# Determination of NTA in Aquatic Toxicity Studies: A Compleximetric Method Using Atomic Absorption Spectroscopy.

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The chelating agent Nitrilotriacetic acid (NTA) has been shown to be completely biodegradable (THOMPSON and DUTHIE 1968, PFEIL and LEE 1968, SWISHER et al. 1967). Evaluation of the toxicity and biological effects of NTA in aquatic systems requires periodic measurement of NTA concentration to ascertain the extent of degradation.

In related investigations on the toxicities of metals and metal-NTA complexes (LAUTENBACHER and BAKER), the technic of atomic absorption spectroscopy (AAS) is used to determine metal concentrations. The proposed method for determination of NTA involves the quantitative solubilization of $PbCO_3$ by NTA and subsequent measurement of the Pb-NTA complex by AAS. The method is designed to facilitate controlled laboratory studies on NTA.

THOMPSON and DUTHIE (1968) report that NTA is degraded through a series of simpler substances to the ultimate end products of $CO_2$, water and inorganic nitrogen. They recommend following the degradation of NTA by measuring colorimetrically its ability to compleximetrically extract zinc from a zinc-Zincon reagent. Although their method does not isolate and measure the intermediate or ultimate products of degradation, it does evaluate the capacity of the remaining NTA for forming chelates. Other instrumental methods for the determination of NTA based on complex formation use polarographic (HABERMAN 1971, AFGHAN and GOULDEN 1971, AFGHAN et al. 1972), high-speed ion exchange chromatographic( LONGBOTTOM 1972) or colorimetric ( SWISHER et al. 1967) measurement technics. Polarography and high-speed ion exchange chromatography offer somewhat greater sensitivity and/or specificity than the colorimetric methods but are more elaborate and time-consuming.

The proposed method is similar in ease and rapidity to the colorimetric methods and does not require special reagent. AAS instrumentation, as routinely used in metal toxicity studies, requires no modification or special attention. A Corning Model 240, AAS instrument, fitted with a Corning Model 845, laboratory recorder and a Fisher, Model CDR, digital concentration readout, was used in this study.

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### Reagents and Supplies:

10,000 ppm $Na_2NTA$ stock solution: Prepared by dissolving 10.00 g of Eastman-Kodak $Na_2NTA$ in distilled water and diluting to mark in a 1 liter volumetric flask.

Powdered $PbCO_3$: Baker, ACS grade.

Filters: 13 mm Versapor filters, 0.90 u pore size, Gelman Inst. Co., Ann Arbor, Michigan, in Swinex-13 filter adapters, Millipore Corp., Bedford, Mass.

## THE METHOD:

The method as described here is designed to determine NTA concentrations of 5 to 100 ppm, as $Na_2NTA$, in distilled or natural waters, including tap water, but not untreated sewer effluents. Concentrations of greater than 100 ppm $Na_2NTA$ can be determined by diluting the sample into the working range with distilled water. The method requires either a sufficient quantity of NTA-free control water for the preparation of blanks and standards, or, that the "method of addition" (REYNOLDS and ALDOUS 1970) be used if control water is not available.

### Preparation of Standards and Samples:

Intermediate stock solutions: Dilute in 100 ml volumetric flasks, 0, 2, 4, 6, 8, and 10 ml aliquots of the 10,000 ppm $Na_2NTA$ stock solution with distilled water. Label these intermediate stocks as 0, 20, 40, 60, 80 and 100 ppm $Na_2NTA$. Note that the labeled concentrations are for convenience of use and not actual concentrations.

Working standards: Pipet 1.0 ml of each intermediate stock into test tubes also labeled as 0 to 100 ppm $Na_2NTA$. Pipet 10.0 ml of control water into each tube.

Samples: Pipet 10.0 ml of each sample into test tubes. Pipet 1.0 ml of distilled water into each tube.

Treatment of Standards and Samples: Check pH, and if not between 6 to 9, adjust with a drop of 1 N NaOH. By means of a measuring scoop, deliver approximately 35 mgm of powdered $PbCO_3$ into each tube. Cap the tubes and shake gently for 15 minutes.

Draw at least 5 ml of the contents of a tube into a 10 ml plastic hypodermic syringe fitted with a 10 cm length of plastic tubing. Remove the tubing and place a filter adapter onto the syringe. Force the solution through the filter, discarding the first ml of filtrate.

Collect the clear filtrate in a clean test tube. Filter each of the standard and sample solutions. There is enough solution in the mixing tubes to permit rinsing the syringe and filter with about 3 ml of new solution before each filtration. One filter disc should be satisfactory for filtration of up to 5 solutions.

