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pH and buffering capacity problems involved in the determination of ammonia in saline water using the indophenol blue spectrophotometric method

Su-Cheng Pai*, Yung-Jin Tsau, Tai-I Yang

Institute of Oceanography, National Taiwan University, Taipei, Taiwan, ROC

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Abstract

Magnesium ions in brackish water or sea water complex with the citrate reagent (CIT) and thus interfere with the indophenol blue spectrophotometric method for ammonia measurement by altering the final pH of the color solution. The complex system, possibly $Mg(CIT)^- + OH^- \Leftrightarrow Mg(OH)(CIT)^{2-}$, shows buffer characteristics and can be identified by acid–base titration. A maximum capacity of ca. 40 μeq pH^{-1} ml^{-1} was found at pH 10.2 when $[Mg^{2+}]$ was 0.045 M. To compensate for this capacity, extra alkali reagent should be added to sea water samples for the optimal IPB color reaction. For samples with a wide salinity range, the results still need to be corrected empirically either for pH, $[Mg^{2+}]$ or salinity.

Keywords: Ammonia; Indophenol blue; pH; Buffering capacity

1. Introduction

The 5th ICES intercomparison exercise for nutrients in sea water was held in 1995 and the results of ammonia determination were reported by Aminot et al. [1] in 1997. They concluded that the indophenol blue (IPB) method [2] is still the most widely adopted for ammonia, but difficulties exist in the oceanographic communities, because the results for three blind samples appeared somewhat erratic. Through a questionnaire, the procedures of 64 participating laboratories were split into different categories (e.g. reagent strengths, contamination, temperature effect, etc.) and problems were diagnosed systematically. Among these, problems on the buffering capacity or the 'pH-shift' phenomenon remain unclear.

The 'pH-shift' in sea water analysis using the IPB method has been demonstrated in many earlier works [3,4]. It is well known that the optimal pH for the IPB reaction should be around 10.5 for freshwater [5], but when the same reagents are added to a sea water sample, the final pH drops to as low as 9.8. The shift to a lower pH results in a lower sensitivity (~12% less absorbance) and a slower reaction rate [6,7]. Some investigators believed that the interference might come from the salt matrix and therefore, termed it the 'salt error', but Mantoura and Woodward [8] indicated that the 'salt error' might be induced by alkalinity difference rather than ionic strength. They reported that by adding extra phenate reagent, the salt error could be minimized to ca. 8% in terms of sensitivity. The addition of other buffers has also been suggested,

* Corresponding author. Tel.: +886-223627358; fax: +886-223632912.
E-mail address: scpai@ccms.ntu.edu.tw (S.-C. Pai).

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including the K_2CO_3–Na_2HPO_4 system [9], the borate system [10], and EDTA, tartrate [1], etc. In our experience none of those buffers can provide satisfactory improvement. In fact, Aminot et al. [1] have advised that adding more buffers "might increase the complexity rather than solve the problem".

Apparently, there is an unidentified buffering system in the sea water sample, and its strength is stronger than the capacity of all existing IPB reagents. The puzzle becomes: 'which system?'. If one examines the major components of sea water, the carbonate system ($pK_2 = 10.3$ in freshwater and $pK_2' = 9.1$ in sea water at 25°C) [11] is the prime suspect. However, the natural concentration of carbonate (~2 mF1) is too low to be significant. In our early experiments, a variety of salt solutions (NaCl, Na_2SO_4, KCl, $MgCl_2$, $CaCl_2$, etc.) were tested individually in the IPB method. The result was surprising: the pH shift occurred only when Mg^{2+} was present. Theoretically, Mg^{2+} does not act as a buffer, but it may do so if it is complexed with some ligands (most likely citrate). To identify the existence of such a buffering system, the acid–base titration technique was applied.

2. Experimental

2.1. Reagents

All reagents were prepared from Merck GR grade chemicals (except for bleach water) and dissolved in fresh Milli-Q water. The recipe used in the following experiment was similar to that suggested by Solórzano [2].

