

10.2.10 Determination of ammonia

In all methods for the determination of ammonia the sum of NH_4^+ and NH_3 is measured. Most of the earlier procedures also included varying amounts of labile organic nitrogen compounds such as trimethylamine and the amino acids in the determination. These methods have been reviewed by Riley (1975).

The blue colour of indophenol formed by phenol and hypochlorite in the presence of NH_3 was first reported by Berthelot (1859). About 30 applications of this reaction have been adopted for the determination of ammonia in various media. To be reasonably sensitive, the reaction requires elevated temperature or a catalyst. A number of transition metals ions have been used as catalyst, including: Mn^{2+} , Ag^+ , Fe^{2+} , Cu^+ , $[\text{Fe}(\text{CN}_6)]^{4-}$, $[\text{Fe}(\text{CN})_5\text{NO}]^{2-}$. The last was suggested by Lubochinsky and Zalta (1954) and seems to yield the highest sensitivity. In this ion NO has a positive charge and Fe is divalent. Mann, Jr. (1963) has applied the Lubochinsky-Zalta technique for NH_3 determination after a micro Kjeldahl digestion but experienced difficulties with the Hg ion used as a catalyst in the digestion.

Sagi (1966) introduced the indophenol blue method with a nitroprusside catalyst for the direct measurement of ammonia in seawater.

Koroleff (1969, 1970) examined this procedure more closely and suggested the method as described below.

10.2.10.1 Principle of the method

Ammonia reacts in moderately alkaline solution with hypochlorite to give monochloramine which, in the presence of phenol, catalytic amounts of nitroprusside ions and excess of hypochlorite, gives indophenol blue. The reaction mechanism is complicated and cannot be fully explained. Quinone chloramine is possibly formed in one of the intermediate stages. The formation of monochloramine requires a pH of between 8 and 11.5. At higher pH ammonia is incompletely oxidized to nitrite, as in the method by Richards and Kleisch (1964). The indophenol formed has a pK value of about 8.9 and is fully oxidized at pH 10.8. The ratio phenol/hypochlorite must be fairly constant at about 25 (mass ratio) of phenol/available chlorine, where 'available chlorine' is the total amount of chlorine at all oxidation levels. Hypochlorite solutions (e.g., commercial bleaching agents) tend to give rather unstable dilute solutions with rapidly decreasing contents of available chlorine (about 3.5 %). Grasshoff and Johannsen (1972) introduced dichloro-s-triazine-2,4,6-(1H,3H,5H)-trione sodium salt (Trione, DTT) as an alternative hypochlorite donor. It has been used by Dal Pont *et al.* (1974) and Liddicoat *et al.* (1975) among others. This reagent has the advantage of being a stable solid, and the formation of hypochlorite when hydrolysed is rapid. The acid form contains 32 % of positive monovalent chlorine which is equivalent to 64 % available chlorine in a bleaching solution (28 and 58 % if the DTT dihydrate is used).

In seawater at a pH > 9.6, Mg and Ca ions may precipitate as hydroxides and carbonates, but these ions can be held in solution by complexation with citrate as suggested by Solorzano (1969). The efficiency of the citrate buffer has been improved considerably by the addition of a small amount of the disodium salt of EDTA (ethylenediaminetetraacetic acid) (Ryle *et al.*, 1981).

With the reagent concentrations suggested by *Koroleff* (1969), the formation of indophenol blue takes several hours at room temperature. The reaction can be accelerated (i) by increasing the concentration of the reagents as has been done in the majority of recent procedures, (ii) by increasing the reaction pH to more than 12, (e.g., *Scheiner*, 1975), (iii) by increasing the reaction temperature as in most automatic determinations and (iv) by irradiation of samples with long-wave ultraviolet light (*Liddicoat et al.*, 1975).

All these alternatives have been studied by *Koroleff* (unpublished), who found that a three-fold increase of the main reagent concentrations is advantageous. Also, it was observed that a reaction pH > 11.0 must be avoided, otherwise erratic blank values with greenish shades are obtained, and finally, that a reaction temperature of 37–40 °C is better than irradiation for complete colour formation within 30 min. The reaction time is 2–6 h at 20 °C depending on the salinity of the sample.

In the flow-analysis method described in Section 10.3, maximum sensitivity is obtained by heating to about 40 °C. Higher temperatures do not noticeably increase the sensitivity but enhance the tendency of turbidity formation by Ca and Mg hydroxides.

10.2.10.2 Range and precision

The molar absorptivity of the indophenol blue is about 20 000. Thus, the absorbance for 1 µmol/L is 0.200 measured in a 10 cm cell. Taking an absorbance of 0.010 as the detection limit, 0.05 µmol/L can be observed. The chemical process allows the determination of up to 150 µmol/L, but such samples must be diluted as Beer-Lambert's law is obeyed only to a concentration of about 40 µmol/L (absorbance 0.8 in a 1 cm cell). Dilution of the coloured solution after reaction is possible.

