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Determination of food colouring candies: implementation of a new activity in an experimental subject

Determinació de colorants alimentaris en caramels: implementació d'una nova pràctica a una assignatura experimental

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Només un ull ignorant assigna un color fixe i immutable a cada objecte.

Paul Gauguin

A l'Héctor i al Dícac. Gràcies per l'orientació, suport i ajuda sempre que la he necessitat. A la meva família i amics, per ser-hi sempre.

REPORT

IDENTIFICATION AND REFLECTION ON THE SUSTAINABLE DEVELOPMENT GOALS (ODS)

This project could impact any of the first three areas in which the 17 Sustainable Development Goals (SDG) are grouped. People, Prosperity and Planet. The objective is to increase the student's interest in developing an experimental subject of the degree. This motivation will improve their learning, increase their knowledge and their ability to solve future problems. The main objective of this project can be related to goal 4.4. of the sustainable Development Goal number 4, Quality Education: "*By 2030, substantially increase the number of youth and adults who have relevant skills, including technical and vocational skills, for employment, decent jobs and entrepreneurship.*"

Motivation and work with real problems promotes the acquisition of the required competences to help achieve the goals of other SDGs.

The problem-solving capacity acquired by the student, in the chemical sector, can help in the solution of other problems such as CO_2 reduction, which would fall under Sustainable Development Goal 13: Climate action, if student ended up in the future as part of a company dedicated to optimizing processes for CO_2 reduction, for example.

The food analysis of this work raises awareness about responsible food consumption and production, which would correspond to the SDG 12: Responsible consumption and production. Since it is necessary to keep in mind all that is contained in the food, for a better nutrition.

Therefore, this project can help to educatively train someone with great capabilities to contribute to make changes in society to reach the goals set by the United Nations.

CONTENTS

1. SUMMARY

Motivation plays a fundamental role in a student's learning. In experimental subjects, it has been seen over the years, that what creates a certain interest and motivation in students is working with real samples that are present in our daily life. In the *Analytical Chemistry Laboratory* subject, students already work with motivational samples for other techniques, but there are few determinations of them that use the UV – Vis Spectrometry technique. Therefore, it is proposed to develop a new activity to enhance the learning of this technique.

It is proposed to make the determination of food colorants present in candies. Food colorants are additives that make foods more attractive, enhance their colour, or give them colour if they are colourless. The presence and concentration of colorants in food is regulated by law and their determination is interesting.

The aim of this work is to develop a new activity for the measurement of the different food colorants that give colour to the analysed candies: E100 (yellow), E120 (red), E133 (blue), E160a and E160e (orange).

To carry out the determination, first the extraction medium was found for each one, making studies of solubility, degradation and pH influence. For E100 and E133 an EtOH/H_2O 70:30 mixture, for E120 a phosphate buffer solution at pH 7.5, and for E160 an EtOH/H_2O/CH_2Cl_2 50:25:25 mixture.

The wavelengths of the corresponding maximum absorbances were established, the linearity intervals of each of the dyes were found and the samples were extracted. Absorbance measurements were carried out and the amount of colorant of each colour sample was found: 0.05 mg/g in yellow samples, 0.09 mg/g in blue ones, 0.07 mg/g in red ones, 0.017 mg/g in orange ones, and in the green samples 0.025 mg E100/g and 0.013 mg E133/g.

It is concluded that an activity for the determination of food colorants in candies has been satisfactorily developed.

Keywords: UV – Vis Spectrometry, food colorants, candies, motivation, motivational samples, solubility, degradation, pH influence

2. RESUM

La motivació juga un gran paper fonamental en l'aprenentatge d'un estudiant. En les assignatures experimentals s'ha vist, durant els anys, que el que crea un cert interès i motivació als alumnes és treballar amb mostres reals i presents al nostre dia a dia. A l'assignatura de Laboratori de Química Analítica, ja es treballa amb mostres motivadores per altres tècniques, però no hi ha gaires pràctiques dedicades a la tècnica d'espectrometria UV – Vis amb les que s'hi treballi. Per això, es proposa desenvolupar una nova pràctica per potenciar l'aprenentatge d'aquesta tècnica.

