## Chapter 2

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# Smartphone-Based Analytical Procedure in the Teaching Lab: A Proposal for Undergraduate Students

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**Abstract**

This chapter's main objective is to serve as a guide for chemistry instructors willing to implement the use of the smartphone in the undergraduate laboratory. Different samples have been studied and the laboratory protocol described, in order to ease the adaptation to the preferences of each reader. Additionally, the obtained results are shown and the theoretical concepts discussed. In this chapter, a proposal to get the students involved in the analysis process is made using the smartphone as an analytical detector. To it, a simple setup was built from locally acquired materials, and phosphate was analysed based on colour parameters extracted from the image. As an analyte, phosphate has been chosen due to its wide appearance in diverse matrices and its importance in the industry. Based on the RGB colour space, phosphate can be easily analysed in water, washing powders, eyedrops and blood matrices. Overall, with this lab practice students can use their own smartphone to carry out the analysis, and optimize the image conditions that best suit their device. Additionally, Green Analytical Chemistry principles are implemented in the approach to ensure that students can identify them.

**Keywords:** Smartphone, phosphate, active learning, green analytical chemistry, digital image colorimetry.

## Introduction

### *The importance of phosphorus*

Phosphorus is one of the most abundant elements in earth, being phosphate ($PO_4^{3-}$) the most common specie. It is involved in many different biochemical processes (such as energy transfer, formation of DNA/RNA, pH buffer…). Also, it plays a major role in the formation of biological membranes, as phospholipids contain phosphate in their composition. It is because of that, that 700 mg of phosphorus is the recommended dietary allowance for adult population (Phosphorus – Health Professional Fact Sheet, 2021). This essential mineral is found in many different foodstuffs: milk, poultry, legumes or vegetables are a source of phosphorus.

Additionally, phosphorus has found a wide variety of industrial applications, especially in the form of phosphoric acid ($H_3PO_4$). Among others, phosphoric acid is used for metal treatment, medicines, food additives or refractory industries (De Boer et al., 2019). Additionally, phosphate can form polyatomic complexes, called polyphosphates, which find use in the detergents industry as a surfactant. However, the most remarkable application is its presence in fertilizers as a nutrient for plants. Since phosphorus is a limiting factor in plant growth, applying it to the crops in fertilizers has increased the productivity to obtain more food per unit

of area (Sharpley & Menzel, 1987). Nonetheless, its excessive use in agriculture has generated a pollution problem in aquatic media: eutrophication. The application of phosphorus (and generally also nitrogen) in agricultural soils, ends up accumulating the excess of these nutrients in the aqueous environments, like lakes. Therein, an unusual growth of microorganisms is induced by the presence of sufficient N and P, distorting the natural equilibrium of the ecosystem. This process generally generates an increased rate of fish death (Smith & Schindler, 2009).

### *Analysis of phosphorus*

As can be deduced from the previous section, phosphorus analysis is of great importance nowadays. Many different analytical procedures have been developed to detect and quantify phosphorus in a wide variety of samples. Due to the chemistry of the phosphorus, many of the methods target its phosphate form, since it is the most stable and common one. These methods can be classified into two major groups: chromatographic methods and colorimetric methods.

Ion chromatography uses an analytical column to separate different ionic species based on electrostatic interactions (Fritz, 1987), and make use of a detector to correlate a physicochemical property (absorption, conductivity…) with the concentration of the analyte. Some analytical methods have been described to quantify phosphate using ionic chromatography using a carbonate buffer as a eluent and a conductimetric detector (Tabatabai & Dick, 1983). In this specific case, the linear range comprised up to 1.2mg $L^{-1}$ of phosphorus, and the limit of detection (LOD) was 0.1mg $L^{-1}$. When it comes to colorimetric procedures, a literature survey proves that many different methods and reactions have been developed. For instance, malachite green has been used to develop a colored signal which is proportional to the concentration of P in the medium, reaching the maximum sensitivity at 630nm and keeping a linear trend up to 0.620mg $L^{-1}$ of P (Kallner, 1975). Similarly, quinine has also been proposed as a reagent to quantify phosphate, with a linearity up to 0.6mg $L^{-1}$ of P and a LOD of 0.005mg $L^{-1}$ of P (Kirkbright et al., 1972).

