

## A Micro-Flow-Batch Analyzer using Webcam for Spectrophotometric Determination of *Ortho*-phosphate and Aluminium(III) in Tap Water

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Neste trabalho é proposto um analisador *micro-flow-batch* ( $\mu$ FBA) usando uma *webcam* para determinação espectrofotométrica de ortofosfato e alumínio(III) em água de torneira. Este microsistema foi feito com uretana-acrilato e lâminas de vidro. As determinações de  $\text{PO}_4^{3-}$  e  $\text{Al}^{3+}$  foram realizadas empregando os métodos do azul de molibdênio e quercertina, respectivamente. Para a construção de curvas analíticas lineares um modelo matemático foi usado com base nos valores RGB (vermelho, verde e azul). As faixas de trabalho foram de 25 a  $500 \mu\text{g L}^{-1}$  para ambos os analitos com limites de detecção, desvio padrão relativo (RSD,  $n = 5$ ) e frequência analítica de  $0,82 \mu\text{g L}^{-1}$ ,  $< 0,8\%$  e  $160 \text{ h}^{-1}$  para  $\text{PO}_4^{3-}$ , e  $0,93 \mu\text{g L}^{-1}$ ,  $< 1,1\%$  e  $160 \text{ h}^{-1}$  para  $\text{Al(III)}$ . Comparando com os métodos de referência, diferenças estatisticamente significativas não foram observadas quando aplicado o teste-*t* pareado com 95% de confiança. O teste de recuperação apresentou resultados entre 98,1 e 102,8%. O  $\mu$ FBA proposto apresentou portabilidade satisfatória, robustez, flexibilidade e reduzido consumo de reagentes.

In this work, a *micro-flow-batch* analyzer ( $\mu$ FBA) using webcam for spectrophotometric determination of *ortho*-phosphate and aluminium(III) in tap water is proposed.  $\text{PO}_4^{3-}$  and  $\text{Al}^{3+}$  determinations were performed by employing molybdenum blue and quercetin methods, respectively. In order to build linear analytical curves, a mathematical model was used on the basis of the RGB (red, green and blue) values. Working ranges were from 25 to  $500 \mu\text{g L}^{-1}$  for both analytes with estimated limits of detection, relative standard deviation (RSD,  $n = 5$ ) and sampling rate of  $0.82 \mu\text{g L}^{-1}$ ,  $< 0.8\%$  and  $160 \text{ h}^{-1}$  for  $\text{PO}_4^{3-}$ , and  $0.93 \mu\text{g L}^{-1}$ ,  $< 1.1\%$  and  $160 \text{ h}^{-1}$  for  $\text{Al(III)}$ . Comparing with the reference methods, no statistically significant differences were observed when applying the paired *t*-test at a 95% confidence level. Recovery study shows results between 98.1 and 102.8%. The proposed  $\mu$ FBA presented satisfactory portability, robustness, flexibility, low-cost device and reduced chemicals consumption.

**Keywords:** micro-flow-batch analyzer, digital images, RGB color system, webcam, tap water

### Introduction

Recently, the *micro-flow-batch* analyzer ( $\mu$ FBA)<sup>1</sup> was proposed in order to improve the features of the traditional flow-batch system,<sup>2</sup> such as portability, lower consumption of reagents and sample, and less waste generation. As the flow-batch analyzers, this microsystem also combines favorable characteristics of both flow and batch analysis systems. The transportation of reagents, samples or other solutions into the micro-chamber ( $\mu$ CH) are carried out in a flow mode and, as in the batch system, the sample processing (mixture/homogenization/reaction to generate

colorful product), and absorbance measurement is carried out into  $\mu$ CH (micro-beaker).<sup>3</sup>

Unlike an usual analog to digital converter employed in analytical instruments affords resolution of 12 bit (4096), recent advances in digital image acquisition technology have offered video cameras (webcam) based on charge-coupled devices (CCD), which are capable to capture digital images with up to 24 bits (16.7 million colors). In fact, by using the RGB (red, green and blue) color system, the primary colors are combined in different intensities with values varying in the range 0-255 (8 bits) *per* color.<sup>4</sup> Thus, procedures based on digital images are very sensitive since they included abilities to identify little difference between the colors of images (the analytical signals).<sup>4-6</sup>

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Mathematical models for digital images and RGB data have also been employed to construct analytical curves in quantitative determinations.<sup>5</sup> Lyra *et al.*<sup>4</sup> proposed a method using the norm in an RGB three-dimensional space concept for analytical response in digital images obtained from flame emission spectrometry (FES). Maleki *et al.*<sup>7</sup> used digital images for simultaneous determination of Al(III) and Fe(III) in alloys using chrome azurol S (CAS) as the chromogenic reagent. The RGB based artificial neural network (ANN) models were built from digital images of the Al(III)-CAS and Fe(III)-CAS complexes.

Recently, Wongwilai *et al.*<sup>8</sup> employed a webcam for real time detection in the course of an acid-base titration by means of color change in phenolphthalein indicator for increasing acid concentration in the solution of an automatic flow system. Lima *et al.*<sup>9</sup> demonstrated that a webcam with CCD sensor can be coupled to  $\mu$ FBA and used as digital image-based method for quantitative tannin determinations in green tea.