Es proposa fer la determinació de colorants alimentaris presents en caramels. Els colorants alimentaris són additius que fan que els aliments siguin més atractius a la vista, potencien el seu color, o els hi donen color en el cas de ser incolors. La presència i concentració dels colorants als aliments està regulada per llei i la seva determinació és interessant.

L'objectiu d'aquest treball és desenvolupar un procediment per a la mesura dels diversos colorants alimentaris que donen color als caramels analitzats: E100 (groc), E120 (vermell), E133 (blau), E160a i E160e (taronja).

Per realitzar la determinació, primer s'ha trobat el medi d'extracció per a cadascun, fent estudis de solubilitat, degradació i influència del pH. Per l'E100 i l'E133 una mescla EtOH/H2O 70:30, per l'E120 una solució tampó de fosfats a pH 7.5 i per l'E160 una mescla EtOH/H2O/CH2Cl2 50:25:25.

S'han establert les longituds d'ona dels corresponents màxims d'absorbància, s'han trobat els intervals de linealitat de cadascun dels colorants i s'ha procedit a fer l'extracció de les mostres. S'han fet les mesures d'absorbància i els resultats obtinguts han estat els següents: 0.05 mg/g en els caramels grocs, 0.09 mg/g en els blaus, 0.07 mg/g en els vermells, 0.017 mg/g en els taronges i en els verds 0.025 mg E100/g i 0.013 mg E133/g.

Es pot concloure que s'ha desenvolupat satisfactòriament una pràctica per a la determinació de colorants alimentaris en caramels.

Paraules clau: Espectrometria d'UV – VIS, colorants alimentaris, caramels, motivació, mostres motivadores, solubilitat, degradació, influència del pH

3. INTRODUCTION

In today's society young people have many more resources and opportunities to study and be educated than their parents and grandparents had. There is much more variety of studies, it is affordable for almost anyone who wants to study, and some resources such as the internet have facilitated many things in the education field.

But despite all the resources, does it mean that learning is better? In the end, learning depends on the student himself or herself and the determination and desire he or she has to learn. Both, before without these resources and now, there must be a motivation on the side of the student to learn.

3.1. LEARNING AND MOTIVATION

3.1.1. Learning

According to Ambrose S, on the Based Principles for smart teaching[1], learning is "*a process that leads to change, which occurs as a result of experience and increases the potential for improved performance and future learning. There are three critical components to this definition:*

- *Learning is a process, not a product. However, because this process takes place in the mind, we can only infer that it has occurred from students' products or performances.*
- *Learning involves change in knowledge, beliefs, behaviours, or attitudes. This change unfolds over time; it is not fleeting but rather has a lasting impact on how students think and act.*
- *Learning is not something done to students, it is something students themselves do. It is the direct result of how students interpret and respond to their experiences – conscious and unconscious, past and present."*

As stated in the definition, learning is a process and this process can be divided into four phases:

1. Sensitization:
   Sensitization is the interest and curiosity, the first contact with the topic to be learned during the learning process. It is related to motivation.
2. Knowledge:
   Knowledge is the recognition of new information, which increases motivation and interest.
3. Mastery:
   Once that new information has been internalized, there is the possibility of using it in closed activities, there is a comprehensive information gathering.
4. Self-integration:
   This new information becomes related to previous concepts incorporated and a control and awareness of learning is produced. There is the possibility of using it in both familiar and unfamiliar activities.

Although learning also depends on the teaching provided to the learner, the learner's personality and attitude towards it have a great impact. These lasts are called intrapersonal conditions. Within the intrapersonal conditions are the cognitive and the affective dimension and these are specific to each student.

3.1.1.1. Cognitive dimension

The cognitive dimension is characteristic of the personality of each student, of the vision he/she has of learning. It could be divided in the approach to learning, the conception of learning, the learning style, and the cognitive style.

Then, it is important to highlight the learning approach and the learning style.

