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# Standard Operating Procedure for available Phosphorus. Olsen Method

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## Standard operating procedure for soil available phosphorus

*Olsen method*

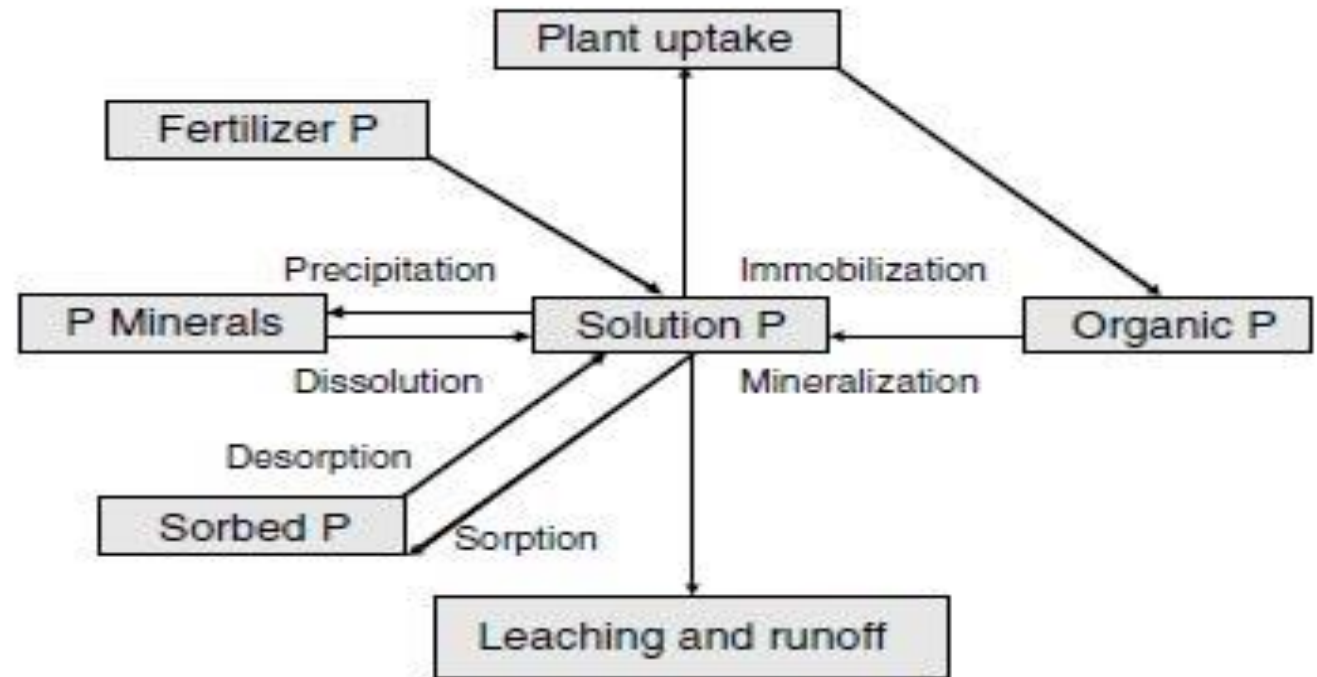
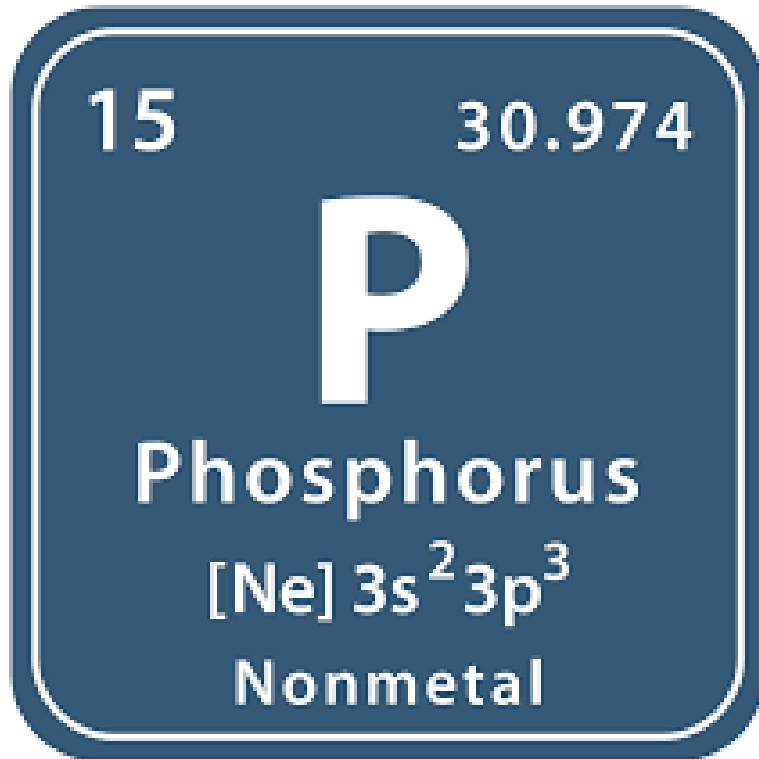


Modify by	Revision	Approval date	Validated date
GLOSOLAN SOP Tech. W.G. Global leaders: Gina Nilo and Bergil Bernaldo, Philippines	By the Review Panel	13 January 2021	13 January 2021

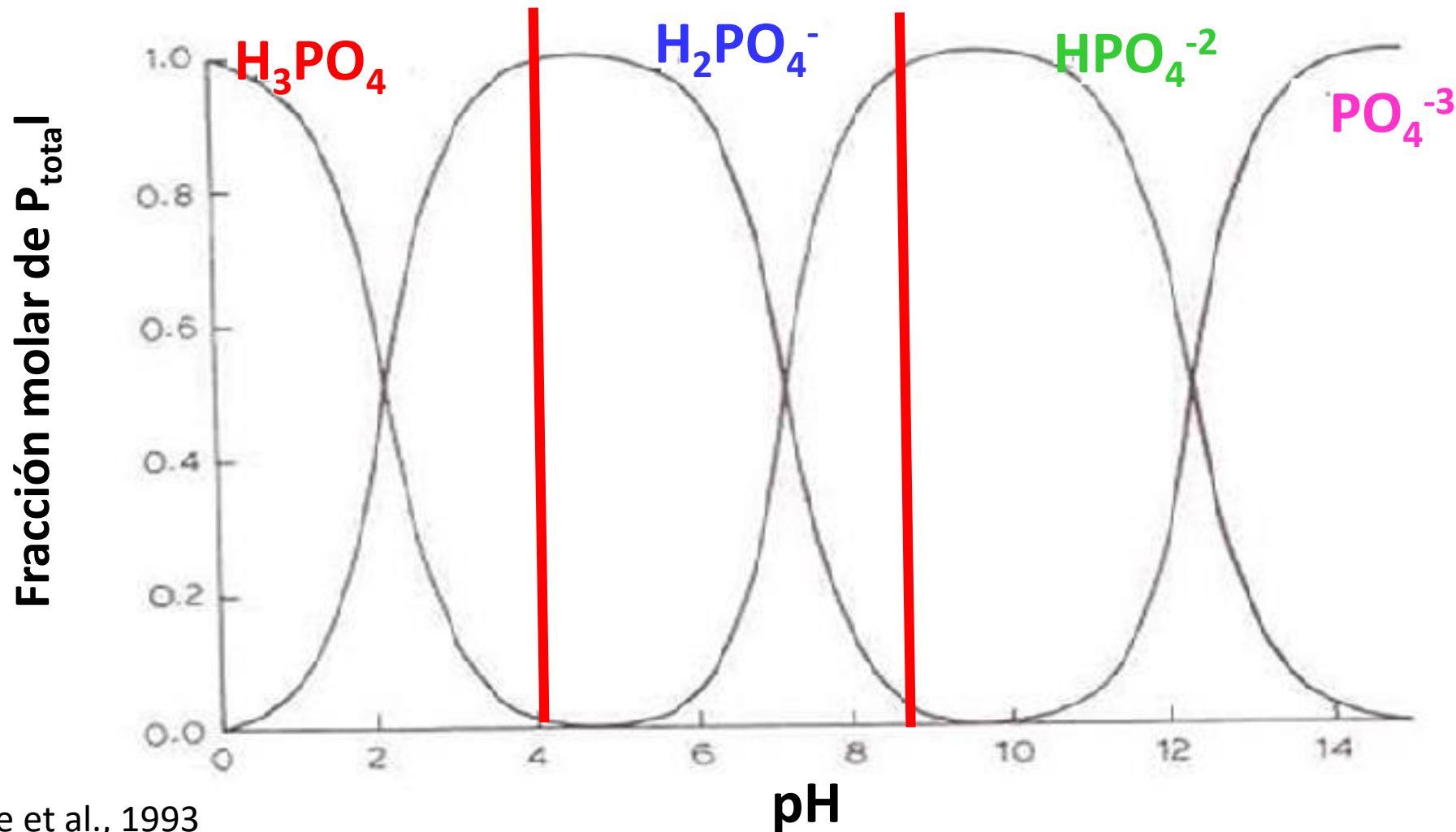
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# 1. Introduction: Phosphorus in Soil



# 1a. Effect of pH on the Distribution of Orthophosphates in Solution



Tisdale et al., 1993

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## 1b. Brief history of P extraction methods from the soil

- First attempt 1845 (Daubeny, Engl.), several subsequent periods
- Soil phosphorus tests formally introduced into agronomic practice in the USA in 1940.
- The main objective of these tests was :
  - a) Identify "optimal" P concentration required for plant growth .
  - b) Define inorganic or organic fertilization needs and the economic return on investment in phosphate fertilizer.