However, the most remarkable one due to its simplicity and common use is the phosphomolybdenum blue method. This analytical procedure is based on the reaction of phosphorus (in the form of phosphate) with a Mo(VI) compound ($MoO_4^{2-}$) in acidic media, following reaction 1. This first reaction produces a pale-yellow complex which can be related to the concentration of phosphate in the sample (Cinti et al., 2016).

$$51H^+ + 7H_3PO_4 + 12Mo_7O_{24}^{6-} \rightarrow 7PMo_{12}O_{40}^{3-} + 36H_2O \qquad [1]$$

Nonetheless, this complex provides a low sensitivity to the method, and higher LODs are obtained. To solve it, a posterior reduction is often done. With it, the P-Mo complex is partially reduced to a Mo(VI) and Mo(V) complex, as can be seen in reaction 2. The resulting complex presents an intense blue color which allows to take more sensitive quantifications, lowering the LOD.

$$PMo_{12}O_{40}^{3-} + 2e^- \rightarrow PMo_{10}^{VI}Mo_2^{V}O_{40}^{3-} \qquad [2]$$

This reduction step can be done with different reagents: as stated in the APHA methods (American Public Health Association), ascorbic acid and stannous chloride are the most suitable ones (Rice et al., 2015). While the latter provides more sensitivity to the method, the former is more reproducible and robust. Either way, the phosphomolybdenum complex that is obtained can be quantified in the visible range, having a broad absorption band around 800nm.

### *Digital image colorimetry*

The application of smartphones in Analytical Chemistry has been a growing tendency during the last few years, thanks to the improvement in their technical properties, and the increasing availability in the market (Capitán-Vallvey et al., 2015; Rezazadeh et al., 2019). Many different analytical problems have been addressed in the field of image treatment using smartphones or capturing devices. On the one side, regarding the inorganic analytes, iron (Mohamed & Shalaby, 2019), calcium (Peng et al., 2019), ammonium ion (Jaikang et al., 2020), lead (Seidi et al., 2014), chloride, nitrite (Sargazi & Kaykhaii, 2020) or pH (Lopez-Ruiz et al., 2014), are some examples of analytes studied with a smartphone as the analytical tool. On the other side, some examples of organic analytes determined using image treatment with smartphone devices are ascorbic acid (Aguirre et al., 2019), ethanol (Böck et al., 2018; Curbani et al., 2020; Marinho et al., 2019), and biomolecules like proteins (Gee et al., 2017). Additionally, different analytical parameters of interest have been also assessed with image treatment like the fermentation degree of cocoa (León-Roque et al., 2016).

All of these are based on the capture of a specific color which is representative of the sample: either as an intrinsic characteristic, or derived from a specific reaction. Color can be defined as a mental perception to a specific part of the electromagnetic spectrum arisen from an object, either by reflection or by emission (Wu & Sun, 2013). The translation of this concept to the mathematical language is done by the *color spaces*, and comprises a part of colorimetry: the science devoted to quantification, analysis and decomposition of color (Wyszecki & Stiles, 2000). A color space is a way of transforming the visual experience of color into a numeric value (Kuehni, 2001). Most commonly, they consist in three different coordinates, which represent a specific quality of color, and are named *tristimulus values*, referencing the three specialized cones in the human eye, which capture three different ranges of wavelengths (Wu & Sun, 2013).

One of the most common color spaces in electronic devices is RGB (Capitán-Vallvey et al., 2015). In it, color is decomposed into red, green and blue components. Each one of these coordinates can take a value between 0 and 255 (even though they are usually normalized from 0 to 1), and hence form a vector (R, G, B). If all of them take the value of 0, the color represented is pure black; if all of them are 255, the color is white. From the RGB coordinates, one can obtain the *grayscale* value. It is calculated as the average value of (R, G, B), and is converting the color signal to a graduation in black and white: if RGB has not been normalized (thus, each channel can take values between 0 and 255), grayscale will vary in that same range; if RGB has been normalized to be comprised in the (0, 1) range, so will grayscale. Equation 3 shows the formula to obtain grayscale value from RGB:

$$grayscale = average([R, G, B]) = \frac{R + G + B}{3} \qquad [3]$$

There exist other color spaces of common use, and they can be interconverted if a reference white is used. CIE XYZ is one of the most noteworthy ones, and was developed back in 1931 by the International Commission on Illumination (CIE, from its name in French). In it, two of the three coordinates represent chroma, understood as the color itself (X and Z), and Y represents luminance. These three coordinates can be obtained by linear combinations of RGB (Hunt & Pointer, 2011; Mohamed & Shalaby, 2019). This color space provides numeric values which, unlike the case of RGB, do not depend on the device of capture, and it receives the name of *uniform* color space. This means that, at a given luminance, the difference between two different colors is the same on both devices (Mohamed & Shalaby, 2019). Additionally, CIE XYZ is often used as an intermediate step in the transformation from RGB to the other color spaces (Capitán-Vallvey et al., 2015).

Among many others, CIE Lab is also worth mentioning. In this case, lightness is represented by L*, which ranges from 0 to 100 (black to white), and color is defined by a* and b*: the change from green to red, and from blue to yellow, respectively. Both can take values between -120 and 120. The obtention of these three parameters requires the previous obtention of CIE XYZ (Mohamed & Shalaby, 2019).

A factor to be considered when applying image treatment in Analytical Chemistry is the lighting that is applied to the sample. Since the observed color will be resulting from an interaction of light with the sample, it must be carefully tuned in order to obtain valuable results. In this sense, different options arise: ambient light, flash from the device, or LED illumination can be chosen as light sources depending on the availability and suitability of each case. Furthermore, the relative position between the light source, the sample and the capture device are also capital parameters, since the result will greatly vary if non-reproducible conditions are used.

### *Active learning*

Active learning can be defined as an approach that aims to get the student involved in the learning process through high order thinking tasks (Armellini et al., 2021). However, getting the student to feel involved in the practice can become a hard task if difficult and sophisticated procedures are meant to be carried out. Hence, in the chapter we propose the application of the student's smartphones to the lab protocol, so that they feel an active part of it.

## Method

In this section, the laboratory protocol to analyze each one of the proposed samples is described. Students must be warned that this practice involves the use of hazardous chemicals, like concentrated sulfuric acid and metals solutions, like antimony and molybdenum. Thus, the adequate safety measures must be taken (lab coat, gloves and eye protection), and the residues generated during the practice disposed in the proper way following the institution's regulations.

### *Reactive mixture preparation*

A reactive mixture must be prepared prior to the analysis of the samples. This mixture is common for any type of sample.

Content:

- 2.5mL of an antimony tartrate solution 0.27% (w/v): prepared weighting 0.135g of $C_4H_4KO_7Sb \cdot 0.5H_2O$ and diluting in 50mL of ultrapure water.
- 25mL of sulfuric acid solution 2.62M: prepared by dilution of 7mL of concentrated sulfuric acid up to 50mL of total volume, with ultrapure water.
- 7.5mL of Mo salt solution (4.12% (w/v), using $((NH_4)_6Mo_7O_{24} \cdot 4H_2O)$: 2g of the salt are dissolved in 50mL of ultrapure water.
- 15mL of ascorbic acid solution 1.79% (w/v), freshly prepared: to it, 0.88g of ascorbic acid need to be dissolved in 50mL of ultrapure water.

It is important that the reagents are added in that specific order to avoid undesired side reactions. The stability of the prepared reactive mixture is 4h.

### *Sample preparation*

Each type of samples needs to be prepared differently, depending on the expected concentration levels and the idiosyncrasy of the matrix. Therefore, each subsection is described independently. Different adaptations are suggested depending on the context of each case: all of the students can analyze one sample, or different ones can be selected and the results compared.

*Blood*

In this case, human blood was used. To obtain it, certified professionals were contacted to extract the blood from two different volunteers. Given the difficulty that this implies, alternatives like rabbit blood can be considered.

The whole blood samples were centrifuged at 1500*g* during 10 minutes to separate the serum from the cellular part of the blood. 20µL of the serum (which deposits on the top part of the tube) were combined with 320µL of the reactive mixture in an Eppendorf flask. A final volume of 2mL was obtained by adding ultrapure water.

