UV-Visible (and IR) Spectrophotometry

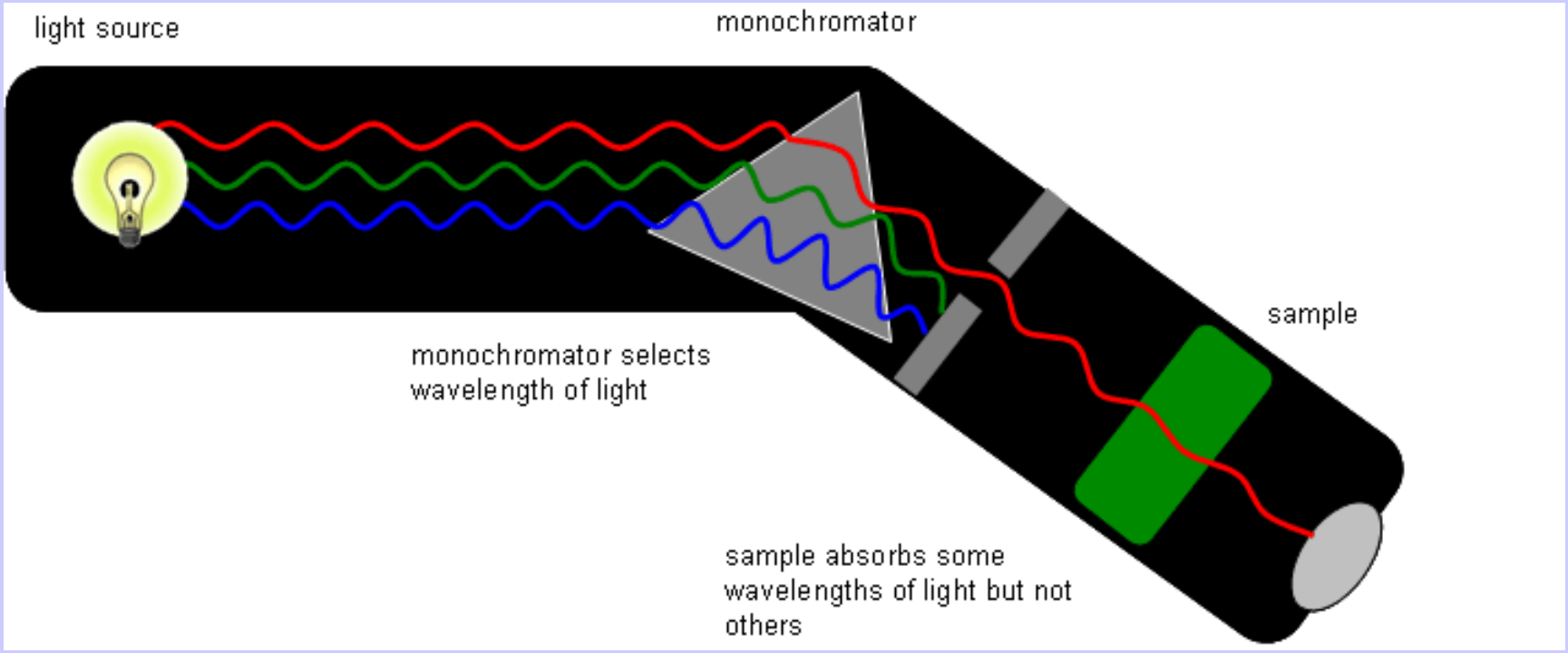
OCN 633

Fall 2013

UV-Visible Spectrophotometry

- Technique based on absorption of light
- Sample (analyte) is exposed to a beam of light
- Sample absorbs light…
- Instrument measures transmitted light
- Concentration of analyte is proportional to the amount of light absorbed

