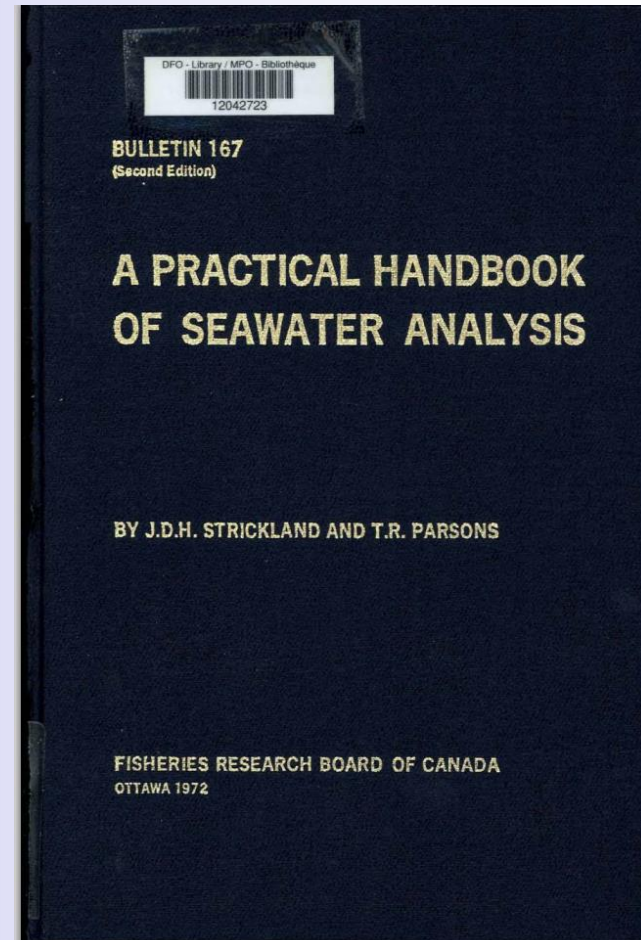
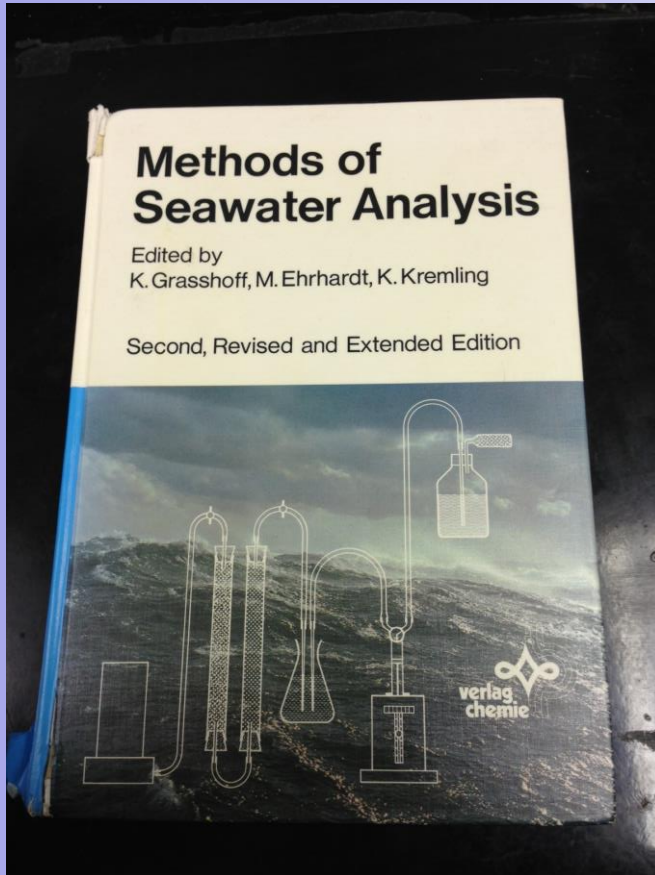


Nutrient Analyses

Biogeochemical Methods
OCN 633

Rebecca Briggs

Methodology



Nutrients typically analyzed on an Autoanalyzer

- PO_4^{3-} (Phosphate)
- Si (Silicate)
- NH_4^+ (Ammonium)
- NO_2^- (Nitrite)
- $\text{NO}_2^- + \text{NO}_3^-$ (Nitrite + Nitrate)
- Total Phosphorus
- Total Nitrogen

Additional Colorimetric analyses:

1. Fe (iron)
2. Ca (calcium)
3. F (fluoride)
4. S (sulfide)

Colorimetric Analysis

- Utilize Beer's law to calculate sample concentration based on a standard solution which is typically made from salts
- A primary standard must:
 - Be obtainable in pure form
 - Must be specific for the reaction (no side reactions)
 - Must be non-hygroscopic
 - Should have a large equivalent weight to reduce error in weighing
- Some labs utilize 'pre-made' standards that can be purchased from companies such as Ricca, OSIL, etc
- WACO standards can be used to verify seawater standards
- Interlab comparisons are used to compare accuracy and precision between analytical labs

Colorimetric Analysis

Utilize specific wavelengths of light to observe adsorption of light by the complex created with the species of interest

Wavelengths and corresponding colour ranges

| Wavelength (nm) | Hue (transmitted)* | Complementary hue of the solution |
|--------------------|--------------------|-----------------------------------|
| < 400 | Ultraviolet | |
| 400–435 | Violet | Yellow green |
| 435–480 | Blue | Yellow |
| 480–490 | Greenish blue | Orange |
| 490–500 | Bluish green | Red |
| 500–560 | Green | Purple |
| 560–580 | Yellowish green | Violet |
| 580–595 | Yellow | Blue |
| 595–610 | Orange | Greenish blue |
| 610–750 | Red | Bluish green |
| > 760 | Infrared | |

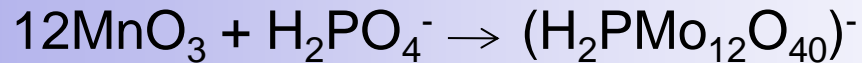
* Hue is one of the three main attributes of perceived colour.

Source: Vogel, 1961.

Determination of Soluble Reactive Phosphorus

Essentially a two step reaction:

1. Orthophosphate reacts with molybdate in an acid solution that forms a yellow-colored phosphomolybdate complex

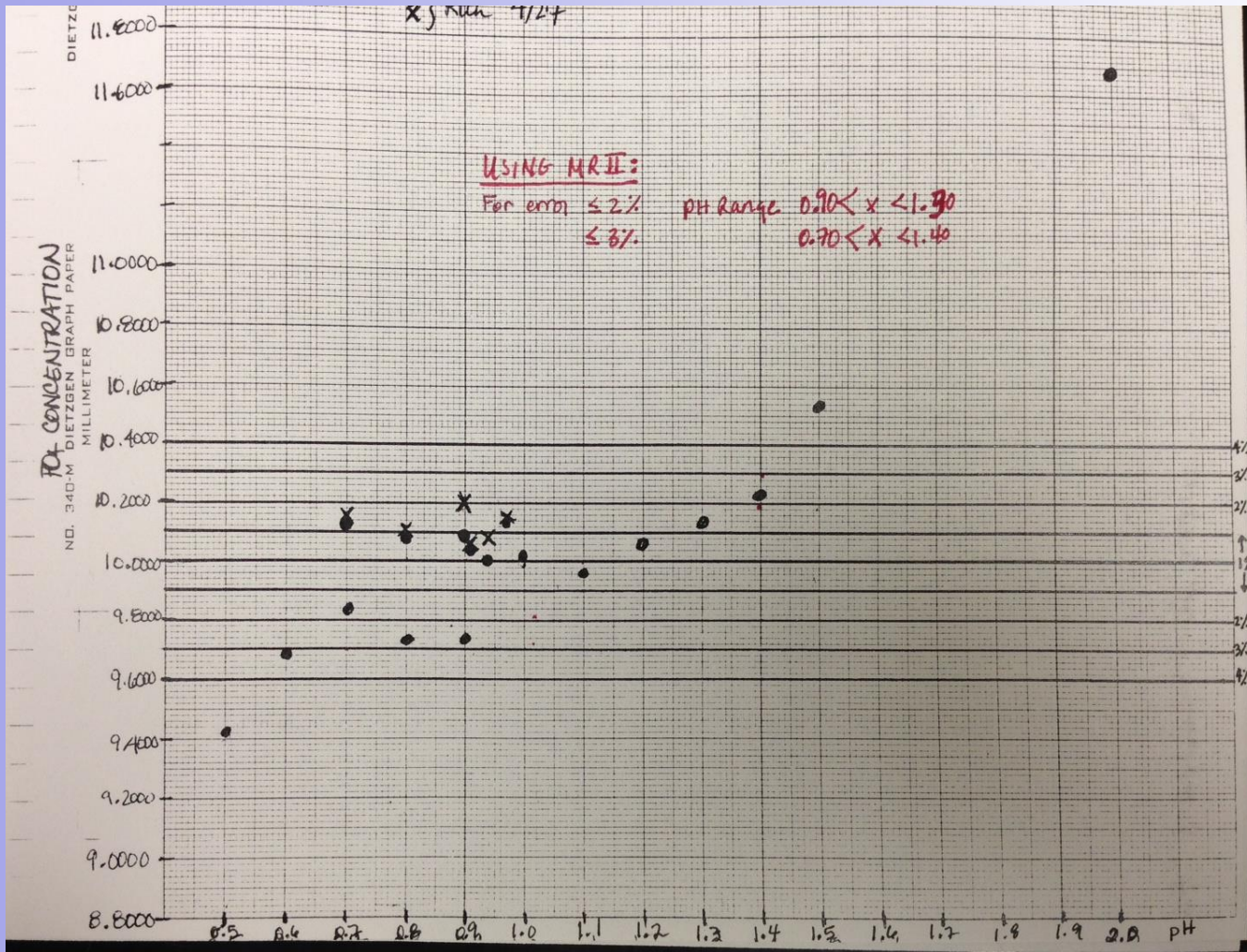


2. Complex is reduced using ascorbic acid to form a blue color and read at 880nm.

Interferences include Silicate, Arsenate, Hydrogen sulphide

pH plays an essential role in dealing with interferences and ensuring rapid color development

Determination of Soluble Reactive Phosphorus



Treat samples identical to standards, particularly with regards to pH!

Matrix effects

- Solution can be used to extract particular elements from solid samples and then analyzed for the species of interest using colorimetric analyses. For example:
 - Sodium acetate can dissolve P bound to carbonates and iron oxides in marine sediments
 - Oxalate can dissolve pools of Fe in marine sediments
- It is important to remember that different matrices effect colorimetric reactions; both with regards to pH and reaction time.
- Some examples with regards to orthophosphate analysis:
 - MgCl_2 solutions are unstable at $>10\mu\text{M}$
 - Sodium acetate is unstable for long periods of time and must be run within 20 minutes of initial reaction
- When working with new matrices always perform tests to ensure maximum recovery and stability
- If matrix effect inhibits the reaction, explore other methods for analysis, or 'clean' the sample using pre-treatment methods

Determination of Total Phosphorus (acid persulphate oxidation)

1. Acidified sample is heated and digested via UV oxidation in the presence of peroxodisulphate to convert organic phosphorus compounds to orthophosphate:

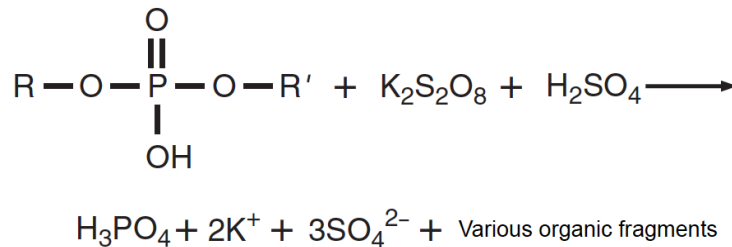


Figure 1 Example of potassium persulfate oxidation of organically bound phosphorus¹

¹ R and R' represent various organic groups

2. Sample is analyzed using molybdate blue method previously described

Alternative method: Alkaline persulfate oxidation

Determination of hydrated silica

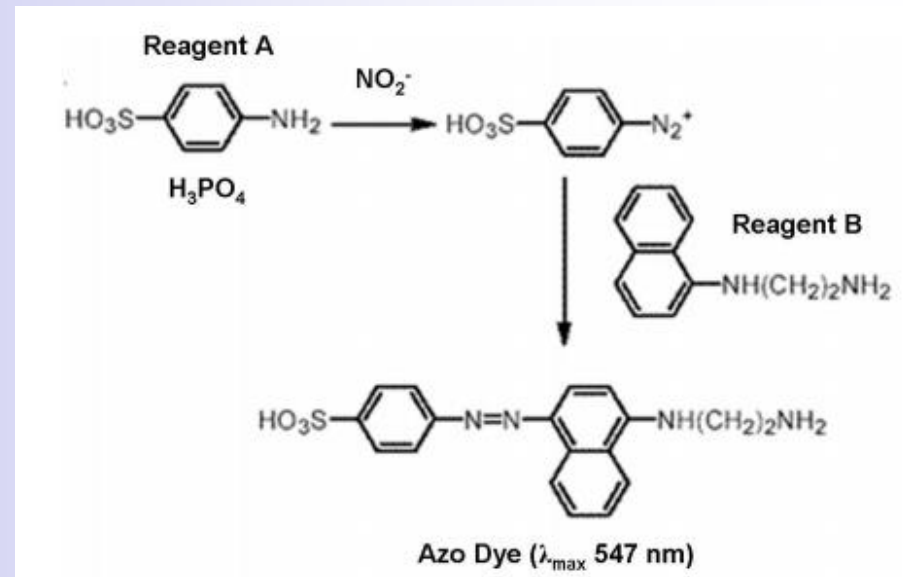
- Very similar to phosphate where dissolved silica is reacted with molybdate form a yellow silicomolybdic acid, which is then reduced to form a blue color.
- In the presence of oxalic acid, there is no influence from phosphate ions.
- Reaction has a large *salt effect*

