Flow Injection / Spectrophotometric Determination of Phosphate in Soybean seed, Soil, and water Samples

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Abstract

A simple flow injection spectrophotometric method is described for the determination of phosphate in soybean [Glycine max (L.) Merill] seeds, soil, and water samples. The absorbance from the molybdenum blue complex is recorded at 720 nm. Various parameters including concentration of reagents, flow rate, mixing coil and sample volume were investigated for phosphate determination. The detection limit is 1.0 μM, the calibration graph is linear over the range of 5.0 - 25 μM with relative standard deviations of 1.2% and sample throughput of 75 h^{-1}.

Keywords:Flow injection, phosphate, soil, seed, water, molybdenum blue, spectrophotometry

1. Introduction

Flow injection analysis (FIA) is a well established technique for rapid, automated and quantitative analysis that combines on-line chemical and physical sample treatment with a range of flow-through detection systems in an enclosed, continuous flow environment. It is particularly well studied to monitor absorbance peak due to the rapid and reproducible mixing of sample and reagent [1].

Phosphorus occurs in most plants in concentrations between 0.1 and 0.4%. This is an essential macronutrient, stimulates early root development, leaf size, tillering, flowering, grains yield and hastens maturity. It is a constituent of certain nucleic acids, phosphatides, phospholipids, chromosomes, coenzymes (NAD, NADP) and ATPs [2]. Much of the phosphorus in soil solution is present in oxoanionic forms, mainly as the phosphate ions, $H_2PO_4^{-1}$. Under very acidic conditions, the monovalent form ($H_2PO_4^{-1}$) is prevalent; the divalent (HPO_4^{-2}) is present in the intermediate pH range, and the trivalent form (PO_4^{-3}) exists under alkaline conditions [3].

Soybean is considered as one of the oldest food crops of the world due to its good quality oil, protein content, and soil enriching properties [4,5]. Its elemental composition is important for human and animal nutrition, much less is known about the determination of phosphate in soybean especially by FIA.

The most frequently used spectrophotometric method for the determination of phosphate is the molybdenum blue method, in which the heteropoly acid formed between phosphate and molybdate is reduced. This method has been used in most of the applications for the determination of phosphate by FIA [6,7,8]. A stopped flow / FIA method has been used with LED based photometer for phosphate determination using phosphate-molybdate-ascorbic acid reaction [9]. Phosphate also forms a yellow heteropoly acid complex with vanadomolybdic acid, which has also been widely used in spectrophotometric determinations of phosphorus [10,11].

The phosphovanadomolybdic acid method is not as sensitive as the reduced phosphomolybdic acid method, but the reaction is very rapid and well suited for adaptation to FIA. Some other methods have also been devised for the estimation of small amounts of phosphate based on spectrophotometry [12], chromatography [13], ion selective electrodes [14, 15] and fluorometry [16].

In the present study, a flow injection/spectrophotometric method is reported for the determination of phosphate in soybean seed, soil and water samples in which the heteropoly acid formed between phosphate and molybdate is reduced with hydroquinone forming phosphomolybdic acid complex which was monitored at 720 nm.

2. Experimental

2.1 Reagents

All solutions and phosphate standards were prepared in distilled/deionized water and all reagents were of analytical grade (Merck, Damstradt, Germany).

Ammonum Molybdate (hepta) Solution (7.5 mM):

This solution was prepared by dissolving 1.86 g of $(NH_4)_6MoO_{24}.4H_2O$ in 200 ml of water containing 5.5 ml of HNO_3 (400 mM).

Hydroquinone solution (200 mM)

This solution was prepared by dissolving 4.4 g of hydroquinone in 200 ml of water at room temperature.

Three composite soil samples at a depth of 0 to 45 cm were taken from five different localities of Agricultural Research Institute (ARI), Quetta. While five different water samples from the tube wells and five soybean seed cultivars were also collected from the same place.

2.2 Seed and soil extraction

Air-dried defatted soybean seed powder (1.0 g) from each cultivar was homogenized separately in 20 ml Tris-HCl buffer (0.1M, pH 7.2) at room temperature for 16 hours, with continuous shaking at 300 min^{-1} (Edmond Bühler 7400 Tübingen). The samples were then centrifuged at 5000 rpm for 20 minutes, filtered through Whatmann filter paper,and the filtrates stored at 4°C for analysis.

