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CALIBRATION OF YELLOW COLOUR SPECTROSCOPIC METHOD OF PHOSPHORUS DETERMINATION FOR WAVELENGTH, WORKING RANGE AND TIME

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Abstract: Yellow colour spectroscopic method to determine phosphorus in solution is a very accurate and reliable tool used world wide in chemistry laboratories. The working range of this method is up to 20 ppm and the absorbance is read at 470 nm wavelength. The yellow chromogen is quite stable for long time. In this study, the method is revised to find the most suitable wavelength at which the yellow chromogen gives the highest absorbance. This study further investigates the working range within which a linear relationship exists between phosphorus concentration and the absorbance. The stability of the chromogen with time was also studied. From the study it is found that, the yellow colourchromogen absorbs only the lower portion of the visible spectrum and 400 nm light is better to use to get highest light absorbance. The absorption values gradually decreases with the increase in wavelength and at 600 nm wavelength absorption value becomes zero. The working range may extend up to 30 ppm instead of 20 ppm. The chromogen was found stable even for 2 months.

Keywords: Spectrophotometer, wavelength, working range, chromogen, absorption.

Introduction

A group of analysis based upon the measurement of the amount of light absorbed or emitted by a substance (mostly coloured) is referred to as spectrophotometry. Absorption spectrophotometryis concerned with measurement of light absorbed by a substance. A substance must fulfill at least two criteria to be determined by spectroscopic method. First, the substance should form a particular chromogen through a suitable chemical reaction and second, the intensity of the chromogen should be proportional to the concentration of the substance (Jackson, 1973a). Absorption spectrophotometry is generally controlled by laws of light absorption. Spectrophotometry is based upon the laws of Lambert, Beer and Bouger.When a light beam falls on a homogenous layer of substances, part of the radiation is reflected (I_r), part is absorbed (I_a) and part is transmitted (I_t). When comparing the intensities of the beams transmitted throughout the solution (containing the substance) and through the solvent, the effect of I_r is neglected in practice, and the relationship is changed into $I_0 = I_a + I_t$ (I_r = negligible). Therefore, the optical density (absorbance) becomes directly proportional to concentration of the substance to be measured. Conformity with Lambert and Beer's law gives a straight line if optical density (absorbance) is plotted against concentration

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(Chemistry libretexts, 2018). The extinction coefficient (molar absorptivity) for any given substance is a constant only so long as the wavelength of light is constant. Hence, the absorbance of a substance changes with wavelength (Wikipedia, 2017).

According to the Beer-Lambert Law absorbance is proportional to concentration at each wavelength. Theoretically one can choose any wavelength for quantitative estimations of concentration. However, the magnitude of theabsorbency is important, especially when we try to detect very smallamounts of material. Themeasurement of light absorbance as a function of concentration is most sensitiveat this wavelength. For this reason, scientists generally select the wavelength ofmaximum absorbance for a given sample and use it in absorbance measurements (Chemistry III lab, 2005). There are some limitations of this law. The linearity of the Beer-Lambert law is limited by chemical and instrumental factors. Causes of nonlinearity include: deviations in absorptivity coefficients at high concentrations (>0.01M) due to electrostatic interactions between molecules in close proximity, scattering of light due to particulates in the sample, fluorescence or phosphorescence of the sample, changes in refractive index at high analyte concentration, shifts in chemical equilibrium as a function of concentration, non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band and stray light (Beer-Lambert Law).

A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected. Depending on the range of wavelength of light source, it can be classified into two different types: UV-visible spectrophotometer**:** uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum. IR spectrophotometer: uses light over the infrared range (700 - 15000 nm) of electromagnetic radiation spectrum (Chemistrylibretexts, 2018).

In visible spectrophotometry, the absorption or the transmission of a certain substance can be determined by the observed color. For instance, a solution sample that absorbs light over all visible ranges (i.e., transmits none of visible wavelengths) appears black in theory. On the other hand, if all visible wavelengths are transmitted (i.e., absorbs nothing), the solution sample appears white. If a solution sample absorbs red light (~700 nm), it appears green because green is the complementary color of red. Visible spectrophotometers, in practice, use a prism to narrow down a certain range of wavelength (to filter out other wavelengths) so that the particular beam of light is passed through a solution sample (Chemistry III lab, 2005).