Salt Effect

- Salt effect is a well established matrix effect for seawater applications of colorimetric analyses. Some analyses are not influenced by salt, others have large corrections (salt factors) that must be applied.
- In the case of silica, salt reduces the color of the blue complex, and for a seawater sample of 35ppt, a salt factor of 1.15 must be applied to all sample concentrations.
- This can be avoided by using seawater standards and baseline, or by correcting for seawater using a refractive index correction (described later)

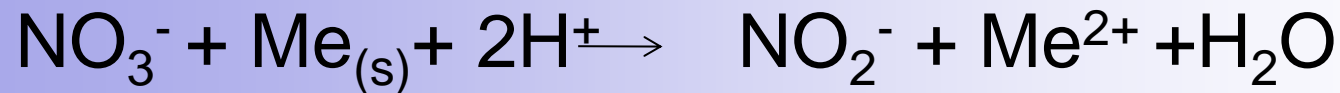
Determination of Nitrite

- Nitrite is measured by employing the Griess reaction:
 - Conversion of sulfanilic acid (reagent A) reacted with nitrite to form a diazonium salt
 - Followed by reaction with N-(1-naphthyl)ethylenediamine (NED; reagent B) to form an azo dye (pink in color) and read at 520nm



Determination of Nitrate

- Reduce nitrate to nitrite using copper-cadmium granules



- Measure using previously described method for nitrite

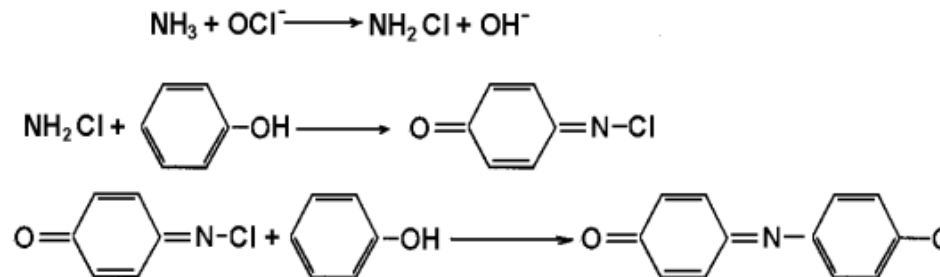
Prepping the cadmium is one of the most difficult parts of this method!

Determination of Total Nitrogen (persulfate oxidation)

- Similar to total P, the sample is heated and digested via UV oxidation in the presence of peroxodisulphate to convert organic nitrogen compounds to nitrate.
- The sample sent through a cadmium column to complete the reduction to nitrite
- In this method an alkaline solution is used to prevent losses from volatilization
- The sample is

Determination of Ammonia

- Traditionally, the indophenol blue method is used to analyze seawater for ammonia:
 - Hypochlorite is added to sample to form mono-chloramines
 - Followed by phenol reaction to produce indophenol blue dye
 - Measured colorimetrically at 860nm



Determination of Ammonia

- Problems with IPB method:
 - Contamination: mainly from the air via cleaning agents or smokers
 - Very large salt effect
 - Variability in replicates due, again, to contamination
 - Phenol is very toxic to work with
- Alternative is the OPA method

Determination of Ammonia

- OPA method utilizes fluorimetry to analyze for ammonia
- Samples are reacted with orthophthaldialdehyde (OPA)-sulfite reagent, fluorescence is measured at 460nm following excitation at 370nm