Three types of learning approach can be classified. The first one is the superficial, in which the student does the minimum to pass the course. The second one is the profound learning approach, in which the purpose of the student is to learn to the maximum. And the last one is the strategic, where the student focuses on achieving the best results [2].

There is also a classification of the learning style. The active learning style, the pragmatic, the reflective and the theorical [3].

A student does not necessarily have to have a particular learning approach or style. In most cases there is a mixture of some of them to a greater or lesser extent.

3.1.1.2. Affective dimension

According to Stevick (1980, p. 4) "*the term affect refers essentially to the area of emotions, feelings, beliefs, moods and attitudes, which greatly influences our behaviour*". [4]

Emotions condition our learning, the importance of affective factors in the teaching and learning processes in the classroom makes success possible beyond the different materials, techniques and procedures used to achieve the curricular objectives, since there is a focus on what happens inside each student [5].

That is why the motivation and attitude of the students towards the subject to be learned is also important. This motivation is not permanent, and it requires a motive and effort.

The affective dimension does not contradict the cognitive dimension. The relationship between them allows the students to learn more firmly and completely. It is possible to understand the human being as whole that articulates these two realities [5].

3.1.2. Motivation

As discussed, motivation plays a big role in a student's learning. When talking in the context of learning, "*motivation influences the direction, intensity, persistence, and equality of the learning behaviours in which students engage. Student's motivation generates, directs, and sustains what they do to learn*". [1]

Motivation can be considered as two main concepts: the expectations for successful in achieving a goal and the subjective value of that goal.

As can be seen in Figure 1, motivation comes from the expectations and the value that the student gives to a goal that has been set [1]. The goals that a learner may have will be determined by his or her

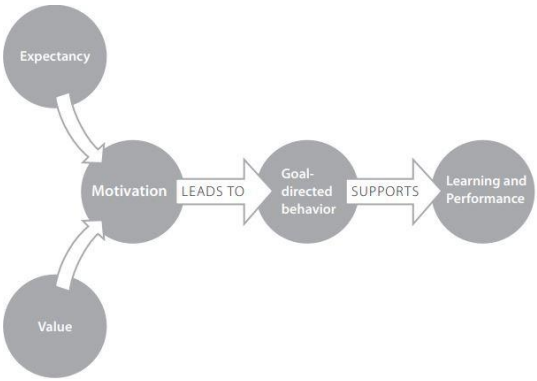

Figure 1. Impact of Expectancy and Value on learning and performance [1]

learning profile, as discussed above.

Therefore, it can be stated that being motivated to learn improves the learning process and that not all students have the same interests, so the motivation to perform a certain task and learn about it is not the same for the different students in a class.

3.2. LABORATORY SUBJECT CONTEXT

This project consists of developing a new laboratory practice for the subject "*Laboratory of Analytical Chemistry*". Over the years, it has been observed that this subject creates a certain motivation and interest in students by working with real samples, when making determinations in products that are present in our daily life. This motivation and interest lead to a better learning [6]. Nowadays, there are practices such as the determination of ethanol in beer by gas chromatography, or the potentiometric determination of fluorides in toothpaste. These are determinations in which students can compare their results with what Is on the label of the beer can or on the toothpaste tube, and allows them to go beyond the determination and analyse day life samples.

However, there are few determinations that use the UV-Vis Spectrophotometry technique where motivational samples are used and that is why a new practise is suggested. A sample with absorption is required to use the UV – Vis technique, so it is proposed to determine the amount of colorant present in the *m&m's* candies. These candies are chosen because the additive is in a superficial layer, and this provides an easy extraction. Moreover, there are candies of different colours, both natural and artificial, in a sachet. Therefore, the determination of different food colorants and even a mixture of them can be done.

Figure 2. m&m's candies

3.3. COLORANTS

The *m&m's* candies are selected as motivational sample because, besides being a sample that the student could eat, it contains dyes. The determination of the amount of dye in a candy is interesting because, nowadays, the presence of additives in food is regulated by law and there is some controversy.