UV-Visible Spectrometry



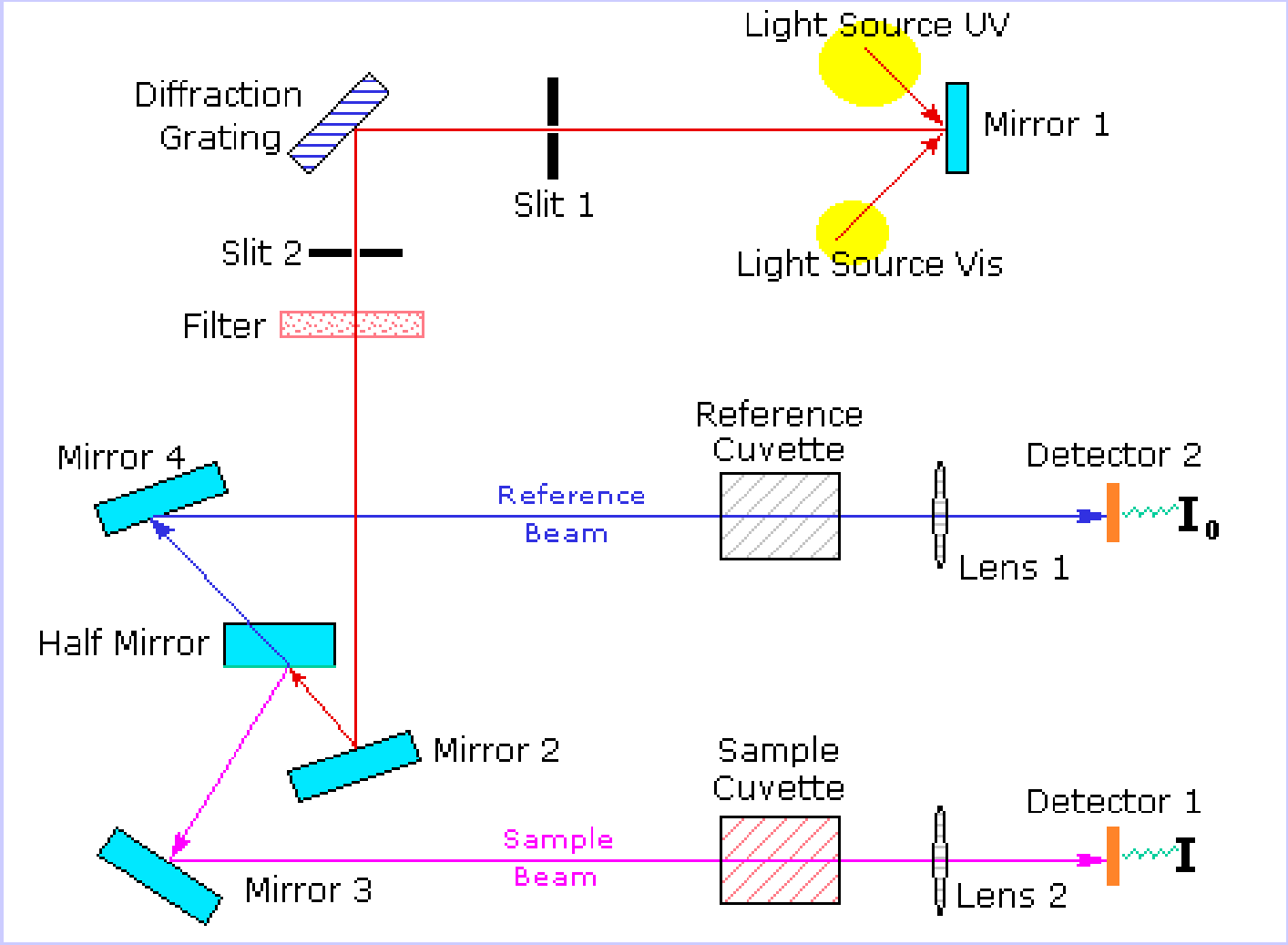
Absorption/Electronic Transitions

- Atoms (and ions) have finite permissible electronic transitions and absorb/emit monochromatic radiation
- Complex ions and molecules have **multiple** possible electronic transitions owing to many overlapping molecular orbitals
- Complex ions and molecules absorb (or emit) light over a wider range of wavelengths.
- This is known as **“broad band”** absorption (emission).

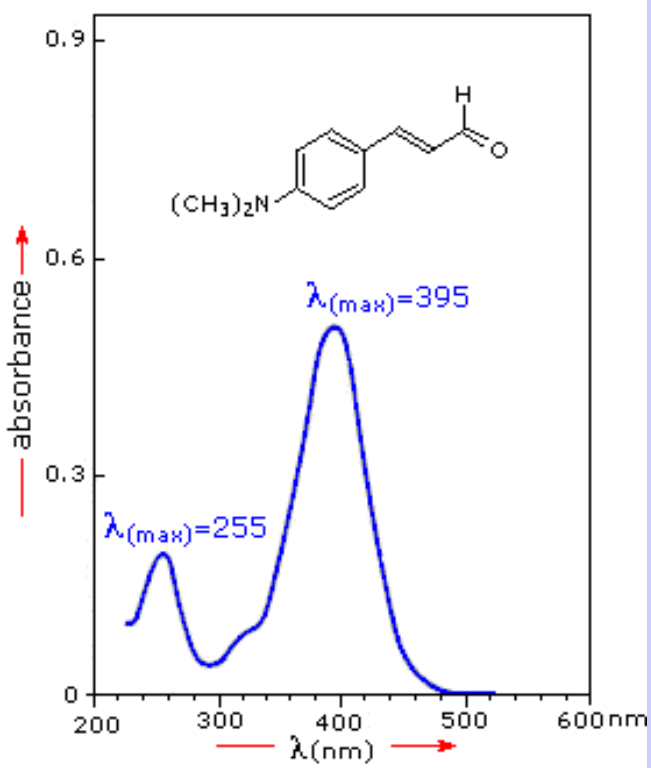
Beer Lambert Law

- States that absorbance of electromagnetic radiation by a given species is directly proportional to the concentration of the analyte.
- It is expressed as: **$A = \varepsilon bC$**
- where A is the absorbance, ε is the molar absorptivity, b is the path length and C is the concentration of analyte.
- Because **ε** and b are fixed under experimental conditions the result is a linear relationship between absorbance and concentration.

Basic UV-VIS instrument



Absorption Spectrum



For the spectrum above, a ($1.42 \bullet 10^{-5}$ M) solution the aldehyde in 95% ethanol was placed in a 1 cm cuvette for measurement. Using the Beer Lambert Law formula, $\varepsilon = 36{,}600$ for the 395 nm peak, and 14,000 for the 255 nm peak.