Spectrophotometer uses wide range of wavelengths starting from ultraviolet to infra red (Chemistry libretexts, 2018). This is important to note that the visible range is only a very small part of the electromagnetic spectrum. Ultraviolet and infrared spectrophotometric methods are suitable for many colorless substances that absorb strongly in the UV or IR spectral regions (Chemistry III lab, 2005). The use of spectrophotometers spans various scientific fields, such as physics, materials science, chemistry, biochemistry, and molecular biology (Rendina, 1976). Common nutrients like P, S, Fe, Mn and others are determined in laboratory in this method. Hence, the spectrophotometer has well been called the workhorse of the modern laboratory (SCRIBD, 2018). This device hence is an essential aid to both research and routine control.

In every spectroscopic method, there are different variables those influence the accuracy of determination. Among these wavelength (at which highest absorbance of the chromogen is achieved), working range (the concentration range of the substance to be determined for the construction of the calibration curve) and time (stability of the chromogen) are three important variables. Every method is needed to be validated for its variables before use them in any laboratory.

In soil, water and environment laboratory, yellow colour (vanadomolybdophosphoric yellow colour method in nitric acid system) spectroscopic method is in common use to determine P present in soil, water, plant, fertilizer, compost etc analysis. The advantages of this method are extreme simplicity, lower sensitivity, stability of colour, freedom from interferences with a wide range of ionic species in concentrations up to 1000 ppm (Jackson, 1973b; Barton, 1948; Kitson and Mellon, 1944). According to Jackson (1958), the working range is up to 20 ppm and the absorbance is read at 470 nm wavelength. The yellow chromogen is stable for infinite time. The present investigation is aimed to validate this method in terms of wavelength, working range and time.

Materials and Methods:

Principle*:* The original method was described by Jackson in 1958. The reaction of phosphoric acid with molybdate ions forms a heteropolymolybdophosphate complex as follows:

$$H_3PO_4 + 12H_2MoO_4 \rightarrow H_3P(Mo_3O_{10})_4 + 12H_2O$$
(yellowcolour)

The exact nature of the yellow chromogen in the system is not known, but the colour is attributed to substitution of oxyvanadium and oxymolybdanum radicals for the oxygen of phosphate radical to give a heteropoly compound that is chromogenic (Jackson, 1973b). The complex is yellow in color, and at high P concentrations forms a yellow precipitate. In the presence of vanadium, the yellow color is intensified (Jackson, 1958).

Reagents

The following reagents are required in this determination.

Standard P solution**:** 0.1098 g KH_2PO_4 is taken into a 500 ml volumetric flask. 2 to 3 ml concentrated H_2SO_4 is added to increase the solubility. About 100 ml distilled water is poured and shake the content till dissolves. The volume is made up to the mark with distilled water and thoroughly mixes the solution. This is a 50 ppm P solution.

Colouring solutions:*Solution-A:* 12.50 g ammonium molybdate is dissolved in 200 ml distilled water at 50^0 C and cool down. *Solution-B:* 0.625 g anhydrous ammonium metavanadate is dissolved in 150 ml hot distilled water. The solution is cooled and then 125 ml of concentrated HNO_3 is added and again cool to room temperature. The content is volume 300 ml with distilled water.*Solution-A+B* (Colouring reagent): This is prepared by mixing solution-A and solution-B in 1: 1.5 ratio. Colouring reagents should be prepared immediate before analysis.

Procedure: To validate the phosphorus yellow colour method for suitable wavelength, working range and stability of the chromogen the following steps were conducted. Standard solutions were prepared from analar grade reagents and the working range were selected double the concentration than that is in common practice (Table 1). Absorbance of the standard solutions was measured by spectrophotometer (Shimadzu UV-Vis spectrophotometer, model: UV-1280, wavelength range: 190 to 1100 nm, Japan) at various wavelengths produced in visible spectrophotometer (400 to 1100 nm) giving 25 nm interval. Correlation coefficients (*r*) for standard solution values and the absorbance at each wavelength were calculated. Calibration curves were constructed by plotting element concentration in X axis and absorbance in Y axis. Calibration curves were presented in two forms. At first all absorbance values at different wavelengths for an element were shown in a fixed scale (Fig. 1). In second form, a series of individual curves were constructed but in various scale (Fig. 2). The absorbance of the standard solutions was measured every week at both 400 and 470 nm wavelength up to 2 months. Calibration curves were prepared to see the variation in absorbance of the chromogen with time.

Table 1: Standard solution composition in yellow colour method for Phosphorus determination.