As described in the Regulation (EC) No 1333/2008 of the European Parliament of the Council of 16 December 2008 a food colouring is an additive that is used for "*making food more*

visually appealing, giving colour to food otherwise colourless, or restoring the original appearance of food of which the colour has been affected by processing, storage, packaging and distribution, whereby visual acceptability may have been impaired". As an additive it is also defined as any substance present in a food that is not normally consumed as a food in itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value, and the addition of which is intended for technological purpose [7].

Food colorants can be classified as natural or artificial. Natural colorants are so called because they are extracted from a vegetable, animal, or mineral substance. Artificial or synthetic dyes are products that have been chemically modified[8].

3.3.1. Natural and artificial colorants

Natural colorants are quite unstable when subjected to thermal treatments, extreme pH, when exposed to light, etc. Changes in taste and smell, colour intensity or composition can occur. They are difficult to work with also because they have low solubility in water.

The opposite happens with artificial colorants. They are characterized by their stability and intense colour. They are soluble in water due to the presence of sulfonic groups[8] and consequently easy to use. The problem with these is that they have a certain chronic toxicity[9] and attempt is made to use them as little as possible and in low quantities.

3.3.2. Regulation

Food dyes began to be regulated because of their detrimental effects on consumers' health [9]. That is why there is currently a lot of controversy with the presence of colorants in food. Therefore it is interesting to propose this laboratory practice because it is a real and current problem.

The presence of additives in the food is regulated by the European Union in Regulation No 1129/2011 of 11 November 2011. Therefore, in order to use a food colouring in the European Union in a food, it must be on the list of authorised ones and must be authorized for that particular product.

All additives are classified by the E – number.

3.3.2.1. E – number

Additives are assigned a number preceded by the letter E by the European Union to identify them, to know their function and their chemical designation in order to allow the free circulation of food products from one country to another. The first number indicates the classification of the additives [10] :

- E 1XX: dyes
- E 2XX: preservatives
- E 3XX: antioxidants
- E 4XX: stabilizers, emulsifiers, thickeners, gelling agents and emulsifiers
- E 5XX: acidulants, acidity correctors, anti-caking agents
- E 6XX: flavour enhancers
- E 9XX: sweeteners, various

E numbers allow additives to be mentioned on food packaging so that they can be identified independently of the language used on the label[8].

Due to the toxicity of some of the dyes there is a value established by law called Acceptable Daily Intake (AID). It determines the amount that a person can take daily throughout his or her life without harming his or her health. This amount is expressed in mg per kg of body weight per day. This value is not valid for babies because their detoxification mechanism is weaker[8], and therefore there is specific legislation for babies.

This proposed practice allows students to compare their results with the current legislation. Therefore, it provides meaningful learning by making the determination closer to real world as a professional.

Dyes can be determined by HPLC with an UV – Vis detector, as well as by UV – Vis Spectroscopy, taking advantage of their colour. But the purpose of this work is for the student to learn about different aspects of UV-Vis Spectroscopy. So both, naturals and synthetic food colorants will be determined by UV – Vis Spectroscopy.

3.4. UV – VIS SPECTROSCOPY

UV-Vis spectroscopy is an analytical technique that measures the absorbance or transmittance of the sample by irradiating a beam of light on the sample [11]. As its name indicates, spectroscopy is performed in the Ultraviolet – Visible range spectrum (380 – 700 nm).

The technique consists of exciting the electrons of a substance to a higher energy state by a beam of light incident on the sample. Light has a certain amount of energy that is inversely proportional to its wavelength. Upon excitation of the electrons, an absorption of energy occurs, and it is this absorption that is detected [12]. This absorption is the reason for the colour of the samples.

Depending on the type of bond and depending on the molecule, it will absorb at one wavelength or another. Both conjugation and aromaticity cause the molecule to absorb significantly[12]. The amount of light absorbed by one mol of substance is called molar absorptivity (ε) and it is characteristic for each substance [11].

A quantitative analysis can be performed with this technique. The amount of light absorbed by the sample can be used to determine its concentration according to Beer-Lambert's law.

3.4.1. Beer-Lambert law

As mentioned above, this law can be used to determine the concentration of a sample through its transmittance or absorbance.

