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Blank and Salinity Corrections for Automated Nutrient Analysis of Estuarine and Sea Waters

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¹Contribution No. UNH-SG-JR-101 from the University of New Hampshire, and No. 3897 from the Woods Hole Oceanographic Institution.

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Blank and Salinity Corrections for Automated Nutrient Analysis of Estuarine and Sea Water

Abstract

Routine measurements of dissolved micronutrients in sea water are performed by numerous laboratories using a Technicon AutoAnalyzer II System. The methods employed are generally the Technicon Industrial Methods with various modifications. Experimentation has shown, however, that there are some problems with these methods in the determination of blanks, which can cause significant errors in estuarine or sea water samples. The blank problems result from: 1) the index of refraction of the sea water sample, 2) reaction products of various wetting agents and sea water, 3) the absorbance of colored substances in the sample, either particulate or dissolved, and 4) the salt error or variable production of reaction products of the nutrient in the sample and the color reagents as a function of sample salinity. These corrections range from 0 to 20% depending upon the salinity of the sample and the method used. Furthermore, recent experimentation has shown that the use of a simple artificial sea water as a blank or in the preparation of standards may result in a determination which is different than natural sea water under the same conditions.

In this paper several methods are recommended for determining the blank and salt correction factors. The method to be used depends on the nature of the samples (fresh, estuarine or sea water) and the nutrient being analyzed.

Introduction

Routine measurements of dissolved micronutrients in seawater including orthophosphate, reactive silicate, nitrite, nitrate, and ammonia are performed by numerous laboratories using a Technicon AutoAnalyzer II System. However, the methods utilized, although primarily Technicon Industrial Methods, are by no means standardized or consistent. One of the major differences concerns the preparation of standards and determination of "blanks" for samples of variable salinity, such as estuarine samples. Experimentation has shown that significant errors in sample values may result if an inappropriate method for determining the blank is used.

The absorbance peak obtained by an automated system for a given nutrient in a seawater sample (when compared to deionized distilled water baseline) represents the sum of absorbances from at least four sources: 1) the light loss due to the differences in the index of refraction of the seawater and deionized distilled water; 2) reaction products (i.e. precipitates) of appropriate wetting agents and the seawater; 3) the absorbance of colored substances in the sample, either particulate or dissolved; and 4) reaction products of the nutrient in the sample and the color reagents. These reaction products may be variable due to a "salt error" caused by a shift in the position of equilibrium as a function of a change in the ionic strength of the solution (1).

In this paper we review the literature methods for determining blank corrections and show how they do not necessarily eliminate all sources of error, we suggest modified correction methods for each of the seawater nutrients, and we discuss how the use of artificial seawater may lead to erroneous correction values.

Materials and Methods

The appropriate salt effect and blank corrections for each of the seawater nutrient methods were determined by analyzing water of different salinities with various combinations of reagents, wetting agents, and distilled deionized water (DDW) in the reagent lines. Sampler wash water contained DDW at all times and was used to set baselines. Seawaters of salinities ranging from 3⁰/oo to 33⁰/oo were made by dilution of low nutrient surface coastal water with DDW. In addition, an artificial seawater (ASW) sample of the following composition was analyzed with seawater samples: NaCl, 31g; MgSO₄·7 H₂O, 10g; NaHCO₃, 0.041 g; dilute to one liter with DDW (2). Salinities for each dilution were determined on a Guildline Autosal Salinometer (Model 8400). The salinity of the above ASW was 33.4⁰/oo when measured conductimetrically using the Autosal and about 33⁰/oo when measured using a refractometer (Endico Model 102).

The contributions to an absorbance peak of a seawater sample were determined using the following methods. First, the seawater dilutions and ASW were analyzed by running DDW only through all reagents and diluent lines. Second, the samples were analyzed by running DDW through the reagent lines, with the appropriate wetting agent and diluent for the method. This is the wetting agent effect. Third, the procedure was repeated using a modified color reagent with one or more of the color-producing chemicals eliminated; in other words, the reaction was run under normal pH, but no color was formed. This determines the total correction due to refractive index, wetting agents, and sample turbidity. Fourth, these same samples were analyzed using all normal reagents and compared to standards prepared in DDW. Finally, the seawater dilutions and ASW were analyzed with several $\mu\text{g-atom l}^{-1}$ standard additions of the appropriate nutrient. This gives the chemical effect of the presence of salt relative to DDW standards. From these experiments correction equations were determined for different salinities, and are discussed individually in the following sections.

These corrections were determined on two individual systems and were shown to be a function of the specific method of analysis used and the standard calibration (STD CAL) setting. The corrections given are intended as a guide to show the extent and type of corrections necessary. It is important to note that the individual analysts should determine the appropriate corrections for their own system and methodology.

Orthophosphate

The method for determination of orthophosphate in seawater depends on the formation of a phosphomolybdate-blue complex (3). The major modifications of the method involve the choice of reducing agents which include ascorbic acid, stannous chloride, or hydrazine. A summary of these methods and their corrections are given in Table I.

We have identified two problem areas with the antimony and ascorbic acid method (4). The first deals with the refractive index correction and the second with the use of Levor IV as a wetting agent. All the methods have been reported to have salt errors of less than 1-2%, so this correction is not necessary.

The refractive index correction in $\mu\text{g-atoms l}^{-1}$ was found to be $\text{RI}(\text{PO}_4) = 0.006 (S^0/\text{oo})$ with DDW in both reagent and diluent lines (4) with a STD CAL setting of 8.00 and full scale value of $5 \mu\text{g-atoms l}^{-1}$ (Fig. 1, Curve 1). This correction should be determined without the wetting agent Levor IV in the diluent because of the following problems.

Levor IV reacts with seawater, producing a precipitate which then contributes to the blank absorbance and hence the apparent phosphate concentration (18). It is a function of both salinity and concentration of Levor IV in the diluent. Technicon recommends that "a seawater blank" be determined by running distilled water only through the reagent lines (4). If only the mixed reagent line is

Table I. Selected salinity-nutrient correction procedures for the automated analysis of orthophosphate in estuarine and seawater.

Analytical Method	Sample Type	Correction Type	Blank or Wash	Standards	Correction Comments	Reference
Phosphomolybdate-blue (ascorbic acid), (3)	Fresh or Salt	RI ¹	DDW ²	DDW	Run blank on sample while running DDW only in reagent lines; salt error <1%; wetting agent, Levor IV.	(4)
Phosphomolybdate-blue (ascorbic acid), (3)	Salt	RI or C ¹	DDW	ASW ³ or NSW ⁴	Run blank on ASW or NSW standard water, slight salt error.	(5)
Phosphomolybdate-blue (hydrazine), (6)	Salt	RI	ASW	ASW	Salt error: less than 2% at 33 °/oo; no correction needed; no wetting agent.	(7,8)
Phosphomolybdate-blue (ascorbic acid), (9)	Salt	RI or C	DDW	NSW	Salt error: less than 1% at 35 °/oo; no wetting agent.	(10)
Phosphomolybdate-blue (SnCl ₂), (11)	Salt	RI	DDW	ASW	Mixed standards in ASW. Identifies degree of refractive index error, but does not routinely correct for it.	(12)
Phosphomolybdate-blue (ascorbic acid), (9)	Salt	RI	ASW, DDW, or 0.075% w/v NaHCO ₃	DDW	Wetting agent, Levor IV.	(13)
Phosphomolybdate-blue (ascorbic acid), (4)	Salt	C	DDW	ASW	No correction mentioned; wetting agent, Levor IV.	(14)
Phosphomolybdate-blue (ascorbic acid), (3)	Fresh or Salt	None	DDW	DDW	Salt error reported less than 1%; no wetting agent.	(15)
Phosphomolybdate-blue (SnCl ₂)	Fresh or Salt	RI or C	not specif.	not specif.	Run samples with sulfuric acid reagent only and subtract.	(16)
Phosphomolybdate-blue (ascorbic acid)	Fresh or Salt	None	DDW	DDW	Salt error less than 1%; no wetting agent.	(17)

