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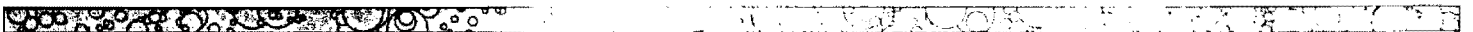
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On-Line and In-Situ Detection of Nitrates with Ultraviolet-Visible Absorption Spectrometry (UVAS)

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INTRODUCTION

Detection and control of nitrates in water supplies is important for protection of the environment and for control of human health. When waste waters that contain nitrates are discharged into surface waters they can promote the unnatural growth of blue-green algae. The consequence, particularly in bodies of water that remain relatively still, is a crowding out of other plant and animal life. The decay of dead algae causes a reduction in the amount of dissolved oxygen available in the water resulting in an upset of the food chain. Eventually, the excess concentration of nutrients in the body of water results in the inability to support any other life forms (eutrophication).[1]

Another significant reason for the control of nitrates is the possible occurrence of infantile methemoglobinemia (blue baby syndrome). Most nitrogen cycle reactions are microbially catalyzed redox reactions, such as the reduction of nitrate $[\text{NO}_3^-]$ to nitrite $[\text{NO}_2^-]$. The nitrite ion is relatively toxic to humans due to its interaction with hemoglobin in the blood to form methemoglobin, which has no oxygen carrying ability. Nitrites are not commonly ingested directly, but nitrate ions that enter the body in food or water can be reduced in the stomach to nitrite, especially in the stomachs of infants where low acidity allows the growth of nitrate reducing organisms. Drinking water supplies that contain high concentrations of nitrates are therefore considered to be a public health danger. [2]

Nitrates can typically enter water supplies in a number of ways, as illustrated in Figure 1. Algae found in soils and surface waters can contribute nitrates through the fixation of atmospheric nitrogen into the form of ammonia which is eventually reduced to nitrate through the action of naturally occurring reducing bacteria. Animal wastes and the decay of organic matter can also provide a source of ammonia for bacterial reduction. Although it is possible for these naturally occurring sources to contribute nitrates in significant quantity to our water supplies, other significant contributions are the result of man made processes. Two principal sources are nitrogen bearing fertilizers and nitrified effluents from sewage treatment plants.

FERTILIZERS AS A SOURCE OF NITRATES

Nitrogen is one of the 16 nutrient elements necessary for plant growth. If any of these elements are not available in the amount required by a particular plant, the growth and yield of the plant will be limited. Many of the 16 elements are required only in trace amounts but nitrogen, phosphorus and potassium are generally extracted from the soil by plants in substantial quantity.

Although nitrogen is abundantly available in the atmosphere (78% by volume) only a few microorganisms are able to assimilate elemental nitrogen directly.[3] Many of the reactions in the nitrogen cycle shown in Figure 1 (nitrification, nitrate reduction and nitrogen fixation) are microbially catalyzed.[4] Among plants, the leguminosae (peas, beans, clover)

have a symbiotic relationship with nodular bacteria which can extract nitrogen directly from the atmosphere for plant growth. Other plants, however, must take up nitrogen in a chemically combined form. Because of the need for abundant quantities of nitrogen for commercial crop production, inorganic fertilizers are used as a source of chemically combined nitrogen in the form of nitrate ions $[\text{NO}_3]^-$, ammonium ions $[\text{NH}_4]^+$ and amino groups $[-\text{NH}_2]$. [3] While inorganic mineral fertilizers are a major reason for modern agricultural productivity, they are also a major source of unwanted nitrates in groundwaters and surface waters.

SEWAGE TREATMENT EFFLUENT AS A SOURCE OF NITRATES

Fecal matter from animals also contain chemically combined forms of nitrogen as well as some other required nutrients, but is mainly composed of carbonaceous materials. While carbon is a required nutrient for plant growth, the quantities available in animal wastes are far beyond the typical amounts required for plant growth, while other required nutrients may not be available at all. Although animal wastes are often used as a natural fertilizer, they are not practical as a source of nutrients for large scale agricultural operations. Human wastes contain pathogens and other disease causing bacteria, which results in this material being undesirable for use as a fertilizer and a potential health hazard if not disposed of properly. Waste waters that contain human wastes are therefore collected and treated prior to discharge into a receiving stream or other body of water.

The contribution of nitrates into the environment from sewage treatment operations is a result of the biological processes used to treat wastewater. These processes convert organic wastes to inorganic forms or to cellular matter. Since nitrogen and phosphorus are essential constituents for cellular activity, wastewater must contain these elements if the biological treatment processes are to be effective. [5] Most of the nitrogen in wastewater originates in the form of ammonia. Discharge of ammonia from wastewater treatment plants will consume oxygen in receiving waters and directly promotes the growth of algae just as effectively as nitrates. Because a major objective for wastewater treatment is to reduce the concentrations of oxygen demanding substances, it is preferable to produce an effluent which has already oxidized ammonia into the form of nitrates. If the receiving streams do not have sufficient hydraulic flow or chemical load carrying capacity to receive a constant flow of nitrates, it may be necessary to provide additional (tertiary) treatment to denitrify the effluent.

Because of the possibility of adverse health and environmental effects from nitrates, it has become necessary to carefully monitor the effluents from wastewater treatment plants for compliance with discharge permit limits. Discharge limits are set on the basis of receiving stream capacity and therefore may differ seasonally.

It has also become necessary to test drinking water for the presence of nitrates, particularly if the source is a surface water that receives agricultural runoffs or treated wastewater effluents. Ground water, particularly from shallow wells at agricultural or industrial sites, can also become contaminated with nitrates and must be regularly tested. The current nitrate limit in drinking water is 10 mg/l (as N). Nitrate contamination is currently the most common reason for shutdown of public water supply wells. [6]

EXISTING EPA APPROVED ANALYSIS TECHNIQUES

Nitrates are currently determined in water or wastewater using one of two basic EPA approved methods. One method measures nitrate only, while the other method uses one of several techniques to measure nitrate and nitrite with eventual subtraction of the nitrite fraction after a separate measurement.

