations since 1976 have shown discrepancies due to an analytical error. The instructions (Major et al. 1976) state that a silicate-free distilled water reagent blank should be subtracted from the spectrophotometric readings of the seawater samples. Several years ago, and we cannot determine when, a mistake was made, which was perpetuated until February 1984. Artificial seawater reagent blank was subtracted. As artificial seawater contains substantial amounts of silicate, all results in a batch were low by a constant amount.

The variability in silicate concentrations of samples when the reagent blank was made with artificial seawater, with distilled water that contained traces of silicate, and Milli-Q de-ionised water are shown in Table 2. The silicate concentration in constitutents of artificial seawater vary from batch to batch. In Figure 7 the large dots and crosses represent the values of some silicate concentrations determined by the manual method when artifical water and silicate-free water, respectively, were used as blanks. In deep-water samples the percentage error is much less significant because of the large concentrations (see Table 2) and small dots in Fig. 8.

Correction procedure

Reagent blanks in silicate-free distilled water were not measured until February 1984. Since then, the mean value of the difference between the silicate-free blank (usually 6 µg/l) and the incorrect blank value of silicate concentration has been 9-124 µg/l. The data can be used unaltered for qualitative comparisons of water masses present only during the period the samples were taken. However, if comparisons of silicate determined before February 1984 are to be made with concentrations listed since then, we recommend making the following adjustments, especially for surface samples, which have a large percentage error.

(1) For recent data, ask the Marine Laboratories for the absorbances for the uncorrected standard curve and the artificial seawater blank. Determine the concentration of silicate in the blank using the calibration curve, subtract the Mill-Q blank (6µg/l) and add the result to each concentration listed in the data bank. Although this will not give a true value, it will be within the accuracy of the technique because the computer listings are converted to µg atoms/l and rounded up.

(2) If the Marine Laboratories cannot supply uncorrected absorbances, either don't make comparisons between new and old data, or get a rough estimate as follows: compare the incorrect silicate concentrations listed for the surface water with correct concentrations analysed by automated methods in samples from that area, from similar depths, during similar seasons. See Table 3 for pre-1984 cruises that used automated methods. A model of seasonal nutrient distributions is being prepared from this data and could be used as reference levels (Airey, in prep.).

Unpreserved samples

Another note of caution. Whenever silicate values are needed, check the nitrate values in the station listing. If the nitrate concentrations in deep waters are negligible (Fig. 8), suspect that a preservative was not used at this station (when this occurs, a whole station will usually be incorrect). We found that silicates analysed in unpreserved samples gave high values (see Fig. 8a). Such data should be discarded. It could be the result of bacterial degradation of diatoms, which would release silicate and nitrate, the latter being subsequently removed by phyto-plankton not requiring silicate.

SUMMARY

Comparisons of the results of manual and automated methods to determine nitrate and silicate in seawater showed discrepancies in the results. Incorrect standardisation caused an error in the manual nitrate determinations, and an incorrect blank value for the reagents caused an error in the manual silicate determinations. If this data is accessed from CSIRO Marine Laboratories Data Bank or the World Data Centre, one should be aware of the order of magnitude of the error and depths where discrepancies have been observed. Comparisons between results determined by the old and new techniques should be made with caution after assessing the importance of the errors described here relative to the question to be answered.

ACKNOWLEDGEMENTS

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Strickland, J.D.H. and Parsons, T.D. (1972) A practical handbook of seawater analysis. (2nd ed.) *Fisheries Research Board of Canada Bulletin* **167**, 310 pp.

TABLE 1 **Nitrate concentrations (µg/l) of duplicate pairs of samples and standards left to react at warm and cool temperatures, as determined by the routine and modified standard addition methods.**

Routine method	Standard addition method	Routine method	Standard addition method
Temperature 21°C		Temperature 12°C	
41	35	42	39
108	114	104	102
245	245	220	212
650	660	660	NA
640	607	590	620

TABLE 2 **Silicate concentrations (µg/l) in seawater when contaminated artificial seawater and silicate-free Milli-Q water were used as reagent blanks.**

Depth (m)	**Artifical seawater**	**Milli-Q water**	**% error**
48	12.5	27.4	119.2
51	16.5	31.3	89.7
82	17.0	31.9	87.7
103	22.7	37.6	65.6
200	109.7	124.8	13.8
400	168.0	184.0	9.5
454	208.0	223.0	7.2
900	901.0	920.0	2.1
1500	2262.0	2286.0	1.1

TABLE 3 **Pre-1984 cruises using automated and manual methods of nutrient determination.**