# 1c. Soil P extraction methods

Method code <sup>a</sup>	Description
PA02	Bray I (dilute HCl/NH <sub>4</sub> F)
<b>PA03</b>	* Olsen (0.5 M NaHCO <sub>3</sub> at pH 8.5)
PA04	Truog (dilute H <sub>2</sub> SO <sub>4</sub> )
PA05	Morgan (Na-acetate/acetic acid)
PA06	Saunders and Metelerkamp (anion-exch. resin)
PA07	Bray II (dilute HCl/NH <sub>4</sub> F)
PA08	Modified after ISFEI method, A.H. Hunter (1975)
PA09	Nelson (dilute HCl/H <sub>2</sub> SO <sub>4</sub> )
PA10	ADAS method (NH <sub>4</sub> acetate/acetic acid)
PA11	Spectrometer method (Brasil)
PA12	North Carolina or Mehlich (0.05 M HCl, 0.025 N H <sub>2</sub> SO <sub>4</sub> )
PA13	Colorimetric in 0.02 N H <sub>2</sub> SO <sub>4</sub> extract, molybdenum blue method
PA14	Dabin (ORSTOM), modified Olsen method for tropical soils (0.5 N NaHCO <sub>3</sub> + 0.5 N NH <sub>4</sub> F buffered to pH 8.5 by NaOH)
PA15	Kurtz-Bray I (0.025 M HCl + 0.03 M NH <sub>4</sub> F)
PA16	Complexation with citric acid (van Renwick)
PA17	NH <sub>4</sub> -lactate extraction method (KU-Leuven)
<b>PA18</b>	Bray-I (acid soils) resp. Olsen (other soils)
PA99	P-method, not defined

## 1d. Brief history of the P-Olsen method

- Introduced by Olsen et al. (1954) ("Estimation of available plant phosphorus in soils by extraction with sodium bicarbonate"). (USDA Circ. 939).
- Use of  $\text{NaHCO}_3$  0.5 M (pH 8.5) to extract the P that plants could extract from calcareous, alkaline and neutral soils. It is used as an indicator of soil P extractability by a 0.5 M  $\text{NaHCO}_3$  0.5 M pH 8.5 solution.
- Method of analysis to predict crop response to P fertilizer inputs in calcareous soils (Olsen et al., 1954).

# Continued. 1d. Brief history of the P-Olsen method

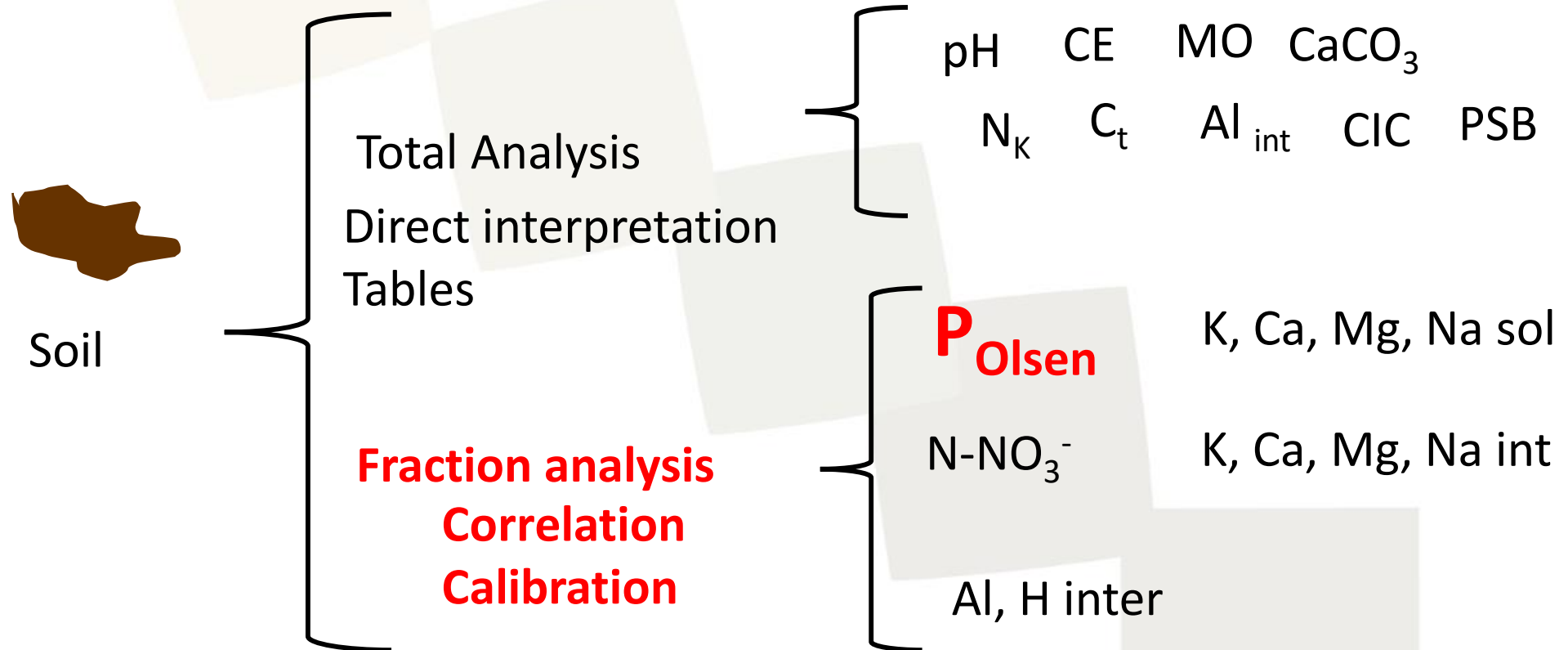
- Crops used: wheat, oats, alfalfa and cotton.
- High correlation between P extracted in the laboratory by the method and that absorbed by these crops.
- Suggested scale of interpretation:
  - < 5 ppm response expected.
  - between 5 and 10 ppm the response was probable
  - < 10 ppm response was unlikely.
- Values were corroborated by other researchers for similar soils and crops and have been erroneously extrapolated for other crops, without taking into account the root density of the crops.