The first method is based on the reaction of the nitrate ion with brucine sulfate in a 13 N sulfuric acid solution at a temperature of 100 degrees centigrade. The color of the resulting complex is measured at 410 nm using a spectrophotometer. Dissolved organic matter, salinity, or the presence of strong oxidizing or reducing agents will cause interferences with this method, resulting in the need for the addition of various reagents

prior to analysis. Absolute temperature control is very important to the proper color development when this method is used.[7]

Two basic methods are approved for measurement of nitrate-nitrite. Both methods use reagents (hydrazine sulfate or a column of granulated copper-cadmium) to reduce nitrate in the sample to nitrite. The nitrite is then diazotized with sulfanilamide and coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride so that the resulting azo complex can be measured at 540 nm using a spectrophotometer. Automated and manual procedures are available for the cadmium reduction method. [8,9,10]

Nitrites are determined by using the azo formation procedure outlined above for spectrophotometric measurement at 540 nm. The presence of strong oxidants or reductants can interfere with these measurements. [11]

None of these methods are well suited for the rapid screening of well water samples in the field or for continuous monitoring of drinking water supplies or wastewater effluents.

Field screening requires a method that does not utilize multiple step sample processing, elaborate glassware, carefully prepared reagents or controlled conditions such as temperature stability. As for continuous measurement, it may be possible to design apparatus that would be able to perform highly automated versions of these procedures but, at best, the analyzer would only be able to perform analysis of side stream samples in batch intervals. Depending upon the procedure in use, the samples would need to be filtered and extensively processed to circumvent possible interferences or contamination of the reagent media. Once time, distance and reagent reactions separate the sample from the body being sampled, it is questionable how well any analytical result will represent actual conditions.

It should also be noted that effluents from community drinking water facilities or wastewater treatment plants almost always will carry residuals of chlorine or other oxidizing agents, which can interfere with many of the standard methods discussed above. If an automated analyzer were to perform an analysis under these conditions, additional sample processing and reagent addition to avoid interference from oxidizing agents would be required.

LABORATORY SPECTROPHOTOMETERS

Some discussion of the laboratory instruments currently used for nitrate analysis may be instructive as a foundation for the coming discussion of newly developed spectrometric methods. Figure 2 shows the typical arrangement of a spectrometer, including a light source, the sample and a detector. In the simplest form, an analysis is performed by measuring the intensity of light transmitted through the sample at a single wavelength. This is typically accomplished by either filtering the light source so that only the desired wavelength passes to the sample or filtering after the sample so that only the desired wavelength passes on to the detector. Frequently, light is split so that one fraction passes through the sample while the other fraction passes through a reference standard or a blank for purposes of comparison with the results from the sample.

Since the analysis is performed using only a single (usually peak) wavelength, a substantial amount of chemical processing must be done prior to analysis so that any possible interference from other chemicals in the sample is suppressed at the wavelength being used for the analysis. Chemical processing is also frequently performed to create or enhance a response from the analyte of interest at the wavelength being used for analysis of the sample. For example, the reagents used in the standard EPA approved method for nitrate analysis are designed to form a complex molecule that shifts the natural absorption spectra of nitrate from the ultraviolet wave range into the visible wave range. These same reagents also act to shift the intensity of the absorption spectra in a manner similar to that illustrated in Figure 3. Reagents of this type are typically called auxochromes. Azo compounds are well recognized for their characteristic nitrogen bonds

measurements of nitrate without the delay caused by extraction, transport and laboratory processing of samples. The system should be capable of continuously analyzing side streams (on-line analysis) or direct detection within the media being analyzed without sample extraction (in-situ analysis). These requirements are difficult to meet using analytical procedures that require the use of reagents, yet the problem of interference from other chemical substances that may be present in the media must somehow be addressed. Even a relatively "pure" well water supply can contain substantial concentrations of calcium, magnesium, iron, manganese, sodium, potassium, bicarbonate, sulfate, chloride, and silica. Surface waters can contain many additional chemical and biological constituents while waste waters will have an even more complex multi-constituent chemistry. Each of these media will present a substantial and highly variable matrix for detection and analysis of nitrates.

IMPROVED SPECTROMETRIC TECHNIQUES

A recently developed technique makes use of the fact that nitrates in water exhibit natural absorption spectra in the ultraviolet wave range. Existing spectrometry techniques use an azo complex to shift this spectra into the visible wave range. The natural nitrate spectra peaks in the vacuum ultraviolet range, but does possess a shoulder that extends into a portion of the ultraviolet range where it is capable of being detected under normal conditions.[13] This is illustrated in Figure 5.

Other chemical substances also exhibit absorption spectra in this wave range. The absorption signature for the water solution being analyzed is a function of the combined spectra from all of the absorbing components in the solution, as illustrated in Figure 6. As noted previously, when interferences are present at a given wavelength either the sample must be manipulated to eliminate the interference or some other portion of the spectra must be used for the analysis. New technology has made it possible to use the entire available portion of the absorption spectra for the solution and to process (manipulate) this information using sophisticated statistical and mathematical models.

A special purpose ultraviolet-visible absorption spectrometer is used for this analysis. This analyzer includes fiber optic probes, silicon photodiode array detectors and embedded processors that apply advanced chemometric algorithms.

The fiber optic probe permits contact with a liquid to be made remote from the analyzer, such as in a flow stream, process tank or monitoring well. The probe can be designed to accommodate a continuous flow from a side stream as shown in Figure 7 or a probe can be designed to be inserted into a well or other reservoir as shown in Figure 8. This allows an analyzer to operated in a relatively benign environment remote from actual contact with the liquid media being analyzed. Only the optical probe is in contact with the media.

Array detectors permit simultaneous scans of absorption spectra over a wide range of wavelengths, without the need to mechanically adjust optical components for each wavelength to be detected. Up to 1024 wavelength intervals from 200 to 800 nm can be simultaneously detected. This allows a larger number of features from the absorption spectra to be captured with excellent resolution and available for nitrate analysis.

Chemometrics makes it possible to accurately estimate the contributions from individual chemical constituents to the absorption spectra for the entire mixture. Chemometric models employ a three step process consisting of the quantification of spectral information for the solution, the preprocessing of this information using one of several available techniques, then the analysis of this processed information using a preselected mathematical model or statistical technique. The selection of wavelengths, preprocessing techniques and analytical techniques that provide the best prediction is performed in a manner similar to that outlined in Figure 9. A number of samples with known concentrations of the analytes of interest and (known or unknown) random

that can result in the shifting of absorption spectra for a chromophore to longer wavelengths and higher intensities. [12]

The ability of auxochromatic reagents to shift absorption spectra for an analyte is particularly important if only a single peak wavelength is being used for the analysis. When only one wavelength is being observed, any other chemical substance in the sample that has natural absorption spectra with features that overlap the wavelength under observation will cause an inaccuracy. Since the absorption at any one wavelength is influenced by all of the components that have some portion of their absorption spectra at that wavelength, there are two basic strategies to overcome this problem. Either the sample must be manipulated to eliminate the interference or the analysis must look at some other some other portion of the spectra for the analyte that is not affected by the other chemicals if one exists.