Analysis of filtered Baltic Sea samples of about 0.5 µmol/L spiked to nominally 1, 2 and 3 µmol/L ammonia and analysed in three independent analytical runs resulted in a mean standard deviation of ± 0.092 µmol/L or ± 2.7 % (*Hansen and Johannsen*, unpublished). Similar results have been reported by *Riley et al.* (1972) and *Solorzano* (1969). The recent ICES intercomparison exercise (*Aminot and Kirkwood*, 1995) showed an overall relative standard deviation of more than 20 %, indicating that, despite good precision of ammonia measurements within one laboratory, the inter-laboratory precision is comparatively poor. This is probably due to the ease of contamination in preparations of zero water and standards as well as in the handling of samples for ammonia determinations.

10.2.10.3 Interferences

The possibility of interference from amino acids and urea has been examined by several workers (e.g., *Zadorojny et al.*, 1973), using similar concentrations of phenol and hypochlorite and amino acid concentrations of about 14 µmol/L. Of 19 amino acids, *L*-phenylalanine caused the greatest interference and decreased the ammonia recovery by 13 %. For 14 other acids the interferences fluctuated around ± 2 % of the 'true' value. The overall content of amino acids in seawater generally is about 0.5 µmol/L, therefore interferences by these substances may be neglected. No interference has been observed for urea.

Zadorojny et al. (1973) tested 25 possibly interfering inorganic constituents of seawater and observed no effects up to levels of 5 mg/L. At the 10 mg/L level a 7–14 % positive error

was estimated for cyanide, thiocyanate and sulphide, and a 6 % negative effect by mercury (II) ions.

Koroleff (1969, 1970) found that mercury(II) ions at a concentration of 10–200 $\mu\text{mol/L}$ (polluted waters in the vicinity of pulp and paper industry) decrease the indophenol blue by about 20 % and that samples containing more than 60 $\mu\text{mol/L}$ of sulphide should be diluted.

The indophenol blue produced by the same amount of ammonia is less in seawater than in pure water. This salt effect is caused by (i) magnesium ions, as also stated by *Grasshoff and Johannsen* (1974) and (ii) by buffering capacity of seawater. Increasing amounts of Mg^{2+} ions and increasing buffer capacity decrease the final reaction pH. Consequently, the salt effect can be assumed to be caused by the pH. The chloride ion has no influence.

If the reagents are adjusted to establish a pH of 11.0 in a pure water sample, the resulting pH in natural seawater samples of salinities S is approximately

$$\text{pH} = 11.0 - 0.500 \cdot S + 0.00045 \cdot S^2$$

Samples from brackish waters, with a wide salinity range, have to be corrected with respect to the salt error. Standards are prepared using a zero water (ZW) of medium sample salinity (S_0).

The experimental linear salinity correction

$$\text{NH}_{3(\text{cor})} = [1 + 0.0073 \cdot (S_s - S_0)] \cdot \text{NH}_{3(\text{unc})}$$

where S_s is the salinity of the actual sample, corrects the salinity error to within ± 1 %. Considering the methodical standard deviation of ± 3 –5 % (see Section 10.2.10.2), this is sufficient for most applications.

For manual analyses, standards may be prepared in pure water and the term ($S_s - S_0$) in the above equation is then displaced by S_s .

10.2.10.4 Reagents

All pure water, either distilled or deionized, should be passed through an ion exchange column immediately before use. Cation-exchange resin (*e.g.*, Permutit RSB 100) is preferable, but 'mixed bed' cation-anion resins are also sufficient. As only traces of ions have to be removed, columns containing about 500 mL of resin are capable of deionizing more than 100 L of pure water. In flow-systems, a small cation-exchange column (about 10 cm long with 1 cm i.d.) can be inserted in the pure water lines, and the segmentation air is aspirated through a small wash bottle containing dilute sulphuric acid.

1. *Sodium hydroxide, 1.0 mol/L*: Dissolve 40 g of sodium hydroxide (NaOH) in pure water and dilute to 1 L. Store in a well-stoppered polyethylene bottle.
2. *Sodium hydroxide, working solution*: Add 2 mL of phenol reagent (reagent 3) and 1 mL of citrate solution (reagent 8) to 50 mL of pure water. Titrate with the NaOH (reagent 1) to a pH of 11.0 using a pH meter. Dilute the 1 mol/L NaOH solution so that the pH is 11.0 when 2 mL are added. The solution thus obtained, contains about 0.8 mol/L NaOH and is used for preparing the hypochlorite reagent. Store in a tightly closed polyethylene bottle.
3. *Phenol reagent (manual method)*: Dissolve 80 g of colourless phenol ($\text{C}_6\text{H}_5\text{OH}$) in 300 mL ethanol and add 600 mL of pure water. Dissolve 600 mg of disodium nitroprus-