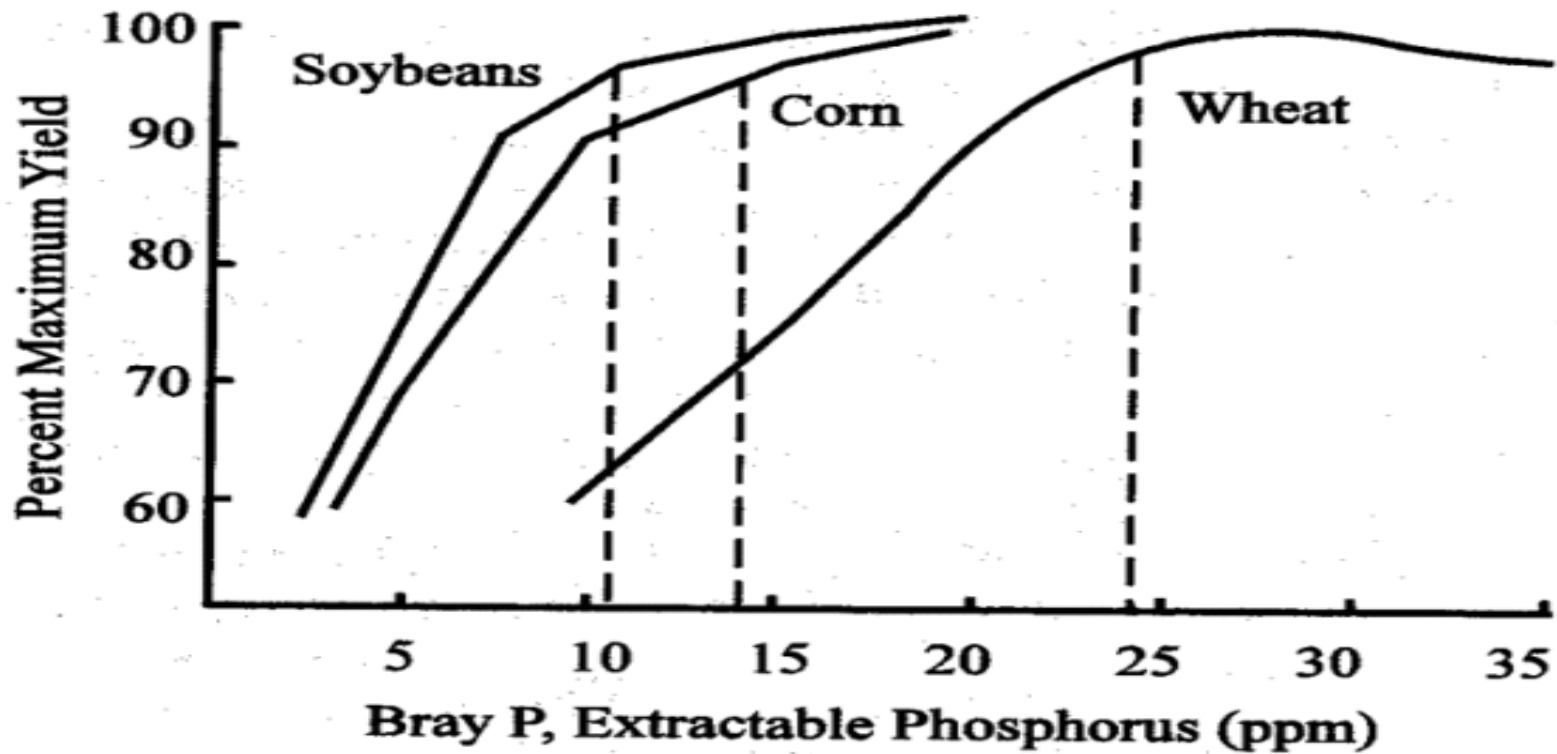
## Continued. 1d. Brief history of the P-Olsen method

- Olsen and Sommers introduced it (S. Olsen & L. Sommers. Phosphorus. In: Methods of soil analysis; chemical and microbiological properties. Part 2. Ed. by A.L. Page, R.H. Miller and D.R. Keeney. American Society of Agronomy/Soil Science Society of America: Madison, Wisconsin, 403-430 (1982)).
- Used in the North Central and Western United States.

# 1e. Fraction analysis: phosphorus



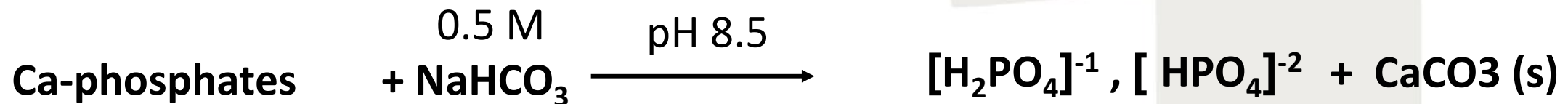
# 1f. Available fraction



## 2. Scope of application

## 2a. Most frequent uses of the P-Olsen method

- Soil fertility diagnosis, environmental assessment, P desorption, soil classification and several others.
- Most frequent use: **diagnosis of soil P availability status.**
- The soils for which it was developed were **calcareous soils**, particularly those with > 2% free calcium carbonate.
- But there is research showing that it is **reasonably effective for moderately acidic soils** (Fixen and Grove, 1990).

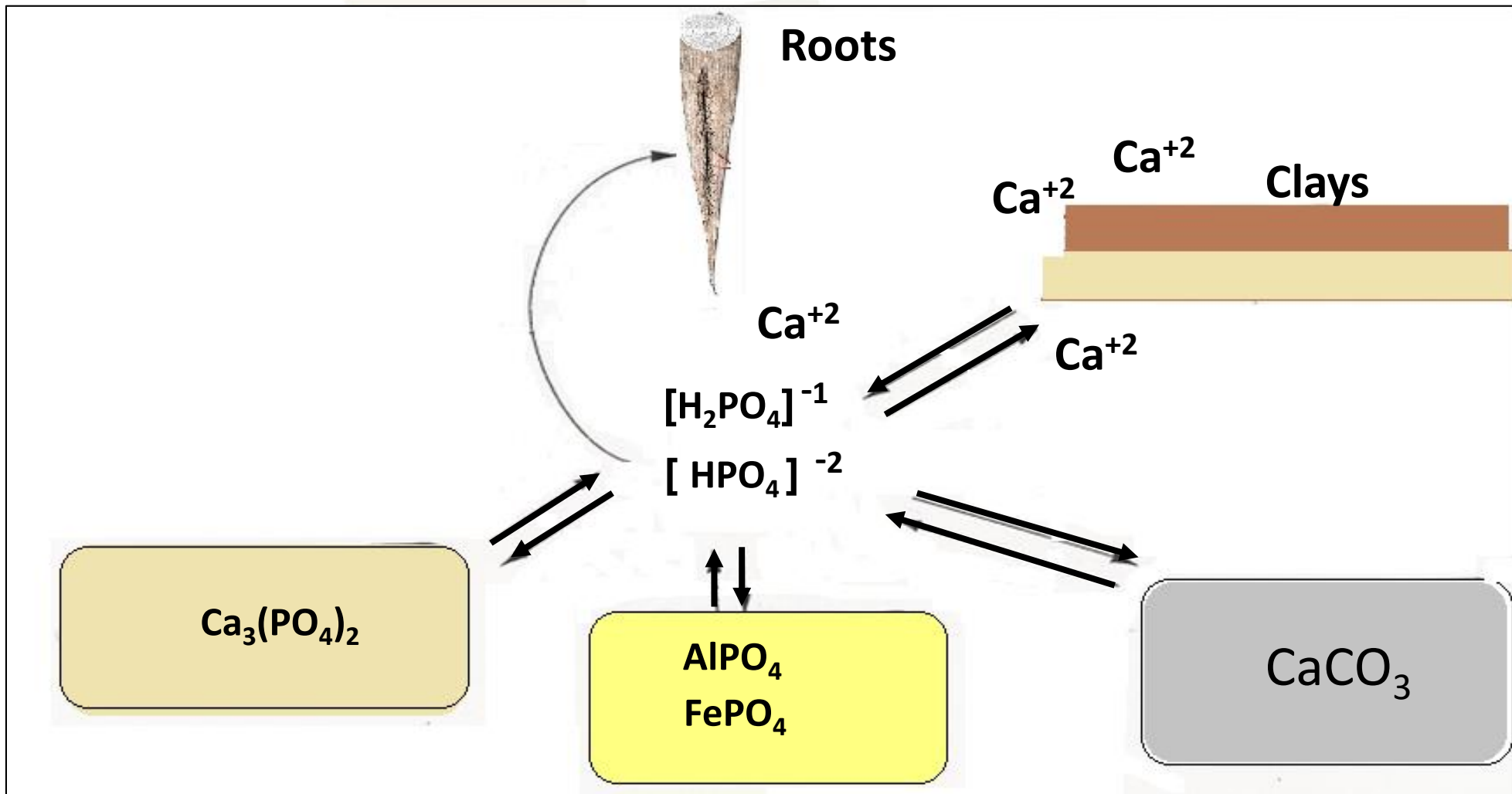


## 2b. Soil properties to consider when selecting a method to determine P and recommended methods (Elrashidi, 2010)

Soil	pH	Minerals	Methods
Acid	< 6.0	Al-P, Fe-P y Mn-P	Bray 1, Miehlich 1, 3, Water, Fe-oxide Impregnated Paper (IIP), Anion Exchange Resin (AER)
Slightly acidic to slightly alkaline	6.0 a 7.2	Al-P, Fe-P y Mn-P Mg-P, Ca-P	Bray 1, Miehlich 1, 3, <b>Olsen</b> , Water, Fe-oxide impregnated paper (IIP), anion exchange resin (AER), Miehlich 1, 3, Olsen, Water, Fe-oxide impregnated paper (IIP), anion exchange resin (AER)
Alkaline and calcareous	> 7.2	Ca-P, Mg-P	Olsen, Water, Fe-oxide Impregnated Paper (IIP), Anion Exchange Resin (AER)