Measurement of Absorbance

- Absorbance is not directly measurable
- Instead measure “transmittance”, the fraction of incident radiation transmitted by the solution

$$\mathbf{T = I/Io}$$

Where T = transmittance, Io = Incident radiation,

I = exiting (transmitted) radiation

- Absorbance is:

$$\mathbf{A = -\log T = \log (Io/I)}$$

Processes affecting T

- Reflection loss at air/cuvette interface
- Scattering losses in solution
- Absorption by analyte
- Absorption by cuvette material
- Absorption by interfering species

Application of Beer's Law to Mixtures

- Beer's law also applies to solutions containing more than one absorbing species
- In such cases the total absorbance is the sum of individual component absorbances

$$\mathbf{A_T = A_1 + A_2 + A_3 + A_4 + \ldots A_n}$$

Limitations to the Applicability of Beer's Law

- Few exceptions to generalization that A is linearly related to path length
- Deviations from direct proportionality between A and C at fixed b are frequent
- Some of these are fundamental and represent real limitations of the law
- Others are a consequence of how the measurements were made… (instrumental)
- Others include chemical changes associated with concentration changes (chemical deviations)

Limitations

- Beer's law typically adhered to if C < 0.01M
- Limitations also depend on value of **ε**
- High **ε** will limit applicability to very low conc.
- Low **ε** will allow application to higher conc.
- Part of concentration limitation is due to potential for species (at high concentration) to interact in solution and change how they interact with light
- Applies to same species and to others (electrolytes)
- Interaction between different species also impacts applicability

Chemical Deviations

- If analyte dissociates (e.g., weak acid/base)

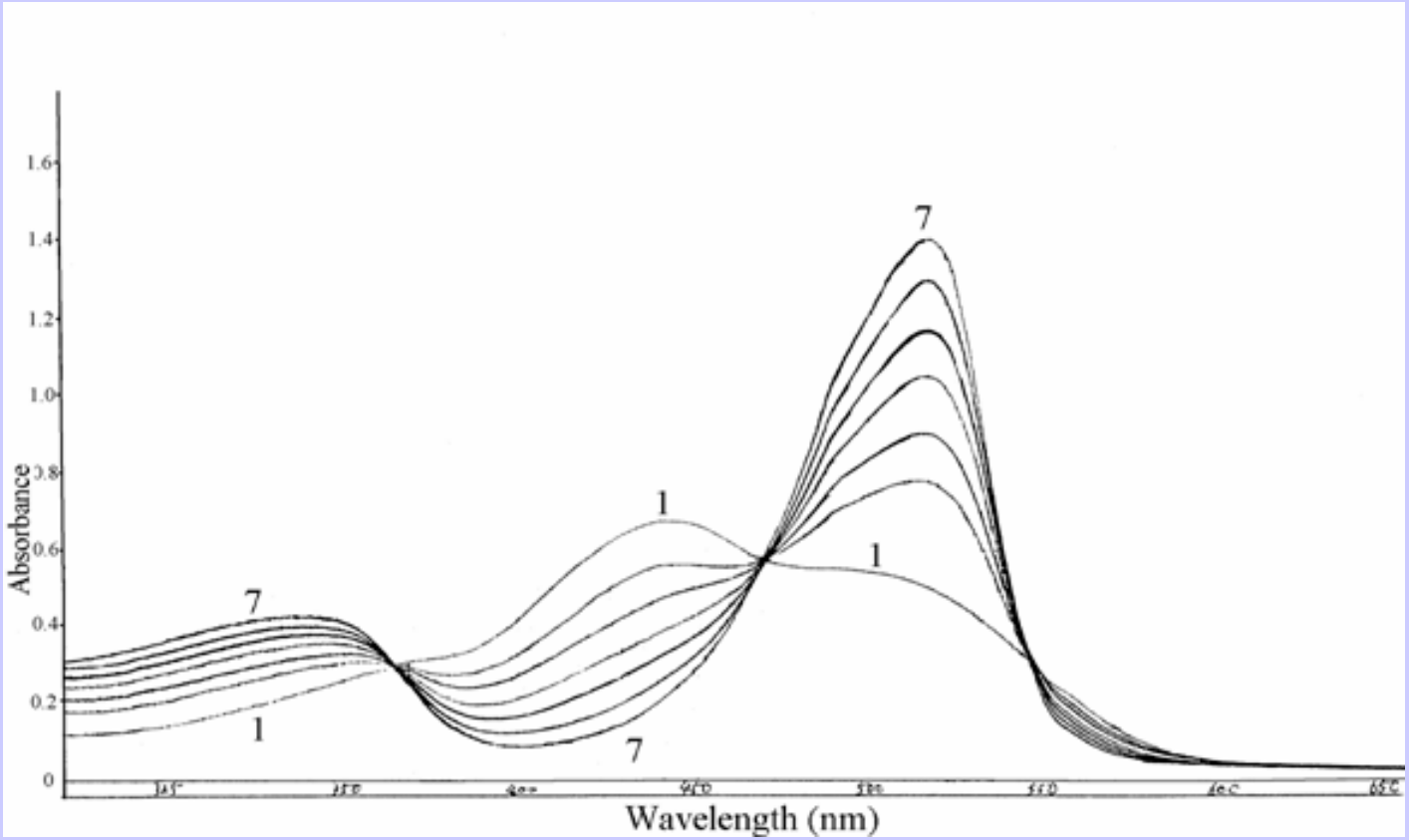
$$HIn \rightarrow In^- + H^+$$

- Undissociated and dissociated forms have different colors

  ➔ different forms absorb differently (i.e., at different wavelengths)

This is used to advantage in the spectrophotometric determintaion of pH

Variations in Spectra as f(composition)