	Nutrients determined							
	Automated					Manual		
Ship and Cruise no.	N3	SI	PO	NH	N2	N3	SI	PO
Atlantis 11/76	*	*	-	-	-	*	*	*
Kimbla 3/77	*	*	-	-	-	-	-	-
Kimbla 6/77	*	*	-	-	-	-	-	-
Sprightly 6/77	*	*	*	-	*	-	-	-
Courageous 6/77	*	*	*	-	*	-	-	-
Courageous 9/77	*	*	*	-	*	-	-	-
Courageous 1/78	*	*	*	-	*	-	-	-
Sprightly 1/78	*	*	*	-	*	-	-	-
Sprightly 3/78	*	*	*	*	*	*	*	*
Sprightly 12/78	*	*	*	-	-	*	*	*
Sprightly 15/78	*	*	*	-	-	*	*	*
Sprightly 16/78	-	-	-	-	*	*	*	*
Sprightly 14/80	*	*	*	*	*	*	*	*
Sprightly 15/80	*	*	*	*	*	*	*	*
Soela 4/81	*	-	-	-	*	-	-	-
Sprightly 14/83	*	*	*	*	*	*	*	*
Sprightly 15/83	*	*	*	*	*	*	-	*

N3 nitrate; SI silicate; PO phosphate; NH ammonia; N2 nitrite.
Cruises are numbered consecutively within each year: e.g., 11/76 is the 11th cruise in 1976.

* samples were analysed.

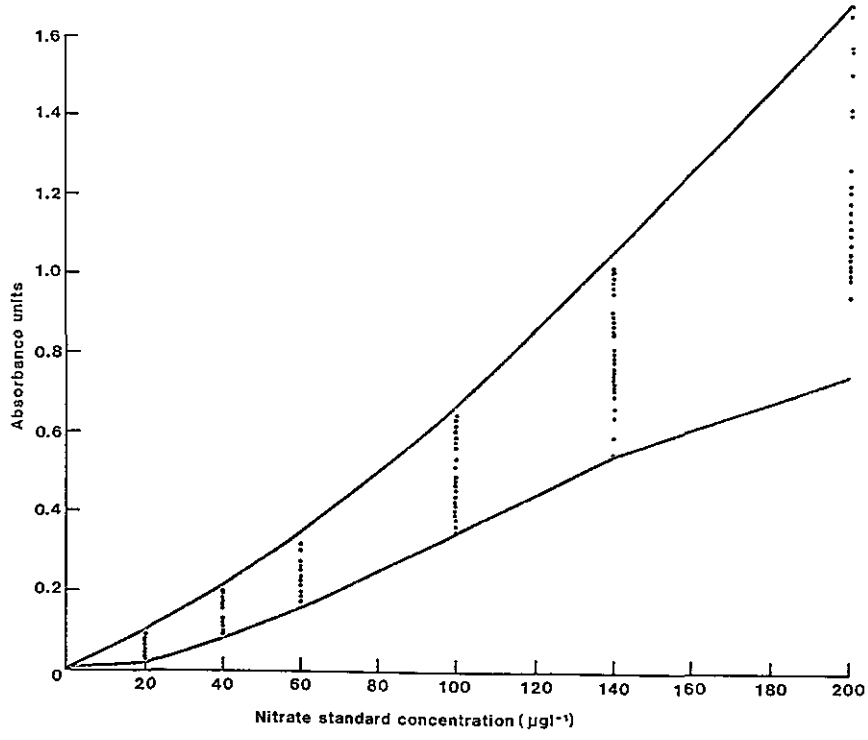

Figure 1 Absorbance versus nitrate concentration (µg/l) to show the variability with time in strychnidine standard curves for nitrate determinations.