*Water*

Rainwater and irrigation water samples were collected and filtered by a 0.22µm nylon filter, and stored at 4ºC until analysis. An appropriate dilution (1, 5 or 7mL in a total volume of 10mL) was made to obtain a sample within the linear range. Before bringing to volume the volumetric flasks, 1.6mL of reactive mixture were added.

Additionally, recovery studies can be carried out with the students to assess the accuracy parameter of the method. To it, we recommend analyzing a sample which is phosphate free (thus, they might need to analyze it need in advance and check if it has a concentration above the LOD of the method) and adulterating it with added phosphorus. For instance, a 1mg $L^{-1}$ of phosphorus sample can be prepared using tap water. From it, a ½ dilution can be carried out to obtain a measuring solution of 0.5mg $L^{-1}$.

*Washing powder*

This kind of sample, which is often in solid state, often contains phosphate in the percentage level, and so it needs a high level of dilution to be analyzed in the mg $L^{-1}$ range. To it, we propose a x25000 times dilution which enables to measure samples within the 0.30 – 13.2%

(w/w) expressed as $Na_3PO_4$, comprising from the limit of quantification of the method up to the upper limit of the linear range. For instance, 0.1g of solid sample can be dissolved in 25mL of ultrapure water, and from it, 100µL transferred to a 10mL volumetric flask in which 1.6mL of reactive mixture are added.

As stated above, there is also the possibility to analyze different samples which are originally phosphate free, by creating a spiked sample with the students. Solid $Na_3PO_4$ or similar can be used as a source of solid phosphorus, and mixed previously with the sample and homogenized in order to obtain reproducible results. Alternatively, students can also take part in this process by spiking the sample and learning how to obtain a spiked mixture of a solid sample to assess accuracy. This, in combination with the water analysis, would allow students to learn how to obtain both liquid and solid spiked samples, a basic skill in Analytical Chemistry.

*Eyedrops*

All of the samples that were selected declared to contain phosphate in their composition, either as an additive or as part of the active compound. Due to it, different dilutions needed to be done in order to have a measuring solution within the linear range. So, an appropriate amount of each liquid eyedrop was diluted up to 10mL with ultrapure water, containing 1.6mL of reaction mixture.

For any given sample, after having added the reactive mixture, an intense blue color started to appear. A reaction time of 10-20 minutes was allowed to pass before measuring the resulting solutions.

### *External calibration*

An external calibrate was prepared using a 1000mg $L^{-1}$ stock solution of phosphorus (prepared from 0.44 g of $KH_2PO_4$ dissolved in 100mL of ultrapure water). From the stock solution, a working solution of 50mg $L^{-1}$ of P was prepared by dilution. The calibration curve was built with the volumes shown in *Table 2.1.* This same working solution can be used to prepare the spiked water samples.

### *UV-Vis analysis*

Measurements were carried out in a HP 8452A diode array. Samples and standards were measured at 820nm to obtain the absorbance values. However, due to the wide absorbance band of the colored complex, this maximum value might be adjusted in each case, depending on the range of the instrument. Instrumental blank was made with ultrapure water, and a reagent blank (prepared as a standard with no added phosphorus, P0 at *Table 2.1*.) was measured to check for any possible phosphate contamination of the reagents. Each sample/standard was measured in individual triplicates and the average value was used. Furthermore, a whole spectrum in the visible range is needed.

***Table 2.1***. Calibration curve preparation.

| *Standard* | *[P] \| mg $L^{-1}$* | *V working solution \| µL* |
|---|---|---|
| P0 | 0 | 0 |

| | | |
|---|---|---|
| P1 | 0.2 | 40 |
| P2 | 0.4 | 80 |
| P3 | 0.6 | 120 |
| P4 | 0.8 | 160 |
| P5 | 1.0 | 200 |
| P6 | 2.0 | 400 |
| P7 | 3.0 | 600 |
| P8 | 4.0 | 800 |
| P9 | 5.0 | 1000 |