Measurement: Prepare the AAS instrument for measurement of Pb concentrations of up to 90 ppm. Adequate sensitivity and linearity should be attained using the 283 nm spectral line. Measure absorbance values for each of the standard and sample filtrates. Plot absorbance versus ppm $Na_2NTA$ for each standard on linear graph paper and determine the unknown concentrations from the calibration curve. If a digital concentration readout (DCR) is available the graphic procedure may be omitted. Set zero on the DCR while aspirating the 0 ppm standard filtrate. Using the highest standard filtrate known to be on the linear portion of the curve, set the concentration vernier to the corresponding NTA concentration. Read the remaining standards to validate the DCR settings before reading the unknowns.

Method of Additions: Pipet 1.0 ml of each intermediate stock solution into a series of test tubes. Pipet 10.0 ml aliquots of the sample solution into each of the tubes. Label the tubes as "plus o" to "plus 100" respectively. Add a measure of powdered $PbCO_3$ to each tube, cap, shake and filter as described earlier. Measure the absorbance of each filtrate by AAS. Use a "method of additions" plot to determine the concentration of $Na_2NTA$.

## DISCUSSION:

The proposed method was empirically designed for use in controlled laboratory studies and is not recommended for field determinations of ambient NTA levels. In theory, however, the proposed method could be used to determine NTA in waters subjected to industrial wastes if the analyst were certain that

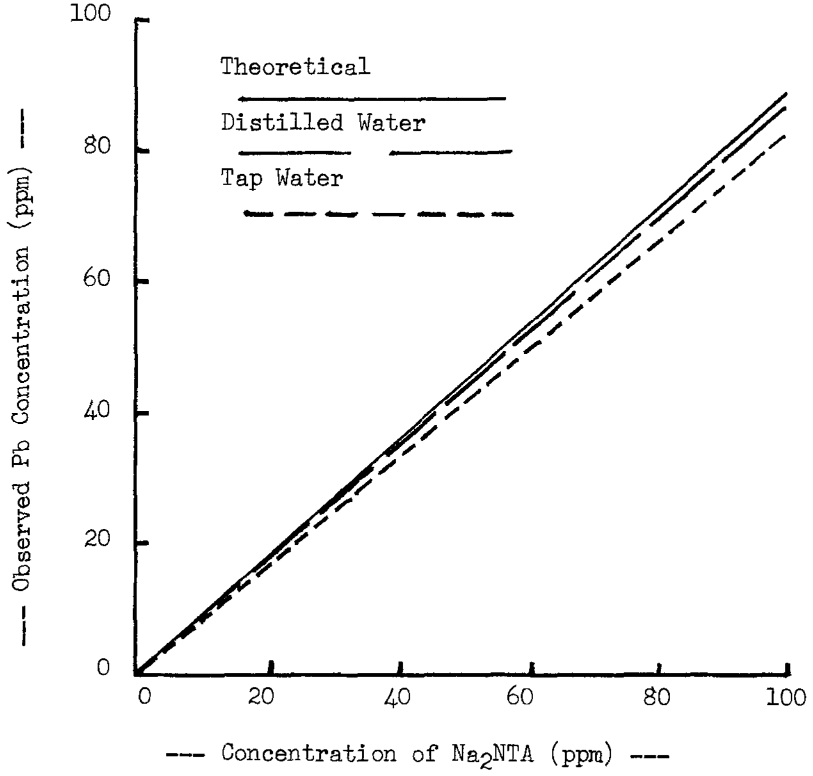

Figure 1. Calibration curves. Matrix effect in tap water causes suppression of observed Pb.

other complexing anions such as EDTA and $CN^-$ were absent. Cations present in natural waters, Ca as an example, also interfere through competition with Pb for the NTA. Existence of a matrix effect in natural waters is demonstrated in Figure 1. Measurements of Pb from $Na_2NTA$ solutions prepared in both distilled and tap water are compared to theoretical values calculated from the molar ratio of

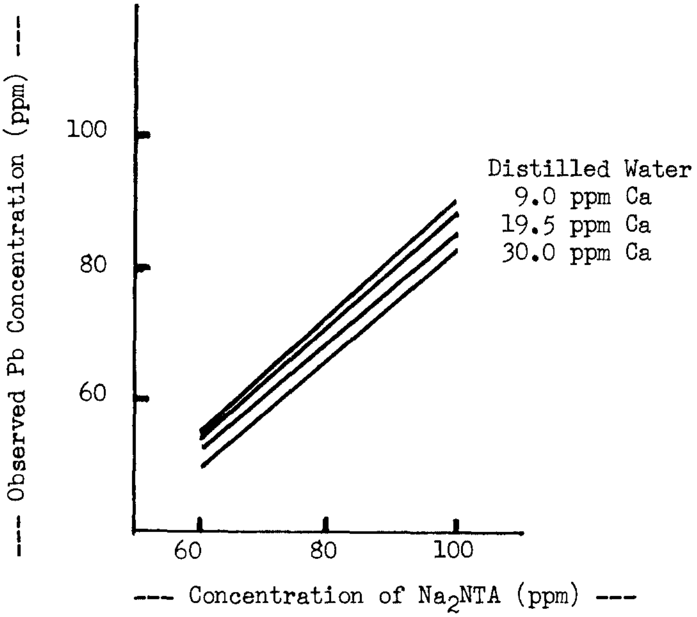

Figure 2. Calibration curves prepared in tap waters of varying Ca concentration show decrease in observed Pb as Ca content increases.

