

# Flow Injection Preconcentration and Spectrophotometric Determination of Orthophosphate in Natural Waters

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Visando melhorar o limite de detecção na determinação espectrofotométrica do íon ortofosfato em águas naturais, desenvolveu-se um procedimento de pré-concentração automática, acoplado-se uma mini coluna com resina aniônica (AG1-X8) "on-line" em um sistema de análise química por injeção em fluxo (FIA). A detecção espectrofotométrica foi feita após reação com molibdato de amônio e cloreto estânico em meio ácido. Velocidade analítica de 80 determinações por hora foi obtida para amostras com concentrações na faixa de 5 a 50 µg/l. Desvio padrão relativo de 1% foi calculado a partir de 10 medidas de uma amostra típica (30 µg/l). Recuperação em torno de 97% foi obtido e um limite de detecção de 2 µg/l foi estimado considerando o mesmo igual a 3 vezes o desvio padrão do branco.

A small column packed with an anion-exchange resin (AG1-X8) was coupled on-line to a flow injection system in order to perform preconcentration of orthophosphate ions in natural waters. Spectrophotometric measurement was performed after reaction with ammonium molybdate and stannous chloride in acidic medium. A throughput of 80 samples per hour was achieved for concentration ranging from 5 to 50 µg/l. Measurements are characterized by relative standard deviation of 1%, for a typical sample (30 µg/l), recovery of 98% and limit of detection of 2 µg/l estimated as three times of blank standard deviation were calculated.

**Keywords:** *flow injection; orthophosphate; spectrophotometry; ion exchange.*

## Introduction

Research in limnology requires the determination of several ions in natural waters, phosphate having a great importance owing to its function as nourish in aquatic media<sup>1</sup>.

The molybdenum blue method is a widespread procedure for determination of orthophosphate. However, depending on the water provenance, the phosphate concentration is lower than the detection limit presented by this analytical method. In this case, it is recommended to concentrate, employing either liquid-liquid extraction with an organic phase or liquid-solid adsorption with an anion-exchange resin. Extraction with an organic solvent after reaction with ammonium molybdate and stannous chloride has been used<sup>2</sup>, but this is a tiresome work. To overcome this drawback, a preconcentration procedure extracting the phosphate ions from the liquid phase by an anion-exchange resin was investigated.

The anion-exchange resin AG1-X8, 200-400 mesh, from Bio-Rad Labs was employed, coupling the resin mini column to the flow injection system. Effects of sample flow rate through the resin column, resin loading time, eluent solution, flow rate and concentration were investigated.

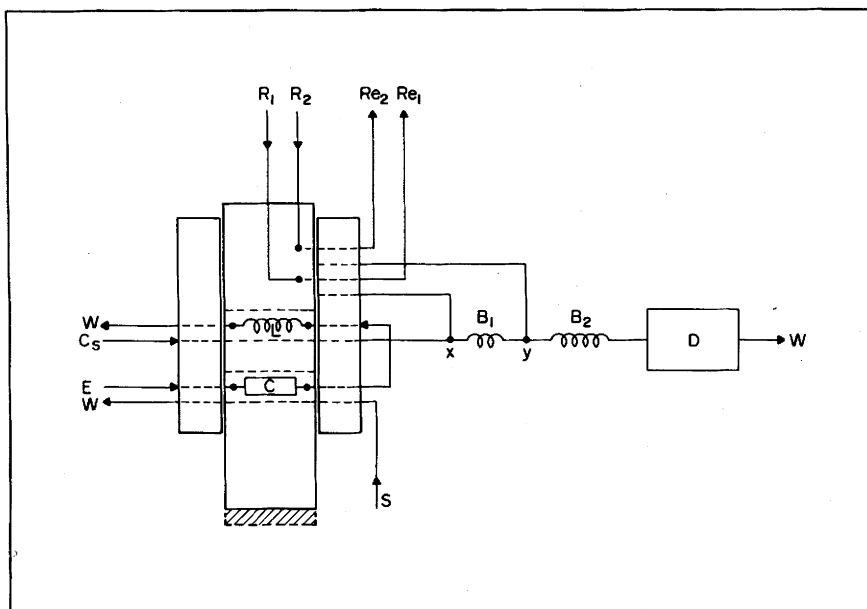
## Experimental

### Apparatus

A Micronal B342II spectrophotometer furnished with a flow cell (14mm optical path) and coupled to a Radiometer REC 61 strip-chart recorder was employed. An Ismatec MP13R peristaltic pump furnished with Tygon pump tubing was employed to propel samples and reagent solutions.