	Standard P solution (ppm)	*50 ppm P solution (ml)*	*Colouring reagent*	*Final volume*
Common	*0*	*0*	*10 ml*	*25 ml*
	5	*2.5*		
	10	*5.0*		
	15	*7.5*		
	20	*10.0*		
Extended	*25*	*12.5*		
	30	*15.0*		
	35	*17.5*		
	40	*20.0*		

Results and Discussion

Absorption values recorded for a series of standard solutions at different wavelengths to determine Phosphorus are discussed in the following sections. The method was investigated for best wavelength at which highest absorbance is obtained, working range that shows highest linear relationship (highest *r* value) and time dynamics (stability of the chromogen). The findings are separately discussed below.

Best wavelength and working range: The calibration curves for the standard solutions and their absorbance are shown in Fig. 1. The graph compares the absorption values recorded at different wavelengths in a fixed scale. Fig. 1 clearly state that standard solutions for yellow colour method absorb only the lower portion of the visible spectrum. The highest absorbance is obtained at 400 nm wavelength. Absorption values gradually decreases with the increase in wavelength and at 600 nm wavelength absorption value becomes zero for all standard solutions. Therefore, no further spectrophotometer reading was taken. As the absorption is about 4 times higher at 400 nm wavelength than that recorded at 470 nm, 400 nm wavelength is recommend to measure the absorbance for this yellow colour method.

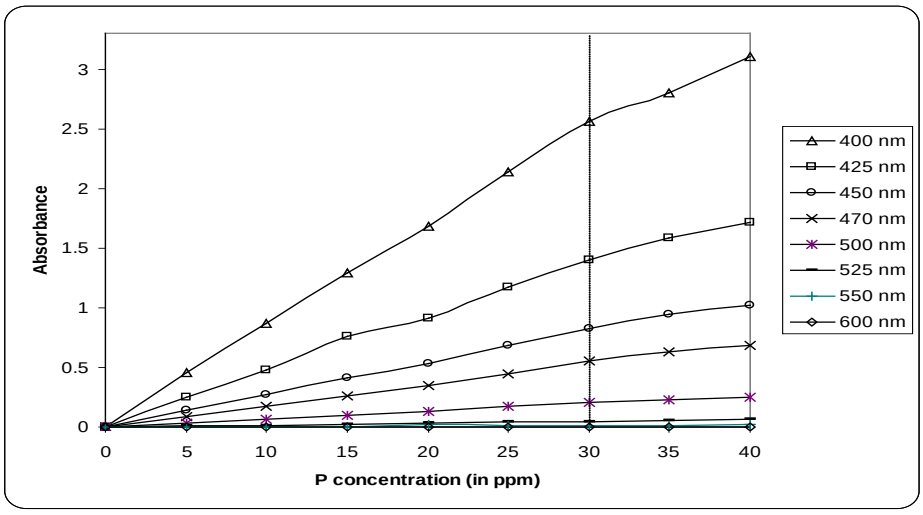

Fig. 1: Calibration curves of yellow colour method at different wavelengths (Fixed scale).

To observe the linear nature of these curves, the data is presented in Fig. 2 in an individual scale. Absorbance taken beyond 525 nm the linear nature of the graph is lost hence higher wavelengths is of no use in this method. The nature of the graphs shows that up to 30 ppm Phosphorus standards the relationship is linear (*r* value) in all curves except for 550 nm. Beyond 525 nm wavelength the linear relationship between phosphorus concentration in solution and their light absorbance is lost.

