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THE ANALYSIS OF AMMONIA IN POLLUTED SEA WATER

by

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ABSTRACT

An indophenol blue procedure with combined internal and external standard type calibration is described. It gives reasonably accurate and precise measurements of ammonia concentrations in polluted waters of the types encountered in fish rearing tanks and in natural sea water. While both amine and nitrite interfere in this reaction, and considerably so in those not too frequent cases where their concentrations exceed the ammonia concentration on a nitrogen atom basis, the interference from both, and from the products which may be formed when the two are present together, is of an indirect "suppressing" type and not of a direct type in which the IPB can be produced other than from the ammonia nitrogen. Such interference can therefore be dealt with quite satisfactorily by the use of internal standards.

INTRODUCTION

Measurements of ammonia in sea water, including polluted areas of the sea and, in particular, water used in fish rearing and fish farming, are of importance in both nutrition and toxicity studies. The sensitivity needs to be high in some cases, e.g. to follow algal and other systems in which ammonia may be a growth limiting nutrient. In other cases reasonable accuracy is essential since the ammonia toxicity threshold of fish is quite sharp. It is also convenient for the method to be able to operate in fresh water, since in fish rearing the fish may be transferred from one medium to the other. The pollutants may vary widely, but those which are most likely to interfere are nitrogen containing compounds, such as amines and amino acids, which may either participate in the chain of reactions employed in ammonia measurement, diverting it, or break down to produce ammonia. Hydrogen sulphide, often present in anoxic waters, is known to interfere and must be removed, (Andersen and Rey, 1972).

Most current methods depend on attack of the ammonia molecule with chlorine, and conversion of the product through a series of intermediates to build up the nitrogen into a fairly large molecule which can be measured colorimetrically. This approach is convenient since the simple procedures, which can be developed using generally available instruments, are suitable for rapid measurements on the numerous samples required to characterise an area which may be subject to rapid natural change, and can be applied before bacterically or otherwise induced extraneous changes occur. However, it has the disadvantage of not being self-checking, and since many kinetic factors are involved, requires careful control and calibration, the more so when interfering pollutants are present.

Several types of reaction producing chromophores have been used in ammonia measurement e.g. reactions leading to formation of a complex of bis-(3-methyl-I-phenyl-5-pyrazolone), (Strickland and Austin, 1959, Johnstone, 1966), and to one of sulphanilamide coupled to N-(I-naphthyl)-ethylene diamine (Richards and Kletsch, 1964, Matsunaya and Nishimura, 1974). Unfortunately all are multi-stage. In recent years the indophenol blue (IPB) reaction has been favoured as the basis of most procedures because of its specificity. This involves conversion of the ammonia to monochloramine at a suitable pH, 9.7 to 11.5 (pK is 8.1, losses occur above pH 11.5 due to conversion to dichloramine, nitrite etc). The monochloramine then reacts with phenol or salicyclic acid to form IPB through a series of intermediates such as quinone chlorimide. Absorbance due to IPB is measured at 640 nm. Harwood and Huyser, 1970, and Harwood and Kalme, 1970, found sodium nitroprusside was a more effective catalyst than acetone, used previously. Koroleff, 1970, used sodium nitroprusside with colour development in the dark, otherwise high blanks developed. The reaction was rather slow and he noticed marked "suppression" of response when his procedure was applied to polluted waters of the Baltic Sea. Undoubtedly a faster main reaction would minimize side effects. Solarzano, 1969, using a similar method to Koroleffs' employed a citrate buffer to prevent precipitation of magnesium hydroxide at the high pH required for the IPB reaction to go rapidly towards completion. Degobbis, 1973, studying temperature effects on reaction rate in a similar system, recommended 25° to 45°C. Dal Pont et al., 1974, improved on Solarzano's method by increasing the citrate concentration and the sodium hydroxide to get a correct pH adjustment for the indophenol blue oxidation (10.4 to 10.5) without any precipitate formation. They also subjected the reaction mixture to a period at high temperature (~ 70°C) to hasten colour development. The main recent improvements were due to Liddicoat, Tibbitts and Butler, 1975, who using Solarzano's citrate buffer replaced the nitroprusside catalyst by ferrocyanide, used sodium dichloro isocyanurate as a more convenient source of chlorine than hypochlorite (as did Dal Pont et al) and subjected the reaction mixture to UV irradiation instead of working in the dark. The ferrocyanide gave a much lower blank absorbance at 640 nm. It is clear that some

