

5. Detection Methods

5.2 Photometry

5.2.5 Vanado-molybdate-yellow: in medium concentration range

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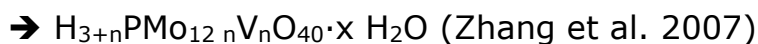
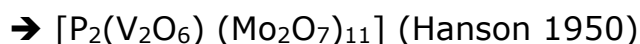
Principle

In the vanadate-molybdate-procedure, orthophosphate and ammonium vanadate react together with molybdate to yellow ammonium phosphorus vanado-molybdate. Misson (1908) is stated as discoverer of this reaction in older publications. This source was electronically not available, but the method was. This method was analysed more detailed, adjusted and assessed as better suited for P determination in steel than the former gravimetric molybdenum blue method and the titrimetric method by Kitson & Mellon (1944), Hill (1947) and Gerricke & Kurmies (1952), since this method was simpler and faster to handle. A compilation of such older gravimetric and titrimetric methods can be found in Huber von Schönenwerd & Solothurn (1950). Such precipitation methods with molybdenum blue are described by Lunge (1905) und Neubauer & Lückner (1912) as well.

The vanado-molybdate colour complex has a slight yellow colour already without P. For this reason, the reagent blank has to be subtracted from all measurement values. The reagent is prepared in HNO_3 , which means that measurement of P in HNO_3 -containing solutions is possible as well. Similar to the molybdenum blue method interferences of for example As(V) have to be taken into consideration (Gee & Deitz 1953). Measurement of the extinction for P determination is done at different wave lengths (e.g. Cavell 1954, Leonard 1946, Quin & Woods 1976, Singh & Ali 1987), since the optimal wave length is between 380 and 450 nm (Gee & Deitz 1953).

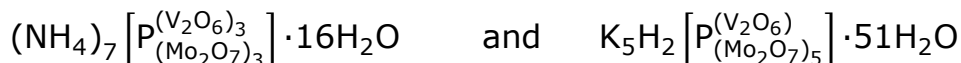
Reaction equation

According to the specific method, reagents and end products vary, which additionally affects limit of quantification, necessary time of reaction and suchlike. The reagent ammonium heptamolybdate is very common: $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 2 \text{H}_2\text{O}$. The general reaction equation and the end products are:



http://vanadium.atomistry.com/heteropoly_acids_with_vanadium.html

if presented with NH_4 : 2 formulas are given:



which is measurable as $\text{PO}_4 \cdot (\text{NH}_4)_3 \cdot \text{VO}_3 \cdot \text{NH}_4 \cdot 16 \text{MoO}_3$ (Huber von Schönwerd & Solothurn 1950)

Concentration range

At a wave length of 430 nm the linear range of the method is between 0.3 (limit of detection) and 20 mg P l^{-1} (9.7...645 $\mu\text{mol l}^{-1}$) and corresponds to the range presented by Singh & Ali (1987). At a concentration of 20 mg P l^{-1} the extinction was 0.8. From concentrations of 40 mg P l^{-1} the extinctions were > 1 (transmission $< 10 \%$) and, therefore, the power range of the photometry is exhausted.

According to the linear concentration range, this method is not suitable for seston (water samples) and needs higher weigh-in for most sediments and soils (compare to chapters 4.1.2 to 4.3.1).

This presented method is used in the working group Agronomy for P determination in plant samples after ashing and digestion with HCl (see chapter 4.3). Currently, phosphorus is determined with other elements at ICP-OES after digestion. The vanadate-molybdate-method is currently only used for P determination in the double-lactate-extract.

The advantage of the vanadate-molybdate-method in comparison to molybdenum blue, instead of much lower sensitivity, is the stability of the used reagents and the colour complex of at least one week (Burns & Hutsby 1986). According to Gericke & Kurmies (1952), the reagent is longer stable than a week in a brown bottle.

Procedure

- ▶ **Determination of wave length:** Measurement of blanks and standards in a range of 370 to 450 nm.
 - ▶ Below 370 nm care for optical quality of the cuvette (OS: Optical special glass or plastic cuvettes for specific wave length range)!
 - ▶ Assessment between high signal-noise ratio (increases in the UV range) and low blanks (increases strongly in the UV range as well (Fig. 5.2.1-1, compare to Ma & McKinley 1953).
 - ▶ Most suited wave length are around 400 nm. The working group Agronomy decided to measure at 430 nm.

