



## Spectrophotometric determination of Phosphate in water and sugarcane sample

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### Abstract

This study is aimed to evaluate the phosphate concentration in parts per million in water and two sugarcane samples from two different Field areas through molybdenum blue phosphorous method in conjugation with UV-Visible spectrometer. Phosphomolybdate complex was formed by the addition of molybdate and subsequently reduced with hydrazine hydrate. This obeys Beers-Lamberts law at 840 nm in 0.1-0.7 ppm concentration range. The reagents used are potassium hydrogen phosphate, ammonium molybdate and hydrazine sulphate. The amount of phosphate present in water and sugarcane is found to be proportional to the color intensity of reduced phosphomolybdate solution. The phosphate concentration in Malir water sample is 0.427 ppm, in sugarcane samples from Chakwal and Larkana district of Pakistan are 0.847 and 0.671 ppm, respectively.

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## Introduction

In the emerging world contamination of ground water, soil, surface water, air and sediments with precarious and noxious chemicals are facing a major problem. This might be owing to poor handling of environmental resources, improper utilization of the environment, technological advancement and increasing population density. Water is one of the major components of the earth. The earth crust is covered with 70% of water (lakes, rivers, sea) according to the graphical analysis. Scientists discovered through their research that many chemical contaminants are present in water. Some of these includes sulphates, nitrates and phosphates amongst other chemicals. Phosphate is an oxide of phosphorous and is one of the common pollutant in water. Phosphorous is the most abundant element on the surface of the earth and occurs in the form of phosphate. Phosphate is an important source for plant growth. Biochemical process need phosphate for nutrition. The phosphate concentration is directly proportional to the rate of plant growth. In the modern world phosphorous have proven to be advantageous in several fields like medical, agricultural, metallurgy and environmental science. When phosphate is present in water, eutrophication occurs (Heavy algal growth) and as such is detrimental. Large quantities of phosphate are using in builders, surfactants, detergents and other varied ingredients including anti redeposition agents, perfumes, brighteners and enzymes (Kharat & Pagar, 2009; Lal & Singh, 1961).

In sugarcane juice manufacturer, Phosphate is also important during sugarcane juice clarification. In this perspective, the chemical action of phosphate assumes a substantial role mainly when the juice is deficient in natural phosphate content in the range of 300-350 ppm. The addition of soluble phosphate is the only alternative to achieve the largest value of adding single or triple superphosphate and ortho phosphoric acid if the less phosphate content are present in sugarcane. Conversely, the routine analysis of phosphate is important in the areas of medicinal, agricultural, environmental sciences etc (Batool *et al.*,

2015; Pradhan & Pokhrel, 2013). Several procedures have developed to observing the phosphate level in the natural water in recent years such as flow injection spectrometry, complexogravimetry, ion chromatography, colorimetry, HPLC, spectrophotometry and atomic absorption spectrometry. Amongst these procedures, spectrophotometric method include ammonium molybdate are the most commonly practiced method (Oladegi *et al.*, 2016).

The spectrophotometric molybdenum blue method is a well-established method for determining phosphate. The absorbance of molybdenum blue is measured spectrophotometrically at a certain wave length that gives maximum absorbance. The intensity of the blue color is proportional to the amount of phosphate present in the sample solution. Several reducing agents have been reported in the literature such as ascorbic acid, stannous chloride and sodium sulphide and so on. Nevertheless most of the reducing agents undergo from some drawbacks such as sensitivity, stability of color, interferences from arsenic, absorption by the blank and the length of the time required for the full color development (Ganesh *et al.*, 2012; Mahadevaiah *et al.*, 2007; Habibah *et al.*, 2018). In this investigation, hydrazine hydrate has been used as the reducing agent for the reduction of molybdophosphoric acid to molybdenum blue. The blank gives no color, which is the major advantage of this method.

## Materials and methods

### Instruments

The spectral measurements were made using ultraviolet visible spectrophotometer (Schimadzu UV 1800) with 1cm matched quartz cells. An electronic balance, model PA 214V, OHAUS and a drying oven, Memmert schwabach FRG Germany Typ UN55 were used.