# 3. Principles of Olsen's extractant for P

### 3a. Understanding the P system in soil: Where is the P in soil located?





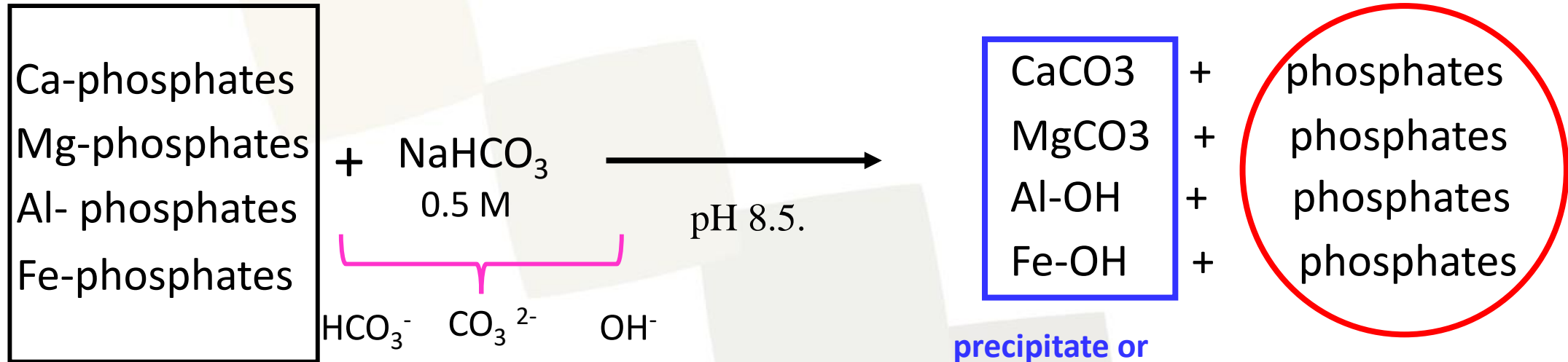
## 3b. What P does the P-Olsen method extract from the soil?

The P extracted by Olsen's method comes from:

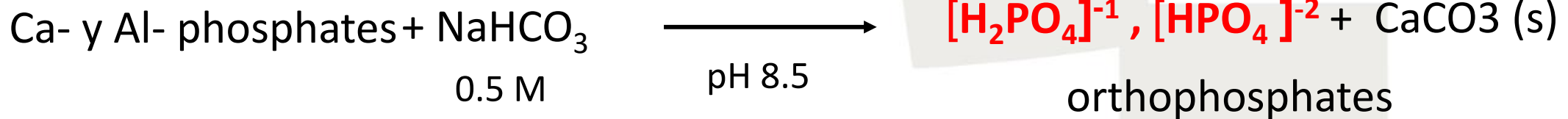
- Mostly from P adsorbed on the surface of Fe and Al minerals with different crystallinity and on calcium carbonate particles, and;
- From organometallic and phosphate complexes of relatively high solubility.

# 3c. Why and how does Olsen's extractant solubilize P?

increase their solubility



precipitate or  
decrease in solution



## 3d. Factors Affecting Extraction

- Extraction solution
- Extraction time, vessel size and type of agitation
- Extraction Temperature
- Spectrophotometric Measurement

## 3e. Effect of extraction solution pH

Table 1. Influence of pH on bicarbonate extractable phosphate.

Soil ID	0.50 N NaHCO <sub>3</sub>		
	pH 8.50	pH 8.75	pH 9.00
94103 <sup>1</sup>	6.0	8.3	10.3
94107	11.3	13.5	14.5
95102	24.0	32.6	40.4
94101	38.9	45.4	49.6

<sup>1</sup> Soils obtained from 1994 and 1995 former Western States Proficiency Testing Program.

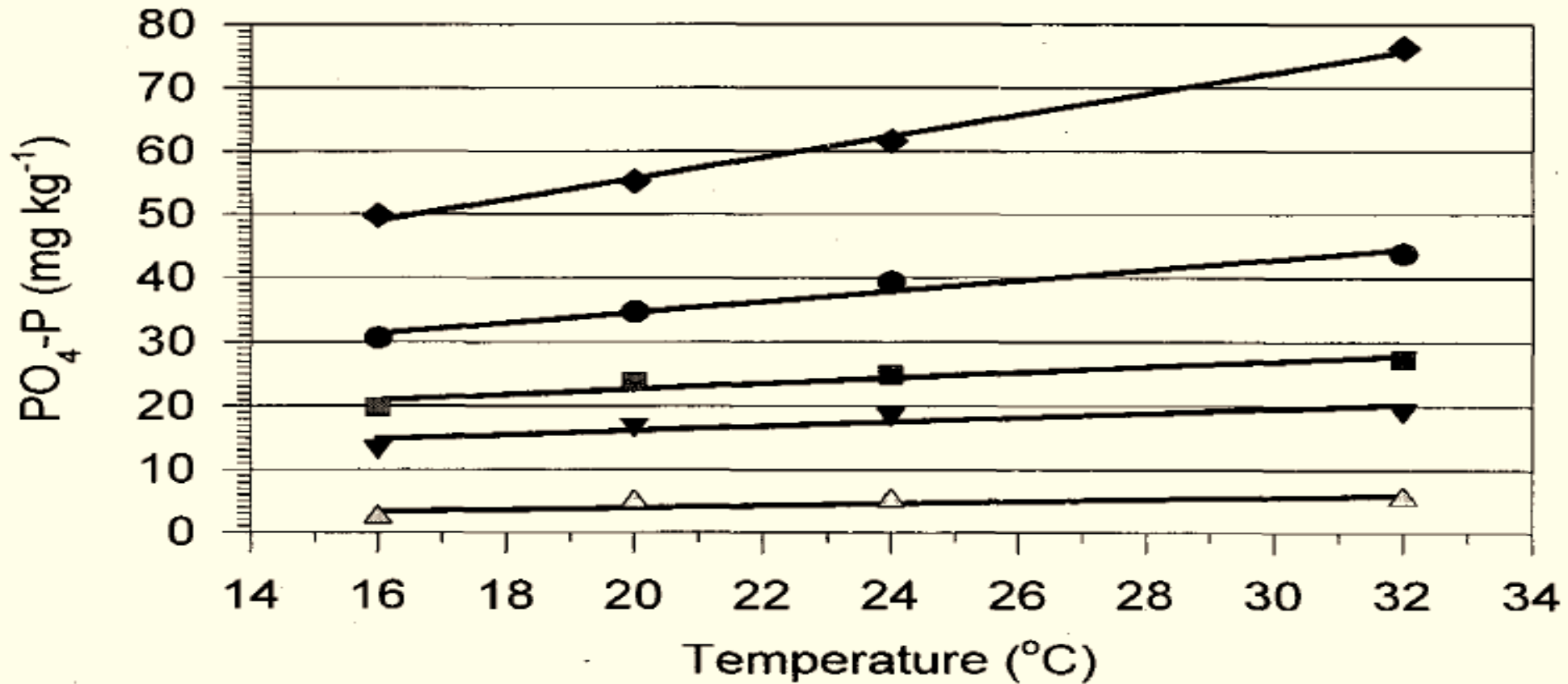
### 3f. Effect of the time, vessel size and type of agitation.

Increasing the extraction time to 40 minutes increases the amount of  $\text{PO}_4\text{-P}$  by 5%.

It is critical that the extraction vessel contain a minimum of **25% dead space** (occupied by air) to provide sufficient agitation.