1. Phenol reagent: dissolve 10 g of phenol in 100 ml of 95% ethanol.
2. Nitroprusside reagent: dissolve 1 g of sodium nitroprusside in 100 ml of water.
3. Citrate reagent: dissolve 100 g of trisodium citrate in 250 ml of water.
4. Sodium hydroxide 0.5 M: dissolve ca. 5 g of NaOH in 250 ml of water.
5. Hypochlorite reagent: commercial bleach water.
6. Mixed oxidizing reagent: mix 50 ml of citrate reagent, 50 ml of sodium hydroxide and 25 ml of bleach water.

2.2. Procedure for titration

Test samples were prepared as shown in Table 1. Before titration, the pH of each sample was adjusted to 7–8 by adding concentration HCl dropwise. The solution was titrated with 1.0 M NaOH using a Radiometer (Copenhagen) ABU80 Autoburette and pH was recorded by a Radiometer PHM85 Precision pH meter.

2.3. Calculation of buffering capacity

The buffering capacity (β) was defined as: in the alkaline direction, the amount of hydroxide (μeq) required to raise one pH unit per unit volume (ml).

$$\beta\ (\mu\text{eq pH}^{-1}\ \text{ml}^{-1}) = \frac{\Delta V_b \times C}{\Delta\text{pH}/(V_s + V_a + V_b)} \times 10^3$$

Table 1
List of samples for the titration test and their concentrations of phenol, magnesium and citrate ions

No.	Test sample	[Phenol] (mF)	[Mg^{2+}] (mM)	[Citrate] (mF)
1	59 ml QW (Milli-Q water)	0	0	0
2	50 ml SW (sea water, $S = 34.1$) + 9 ml QW	0	45	0
3	57 ml QW + 2 ml phenol reagent	36	0	0
4	50 ml SW + 2 ml phenol reagent + 7 ml QW	36	45	0
5	50 ml QW + all IPB reagents (9 ml)[a]	36	0	46
6	50 ml SW + all IPB reagents (9 ml)[a]	36	45	46
7	48 ml SW + 2 ml 1 M $NaHCO_3$ + all IPB reagent (9 ml)[a]	36	44	46
8	56 ml QW + 3 ml 0.9 M Mg^{2+}	0	46	0
9	57 ml QW + 2 ml citrate reagent	0	0	46
10	54 ml QW + 3 ml 0.9 M Mg^{2+} + 2 ml citrate reagent	0	46	46
11	52 ml QW + 3 ml 0.9 M Mg^{2+} + 2 ml citrate reagent + 2 ml phenol reagent	36	46	46

[a] Following Solózano's procedure: to sample (50 ml) is added 2 ml of phenol reagent, 2 ml of nitroprusside reagent and 5 ml of mixed oxidizing reagent to make a final volume of 59 ml.

where V_s is the initial volume of the sample (59 ml), V_a the volume of acid added to decrease the pH, V_b the accumulated volume of titrant and C titrant concentration (1 M). Accordingly, each data point on the titration curve was calculated for β and plotted on the 'β versus pH' diagram.

3. Results and discussion

3.1. Titration of sea water

The titration curves together with the buffering capacity plots for a Milli-Q water (test 1) and sea water (test 2) are shown in Fig. 1. For pure water the capacity increased sharply at pH > 11. This capacity is induced by the alkali itself and can be referred to as a "pseudo buffering capacity". For sea water, precipitation started at ca. pH 9.8. When this happened, further addition of NaOH did not increase the pH of the solution, and the capacity became infinite. A little bump at pH 9.0–9.8 can be seen on the capacity curve for sea water, which should be the contribution of carbonate and borate systems. The scale, however, is apparently $<2\,\mu eq\,pH^{-1}\,ml^{-1}$.

3.2. Titration of phenol

The phenol reagent itself is a strong buffer ($pK_a = 10$) in the IPB method. The results of tests 3 and 4 refer to phenol (final concentration 36 mF) in Milli-Q water and sea water, respectively. In a freshwater medium, the buffering capacity has a peak of ca. $20\,\mu eq\,pH^{-1}\,ml^{-1}$ at pH 10. The height is slightly lower than expected and the reason is not clear (empirically each mF ligand produces a capacity peak of ca. $0.8\,\mu eq\,pH^{-1}\,ml^{-1}$ depending on the experimental conditions). In sea water, only half of the phenate peak can be seen, because precipitation occurred above pH 9.8.