### *Image capture setup*

A Samsung Galaxy Edge S7 model SM-G93F was used to capture the images in the optimization step. This device has a 12.2MP camera sensor, and the native camera app was used in the 'pro' mode. More specifically, the image acquisition parameters were: ISO 50, white balance 5700K and aperture 1/1000. The smartphone was placed on a methacrylate structure made in the lab, with the main camera pointing to the 96 microwell plate containing the sample solutions. As a light source, a desktop lamp was used with the light bulb pointing up. Above the light source, a diffusive material was placed to ensure homogenic lighting of the sample. The plate was placed on top of the lamp using the diffusive material as a base, so that light could go through it and reach the smartphone. That microwell plate consisted in a 96 positions plate, with a maximum volume of 350µL, and a transparent base. The walls were made out of black material to avoid interferences of the light source. *Figure 2.1.* shows a scheme of the setup.

Zoom was made to cover 5 x 4 wells of the microplate. The obtained image, in .jpg format, was transferred to a computer to obtain the different image parameters. *Figure 2.1* shows the proposed setup. In this case, Colorlab tool (Malo & Luque, 2002) for Matlab® was used. However, different alternatives cost free can be used, being ImageJ, a free-to-use tool developed by the National Institute of Health (NIH) a recommendable option (Schneider et al., 2012).

To obtain the color parameters, it is important that the selected region of the photograph, which is commonly known as Region Of Interest (ROI), contains a sufficient part of the solution to capture a representative color of the sample. Additionally, it must be avoided to include any part of the microplate within the ROI.

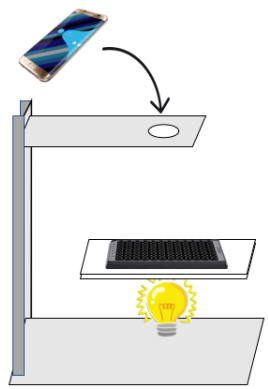

***Figure 2.1.*** Proposed setup to analyze phosphate by image colorimetry.

## Results

In this section of the chapter, the different results that are expected to be obtained by the students are addressed. Firstly, a qualitative study is proposed for them to get a grasp on the different concepts regarding image analysis and colour spaces, connecting those with chemical information. Second, the results of analysed samples are shown, both for the colorimetric and the reference method (UV-Vis spectroscopy). All in all, each instructor might select different parts of the proposed studies, or decide to take on the whole experience, depending on the specificities of each laboratory module.

### *Qualitative study: RGB color space interpretation*

After all the images have been taken, colour parameters can be extracted and analysed. Additionally, some parameters of interest can be also studied, like the influence of well volume. As an example, the RGB parameters for different well volumes are plotted in *Figure 2.2.*

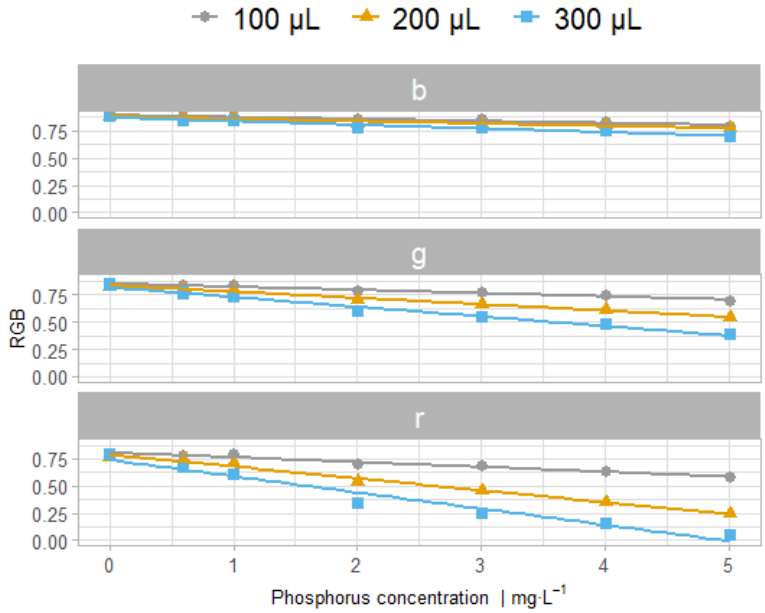


***Figure 2.2.*** RGB parameters trend for different well volumes in a 96 microwell plate.

The results indicate that the maximum sensitivity is obtained using the red (R) channel and 300µL of well volume. In this sense, students are encouraged to make a reasoned interpretation of the results. Some possible questions to state the problem could be:

- For a given well volume (300µL), how is it that sensitivity is decreasing in the specified order: R > G > B?
- For a given color parameter (for instance, R), why is it having more and more sensitivity as volume increases?

