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DETERMINATION OF DYES IN CANDIES IN AN ANALYTICAL CHEMISTRY LABORATORY SUBJECT

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Abstract

The use of everyday life samples in laboratory subjects enhances motivation among students and, therefore, improves their learning outcomes. Candies are a common sample in students' everyday life, which makes their dye content an interesting analyte to determine. Food dyes are usually found in candies to increase their attractiveness by providing a bright, intense colour. Their determination is interesting due to increasing concerns in society about the use of food additives. Therefore, this learning experience aims to quantify the content of food dyes in candy samples by UV-Vis spectrometry, taking advantage of their intrinsic colour. For this determination, students need to extract the dye from the candy, take the solution to an appropriate volume and measure the absorbance. Using the external calibration curve method, students calculate the dye concentration and then use this information to compare it with the limits established by the legislation. During this practical, students have optimized the experimental procedure, addressing the different problems that appeared. Also, several points were detected as good learning opportunities to discuss important aspects of the analytical chemistry. From this practical, students learn about the UV-Vis spectrometry technique, improve their practical skills of laboratory working and enhance their critical reasoning and information searching skills. This enables students to contextualize the analysis performed by understanding the importance of a concentration value obtained to solve a real problem and makes the acquired knowledge more significant, profound and constructive.

Keywords – Analytical chemistry, UV-Vis spectrometry, Dyes, Motivation, Food additives.

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1. Introduction

Students need to acquire significant, profound and constructive knowledge to prepare them for their professional life (Hofstein & Lunetta, 2004). Experimental subjects provide a good opportunity to achieve this, if the activities performed in the laboratory are close to students' future professional practice. Therefore, it is preferable to use real samples related to resolving real-life problems for determinations in an analytical chemistry laboratory (Steinert, Heikes, Mitchell-koch, Baker & Mitchell-Koch, 2022).

Students show a passive attitude when dealing with synthetic samples or just salts, in this sense, the use of real samples has an additional benefit: it increases motivation among students (Bagán, Sayós & García, 2015; Bagán, Serra, Tent, Tarancón & García, 2019; Hartel & Moore, 2014; Hobbs, Patel, Kim, Rugutt & Wanekaya, 2013; Steinert et al., 2022). Motivation positively impacts both, the affective and cognitive dimensions of learning. This, in turn, facilitates the achievement of learning goals (Ambrose, Bridges, DiPietro, Lovett & Norman, 2010).

In the Analytical Chemistry Laboratory of the Chemistry degree, a wide variety of real samples, such as beer, carrots, medicines, and toothpaste, are regularly used. However, this is not the case for the UV-Vis technique, which typically uses synthetic or spiked samples. This situation has led to the detection of a learning problem: a lack of motivation among students stemming from the samples used, as well as an insufficient contextualization of the analysis performed. Consequently, there is a rising necessity to design new practicals for UV-Vis determinations, that incorporate real samples. In this sense, the determination of food dyes is an interesting case to study (Bayline, Tucci, Miller, Roderick & Brletic, 2018; Sigmann & Wheeler, 2004).

Food dyes are additives used in the food industry to enhance the visual appeal of foodstuffs. In the European Union, they are regulated by code E-1XY. The digit '1' identifies food dyes. The other two digits denote the code assigned to a specific compound, being the X digit, the colour family of the dye. Dyes may be synthetic or natural. Natural dyes come from various sources, such as animals or plants (European Union, 2008).

Public opinion has worsened about the use of additives, particularly synthetic ones, and their use has been reduced (Durazzo, Carocho, Heleno, Barros, Souto, Santini et al., 2022). However, they are still widely employed in certain food industries, such as the candy industry, in which a bright, attractive colour is highly valued (Saltmarsh, 2020; Stevens, Burgess, Stochelski & Kuczek, 2014). Their presence must be specified with the above-mentioned code to ensure, for example, that a candy is vegan. Also, their quantities must be controlled according to regulations because the consumption of high quantities of some dyes would produce health issues. For these reasons, dye analysis in the food industries is a routine practice (Saltmarsh, 2020; Buchler, Smith & Lawrence, 2010).