While conventional spectrometric methods commonly observe only a single peak wavelength, it should be recognized that the absorption spectrum for a chemical substance in a liquid solvent exists over a range of wavelengths as illustrated in Figure 4. There is, therefore, a substantial amount of information that is available for analysis but not used for simple forms of spectrometry. If more of the available information is to be used, a more sophisticated instrument is needed to detect and analyze multiple wavelength information.

A more sophisticated laboratory spectrometer may be able to be adjusted for operation at several different wavelengths through the use of several alternative light sources, several alternative detectors, mechanical adjustment of the filters, or some combination of these techniques. The purpose of these adjustments is to permit multiple wavelength analysis of samples and also for the flexibility of being able to accommodate many different analytical procedures using the same instrument.

Highly sophisticated research spectrometers use broad band light sources with diffraction gratings to separate light into individual wavelengths after transmission through the sample. Small mechanical adjustments to the optical components, usually controlled by a computer, allow the operator to select a specific wavelength of interest or to sequence through a range of individual wavelengths. As one would expect, these capabilities add appreciably to the cost and delicacy of the instrument.

REQUIREMENTS FOR REAL TIME NITRATE ANALYSIS

There are several reasons why a need exists for analysis of nitrates using "real time" (on-line or in-situ) techniques:

1. for continuous monitoring of nitrification/denitrification processes at wastewater treatment plants so that real time information can be used for process optimization and control.
2. to predict chloramine formation in conjunction with chlorine disinfection at water and wastewater treatment facilities.
3. to monitor compliance with nitrate discharge limits and maintain a continuous record of discharge concentration fluctuations at wastewater treatment facilities.
4. to continuously monitor the quality of drinking water supplies and initiate treatment or diversion of nitrate contaminated water.
5. for rapid screening of surface or well waters for the presence of nitrates.
6. for continuous monitoring and/or control of other chemical processes where nitrates are a significant control variable.

These needs call for an analytical system that is capable of providing reliable

variations of other constituents that may be present are processed as a learning (calibration) set to establish spectral differences against a variable background. This information is also used to establish the coefficients and weighting factors that will be used in the various analytical models. Preprocessing, such as illustrated in Figure 10 is used to reduce noise and to amplify differences in otherwise closely grouped spectra.

The BI-800 Ultraviolet-Visible Absorption Spectrometer (UVAS) was developed specifically for the purpose of performing real time multiple component chemical analysis in liquids. A block diagram of the analyzer is shown in Figure 11. One of the first applications for this technology was to demonstrate the feasibility of performing on-line analysis of certain chemical constituents in a hydroponic plant growth nutrient solution for NASA. This hydroponic solution includes all 16 of the nutrients that are necessary for plant growth, including nitrates.

RESULTS FROM ANALYTICAL EXPERIMENTS

Under a recent NASA study [14] an experiment was performed to evaluate the ability of UVAS methods to identify nitrates in a multiple component nutrient solution without the use of reagents. The experiment used a total of thirty hydroponic nutrient solution samples that contained known nitrate concentrations ranging from 0 to 500 ppm and random concentrations of the other 15 nutrients. A total of 26 samples were processed as a learning set and the remaining four were used as a test set. Following processing of the learning set, it was concluded that excellent results were being obtained with a model that used stepwise regression of untransformed absorbance data, and very good results were also being obtained with regression of first derivatives and regression of Walsh transforms. The test sets were processed using stepwise regression of raw, first derivative and Walsh transformed absorbance data, resulting in some remarkably accurate predicted concentrations for nitrate in the test set. The average error of prediction for both raw (untransformed) and first derivative transformed absorbance was less than 1.0 ppm over a range of 10 to 400 ppm in the test set, although slightly better results were obtained using the first derivative. Selected spectra from the nutrient solution learning set for this experiment is shown in Figure 12.

Based on the NASA experiments, the researchers concluded that it was possible to accurately predict nitrate concentrations in multi component solutions using absorption spectrometry in the ultraviolet wave range without the assistance of reagents. In a related experiment for the same project, dissolved iron concentrations were also accurately predicted in nutrient solutions using UVAS methods without reagents.

In an independent series of experiments, untreated ground water was obtained from a well in southeast Wisconsin with a depth in excess of 50 meters. Samples of this water were separately spiked with known concentrations of ammonia and other contaminants, then scanned for absorption spectra in the ultraviolet wave range. Relatively weak spectra was observed for ammonia even though the concentrations analyzed were fairly substantial (25 to 100 ppm). Most of the feature variation was observed in the 200 to 250 nm range. The spectra appeared to show increased quenching of absorption spectra with decreased concentrations of ammonia. It should be noted that the foregoing is based on visual observation of the spectra and that the use of chemometric techniques is often able to reveal information that is otherwise unable to be detected through visual observation. A plot of ammonia spectra from this experiment is shown in Figure 13.

In the most recent experiment, groundwater samples from the same well as the ammonia experiment above were spiked with known concentrations of nitrate, then scanned for absorption spectra in the ultraviolet wave range. Observable shifts in the absorption spectra for the groundwater were observed with as little as 1 ppm of nitrate added. The spectra exhibits excellent separation and substantial intensity over the 1 to 10 ppm range, which is the concentration range of greatest interest for field screening of well water. The 220 to 250 nm range appears to show good potential for this analysis. A plot of nitrate spectra from this experiment is shown in Figure 14.

The ammonia and nitrate experiments are additional confirmation that on-line and in-situ analysis of nitrates using UVAS techniques without reagents is a feasible technology.

In order to use this technique it is currently necessary to "calibrate" the analyzer by observing a fair number of samples with known nitrate concentrations and varied background contributions, similar to the method used in the NASA experiments. This requirement renders the technique impractical for quick site to site screening, since it is unlikely that a learning set would be able to be obtained and processed for each site. Calibration could be performed for use of the technique at fixed locations, even locations with highly variable backgrounds such as water and wastewater treatment plants, since the benefits from long term continuous use greatly outweigh the initial investment in a learning set that is large enough to incorporate a wide range of analyte and background variations.