### Preparation of Standard Solutions

The solutions were prepared in double distilled water and all the chemicals used were of analytical reagent grade. In 500mL volumetric flask potassium dihydrogen phosphate (0.717g) was dissolved in double distilled water.

Further dilutions were also prepared from this phosphate solution. 5% ammonium molybdate reagent (50mL) was diluted in a 100mL of volumetric flask to make this solution 2.5%. 10N Sulphuric acid solution was prepared from concentrated sulphuric acid (28mL) in a 100mL volumetric flask. 0.5M hydrazine hydrate solution was prepared from 2.44mL concentrated hydrazine hydrate in a 100mL volumetric flask.

*Preparation of Samples*

*Water sample preparation*

The water sample collected from Malir Karachi was used for the phosphate analysis. To remove the impurities Whatmann-41 filter paper was used for the filter this sample. The filtrate contains all forms of phosphate which might be present in soluble form and in suspension. By acidification (2NH<sub>2</sub>SO<sub>4</sub>) followed by heating for about 30 minutes poly, tripoly and pyrophosphate were totally hydrolyzed to ortho phosphate and it was used for phosphate analysis after this process.

*Sugarcane juice sample preparation*

The sugarcane was collected from the Larkana Sindh and Chakwal Punjab. Sugarcane juices was obtained for the phosphate analysis without any extra modification. In a 100mL volumetric flask 5mL of the sample juice was taken and diluted with double distilled water for the phosphate analysis.

*Adapted procedure for the determination of phosphate in various samples*

The following procedure has been used for the determination of phosphate: Take 1mL of sample in 25mL volumetric flask and add 2mL ammonium molybdate (2.5%) and 0.5mL of sulphuric acid (10N) solutions. After shaking the reaction mixture add 1mL hydrazine hydrate solution (0.5M) and make up the volume up to the mark by double distilled water. Leave the solution for about 45 minutes upto maximum color change and find out the absorbance at 840 nm. Draw a calibration curve to calculate the amount of phosphate in ppm and dilute the sample solution if absorbance becomes out of the range.

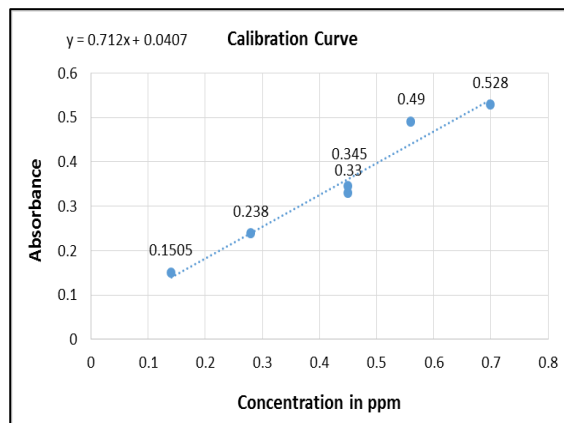
**Result and discussion**

The absorption data of potassium dihydrogen phosphate at 840nm used as standard against the blank (water). The absorbance of blank is negligible for all measurements the position of the λ<sub>max</sub> remains unaffected on changing the concentration of potassium dihydrogen phosphate. To find out the relation between absorbance and the phosphate content calibration curve is necessary for spectrophotometric analysis.

Under the optimized experimental condition, the calibration graph was determined in the concentration range of 0.1-0.8 ppm at 840 nm. A good linear relationship in Fig. 1 was find to exist between the absorbance of system and concentration of phosphate in Malir (Karachi) water sample (MW) with slop 0.712 and intercept 0.0074. The results were obtain at five different concentrations 0.14, 0.28, 0.45 and 0.56 and absorbance were record at 0.151, 0.238, 0.330, 0.490 and 0.528, respectively. The Malir water sample showed absorbance at 0.35 with 0.45-ppm concentration (Table 1).