1. RI - Refractive Index correction,

C - Chemical Interference

2. DDW - Deionized Distilled Water

3. ASW - Artificial Sea Water

4. NSW - Natural Sea Water low in nutrients

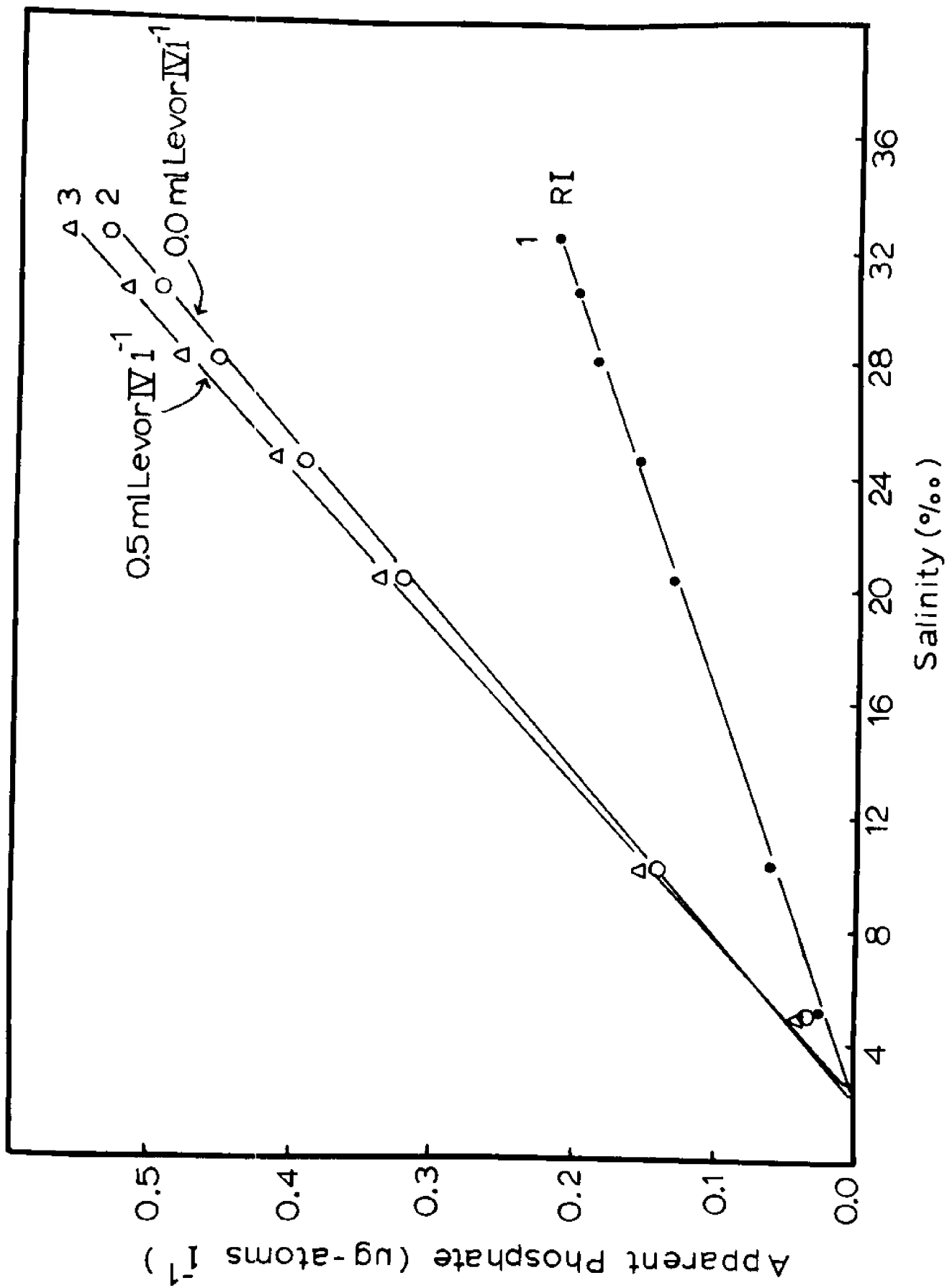


Figure 1. Plot of apparent phosphate concentrations at different salinities under varying experimental conditions relative to DDW standards. Curve 1: DDW in diluent line and reagent line; Curve 2: DDW in diluent line and normal reagents in reagent line; Curve 3: 0.5 ml Levor IV 1⁻¹ in diluent line and normal reagents in reagent line.

replaced with distilled water and not both the mixed reagent and diluent lines, then the Levor IV in the diluent reacts with the seawater producing an absorbance equivalent to a phosphate concentration of about $0.8 \mu\text{g-atoms P l}^{-1}$ at $30^{\circ}/\text{oo}$ for 2 ml l^{-1} Levor IV (Fig. 2). If a concentration of Levor IV above 1.0 ml l^{-1} is used, then the seawater blank could appear to be greater than the sample (Fig. 2). In addition the shape of the apparent phosphate-salinity curve becomes non-linear above 2.0 ml l^{-1} of Levor IV. Obviously then Levor IV should not be used in the diluent when determining the seawater blank or the index of refraction correction.

The high values of apparent phosphate due to Levor IV -seawater reactions are reduced with the addition of the mixed reagent due to its acidic nature. However, the apparent phosphate still increases when Levor IV is present. Fig. 1 illustrates the differences in apparent phosphate concentrations at different salinities between using a diluent without (curve 2) or with Levor IV (curve 3), relative to DDW standards with normal reagents in the reagent line. Gordon (19) found a similar effect with Levor IV using the hydrazine phosphate method of Atlas et al. (8).

Technicon suggests using a concentration of $2 \text{ ml Levor IV l}^{-1}$ in the diluent (4), while some other analysts use a diluent with a concentration of 0.5 ml l^{-1} Levor IV and 1.0 ml l^{-1} Wetting Agent A or no wetting agent at all. Fig. 3 illustrates the effect of variable quantities of Levor IV in the diluent on the apparent phosphate concentrations for different salinity samples under normal reagent conditions. As can be seen, the effect on sample concentration is negligible in DDW, but becomes significant as the salinity increases. For example, a difference in Levor IV concentrations of 0.5 to 4.0 ml l^{-1} can result in the difference in a sample value of up to $0.2 \mu\text{g-atom P l}^{-1}$ at a salinity of $30^{\circ}/\text{oo}$.