The content of phosphorus in tap water sample is an important index to evaluate the quality of water in environmental monitoring. Molybdenum blue spectrophotometric method<sup>10</sup> was a general method to the determining of phosphorus in the *ortho*-phosphate form ( $\text{PO}_4^{3-}$ ), which had the advantages of high sensitivity, less interference and better stability.<sup>10,11</sup> Therefore, the molybdenum blue method seems promising to be adopted as a basis for the development of a miniaturized automatic method using digital images for *ortho*-phosphate determination.

Aluminium(III) ion occurs into the environment by natural processes and also from anthropogenic sources.<sup>12</sup> The toxic effects (it provokes encephalopathy, osteomalacia and microcytic anemia) are well-known also to patients with chronic renal failure.<sup>13</sup> This ion is considered to be one of the risk factors to Alzheimer's disease.<sup>14</sup> Although it is present in low concentrations in tap water, Al(III) is commonly used as flocculating agent in potable water treatment units, and produces problems due to excess (or insufficient) coagulant, particularly during periods of fast variation in water quality.<sup>15,16</sup>

Quercetin is a well-known photometric reagent for determination of Al(III) traces in water and in biological samples. As described by Norfun *et al.*,<sup>17</sup> it forms a stable complex with Al(III) and is relatively free from interfering species commonly present in water. Thus, quercetin seems promising to be adopted as a basis for the development of a  $\mu$ FBA method using digital images for determination of Al(III) in water.

In the present study, an automatic  $\mu$ FBA method using digital images with solenoid micro-pumps is proposed for

determining of *ortho*-phosphate and aluminium(III) using a webcam and RGB data in tap water. The phosphorus and aluminium determinations were performed by employing the molybdenum blue and quercetin methods, respectively. The colored complexes formed by these methods can be quantitatively analyzed by digital images from webcam as detector.  $\mu$ FBA is a novel alternative for automatic determination of these ions in tap water, comparing satisfactorily to the recently methods described in the literature.<sup>10,11,17,18</sup>

## Experimental

### Reagent solutions

All reagents were of analytical grade and freshly distilled and deionized water ( $> 18 \text{ M}\Omega \text{ cm}^{-1}$ ) was used to prepare all solutions.

For spectrophotometric determination of aluminium(III), a stock solution of  $100.0 \text{ mg L}^{-1}$  aluminium(III) was prepared as previously<sup>17,19</sup> from pure ( $> 99.0\% \text{ w/w}$ , Sigma) product by dissolving the appropriate mass of aluminium chloride hexahydrate in  $0.10 \text{ mol L}^{-1}$  hydrochloric acid solution.

Quercetin stock solution ( $1000.0 \text{ mg L}^{-1}$ ) was prepared as previously<sup>17,19</sup> from the pure product (Sigma) dissolving an appropriate weight and using an ethanol/water solution ( $60\%, \text{ v/v}$ ). The surfactant solution was prepared by dissolving  $0.0127 \text{ g}$  of cetyltrimethylammonium bromide (CTAB) (Sigma), in an acetate buffer solution with a concentration of  $0.10 \text{ mol L}^{-1}$ , at a pH of 5.5.

The stock phosphorus solution ( $100 \text{ mg L}^{-1}$ ) was prepared by appropriate dilution of potassium dihydrogen phosphate (KDP) (Sigma). The standard solutions (ranging from  $0.10$  to  $10.0 \text{ mg L}^{-1}$  of phosphorus) were prepared by mixing the stock solution with the acid solutions ( $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$ , all  $1\% \text{ v/v}$ ) (Sigma) and were used for plotting the analytical curve in  $\mu$ FBA.

A  $340 \text{ mmol L}^{-1}$  solution of ascorbic acid ( $\text{C}_6\text{H}_8\text{O}_6$ ) (Sigma) was prepared by weighing  $6 \text{ g}$  of ascorbic acid and dissolving it in  $100 \text{ mL}$  of distilled water. A  $5.4 \text{ mol L}^{-1}$  sulfuric acid solution was prepared by transferring an aliquot of  $30 \text{ mL}$  of concentrated  $\text{H}_2\text{SO}_4$  in  $100 \text{ mL}$  of water.

As described by Norfun *et al.*,<sup>17</sup> aluminium(III) determination required the addition of a masking agent, which consisted of  $0.625 \text{ g}$  of thiourea (Sigma),  $0.1 \text{ mol L}^{-1}$  ascorbic acid ( $15.0 \text{ mL}$ ) and  $0.1 \text{ mol L}^{-1}$  1,10-phenanthroline ( $25.0 \text{ mL}$ ). Then, the pH of the sample was adjusted to 5.5 with  $1 \text{ mol L}^{-1}$  sodium hydroxide (Sigma), transferred into a  $250 \text{ mL}$  volumetric flask and made up to the mark with deionized distilled water. Finally, it was well mixed and

subsequently analyzed by the proposed  $\mu$ FBA method.

For the preparation of the stock solution of 32 mmol L<sup>-1</sup> ammonium molybdate (Sigma), 20 g of salt were weighed and dissolved in 400 mL of water. After 4 h agitation, the solution was transferred to a 500 mL volumetric flask and the volume was completed with water.

The stock solution of 9 mmol L<sup>-1</sup> potassium antimony tartrate (Sigma) was prepared by weighing 3.0 g of potassium antimony tartrate and dissolving them in distilled water. The solution was then transferred to a 1000 mL volumetric flask and the volume completed with distilled water. This solution was stored in amber glass bottles under refrigeration.