**Table 1.** Determination of Phosphate in Malir water (MW).

SL	Phosphate Concentration (ppm)	Absorbance
1	0.14	0.151
2	0.28	0.238
3	0.42	0.330
4	0.56	0.490
5	0.7	0.528
Sample (MW)	0.45	0.345

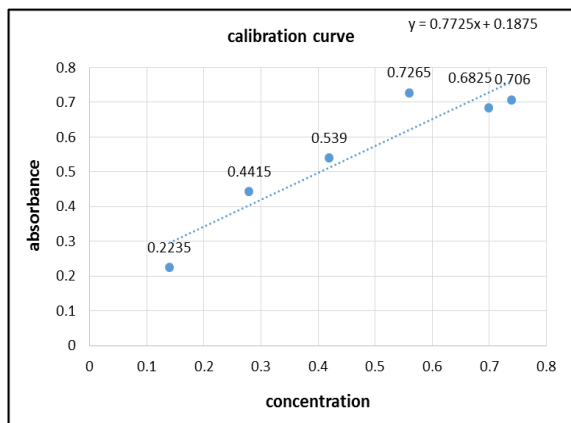


**Fig. 1.** Calibration curve for the determination of phosphate in Malir water (MW).

Table 2 shows the absorbance, amount of phosphate determined by spectrophotometer for the sample of Chakwal sugarcane (CS). From Table 2, it is observed that at the same concentrations as the Malir water sample the absorbance were 0.365, 0.497, 0.504, 0.581 and 0.545 absorbance at 0.745 and its concentration was calculated from the graph was 0.4434 and intercept was 0.3252 (Fig 2).

**Table 2.** Determination of Phosphate in Chakwal sugarcane (CS).

SL	Phosphate Concentration (ppm)	Absorbance
1	0.14	0.365
2	0.28	0.497
3	0.42	0.504
4	0.56	0.581
5	0.7	0.545
Sample (CS)	0.8	0.745



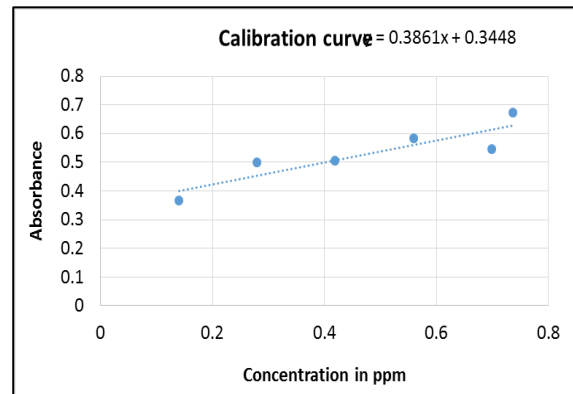
**Fig. 2.** Calibration curve for the determination of phosphate in Chakwal sugarcane (CS).

Table 3 recorded the absorbance, to determine the amount of phosphate by spectrophotometer for the Larkana sugarcane sample (LS). From this table it is cleared that the concentration was same as the previous samples and observed absorbance were 0.224, 0.442, 0.539, 0.727 and 0.638, respectively. The sample showed absorbance at 0.706 and its concentration is 0.74 ppm.

From the Fig. 3 we got slop at 0.7725 and intercept at 0.1875 for the Larkana sugarcane sample. These all results revealed that concentration of phosphate in both the sugarcane samples (CS and LS) are higher than the water sample (MW).

**Table 3.** Determination of Phosphate in Larkana sugarcane (LS).

SL	Phosphate Concentration (ppm)	Absorbance
1	0.14	0.2235
2	0.28	0.4415
3	0.42	0.539
4	0.56	0.7265
5	0.7	0.6825
Sample (LS)	0.74	0.706



**Fig. 3.** Calibration curve for the determination of phosphate in Larkana sugarcane (LS).

**Conclusion**

The molybdenum blue method for phosphate determination by using UV-Visible spectrophotometers is a simple method that can be performed in common laboratories.

As compare to the other methods, this method is cheap and simple. The phosphate concentration in samples was measured at 840 nm. The phosphate concentration of water is high as compared to the recommended limit by the environmental protection. Phosphate concentration of sugarcane samples are higher than water sample.

The system obeys Beers-Lamberts law in the range of 0.1-0.8 ppm for phosphate concentration. This method depends on the stability of molybdenum blue complex, freshness of the reagents used and temperature. For the formation of phosphomolybdenum complex at a temperature 30°C, the maximum time was 45 minutes. The rate of formation of complex was slow at low temperature and hence low absorbance value was observed.

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