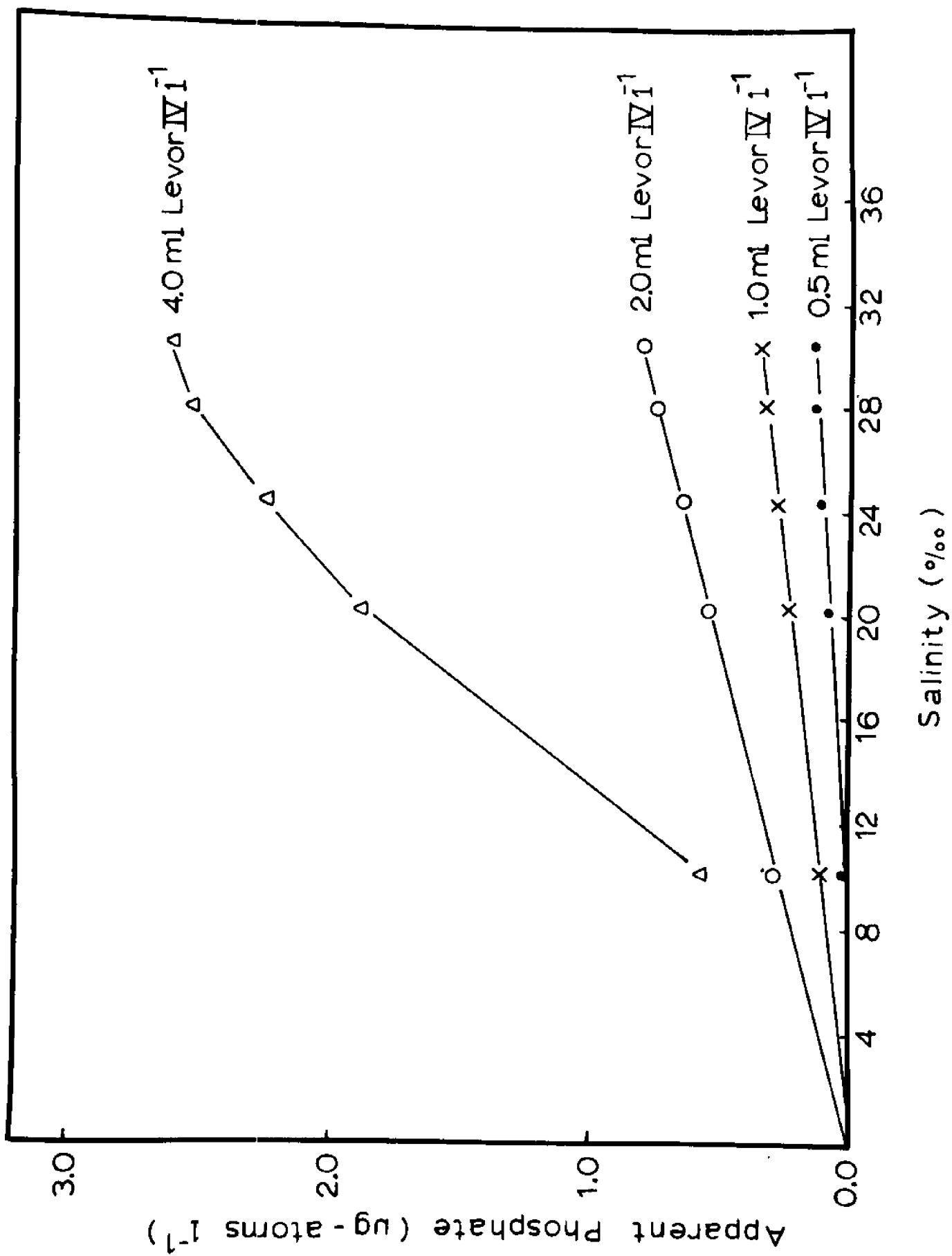


Figure 2. Plot of apparent phosphate concentration at different salinities using DDW only in the reagent line and various concentrations of Levor IV in the diluent line.

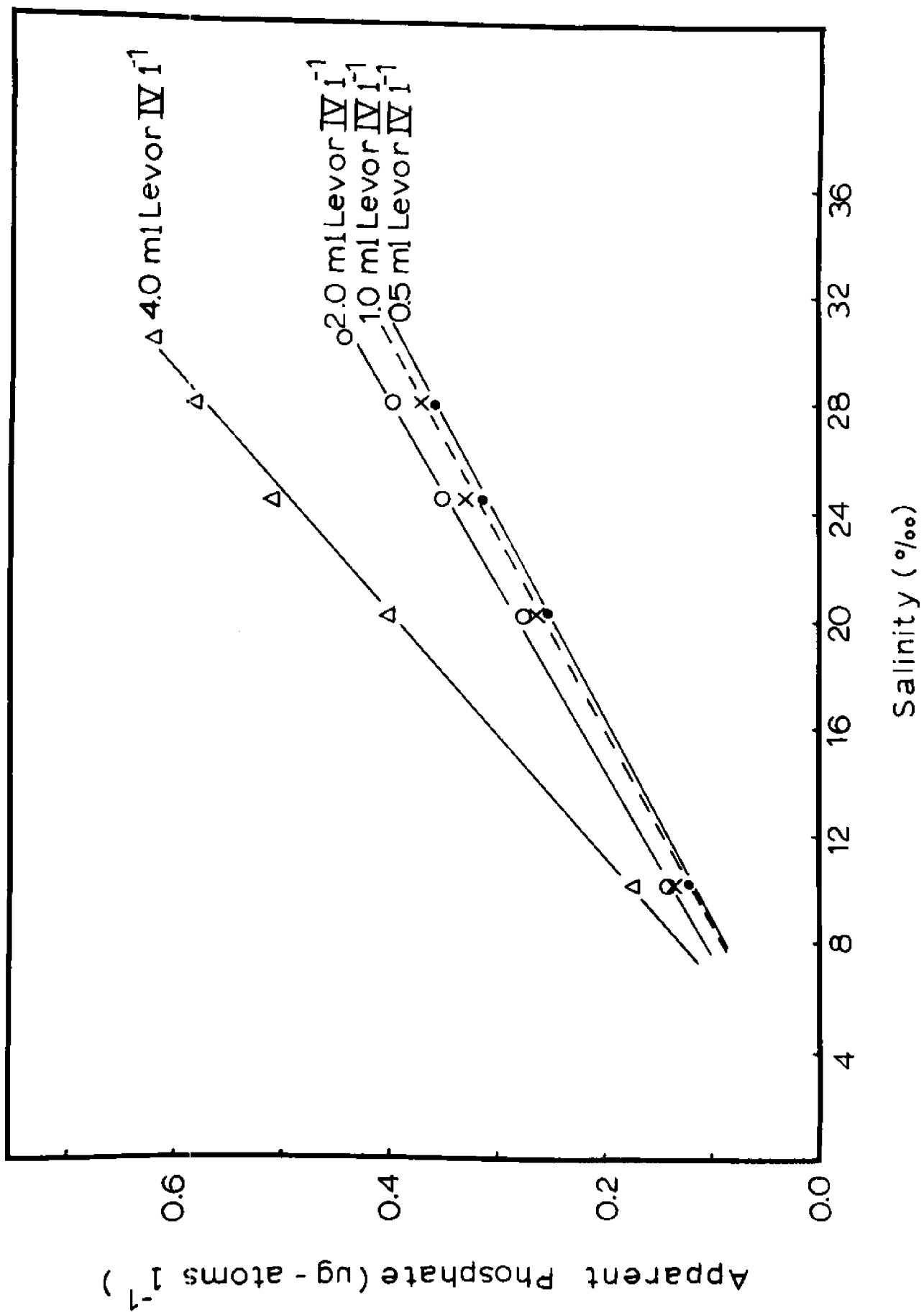


Figure 3. Plot of apparent phosphate concentration at different salinities using normal reagents in the reagent line and various concentrations of Levor IV in the diluent line.

Finally, standards prepared in ASW resulted in the same apparent phosphate concentration as standards prepared in DDW when run with a diluent containing only 0.5 ml Levor IV and 1.0 ml Wetting Agent A.

Reactive Silicate

The method for silicate analysis involves the formation of a silicomolybdate-blue complex, using stannous chloride, metol-sulfite, or ascorbic acid as reducing agents (20, 13, 2). A salt error has been recognized, resulting in about a 5% reduction of the apparent silicate concentration at a salinity of 35⁰/oo. A summary of these methods and their corrections are given in Table II. A detailed discussion of these problems is presented by Atlas et al. (8).

We determined a salt effect for the Technicon method (2) by analyzing 5 $\mu\text{g-atoms Si l}^{-1}$ standard additions to DDW and ASW as well as water ranging in salinity from 5-31⁰/oo. This was shown to be linear with salinity (Fig. 4); the corrected silicate = $(1 + 0.00129 S^0/\text{oo})(\text{observed silicate})$. These corrections were determined using a STD CAL setting of 8.00 with a full scale value of 23 $\mu\text{g-atoms Si l}^{-1}$.