nitrogen from the nitroprusside catalyst of the earlier procedures enters the IPB molecule. In the absence of this wayward nitrogen, the reaction can be conveniently accelerated by photon energy instead of having to be retarded in the dark. However, Liddicoat et al appeared unaware of Dal Pont et al's work, both being done at the same time.

Serious colour suppression had been noted when IPB type methods such as those of Koroleff and Solarzano had been applied to fish rearing tank waters in the Lowestoft Laboratory. The method described here, which combines Liddicoat et al's use of ferrocyanide catalyst and UV irradiation with Dal Pont et al's precise pH control, aims at exact control of all conditions of temperature, reagent concentrations and ionic activities and UV irradiation to get stability and reproducability as far as possible in a kinetic system. A combination of external and internal standard calibration was used to deal empirically with reagent blank and all types of interference effects except direct ones (if any). Temperature was held at $30^{\circ} \pm 1^{\circ}C$, thus differing from Dal Pont et al., to avoid alkaline hydrolysis of amino acids to ammonia, a process which has been shown to occur extensively at higher temperatures (Grasshof and Johanssoen, 1974). Instead the reaction was selectively accelerated by activation energy derived from UV radiation.

PROCEDURE

50 ml samples were filtered through glass fibre paper (Whatman GF/F) into 150 ml pyrex conical flasks. The following solutions were added in this order, with mixing between each addition:-

1. 2.00 ml of 10% phenol (A.R.) in ethanol (A.R.).
2. 5.00 ml of chlorinating agent: to make this
   A. Dissolve 200 mg sodium dichloro isocyanurate ($C_3 Cl_2 N_3 O_3 Na$) in about 10 ml of water containing 3.2 g of sodium hydroxide (A.R.)- dissolved and cooled.
   B. Dissolve 40 g of tri sodium citrate (A.R.) in 80 ml of water. Combine A and B, and make up to 100 ml with water.
3. 2.00 ml of 0.5% solution of potassium ferrocyanide (A.R.). All solutions were freshly prepared each day using glass distilled deionized water.

The flasks were covered with pyrex or polystyrene covers, and maintained at $30^{\circ}C \pm 1^{\circ}C$ on a thermostated hot plate covered with a reflecting surface. All flasks were irradiated uniformly with UV light (e.g. by exposing at $\frac{1}{4}$ m below an array of two "Allen" 365 nm UV lamps model A 409, each having two 8", 15 watt tube type elements before a reflector). The absorbance was measured at 640 nm after at least 40 minutes. Internal and external calibration

lines were prepared for each sample by adding known additions of standard ammonium sulphate solution. "Low ammonia" sea water was used for the external standard lines, and for sample dilutions when necessary to obtain suitable absorbance readings (1, 2, 4 or 10 cm cuvettes were selected). When fresh water was analysed sea water ionic conditions were produced, when desired, by additions of pure reagents (Internal and external standards must be in the same medium.). Sample and reagent blank measurements were derived from the intercepts. Three to five sets of absorbance measurements were made at, say, hourly intervals. Good precision could be obtained in this way, by taking mean ammonia values.

PERFORMANCE

Colour development is essentially complete in 40 minutes, and remains reasonably constant for many hours. A single set of measurements may be made at any time after 40 minutes, though better precision is obtainable by averaging several sets.

About 3% coefficient of variation is obtainable with care and good instrumentation at ammonia concentrations above 0.1 ppm N. In routine daily use for ammonia measurements in fish rearing tank water using simple instrumentation, the precision was as in Table 1.