3.3. Titration of samples with all IPB reagents

A Milli-Q water and a sea water sample were treated equally with all IPB reagents (Solózano's procedure) along with the amount of HCl to bring the pH to ca. 7, and then each sample was titrated with NaOH (Fig. 1, tests 5 and 6). For freshwater, an obvious phenate peak appeared at pH 10 with a height of ca. $20\,\mu eq\,pH^{-1}\,ml^{-1}$, which was no different from the result in test 3. For the sea water sample, the maximum peak shifted to pH 10.2, with a peak height of ca.$60\,\mu eq\,ml^{-1}\,pH^{-1}$. This peak is apparently an overlapped peak composed of both phenol and an unknown system. Precipitation of sea water occurred at around pH 11. A rough estimate was made by subtracting the phenol peak from the combined peak. The net value was ca. $40\,\mu eq\,pH^{-1}\,ml^{-1}$ at pH 10.2, corresponding to ca. 50 mF of ligand in sea water. It can be neither carbonate nor borate, judging from their concentrations. A further identification was made by titrating a sea water sample to which 34 mF sodium bicarbonate and all IPB reagents had been added (test 7). The peak was found at pH 10.4 no higher than that of test 6. A buffering capacity bump at pH 9.0–9.3 can be observed at the position of the peak shoulder, which matches the $pK_2' = 9.1$ of the carbonate system. Based on the above evidence, the contribution of natural-occurring alkalinity to the capacity of the IPB method can be considered insignificant.

3.4. Titration of Mg^{2+} and citrate

The titration curve of a solution containing 46 mM Mg^{2+} (test 8) is shown in Fig. 2. As expected, Mg^{2+} does not provide any buffering capacity, but does cause a plateau above pH 9.8 on the titration curve when $Mg(OH)_2$ starts to precipitate ($pK_{sp} = 10.47$). A 46 mF citrate solution was titrated (test 9), and the capacity plot is similar to that of Milli-Q water (test 1). Although the citrate system has three pK_a values (3.13, 4.76, and 6.40), there is no capacity above pH 8. However, when these two components (Mg^{2+} and citrate) were mixed and titrated, a peak of ca. $40\,\mu eq\,pH^{-1}\,ml^{-1}$ at pH 10.2 appeared (test 10). The peak should refer to a Mg–citrate complex (46 mF), which prevents magnesium ion from precipitating between pH 9.8 and 11. Above pH 11, $Mg(OH)_2$ still precipitates. In a further test using half the amount of Mg^{2+}, the peak height decreased proportionally, and its position shifted to a slightly higher pH (ca. 10.5). It is worth noting that the concentration of citrate must exceed that of Mg^{2+} otherwise a small amount of precipitate would form. This confirms the mole ratio of the Mg–citrate complex to be 1:1.