Techniques are currently under development that will permit field analysis of nitrates and other analytes using UVAS but without the need for the processing of a large number of site specific learning sets. These techniques involve the use of mathematical techniques to simulate background variations during calibration and dampen the effects of background variations encountered in actual use. If successful, these techniques should permit the use of UVAS methods for field screening of well water and surface water as well as intermittent screening of process flows without the need for elaborate site specific learning sets.

Although additional testing in the field needs to be conducted, the researchers have concluded that the analysis of nitrate in waters that contain multiple chemical constituents is possible using Ultraviolet-Visible Absorption Spectrometry (UVAS) without reagents if algorithms that allow for background variations are used for the analysis.

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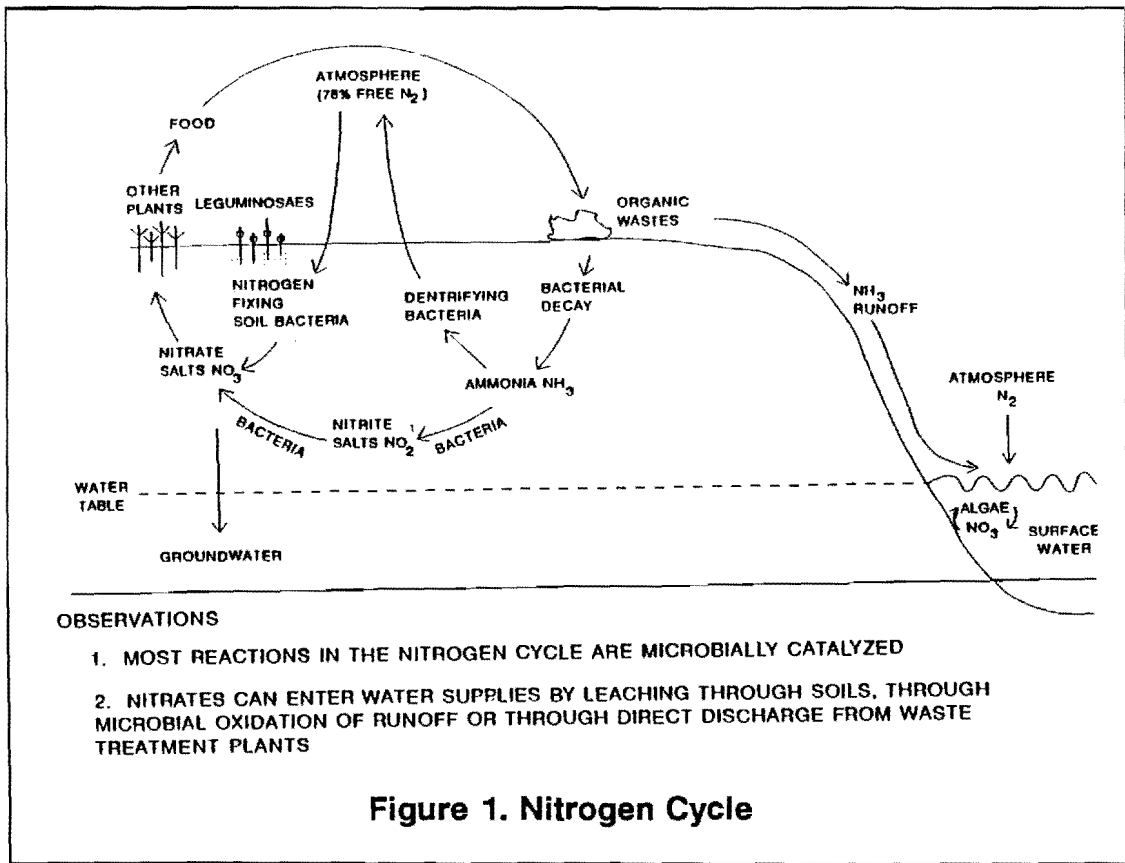


Figure 1. Nitrogen Cycle

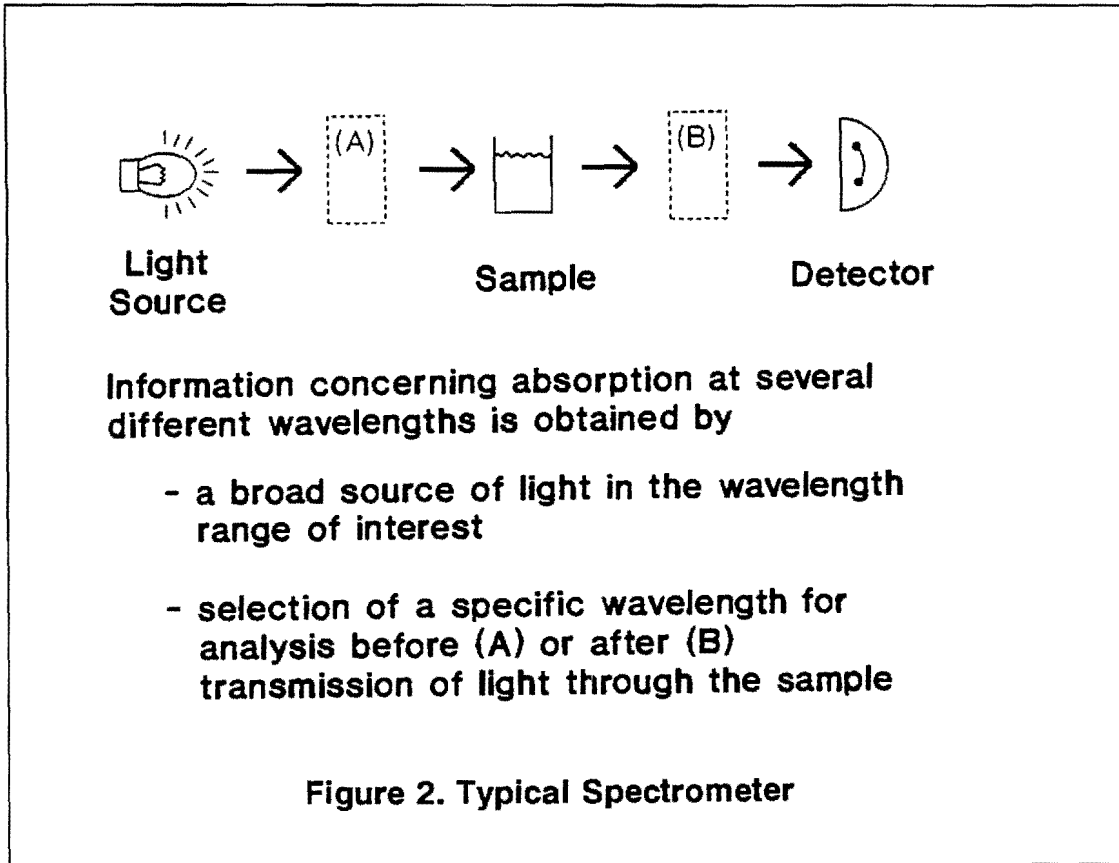
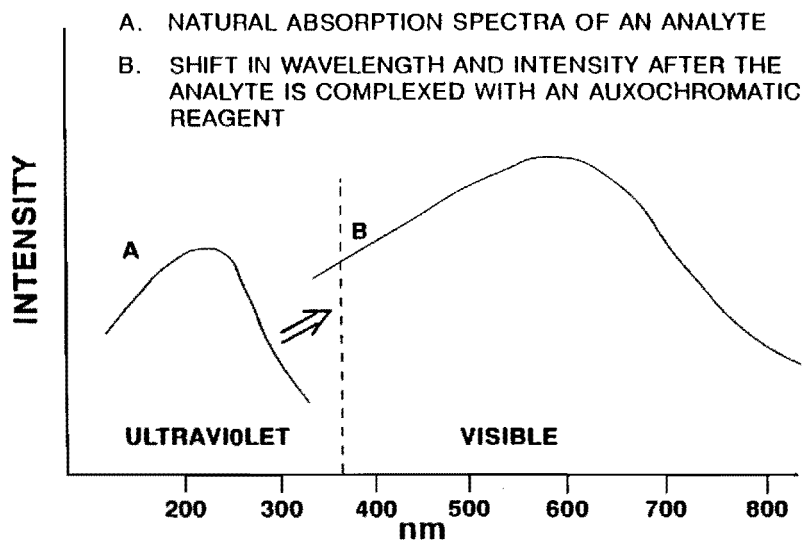