Comparisons were made between this IPB method and an ammonia gas electrode (E.I.L. model 8002-2, used with Radiometer model PHM64 pH/pX meter) on a series of aerated fish tank waters and on near anoxic sea waters pumped from 30 feet below the beach; both were likely to contain organic nitrogen compounds and nitrite. Ammonia measurement by the electrode is expected to be free of interference except from simple semi-volatile amines, which may penetrate the membrane and cause erroneously high ammonia values. The electrode was used with modifications to the inner filling solution for use in sea water as recommended by the makers, and with the recommended high pH buffer. The latter was mixed with sea water on an equal volume basis, because some precipitation occurred at the recommended 1:2 ratio with apparent loss of ammonia, perhaps as insoluble magnesium ammonium phosphate. There is excellent agreement between the two methods (± 4%) at ammonia concentrations in the undiluted sea water above 0.3 ppm N, but at concentrations down to 0.1 ppm some apparently random differences occur as the limit of detection of the electrode is approached.

A linear absorbance/concentration relationship is obtained with about 1 absorbance unit from 0.1 ppm ammonia N when 10 cm cuvettes are used. Reagent blanks were equivalent to about 0.01 ppm ammonia N. The detection limit of ammonia in fresh water or sea water is about .001 to .002 ppm N.

The method has proved suitable for use at sea on research vessels.

INTERFERENCES

Investigation of interferences was then carried out, attempts being made to identify causative compounds, and to elicit information about the characteristics of interfering reactions. Discrimination between direct and "suppression" type interference was made since the latter can be dealt with in principle, e.g. by control of conditions or by yield tracing, while the former cannot. The procedure was tested in the presence of some interferants both in the laboratory with fish tank waters, and at sea in polluted estuaries, and areas of high and low plankton density.

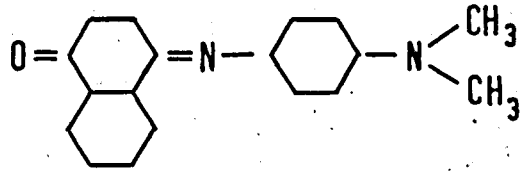
Simple aliphatic amines - primary, secondary and tertiary, which may be derived from excretary products or bacterial or autolytic decomposition products of fish and of many other organisms were considered to be likely interferants in the ammonia-indophenol blue reaction. They are more readily attacked by chlorine than is ammonia as a result of the enhanced nucleophilicity of the nitrogen atom, and the resulting products have structures which might participate at various stages in the chain of reactions leading to IPB.

The methyl and ethyl primary, secondary and tertiary amines were examined for possible interference in the ammonia→IPB reaction by allowing the reaction to take place in pure sea water containing a known addition of ammonia convenient for measurement (0.40 ppm ammonia N), in parallel paired experiments with and without a known addition of each amine in turn. The amines were present at 100 fold excess over the ammonia, all concentrations being expressed on a N atom basis. All the amines suppressed the indophenol blue reaction with ammonia very strongly, and in the general order

primary amines < secondary amines < tertiary amines.

It may be noted that with amines at these concentrations (and also in fact at ten-fold higher concentrations) the concentrations of the reagents used in the reaction are not significantly affected.

The nature of the interference was then investigated in more detail using one of the amines only, dimethylamine, chosen because the nitrogen dimethyl group constitutes a component part of the indophenol blue molecule.



Indophenol Blue

The effect of varying concentrations of amine in the range 0 to 1000 ppm N on the IPB response to ammonia was investigated using pure sea water containing 0.40 ppm ammonia N. The response of the IPB reaction to ammonia in the presence of amine was expressed as a percentage reduction in colour (Figure 1). Very strong suppression is noted, even at low amine concentrations, and with even the IPB formation from the reagent blank suppressed at amine concentrations above 25 ppm N. Numerical analysis of the type of relationship between amine concentration and IPB response to ammonia suggests a third, or possibly fourth power polynomial function (as distinct from a simple power or exponential relationship). This is consistent with only a small or slight suppression effect when ammonia is present in excess, but a very strong suppression of progressively increasing intensity as soon as amine becomes present in excess. It may also imply the molecularity of the process. (Number of atoms of amine N reacting with a molecule of "intermediate").

None of these amines gave any IPB response when pure sea water (ammonia free), to which they had been added, was subjected to the standard analytical procedure.