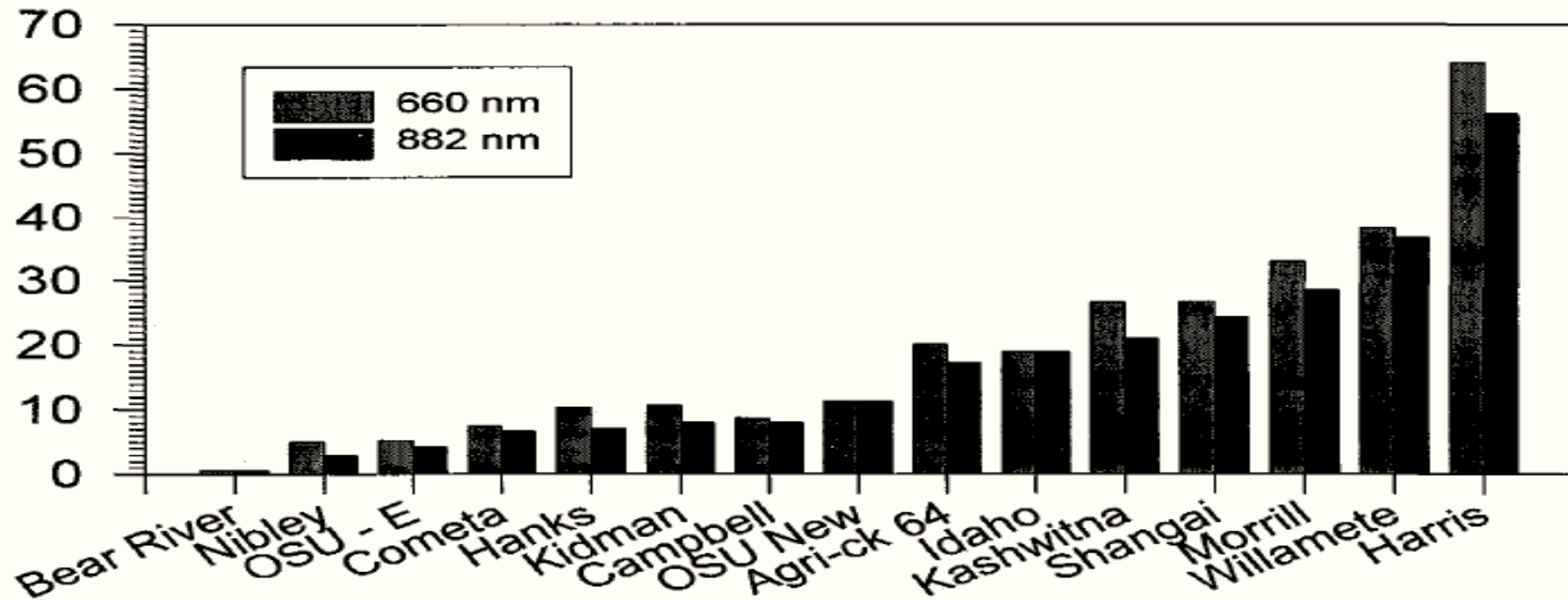
It is recommended that a **reciprocation shaker** be used for the extraction.

### 3g. Effect of Extraction temperature



# 3h. Spectrophotometric Measurement

Figure 2. The influence of spectrophotometric wavelength on PO<sub>4</sub>-P content.

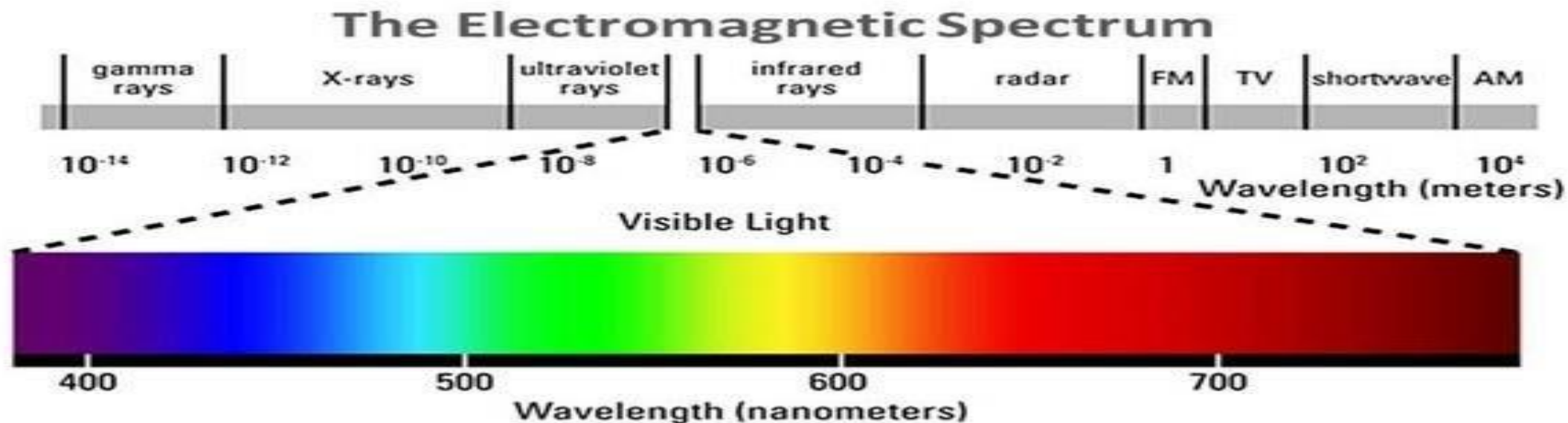


# **3i. Measurement or quantification, part two**

## **Brief introduction to UV-VIS spectroscopy**



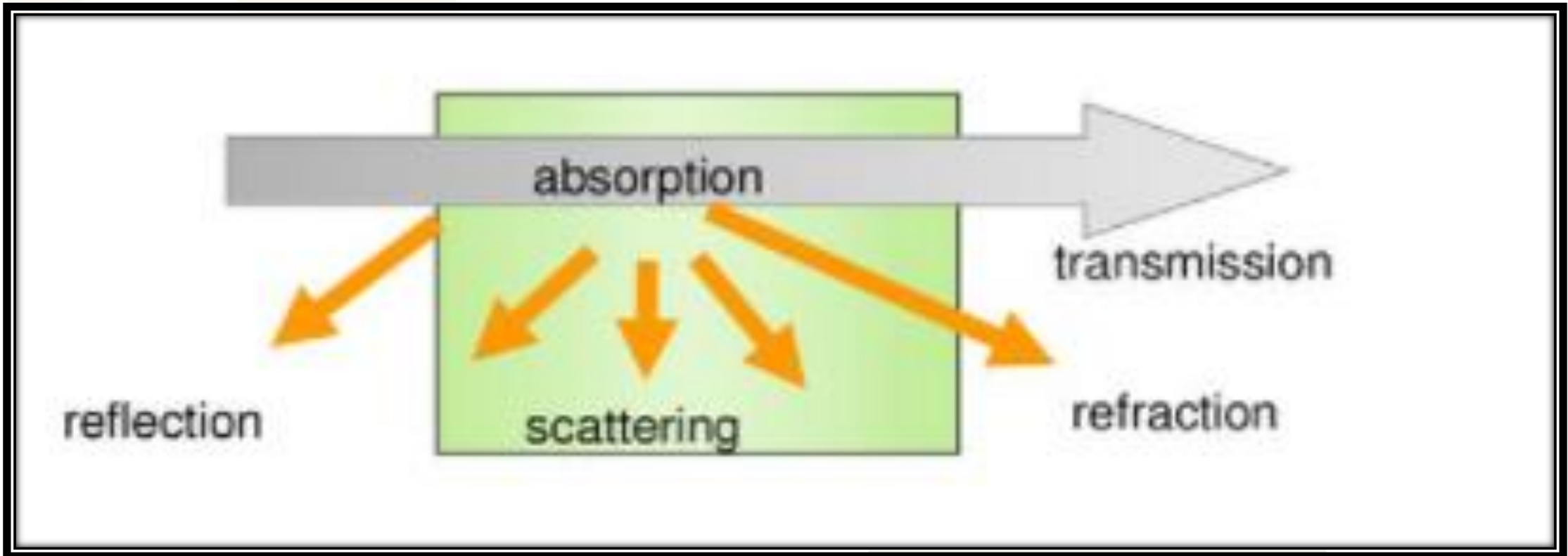
# 3j. EMR spectrum



$\lambda_1 = 660 \text{ nm}$

$\lambda = 882 \text{ nm}$

## 3k. Processes that occur when EMR affects matter



# 3I. Ratio between incident ( $I_o$ ) and transmitted ( $I_t$ ) radiation

$I_o$  = intensity of the incident beam (passes through target or analyte solution)

$I_t$  = intensity of the transmitted beam (what is not absorbed)

Absorbance (energy absorbed by the sample) (A)