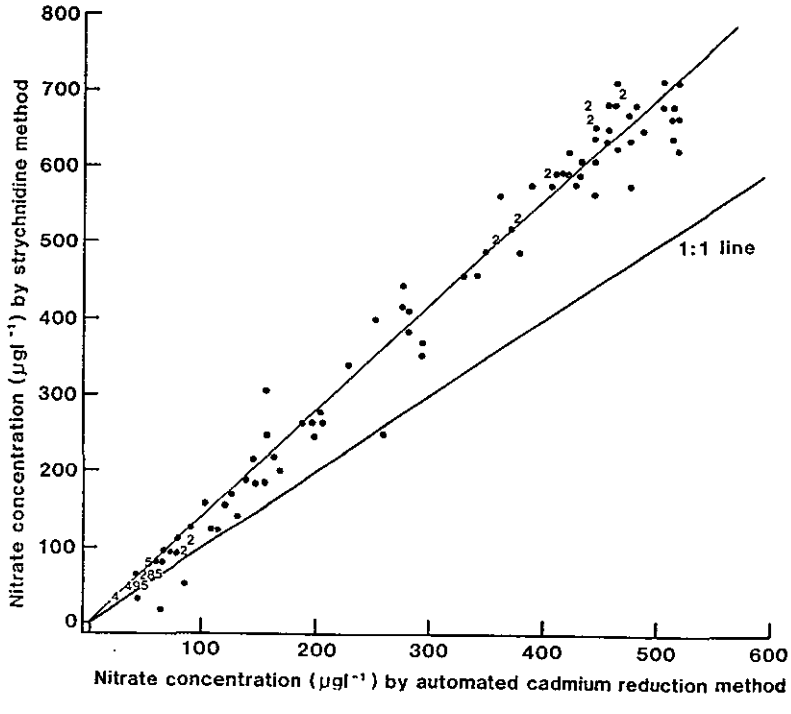

Figure 2 Comparison of nitrate concentrations (µg/l) determined by the automated cadmium reduction method and those determined by the strychnidine method. The samples were taken and preserved in separate bottles as described in the appropriate manuals.

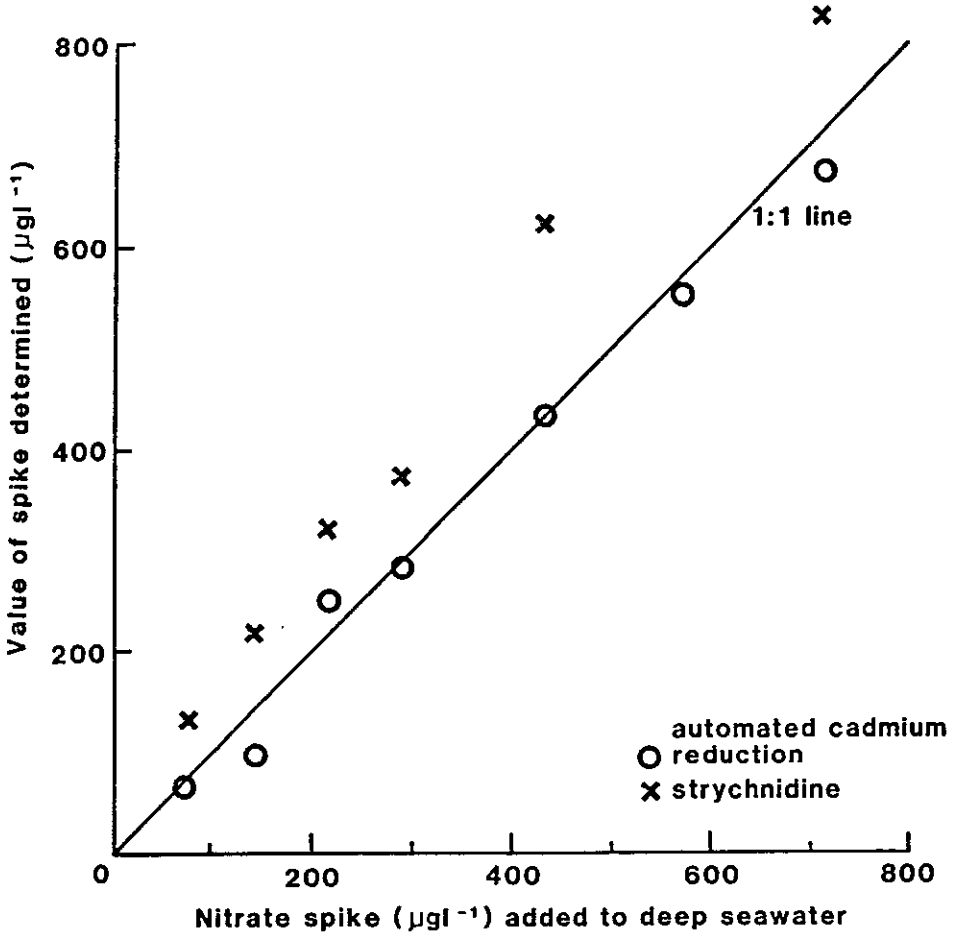

Figure 3 Nitrate concentrations (µg/l) added to seawater sample taken from 500 m versus the spike concentration by the automated cadmium reduction method (circles) and by the strychnidine method (crosses).