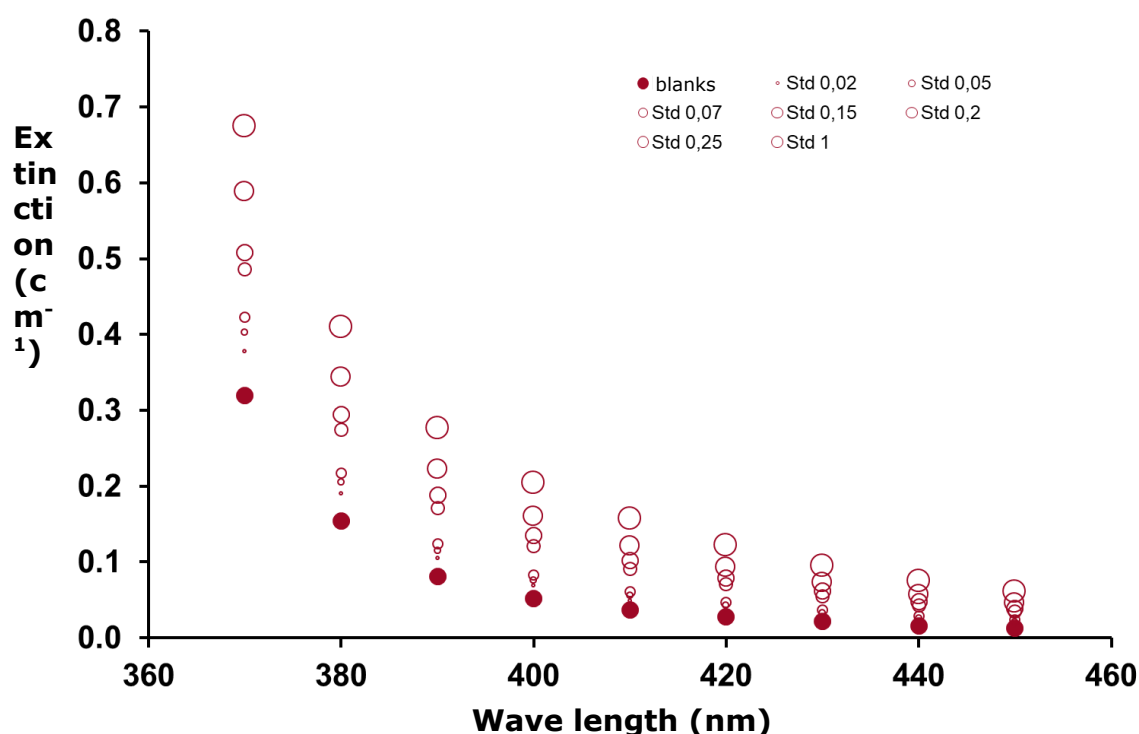


Figure 5.2.5-1 Extinctions of the mean of the blanks and P standards for calibration line at wave lengths between 370 and 450 nm in the experiment for determination of the linear range of the method

- ▶ **Calibration lien for high concentrations (1...20 mg P l⁻¹):** pipette the specific volume in a 100 ml volumetric flask (Tab. 5.2.5-1).
 - ▶ Add the corresponding volume of the sample matrix, e.g. 2 ml of 25 % HCl, if plant ash was digested in boiling HCl (chapter 4.3.1) and
 - ▶ fill flask with RW to calibration mark.
 - ▶ For the determination of the measurement range only ultra-pure water (without matrix, Fig. 5.2.5-3 a) mixed with working solution (see below) also can be used.

Table 5.2.5-1 Addition of working solution (in ml), contained mg P and concentration of P (in mg and mmol P l⁻¹) in 100 ml volumetric flasks for standards of calibration line, as used in the working group Agronomy

Standard No.	0	1	2	3	4	5
Addition working solution in ml	0	1	2.5	5	10	20
mg P absolute	0	0.1	0.25	0.5	1	2
mg P l ⁻¹	0	1	2.5	5	10	20
mmol P l ⁻¹	0	0.032	0.081	0.161	0.323	0.646

- **divergent calibration line for low concentrations (< 1 mg P l⁻¹):**
- the increases of the calibration lines until 1 mg P l⁻¹ and those beyond varied by more than 10 % (Fig. 5.2.5-2). For this reason, values for > 1 mg P l⁻¹ have to be calculated with the second factor (Fig. 5.2.5-2 b).
 - In dependency to the measurable concentration range, the weigh-in of the samples have to be adjusted.

