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Comparison of Nessler, phenate, salicylate and ion selective electrode procedures for determination of total ammonia nitrogen in aquaculture

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ABSTRACT

An assessment was conducted to compare the precision and accuracy of Nessler, phenate, salicylate, and ammonia electrode procedures for total ammonia nitrogen (TAN) concentration determination in waters of aquaculture. Salicylate method was selected as a standard method for its high precision and accuracy. In replicate analyses of water samples for precision estimate, Nessler method usually gave higher mean concentration of TAN than other methods and it is not recommended for use for its high coefficient of variance. Results from Nessler's with Rochelle salt method were more accurate than Nessler without Rochelle salt method. Electrodes for sensing NH_3 and NH_4^+ usually gave higher mean concentration of TAN than salicylate method. NH_4^+ electrode, although with high accuracy, was strongly affected by sodium and potassium and not recommended to use especially in waters of high salinity. The finding also suggested that the salicylate kit by using YSI photometer also was a preferable alternative to salicylate method while Nessler kit was not. TAN concentrations measured in 27 water samples by all methods were highly correlated ($R^2 = 0.919$ to 0.996) with salicylate method. All of the slopes except phenate I and II method were different from 1.0 ($P < 0.05$); all of the intercepts except phenate I and salicylate kit were different from 0.0 ($P < 0.05$).

Statement of relevance: In this study, we evaluated the accuracy and precision of different possible ways of measuring TAN concentration at aquaculture facilities. These findings will be useful because producers need reliable measurement of TAN concentration to effectively manage ponds and other culture systems. The conclusions made in this manuscript provide valuable information when researchers and farmers need to measure TAN concentration.

1. Introduction

Feed-based aquaculture is becoming increasingly intensive, because efficiency is improved by concentrating culture animals in a small volume of water to facilitate feeding, mechanical aeration, use of water quality amendments, and harvest. It is not unusual for intensive ponds to have standing crops of 8000 to 10,000 kg ha^{-1} and daily feeding rates up over 100 kg ha^{-1} as encountered in ictalurid catfish farming in the United States (Boyd and Tucker, 2014). Mechanical aeration is effective in avoiding excessively low dissolved oxygen concentration in intensive ponds, but high concentration of total ammonia nitrogen (TAN) commonly occurs. For example, intensive ictalurid catfish ponds in Alabama (United States) typically had TAN concentrations above 2 mg L^{-1}, and concentrations above 10 mg L^{-1} were measured in some ponds (Zhou and Boyd, 2015).

Analytical methods for ammonia nitrogen in water measure TAN that consists of un-ionized ammonia (NH_3) and ammonium (NH_4^+) in a pH- and temperature-dependent equilibrium, un-ionized ammonia contributes primarily to ammonia toxicity, but high NH_4^+ concentration has some degree of toxicity because it interferes with the outward movement of ammonia through the gills (Liew et al., 2013). The proportion of NH_3 increases with rising pH and temperature (Trussell, 1972), and the NH_3 concentration must be estimated from the TAN concentration. Tables of factors for calculating NH_3 from TAN, pH, and water temperature are available (Boyd and Tucker, 2014; Emerson et al., 1975; Trussell, 1972) and on-line ammonia calculators such as the one found at http://www.hbuehrer.ch/Rechner/Ammonia.html may be used. Total ammonia nitrogen concentrations in culture systems are sometimes great enough to stress culture animals but seldom high enough to cause direct mortality (Boyd and Tucker, 2014; Zhou and Boyd, 2015).

The threat of ammonia stress increases in intensive aquaculture and there is a need to monitor TAN concentration in culture systems. Several methods of measuring TAN concentration are used in aquaculture to include standard laboratory colorimeter procedures (Nessler, phenate, and salicylate methods), either NH_3 or NH_4^+ sensing electrodes (Eaton et al., 2005), and test kits that rely on either the Nessler technique or the salicylate methods. Elevated calcium and magnesium (hardness) concentration and salinity can interfere with the determination of TAN

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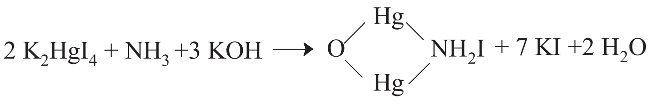
concentration (Eaton et al., 2005). Aquaculture is conducted in freshwater, estuarine water, and seawater, and there is increasing production of marine shrimp in low salinity (1 to 10 g L^{-1}) inland waters (Roy et al., 2010). The salicylate method for TAN is commonly used for seawater, but a recent study (Le and Boyd, 2012) revealed that this method also gave highly satisfactory results for water ranging from salinities of 0.1 to 24 g L^{-1}. Thus, the purpose of the present study was to compare the salicylate method to other methods of determining TAN concentration in freshwater of different hardness and in low-salinity inland water.

2. Background on analytical methods

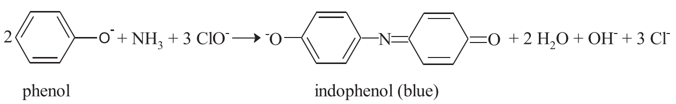
Nessler's reagent – named after Julius Nessler, the German chemist who first made this reagent – is a solution consisting of mercury (II) iodide and potassium iodide in highly alkaline solution. It will react to form a yellow color in proportion to TAN concentration that may be assessed colorimetrically (Krug et al., 1979; Leonard, 1963; Remy, 1956). The basic reaction is



$$2\,K_2HgI_4 + NH_3 + 3\,KOH \longrightarrow O\begin{matrix}Hg\\ \\ Hg\end{matrix}NH_2I + 7\,KI + 2\,H_2O \qquad (1)$$

Nessler's reagent raises the sample pH causing precipitation of calcium and magnesium (hardness cations) as hydroxides creating turbidity that interferes with colorimetric measurement of the yellow color — especially in harder waters. Distillation of a water sample at high pH results in a distillate containing the ammoniacal nitrogen of the sample free of hardness cations. But, this method is time consuming and tedious. The more common means of removing interference by hardness cations is to treat samples with alkaline zinc sulfate solution followed by filtration. Rochelle salt [potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$)] solution is then added to remove residual hardness cations that might react with Nessler's reagent.