A resin column (15mm length x 5mm i.d.) constructed as described elsewhere<sup>3</sup>, and filled with the anionic AG1-X8 resin was connected to the sliding bar of the injector, by means of 3 cm polyethylene tubing (0.8 i.d.). Manifold was made with (0.8mm i.d.) polyethylene tubing.

An automatic sliding bar injector with three commutation sections controlled by a homemade microcomputer was employed<sup>4</sup>. The microcomputer, based on the Intel 8085 microprocessor<sup>5</sup> drove the injector from the column loading to the elution position, and vice-versa, following a software written in assembly language. The software provides facilities to program different time delays for the loading and elution steps. Details of software are available upon request.



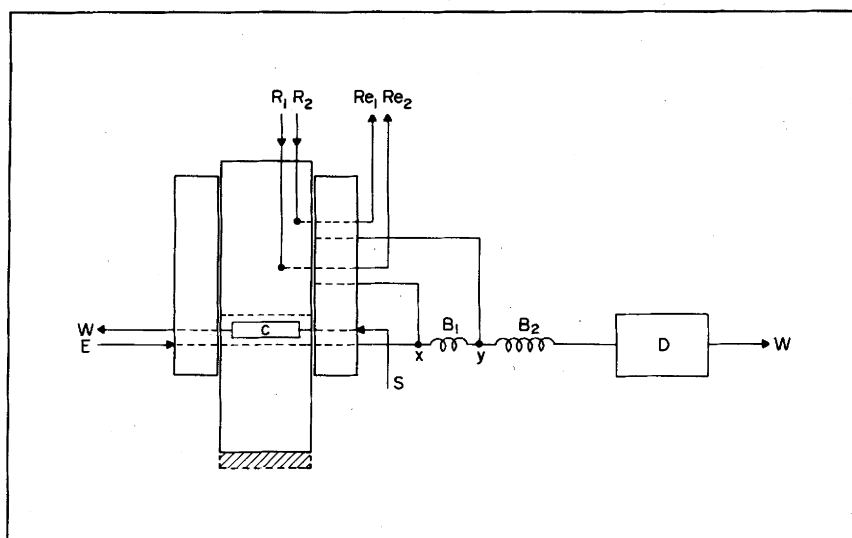
**Figure 1.** Flow diagram of the system to investigate preconcentration parameters. The three rectangular pieces corresponded to an over view of the sliding bar injector. S sample, C resin column (15 x 5mm), E eluent, R<sub>1</sub> ammonium molybdate solution at flow rate of 0.8ml/min, R<sub>2</sub> stannous chloride and hydrazine sulphate solution at flow-rate of 0.8ml/min, Re<sub>1</sub> and Re<sub>2</sub> recovery for R<sub>1</sub> and R<sub>2</sub> respectively, x and y confluence points, B<sub>1</sub>(15cm) and B<sub>2</sub>(100cm) reaction coils, D spectrophotometer at 690nm. Dashed lines indicates inner hole, and the hatched surface indicates the injector eluting position. Arrows indicates the direction of flows.

#### Reagents

All chemicals were of analytical grade and freshly distilled and deionized water was used throughout. The phosphate stock solution (1000 mg/l) was prepared by dissolving 2.1965g KH<sub>2</sub>PO<sub>4</sub> in 200ml water, adding 2ml concentrated nitric acid, and making the volume up to 500ml. Working standards were prepared daily by appropriated water dilutions. The ammonium molybdate reagent was prepared by dissolving 1.9g in about 200ml water, adding 5.8ml concentrated sulfuric acid, and completing the volume up to 500 ml. The stannous chloride reagent 0.01% (w/v) and 0.35% (w/v) in hydrazine sulphate was prepared, in 0.15M sulfuric acid. This solution is stable for at least one week under refrigerator.