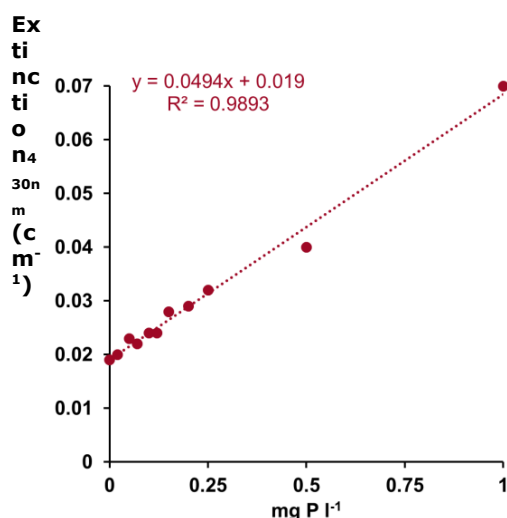


Figure 5.2.5-2 a Calibration line of phosphorus vanado-molybdate complexes in lower concentration range in ultra-pure water. Factor for calculation of concentration (mg P l⁻¹) from extinction at 430 nm (1 cm cuvette) is 20 reciprocally to the increase of the line equation.

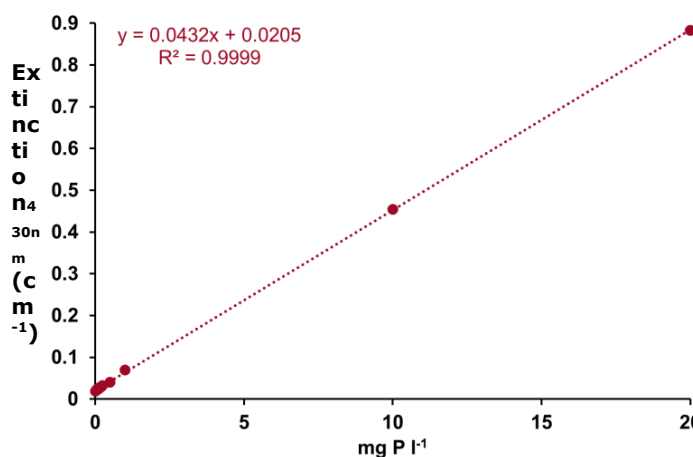


Figure 5.2.5-2 b and in the higher concentration range in the same experiment for determination of the linear range. Factor for calculation of concentration (mg P l⁻¹) from extinction is 23 mg P l⁻¹ E_{430nm}⁻¹.

- **Calibration line for other matrices (1-20 mg P l⁻¹):**
- The increases of calibration lines in colour-changing matrices (Fig. 5.2.5-3 b compared to Fig. 5.2.5-2) can differ strongly from each other.

- ▶ The colour depth of reagents is strongly affected by the pH value of the solution and a bit less from the salt concentration and other ions.



Figure 5.2.5-3 a Line of calibrants of vanado-molybdate-yellow in a double-lactate-extractions-matrix

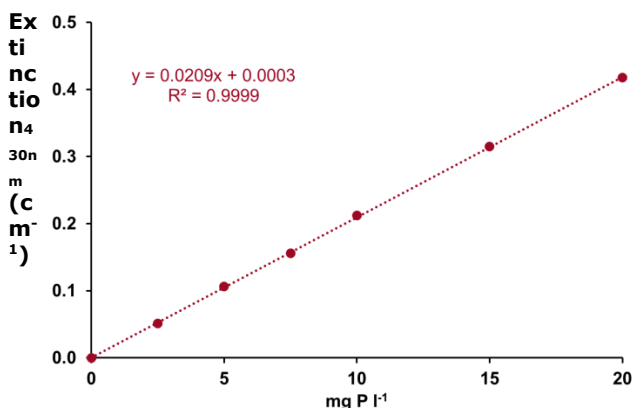


Figure 5.2.5-3 b Calibration line of vanado-molybdate-yellow (same matrix as in Fig. 5.2.5-3 a). Factor for calculation of concentration (mg P l⁻¹) from extinction is 48 mg P l⁻¹ E_{430nm}⁻¹. Spectral photometer Specord 40, 1 cm glass-flow through cuvette, wave length 430 nm.