The reagent solution was prepared by mixing 35 mL of concentrated sulfuric acid in 500 mL of water, then adding 215 mL of stock solution of 0.032 mol L<sup>-1</sup> ammonium molybdate and 72 mL of 9 mmol L<sup>-1</sup> potassium tartrate and antimony. The mixture was transferred to a 1000 mL flask and the volume completed with water. A solution of nitric acid (10% v/v) was used to clean the microsystem fabricated.<sup>17</sup>

#### Sample preparation

Tap water samples were collected from different sources in the city of João Pessoa, Paraíba, Brazil. The samples were filtered through 0.45  $\mu$ m cellulose membrane filters, acidified with nitric acid (1% v/v), and stored in polyethylene bottles before analysis.

#### Apparatus

To fabricate the micro-chamber ( $\mu$ CH) in urethane-acrylate resin with glass slides, it was used a commercial UV light source (Fotolight-MD2-A4, Carimbos Medeiros Ltda, Brazil), with two sets of mercury lamps (BLB-15W-T8, SCT black light).<sup>1</sup>

For layout design of the  $\mu$ CH, the CorelDraw<sup>®</sup> X5 program was used. The layout printing was on polyester transparency films for laser printing using an HP LaserJet P2014. After UV exposure, channels on the substrate were revealed by the removal of the non-exposed resin with an ultrasonic bath (model UltraCleaner 800, Unique, Brazil), as previously.<sup>3</sup>

A spectrophotometer model 8453 Hewlett-Packard diode array UV-Vis, equipped with cuvette (with an inner volume of about 4 mL and an optical path of 1.0 cm), was used for absorbance measurements when employing the reference method.

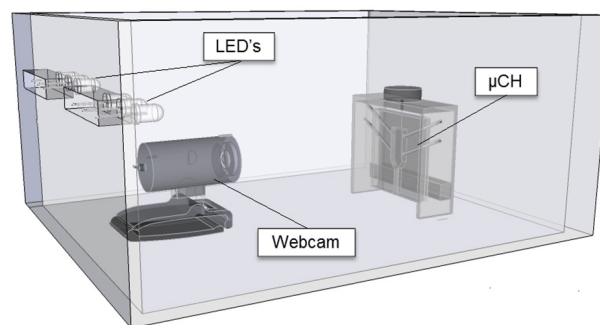
A Microsoft LifeCam Cinema 720p HD 30FPS was used in conjunction with LabVIEW<sup>®</sup> 7.1 software (National

Instruments<sup>®</sup>) to control the  $\mu$ FBA system. The images were captured by means of the software written in Delphi (version 3.0).

The webcam was connected to the USB port of an Intel Core2Duo 2 Gigabytes notebook (PC) and configured to capture 24-bit digital images (16.7 million colors) at a rate of 30 frames s<sup>-1</sup> and a 640 pixels  $\times$  480 pixels spatial resolution.

#### Fabrication process and assembly of the $\mu$ FBA system

The homemade  $\mu$ CH with two glass slides and total volume about 110  $\mu$ L was built in urethane-acrylate photoresist using the methodology described elsewhere.<sup>9</sup> This microsystem is mounted onto a suitable support in a black (darkroom) box measuring 17 cm  $\times$  11 cm  $\times$  9 cm to preserve the system from the effects of spurious environmental radiation while in operation (Figure 1).



**Figure 1.** Schematic diagram of the  $\mu$ FBA lab-made system. The micro-chamber ( $\mu$ CH) was fabricated with glass slides and urethane-acrylate resin.<sup>1</sup>

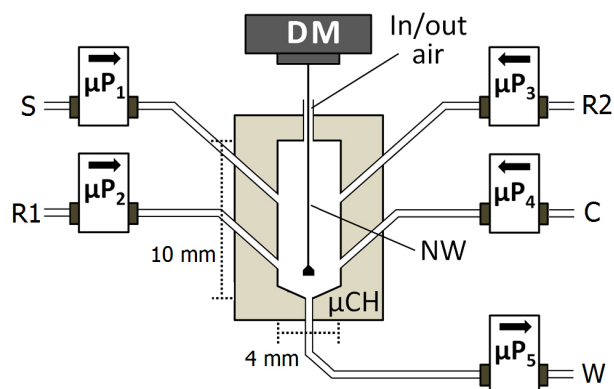
Six white high intensity LEDs (NPE, Thailand) were arranged in the wall behind the webcam in order to give a constant light intensity throughout the experiment. The interior walls of the box were covered with white paper in order to provide uniform illumination and reduce glare.

The fluids are added with solenoid micro-pumps with nominal values of 8  $\mu$ L ( $\mu$ P<sub>1</sub>- $\mu$ P<sub>4</sub>) and 20  $\mu$ L ( $\mu$ P<sub>5</sub>) *per* pulse (Biochem Valve Inc., Boonton, NJ, USA). Teflon<sup>®</sup> tubes with 0.5 mm internal diameter were used for fluid transport. A 0.4 mm nylon wire was used within  $\mu$ CH to ensure an efficient homogenization. The nylon wire was coupled to a CD/DVD-ROM motor drive (model MDN3GT3CPAC, 2000 rpm, 5 V dc).

#### Analytical procedure

The  $\mu$ FBA manifold is shown in Figure 2. The analyzer was operated as described in Table 1 for the different

determinations. The solenoid micro-pumps were actuated at 5 Hz, yielding flow rates from  $0.96 \text{ mL min}^{-1}$  ( $\mu\text{P}_1$  and  $\mu\text{P}_4$ ) and  $2.4 \text{ mL min}^{-1}$  ( $\mu\text{P}_5$ ).