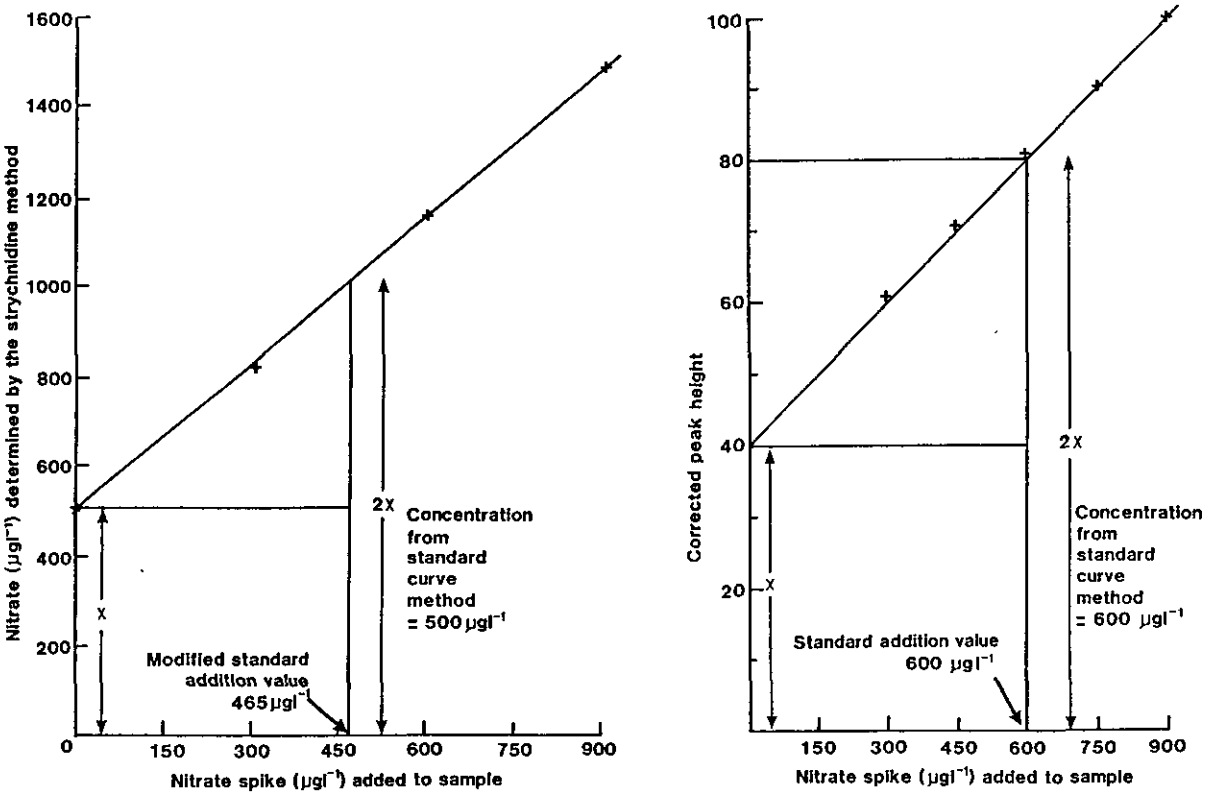

Figure 4 (a) Standard addition technique to determine nitrate by the cadmium reduction method; and

(b) modified standard addition technique to determine nitrate by the strychnidine method.

Values are means of duplicates.