A refractive index correction relative to DDW standards was also determined. This correction in $\mu\text{g-atoms l}^{-1}$ was found to be $\text{RI}(\text{SiO}_4) = 0.012 (S^0/\text{oo})$ under the above analysis conditions. We found no difference between determining this correction with DDW replacing only the molybdate reagent or all of the reagents.

No effect due to Levor IV was found. This is due to the fact that the concentration of Levor IV relative to the amount of seawater in the reaction mixture is lower and the pH is lower than for the phosphate method.

When ASW was used to prepare standards the apparent silicate concentration was 10 to 20% lower than those prepared in DDW. Therefore, it is suggested that for routine analyses standards be made up in DDW (and both a salt correction and

Table II. Selected salinity-nutrient correction procedures for automated analysis of reactive silicate in estuarine and sea water.

Analytical Method	Sample Type	Correction Type	Blank or Wash	Standards	Correction Comments	Reference
Silicomolybdate blue (metolsulfite), (21)	Fresh or Salt	C	DDW ²	DDW	Salt error: 5% at 35 ⁰ /oo; corrected optical density = optical density · (1 + 0.0028 Cl ⁰ /oo).	(20)
Silicomolybdate blue (SnCl ₂)	Salt	RI or C ¹	ASW ³ or 0.075% w/v NaHCO ₃	NSW ⁴	Recognizes salt effect, does not identify degree.	(13)
Silicomolybdate blue (SnCl ₂), (22)	Salt	RI or C	DDW	ASW or NSW	Slight salt error, no corrections given	(5)
Silicomolybdate blue (SnCl ₂), (22)	Salt	RI or C	ASW	ASW	DDW used for blank check, run blank on ASW; no wetting agent.	(7)
Silicomolybdate blue (SnCl ₂), (13)	Salt	RI or C	DDW(?)	ASW	Salt error for 0-50 µg-atom Si l ⁻¹ range: corr ⁰ value = (obs. value) · (1 + .000865/oo; no wetting agent.	(8)
Silicomolybdate blue (SnCl ₂), (13)	Salt	RI or C	DDW	ASW	Mixed standards in ASW, identifies degree of refractive index error, but does not routinely correct for it.	(12)
Silicomolybdate blue (metolsulfite), (20)	Salt	C	DDW	DDW	Salt error: corr. value = (obs. value) (1 + .0015 S ⁰ /oo); no wetting agent.	(10)
Silicomolybdate blue (ascorbic acid)	Fresh or Salt	RI or C	DDW	ASW	Blank reading for S ⁰ /oo of interest with DDW in reagent lines; wetting agent, Levor IV.	(2)
Silicomolybdate blue (ascorbic acid), (2)	Salt	none	DDW	ASW	No corrections mentioned; wetting agent, Levor IV.	(14)

1. RI - Refractive Index Correction
- C - Chemical Interference
2. DDW - Deionized Distilled Water

3. ASW - Artificial Sea Water
4. NSW - Natural Sea Water low in nutrients

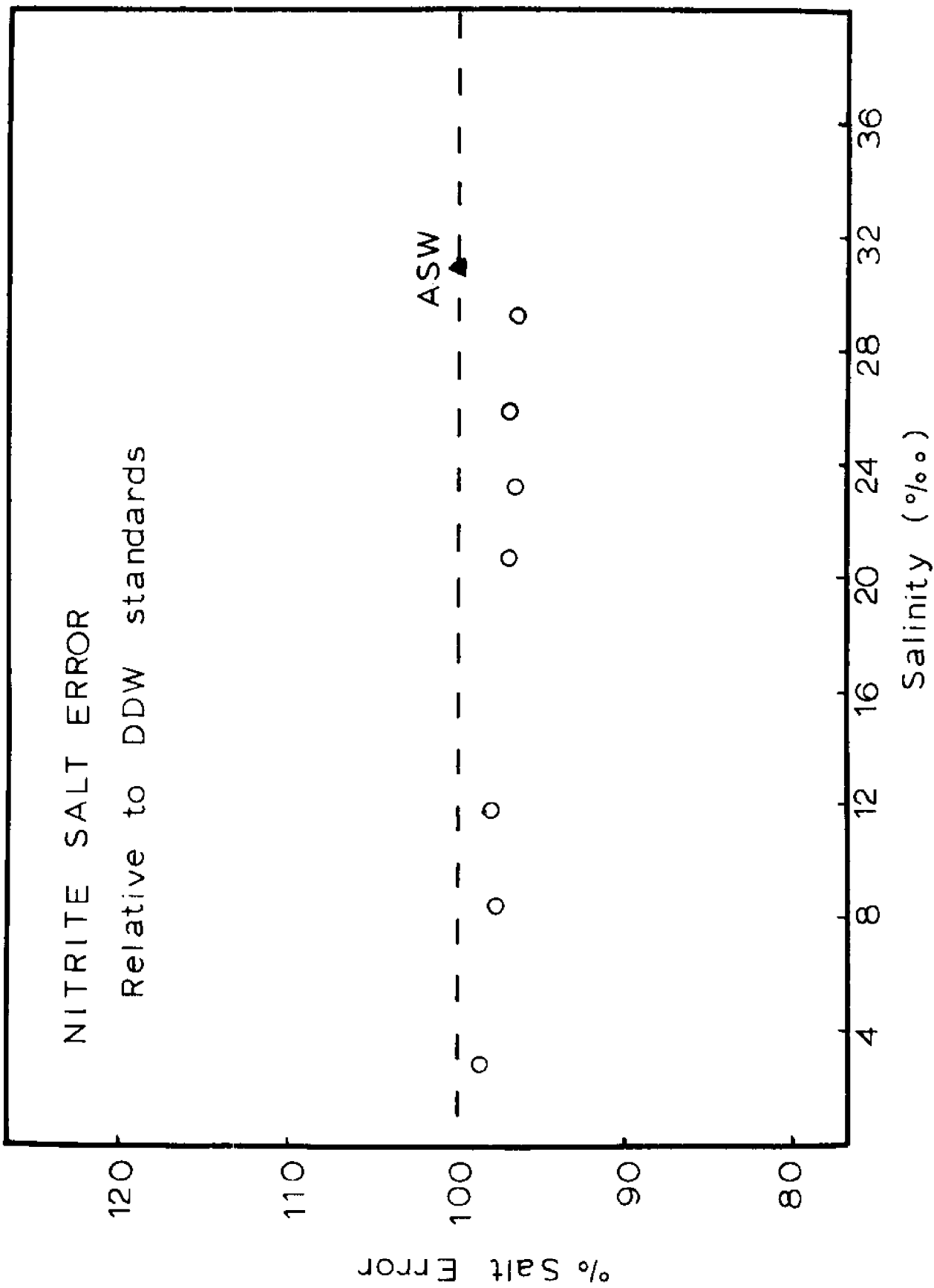


Figure 5. Plot of percent recovery or apparent nitrite relative to DDW standards (percent salt error) at different salinities for the nitrite method (24) based on 1 $\mu\text{g-atom NO}_2$ 1⁻¹ standard additions.

a refractive index correction applied), or natural seawater (NSW) and not ASW of this formula, as it will lead to erroneous correction values. An ASW of a composition closer to that of seawater will give results similar to NSW (17).