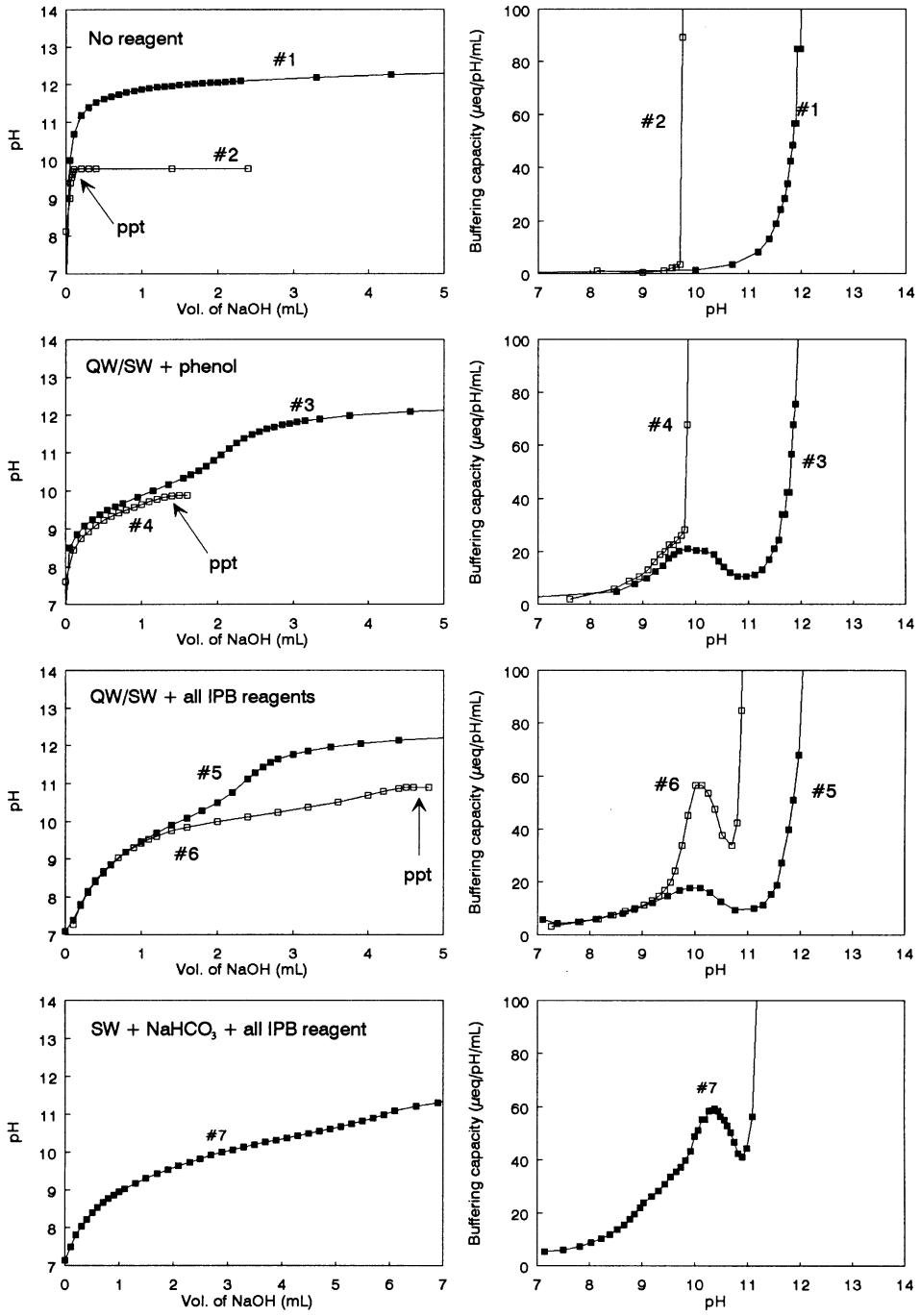

Fig. 1. Titration curves and buffering capacity plots for tests: (1) Milli-Q water; (2) sea water; (3) phenol in Milli-Q water; (4) phenol in sea water; (5) Milli-Q water with all IPB reagents; (6) sea water with all IPB reagents; (7) sea water with bicarbonate and all IPB reagents. Arrows marked with 'ppt' denote precipitation. For details on concentrations, see Table 1.