Figure 2. Typical Spectrometer



REAGENTS ARE OFTEN USED IN PEAK WAVELENGTH ANALYSIS TO SHIFT THE ABSORPTION SPECTRA OF AN ANALYTE FROM ONE WAVEBAND TO ANOTHER (OFTEN FROM THE ULTRAVIOLET TO THE VISIBLE) OR TO INCREASE THE INTENSITY OF SPECTRA BY CHANGING THE POSITION OR SHAPE OF THE SIGNATURE.

Figure 3. Auxochromatic Shifts

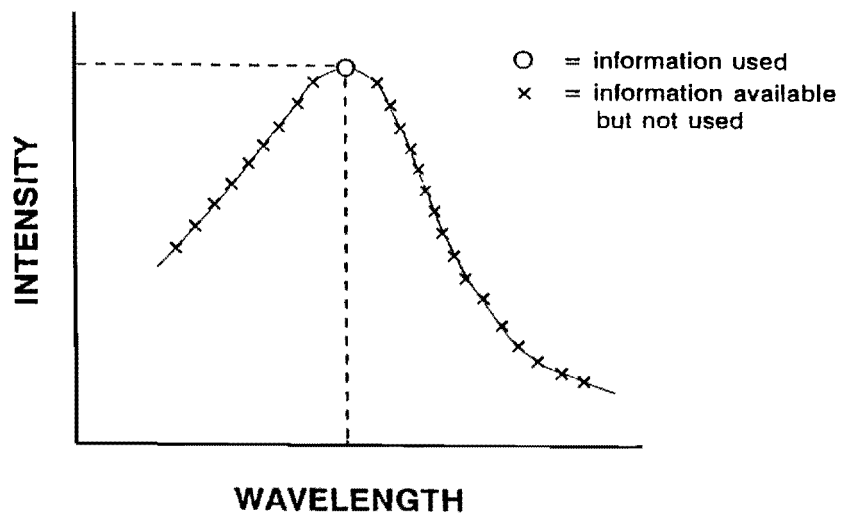


Figure 4. Peak Analysis

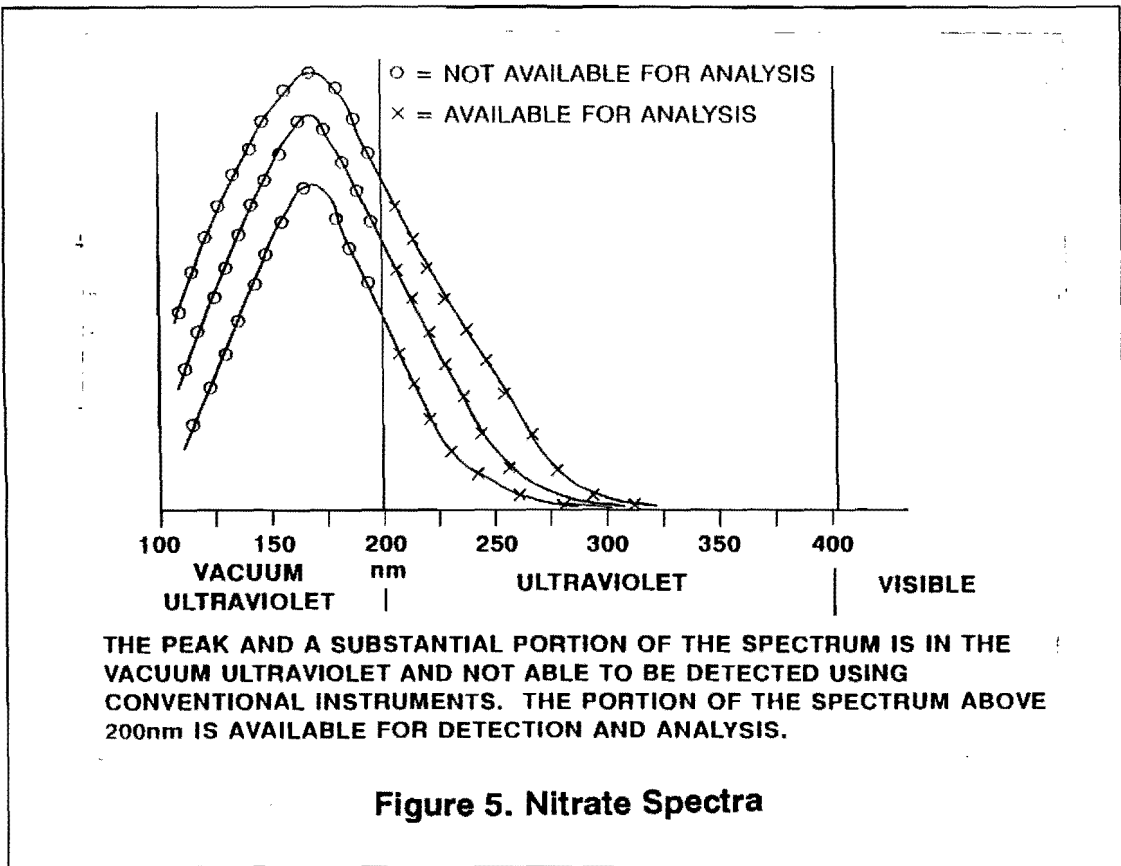


Figure 5. Nitrate Spectra

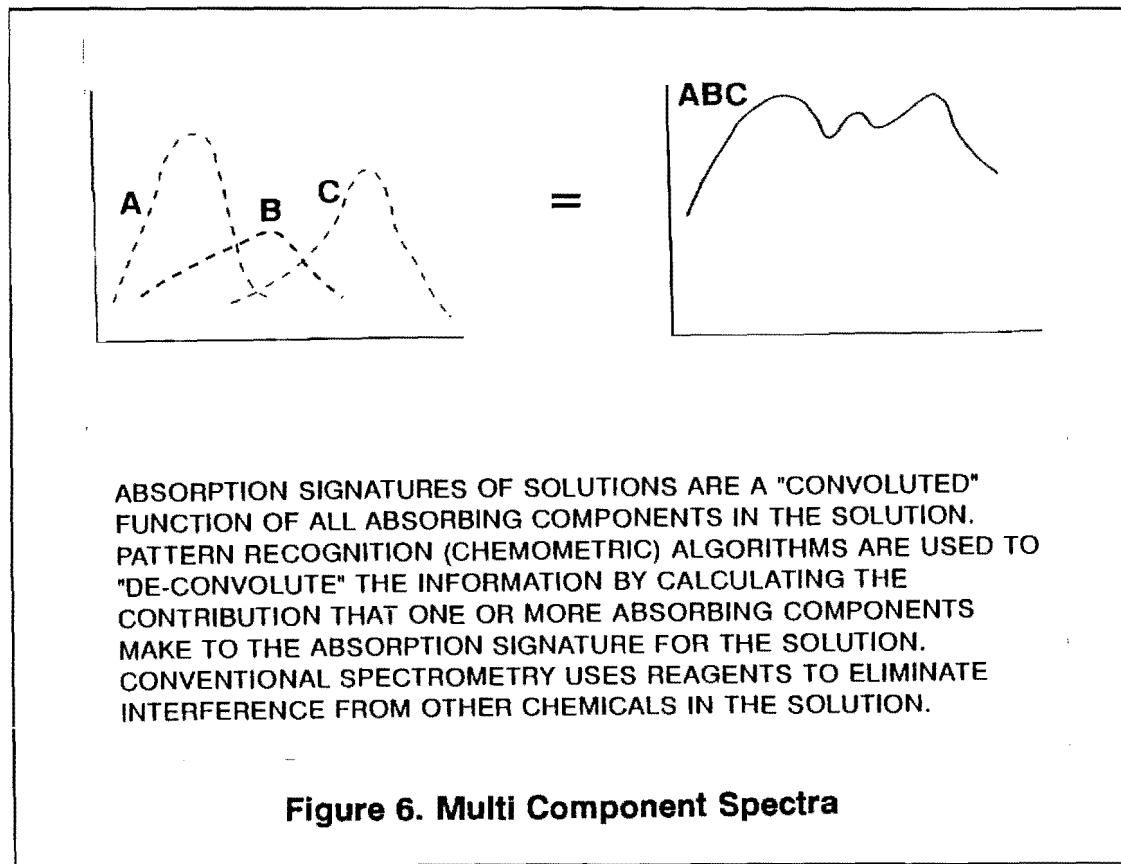


Figure 6. Multi Component Spectra

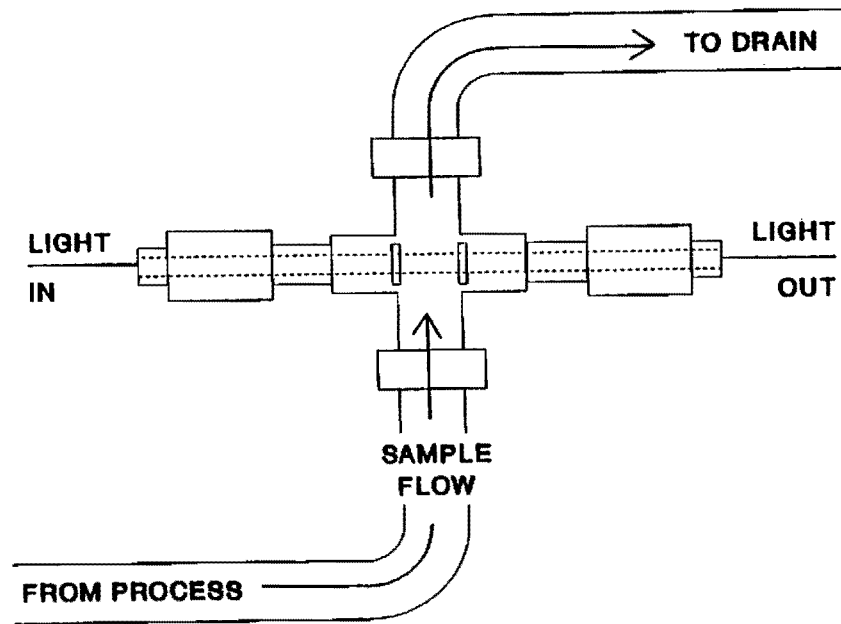


Figure 7. Flow Through Cell

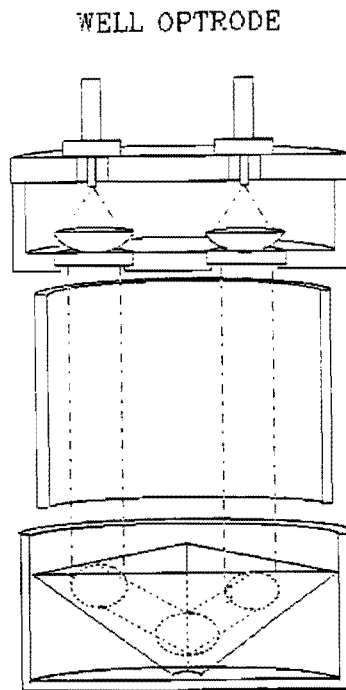


Figure 8. Well Optrode