Transmittance is the fraction of light (%) passing through the sample and the absorbance is defined by the formula $A = -\log(T)$ and follows Lambert Beer's law:

$$A = \varepsilon bc$$

Where: ε is molar absorptivity, b is path length and c is analyte concentration.

Beer-Lambert's law tells us that there is a linear dependence between the concentration of the analyte and its absorbance. This relation is only fulfilled if the solution is homogeneous and the concentration of the substance is not very high, because above certain concentration linearity is lost.

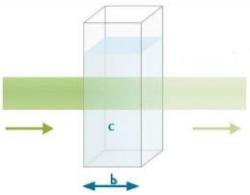

Figure 3. Cuvette [11]

In the case of a multicomponent mixture, as will be one of the cases of this project, the absorbance obtained at a certain wavelength is the addition of the absorbance of the different substances and can be used to determine their concentration separately with an equation system, measuring at different wavelengths.

$$A = A_X + A_Y = \epsilon_X b[X] + \epsilon_Y b[Y] + \cdots$$

The measurements are performed in a UV – Vis Spectrophotometer.

3.4.2. UV – Vis Spectrophotometer

Figure 4 shows a schematic diagram of the basic elements of an UV – Vis Spectrophotometer.

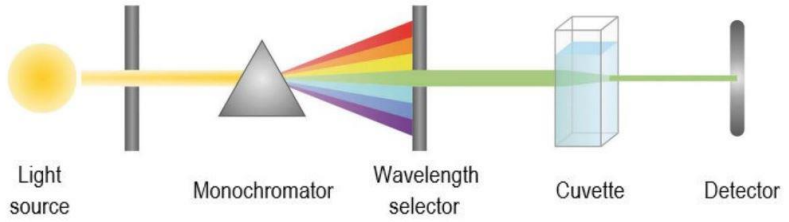

Figure 4. Main components in a UV - Vis Spectrophotometer [11]

In this technique the light beam, generated by a lamp, passes through a wavelength selector to isolate a wavelength. Then, the light passes though the sample, and some energy is absorbed. After the light passes through the sample, a detector is used to convert the light into a readable electronic signal to measure the amount of light absorbed.

Before analysing the sample, a "blank" must be measured. In this way it is subtracted the signals that could be given by the solvent and avoided some dispersion provided by the cuvette.

3.4.2.1. UV – Vis Spectrophotometer equipment

Source of ligth:

Halogen or tungsten lamps are used for wavelengths above 350 nm and hydrogen or deuterium lamps for the ultraviolet spectrum.

Wavelength selector:

The selectors can be monochromators, that allows selection of each wavelength and are the most common [11]. Or absorption filters, and interference filters, mostly used in field or sensors for specific analytes determination.

Cuvette:

The sample cuvettes can be made of plastic, glass or quartz, depending on the wavelength at which the measurement has to be made. Quartz cuvettes absorb less types of wavelengths and are therefore the most commonly used. The plastic ones absorb UV light, and the glass

ones absorb the majority of UVC (100-280 nm) and UVB (280-315 nm) but allow some UVA (315-400 nm)[12].

Detector:

The most used detectors are photomultipliers, which produce a continuous flow of electric current, increasing the signal. Others are the diode detectors, which detect several wavelengths simultaneously and are used to obtain the spectra in a very short time but are less sensitive.

3.4.3. Motivation

The purpose of this practise is that the student learns to use and understand the application of the UV – Vis Spectroscopy technique to the quantification of analytes. Motivational samples will make the student curious and interested in how to make the determination. Thus, he or she will be able to reach not only the mastery phase of learning, but also the self-integration phase.

4. OBJECTIVES

Develop a method to determine the amount of colorant on *m&m's* candies, in the subject of "*Laboratory of Analytical Chemistry*", in the most suitable way for the students.

The method has to be relatively simple and practical so that it is affordable to do it in the hours dedicated to the subject, and with the material available, with the aim that the students learn about the UV – Vis Spectrophotometry technique.