**Figure 2.** (a)  $\mu\text{FBA}$  manifold with its dimensions. Micro-chamber ( $\mu\text{CH}$ ); solenoid micro-pumps ( $\mu\text{P}_1$ - $\mu\text{P}_5$ ); drive motor (DM); nylon wire (NW); sample or standard solutions (S); waste (W). For *ortho*-phosphate determination: R1, ammonium molybdate with antimony and potassium tartrate; R2, ascorbic acid; C, cleaning solution (10%  $\text{HNO}_3$ ). For aluminium(III) determination: R1, quercetin; R2, CTAB; C, water.

#### *Ortho*-phosphate determination

The reagents ammonium molybdate with potassium antimony tartrate (4 pulses, adding  $32 \mu\text{L}$  by  $\mu\text{P}_2$ ), ascorbic acid (4 pulses, adding  $32 \mu\text{L}$  by  $\mu\text{P}_3$ ) and sample or standard solution (4 pulses, adding  $32 \mu\text{L}$  by  $\mu\text{P}_1$ ) were simultaneously added. The homogenization was performed by the drive motor (DM) coupled to the nylon wire (NW) by 2 s. After these steps, digital images are then captured and  $\mu\text{CH}$  is emptied (5 pulses, removing  $96 \mu\text{L}$  by  $\mu\text{P}_5$ ).

Afterwards,  $\mu\text{CH}$  is cleaned by activation of  $\mu\text{P}_4$  (12 pulses), adding  $96 \mu\text{L}$  of cleaning solution (10% v/v  $\text{HNO}_3$ ) while activated, and DM is activated for 2 s performing the agitation. Then,  $\mu\text{P}_5$  is activated (5 pulses) to discard the contents of the  $\mu\text{CH}$ . This cleaning and discard procedure must be done twice to effectively clean the  $\mu\text{CH}$ .

The procedure for in line blank preparation is similar to described for the sample analysis. The difference is that  $0.1 \text{ mol L}^{-1} \text{H}_2\text{SO}_4$  solution is used instead of the sample or standard solutions.

#### Aluminium(III) determination

The sample or standard solutions (4 pulses, adding  $32 \mu\text{L}$  by  $\mu\text{P}_1$ ), quercetin solution (4 pulses, adding  $32 \mu\text{L}$  by  $\mu\text{P}_2$ ) and CTAB solution (4 pulses, adding  $32 \mu\text{L}$  by  $\mu\text{P}_3$ ) were simultaneously added. The homogenization was performed by the drive motor (DM) coupled to the nylon wire (NW) by 2 s. After these steps, the digital images are then captured and  $\mu\text{CH}$  is emptied (5 pulses, removing  $96 \mu\text{L}$  by  $\mu\text{P}_5$ ).

Afterwards,  $\mu\text{CH}$  is cleaned by activation of  $\mu\text{P}_4$  (12 pulses), adding  $96 \mu\text{L}$  of water and DM is activated for 2 s performing the agitation. Then,  $\mu\text{P}_5$  is activated (5 pulses) to discard the contents of  $\mu\text{CH}$ . This cleaning and discard procedure must be done twice to effectively clean  $\mu\text{CH}$ .

The procedure for in line blank preparation is similar to described for the sample analysis. The difference is that water of  $\mu\text{P}_4$  (12 pulses) is used instead of the sample or standard solutions.

#### The treatment of digital images and mathematical model

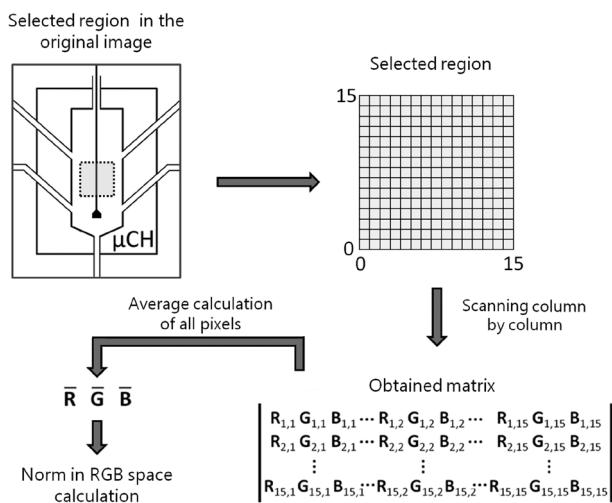
The treatment of the captured digital images was made by means of a second software also written in Delphi (version 3.0). The routine with the working stages of this software is similar to that used elsewhere<sup>6,9</sup> and illustrated in Figure 3. Initially the user selects the most homogeneous region in the image which will define the coordinates of the selected region, and will also be used for all other images. Then, the software scans all the pixels, column by column, to extract the RGB component for each pixel and calculate a mean integer value of each RGB component. These mean values are used in the

**Table 1.** Switching course of the solenoid micro-pumps for the determination of *ortho*-phosphate and aluminium(III) in the proposed  $\mu\text{FBA}$ <sup>a</sup>