First question is expected to be answered based on the absorption spectra of the complex (which is measured during the practice, in the UV-Vis instrument) and the visible colour of the

complex (blue). In RGB colour space, as the sample gets darker and darker (in this case, as the concentration increases, the samples get a more intense blue colour), the average of the three components will tend to 0. This concept is can be easily explained in grayscale terms: as the sample gets more concentrated, its aspect becomes darker, and the grayscale (*Figure 2.3.*) is closer to 0. See Equation 3 in the Introduction section.

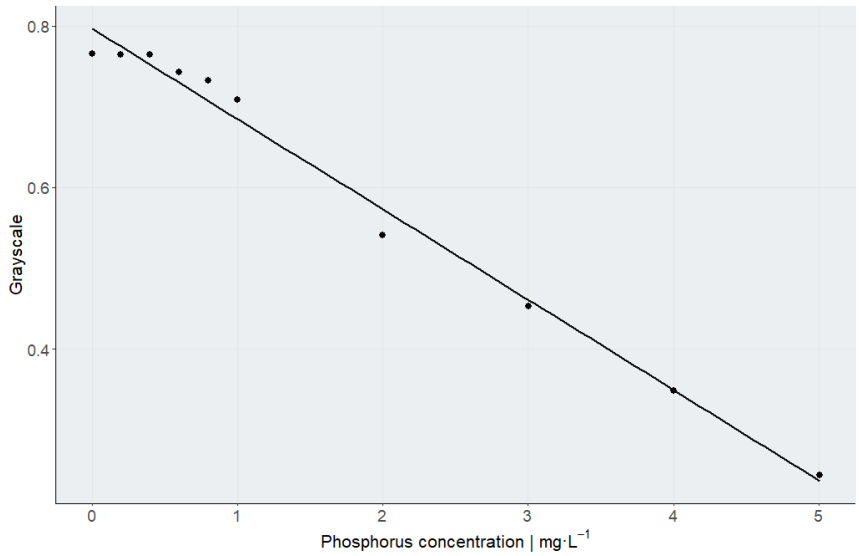


***Figure 2.3.*** Grayscale values for 200µL as volume sample in the 96 microwell plate.

Thus, this is why we find that all three go down. However, it might be counterintuitive that R is the parameter showing more sensitivity, since the observed colour is blue. Hence, this is a great chance for them to correlate RGB colour space with absorption spectra: visible spectrum shows that the wide absorption band of the complex becomes more significant from 600 to 800nm. As can be seen in *Figure 2.4.*, as the absorption band gets more intense (due to higher concentration), the effect is taking place around 700-800nm, and hence it is being represented by R.

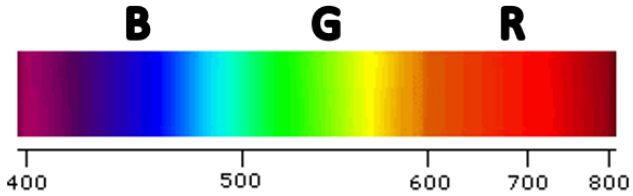


***Figure 2.4.*** RGB parameters covering the visible part of the electromagnetic spectrum.

Regarding the second stated question, students are thought to correlate this concept with Lambert-Beer's law. Since the setup is lighting the samples from the bottom part, and the detector is placed on top, the system is behaving very similarly to a UV-Vis instrument. So, the thicker the layer of solution, the higher the interaction with the analyte and the higher the sensitivity.

To this point, only RGB has been assessed since it is the easiest to obtain with common software. As this chapter focuses on the introduction of undergraduates to the application of smartphones in Analytical Chemistry, we find that it is a good starting point. However, if interested, this same procedure can be done with CIE Lab colour space, studying and extracting reasoned conclusions from the data.

### *Quantitative study of samples*

Once the samples have been analysed both with the smartphone and with the ultraviolet-visible spectroscopy, a comparison between them can be made. Students are expected to plot their results in a figure like *Figure 2.5.* or similar.