Transmittance (energy passing through the sample) (T)

$$T = \frac{I_t}{I_o}$$

$$A = \log \frac{I_o}{I_t}$$

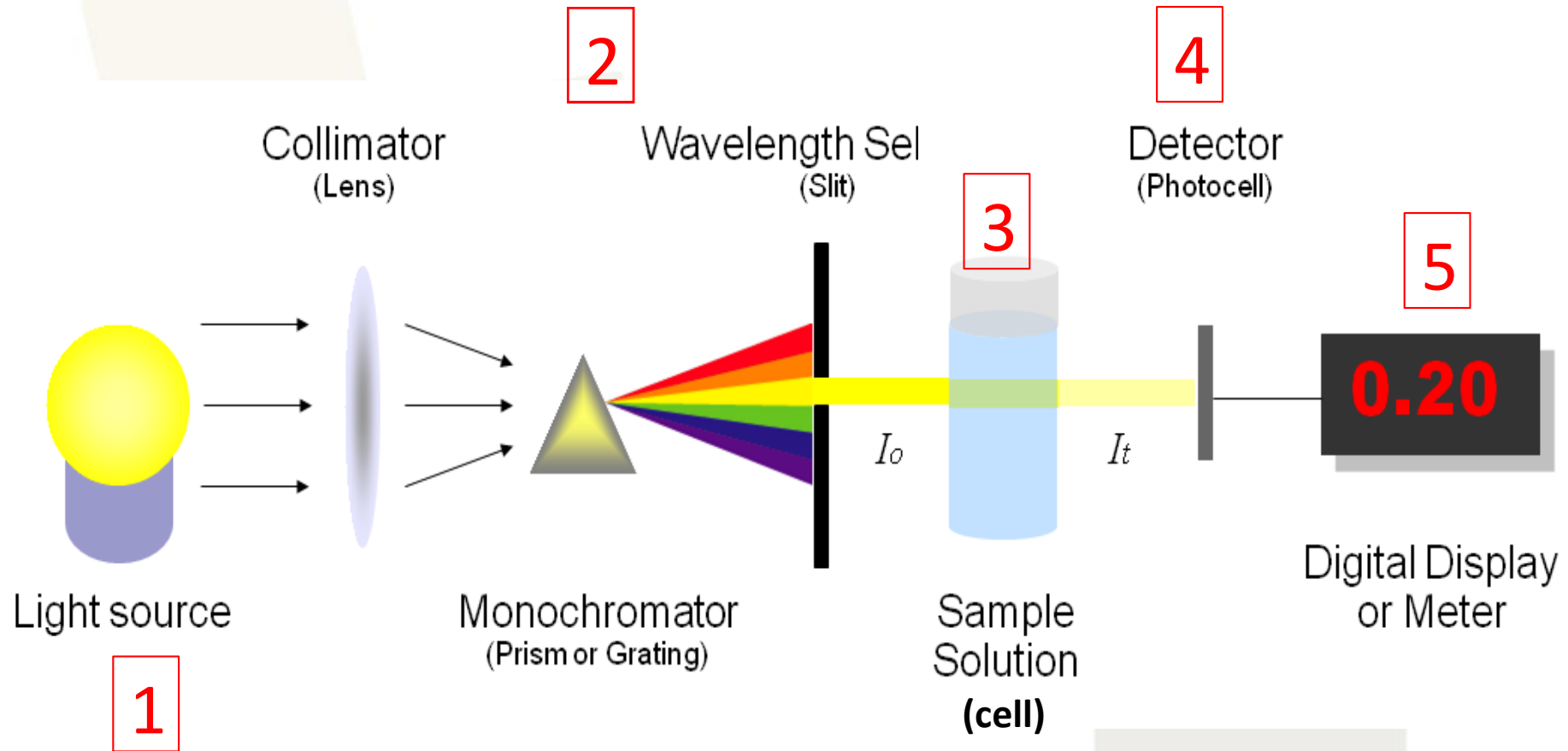
$$A = \log \frac{1}{T}$$

$$T = \frac{I_t}{I_o} * 100 = \% T$$

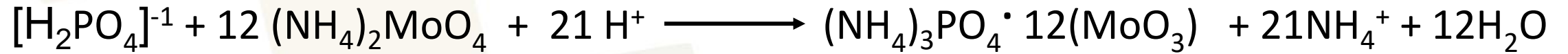
Como:  $T = \frac{I_t}{I_o}$

$$A = - \log T$$

# 3m. Basic structure of a spectrophotometer



# 3n. Chemical reactions in the colorimetry of P



Ammonium phosphomolybdate



**Complex**

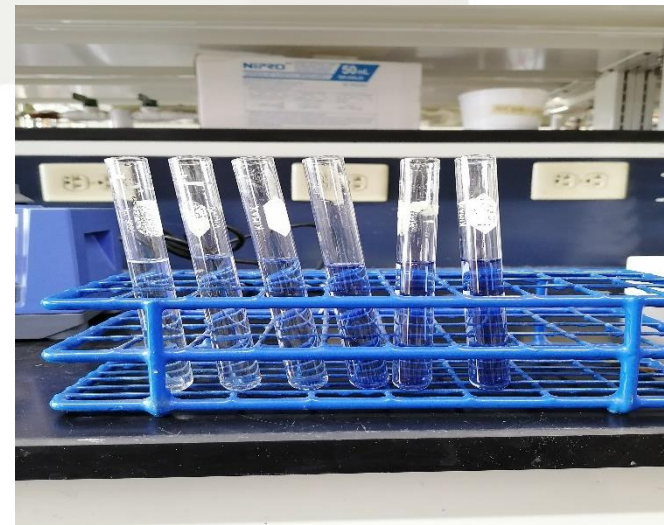
**Ac. Phosphomolybdic  
heteropolyacid  
yellow**

(ascorbic acid)

**molybdenum blue complex  
(phosphomolybdenum)**

Vanadomolybdophosphoric acids

Abs



$\lambda_1 = 660; \lambda_2 = 882 \text{ nm}$

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## 30. Caution in measurement

- Make sure that the phototube of the instrument is suitable for reading at that LO.
- Correlation coefficient between the concentration of the standards and the absorbance of the colored solution on the calibration curve should be  $\geq 0.995$ .
- \*If the absorbance of the problem samples is greater than that of the peak of the curve, dilute the extract with the extractant solution and record the dilution factor. Take a new aliquot and repeat the operation.
- Verify the preparation of the reagents, standards, and that the instrument configuration and operation is correct.
- In P-Olsen there are frequent contaminations that are detected by a slight coloration of the blank (zero point CC), take special care in the washing of the material.

## 3p. Interferences in the colorimetric method of P-Olsen

### 1) Organic matter (agitation or filtering with activated carbon)

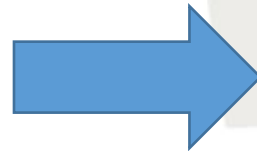


# Continued 3p. Interferences in the P-Olsen colorimetric method

## 2) Ion solution $(\text{ArO}_4)^{3+}$ , $(\text{SiO}_4)^{4+}$

- Sb and K tartrate form the colored heteropolyacid complex or Mo blue.
- Sb accelerates the development of the blue color and stabilizes it for 24 hours, no interference from Si is expected.

- The final pH of the colored solution remains within the "plateau" of maximum stability of the complex.
- The final acidity of the solution contributes to the precipitation of suspended material (Watanabe and Olsen).



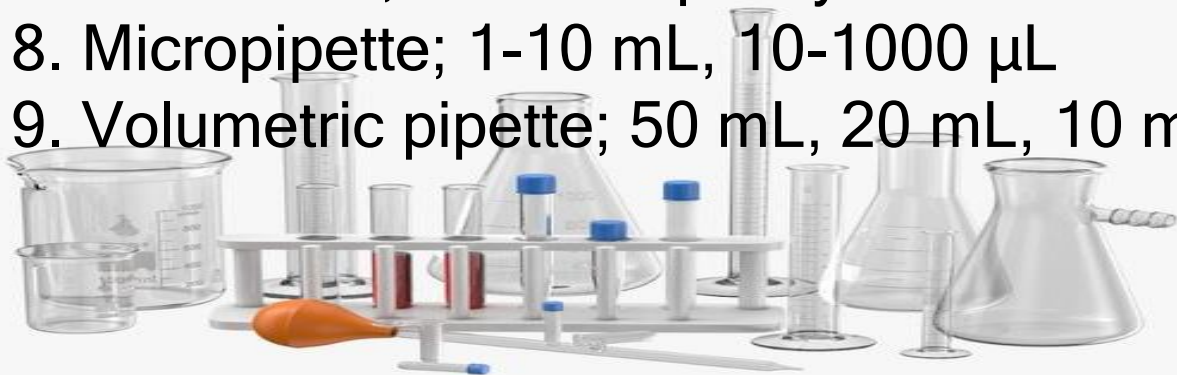
Importance of achieving the required degree of acidity required in solution



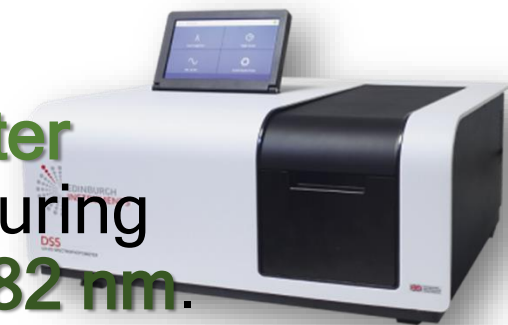
# 4. Equipment and Laboratory Wares

## 4a. Equipment and Laboratory Wares for P-Olsen Analysis

1. Erlenmeyer flasks, 125 mL
2. Funnel
3. Beakers; 5L, 1L, 500 mL
4. Polyethylene bottles with lid, wide-mouth type, 125 mL capacity
5. Volumetric flasks; 1L, 250 mL, 100 mL, 50 mL
6. Graduated cylinder; 100 mL, 50 mL
7. Test tubes, 20 mL capacity
8. Micropipette; 1-10 mL, 10-1000  $\mu\text{L}$
9. Volumetric pipette; 50 mL, 20 mL, 10 mL



1. **UV-VIS spectrophotometer** capable of measuring absorbance at **882 nm**.