#### Flow diagram

Influence of sample flow rate and column loading time on the resin column efficiency was investigated with the system outlined in Fig. 1. In the position indicated, the sample (S) is pumped through the resin column (C), where the phosphate ions are withdrawn from the sample solution. The sample depleted in the analyte ions is directed towards waste (W), while R<sub>1</sub> and R<sub>2</sub> reagent are directed to the respective store vessels. Displacing the sliding bar of the injector to the other position, the resin column is inserted in the analytical path, and the eluent solution (E) is pumped through it, causing the eluting process. At the same time, R<sub>1</sub> and R<sub>2</sub> reagents are directed towards confluences x and y, merging with the analyte ions released from the resin. The



**Figure 2.** Flow diagram of the system to implement the zone-sampling process. L(20cm) sampling loop, Cs sample carrier stream at 2,6ml/min. Other symbols as in Figure 1.

chemical reaction to produce the compound spectrophotometrically detectable, occurs in B1 and B2 reactors, while the zone formed by the eluted band and reagents is transported by the carrier stream towards the detector (D). Switching back the injector, other analytical cycle is initialized.

Effects of varying flow rate and eluent concentrations were investigated by employing a zone sampling process<sup>6</sup>, implemented as in Figure 2. In the position indicated, the previously adsorbed analyte ions, were eluted and directed towards waste through loop L. By switching back the sliding bar of the injector, this loop was inserted into the analytical path, so that a portion of the eluted band sampled by loop L, was transported by the carrier stream towards the detector, being the reagents added as in Fig.1. The microcomputer was programmed to increase each eluting time interval with a constant value; thus, for each eluting step, a different portion of the eluted band was sampled.

#### Procedure for investigation of experimental variables

To investigate the resin adsorbing dependence on sample flow rate and column loading time, the system of Figure 1 was employed, with a 50 µg/l phosphate solution as standard, and 0.02, 0.05, 0.1, 0.25 and 0.5M nitric acid solutions as eluent.

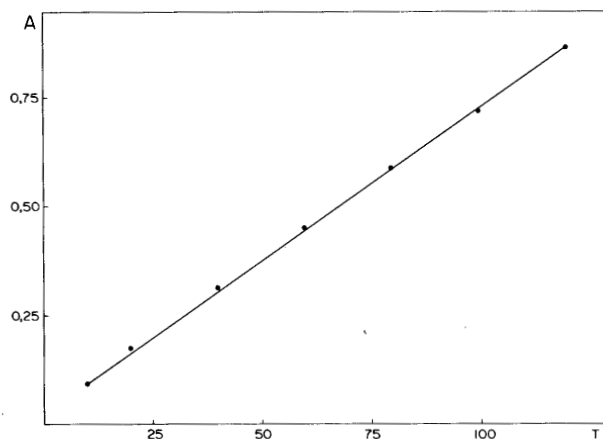
Effects of sample flow rates from 1.0 to 12.0 ml/min were investigated by using a loading time interval of 25s, and a 0.05M nitric acid solution at 2.6 ml/min as eluent. Effects of loading time were investigated with the same system. The sample flow rate was fixed at 6.2 ml/min and the microcomputer was programmed to increase the loading time interval in steps of 20s from 20 up to 120s.

Effects of eluent concentration and flow rates were investigated with the flow system of Fig. 2. Sample flow rate and column loading time interval were previously fixed at 6.2 ml/min. and 25s respectively, 0.05, 0.10, 0.25, 0.5 and 1.0M nitric acid or sodium nitrate solution were used as eluents at flow rates of 1.6, 2.6, 3.2 or 4.2 ml/min. The eluting time was programmed for each eluent flow rate in order to fill exactly the sampling loop L. After the first run, this time interval was increased two fold for the second run, three fold for the third one and so on.