These analyses can be performed by high pressure liquid chromatography (HPLC) (Chung, 2021). However, to perform these determinations, one can take advantage of the intrinsic colour and carry out the determination using UV-Vis spectrometry. In this way, students can learn about this technique, but also, go further and learn about the legislation that regulates the amounts of dyes allowed and assess whether the consumption of these candies is safe or not.

Regarding sample selection, careful consideration was given to both motivate students and facilitate the successful execution of the analytical process. Specifically, the chosen sample needed to possess qualities that would engage student interest while requiring a pretreatment procedure of moderate complexity, thereby ensuring a meaningful learning experience. For this purpose, M&M's candies were selected as sample (Birdwhistell & Spence, 2002; Kandel, 1992). They were chosen because the dye is present in a surface layer, which simplifies extraction, and because the candies come in different colours, each containing a different dye that can be analysed, thereby increasing sample diversity. The blue candies contain erioglaucine (E-133), a synthetic dye with high colour intensity and solubility. The yellow ones contain curcumin (E-100), a natural pigment extracted from strains of Curcuma longa L. The green ones contain a mixture of these two dyes, blue and yellow, as no stable, bright and intense green dye, that accomplish the candies requirements, is available in the food industry (Coultate & Blackburn, 2018; Viera, Pérez-Gálvez & Roca, 2019). The red ones contain carminic acid (E-120), a natural colorant extracted from the female insect Dactylopius coccus costa or cochineal. The orange ones contain β-carotene (E-160a), a natural mixture of carotene extracted from different sources such as palm oil or carrots. Finally, titanium dioxide (E-171) is present as the white dye beneath the surface colour layer, which was not considered in this learning experience (Alexandru-Grumezescu, 2018; Kendrick, 2012).

The objective of this work is to develop a new practical for the UV-Vis spectrometry technique that motivates students and allows the application of this quantification to address a real-life problem. This was designed with the following specific objectives:

- Develop and optimize an experimental method for dye determination using UV-Vis spectroscopy in an undergraduate practical subject on Analytical Chemistry.
- Enhance students' motivation and, consequently, their engagement in their learning process.
- Improve critical reasoning skills and information retrieval, particularly by contextualizing the concentration values obtained at the end of the experiment.

2. Design/Methodology/Approach

2.1. Overview of the Subject

This laboratory practical was conducted in the subject Analytical Chemistry Laboratory, a compulsory third-year subject of the Chemistry degree. Before enrolling in this subject, the students must complete a theoretical subject about instrumental analysis. This allows them to apply the theoretical knowledge that they have gained about sample analysis in the laboratory.

The students work in pairs to conduct various experiments related to instrumental techniques. The ratio of students to teachers is approximately twenty to two. The subject takes place in a laboratory, over four consecutive weeks, for four hours a day. Typically, the teacher assigns an experiment, involving the determination of one or several analytes in a sample. Students are required to read and understand the procedure, perform preliminary calculations and present them to the teacher. Once deemed correct, students conduct the experiments and the required calculations and evaluate the results, always under the guidance and supervision of a teacher, to finally present the results in an interview.

During the experimental work, the teachers are in the laboratory alongside students, observing their work and solving any doubt. In the final interview, students are questioned about both practical and theoretical aspects related to the practical performed. In this way, students are assessed regarding their answers and critical reasoning capability along with the observation of the teacher during their laboratory work. For some specific determinations, such as that presented in this article, students must give an answer to a raised question. For instance, determine whether the analyte concentration is in agreement with the current legislation levels. Therefore, they must search for information in standards and legislation. In these cases, a report on the experiment is prepared by the students and is also used for the assessment.

2.2. Design

Before introducing this new laboratory practical, a series of preliminary experiments were conducted to establish the essential parameters necessary for its development. These included defining linearity intervals, selecting appropriate extraction solvents, and assessing the stability of standards. These essential parameters were provided to the students as the basis for conducting the laboratory practical. Through the initial development, students encountered various experimental issues, which they successfully solved. The corresponding modifications were incorporated into the practical procedure. Similarly, the practical procedure was adjusted during subsequent performances, whenever some issue was detected.

The evaluation of this laboratory exercise was multifaceted. It involved, on the one hand, direct observation by the teacher, who was consistently present in the laboratory to offer guidance and support, fostering a supportive and conducive learning environment. On the other hand, the assessment also involved individual interviews with students, analysis of their experimental results, review of their final reports, and feedback gathered through institutional evaluation forms related to the subject.