Consideration of means of preventing this interference by destruction of the amines, or by their conversion to non-interfering substances leads to the idea of treatment with nitrous acid or nitrite. In this way the primary amines may be converted to non-interfering alcohols, the secondary amines to nitroso compounds (whose behaviour in the IPB reaction would need investigation), and the tertiary amines to a mixture of nitroso compound and alcohol. It has generally been thought that nitrite in moderate amounts does not in any way affect the ammonia→IPB reaction. It has been the custom to use most of the above mentioned IPB procedures for ammonia measurements in waters which are known to normally contain nitrite (frequently measured alongside the ammonia), sometimes in concentrations up to 10 times the ammonia concentration (on a N atom basis). However, before proceeding with work aimed at destroying amines, the possibilities of nitrite interference and suppression of the ammonia→IPB reaction were tested directly by a set-up exactly similar to that described above for examining amine suppression. Figure 2 indicates that very considerable suppression by nitrite does occur, though it is not as strong as in the case of any of the amines. Again, it is not great so long as the nitrite N concentration is less than the ammonia N concentration, but rapidly increases as the nitrite concentration exceeds the ammonia concentration. This suppression effect was confirmed by looking in the same manner at the effect of increasing nitrite concentration on the IPB reaction when the standard IPB procedure is applied to fish tank water containing ammonia only from biota (fish and bacterial filter). In this case apparent ammonia concentrations varied in the different experiments between .16 ppm N and .28 ppm N, averaging .21 ppm N. Figure 3 shows suppression of a very similar nature to that of Figure 2.

To evaluate the procedure for use at sea it was tested out and found satisfactory on a range of sea water types from various polluted estuarine situations, and on water from the open sea with high and low plankton density. In all cases nitrite measurements were made on the same sample, and a parallel ammonia measurement was made on a sample portion to which additional 0.5 ppm nitrite N had been added. The ammonia concentration in the sea water varied from < .002 to .275 ppm N, and the naturally occurring nitrite from .00003 to .08 ppm N, depending on location. The added nitrite caused some suppression of the IPB formed. (Approximately 2%, P .25).

DISCUSSION

The effects of amines and nitrite indicate sources of interference to IPB formation from non-ammonia nitrogen which may arise in fish rearing tank water and in the sea. The levels of nitrite used in the above tests were well in excess of the levels normally occurring in the sea and in fish tanks which are fitted with bacterial filters. Interference from this source will normally be small, though from the nature of the effect a sharply increasing suppression can occur when nitrite is present in excess of ammonia. A ten-fold excess may sometimes develop in the pre-establishment phase in fish tanks. It is more difficult to evaluate possible interference from amines, particularly because of their more pronounced effects. It was unlikely during the present studies since external and internal standards agreed, but there is a need to know more about the occurrence and concentration of amines in both natural sea water and fish cultivation units. Further studies to evaluate possible interference from nitrite and amines to ammonia analysis are desirable at levels at which all three constituents typically occur.

The significance of experiments with the ternary system at higher concentrations is that the possible intermediates formed, especially reactive nitrosoamines from di- and tri-methylamine, apparently do not condense (e.g. at the p-position of indophenol residues), or otherwise cause introduction of extraneous N to the IPB molecule. The IPB reaction could therefore be the basis of self-checking ammonia procedures utilizing the isotope dilution or N-tracing principle.

A further development envisaged is the use of high intensity UV irradiation, at an appropriately chosen activation frequency, to reduce IPB development time and increase sensitivity of measurements by this reaction, higher sensitivity being desirable occasionally.