2. Analytical Balance, precision of at least 0.0001g
3. Reciprocating shaker
4. Vortex mixer

# 5. Reagents and Required Chemical Solutions

# 5a. Reagents and chemical solutions for P-Olsen

**01**

## EXTRACTING SOLUTION

- ❑ Sodium Bicarbonate solution, 0.5 M, pH 8.5 (extracting solution). Adjust the pH to 8.5 by adding NaOH 1 M (4 g/100 mL).

**02**

## MIXED REAGENT

- ❑ 200 ml deionized water
- ❑ 50 ml 4 M H<sub>2</sub>SO<sub>4</sub>
- ❑ 15 mL of 4 % NH<sub>4</sub>-molybdate solution
- ❑ 30 mL of 1.75 % ascorbic acid solution
- ❑ 5 mL of 0.0275% KSb-tartrate solution

**03**

## DEIONIZED WATER

- ❑ (EC < 0.001 dS m<sup>-1</sup> (ASTM D1193-91 and ISO 3696:1987)).

**04**

## PHOSPHATE FREE ACTIVATED CHARCOAL

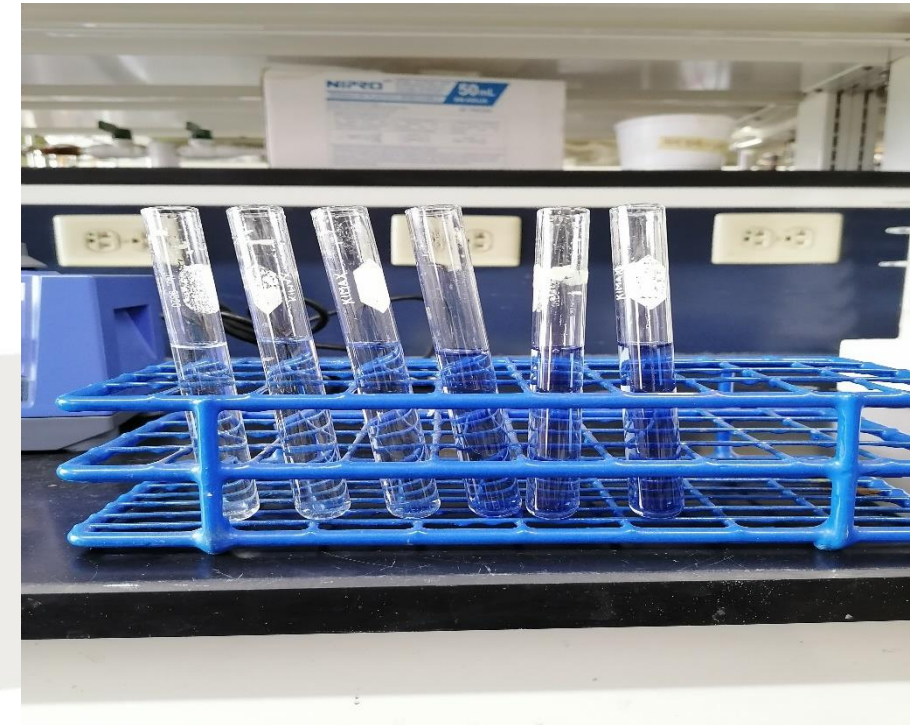
**05**

## STANDARDS SERIES

- ❑ (0-0.4-0.8-1.2-1.6-2.0 mg/L P)

## 5c. Preparation of standard solutions of P

- Standard solution of P  $100 \text{ mg P L}^{-1}$ 
  - Using standard (NIST or traceable equivalent) of  $1000 \text{ mg P L}^{-1}$
  - Prepare the standard from  $0.4390 \text{ g KH}_2\text{PO}_4$  (dried for 2h at  $110 \text{ }^\circ\text{C}$ ) in extractant solution and make up to 1L in volumetric flask.
- Phosphate secondary standard solution of  $4 \text{ mg P L}^{-1}$
- Working standard series (calibration curve):  
 $0, 0.4, 0.8, 1.2, 1.6$  and  $2.0 \text{ mg P L}^{-1}$
- NOTE: The range of the standard series can be adjusted according to equipment specifications and soil P concentration.



# 6. Caution in the preparation of reagents

## 6a. Caution and safety

- ✓ Wear proper personal protective equipment.
- ✓ Observe careful and proper handling of chemicals when using strong alkali bases, strong acids and oxidizing agents.
- ✓ Avoid mixing incompatible chemicals to reduce risks of fire and explosion inside the laboratory.
- ✓ Always pour the acid into water to avoid splattering.
- ✓ Refer to the Safety guidelines or the Safety Data Sheet (SDS) before proceeding.



## 6b. Caution in the preparation of reagents. Chemical risks

6a1.  $\text{H}_2\text{SO}_4$  is a clear, colorless, odorless liquid. It is extremely corrosive and causes severe burns if not handled properly. Dilute it by adding a small portion of acid to a large amount of water and work under a fume hood to avoid inhaling its vapors.

6a2. Ammonium molybdate solution is a corrosive liquid. Contact with eyes or body may cause serious health injuries. Reaction with metals produces hydrogen and, in a fire, produces sulfur oxides.



## 6b. Caution in the preparation of reagents: Chemical Risks

6a3. Ascorbic acid has no known effect on the skin/body; it should be stored in light resistant containers because it is light sensitive and kept away from incompatible materials (oxidizing agents).

6a4. Potassium antimony tartrate is hazardous in case of contact with skin or body. Do not dispose of waste down the drain. Incompatible with strong acids, bases and oxidizing agents. Do not expose to direct sunlight.

6a5 Activated charcoal is hazardous in case of contact with skin or body. Causes eye and respiratory irritation if inhaled. Keep away from heat, sparks, open flames and hot surfaces.

# 7. Sample preparation

## 7a. Drying, milling and sifting

- Air dried soil (or oven with air circulation)  $35 \pm 5^\circ\text{C}$
- Milled and sifted sample to  $\leq 2.0$  mm (mesh 10) (to conserve sample representativeness, to obtain subsample by quarticulation or other procedure)