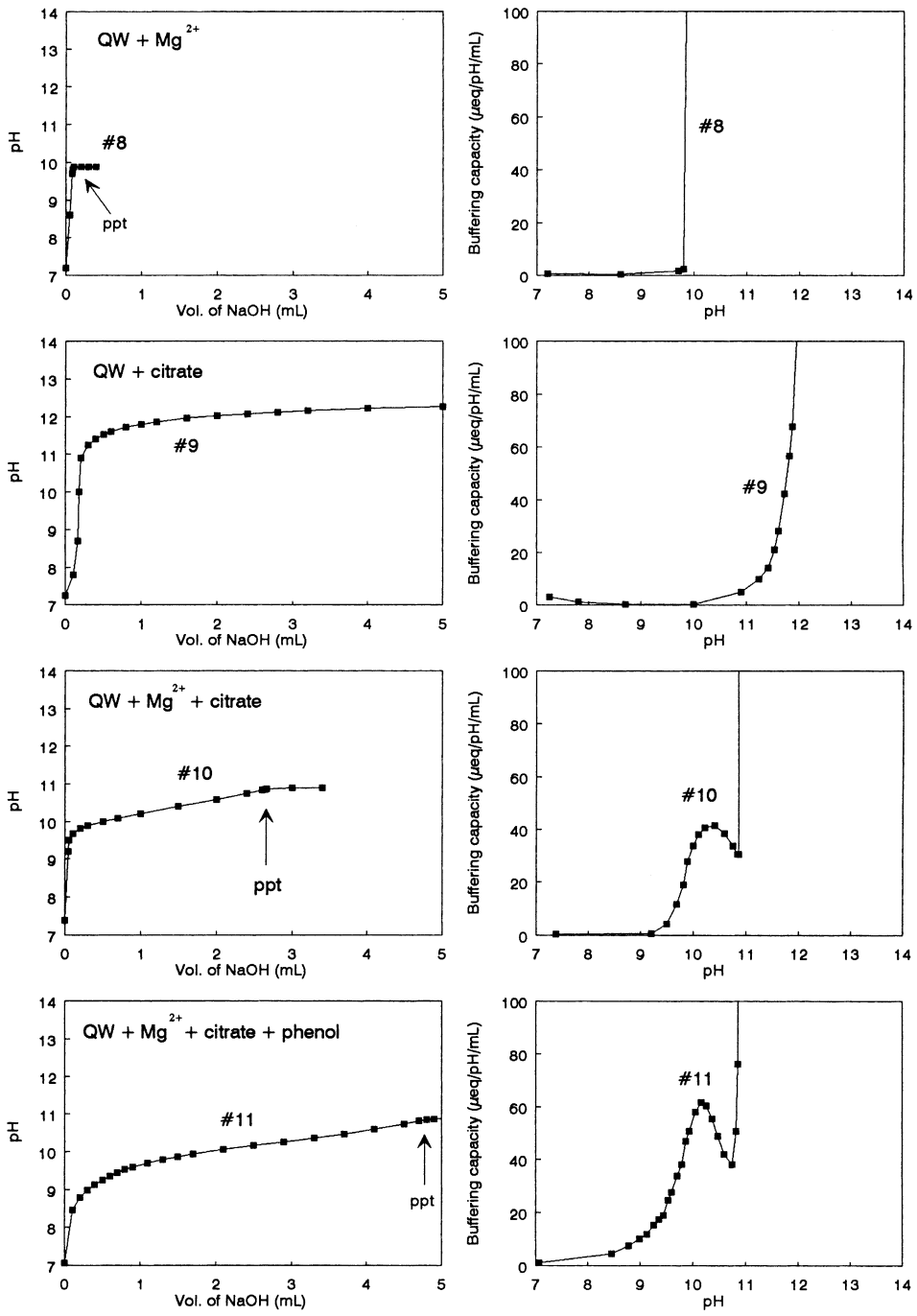

Fig. 2. Simulation of buffering capacity for sea water using the IPB method. Titration curves and buffering capacity plots for tests: (8) Mg^{2+} in Milli-Q water; (9) citrate in Milli-Q water; (10) Mg^{2+} + citrate in Milli-Q water; (11) Mg^{2+} + citrate + phenol in Milli-Q water.

3.5. Titration of mixture of Mg^{2+}, citrate and phenol

In test 11 a mixture of 46 mM Mg^{2+}, 46 mF citrate and 36 mF phenol was titrated. The buffering capacity plot of this mixture gives a pattern (Fig. 2) that is almost identical to that from sea water with all IPB reagents (see Fig. 1, test 6). This is clear evidence that the strong buffering capacity in the IPB method for sea water analysis is mainly provided by the combination of the phenate system ($pK_a = 10$) and the Mg–citrate complex system (apparent $pK = 10.2$).

3.6. The Mg–citrate buffer system

There are two types of chemical equilibrium that can provide the buffering capacity peak as shown in test 10.

