5. Detection Methods

5.2 Photometry

5.2.3 Molybdenum blue: in lowest concentration range, in water analytics
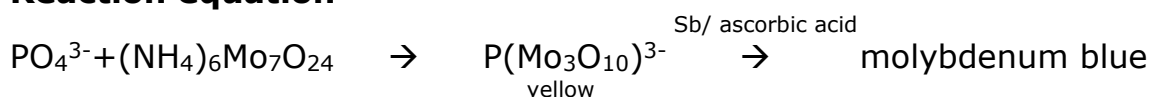
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Principle

Phosphate ions react in acid solution with molybdate to yellow phosphorus molybdic acid, which can be reduced with ascorbic acid to molybdenum blue (Denigès 1921, in the variant of Murphy & Riley 1962, as textbook chapter by Hansen & Koroleff 1999). Antimonyl tartrate stabilises the dye. Molybdenum blue is a colloidal mixed oxide, in which the molybdenum has oxidation states between V and VII. Molybdenum blue is quantified photometrically.

Interfering ions, such as arsenate (chapter 5.2.1, cf. Review: Blomqvist et al. 1993) e.g. in waste water treatment plants with industrial waste water, have to be eliminated. A further and serious disturbance is silicate, an omnipresent ion in sand and glass. Very acidic reaction conditions hinder the reaction of silicate with molybdate (Gripenberg 1929). However, such acidic condition may cause decomposition of acid-labile organic phosphate compounds such as glucose-6-phosphate, which reacts to phosphate and is measured as phosphate as well. For this reason, the result of the molybdenum blue reaction under these conditions is not called (ortho-)phosphate but soluble reactive phosphorus (SRP). Probably, it is a bit overestimated in comparison to phosphate.

Reaction equation

$$PO_4^{3-} + (NH_4)_6Mo_7O_{24} \quad \rightarrow \quad \underset{\text{yellow}}{P(Mo_3O_{10})^{3-}} \quad \overset{\text{Sb/ ascorbic acid}}{\rightarrow} \quad \text{molybdenum blue}$$

Concentration range

The disadvantage of this method in comparison to vanado-molybdenum-yellow and probably the malachite-green (both contain molybdate as well in the reagent) is the low stability of the used reagents. The ascorbic acid as well as the molybdenum mixed reagent have a short stability time. The ascorbic acid solution changed to a greenish solution (after around 10

days) or loses its effect and the mixed reagent is extremely sensitive to impurities. These impurities originated from ultra-pure water for the reagent or the vessels, e.g. dishwasher-cleaned vessels. Additionally, pipetting errors can transfer discolorations. That means that it is better to pipette from small vessels of 5 to 20 ml (for around 20…30 samples) than the original 100 to 200 ml reagent vessel. Reagents are ageing by light (change to blue-green within 2 to 3 days) as well, causing false-positive results.

The linear range of the method at a wavelength of 885 nm and a cuvette length of 5 cm is between 0.05 µmol l^{-1} (limit of quantification) and 10 µmol l^{-1} (around 0.002 and 0.31 mg P l^{-1}). For this reason, the method is well suited for water samples, but for P-rich material it has to be adjusted to the measurement range (strong or stepwise dilution).

The molybdenum blue reaction is one of the most sensitive methods, even though it is not free from matrix effects, which are caused, for example, by salt in seawater. For this reason, standards for seawater samples have to be set in seawater salinity (chapter 5.2.2). The limit of detection is normally 0.05 µmol l^{-1} (1.6 µg l^{-1}), if a 5 cm long cuvette is used. Two options exist to bring down the limit of detection into the nano-molar range: concentration of the analyte (chapter 5.5.1) and the improvement of detection, e.g. by longer cuvettes (Review: Patey et al. 2008, chapter 5.5.2).

Procedure

► **Calibration line**: The samples volume has to be at least 15 ml to fill a 5 cm cuvette. 20 or 25 ml are possible as well.
  ► A further miniaturisation is only possible by a higher limit of detection (4 ml for a 1 cm cuvette). Impurities and errors of pipetting have a much larger effect for smaller sample volumes.
  ► Vessels can be 25 or 50 ml Erlenmeyer flasks (best wide-neck flasks) or 50 ml centrifuge tubes (multiple reusable) (Fig. 5.2.3-1).
  ► In water analytics equidistant calibration lines with 10 measurement points are usual (chapter 6.1). The coefficient of determination should be $r^2 > 0,995$.