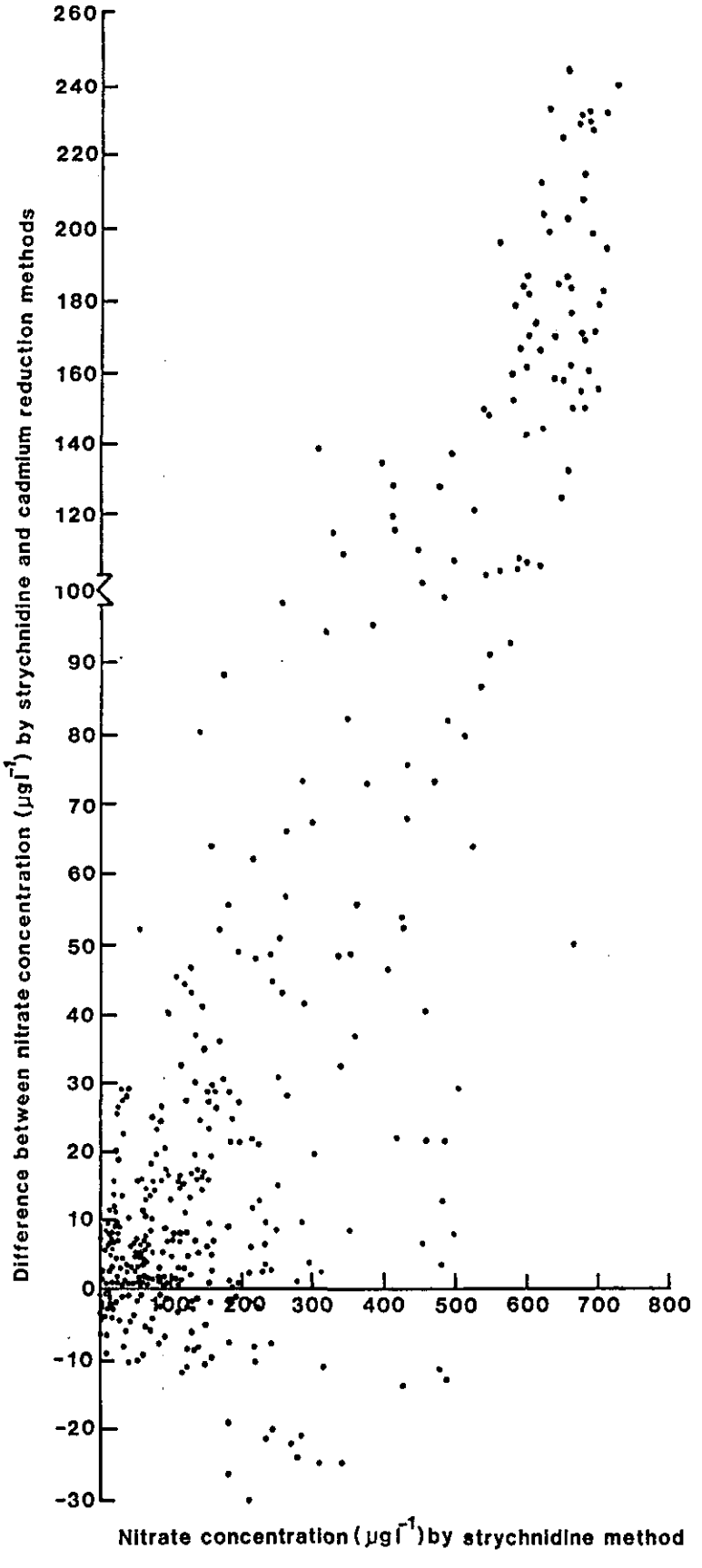

Figure 5a Strychnidine values versus the anomaly between them and the autoanalyser values (µg/l).

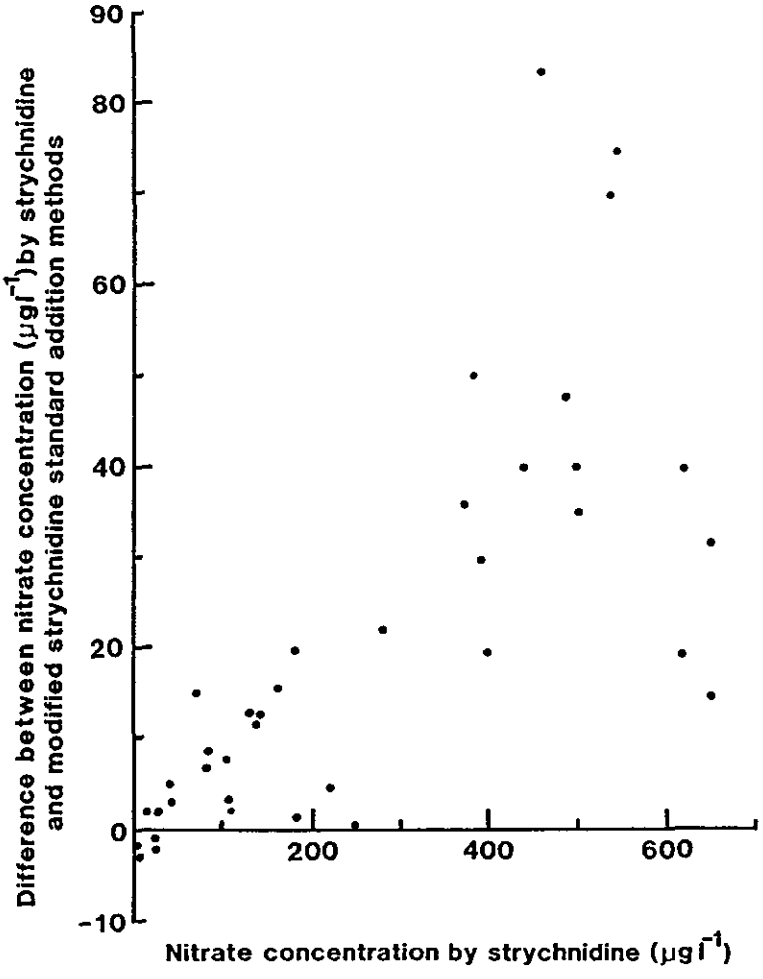

Figure 5b Strychnidine values versus the anomaly between them and the values determined by the modified standard addition method (µg/l).