Analytical Applications

- pH determination (use of indicator dye)
- Nutrient analysis
- Organic compound analysis
- Metals analysis (complexes)
- Gas analysis (IR, e.g., CO_2)
- Pharmaceutical Industry

Spectrophotometric pH Determination

- This application depends upon addition of a very small amount of indicator (HIn) dye to the solution whose pH you wish to determine.
- The organic indicator used is a weak/acid base which dissociates depending on its K_a and the solution conditions

“Spec” pH

- Many organic dyes are weak acids/bases ➔ the partitioning between HA and A^- is a function of pH

$$HA + H_2O \leftrightarrows H_3O^+ + A^-$$

$$K_a = [H_3O^+][A^-]/ [HA]$$

$$pH = pK_a + \log [A^-]/[HA]$$

- Thus if the K_a is known and you can measure the [HA] and $[A^-]$ the pH can be calculated

“Spec” pH

- Typically the dissociated and undissociated forms of the dye have different colors… i.e., they absorb light at different wavelength

- The ratio of the two forms of the dye can be determined by measuring the absorbance of the solution at the two wavelengths characteristic of the undissociated and dissociated “colors”

“Spec” pH

- The absorbance of the solution at a wavelength is equal to the sum of the absorbances of the individual components in a mixture. For two overlapping components the absorbance must be measured at two wavelengths

$$A_1 = \varepsilon_{a1} b C_a + \varepsilon_{b1} b C_b$$
$$A_2 = \varepsilon_{a2} b C_a + \varepsilon_{b2} b C_b$$

subscripts 1 and 2 indicate the two wavelengths and the subscripts a and b indicate the acid and base forms of the dye

“Spec” pH

- See SOP 7 from the Guide for OA Practices (I sent you the pdf)
- Theoretically you should be able to determine pH to 0.001-0.002 precision
- Accuracy, however, is usually 5 to 10X worse
- In lab, Bobby will demonstrated our simple system…

Applications of UV-VIS Spectrometry to Nutrient Analysis

- Applicable to natural waters or wastewater
- High regulatory and research importance
- Analysis of N species (NO_2^-, NO_3^-, NH_4^+)
- Analysis of PO_4^{-3}
- Analysis of $Si(OH)_4$
- Determination of Organic N and P
- Various operationally defined forms (TKN, or total N by persulfate oxidation)

Autoanalyzer Detection Limits in Seawater

- Ammonia 0.025 μM
- Nitrate 0.005 μM
- Nitrite 0.005 μM
- Phosphate 0.0014 μM
- Silicate 0.015 μM

Note: actual limits of quantification are typically 10x greater than listed above

Silica

- $Si(OH)_4$ chemistry is based on formation of silico-molybdate blue complex by reduction with ascorbic acid
- Oxalic acid is added to remove interference from PO_4^{-3}, which also forms a molybdate blue complex
- Tannins, high Fe and sulphide interfere
- Reaction chemistry is temp. sensitive
- Absorption maximum at 820 nm

Phosphate

- PO_4^{-3} chemistry is based on formation of a phospho-molybdate blue complex
- Also use ascorbic acid reduction
- Heating sample increases rate of color development
- Absorption naximum at 880 nm
- Need to prepare blanks by precipitating out any PO_4^{-3} with hydrolyzed Fe^{3+} solution

Nitrate

- NO_3^- analysis requires reproducible reduction to NO_2^-
- Reduction is performed by passing sample across a Cd-Cu column
- Most surface seawater has <0.3 µM NO_2^- and it is generally non detectable in deep water
- NO_2^- color forming chemistry is based on reaction with sulfanilamide and naphthylethylenediamine-dihydrogen chloride (NED)
- Absorption maximum at 540 nm

Ammonia

- Method based on Berthelot-reaction where NH_4^+, phenol and hypochlorite (ClO^-) react, under alkaline conditions, to form indophenol blue
- Cigarette smoke is a source of NH_4^+ and should be avoided
- NH_4^+ is volatile so should minimize exposure of samples, reagents and standards to air
- Sample storage is thought to be an issue…
- Cannot analyze samples low in NH_4^+ when doing NO_3^- analysis because of use of NH_4Cl buffer

Special Precautions

- Multiple precautions are necessary for (low nutrient level) seawater analysis
  - Acid washing of all labware with HCl
  - No use of HNO_3 anywhere in lab
  - Avoid use of glass (SiO_2 problems) except for NH_4^+ analysis
  - Matrix matching of samples and standards (either use 3.2% NaCl or nutrient-free seawater)
  - Note that human skin is a potentially significant source of contamination
  - Store filtered samples frozen (if not analyzed immediately)
  - High SiO_2 samples should be thawed for at least 24 hrs because of polymerization issues (or diluted prior to storage)

Continuous Flow Analyzers (CFA)

- Chemistry is controlled by multi-channel peristaltic pump to regulate flow of sample and reagents
- Sample flow is “segmented” with air bubbles to enhance mixing of reagents and samples and to reduce smearing of samples
- Between 20 and 100 segments of liquid are separated by bubbles as they flow sequentially through the tubing

CFA (II)

- An autosampler probe moves between sample cups and a reservoir of wash solution, which also serves to generate a baseline response
- The sample/reagent mixture flows through mixing coils & reacts to produce color complexes in proportion to the concentration of the nutrient
- Depending on the method, a heated coil increases reaction temperature & helps develop color

CFA (III)