1. Dissociation of hydrogen ion from a weak acid ($HL \Leftrightarrow H^+ + L^-$); in the present case it might be

   $$HMgCIT^0 \Leftrightarrow H^+ + MgCIT^-$$

   Since the formation constant for $HMgCIT^0$ is $10^{3.29}$ [12], then the buffering capacity peak of this system should appear at pH 3.29. Therefore, the pH 10.2 peak cannot be explained by the dissociation of the magnesium hydrogencitrate complex.
2. Hydrolysis of a species (marked as L) that binds a hydroxide ion ($L + OH^- \Leftrightarrow LOH^-$), so that the addition of hydroxide ion does not increase the pH correspondingly. Thus, the system acts like a buffer. In the present case the most probable system would be the hydrolysis of $MgCIT^-$ between pH 9 and 11.

   $$MgCIT^- + OH^- \Leftrightarrow Mg(OH)CIT^{2-}$$

   Further addition of NaOH (at pH > 11) destroys the complex due to the competition for OH^-, and magnesium hydroxide ($pK_{sp} = 10.47$) starts to form. It may be described as

   $$Mg(OH)CIT^{2-} + OH^- \Leftrightarrow Mg(OH)_2 \downarrow + CIT^{3-}$$

3.7. Recommended procedure

In order to overcome the extra buffering capacity produced by the Mg–citrate system and to eliminate the pH difference between freshwater and sea water sample sets, the following procedure is proposed. It is similar to procedure E described by Ivančič and Degobbis [7], but the volumes of reagents has been altered, so that all can be added in equal volume (25 ml sample plus 1 ml of each reagent). This modification is for the convenience of shipboard operation, because reagents can be delivered rapidly by dispensers. Dichloroisocyanurate was used instead of hypochlorite owing to its stability [10]. The final concentration of citrate reagent is raised by 20% to ensure it is in excess of [Mg^{2+}]. Merck GR grade chemicals were used unless stated otherwise.

3.8. Reagents

- Phenol reagent (R1): dissolve 10 g of phenol in 100 ml of 95% (v/v) ethanol.
- Citrate reagent (R2): dissolve 50 g of trisodium citrate in 100 ml of water.
- Alkaline dichloroisocynurate reagent (R3): dissolve 1 g of dichloroisocyanuric acid sodium salt dihydrate ($C_3Cl_2N_3NaO_3 \cdot 2H_2O$, Fluka Chemika, 35915) and 3.6 g of sodium hydroxide in 100 ml of water. This reagent should be freshly prepared.
- Sodium hydroxide (R4): dissolve 4 g of sodium hydroxide in 100 ml of water.
- Nitroprusside reagent (R5): dissolve 0.5 g of sodium nitroprusside in 100 ml of water. The reagent should be stored in an opaque bottle.

3.9. Procedure

To 25 ml of sample is added sequentially without delay, and mixed thoroughly, 1 ml of R1, 1 ml of R2, 1 ml of R3, X ml of R4 ($X = 0$ for freshwater and 1 for sea water), and 1 ml of R5. The reaction can be complete within 1 h at room temperature and the reading is stable for up to 24 h. The absorbance is measured at 640 nm. Following colorimetric measurement, the final pH is also checked, and should be 10.5 ± 0.1.

3.10. Reagent blank and sensitivity

A spiking experiment was carried out on board a research vessel using the proposed procedure. Sea water was collected from a depth of 500 m, which provided a low ammonia concentration background.

Table 2
Reagent blank and spiking test for Milli-Q water and a 500-m deep sea water

Medium	$[NH_3]$ spiked (μM)	Final pH	Abs (640 nm, 1 cm)	$[NH_3]$ found[a] (μM)	R.S.D. (%) ($n = 7$)
Milli-Q water	0	10.49 ± 0.03	0.009 ± 0.0005	0.00 ± 0.03	
	5.00	10.49 ± 0.02	0.101 ± 0.001	5.08 ± 0.05	1
Sea water	0	10.51 ± 0.02	0.009 ± 0.0007	0.00 ± 0.04	
	5.00	10.52 ± 0.02	0.097 ± 0.001	5.03 ± 0.05	1

[a] Calculation was based on the molar absorptivity of $2.1 \times 10^4\ M^{-1}\ cm^{-1}$ at pH 10.5. All values are mean ± 1 S.D.