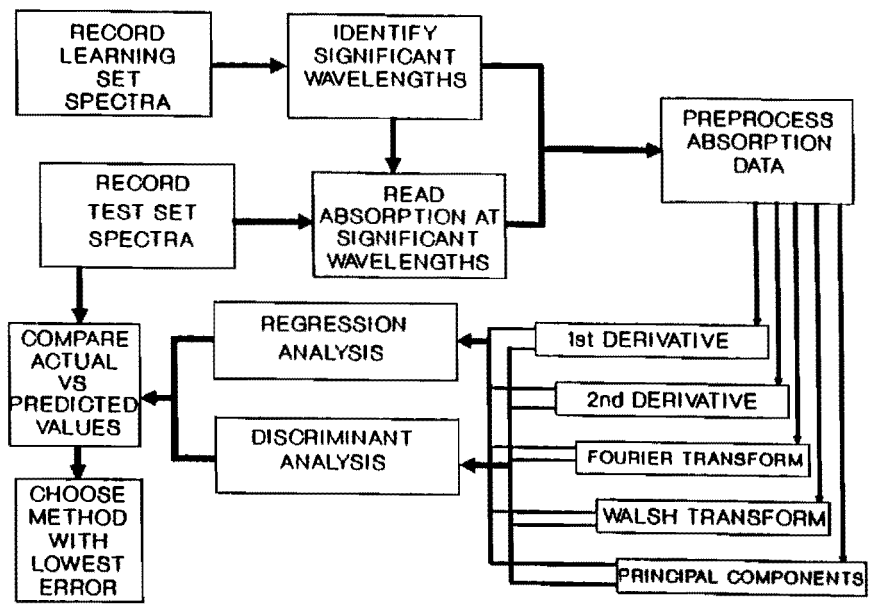


Figure 9. Analytical Model

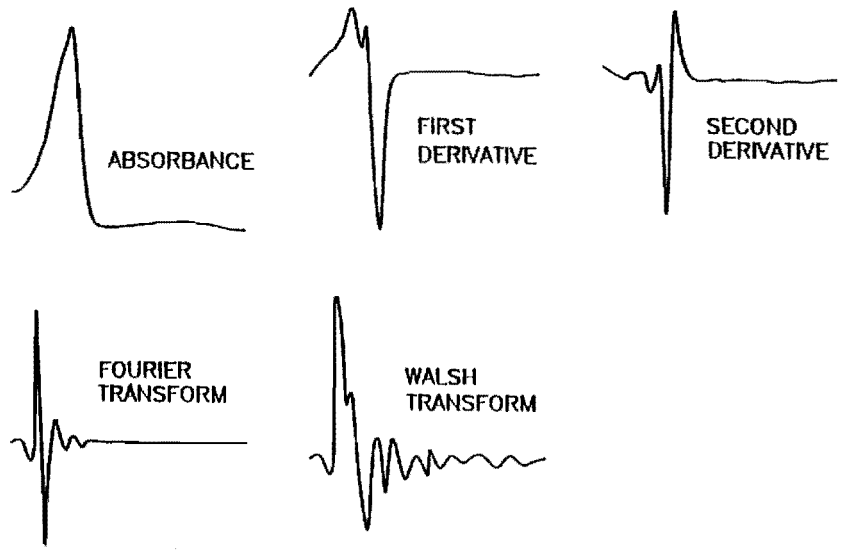


Figure 10. Preprocessed Spectra

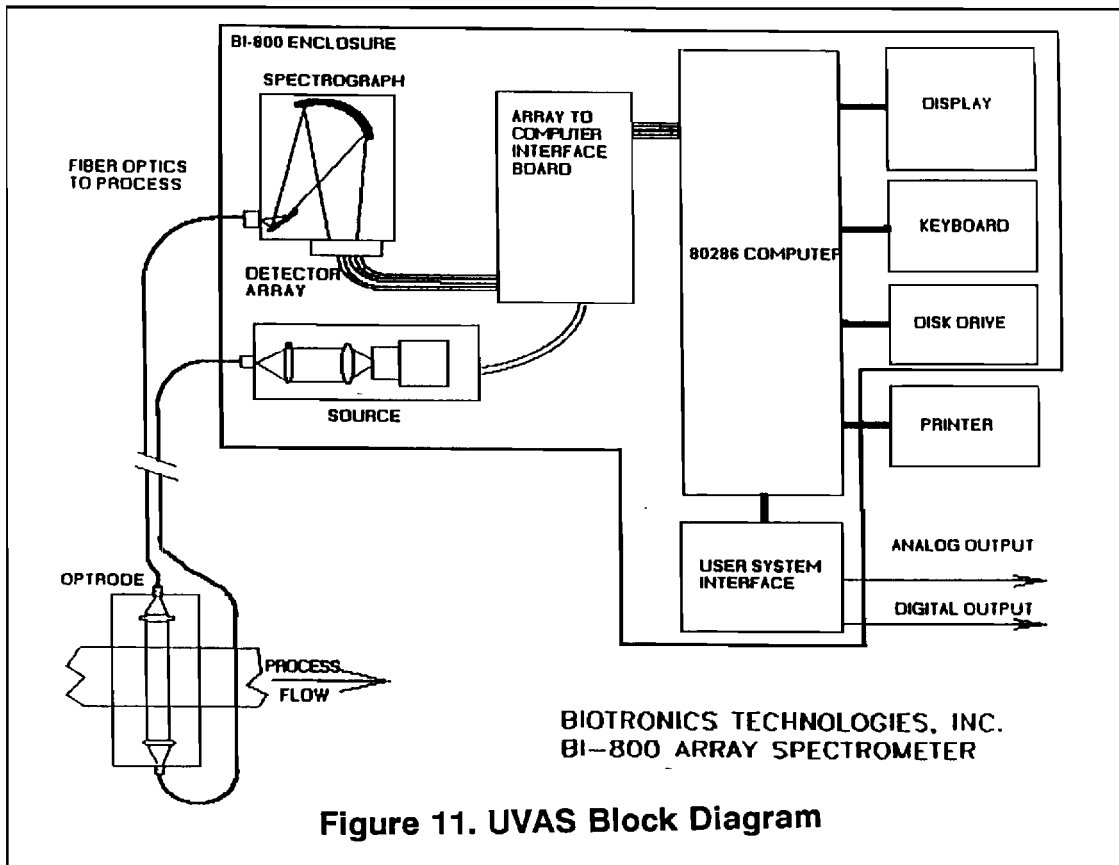


Figure 11. UVAS Block Diagram

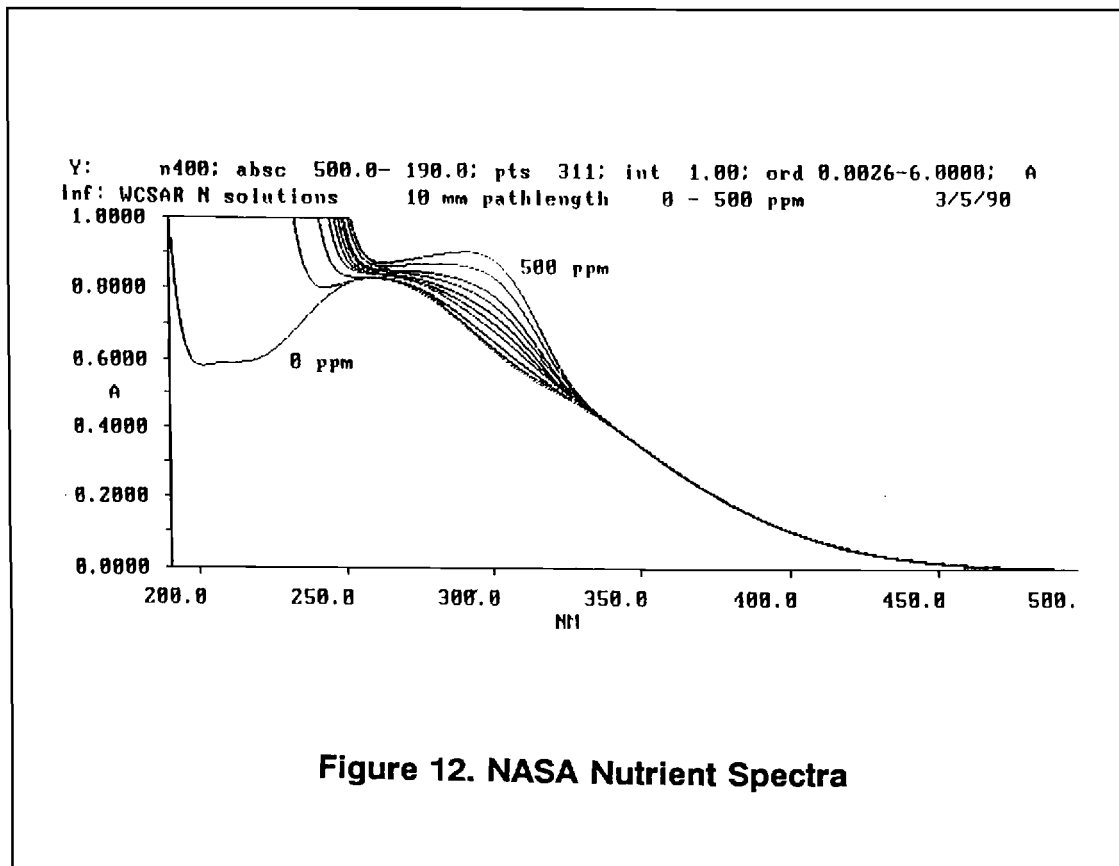


Figure 12. NASA Nutrient Spectra