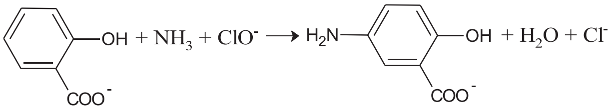
The phenate (or phenol) method is based on the Bethelot reaction in which ammonia reacts with phenol and hypochlorite under alkaline condition. Ammonia is converted to monochloramine at pH 9.7–11.5, which reacts with phenol in the presence of hydrochlorine to form blue-colored indophenol in proportion to the ammoniacal nitrogen concentration in the sample (Searle, 1984), the reactions are:



(2)

Sodium nitroprusside is generally used to catalyze indophenol reaction and intensity the blue color (Lubochinsky and Zalta, 1954; Mann, 1963). A citrate buffer often is added to stabilize pH and prevent precipitation of hydroxides (Hampson, 1977; Solorzano, 1969; Verdouw et al., 1978). Rochelle salt solution with manganese sulfate catalyst sometimes added to samples before applying the other reagents to lessen the inference of calcium hydroxide precipitation in hard waters (Boyd and Tucker, 1992).

The salicylate method actually is a modification of the phenate method in which sodium salicylate is substituted for phenol. This modification eliminates production of ortho-chlorophenol that is toxic and highly volatile (Lammering, 1960; Roberts et al., 1977; Verdouw et al., 1978). In the salicylate method, monochloramine formed by the reaction of ammonia and hydrochlorine reacts with salicylate to form blue-green colored 5-aminosalicylate in proportion to the amount of ammoniacal nitrogen presented as follows:



(3)

Sodium nitroferricyanide acts as a catalyst to intensify the color of 5-aminosalicylate.

An ion-selective electrode (ISE) – often called a specific ion electrode – responds to the concentration of a particular ion or gas in solution. The result is a potential difference that can be measured by a high impedance voltmeter (i.e. pH meter). The voltage, in accordance with the Nernst equation, depends upon the common logarithm of the activity of the ammonium ion or gaseous ammonia (NH_3) in solution.

There are two types of electrodes for sensing TAN concentration. One senses NH_3 using a hydrophilic gas-permeable membrane to separate the sample from a solution of ammonium chloride within the electrode (internal solution). A strong base such as lithium hydroxide added to the sample converts essentially all NH_4^+ to NH_3. Ammonia diffuses through the membrane until the partial pressure of ammonia is equal on both sides. The pH change in the internal solution is sensed by the electrode and is proportional to the partial pressure (or concentration) of NH_3 in the sample.

The NH_4^+ sensing electrode has a polyvinylchloride (PVC) membrane containing an ammonium-carrier. The water sample is acidified to lower the pH and convert essentially all NH_3 to NH_4^+. The electrode potential of the sample relative to the reference electrode of the NH_4^+ sensing probes is proportional to the NH_4^+ activity (or concentration) in the sample. The upper limit of sensitivity of ISE was 17,000 mg L^{-1} in this study – a concentration limit much higher than those of 1 to 5 mg L^{-1} measurable by colorimetric procedures. The ability to measure high concentrations avoids diluting water samples and saves time when there are many water samples with TAN concentrations exceeding the upper limit of sensitivity of the colorimetric procedures. The lower concentration limits of ISE – 0.04 mg L^{-1} for the NH_3 probe and 0.05 mg L^{-1} for the NH_4^+ probe – are below the concentrations that can be detected by colorimetric procedures. The ability to detect low concentrations is important in many applications such as cation exchange capacity measurement (Borden and Giese, 2001), flow rejection technique to rapidly determine low ammonia concentrations (Meyerhoff and Fraticelli, 1981; Radomska et al., 2004), and urea determination in human sera (Palleschi et al., 1988)

Field kits for measuring TAN concentration in freshwater rely on the Nessler method. Kits for brackish water and seawater typically rely on the salicylate method.

3. Materials and methods

The TAN procedures evaluated in this study, the concentration ranges for the methods without sample dilution, and references for analytical protocol are given in Table 1. The water analysis kits, NH_3 and NH_4^+ sensing electrodes, the prepackaged dry reagents and solutions for these procedures were purchased new. Most reagents for other procedures were prepared from ASC-grade laboratory reagents and high-quality, ammonia-free distilled water. The TAN standards were prepared from ammonium chloride (the salinity increase resulting from ammonium chloride in standards can be ignored, because at the maximum TAN standard concentration of 1 mg L^{-1}, the salinity contributed by ammonium chloride was only 3.81 mg L^{-1} (0.0038 ppt)). The absorbances of samples – except in analyses made by test kits – were measured (1-cm path length) with an Aquamate Spectrophotometer (Thermo Fisher Scientific, Atlanta, GA USA). A VWR symphony model B20PI Benchtop meter (VWR Scientific Products, West Chester, PA,