Step	Description	$\mu\text{P}_1$	$\mu\text{P}_2$	$\mu\text{P}_3$	$\mu\text{P}_4$	$\mu\text{P}_5$	Pulse	time / s
1	addition of the sample and reagents (S, R <sub>1</sub> and R <sub>2</sub> ) <sup>b</sup>	1/0	1/0	1/0	0	0	4	1.6
2	homogenization using the drive motor (DM)	0	0	0	0	0	0	2.0
3	capture digital images	0	0	0	0	0	0	1.0
4	Waste (W)	0	0	0	0	1/0	5	2.0
5	Cleaning (C) <sup>c,d</sup>	0	0	0	1/0	0	12	4.8
6	homogenization using the drive motor (DM) <sup>d</sup>	0	0	0	0	0	0	2.0
7	waste of cleaning (W) <sup>d</sup>	0	0	0	0	1/0	5	2.0

<sup>a</sup>Codes 1/0 and 0 indicate actuation of the solenoid micro-pumps and that the devices remain inactive, respectively. <sup>b</sup>2 pulses for each actuation of the micro-pump. <sup>c</sup>Cleaning of the micro-chamber with water (for the aluminium) or 10%  $\text{HNO}_3$  solution (for the *ortho*-phosphate). <sup>d</sup>The step 5 to 7 are repeated twice for every sample.

RGB-based value calculation (analytical response) as described below.



**Figure 3.** Routine of the software for digital image treatment.

The RGB-based values were calculated by means of a mathematical model developed from the concept of vector norm “ $\|v\|$ ”,<sup>6</sup> calculated as shown in equation 1:

$$\|v\| = \sqrt{\bar{R}_{s-b}^2 + \bar{G}_{s-b}^2 + \bar{B}_{s-b}^2} \quad (1)$$

where  $\bar{R}_{s-b}$ ,  $\bar{G}_{s-b}$  and  $\bar{B}_{s-b}$  result from the difference between the  $\bar{R}_s$ ,  $\bar{G}_s$  and  $\bar{B}_s$  average values obtained from digital images of the standard solutions and samples and  $\bar{R}_b$ ,  $\bar{G}_b$  and  $\bar{B}_b$  from the blank.

As a result, a linear relationship was observed between the analyte concentration ( $C$ ) in the standards solution (or sample) and RGB data, for which the following equation is valid:

$$\|v\| = kC \quad (2)$$

As demonstrated in previous work,<sup>6</sup> equation 2 provides the basis for building  $\mu$ FBA analytical curves, establishing a linear relationship between  $\|v\|$  (RGB data value adopted as

analytical response) and analyte concentration in standards solutions. Furthermore, the vectors associated with the digital images from each analyte should be positioned on the same support line in the RGB three-dimensional space.

### Batch reference method

For comparison, the proposed  $\mu$ FBA performance was evaluated against a molybdenum blue method (reference method).<sup>20</sup> Standard solutions were prepared from 1.0 to 10.0 mg L<sup>-1</sup>. The analytical signals (absorbance) were measured at a maximum absorbance of around 882 nm. The analysis of each sample was performed in triplicate and the concentrations were calculated from the analytical curve.

The reference spectrophotometric procedure for aluminium(III) in water analysis was carried out according to standard methods (Standard Methods, 20<sup>th</sup> ed., Method 3500-Al B, Eriochrome cyanine R method).<sup>21</sup> The analyses were performed at a maximum absorbance of around 535 nm.

## Results and Discussion

### $\mu$ FBA parameters

Volumes of reagents and samples to be added inside  $\mu$ CH during the automatic procedure were evaluated in order to improve the sensitivity and reproducibility of the analytical signal. The system value selection (univariate method) was carried out in conformance with studies by Chuan-Xiao *et al.*<sup>10</sup> (for PO<sub>4</sub><sup>3-</sup>) and Norfun *et al.*<sup>17</sup> (for Al<sup>3+</sup>).

For both methods, volumes of 32  $\mu$ L of sample (or standard solution) and reagents were selected as the best compromise between reproducibility and sensitivity considering the volume used in the total mixing chamber (about 96  $\mu$ L). The range evaluated and values selected for each parameter are shown in Table 2. If need, other sample dilutions may be carried out in line by simply changing the operational parameters in  $\mu$ FBA control software.

**Table 2.** Selected parameters of the proposed  $\mu$ FBA for determination of PO<sub>4</sub><sup>3-</sup> and Al(III) using digital images

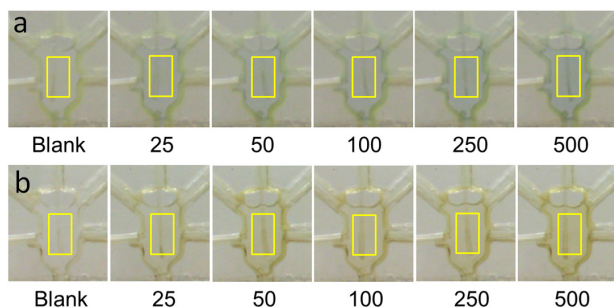
Parameter	PO <sub>4</sub> <sup>3-</sup>		Al(III)	
	Evaluated range	Selected value	Evaluated range	Selected value
Sample volume / $\mu$ L	8-80	32	8-80	32
Volume of reagent 1 <sup>a</sup> / $\mu$ L	8-64	32	8-64	32
Volume of reagent 2 <sup>b</sup> / $\mu$ L	8-64	32	8-64	32
Total volume / $\mu$ L	48-112	96	48-112	96

<sup>a</sup>Ammonium molybdate with potassium antimony tartrate (for PO<sub>4</sub><sup>3-</sup> determination) or quecetin (for Al(III) determination). <sup>b</sup>Ascorbic acid (for PO<sub>4</sub><sup>3-</sup> determination) or cetyltrimethylammonium bromide (for Al(III) determination).