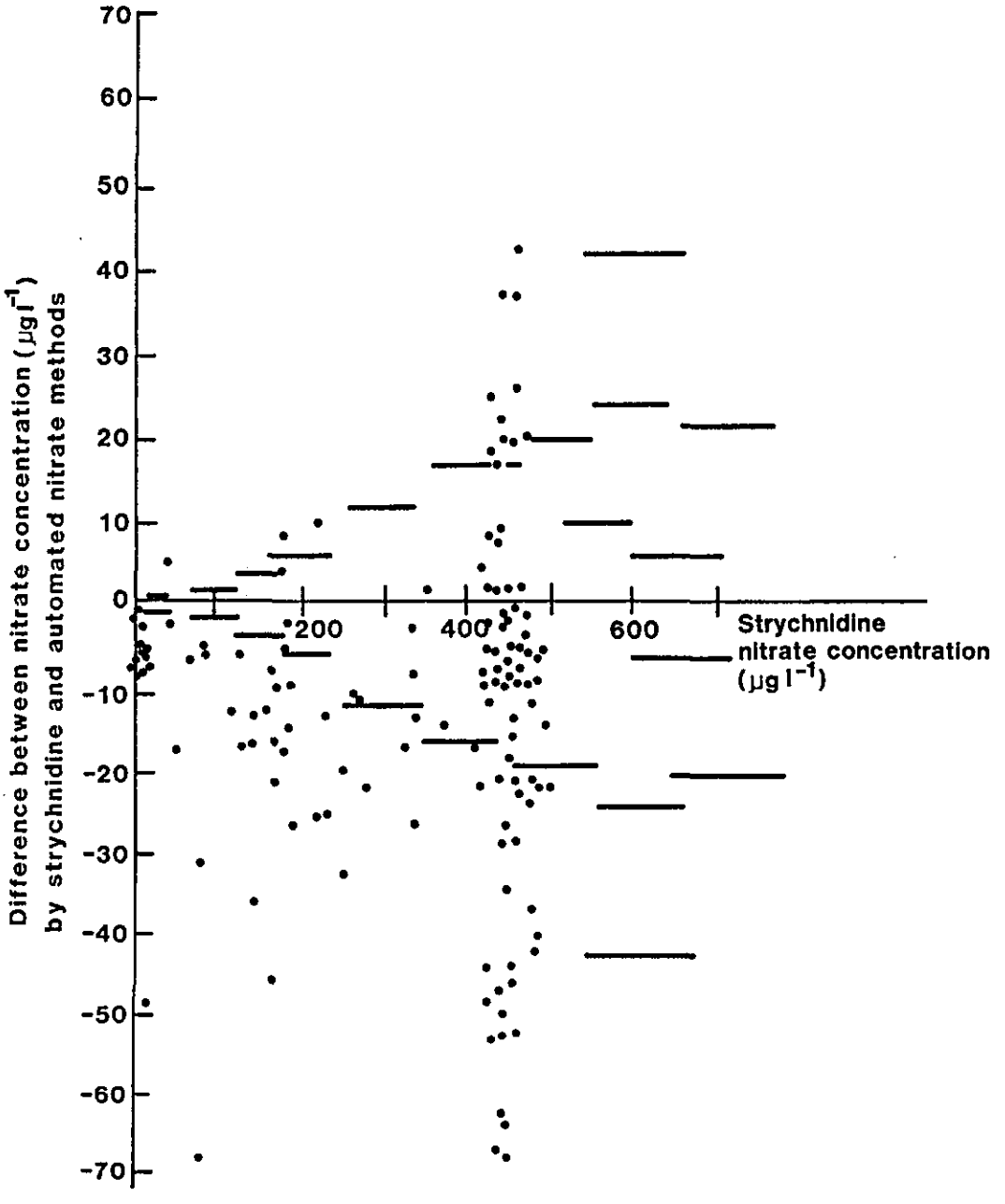

Figure 5c Strychnidine values (single determinations) versus the difference between them and the autoanalyser values for a set of samples determined using correct standard procedures. The horizontal bars show some standard deviations µg/l for the strychnidine method (5-10 replicates).