Nitrite

All the nitrite analysis methods use some modification of the Griess-Ilosvay method as described for use with seawater by Bendschneider and Robinson (23). This method is reported by most authors to be free of any salt error, as summarized in Table III. We found, however, that $1 \mu\text{g-atom NO}_2 \text{ l}^{-1}$ standard additions to NSW samples of various salinities were on the average $2.5 \pm 0.7\%$ lower than DDW with the same standard addition using the Technicon method (24). The effect was not linear with salinity (Fig. 5). Consequently for very precise work this slight salinity depression on apparent nitrite concentration should be checked out by the analyst.

Since nitrite analyses are often run at a high STD CAL setting, the index of refraction correction must be made if standards are made with DDW and DDW is used to set baseline. We found this correction in $\mu\text{g-atoms l}^{-1}$ to be about $\text{RI}(\text{NO}_2) = 0.00193 (S^0/\text{oo})$ at a STD CAL = 7.70 for a full scale value of $2 \mu\text{g-atom l}^{-1}$. This correction should be determined for a known salinity sample by replacing the mixed reagent with a 10% phosphoric acid solution, as using DDW alone will result in a RI correction that is too high.

Finally, ASW standards did not vary significantly from the DDW standards suggesting that whatever is present in seawater that depressed the absorbance may not be present in ASW. Consequently, for very precise work the same salt corrections must be applied to ASW standards as would be applied to DDW standards, since they both differ from NSW standards.

Table III. Selected salinity-nutrient correction procedures for the automated analysis of nitrite in estuarine and sea water.

Analytical Method	Sample Type	Correction Type	Blank or Wash	Standards	Correction Comments	Reference
Griess-Ilosvay (23)	Salt	RI or C ¹	ASW or 0.075% w/v NaHCO ₃	NSW ⁴	Recognize salt effect; does not mention degree; wetting agent used.	(13)
"	Salt	none	ASW ³	ASW	Used nitrate system without reduction column; wetting agent used.	(7)
"	Salt	none	DDW ²	DDW	No corrections mentioned.	(5)
"	Salt	none	DDW	ASW	Used nitrate system without reduction column (no wetting agent mentioned).	(12)
"	Salt	none	DDW	DDW	Used nitrate system without reduction column; no wetting agent.	(10)
"	Fresh or Salt	RI	Not Specif.	Not Specif.	Run samples without color-forming reagent and subtract.	(16)
"	Salt	RI	DDW	DDW	Run blank on sample with DDW in reagent lines.	(24)
"	Salt	RI or C	DDW	ASW	Uses nitrate system without column; wetting agent used.	(15)

1. RI - Refractive Index correction,

C - Chemical Interference

2. DDW - Deionized Distilled Water

3. ASW - Artificial Sea Water

4. NSW - Natural Sea Water Low in nutrients

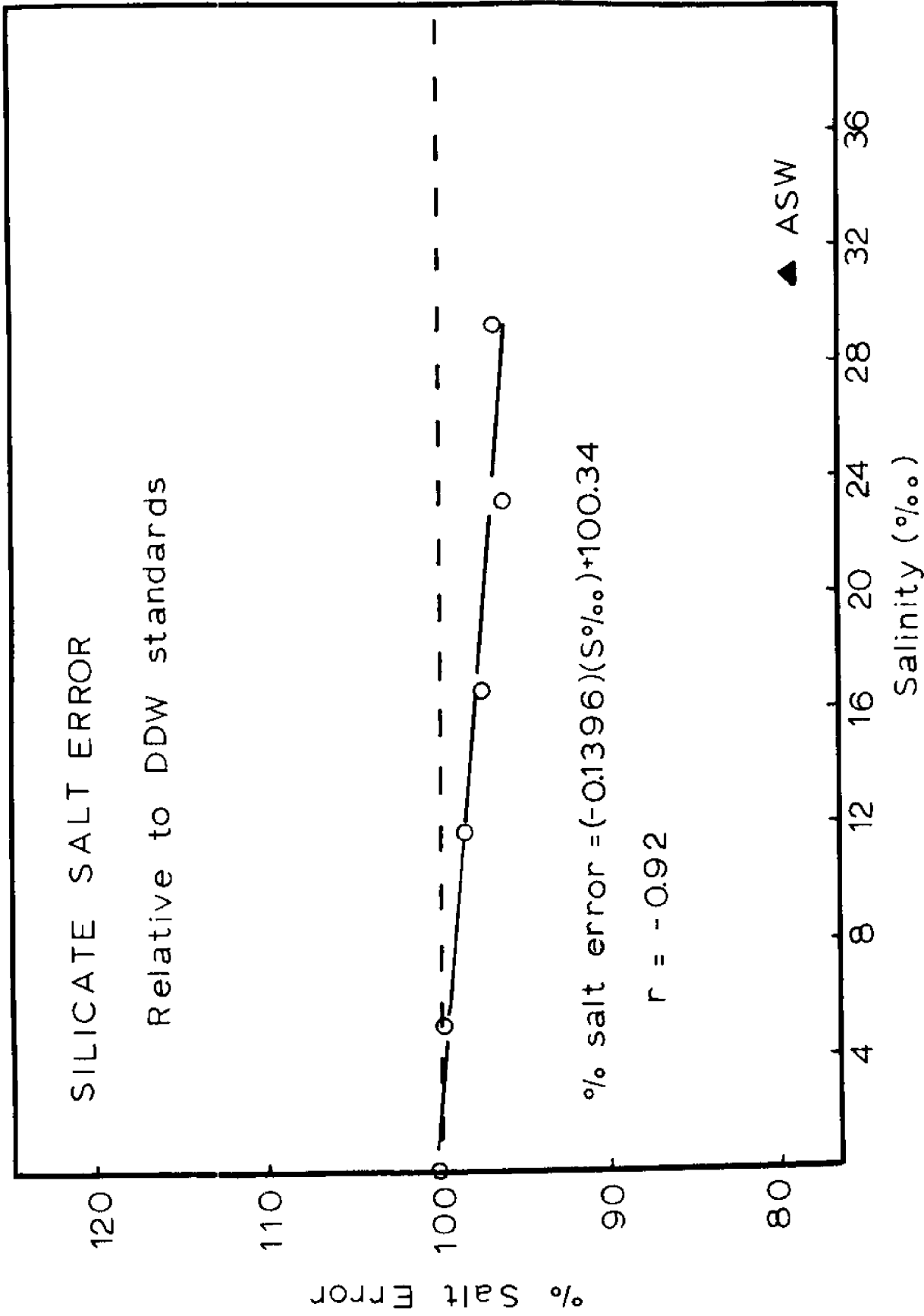


Figure 4. Plot of percent recovery or apparent silicate relative to DDW standards (percent salt error) at different salinities for the silicate method (2) based on 5 $\mu\text{g-}$ atoms Si l^{-1} standard additions.

Nitrate

Nearly all nitrate in seawater methods utilize a copper-cadmium reduction column to reduce the nitrate to nitrite. The resulting nitrite is then analyzed using the Greiss-Ilosvay method just described. Minor variations in the method include the use of EDTA instead of NH_4Cl to activate the column, although most recent workers are using NH_4Cl for this purpose (Table IV).

A salt error has been recognized by some workers and is a function of the buffer used: for EDTA the apparent nitrate concentration relative to DDW standards decreases by 16 to 19% (1); for NH_4Cl the apparent nitrate concentration relative to DDW standards increases by about 10% (Fig. 6).

We determined the salt effect for the Technicon NH_4Cl -buffer method (26) by analyzing 5 μg -atoms NO_3^- standard additions to DDW, ASW, and NSW of various salinities. This effect may or may not be linear with salinity depending on the age and condition of the reduction column. For a new column this effect is linear; the corrected nitrate = $(1.00 - 0.00295 S^0/\text{oo})(\text{observed nitrate})$ at a STD CAL of 8.00 and a full scale value of 7.6. Consequently, it is important frequently to check this correction factor on the column and chemical system being used since this correction will vary depending on the age of the column.