### The captured digital images

Figure 4 shows the digital images obtained from six standard solutions with different concentrations. The first image of the sequences is the blank solution. All images represent a selected area equal to  $35 \times 55$  pixels. As can be seen in Figure 4, the images present an increase in the intensity for the color dark purple with analytical concentration in each standard solution.

Each sampled image was a matrix with  $640 \times 480$  pixels and the delimited region by the user has been a matrix with about  $35 \times 55$  pixels. The pixel resolution was  $87 \times 87$  dots  $\text{in}^{-1}$ . The RGB-based value calculations are based on the product  $2^R \times 2^G \times 2^B$ , where R, G and B are the red, green and blue color components, respectively. These components may assume integer values in a range from 0 to 7, reaching up to 16,777,216 colors.



**Figure 4.** Digital image captured and selected area ( $35 \times 55$  pixels), the complex color in some standard solutions ( $\mu\text{g L}^{-1}$ ) and blank: (a) *ortho*-phosphate and (b) aluminium(III).

Other linear relationships between complex color and the ratios  $B/R$ ,  $B/G$ ,  $B/I_{\text{tot}}$  ( $I_{\text{tot}} = \text{RGB}$ ), and  $\log(B)$  were also studied to possibly maximize precision, as already done in other works.<sup>19</sup> However, in all cases, the results were poor when compared to using vector norm for concentration of the complex formed. The statistical quality of the regression was verified by the residuals and analysis of variance (ANOVA). They confirmed the homoscedastic distribution of residuals and a significant linear regression.

### Potential interfering ions

Natural water is a complex matrix presenting several chemical species, as ions, which could cause interferences in the analysis of interest. Thus, it is necessary to test the effect of the ion coexistence on the color reaction for the determination of the studied ions. In this sense, a set of assays was performed in order to establish the tolerance limit of the proposed methods. It was considered as a tolerable limit the interferent concentration of foreign ions that produced an error up to a 5% deviation in the

determination of *ortho*-phosphate and aluminium(III) alone on comparing with measurements obtained in the absence of the assayed substance.

For the determination of *ortho*-phosphate, some ions usually present in water were selected according to the literature<sup>17</sup> and results obtained are summarized in Table 3. As can be seen, the spectrophotometric procedure has a wide tolerance for the assayed chemical species and severe interferent effects do not take place, at a relatively low concentration level. Compared with the spectrophotometric method, factors that do not influence the determination would not have any effect for the present method. Therefore, the proposed spectrophotometric method using digital images has a satisfactory practicability.

**Table 3.** Summary of the interference effects of possible ions on the peak height obtained from  $50 \mu\text{g L}^{-1}$  of *ortho*-phosphate and aluminium(III) ions

	Foreign ion	Tolerable concentration ratio <sup>a</sup> / ( $\mu\text{g L}^{-1}$ )
<i>Ortho</i> -phosphate	$\text{Ca}^{2+}$ , $\text{Mg}^{2+}$	$5.0 \times 10^5$
	$\text{Al}^{3+}$ , $\text{Fe}^{3+}$	$3.0 \times 10^5$
	$\text{Mn}^{2+}$ , $\text{SO}_4^{2-}$	$2.5 \times 10^5$
	$\text{Co}^{2+}$	$2.0 \times 10^5$
	$\text{Cu}^{2+}$	$5.0 \times 10^4$
	$\text{Cd}^{2+}$	$4.0 \times 10^4$
Aluminium(III)	$\text{Na}^+$ , $\text{Cl}^-$	$1.0 \times 10^6$
	$\text{Mn}^{2+}$ , $\text{Ni}^{2+}$ , $\text{SO}_4^{2-}$	$5.0 \times 10^5$
	$\text{Mg}^+$	$2.0 \times 10^5$
	$\text{PO}_4^{3-}$	$1.5 \times 10^5$
	$\text{Ca}^{2+}$ , $\text{Zn}^{2+}$	$5.0 \times 10^4$
	$\text{Fe}^{2+ \text{ b}}$ , $\text{Fe}^{3+ \text{ c}}$ , $\text{Cu}^{2+ \text{ d}}$	$1.0 \times 10^4$

<sup>a</sup>The concentration of an ion is considered to be interfered when causing a relative error of more than  $\pm 5\%$  with request to the signal  $\text{PO}_4^{3-}$  and  $\text{Al}^{3+}$  alone. <sup>b</sup> $0.01 \text{ mol L}^{-1}$  1,10-phenantroline as masking agent. <sup>c</sup> $0.006 \text{ mol L}^{-1}$  ascorbic acid +  $0.01 \text{ mol L}^{-1}$  1,10-phenantroline as masking agent. <sup>d</sup> $0.25\%$  thiourea as masking agent.