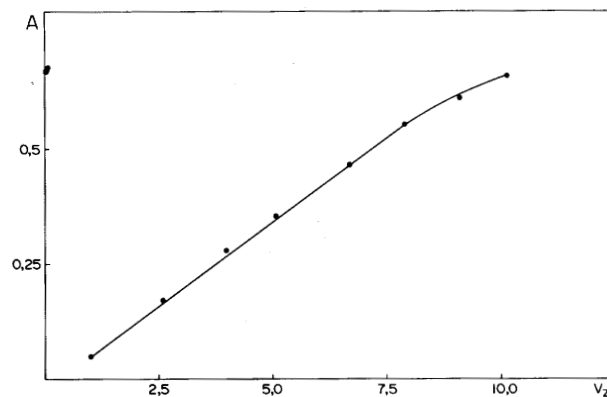
## Results and Discussion

Photometric measurements were performed employing the blue compound formed by reacting the orthophosphate ions with ammonium molybdate and stannous chloride in acidic medium. Previous tests yield better results when nitric acid solution was used. Therefore, nitric acid solution was established as carrier stream and eluent.

The amount of analyte adsorbed on the anion exchanger is dependent of both loading time and sample flow rate through it (Figs.3 and 4). The loss in linearity for sample flow rates higher than 8 ml/min, can be ascribed to the resin difficult to withdraw the ions from the sample. In this condition, the ions are pushed more quickly through the column, decreasing the time of contact with resin exchanger sites, thus hindering the adsorbing process. This is in agreement with other authors<sup>7</sup> who which reported that a loss of about 30% on the resin adsorbing efficiency was observed for 10 ml/min sample flow rate. On the other hand, the curve shown in the Fig. 4 is linear on the overall tested interval. In order to improve the limit of detection, then, it is



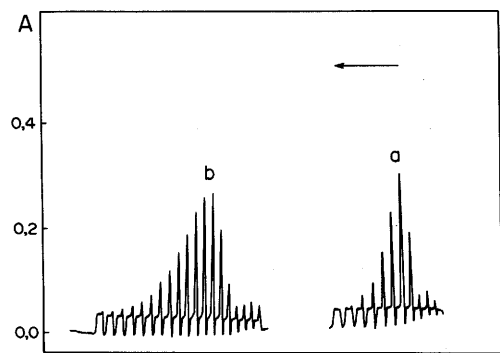
**Figure 3.** Effect of sample flow rate. A absorbance,  $V_z$  sample flow-rate in ml/min. Results obtained using a 0.5mg/l phosphate standard solution, employing the flow diagram of Figure 1 with column loading time of 25s, eluent 0.05M nitric acid solution at flow-rate of 2.6ml/min.



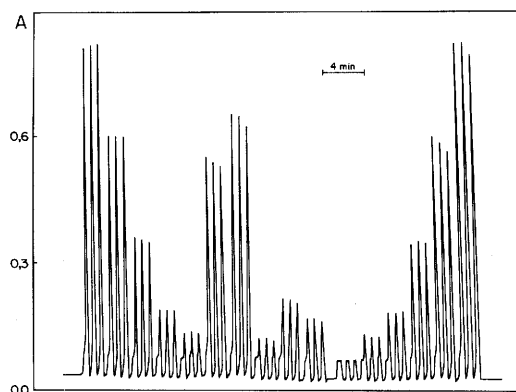
**Figure 4.** Effect of column loading time. Sample flow-rate 6.2ml/min. Other working parameters as in Figure 3.

more profitable to increase the column loading time instead of sample flow rate.