Air dried soil sample of each locality was extracted in water following the procedure described in the reference [17]: 50 g of soil sample (crushed to pass through a 40-mesh sieve) was weighed accurately into a beaker, pasted with deionized water and left for 6 hours. The dissolved material containing phosphate was extracted from the soil paste by using suction pump. The filtrate was than centrifuged at 5000 rpm (IEC B-20A Centrifuge, Damon/IEC Division) and the supernatant was used to analyse phosphate.

2.3 Instrumentation and procedure

Figure 1 shows a flow injection manifold for the determination of phosphate. A peristaltic pump (Ismatec Reglo 100) was used to propel the sample, carrier and reagent streams through PTFE tubing (0.8 mm, i.d.) at 0.8 ml min^{-1}. Phosphate standard solutions (60 µl) were injected into the ammonium molybdate stream via a rotary valve (Rheodyne 5020) and merged with reducing agent in a mixing coil (20 cm length). The absorbance of the molybdenum blue was monitored at 720 nm using a spectrophotometer (Hitachi-1100, Japan) with a flow through cell (30 µl), connected to a chart recorder (Kipp and Zonen BD 40).

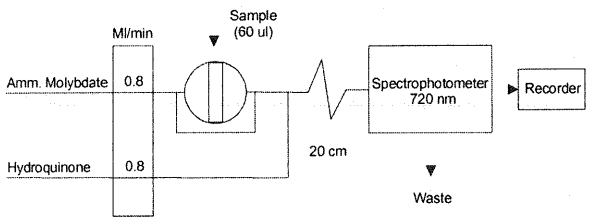

Fig. 1. Flow injection spectrophotometric manifold for phosphate.

3. Results and discussion

3.1 Optimization of FIA manifold

In order to obtain a system with optimal conditions for the determination of phosphate, various parameters were studied, including concentration of reagents, flow rate, mixing coil length and the presence of foreign ions. All these studies were performed with 10 µM phosphate solution. The results are shown in Table I.

The effect of ammonium molybdate concentrations was studied over the range of 1.0 - 12.5 mM. The maximum absorbance was obtained at 7.5 mM of ammonium molybdate, above this concentration no appreciable increase was observed and therefore was used for subsequent studies.

The effect of hydroquinone concentration on the reduction of the complex was investigated over the range of 150 - 300 mM. The maximum absorbance was recorded at a concentration level of 200 mM. Further increase in hydroquinone concentration did not show any significant increase in absorbance and therefore was selected for further studies.

The hetropoly acid between phosphate and molybdate depends on the acidity of aqueous medium. The effect of nitric acid concentration was studied over the range (200-1000 mM). The absorbance was maximum at 400 mM. Further increase in nitric acid concentration result in lowering the absorbance, probably due to its interference in the reduction of phosphomolybdenium blue by hydroquionone.

The flow parameters were optimized in terms of speed and sensitivity. The flow rates and mixing coil length had a large influence on the system performance, since both affected the contact time between the reagents and sample solution. The flow rate and mixing coil length were studied over the range of 0.4 - 2.0 ml min^{-1} and 10 - 120 cm. Both channel at a flow rate of 0.8 ml min^{-1} and mixing coil length of 20 cm, gave the maximum absorbance with steady baseline and therefore were used for further studies.

Table I. Effect of variables on the determination of phosphate.

Amm. molybdate (mM)	1.0	2.5	5.0	7.5	10.0
Absorbance*	0.030	0.030	0.046	0.060	0.050
Nitric acid (mM)	200	400	600	800	100
Absorbance*	0.042	0.060	0.056	0.050	0.050
Hydroquinone (mM)	150	200	250	300	350
Absorbance*	0.050	0.065	0.055	0.050	0.040
Flow rate ml min-1	0.4	0.8	1.2	1.6	2.0
Absorbance*	0.030	0.050	0.035	0.030	0.020
Mixing coil length (cm)	10	20	40	80	120
Absorbance*	0.060	0.065	0.060	0.050	0.040
Sample volume (µl)	30	60	90	120	150
Absorbance*	0.065	0.095	0.084	0.070	0.050

*Mean of four injections

3.2 Calibration graph

Under the optimum conditions established, a calibration graph was obtained for phosphate determination in the range of 0.5 - 2.5 mM as shown in Fig. 2 & 3. The correlation coefficient (r) was 0.9995 (N=6) and regression equation y = 0.065x – 0.004 [y = absorbance, x = concentration).The limit of detection (2 × blank signal) was 0.001 mM with a sampling throughput of 75 h^{-1}. The relative standard deviation for six injections of 0.1 mM of phosphate was lower than 1.2%.