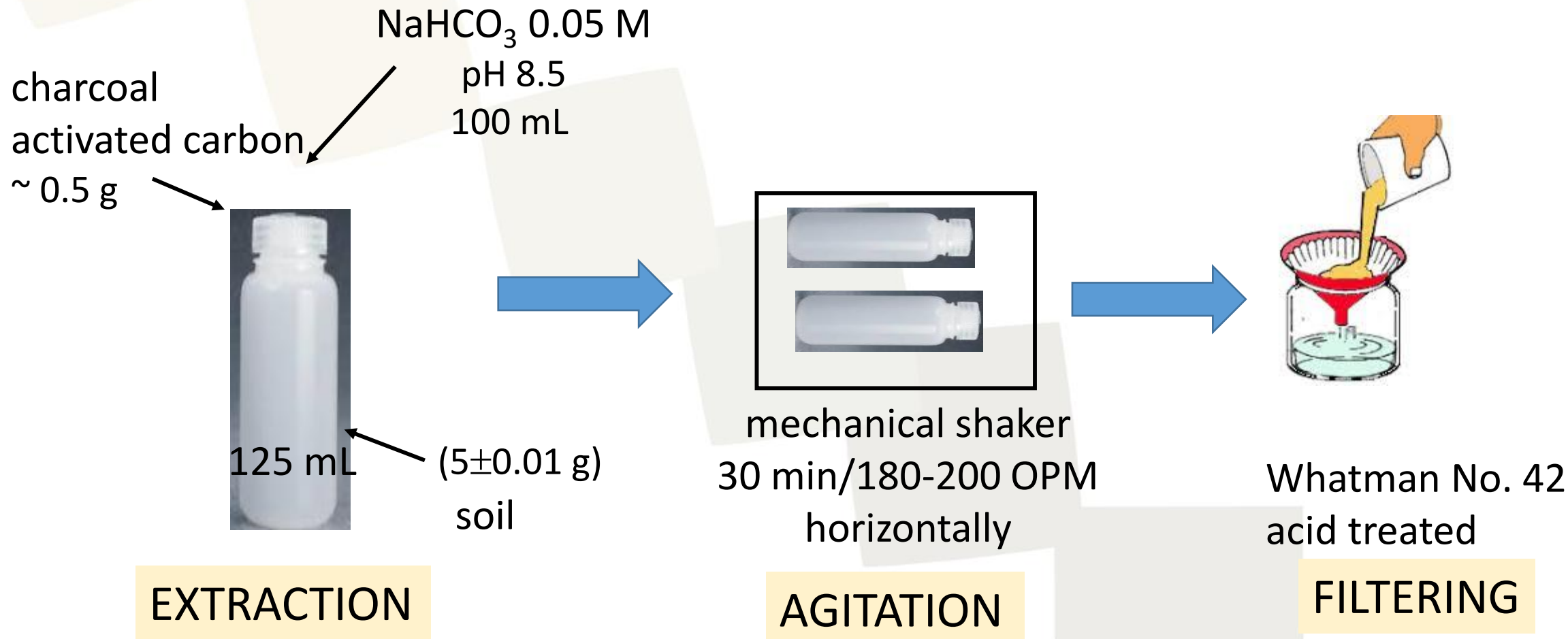
# 8. Procedure

**The P-Olsen analytical procedure is divided into two parts**

**EXTRACTION  
(SOLUBILIZATION)**

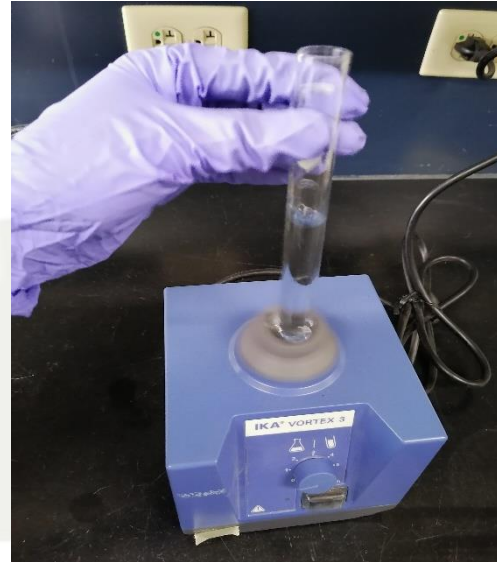
**QUANTIFICATION  
(MEASUREMENT)**

# Extraction-Solubilization, First step



\* Include 2 blanks and three quality control materials (QCMs).

# Spectrometric measurement or quantification of P

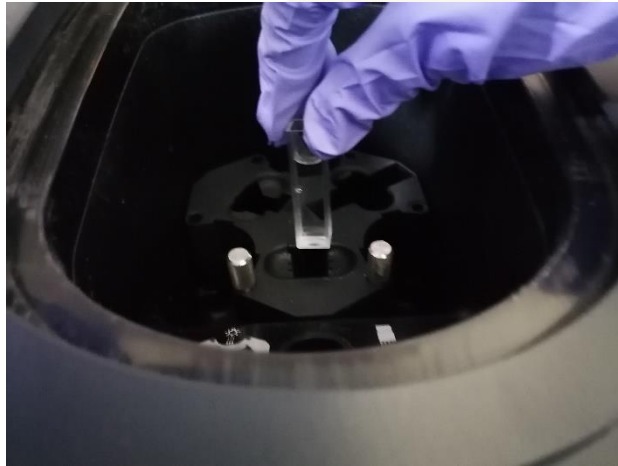


Stand for 30 min  
max. 1 hour

3 mL aliquot of extract  
+ 3 mL of mixed reagent

vortex

# Colorimetric determination of P

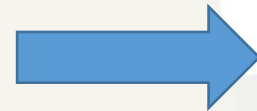


$\lambda_2 = 882 \text{ nm}$



$[P_{\text{sol}}]$

0, 0.4, 0.8, 1.2, 1.6 and 2.0 mg P L<sup>-1</sup> (3 mL standard + 3 mL mixed reagent)

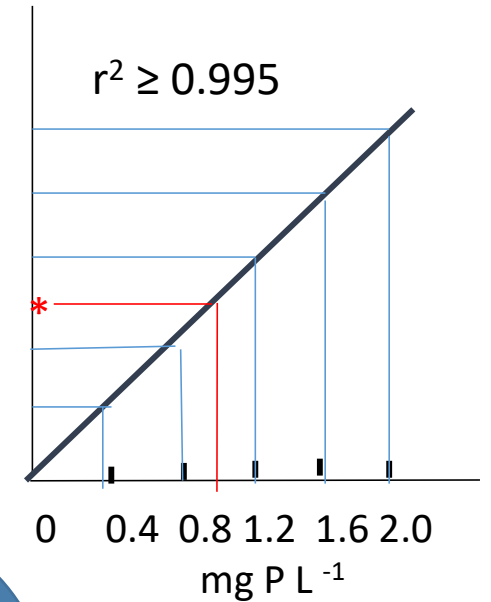


Abs

+

P soil mg kg<sup>-1</sup>

Abs



Law of Lambert and Beer

$$\text{Abs} = abC$$

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# 9. Calculations required in P-Olsen's method

## 9a. Calculation of P present in air-dried soil

$$\begin{aligned}\text{mg P kg}^{-1} &= (a - b) \times \frac{V \times 1\text{L} \times 1000 \text{ g} \times \text{DF} \times \text{mcf}}{W \times 1000 \text{ mL} \times 1\text{kg}} \\ &= (a - b) \times \frac{V \times \text{DF} \times \text{mcf}}{W}\end{aligned}$$

a = P concentration in extracted sample ( $\text{mg L}^{-1}$ )

b = P concentration in the target ( $\text{mg L}^{-1}$ )

V = extraction volume (mL)

W = soil sample weight (g)

DF = dilution factor = total solution volume/aliquot (extract)

mcf = humidity correction factor

\* Factor to report in  $\text{P}_2\text{O}_5 = 2.29 \times \text{P} (\text{mg kg}^{-1})$

\*\* Report in  $\text{mg P kg}^{-1}$  and on oven-dry weight basis to two decimal places.

# 10. Quality assurance/quality control

## 10a. Accuracy test



Participate in an **Inter-Laboratory Proficiency Test Program** (Inter-Laboratory Proficiency Test) at least once a year.

Perform replicate **analysis of the Certified Reference Material (CRM)** and compare the results with those of other laboratories, as indicated in the certificate analysis report.

## 10b. Precision test



- ✓ Perform **replicate analysis** of 10 percent of the samples in a test batch.
- ✓ Calculate the **percent relative standard deviation (%RSD)** to determine if the precision of the replicate analyses is within specifications.

Compare the result with the target precision for the analyte concentration (Table 1).

$$\% RSD = \frac{s}{\bar{x}} \times 100$$

Where:  $s$  = standard deviation of the replicated result  
 $\bar{x}$  = mean

**Tabla 1. Expected accuracy (repeatability) as a function of analyte concentration.**

Analyte, %	Analyte ratio	Unit	RSD, %
100	1	100%	1.3
10	10 <sup>-1</sup>	10%	1.9
1	10 <sup>-2</sup>	1%	2.7
0.01	10 <sup>-3</sup>	0.1%	3.7
0.001	10 <sup>-4</sup>	100 ppm (mg/kg)	5.3
0.0001	10 <sup>-5</sup>	10 ppm (mg/kg)	7.3
0.00001	10 <sup>-6</sup>	1 ppm (mg/kg)	11
0.000001	10 <sup>-7</sup>	100 ppb (µg/kg)	15
0.0000001	10 <sup>-8</sup>	10 ppb (µg/kg)	21
0.00000001	10 <sup>-9</sup>	1 ppb (µg/kg)	30

**Source:** AOAC Peer Verified Methods Program. Manual on Policies and Procedures (AOAC, 1998)

## 10c. Control chart



- ✓ Analyze at least a triplicate of the **quality control material (external or internal)** per batch.
- ✓ Construct a **control chart** with the results.
- ✓ Monitor for out of specified limits.

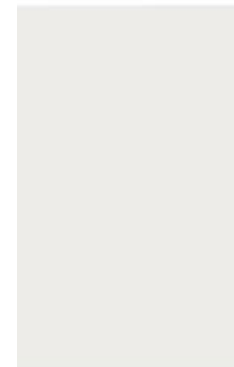
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**Inventory of P-Olsen data in the  
ISRIC-WISE soil database  
for use with QUEFTS**  
(Version 1.0)

**Niels H. Batjes**  
(May 2010)



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**Thank you very much**

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