2.3. Experiment

The final optimized determination of each dye follows the same general procedure. To prepare the calibration curve, a standard stock solution of 100 mg/L (1000 mg/kg for β-carotene) is prepared from the solid standard. Then, students prepare, volumetrically, a dilution tree to make five standards within the working interval, except for β-carotene, which is prepared by weight.

To prepare the samples, one of the M&M's is placed in a beaker with the desired solvent and shaken by hand until all the dye is extracted from the candy, and the white underlayer becomes visible. Then, the solution is decanted and placed in a volumetric flask of the desired volume. For the red dye, the solution has to be boiled before placing it in the volumetric flask to break the dye complex with aluminium. This procedure is performed in triplicate. Finally, students wait until the solid decant in the volumetric flask and then filtrate the desired volume (3-6 mL), using a disk filter, to remove any remaining solids from the white layer.

Both samples and standards are then measured in a UV-Vis spectrometer. The most concentrated standard is used to register the spectra and determine the measurement wavelengths that are used for the determination, corresponding to the maximum absorbance intensity in the visible spectrum. Finally, the concentrations are calculated using the external calibration curve method.

This general procedure has some variations depending on the dye being analysed. For erioglaucine, curcumin and the mixture of both dyes (green M&M), the extraction and standard medium is H_2O:MeOH 30:70, with the exception of the 100 mg/L standard of curcumin, which is prepared in ethanol (96%), with subsequent dilutions prepared in the aforementioned medium. For the carminic acid dye, the extraction and standards preparation medium is a 0.1 M phosphate buffer at pH 7.5. Finally, for β-carotene dye, the extraction and final standards media is a mixture of EtOH:H_2O:CH_2Cl_2 50:25:25. However, as for curcumin, the first β-carotene standard (1000 mg/kg) and intermediate standards with concentrations greater than 17 mg/kg must be dissolved in dichloromethane. Due to the volatility of dichloromethane, a volumetric measure is not suitable. In this case, the solutions are prepared by weight in closed vials.

Table 1 shows the working intervals determined experimentally and their corresponding measurement wavelengths for each dye determination. Typically, students used a general interval from 1 to 10 mg/L for all dyes.

	Blue (E-133)	**Yellow (E-100)**	**Green (E-133 + E-100)**	**Red (E-120)**	**Orange (E-160a)**
Work interval (mg/L) *(mg/kg)	1-20	1-20	–	1-23	1-13*
Measure wavelength (nm)	628	430	628/430	553	484

Table 1. Working intervals (in mg/L, or mg/kg in the case of the orange dye) and wavelengths for the UV-vis measurement (in nm) for the dyes studied

The sample and standard solutions are stable throughout the practical sessions, but some recommendations need to be followed. For β-carotene, solutions must be stored in the fridge to avoid degradation and loss of solvent. Solutions containing curcumin should not be directly exposed to sunlight, as exposure causes the dye to degrade.

3. Results and Discussion

This laboratory experiment has been regularly conducted annually since 2019 by part of the students enrolled in this subject. Aligned with the objectives of the article, and using the practical development as a guide, this section presents the challenges encountered and solved during students' experimental work, the critical points identified to enhance their reasoning skills, and the ways in which engagement and motivation were fostered.

First, students are typically assigned to analyse two or three different dyes, rarely all of them. This is because all the dyes are measured using the same analytical technique, and it was observed that repeatedly analysing the same type of samples could diminish the initial motivation students experienced with the candy samples, due to the repetitive nature of the task, as noted in previous studies (Bagán et al., 2015). Initially, students present their preliminary calculations to the teacher. Generally, they demonstrated proper calculations for preparing both the samples and standards. However, some students encountered difficulties with β-carotene calculations, as these solutions are prepared by weight. This presented a valuable learning opportunity, highlighting the importance of concentration control in analytical chemistry and the challenges associated with using organic solvents.

Next, students prepare all the solutions, both samples and standards, before proceeding with the measurements. Their performance was generally acceptable, except for β-carotene, due to the complexity of weighing a volatile solution and working with a ternary solvent mixture. Despite these challenges, students ultimately succeeded in preparing the solutions, demonstrating an excellent opportunity to enhance their laboratory skills.