For the determination of Al(III) effects of interfering ions (Table 3) were again evaluated in a similar way as made by Norfun *et al.*<sup>17</sup> Briefly, the maximum w/w ratio of interfering solutions containing  $50 \mu\text{g L}^{-1}$  Al(III) and different concentrations of some metals which might be present in waters were tested, and the absorbances were measured. All tested cations and anions caused interference  $< 5\%$  for the determination of the analyte of interest. However, Cu(II), Fe(II) and Fe(III) were again observed as the most serious interferences. Thus, a solution containing thiourea, ascorbic acid and 1,10-phenanthroline was also used as masking for Cu(II), Fe(II) and Fe(III), as adopted by Norfun *et al.*<sup>17</sup>

### The $\mu$ FBA applications

For the *ortho*-phosphate determination in tap water, the regression equation was  $A = 15.4796 + 0.0214 C$ , where  $A$  is the analytical response and  $C$  is the analytic concentration in  $\mu\text{g L}^{-1}$  of  $\text{PO}_4^{3-}$ . The linear correlation coefficient ( $r$ ) was 0.9995 ( $n = 5$ ) in the range between 25.0 to 500  $\mu\text{g L}^{-1}$  (25, 50, 100, 250 and 500  $\mu\text{g L}^{-1}$ ). For the determination of  $\text{Al}^{3+}$  in tap water using the  $\mu$ FBA system, the regression equation was  $A = 21.1871 + 0.0298 C$ , where  $C$  is the aluminium(III) concentration in  $\mu\text{g L}^{-1}$  in the measuring solution. The squared linear correlation coefficient was 0.996 ( $n = 5$ ) in the range between 25.0 to 500.0  $\mu\text{g L}^{-1}$  (25, 50, 100, 250 and 500  $\mu\text{g L}^{-1}$ ).

All analytical curves were statistically validated by analysis of variance (ANOVA), showing no significant lack of fit in the proposed models at a 95% confidence level. The limit of detection (LOD) and the limit of quantification (LOQ) for both methods were estimated based on the criteria established by International Union of Pure and Applied Chemistry (IUPAC).<sup>22</sup> LOD was evaluated as 3 times the standard deviation of the blank measurement and LOQ was evaluated as being 10 times the standard deviation of the blank measure. For the  $\text{PO}_4^{3-}$  determination, LOD and LOQ were 0.82 and 2.75  $\mu\text{g L}^{-1}$ , respectively; and for the  $\text{Al}(\text{III})$  determination, LOD and LOQ were 0.92 and 3.09  $\mu\text{g L}^{-1}$ , respectively.

Table 4 presents the results for the proposed  $\mu$ FBA, and the reference spectrophotometric method for  $\text{PO}_4^{3-}$  and  $\text{Al}(\text{III})$  determinations in tap water.<sup>20,21</sup> No statistically significant differences were observed between the results at a confidence level of 95% when applying the paired  $t$ -test. The relative standard deviation (RSD) was less than

1.1 and 0.8%, for the *ortho*-phosphate and aluminium(III), respectively, from three replicates.

Recovery tests were also performed and, in this case, three samples were used for each analyte. 1.0 mL standard solution with known concentrations of 10.0, 25.0 and 50.0  $\mu\text{g L}^{-1}$  for each analyte was added to 9.0 mL of the tap water (21.4, 85.4 and 109.2  $\mu\text{g L}^{-1}$  of  $\text{PO}_4^{3-}$ , and 45.8, 69.1 and 112.1  $\mu\text{g L}^{-1}$   $\text{Al}(\text{III})$ ), for measurement using the proposed  $\mu$ FBA. The recovery values obtained are shown in Table 5. As can be seen, the recoveries obtained for each of the samples were within the 98.1-102.8% range.

**Table 5.** Recoveries of *ortho*-phosphate and aluminium(III) in tap water ( $n = 3$ )

Sample	Recovery / %		
	10.0 ( $\mu\text{g L}^{-1}$ )	25.0 ( $\mu\text{g L}^{-1}$ )	50.0 ( $\mu\text{g L}^{-1}$ )
<i>Ortho</i> -phosphate			
1	99.1 $\pm$ 2.1	99.4 $\pm$ 2.4	100.3 $\pm$ 2.2
2	98.6 $\pm$ 2.3	100.9 $\pm$ 2.2	98.7 $\pm$ 2.1
3	102.6 $\pm$ 2.3	102.8 $\pm$ 2.5	101.6 $\pm$ 2.3
Aluminium(III)			
1	98.4 $\pm$ 2.5	102.3 $\pm$ 2.5	98.1 $\pm$ 2.2
2	101.9 $\pm$ 2.4	99.2 $\pm$ 2.1	101.5 $\pm$ 2.6
3	99.7 $\pm$ 2.2	98.8 $\pm$ 2.4	102.4 $\pm$ 2.1

It is worth noting that a solution containing thiourea, ascorbic acid and 1,10-phenanthroline was used as masking for Cu (II), Fe (II) and Fe(III). Nevertheless, the question concerning to the use of 1,10-phenanthroline as a masking reagent for iron needs to be commented. Ascorbic acid is an efficient reducing reagent for Fe

**Table 4.** Results for *ortho*-phosphate and aluminium(III) determinations in tap water using the  $\mu$ FBA proposed and the reference method. Mean values and uncertainties are based on three analytical determinations