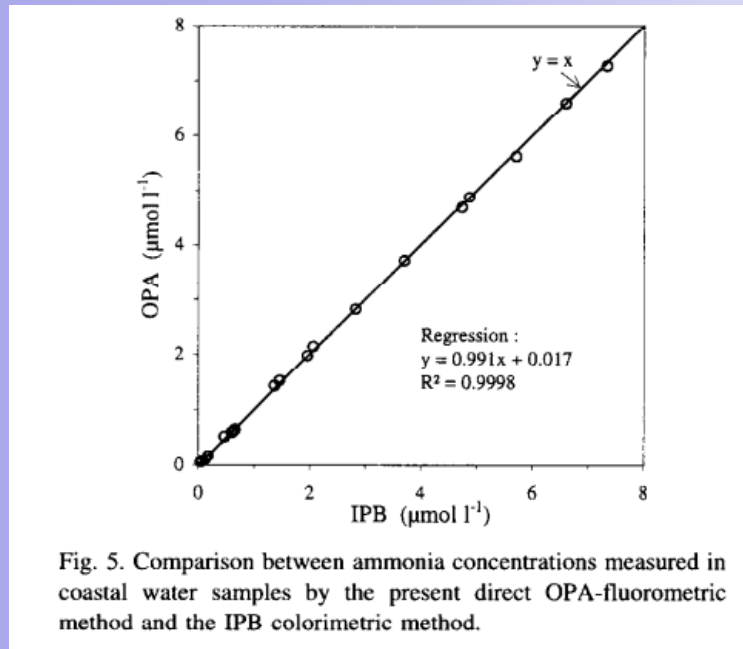
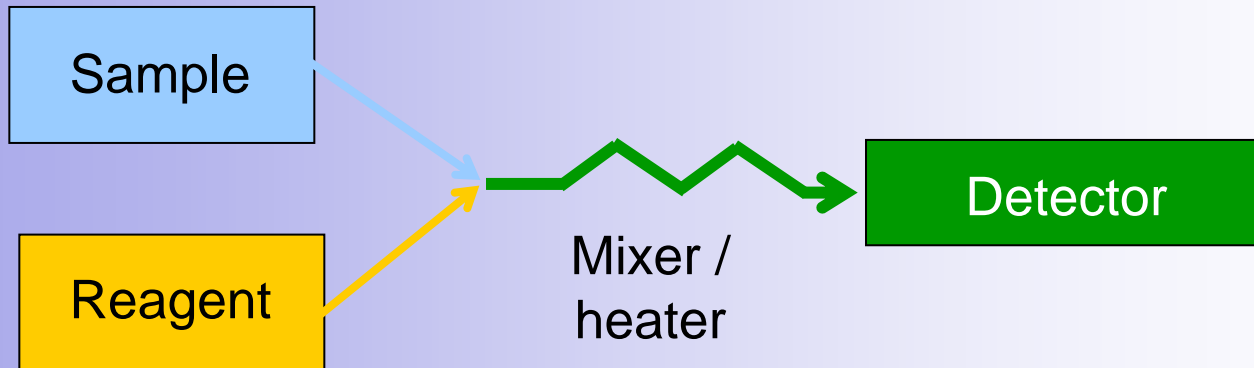


Fig. 5. Comparison between ammonia concentrations measured in coastal water samples by the present direct OPA-fluorometric method and the IPB colorimetric method.

Superior method because:

- Less interference/contamination issues
- No refractive index problems (matrix effects) with seawater
- Detection limit is 1-3 times better (good for low level seawater concentrations)
- Reagents are less toxic

Continuous flow analyzer



- Flow-Injection Analysis (FIA)
- Segmented Flow Analysis (AutoAnalyzer)

Segmented Flow AutoAnalyzer

SEAL Analytical five-channel segmented-flow continuous analyzer consisting of a sampler, a pump, mixing and reaction manifolds and photometers. S-LAB also has a Jasco Fluorescence detector and chemistry manifold for analyzing ammonium by fluorescence

MT19 chemistry manifold is multi-test manifold and interchangeable for seawater and low level water



aluminum,
ammonia, colour,
chloride, copper,
iron, manganese,
nitrate, total N,
phosphate, total
P, silicate, sulfide,
and zinc

Segmented Flow AutoAnalyzer

| Color Code | Abbreviation | Flowrate* ml/min |
|-----------------|--------------|------------------|
| Orange / Blue | (orn/blu) | 0.05 |
| Orange / Green | (orn/grn) | 0.10 |
| Orange / Yellow | (orn/yel) | 0.16 |
| Orange / White | (orn/wht) | 0.23 |
| Black / Black | (blk/blk) | 0.32 |
| Orange / Orange | (orn/orn) | 0.42 |
| White / White | (wht/wht) | 0.60 |
| Red / Red | (red/red) | 0.80 |
| Grey / Grey | (gry/gry) | 1.00 |
| Yellow / Yellow | (yel/yel) | 1.20 |
| Yellow / Blue | (yel/blu) | 1.40 |
| Blue / Blue | (blu/blu) | 1.60 |
| Green / Green | (grn/grn) | 2.00 |
| Purple / Purple | (pur/pur) | 2.50 |
| Purple / Black | (pur/blk) | 2.90 |
| Purple / Orange | (pur/orn) | 3.40 |
| Purple / White | (pur/wht) | 3.90 |