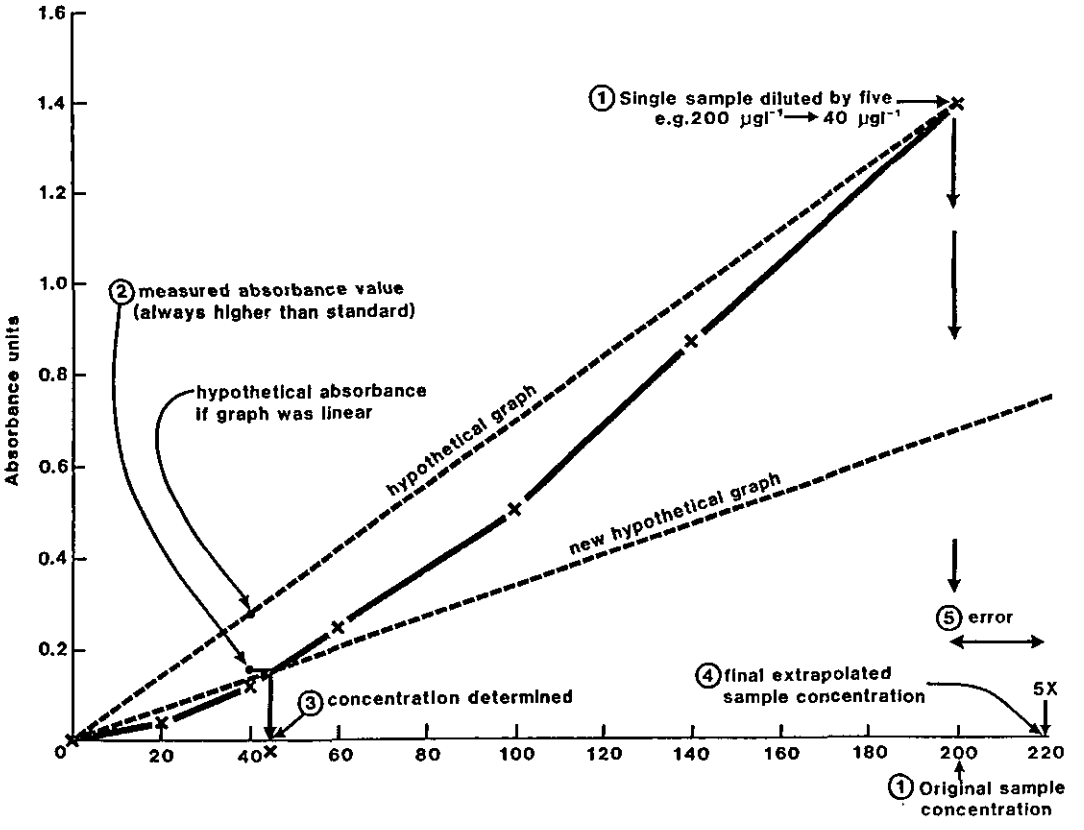

Figure 6 Schematic graph to show how errors occur when dilutions are used for nonlinear standard curves.