Sample	Proposed		Reference		Proposed		Reference	
	$\text{PO}_4^{3-} \pm \text{SD}^a / (\mu\text{g L}^{-1})$	RSD <sup>b</sup> / %	$\text{PO}_4^{3-} \pm \text{SD}^a / (\mu\text{g L}^{-1})$	RSD <sup>b</sup> / %	$\text{Al}(\text{III}) \pm \text{SD}^a / (\mu\text{g L}^{-1})$	RSD <sup>b</sup> / %	$\text{Al}(\text{III}) \pm \text{SD}^a / (\mu\text{g L}^{-1})$	RSD <sup>b</sup> / %
1	65.2 $\pm$ 0.3	0.4	64.9 $\pm$ 0.2	0.3	28.4 $\pm$ 0.3	0.9	29.3 $\pm$ 0.1	0.4
2	46.3 $\pm$ 0.2	0.5	45.8 $\pm$ 0.2	0.4	33.8 $\pm$ 0.2	0.5	33.5 $\pm$ 0.1	0.4
3	52.7 $\pm$ 0.2	0.4	52.8 $\pm$ 0.2	0.3	21.3 $\pm$ 0.2	1.0	21.4 $\pm$ 0.2	0.9
4	56.6 $\pm$ 0.3	0.5	56.9 $\pm$ 0.1	0.2	48.6 $\pm$ 0.3	0.6	48.9 $\pm$ 0.3	0.5
5	111.8 $\pm$ 0.4	0.3	112.1 $\pm$ 0.3	0.2	44.1 $\pm$ 0.4	0.9	43.9 $\pm$ 0.3	0.7
6	68.7 $\pm$ 0.3	0.4	69.1 $\pm$ 0.2	0.2	123.7 $\pm$ 0.6	0.4	124.1 $\pm$ 0.5	0.4
7	125.1 $\pm$ 0.4	0.3	124.9 $\pm$ 0.3	0.3	109.4 $\pm$ 0.3	0.2	109.2 $\pm$ 0.1	0.1
8	45.9 $\pm$ 0.3	0.8	45.3 $\pm$ 0.2	0.5	85.3 $\pm$ 0.2	0.3	85.4 $\pm$ 0.2	0.2
9	113.8 $\pm$ 0.3	0.2	114.0 $\pm$ 0.2	0.2	97.6 $\pm$ 0.3	0.3	97.4 $\pm$ 0.2	0.2
10	80.0 $\pm$ 0.2	0.3	80.4 $\pm$ 0.2	0.2	112.9 $\pm$ 0.4	0.4	113.4 $\pm$ 0.4	0.3

<sup>a</sup>SD: standard deviation of three replicates. <sup>b</sup>RSD: relative standard deviation. <sup>c</sup>ND: not detected.

**Table 6.** Analytical features of the proposed  $\mu$ FBA and other recently procedures for determination of *ortho*-phosphate and aluminium(III) in natural water

Parameter	<i>Ortho</i> -phosphate			Aluminium(III)		
	$\mu$ FBA	Batch <sup>10</sup>	FIA <sup>a 11</sup>	$\mu$ FBA	FIA <sup>a 17</sup>	SIA <sup>b 18</sup>
Detection limit / ( $\mu\text{g L}^{-1}$ )	0.82	5.36	1.0	0.93	7.0	2.0
Working range / ( $\mu\text{g L}^{-1}$ )	25-500	0-10.0	0-200	25-500	20-500	40-500
RSD <sup>c</sup> / %	< 0.8	< 3.5	4.6	< 1.0	1.1	2.8
Sampling rate / h <sup>-1</sup>	160	–	115	160	60	57
Sample consumption / $\mu\text{L}$	32	25000	950	32	125	470
Reagent consumption / $\mu\text{L}$	64	1000	1000	64	125	115
Method	molybdenum blue	molybdenum blue	molybdenum blue	quercetin	quercetin	CAS <sup>d</sup>
Integrated detection	present	absent	absent	present	absent	absent
Carrier fluid	absent	absent	present	absent	present	present

<sup>a</sup>Flow injection analysis. <sup>b</sup>Sequential injection analysis. <sup>c</sup>Relative standard deviation. <sup>d</sup>Chrome azurol S.

(III), while 1,10-phenanthroline reacts with Fe(II) and forms a compound that absorb radiation around 530 nm, so that it should cause interference. The results shown that no significant interfering effect was observed, thus for analytical proposal, the procedure was ready to use. According to Meyer *et al.*,<sup>23</sup> thiourea is a versatile ligand for iron, thus this feature would inhibit the formation of the complex with 1,10-phenanthroline. In this sense, the use of 1,10-phenanthroline would be not necessary. However, such finding was not investigated in this work.

Table 6 presents selected analytical features of the proposed  $\mu$ FBA, and other automated systems as described in the literature for *ortho*-phosphate<sup>10,11</sup> and aluminium(III)<sup>17,18</sup> determinations in water. In general,  $\mu$ FBA (compared to recently methods) presents satisfactory parameters such as limit of detection, relative standard deviation, working range, sampling rate, in line preparation of calibration solutions, elimination of the carrier fluid and no associated fluid dispersion problems, such as loss of sensitivity.

## Conclusion

This study proposes a portable  $\mu$ FBA for photometric determination of water quality parameters. For the illustration, the analyzer was applied for determination of two important quality parameters in tap water:  $\text{PO}_4^{3-}$  and  $\text{Al}^{3+}$ . This system presents a high sampling throughput, thanks to optimizations in the addition times and volumes of the fluids in the microsystem that allowed homogenization efficient and discrete measurements, with lower waste generation, contributing to the basic principles of green chemistry and the advancement of microanalysis.

By the use of an inexpensive webcam for analytical detection, the proposed  $\mu$ FBA offers an alternative to

traditional spectrophotometric analysis. The use of the webcam dispenses the wavelength selection, which allow reduces costs and simplifies the instrumentation. With the webcam it is also possible to implement chemometric treatments due to the trivariate nature of the detection (when used RGB data), as well as the spatial-resolution characteristics inherent in digital images.

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