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Table 1 Precision in routine use of IPB procedure for ammonia determination in fish tank water

Regime	Type of water	No. of days on which sets of ammonia determinations were made	Range of ammonia concentrations ppm N	Mean coefficient of variation %	Average no. of determinations on each sample i.e. per day	Standard error of mean of each day's measurements %
A	Fresh water during establishment of bacterial filter i.e. high concentrations of organic nitrogen compounds and nitrite	13	.75 to 5.4	18.5	4.7	8.5
B	Fresh water with established bacterial filter i.e. low concentrations of organic nitrogen compounds and nitrite	23	.045 to .13	13.4	3.4	7.3
C	Sea water during establishment of bacterial filter i.e. high concentrations of organic nitrogen compounds and nitrite	20	.25 to .98	9.2	3.5	4.9
D	Sea water with established bacterial filter i.e. low concentrations of organic nitrogen compounds and nitrite	80	.06 to .33	11.2	2.8	6.7

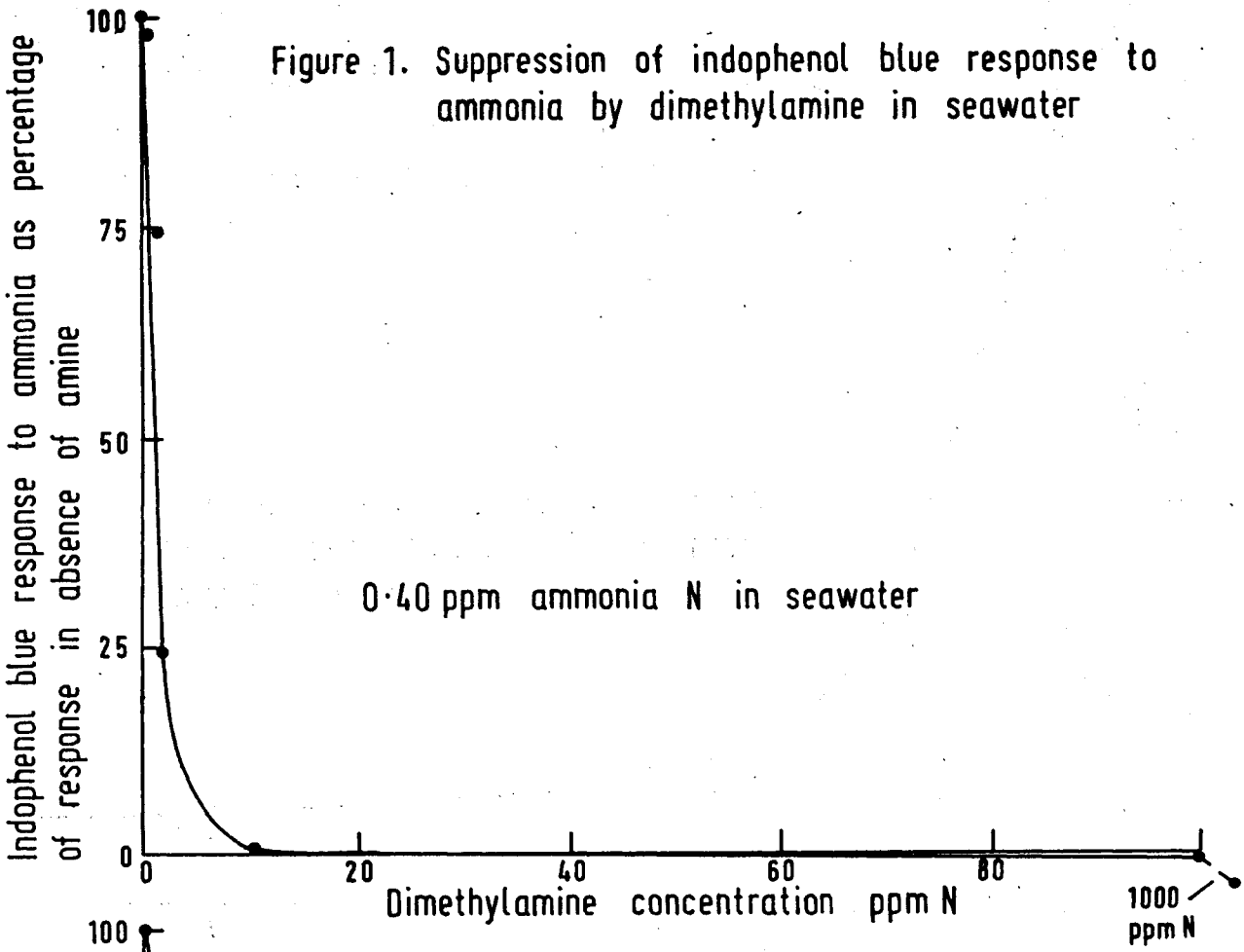

Figure 1. Suppression of indophenol blue response to ammonia by dimethylamine in seawater