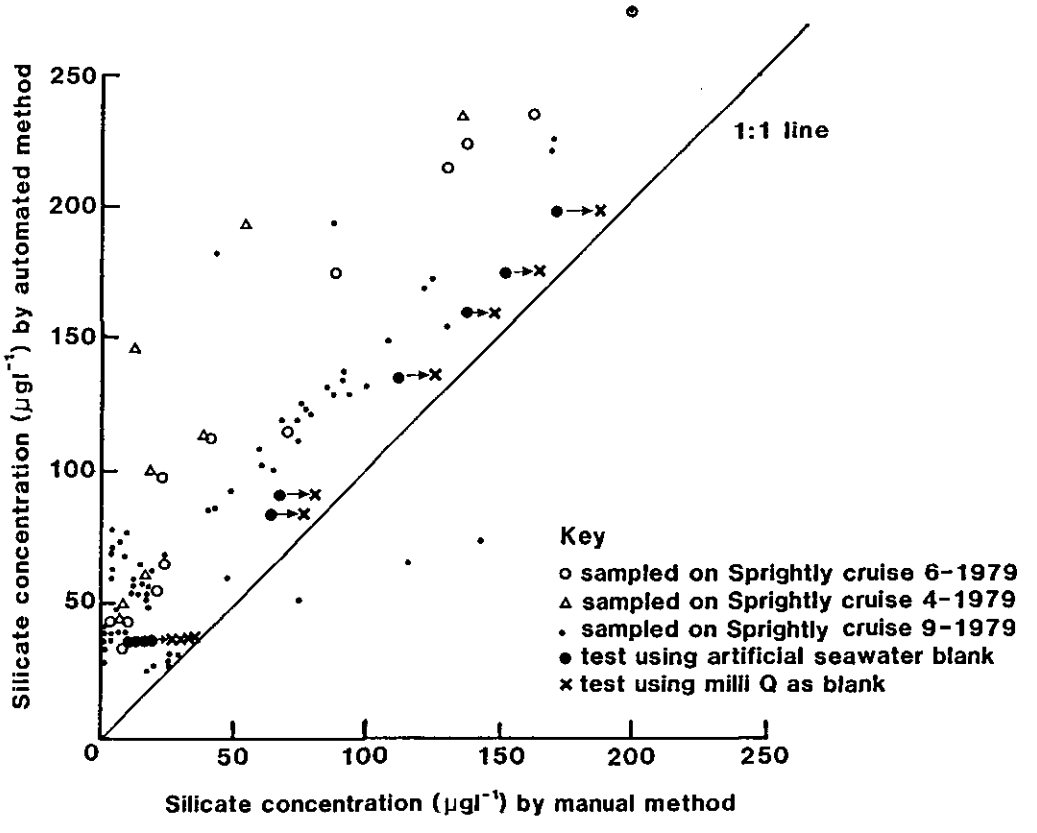

Figure 7 Silicate concentrations (µg/l) in samples from several cruises determined by the automated method against those determined by the manual method (small dots), using the artifical seawater blank (large dots), and the correct silicate-free de-ionised water blank (crosses).

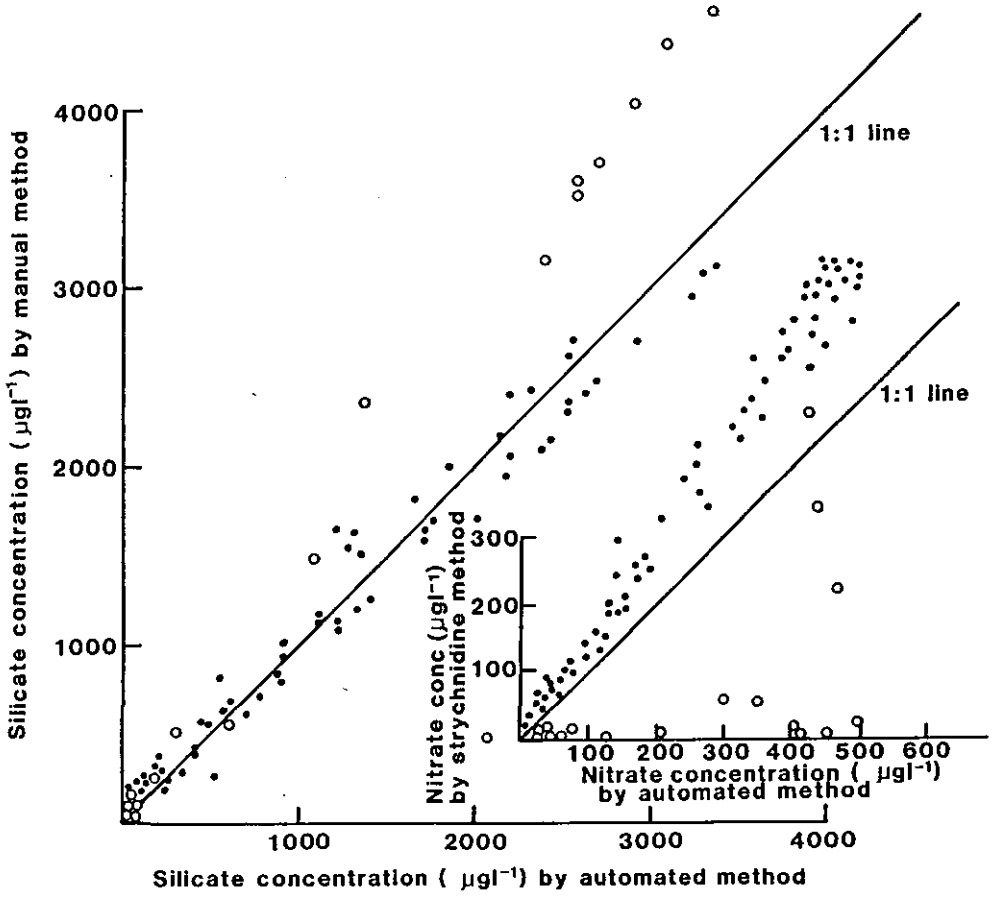

Figure 8 Silicate concentrations (µg/l) determined by the automated method against those determined by the manual method. The small dots represent samples with preservative; the open circles show increase in silicate due to the absence of the preservative. (The smaller graph shows the depleted nitrate values (open circles) measured in the same samples, indicating that the preservative was absent).

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