▶ Set and measure samples

- ▶ Set the calibration line (Tab 5.2.5-1) in a 50 ml volumetric flask for each sample, each blank and each standard.
- ▶ Pipette in each flask 15 ml of the VM-mixture.
- ▶ Fill the flasks with the specific filtrate (sample, blanks) and the standard.
- ▶ Close each flask with a plug, swing and wait for 2 hours.
- ▶ Swing the solution directly before measurement.
- ▶ Measure extinction at spectral photometer in a 1 cm cuvette at 430 nm (Fig. 5.2.5-5).
- ▶ If the measurement value is outside the calibration range, the sample extract has to be diluted und mixed again with the VM-Mix reagent and measured again.

Quality management

- ▶ Determine optimal wave length for used extraction matrix (see Fig. 5.2.5-1).
- ▶ Use at least 1 blank for 10 samples.
- ▶ If no real sample repetitions are planned, measure 2 repetitions for every 10th sample.
- ▶ Measure only within the calibration range.
- ▶ Monitor by control charts according to chapters 6.3 to 6.5.



Figure 5.2.5-4 P extracts (DL: double-lactate and vanado-molybdate-yellow method). There are barely soils in Mecklenburg having < 3 mg total P or DL P kg soil dry matter⁻¹.

Calculation

The strong dilution of the samples (35 ml) by the reagent mixture (15 ml) means that both volumes have to be measured exactly. This is less critical in other common reagent amounts (< 5 % of total volume). This mixture-relation has to be stable for all measurements to calculate the correct concentration according to the calibration line.

Chemicals

The necessary amount of the vanadate-molybdate-mixture depends on the number of samples. In the working group Agronomy, a 12 litre vanadate-molybdate-mixture is prepared, since after harvest of pot and field experiments a lot of samples have to be measured. If a few samples must be measured, less chemicals are necessary.

- ▶ Ultra-pure water (RW)
- ▶ **diluted HNO_3** : fill 2776 ml RW in a 15 l glass vessel and subsequently 1224 ml concentrated HNO_3 . The solution is warmed up only a bit.
- ▶ **4 l vanadate solution**: produce 2 x 2 litre.

- ▶ Weigh in 5 g ammonium vanadate (NH_4VO_3) in a 2000 ml volumetric flask and dissolve in around 1000 ml of hot RW.
- ▶ Add 40 ml of concentrated HNO_3 and, after cooling down, fill with RW to the calibration mark.
- ▶ **4 l molybdate solution:** produce 2 x 2 litre.
 - ▶ Weigh in 100 g ammonium molybdate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 2 \text{H}_2\text{O}$) in a 2000 ml volumetric flask and dissolve in around 1000 ml of warm RW.
 - ▶ Fill to calibration mark with RW after cooling down.
- ▶ **VM-reagent** (vanadate-molybdate-mixture) for a 12 l stock solution
 - ▶ Mix 4 litres diluted HNO_3 , 4 litres vanadate solution and 4 litres molybdate solution.
 - ▶ Store the mixture in the dark. Wait at least 3 days before first usage, waiting for a week would be better.
 - ▶ Mixture can be used for around 3 months.
- ▶ **Standards**
 - ▶ Dry KH_2PO_4 at 105 °C and cool down in a desiccator.
 - ▶ P stock solution (1 g P l^{-1}): weigh in 4.394 g of dried KH_2PO_4 in a 1000 ml volumetric flask (molecular mass of KH_2PO_4 = 136,09 g l^{-1})
 - ▶ Add 500-700 ml of RW, dissolve KH_2PO_4 and fill with RW to calibration mark.
 - ▶ Alternatively, use commercial P standards.
 - ▶ P working solution (100 mg P l^{-1}) for calibration line from P stock solution (1 g P l^{-1}) dilute 1:10: pipette 10 ml of P stock solution in a 100 ml volumetric flask and fill with RW to calibration mark.

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