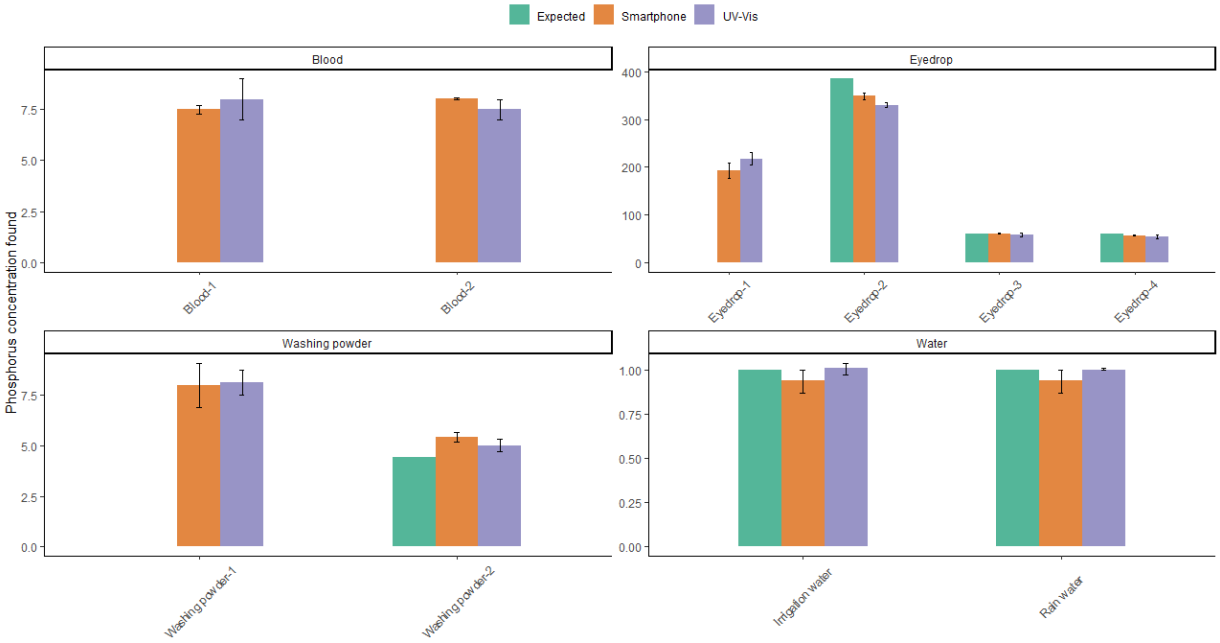


***Figure 2.5.*** Results obtained for the reference method (UV-Vis spectroscopy) and the colorimetric analyzer (smartphone). When a expected value of concentration was known, it has been added. Blood samples are expressed in mg $dL^{-1}$; Washing powders in w/w percentage of $Na_3PO_4$; Eyedrops and water in mg $L^{-1}$ of phosphorus.

As can be observed, results obtained with the proposed setup are comparable to those obtained with the reference method, validating the procedure. Additionally, when a sample had a known expected value, it was accordant to the experimental data.

It is interesting to compare the analytical performances of both techniques. For instance, in terms of linearity. *Figure 2.6.* plots a comparison of both instruments. It can be observed that, while UV-Vis only keeps linearity until 1mg $L^{-1}$ of phosphorus, the smartphone setup allows to keep a wider linear range.