- Samples with developed color flow through a colorimeter to measure the color intensity of the solution
- Output of instrument is an analog voltage, which is proportional to absorbance
- Response (color) is calibrated with solutions of known nutrient concentrations…

Autoanalyzer Components

- Technicon AA-II (or III) consists of six components
  - Automated sampler
  - Perstaltic pump
  - Analytical cartridges
  - Colorimeters
  - Chart recorders (or electronic counterpart)
  - Computer to drive system/record data

Technicon (now Bran-Leube) AutoAnalyzer

2-channel AutoAnalyzer 3

UH APNA

- The high maintenance toy…
- *In-situ* system designed for profiling but now being used for moored applications
- Simultaneously runs five channels
- Five analytical spectrometers, 2 reference spectrometers
- Deployable (ideally) for several weeks (not in Hawaii because of warm waters)
- Important for coastal projects examining transfer of materials between land and sea, productivity

Choice of Optical Cell Path Length and Analytical Ranges

Analyte	1cm Cell	15 cm
Nitrite	**0 - 60.0 μM**	**0 - 10 μM**
Nitrate	**0 - 60.0 μM**	**0 - 10 μM**
Phosphate	**0 - 60.0 μM**	**0 - 5 μM**
Silicate	**0 - 60.0 μM**	**0 - 10 μM**
Iron (II)	**0 - 60.0 μM**	**0 - 5 μM**
Ammonium	**Fluorescence**	**0 - 20 μM**

APNA Wavelengths

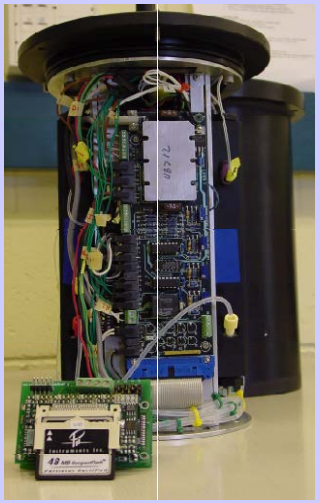
Nutrient	Wavelength
Nitrite	540 nm
Nitrate	540 nm
Iron (II)	560 or 540 nm
Phosphate	880 nm
Silicate	820 nm
Ammonia	Excite 370, Emit 470 nm

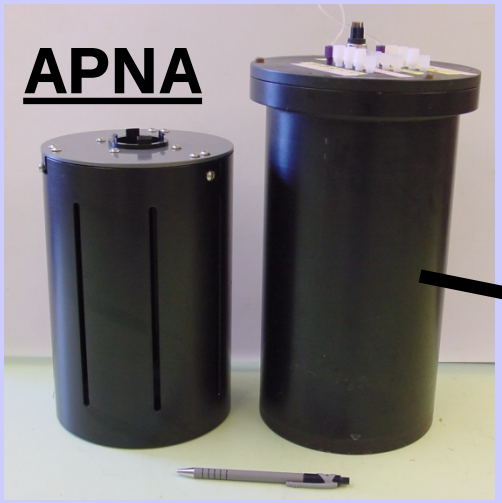
APNA Components

APNA consists of several components:

- Autonomous Profiling Nutrient Analyzer (APNA) - submersible multi-channel reagent delivery module with multiple ChemStar electro-optical detectors
- A submersible flooded, reservoir for reagents and standards
- The LabView Graphical Interface (MS Windows) operating on a host computer
- A Deckbox with test cable for power and communications
- A Pelican Case for storage and shipping