The refractive index correction in μg -atoms l^{-1} was found to be $\text{RI}(\text{NO}_3^-) = 0.0045 (S^0/\text{oo})$ at a STD CAL of 8.00. This correction should be determined for known salinity samples by replacing the mixed reagent with a 10% phosphoric acid solution, as using DDW alone will give a RI correction that is too high. This correction is also dependent on the condition of the column and the full scale range. No effect due to the wetting agent Brij-35 was found.

Finally, standards made up in ASW showed an increase in apparent nitrate which was equivalent to that found for a seawater of about 35⁰/oo. Consequently, DDW or ASW standards are acceptable, if the appropriate corrections are made.

Table IV. Selected salinity-nutrient correction procedures for the automated analysis of nitrate in estuarine and sea water.

Analytical Method	Sample Type	Correction Type	Blank or Wash	Standards	Correction Comments	Reference
Cd reduction (EDTA)	Fresh or Salt	C	DDW ²	DDW or NSW	Salt error: about 16 to 19% decrease in apparent NO ₃ relative to DDW standards; no wetting agent.	(1)
Cu-Cd reduction (NH ₄ Cl)	Salt	RI or C ¹	ASW ³ or DDW or 0.075% w/v NaHCO ₃	NSW	Recognizes salt effect, does not mention degree; wetting agent used.	(13)
Cu-Cd reduction (NH ₄ Cl) (13)	Salt	RI or C	ASW	ASW	Salt error: negligible at high range; no corrections necessary; wetting agent used.	(7,8)
Cu-Cd reduction (NH ₄ Cl) (25)	Fresh or Salt	RI or C	ASW then DDW	ASW	Standardize with ASW in wash and standards, then DDW wash for samples; wetting agent used.	(26)
Cu-Cd reduction (NH ₄ Cl) (25)	Salt	C	DDW	ASW or NSW	Salt effect causes no significant error, blanks must be run on ASW.	(5)
UV irradiation (27)	Fresh or Salt	RI or C or reagent	not specifi.	not specifi.	Run samples without color-forming reagent and subtract.	(16)
Cu-Cd reduction (NH ₄ Cl) (13)	Salt	RI	DDW	ASW	Mixed standards in ASW. Identifies degree of RI error but does not routinely correct for it.	(12)
Cd reduction (EDTA), (1)	Salt	none	DDW	DDW	No corrections mentioned; no wetting agent.	(14)
Cu-Cd reduction (NH ₄ Cl) (13)	Salt	none	DDW	ASW	No corrections mentioned; wetting agent used.	(10)
Cu-Cd reduction (NH ₄ Cl)		RI or C	DDW	ASW	No mention of variable response to salinity; wetting agent used.	(15)
Cu-Cd reduction (NH ₄ Cl)	Fresh or Salt	RI or C	DDW	ASW	Wetting agent used.	(17)

1. RI-Reactive Index Correction
 C-Chemical interference
 2. DDW-Deionized Distilled Water
 3. ASW - Artificial Sea Water
 4. NSW - Natural Sea Water low in nutrients

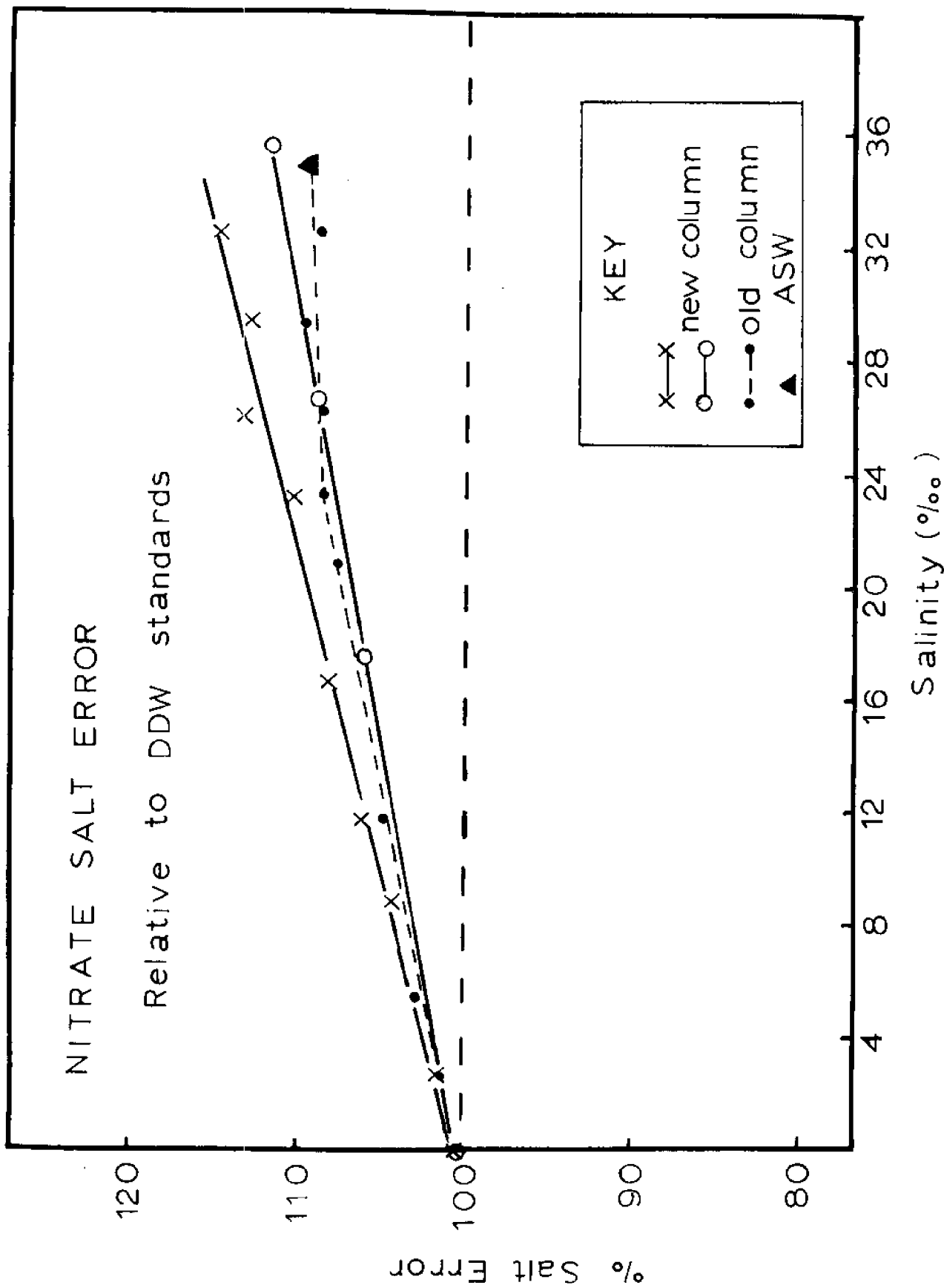


Figure 6. Plot of percent recovery or apparent nitrate relative to DDW standards (percent salt error) at different salinities for the nitrate method (26) showing the effect of column condition on the apparent nitrate. Standard additions of $5 \mu\text{g-atoms NO}_3 \text{ l}^{-1}$ were used.

Ammonia

Most of the automated ammonia methods utilize the Berthelot reaction in which ammonia reacts with sodium phenate and hypochlorite to produce an indophenol-blue compound (28, 5, 29, 17). Recent changes in the method, reported to increase stability, include changes in the buffer and the use of sodium isocyanurate (sodium dichloro-S-triazine-2,4,6-(1H, 3H, 5H)-trione) as the hypochlorite donor instead of sodium hypochlorite (Chlorox) commonly used (32, 33). A summary of all these methods is given in Table V. We are presently using the O'Connor and Miloski (33) method described by Adamski (34) and will report our findings on this method.