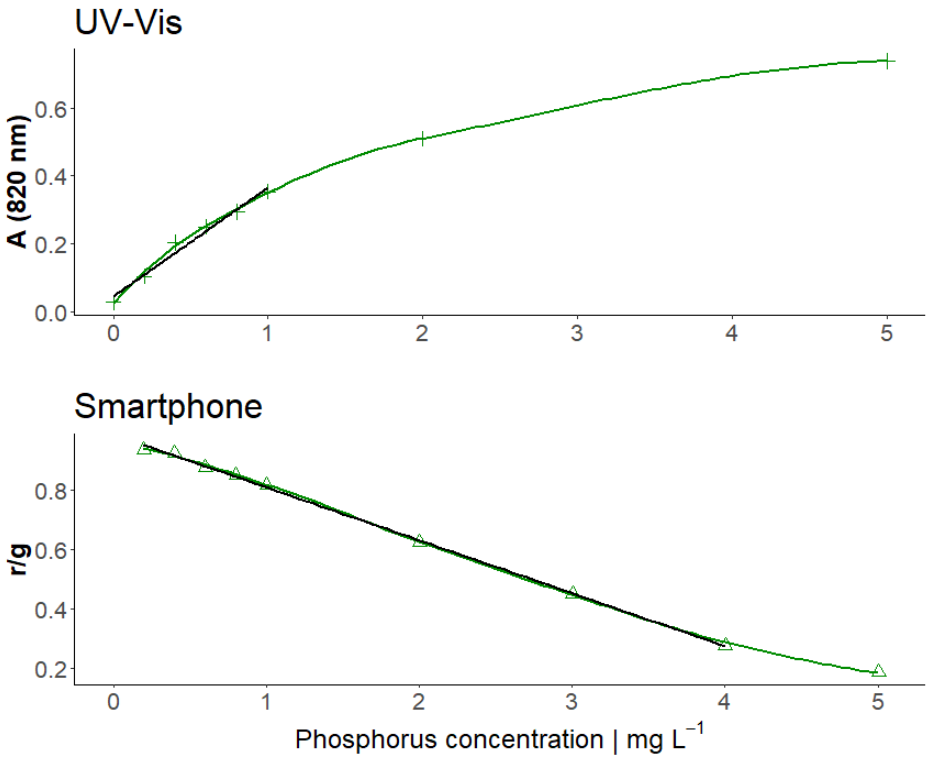


***Figure 2.6.*** Calibration graphs for UV-Vis spectroscopy and Smartphone device in the (0, 5)mg $L^{-1}$ of phosphorus. The linear range is shown in black.

### *Green analytical chemistry parameters discussion*

As a final step, Green Analytical Chemistry is assessed by the students. To it, we propose to follow the 12 different parameters of a method to be considered as green, described by Gałuszka *et al.* (Gałuszka et al., 2013). Namely, they can be summarized as:

- Prioritize direct methods
- Integrate different analytical processes and operations
- Reduce the waste generation, and treat it conveniently
- Reduce the energy waste
- Automatize and miniaturize methods
- Prioritize reagents which are obtained from renewable sources
- Increase safety for operators
- *In-situ* analysis are preferred
- Avoid derivatization steps
- Size and number of samples should be reduced
- Multi-analyte or multi-parameter methods are preferable
- Eliminate or reduce toxic reagents

Hence, they should identify which are the parameters that this method has, and justify why. In this case:

- Reduce the waste generation, and treat it conveniently: when compared to the reference method described in the APHA (Rice, 2015), a reduction of 160 times is obtained.
- Reduce the energy waste: in this case, the energy consumption of the process is much lower, since no instrument is needed other than the smartphone.

- Automatize and miniaturize methods: this parameter is explained the same way was the first one discussed.
- Increase safety for operators: since this method requires lower reagent volumes, the danger associated to their handling is reduced.

Additionally, different variations to the setup can be made, as it can be a good source of discussion with the students. For instance, if the parameter *'Multi-analyte or multi-parameter methods are preferable'* wanted to be accomplished, what adaptations should be done? Different colorimetric reactions can be carried out in the same setup and analysed in the same photograph, saving time and resources.

## Conclusions

The implementation of smartphones in Analytical Chemistry has been a growing trend during the last few years. Thanks to their wide availability, lower cost and improved camera sensors, they have become a useful tool in the laboratory of analytical chemists. Hence, it is a topic which needs to be addressed in the chemistry undergraduates curricula in order to prepare future chemists in the communications era. In this chapter, a procedure to easily implement smartphones in the practice laboratory has been developed and applied to four different samples: washing powders, water, eyedrops and blood. The results proved to be comparable to those obtained with the UV-Vis reference method. With it, students are able to use their own devices in the lab, promoting their involvement with the practice as stated in the principles of active learning. Additionally, a part of the procedure has been designed to allow the students to identify the different principles of Green Analytical Chemistry.

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