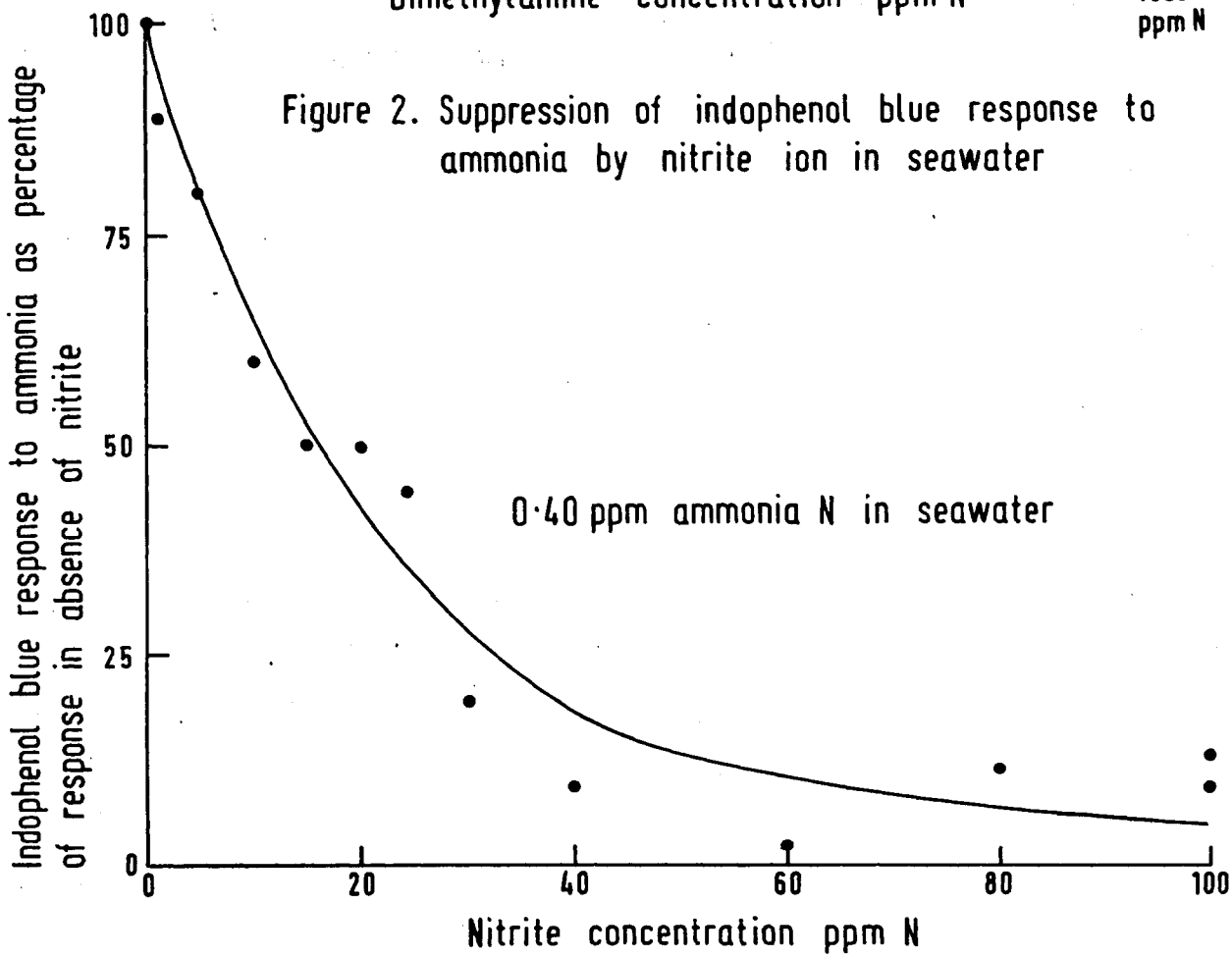

Figure 2. Suppression of indophenol blue response to ammonia by nitrite ion in seawater