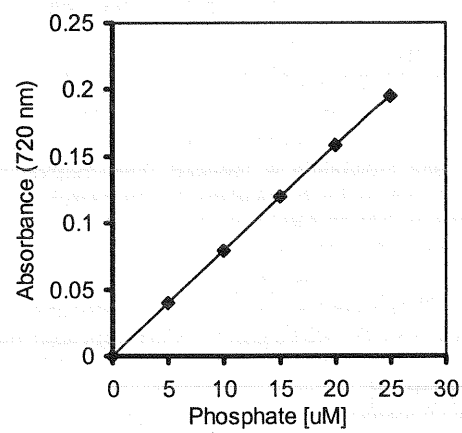

Fig. 2. Calibration graph for phosphate.

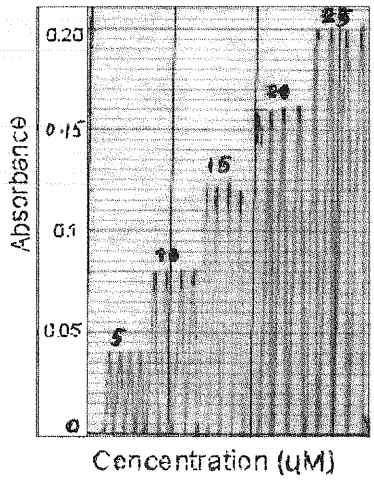

Fig 3. Typical flow injection peaks for phosphate determination.

3.3 Interferences

The effect of various anions and cations (0.1 mM) on the absorbance was compared with phosphate ion (1.0 mM). The following ions did not interfere such as; calcium, magnesium, cadmium, chromium, ammonium, copper (II), cobalt (II), chloride, acetate and fluoride. However, the only significant interference observed was from lead (II) and barium, which has to be removed from the sample by physical or chemical means.

Table II. Determination of phosphate in soil, soybean [*Glycine max* (L.) Merill.] seed extracts and irrigation water samples.

Samples	Found	
	Flow Injection method*	Reference Method**
Seeds extract (g%)		
1 cv^. Williams82	0.605 (± 0.077)	0.592 (± 0.054)
2 cv. Swat-84	0.205 (± 0.054)	0.225 (± 0.008)
3 cv. NARC I	0.290 (± 0.090)	0.305 (± 0.065)
4 cv. NARC II	0.300 (± 0.070)	0.297 (± 0.070)
5 cv. NARC III	0.295 (± 0.045)	0.329 (± 0.058)
Soil extract (ppm)		
1	0.54 (± 0.020)	0.52 (± 0.04)
2	0.48 (± 0.015)	0.45 (± 0.02)
3	0.20 (± 0.020)	0.22 (± 0.01)
4	0.35 (± 0.030)	0.38 (± 0.02)
5	0.45 (± 0.025)	0.43 (± 0.03)
Water samples (ppm)		
1	0.142 (± 0.010)	0.125 (± 0.050)
2	0.140 (± 0.033)	0.136 (± 0.065)
3	0.150 (± 0.042)	0.148 (± 0.047)
4	0.154 (± 0.023)	0.135 (± 0.015)
5	0.154 (± 0.040)	0.150 (± 0.035)

*Data is the mean of three replicates/injections ± standard error in parentheses.
**Charlot,(1964). Ref. 12.
cv^. Cultivar.

3.4 Analysis of seed, soil, and water samples

The flow injection spectrophotometric method has a great potential for the analysis of real samples. This was confirmed by the results obtained for phosphate in soil, water, and soybean seed samples as shown in Table II. The results were in good agreement with the reference spectrophotometric method. This indicates the validity of the method for phosphate ion determination.

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