APNA on URI Profiler



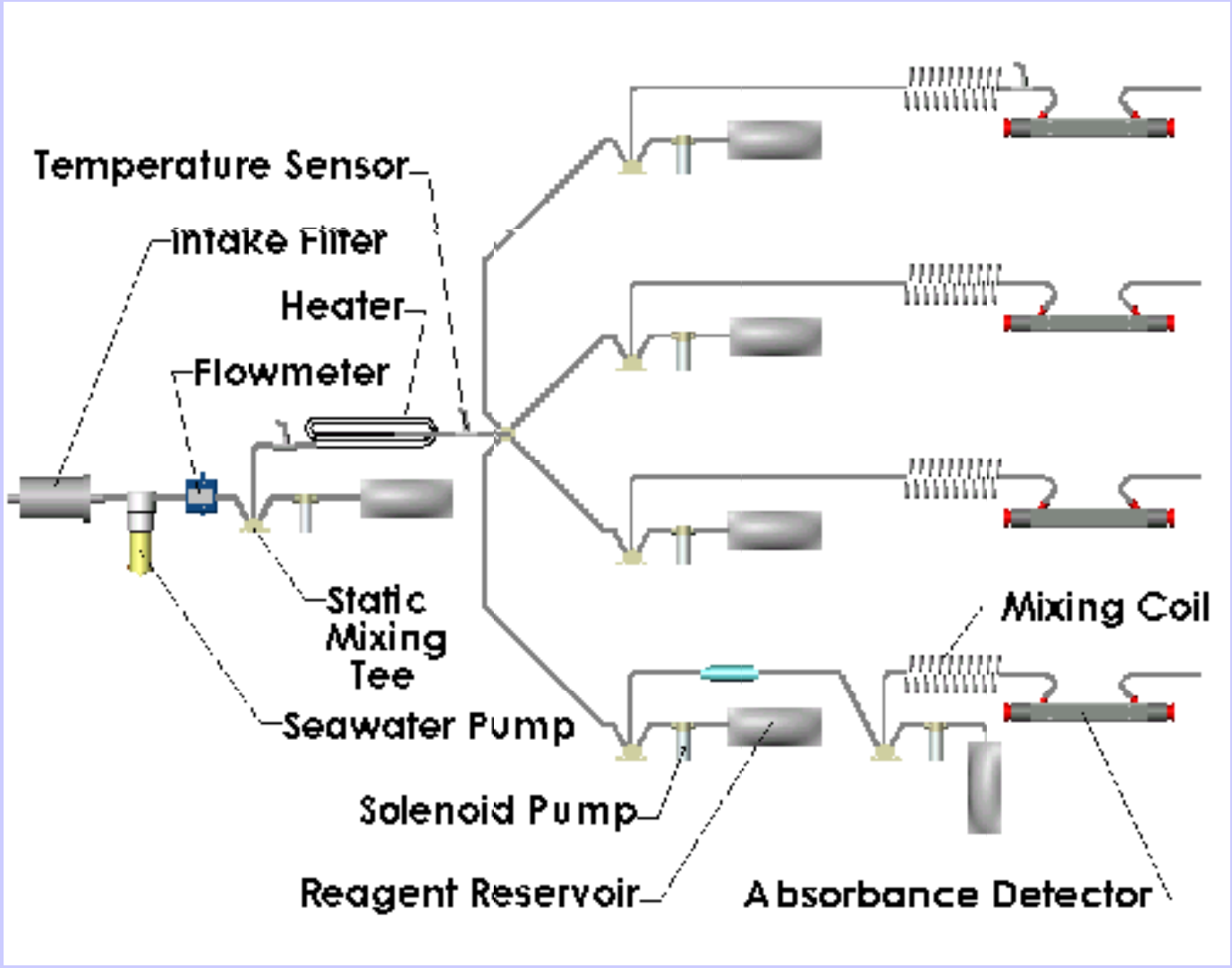


2005

ORCAS IOPC_ Profiler

- **Multi-channel chemical analyzer**
- **Autonomous or cabled profiling**
- **NO_3^-, NO_2^-, PO_4^{-3}, $Si(OH)_4$, NH_4^+**
- **2-4 week duration**
- **Nutrient data telemetry**