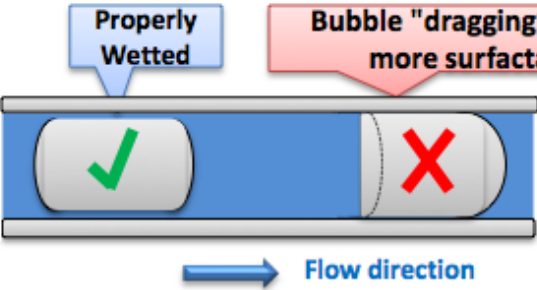
The Pump

High precision peristaltic pump with flow-rated pump tubes which provide different delivery rates


Segmented Flow AutoAnalyzer




- Air is pumped into the lines to prevent smearing of samples during the flow
- Bubble pattern is an indicator of how well everything is running

• **Bubble shape** – Bubbles in Tygon tubing must be round at the front and back. If the back of the bubble looks straight, the tubing has not be properly wetted and more surfactant is needed.



• **Observe the addition of fluids at each injection point.** The addition must be smooth and continuous with out hesitation or surging.



| | |
|---|----------------------------------|
|  | Too Small |
|  | Optimum: length = 1.5 x Diameter |
|  | Too Large |

Segmented Flow AutoAnalyzer

- Mixing coils are used to ensure adequate mixing of each segment. Mixing time is determined based on viscosity and density of reagents, flow rate, and coil diameter

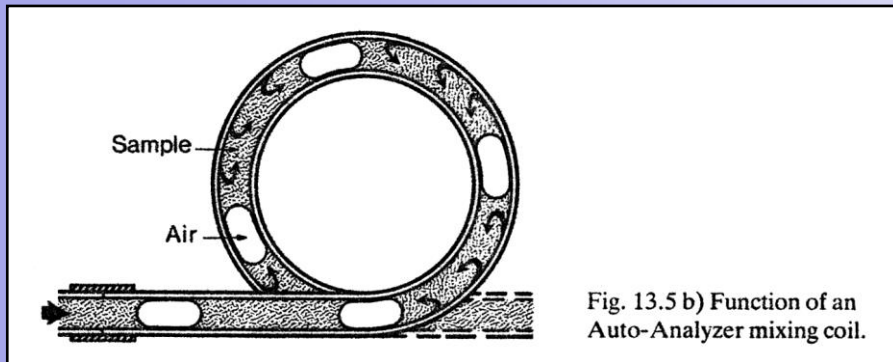
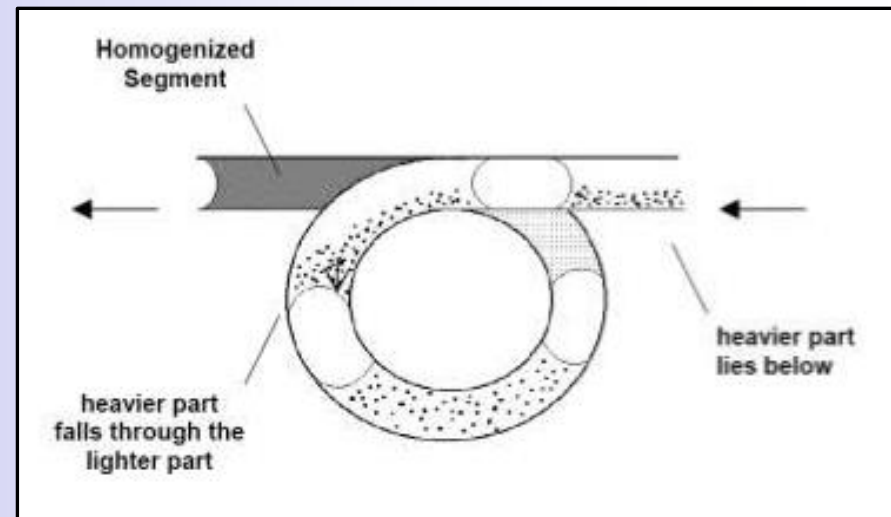
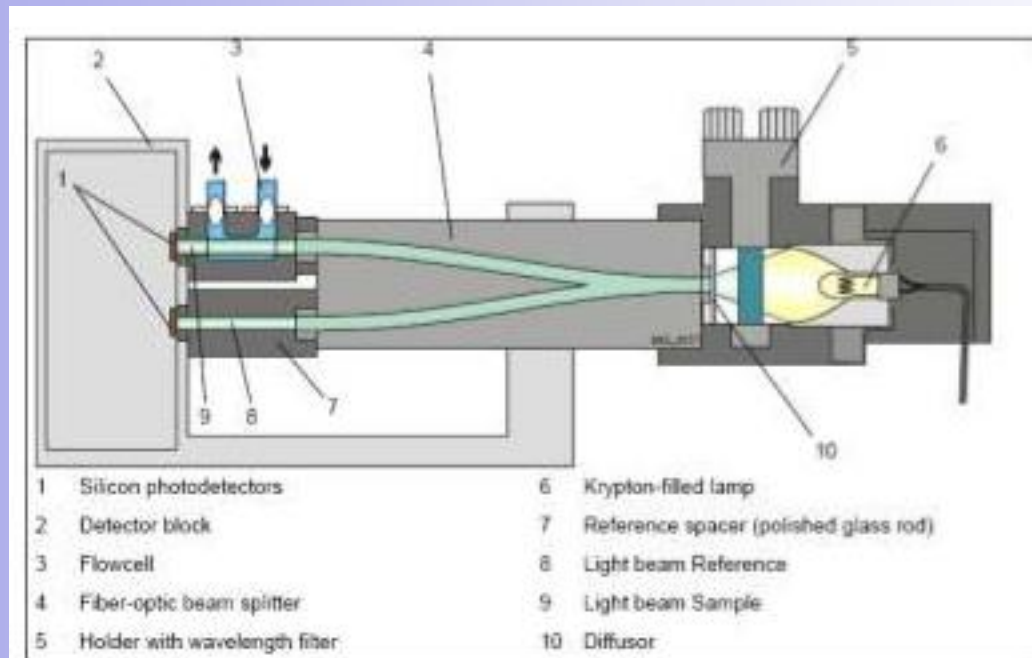