Grasshoff and Johannsen (32) reported finding an unavoidable interference from salinity due to a change in pH of the reaction solution at different salinities. They found an increase in sensitivity at intermediate salinities and almost no increase at normal seawater salinities.

We determined the salt effect for the O'Connor and Miloski (33) method by analyzing 2.5 $\mu\text{g-atoms NH}_4^+ \text{l}^{-1}$ standard additions to the various salinity waters previously described. We found an increase in sensitivity relative to DDW standards (nonlinear relationship) up to about 15 to 18‰ salinity and a decrease at higher salinities (Fig. 7). The salt error was approximately linear between about 6‰ and 35‰ with the corrected ammonia = $(0.0157S^{\circ}/\text{oo} + 0.781)(\text{observed ammonia})$ for this range. These corrections were determined with a STD CAL setting of 8.00 and a full scale value of 5.4 $\mu\text{g-atoms NH}_4^+ \text{l}^{-1}$. We found the amount of salt error to vary as a function of the age of the buffer and isocyanurate reagents. If the salinity of samples to be run is between 0‰ and 10‰ the analyst should carefully determine the increase in sensitivity in this range. We found a maximum of about 15% increase at 5 to 7‰ (Fig. 7).

Table V. Selected salinity-nutrient correction procedures for the automated analysis of ammonia in estuarine and sea water.

Analytical Method	Sample Type	Correction Type	Blank or Wash	Standards	Correction Comments	Reference
Indophenol-blue (hypochlorite), (30)	Fresh or Salt	RI or C	ASW	ASW	Seawater samples 22% more sensitive than fresh water.	(28)
Indophenol-blue (Hypochlorite), (30)	Salt	RI or C ¹	DDW	ASW	No salt error if standards prepared in ASW.	(5)
Indophenol-blue (isocyanurate), (31)	Salt	RI or C	DDW	not specif.	Interference of salinity is due to pH change in reaction solution at diff. S/00's, error=+7.5 - 2%; correction curve given.	(32)
Indophenol-blue	Fresh or Salt	RI or C or reagent	not specif.	not specif.	Run samples without color forming reagent and subtract.	(16)
Indophenol-blue (hypochlorite)	Fresh or Salt	RI	DDW	DDW	Run blank on sample with DDW in reagent lines; wetting agent used.	(29)
Indophenol-blue (hypochlorite), (28)	Salt	RI or C	DDW	ASW	No correction mentioned; wetting agent used.	(14)
Indophenol-blue (hypochlorite)	Fresh or Salt	RI or C	DDW	ASW	No corrections mentioned.	(15)
Indophenol-blue (isocyanurate), (33)	Salt	None	DDW	not specif.	No salt correction mentioned; method used for Kjeldahl nitrogen.	(34)
Indophenol-blue (hypochlorite)	Fresh or Salt	RI or C	DDW	ASW	No correction mentioned; wetting agent used.	(17)

1. RI - Refractive Index correction

C - Chemical Interference

2. DDW - Deionized Distilled Water

3. ASW - Artificial Sea Water

4. NSW - Natural Sea Water

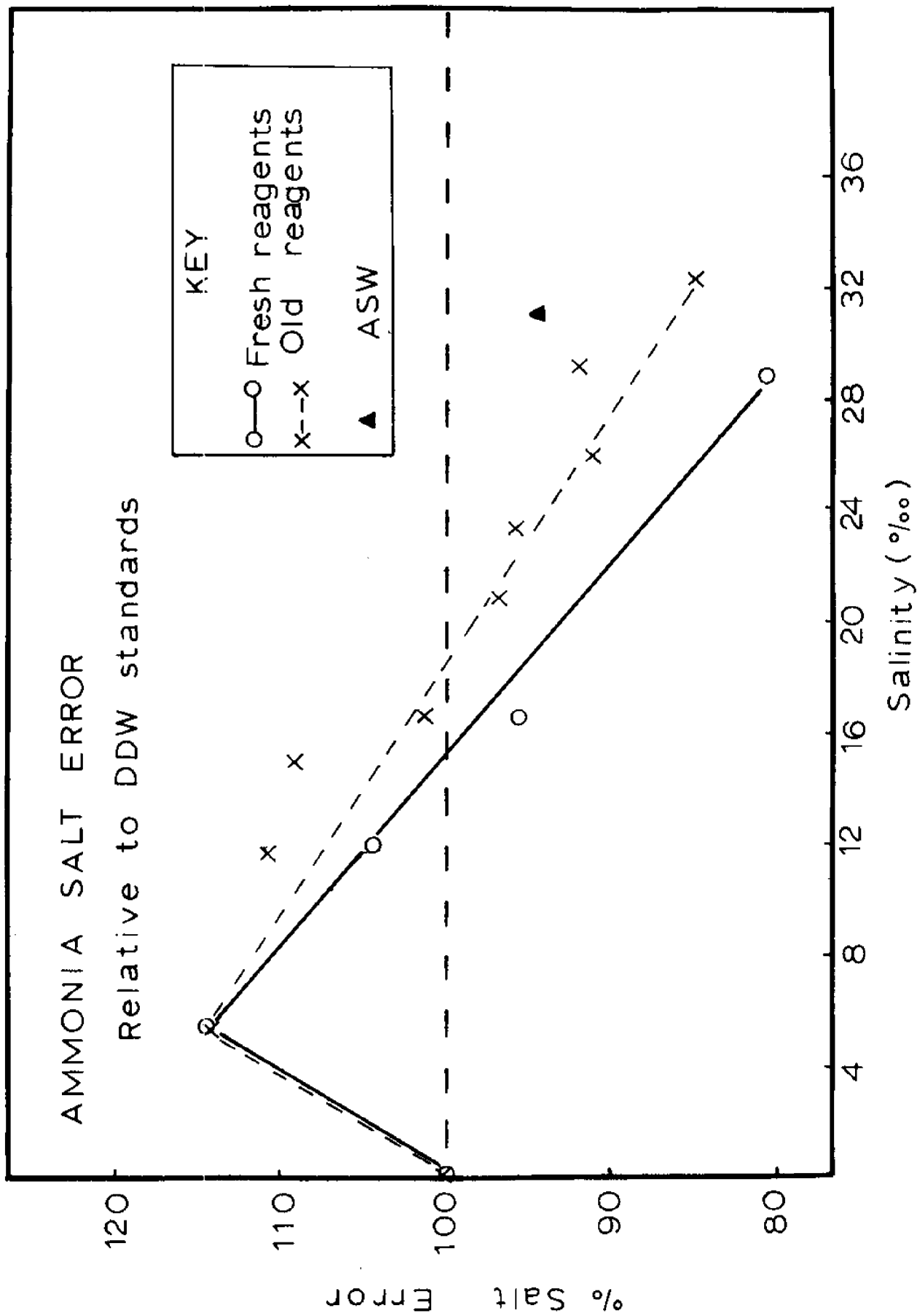


Figure 7. Plot of percent recovery or apparent ammonia relative to DDW standards (percent salt error) at different salinities for the ammonia method (33) showing the difference in curve position as the old reagents were replaced with new ones. Both curves were based on 2.5 μg -atoms NH_4^+ 1-l standard additions.