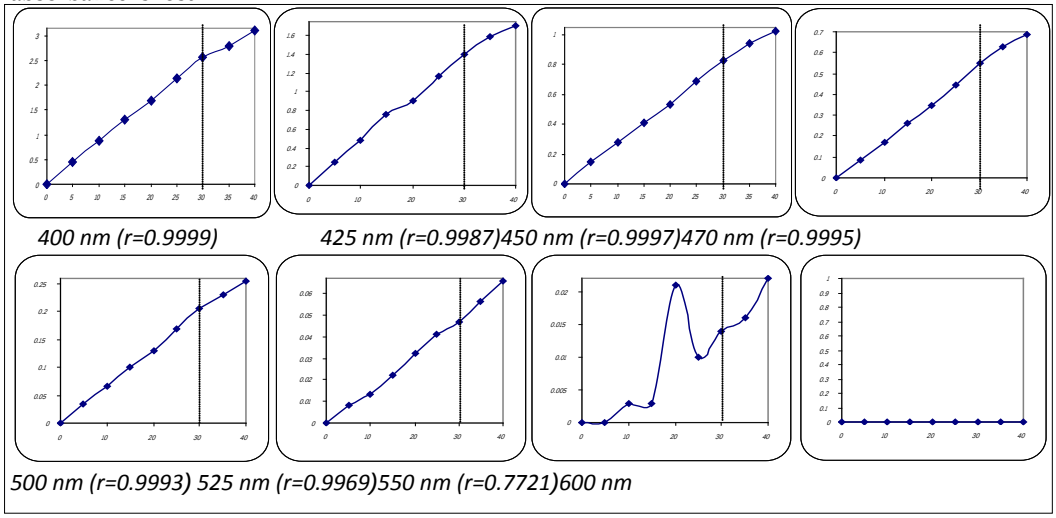

Fig. 2: Absorbance pattern of phosphorus yellow colour method at different wavelengths. (X= Concentration of P in ppm, Y= Absorbance & r is calculated up to 30 ppm P)

Wavelength that produces highest absorbance and shows highest *r* value should be the best point of the visible spectrum. Hence, 400 nm is found the best spectrum for this method. As standard solutions up to 30 ppm of Phosphorus shows linear relationship (*r* =0.9999) at 400 nm wavelength, it may be recommended from the present study that yellow colour standard solutions can be extended up to 30 ppm of Phosphorus. Jackson (1958) stated that the colour is read in spectrophotometer with a light maximum from 400 to 490 nm, according to the sensitivity needed and this choice of wavelength depends upon the concentration of Phosphorus in standard solutions. The working range is limited within 20 ppm in the method originally described by Jackson (1958).

Time dynamics: The present investigation finds 400 nm at which highest absorbance of standards is obtained whereas 470 nm is the commonly used wavelength for yellow colour method. Therefore, to see the time dynamics both wavelengths were considered for comparison. The findings are presented in Fig. 3.

Fig. 3: Calibration curves up to 2 months for yellow colour method at 400 nm & 470 nm.

Absorbance recorded in case of 400 nm wavelength is much higher compared with that observed in case of 470 nm. Up to 2 month time the chromogen was found stable in both wavelengths. No precipitate observed during this period. However, after 15 days some of the reading at 400 nm wavelength became unstable. On the other hand, all readings were found stable in case of 470 nm wavelength. Though the absorbance is about 4 times higher in case of 400 nm wavelength over 470 nm and the reading is stable for 15 days, this study suggest 400 nm light to be used in this method. Beyond 15 days it is of rare practical need to use the method.

Conclusion

The chromogen absorbs only the lower portion of the visible spectrum and 400 nm light is better to use to get highest light absorbance. The absorption values gradually decreases with the increase in wavelength and at 600 nm wavelength absorption value becomes zero. The working range may extend up to 30 ppm instead of 20 ppm that is in common practice for this method. Though the chromogen is stable even for 2 months, it is better to take reading within 15 days if we use 400 nm light to get stable reading.

References

Barton, C.J. 1948. Photometric analysis o f phosphate rock, Anal. Chern. 20:1068-1073.

Beer-Lambert Law (online), available at <http://life.nthu.edu.tw/~labcjw/BioPhyChem/Spectroscopy/beerslaw.htm> (15-04-2019).

Chemistry III lab. 2005. Spectrophotometry: Absorption measurements & their application to quantitative analysis, pp: E-1-7.

Chemistry libretexts, 2018. Specphotometry (online), available at <https://chem.libretexts.org /Core/Physical_and_Theoretical_Chemistry/Kinetics/ReactionRates/Experimental_Determination_of_Kinetcs/Spectrophotometry> (17-04-2019).

Jackson, M.L. 1958. Soil chemical analysis, Prentice-Hall Inc., pp: 51-53.

Jackson, M.L. 1973a. Soil chemical analysis, Prentice-Hall Inc., pp: 430.

Jackson, M.L. 1973b. Soil chemical analysis, Prentice-Hall Inc., pp: 151.

Kitson, R.E. and M.G. Mellon. 1944. Colorimetric determination of phosphorus as molybdivanado-phosphoric acid. Ind. Eng. Chem. Anal. Ed. 16:379-383.

Rendina, George. 1976. Experimental Methods in Modern Biochemistry, W. B. Saunders Company, pp: 46-55.

SCRIBD, 2018. Spectrophotometer Modern (online), available at

<https://www.scribd.com/document/296189873/Spectrophotometer-Modern> (15-01-19)

Wikipedia, 2017. Extinction coefficient (online), available at <https://en.wikipedia.org/wiki/ Extinction_coefficient> (15-04-19)