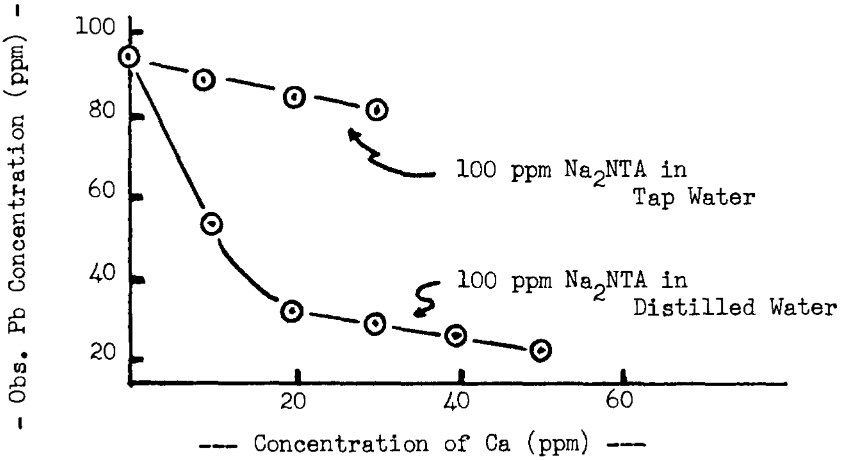

Figure 3. Calcium solutions prepared in distilled water cause greater reduction in observed Pb than do tap waters with similar Ca content.

$Na_2NTA$ to Pb. The quantity of Pb dissolved in distilled water by $Na_2NTA$ approached the theoretical much more closely than did that in the tap water. It was surmised that suppression of the Pb concentration in tap water was due in part to Ca-NTA complexation.

Figure 2. was prepared from data obtained by varying the concentration of Ca in tap water. The problem involves more than Ca ion, however, as indicated by the results of an experiment in which solutions of similar Ca ion content were prepared with both tap and distilled water, Figure 3. No further attempt was made to determine or explain the interactions occurring in the natural waters which give rise to the matrix effects. It was sufficient that an equilibrium was obtained between the Pb-NTA complexes and the interferences, as indicated by the linearity of the calibration curves when all solutions were prepared in control waters.

The "method of additions" may be used to compensate for matrix interferences when no control water is available. Samples of natural waters, spiked with 30 ppm $Na_2NTA$, were assayed by the proposed method. The "method of additions" was used to compute the results presented in Figure 4. Lake water used in the experiment dissolved 1 ppm Pb before addition of NTA.

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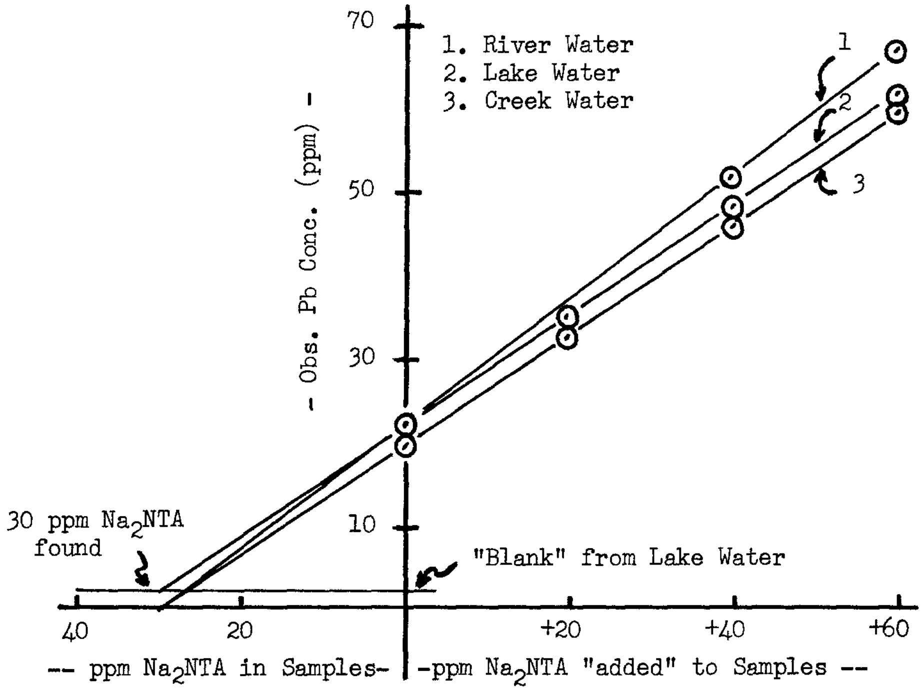

Figure 4. "Method of Additions" determination of NTA in Surface Waters spiked with 30 ppm $Na_2NTA$.