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inf: Ammonia in wellwater 10 mm path 5/6/91

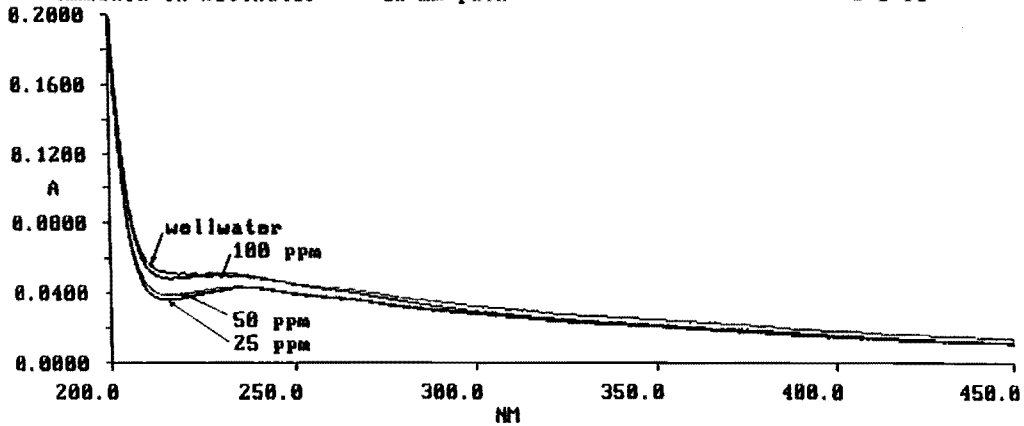


Figure 13. Ammonia in Groundwater

X: well1; absc 450.0- 200.0; pts 251; int 1.00; ord 0.0094-0.5022; A
inf: Nitrates in Wellwater 10 mm pathlength 11/6/91

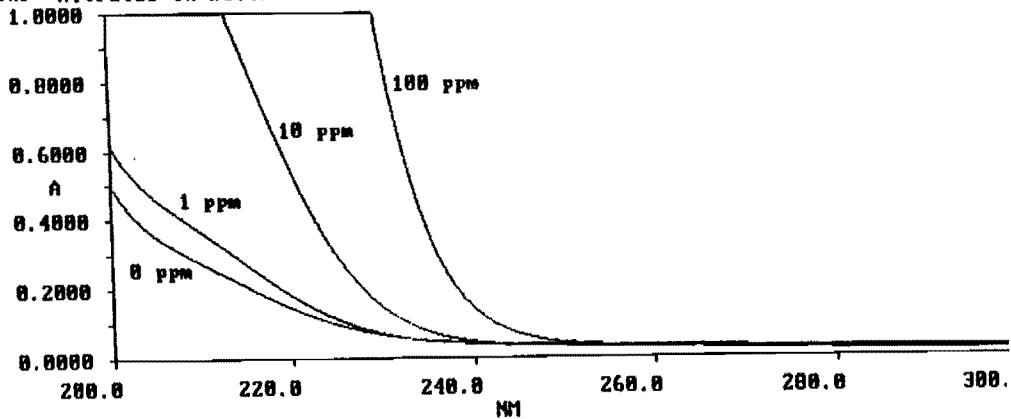


Figure 14. Nitrate in Groundwater