APNA Continuous Flow Microfluidics



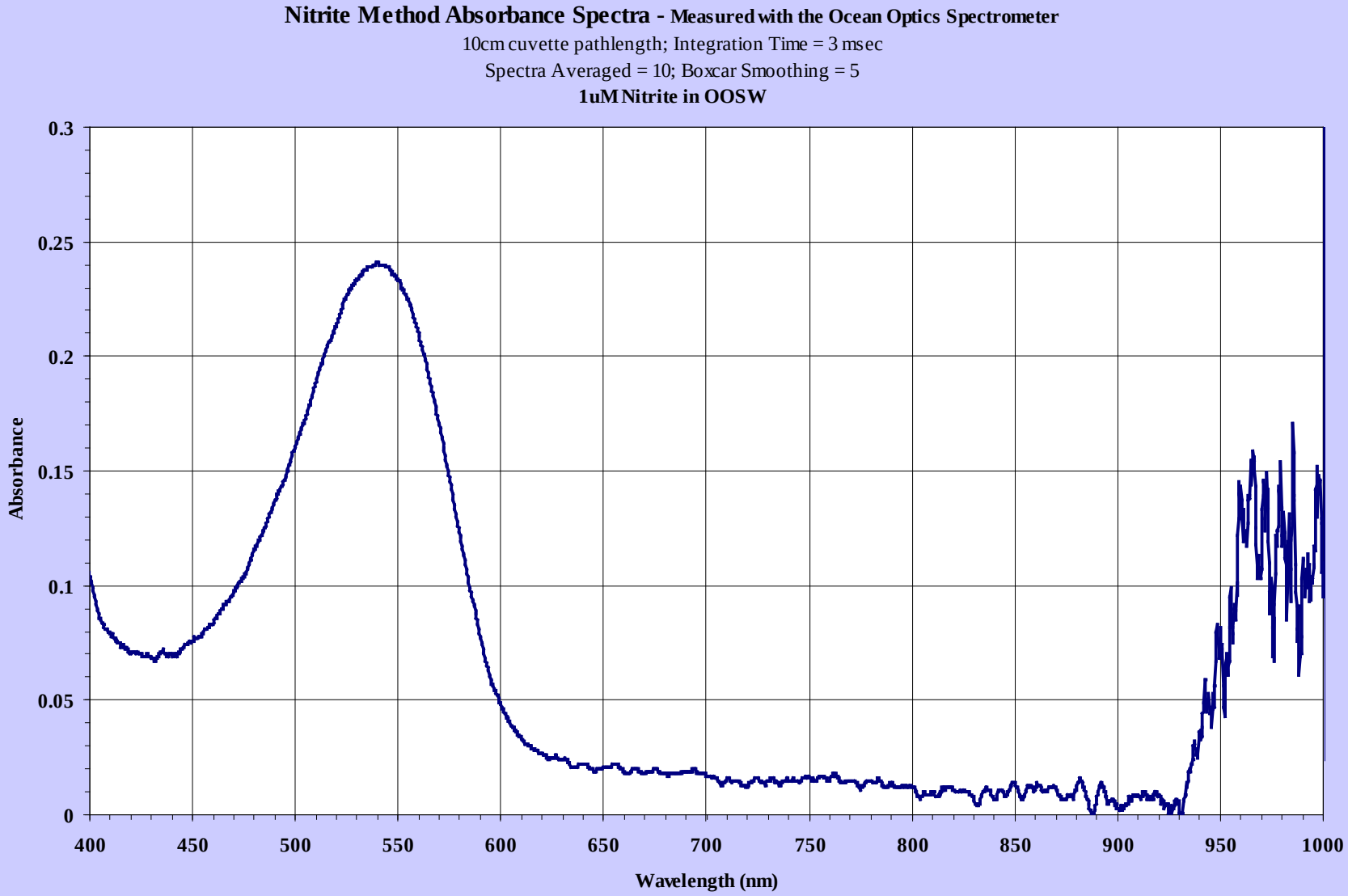
Spectrophotometric Methodologies

Nitrite: Based on the formation of a colored azo dye. Nitrite reacts with sulfanilamide to form a diazonium ion that is subsequently coupled with N-(1-napthyl)-ethylenediamine dihydrochloride to form a highly colored product (pink - 540 nm).

Total Nitrate + Nitrite: Based on the quantitative reduction (>95%) of nitrate to nitrite which is then determined colorimetrically at 540 nm, as described above. The reduction is made by passing seawater through a reducing column containing copper coated cadmium granules

Iron(II): Reduced iron is determined using the classical Ferrozine complex (pink - 560 nm).

Nitrite Spectra



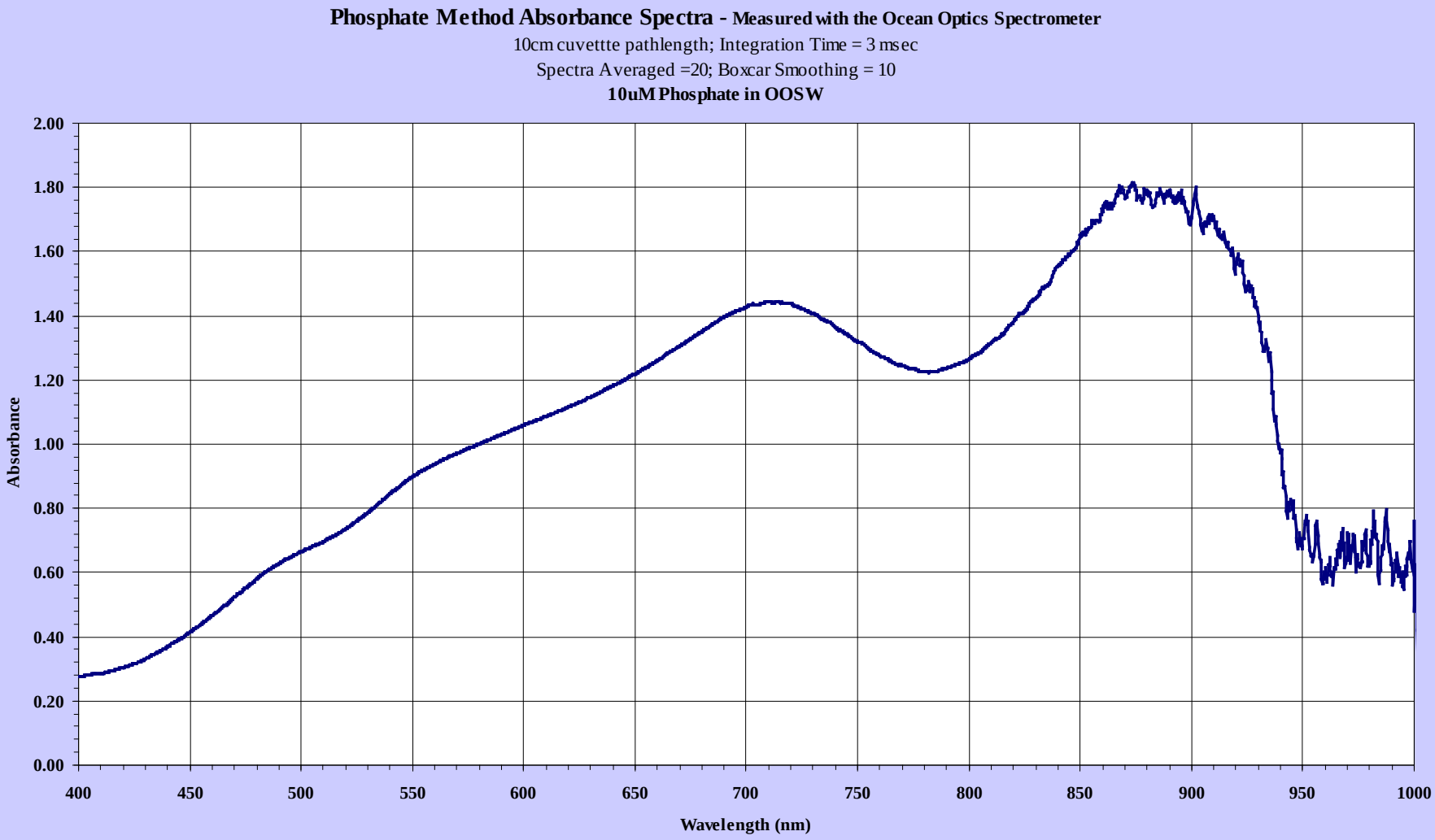
Spectrophotometric Methodologies

Phosphate: A phospho-molybdate complex is formed by interaction between ammonium molybdate and orthophosphate with presence of antimony. This complex is then reduced with ascorbic acid to form a blue compound (880 nm).