Throughout the experimental work the pH of standard and test solutions remained in the proximity of pH 7. In anticipation that a change in pH might be incurred due to the variability of natural waters, the effect of altering pH was determined. Solubilization of $PbCO_3$ by 100 ppm $Na_2NTA$ as a function of pH is shown in Figure 5. Satisfactory results were obtained between pH 6.5 and 9.5.

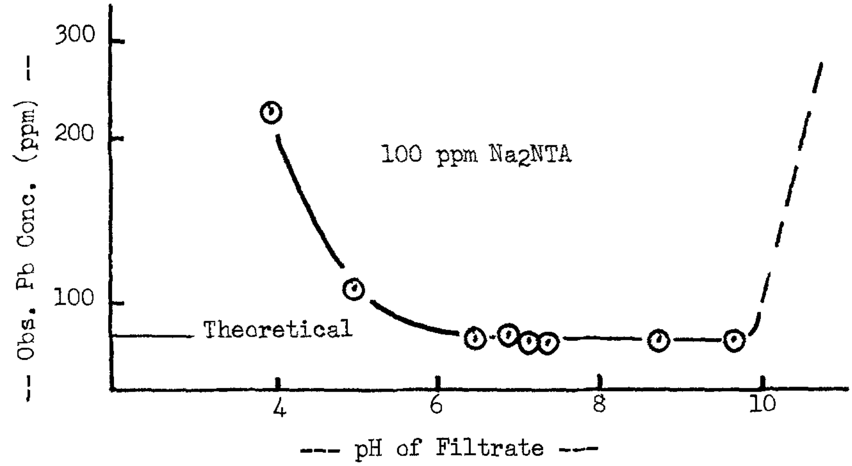

Figure 5. Effect of pH on observed Pb for solutions containing 100 ppm $Na_2NTA$.

The additional step of converting absorbance to ppm Pb before plotting the calibration curve was used throughout the experimental work for comparative purposes. A plot of absorbance versus ppm $Na_2NTA$ is sufficient for determination of NTA concentrations in routine work. Figure 6.

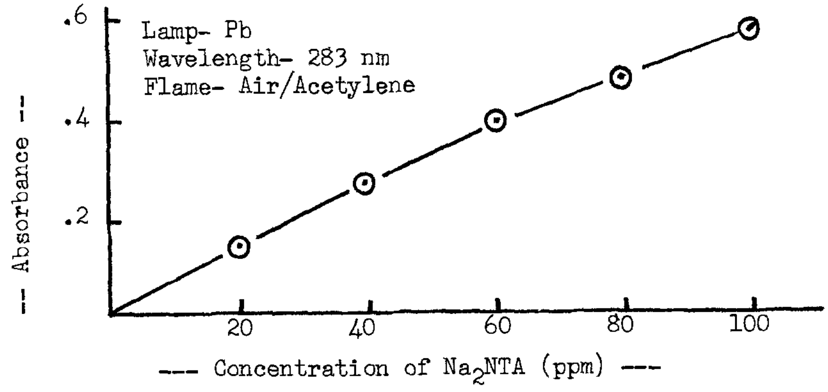

Figure 6. Typical calibration curve for graphic computation of NTA in Natural Water by the proposed method.

Standard solutions in this study were prepared from, and the concentrations specified as, $Na_2NTA$. The purity of the reagent was demonstrated by its ability to complex a nearly stoichiometric amount of Pb. Results of trials using NTA as the free acid were not as accurate, possibly due to presence of extra-formular impurities in the reagent.

## SUMMARY:

A compleximetric-AAS method for the determination of NTA in controlled aquatic systems is proposed as an expedient in the toxicological studies of NTA. The method is based on the formation of a soluble Pb-NTA chelate equivalent to the amount of NTA present in the original sample. The amount of Pb-NTA chelate is determined by AAS measurement of Pb. Concentration of NTA is determined graphically or by digital concentration readout. Matrix effects on the formation of Pb-NTA chelate are counteracted by the use of control water in preparation of standards, or by using the "method of additions." The proposed method is rapid and easy to perform. Pre-treatment of the sample is minimal, requiring only pH adjustment and/or dilution.

## AKNOWLEDGEMENT:

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