Another challenging step identified during the optimization of this practical was the filtration of the sample extract. Initially, students attempted to filter the extract using various methods, such as paper filters, Buchner funnels, or disk filters. However, in all cases, the systems clogged due to the suspension formed by the white layer of the candy. Ultimately, it was concluded that the most effective procedure was to transfer the extraction solution to the volumetric flask, allow the solids to decant, typically until the next session, and then filtrate just a small volume of the upper part with a disk filter. This step also provided a valuable learning experience. While students are usually discouraged from transferring solids into volumetric flasks, in this case, it could be acceptable, as the resulting volume error is negligible, saving also time.

An additional learning scenario emerged with the solvents used for the extraction, particularly in the determination of β-carotene. In this case, the dye is insoluble in water but soluble in dichloromethane. However, if students attempt the extraction in dichloromethane, the dye is not successfully extracted. If they try it with water, the dye is solubilized. This unexpected result typically caught students' curiosity and captured their attention. The reason is that the surface of the candy does not just contain the dye: it also contains sweeteners and Arabic gum, to immobilise the dye on the surface. Both of them soluble in water. The need for a consistent matrix in the standards and the samples, which required the use of a ternary solvent mixture to ensure a single phase, suitable for both sample extraction and standard preparation, became a key learning point for the students.

Finally, students measured all the solutions using the UV-Vis spectrometer. While the instrumental measurements were generally performed correctly, some students neglected to determine the specific wavelengths for measurement, and instead used the theoretical ones provided in the procedure. This served as another important learning moment, reinforcing the need to measure the entire absorbance spectrum beforehand to identify the specific wavelengths.

Figure 1 shows the spectra of all the dyes present in the different candies.

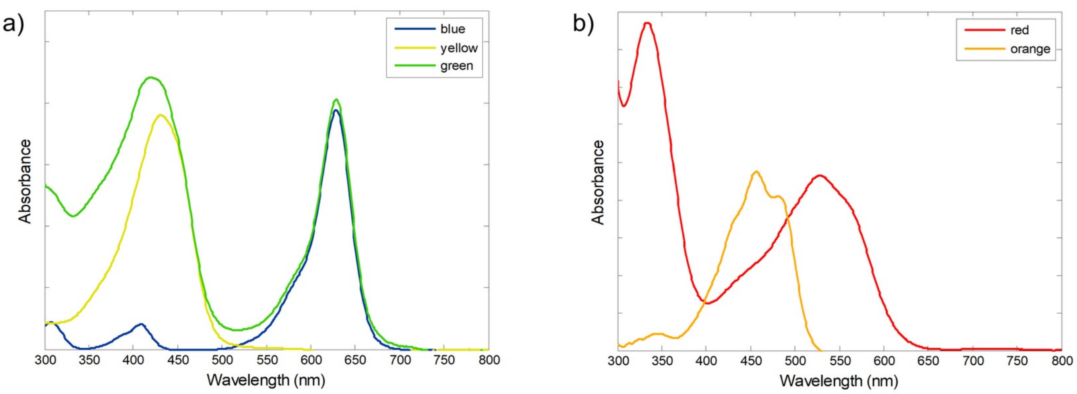

Figure 1. Absorbance spectra of a) blue and yellow dyes, as well as green M&M's extract and b) red and orange dyes, rescaled to improve visualization

At this point, a new learning opportunity arises with the analysis of the green sample, the only candy containing two dyes. Students must decide how to quantify these two dyes, using knowledge from their previous theoretical subject, considering the characteristics of the technique and potential interferences. Most students opted to use single wavelengths for each dye, as the spectral analysis (Figure 1a) reveals only minimal interference from erioglaucine in the curcumin spectrum and no interference from curcumin in the erioglaucine spectrum. This allows for the selective analysis of both dyes by choosing the appropriate wavelength for each. However, students are also encouraged to use a system of equations to determine the concentrations and compare the results. Regardless of the method used, this approach provides a valuable opportunity for them to reason about the selection of different quantification methods in this technique, considering both the interfering wavelengths and the possible errors associated with each method. The equation system for green sample determination is shown below:

$$A_{430\,nm} = \varepsilon_{curcumin(430\,nm)} \cdot [curcumin] + \varepsilon_{erioglaucine(430\,nm)} \cdot [erioglaucine] \quad (1)$$

$$A_{628\,nm} = \varepsilon_{curcumin(628\,nm)} \cdot [curcumin] + \varepsilon_{erioglaucine(628\,nm)} \cdot [erioglaucine] \quad (2)$$

Usually, students spend 1 to 2 laboratory sessions to complete the determination of a single dye and 2 to 3 sessions if they determine the dyes in the blue, yellow, and green candies together, or the orange candies.