The eluting process was investigated employing the zone-sampling technique<sup>6</sup>, that provides facilities to study both eluent solution flow-rate and contraction without causing any change in the reaction condition. These tests were implemented with the system of Fig. 2, and results are shown in Fig. 5. The resampling loop was 20 cm long (100 µl), thus a time interval of 2.3s is necessary to fill the sampling loop with a fraction of the eluted band. The microcomputer was programmed in such a way that each eluting time interval was 2.3 seconds higher than the previous one, thus each slice sampled from the eluted band did not contain any portion before exploited, but avoiding also lost between the fractions sampled. Looking at Fig. 5a from right to left, the first three recorded peaks are related to the analyte present in the solution inside column but not adsorbed. The following six peaks are related with the slices of eluted band sampled during the time intervals of 9.2, 11.5, 13.8, 16.1, 18.4, 20.7 and 23.0 respectively. So, phosphate ions are completely eluted within a volume of 0.6 ml. The resampling loop was 20cm length and 0.8 mm i.d., therefore the analyte eluted has a length of 120 cm. This can be confirmed by an-



**Figure 5.** Recorder tracing for sampling the eluted band. Results obtained with flow diagram depicted in Fig.2. Sample flow-rate, 6.2ml/min; column loading time, 25s. Standard 0.5mg/l phosphate solution and eluent 0.05M nitric acid solution, at flow rate of 2.6ml/min. For the recorder tracing sets a and b the eluting time intervals were 2.3s and 1.1s respectively.



**Figure 6.** Recorder tracing of routine analysis. From right to left, 5 phosphate standard of 0,5, 10, 25 and 50 mg/l, 5 samples and again the standard, all in triplicate. Working parameters to flow diagram of figure 1; sample flow rate at 6.0ml/min., column loading time at 25s, eluent 0.05M nitric acid solution at flow rate 2.6ml/min, and eluting time interval at 20s.

alysing Fig. 5b, where the eluting time was half from that employed before, yielding twelve peaks instead of six.

By enveloping both recorder tracing sets one can observe that the peak profile distribution resemble a skewed gaussian curve, similar to that obtained in usual flow injection systems. The eluting process was done in reverse flow configuration to minimize the analyte dispersion by the column dead volume. The fluid conduits connecting the column outlet with the resampling loop, were made with a 30 x 0.3mm of polyethylene tubing, thereby its contribution for analyte dispersion has little significance. So, the peak profile distributions are due to the resin feature. Experiments changing either the eluent flow rate from 1 to 10 ml/min or concentration from 0.05 to 1M of HNO<sub>3</sub>, yield results identical to these shown in Fig. 5.

Considering these results, the following working parameters were fixed: 6.2 ml/min for sample flow rate; 0.05M nitric acid solution at flow rate 2.6 ml/min as eluent. Summing the eluent and reagents streams, the overall flow rate was 4.2 ml/min, yielding a sample residence time of 8s. The reaction is not so fast, and was only 80% completed during transport to the detector. The analytical path was increased

to 200cm, but the gain in signal was not significant, probably the gain in compound forming was lost by dispersion. The sample residence time can be increased by diminishing the overall system flow rate, thereby causing decrease in sample throughput. Whereas the time to complet the reaction is three time higher than that observed for the sample residence in the proposed FIA network, of course, it is more profitable to increase the column loading time.

Nitric acid solution was selected as eluent considering the reaction features, albeit the resin AG1-X8 selectivity for nitrate ions is about eleven times higher than that for phosphate ions<sup>8</sup>, owing to the adsorbing process should be partial. To overcome this doubt the resin column effluent was collected during several column loading steps and analysed in the same way as the sample. As the result was equal to that observed for the blank measurement, resin efficiency to withdraw phosphate ions from the sample was found to be practically 100%.

Once settled the working parameters for the proposed FIA system, a set of water samples from Ibitinga-SP dam were analysed, yielding the recorder tracing shown in Fig. 6. A throughput of 80 samples per hour can be deduced from this figure for concentration ranging from 5 to 50 µg/l.

Other profitable features such as relative standard deviation of 0.5% and recovery better 98% were also calculated. A detection limit of 2 µg/l was stimated as suggested by IUPAC<sup>9</sup>, that is better than those presented by other spectrophotometric methods based on FIA<sup>10,11</sup> and by ion chromatography<sup>12</sup>. The consumption of reagents stanous chlorid and ammonium molibdate were 260µl per determination, owing to the intermitent streams configuration<sup>13</sup> employed for the reagents instead the continuos streams.

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