Fig. 13.5 b) Function of an Auto-Analyzer mixing coil.



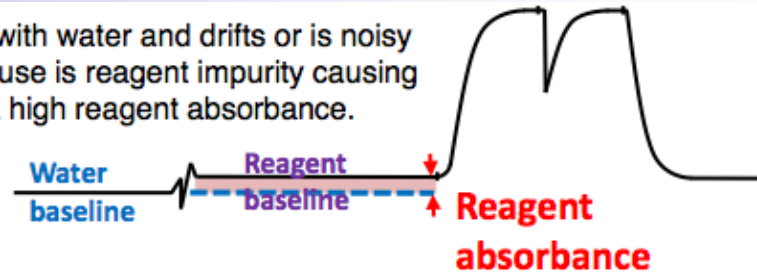
Segmented Flow AutoAnalyzer

- Seal AutoAnalyzer uses a dual-beam photometer
 - Reference beam compensates for changes in lamp output, temp, voltage, and other variables
- Light source is a high-pressure krypton-filled lamp
- Light beam is directed onto a flow cell through which sample flows

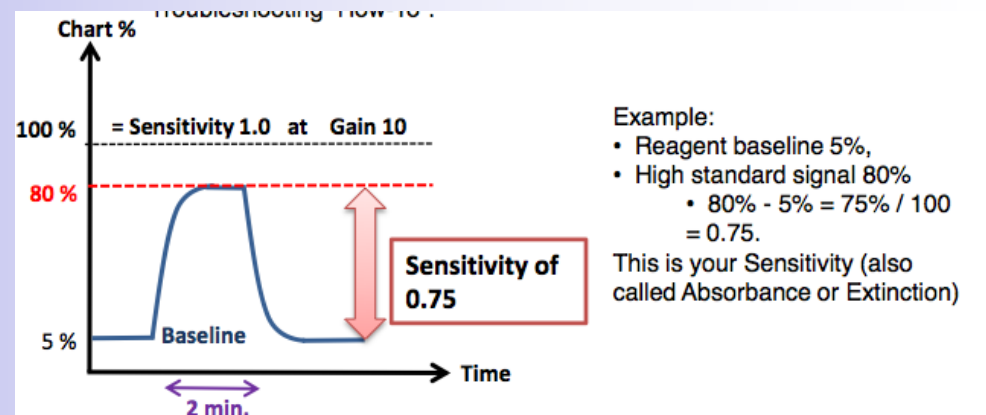


Segmented Flow AutoAnalyzer

- If the baseline was stable with water and drifts or is noisy with reagents, a common cause is reagent impurity causing a deposit in the flow cell or a high reagent absorbance.