Replicate measurements (Table 2) show that the readings for this sea water were almost identical to that of the Milli-Q reagent blanks (RB). Therefore, the raw readings for the spiked sea water sample can be deducted directly. The molar absorptivities ($\varepsilon_{640\,nm}$) of the IPB for both media gave an almost same value of $2.1 \times 10^4\ M^{-1}\ cm^{-1}$ at pH 10.5. This confirms the finding by Mantoura and Woodward [8] that the so-called 'salt-error' is caused by pH difference, and not ionic strength. Thus, the salt-error can be eliminated completely at the same final pH. The concentration of ammonia in a sample is calculated by

$$[NH_3]\,(\mu M) = \frac{Abs - RB}{b \times \varepsilon} \times \frac{29 + X}{25} \times 10^6$$

where $(29 + X)/25$ is the volume factor ($X = 0$ for freshwater and 1 for sea water), b the light path length. Following the proposed procedure, a freshwater sample containing 5 μM ammonia should give a net absorbance of 0.092 at 640 nm using a 1 cm cell, whereas for a sea water of the same spiking concentration, it should be 0.088. Keeping time-duration between reagent additions as short as possible was found essential to prevent decomposition of monochloramine. The precision (R.S.D. (%)) of the proposed procedure was consistently around 1% at the 5 μM level. In our experience, the use of dichloroisocynurate has been always better than hypochlorite as oxidant.

4. Conclusions

The pH shift problem in the IPB method for ammonia determination in sea water cannot be clarified without quantitatively identifying the 'hidden' buffer. In this study the 'puzzle' was unravelled by titration experiments. The hidden system was found to be the hydrolysis of the magnesium–citrate complex, which changes the final pH and interferes with the color formation reaction. The effect of alkalinity is comparatively insignificant. This finding suggests that for sea water analysis more NaOH should be added to overcome the buffering capacity. This work also explains that the addition of an external buffer system does not help to solve the problem, because the combined capacity produced by 36 mF phenate and ca. 50 mF Mg–citrate systems is comparatively high, which limits the strength of any additional buffer system.

More recently, we have tried to use other complexing agents to replace citrate but without success. Oxalic acid, which has strong complex binding with Mg^{2+} and does not provide any buffering capacity between pH 7 and 11, sounds hopeful for high Mg^{2+}-containing samples, cannot be used for sea water as it precipitates Ca^{2+} readily. Before other substitutes are found, the best solution is to examine the final pH of each sample to ensure it is in the optimal range of pH 10.5 ± 0.1. For samples with a wide salinity range, the analyst can make calibration graphs at various pH values and salinities to create empirical equations to correct the results.

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References

[1] A. Aminot, D.S. Kirkwood, R. Kérouel, Mar. Chem. 56 (1997) 59.

[2] L. Solórzano, Limnol. Oceanogr. 5 (1969) 899.
[3] J.D.H. Strickland, T.R. Parsons, A Practical Handbook of Seawater Analysis, Fisheries Research Board of Canada, Ottawa, 1972, p. 87.
[4] M.I. Liddicoat, S. Tibbits, E.E. Butler, Limnol. Oceanogr. 20 (1975) 131.
[5] C.J. Patton, S.R. Crouch, Anal. Chem. 49 (1977) 464.
[6] K. Sasaki, Y. Sawada, Bull. Jpn. Soc. Scientific Fisheries 46 (1980) 319.
[7] I. Ivančič, D. Degobbis, Water Res. 18 (1984) 1143.
[8] R.F.C. Mantoura, E.M.S. Woodward, Estuarine, Coastal Shelf Sci. 17 (1983) 219.
[9] Y. Nimura, Bull. Jpn. Soc. Scientific Fisheries 39 (1973) 1315.
[10] K. Grasshoff, H. Johannsen, J. Conseil Int. Pour l'Exploration Mer. 34 (1972) 516.
[11] J.P. Riley, R. Chester, Introduction to Marine Chemistry, Academic Press, London, 1971, p. 121.
[12] J.A. Dean (Ed.), Lange's Handbook of Chemistry, 14th Edition, McGraw-Hill, New York, 1992, p. 91.