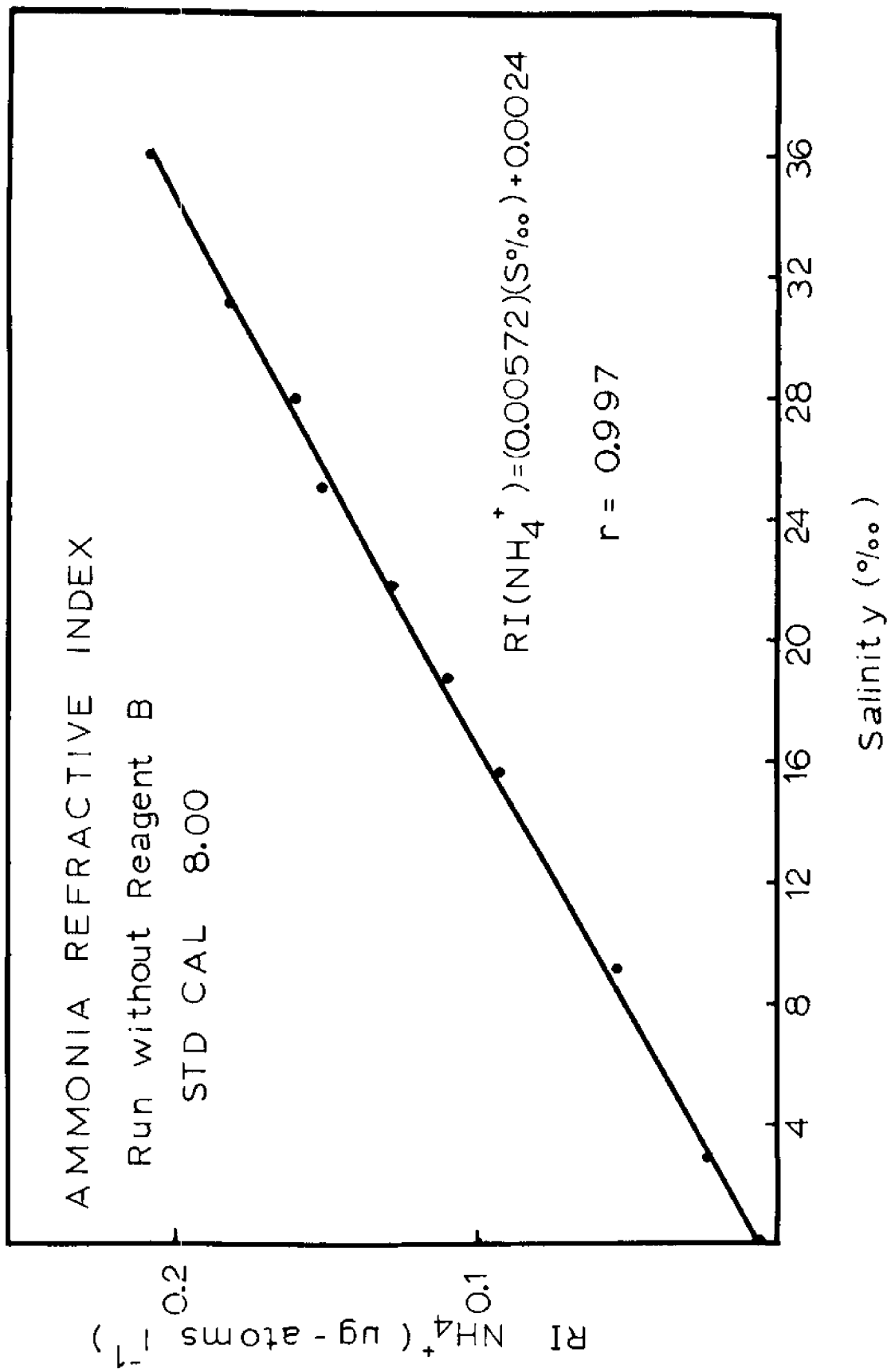


Figure 8. Plot of the apparent ammonia concentration as a fraction of salinity due to the difference in refractive index of the sea water and the DDW wash. This was determined with the reagent containing sodium hydroxide and isocyanurate replaced with DDW (33).

The refractive index correction in $\mu\text{g-atoms l}^{-1}$ relative to DDW standards was found to be $\text{RI}(\text{NH}_4^+) = 0.0057 (S^0/\text{oo})$ under the previously mentioned conditions without reagent B (sodium hydroxide and isocyanurate)(Fig. 8).

Standards prepared in ASW showed a decrease in apparent ammonia which was equivalent to that found for a seawater of only $30^0/\text{oo}$. Consequently, if a system is calibrated with ASW standards and if natural seawater samples of $35^0/\text{oo}$ salinity are analyzed, the final values will be about 6% too low. This decrease is apparently due to a deficiency in Technicon ASW. We suggest for samples with 10 to $35^0/\text{oo}$ salinity range that standards be prepared with two different salinities encompassing the range of salinities expected and a regression determined to correct the sample values.

Summary of Correction Procedures

We suggest that one of the following two methods be used depending on the salinity range of the samples being analyzed. In both methods DDW is used to set the baseline and as a wash between samples.

Method 1: Open ocean or narrow salinity range samples. Prepare standards with filtered low nutrient natural seawater (NSW). Technicon artificial seawater (ASW) may be used instead of NSW only for nitrate standards; it will lead to erroneous values in all the other chemistries. APHA (17) artificial seawater may be acceptable for other chemistries. Prepare standards in volumetric flasks using precision small volume auto pipets; in this way the standard addition does not significantly alter the salinity. Silicate seawater standards must be prepared in polypropylene volumetrics to avoid leaching of silica from the glass.

Analyze standards using normal reagents and run a blank on the water used to make the standards. Subtract the blank from the standards and determine the

full scale value for the analysis = $[(\text{conc. of std.}) \div (\text{peak height of std.} - \text{peak height of blank})] \cdot 100$. This corrects for both the salt error in the method and the wetting agent error (for phosphate) at the sample salinity.

Determine the refractive index correction for the samples by analyzing representative samples with only deionized water in the reagent and diluent lines or as described under the individual analysis sections. These values are summarized in Table VI. The concentration of the nutrient in the samples is then determined: $\text{corrected concentration} = [(\text{peak height of sample}) \cdot (\text{full scale value}) \div 100] - [\text{Refractive Index corr. in conc. units}]$.

Method 2: Estuarine or variable salinity samples. For samples with a wide range of salinities we suggest that routine standards be prepared in DDW and that a separately determined salt error correction factor be applied to the observed concentration to obtain the corrected value. The type and extent of these salt error corrections were discussed under the individual nutrient sections and do not appear necessary for phosphate.

We suggest the following procedure to determine the salt error correction factor: Dilute low nutrient NSW with DDW to make a range of salinities. Prepare standard additions as described in Method 1 above, as well as DDW standards, using a precision small volume auto pipet. Analyze the DDW standards and each dilution as well as the dilutions with the standard additions.

Determine the difference in concentrations between the seawater dilutions and those with the standard additions. Calculate the change in apparent nutrient concentration relative to the DDW standards as a function of salinity. Finally, to obtain the corrected concentration, subtract the appropriate refractive index correction, as described in Method 1, from the observed apparent concentration and multiply by the salt error factor.

Table VI. Summary of Refractive Index (RI) corrections for methods discussed in text.

Method and Reference	STD CAL	Full Scale Value ($\mu\text{g-atoms l}^{-1}$)	RI correction $f(S^0/\text{oo})^{**}$ ($\mu\text{g-atoms l}^{-1}$)
Phosphate (4)	8.00	5	$0.006(S^0/\text{oo})^*$
Silicate (2)	8.00	23	$0.012(S^0/\text{oo})$
Nitrite (24)	7.70	2	$0.0019 (S^0/\text{oo})$
Nitrate (26)	8.00	7.6	$0.0045 (S^0/\text{oo})$
Ammonia (33)	8.00	5	$0.0057 (S^0/\text{oo})$

*Includes effect of Levor IV at 0.5 ml l^{-1} concentration in the diluent.

**These values can be approximated at different STD CAL settings if the dilution ratios remain the same. Multiply f by the ratio: full scale absorbance at STD CAL give above \div full scale absorbance at new STD CAL.

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