Figure 5.2.3-1 Erlenmeyer flasks with different phosphate-containing and phosphate-free samples (molybdenum blue, standard= 5 µM l^{-1}). Filters are strongly discoloured partly by the filtrated seston.

► **Setting and measuring of samples**

   ► Samples with high turbidity (seston, milled sediment material) have to be filtrated with vacuum filtration by GF 6 (nominal pore size 1-2 µm) or if containing very small particles by cellulose acetate (nominal pore size 0.45 µm). Without negative pressure folded phosphate-free filters (e. g. MN 616 G) can be used (Fig. 5.2.3-2).

   ► If necessary, measure turbidity or discolouration of the filtrates at 885 nm (not necessary in persulfate digestions),

   ► add 0.25 ml ascorbic acid solution to 25 ml of the filtrated sample,

   ► add 0.5 ml Mo-mix-reagent, wait 20 minutes,

   ► make reagent blank RBW analogous to samples with 25 ml of ultra-pure water and

   ► measure all samples at 885 nm in a 5 cm cuvette.

Figure 5.2.3-2 a Filtration trestle with filter (GF 6, 47 mm). Connection to pump (-200 mbar pressure) on the left.

Figure 5.2.3-2 b Bodden samples filtrated by folded filters.

Quality management

► Per 10 samples at least 1 blank
► If no real samples repetitions are planned, set 2 measurement repetitions for every 10th sample.
► Measure only in the calibrated range.
► Make control cards such as in chapters 6.3-6.5 explained.

Calculation

$$PO_4^{3-} = F \cdot (E_{sample} - E_{RBW} - E_{FBW})$$

PO_4^{3-}	Phosphate concentration (µmol l^{-1})
F	Factor of calibration line[1]
E	Extinction[2]
RBW	Reagent blank value
FBW	Absorption of filtrates

► The RBW can be subtracted, if the signal ("noise") originates from the reagent.
► FBW can only be subtracted, if the sample is turbid and cannot be filtrated or if an appreciable discolouration is visible.

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[1] Increase of calibration line, if extinction is on the x-axis and concentration on the y-axis.

[2] Always set the same cuvette length!

Handbook on the selection of methods for digestion and determination of total P in environmental samples

Chemicals

- ► **Ultra-pure water** (RW) is deionized silicate-free water: molybdate is reacting with silicate, which can origin from certain glasses or ion exchangers in larger amounts. Measurement of natural (low) silicate concentrations is suppressed by measurement conditions. In each case, deionat from silicate-free ion exchangers has to be used e.g. from Rostocker Kraftwerk or from very high-quality ultra-pure water systems, e.g. Milli-Q.
- ► **Molybdate-reagent:** dissolve 100 g ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot H_2O$ in 1 litre ultra-pure water; stable for several months.
- ► **Antimony-reagent:** dissolve 2.7 g potassium-antimonyl-tartrate in 200 ml 50 % sulfuric acid; stable for several months.
- ► **50 % sulfuric acid:** mix the same volumes of RW and concentrated sulfuric acid carefully by very slowly stirring (Heat development! Very corrosive! Collection tray!).
- ► **Mix-reagent:** mix 12.5 ml Mo-reagent, 36.5 ml 50 % sulfuric acid and 1 ml antimony-reagent. If the colour of the mix-reagent turns to blue, it has to be mixed again.
- ► **Ascorbic acid-reagent:** dissolve 8 g ascorbic acid in 100 ml RW. Storable in a dark flask for 1 to 2 weeks.
- ► **Standards**
  - ► Dry KH_2PO_4 at 60 °C and cool down in a desiccator.
  - ► prepare 1 mM phosphate stock solution (molar mass: 136.19 g mol^{-1}): weigh in 136.19 mg in a 1 l volumetric flaks, dissolve in RW, fill to calibration mark.
  - ► Prepare 10 µM phosphate working solution: pipette 5 ml of 1 mM stock solution in a 500 ml volumetric flask, fill to calibration mark with RW, shake well.
  - ► Alternatively: use commercial P-standards.

References

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