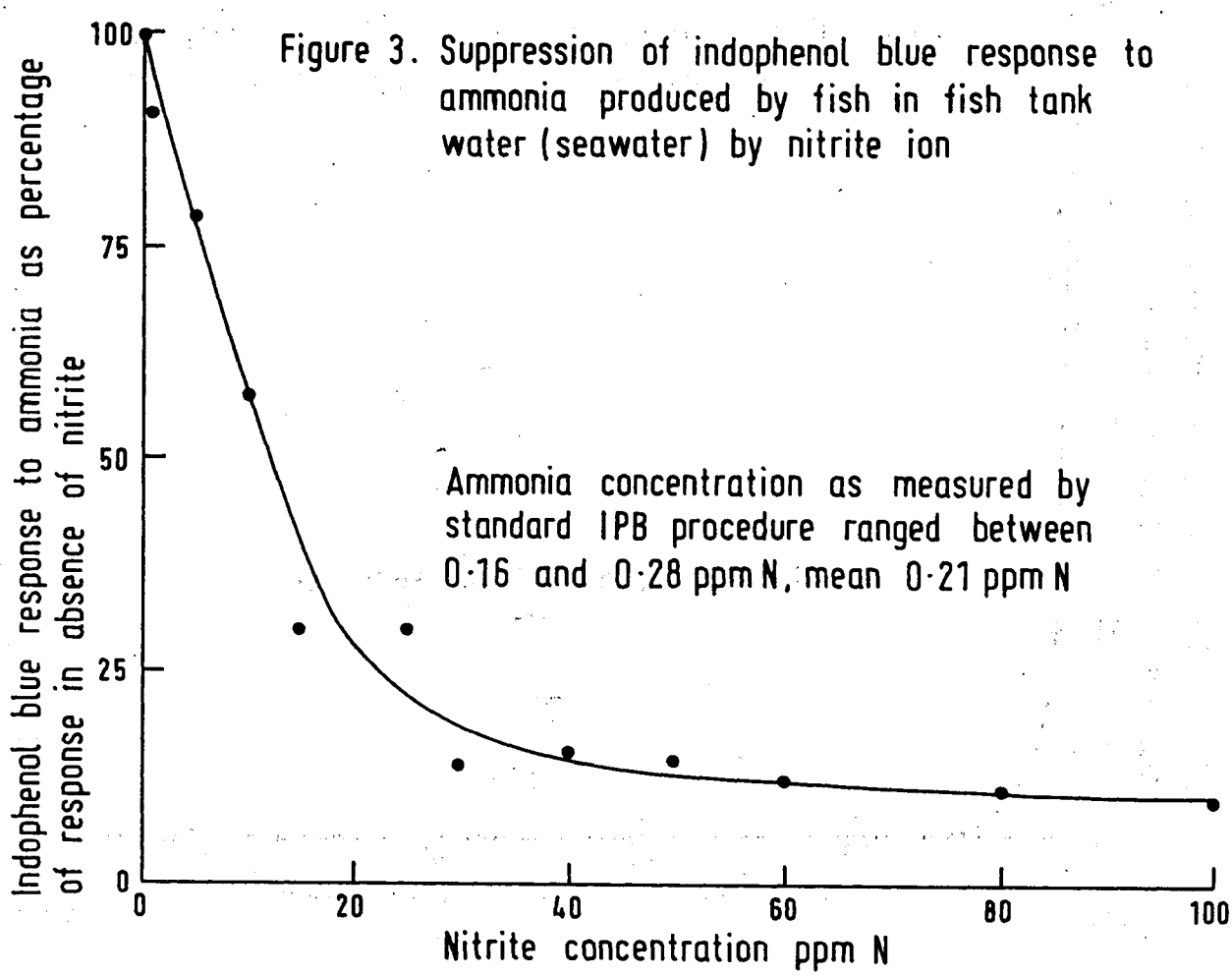

Figure 3. Suppression of indophenol blue response to ammonia produced by fish in fish tank water (seawater) by nitrite ion