The final step of the laboratory sessions is an interview with the students where they present their results. The average dye concentrations obtained during the method setup, prior to the incorporation of the practice into the subject, and for a sample of 20 candies of each colour are summarized in Table 2. The values obtained by students over the years align with these reference intervals, in nearly all cases, indicating that the practical work is achievable for all students.

Concentration	Candie analysed				
	Blue (E-133)	**Yellow (E-100)**	**Green (Blue/Yellow) (E-133 + E-100)**	**Red (E-120)**	**Orange (E-160a)**
mg/sample unit	0.23(0.15)	0.13(0.09)	0.026(0.014)/0.051(0.030)	0.18 (0.11)	0.042(0.019)
mg/g	0.10(0.03)	0.05(0.03)	0.013(0.006)/0.025(0.009)	0.08 (0.04)	0.019(0.006)

Table 2. Reference interval concentration of the different dyes in the M&M's samples in mg of dye per unit of candy and mg of dye per g of candies. Data was expressed as mean followed by three times the standard deviation, in brackets.

As shown in Table 2, students must express their results in milligrams of dye per unit or per gram of candy. Reporting the content in milligrams per unit makes it easy to calculate the legal limits of the dyes or even the number of candies that must be consumed to be detrimental to health. Additionally, expressing the content in milligrams per gram shows a reduction in the associated standard deviation. This is due to the different sizes of the samples, as larger samples contain a higher amount of dye. This is not considered when the results are expressed in milligrams per unit, but is partially addressed in milligrams per gram, as the weight of the sample is proportional to the size. However, the relative standard deviation is not as low as typically found in other determinations conducted on this subject. This discrepancy serves as another valuable learning opportunity for students to explore the uncertainty of measurements and the various contributing factors, particularly those related to sample homogeneity, which is usually negligible in other samples used in the course. It is worthy to mention that this concept was often challenging for students, as they tend to associate standard deviation with the quality of their results. Therefore, this context presents an excellent opportunity to encourage debate and reasoning with them.

At the end of the course, students are also required to prepare a report as part of their final assessment. This report must be 4-6 pages long and include: a brief introduction about dyes and the UV-Vis spectroscopy technique; an experimental section detailing the experimental steps, equipment used, wavelengths of measurement, etc.; a results and discussion section that not only shows the concentrations obtained, but also the problems encountered during the experiment; and, especially, an evaluation of the results in terms of candy ingestion regarding the dye legislation. The report must also include a conclusion and a bibliography section.

In this case, the conclusion section is particularly crucial, as it encourages students to contextualize their concentration values, in the framework of real problem-solving scenarios, to achieve more significant knowledge. In this way, more professional skills as analytical chemists are gained, and students' motivation is enhanced by seeing the real use of their work.

Finally, all students demonstrated high motivation during the practical session under the supervision of the teacher, as well as when presenting their results in the final interview. These observations were also reflected in the results of institutional forms, which indicated high motivation in conducting the practical work, directly linked to the use of real samples.

4. Conclusions

Through this learning experience, students learned the theoretical fundaments and practical applications of UV-Vis spectroscopy through the determination performed. Students' reviews were positive, as the candy sample is something present in their everyday lives. This factor motivated them to carry out the determination, improving the practical skills of laboratory working. Additionally, critical reasoning and search for information skills were improved, as shown in the reports prepared. This made the determinations more similar to their professional work, which made the knowledge more significant.

Finally, other aspects of the determinations, such as the deviation due to the heterogeneity of the samples, facilitated discussion of other analytical chemistry concepts, allowing students to gain more significant knowledge through practical application.

Consequently, the practical presented involving the determination of dyes in candies using UV-Vis spectrometry is considered an effective learning experience as it enhances students' motivation, ensures that all students can successfully complete the practical and provides valuable opportunities for the discussion of important concepts in analytical chemistry.

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