- Reagent absorbance is calculated whenever new reagents are made to ensure the reagents are clean.
- Sensitivity can be used to ensure that method is working optimally



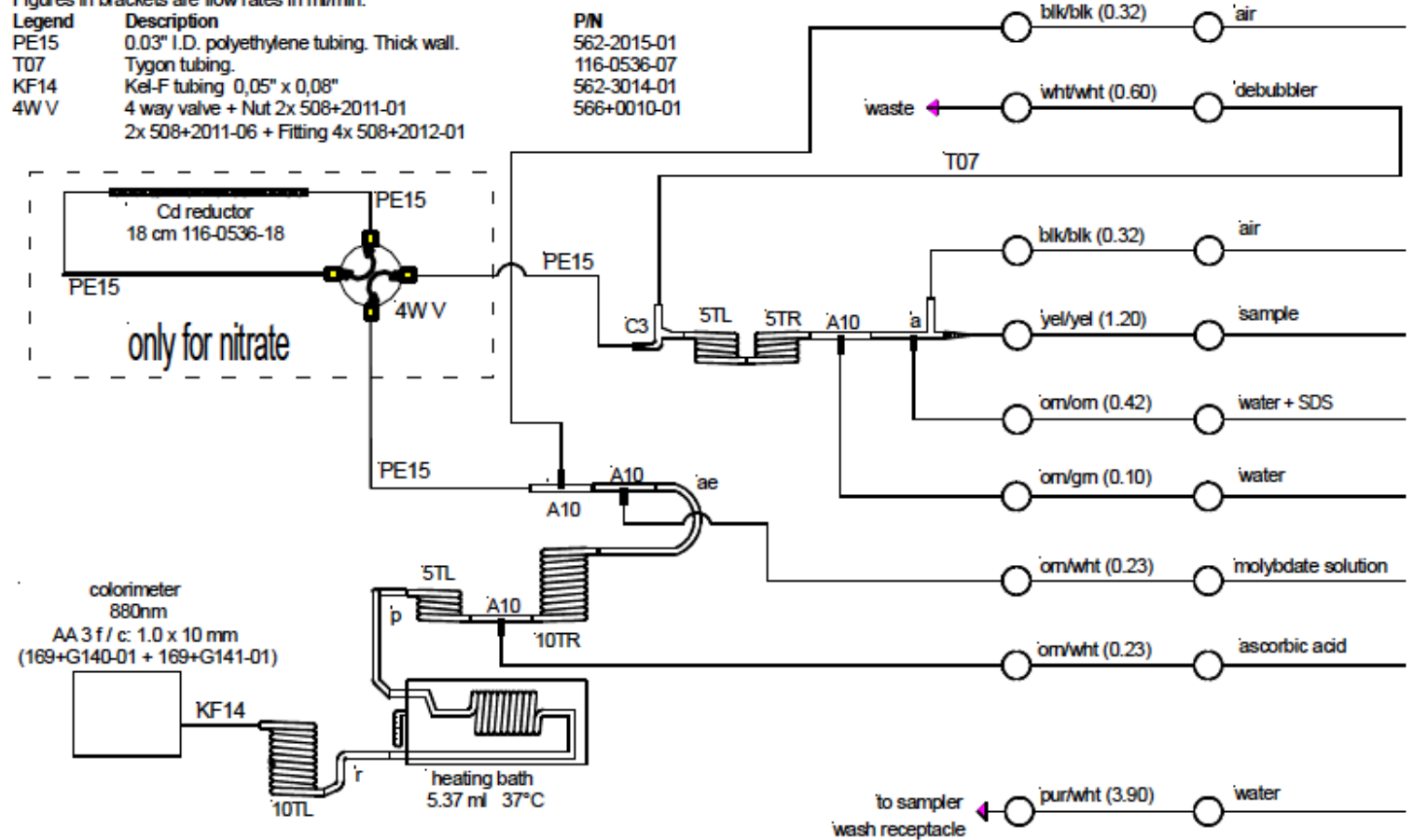
Typical Flow chart for AA3

NOTES:

Figures in brackets are flow rates in ml/min.

| Legend | Description |
|--------|---|
| PE15 | 0.03" I.D. polyethylene tubing. Thick wall. |
| T07 | Tygon tubing. |
| KF14 | Kel-F tubing 0,05" x 0,08" |
| 4W V | 4 way valve + Nut 2x 508+2011-01 2x 508+2011-06 + Fitting 4x 508+2012-01 |

| P/N |
|-------------|
| 562-2015-01 |
| 116-0536-07 |
| 562-3014-01 |
| 566+0010-01 |



| | | | | | | |
|----------|--------------|------------|------------|----------------|-----------|-----------------------------|
| DRAWN | B.Dettmer | 08.12.2003 | SYSTEM | AA 3 | PARAMETER | Phosphate |
| CHANGED | S.Giedigkeit | 26.02.2010 | METHOD NO. | G-297-03 | MATRIX | Water |
| RELEASED | U. Grummisch | 26.02.2010 | REMARK | Multitest MT19 | RANGE | 0 - 50 to 0 - 500 µg/L as P |

SEAL Analytical

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