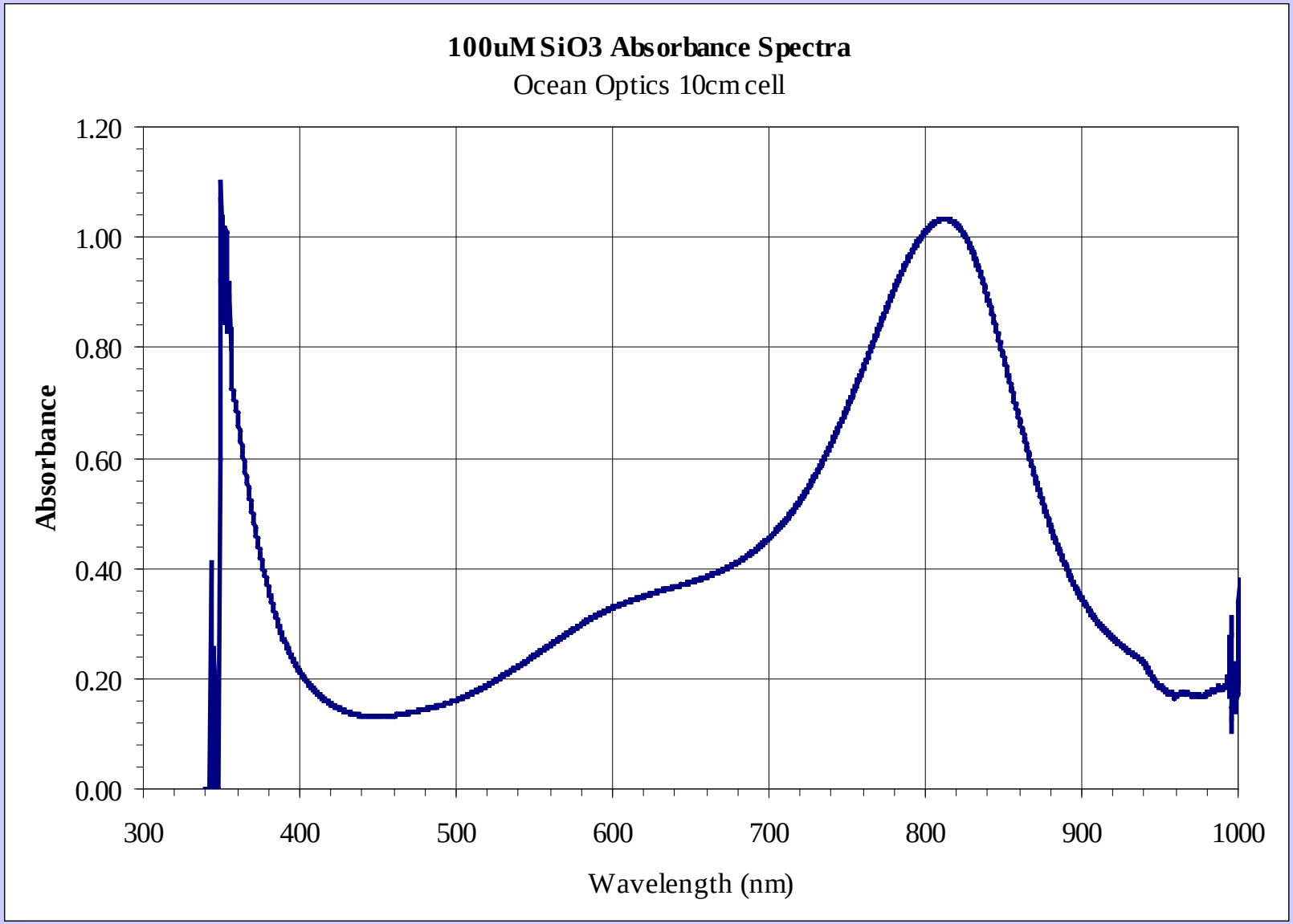
Silicate: Determination is based on the methodology of Grasshoff and Koroleff (1983).

- Molybdic acid reacts with silicic acid to form silicomolybdic acid.
- Oxalic acid is added to limit interference from phosphate.
- Silicomolybdic acid is reduced to silicomolybdous acid, or "molybdenum blue", using *L*-ascorbic acid as the reductant
- Maximum absorbance occurs at 820nm.

Ortho-Phosphate Spectrum



Silicate Spectrum



Ammonia Determination by Fluorescence

NH_4^+ is reacted with *o*-phthalaldehyde (OPA) and sulfite to yield a product that can be detected fluorometrically (Genfa and Dasgupta, 1989; Holmes et al., 1999; Aminot et al, 2001).

The fluorophore has an excitation spectrum that covers a 100 nm band from 300-400 nm with a peak maxima of 365 nm.

The emission spectrum has a bandwidth of 165 nm from 385 nm to 550 nm and peak maxima at 420 nm.

The NH_4^+ – OPA – sulfite reaction is pH and temperature dependent. Optimum conditions are a pH of ~11 and 30-60^{o}C.

APNA procedure achieves a high reaction pH near 11 in seawater by using EDTA and a sodium phosphate buffer (Na_2HPO_4 & NaOH) solution.

Ammonia - OPA Fluorescence Spectrum