Study the solubility of the different food colorants, study their linearity interval, the process of extraction in the sample, and determine the amount of colouring agents present in *m&m's* candies by UV – Vis Spectrophotometry.

5. EXPERIMENTAL SECTION

5.1. REAGENTS AND APPARATUS

- Curcmin from Sigma-Aldrich
- Erioglaucin from Sigma-Aldrich
- Carminic Acid from Sigma-Aldrich
- Beta carotene from Sigma-Aldrich

An UV – Vis Spectrophotometer, Flexible UV – Vis, Cary 60 Agilent has been used to do all the measures. The spectra are measured in a medium scan rate and in a range of wavelengths from 200 to 800 nm.

5.2. STUDY OF CURCUMIN (E100) AND ERIOGLAUCINE (E133)

5.2.1. Curcumin solubility study

Six vials containing 20 mg of curcumin each have been prepared. To each of them 20 ml of each of the following solvents have been added:

- Double deionized H_2O
- EtOH/H_2O 40:60 mixture
- EtOH/H_2O 60:40 mixture
- EtOH
- Acetone
- NaOH 0.1 M

Vials with 2 mg of curcumin each have been also prepared. To each of them 20 ml of the following solvents have been added:

- EtOH/H_2O 20:80 mixture
- EtOH/H_2O 80:20 mixture
- EtOH/H_2O 90:10 mixture
- EtOH/H_2O 95:05 mixture

5.2.2. Erioglaucine solubility study

Six vials each containing 20 mg of erioglaucine and six more containing 10 mg were prepared. To each of them 20 ml of each of the following solvents have been added:

- Double deionized water
- EtOH/H_2O 40:60 mixture
- EtOH/H_2O 60:40 mixture
- EtOH
- Acetone
- NaOH 0.1 M

5.2.3. Study of the degradation and influence of pH in EtOH and H_2O

Vials were prepared with 5 mg/L solutions of curcumin. Five vials in ethanol, five vials in double deionized water, and other five in a mixture of EtOH/H_2O 70:30. With each of the solvents at pH: 1, 4, 7, 9, 13. (Equivalent molar concentrations in the case of ethanol and the mixture as solvent.)

The UV – Vis spectra of the solutions were measured.

For the degradation study, the solutions at pH 7 and two more solutions of 5 mg/L with EtOH/H2O 40:60 and EtOH/H2O 60:40 mixtures as solvents were used.

Spectra were recorded during the first 4 days and after one week.

5.2.4. Photodegradation of curcumin

A vial containing 10 mg of curcumin in 10 ml of EtOH was prepared. A first dilution of 0.5/5 was made from this solution. In two vials labelled as dark vial and light vial a 0.5/10 dilution has been made for each of the previously diluted solution.

One vial was left under natural light irradiation and the other was left inside an opaque bottle.

The spectra of the two vials were recorded on the same day of preparation and after 14 days.

5.2.5. Study of the absorbance in small variations in the matrix of an EtOH/H_2O 70:30 mixture

Starting from a stock solution of 1000 mg/L curcumin in EtOH the corresponding dilutions were made to reach three solutions of 5 mg/L. The solvents used have been mixtures of EtOH/H_2O The first one with proportions 69:31, the second 70:30 and the last 71:29.

The UV – Vis spectra of the three resulting solutions have been measured.

5.3. STUDY OF CARMINIC ACID (E120)

5.3.1. Study of the solubility of carminic acid and the influence of pH

Two vials have been prepared. One with 10 mg of carminic acid in 10 ml double deionized water, and another more diluted with 2 mg of acid in 20 ml of water.

Solutions have been prepared at different pH: 1.3, 2.5, 4.3, 6.2, 9.8, and 11.

A total of 2 mg was weighed into a vial and 20 ml of double deionized water was added. From this solution, 6 vials of 50 mg/L were prepared adding acid (HCL 1M) or base (NaOH 1M) to the vials, measuring the pH with the pH-meter, until the desired pH was reached.

The absorbance spectra of the solutions at different pH, were measured.

5.3.2. Preparation of phosphate buffer solution at pH 7.5

A solution of $NaH_2PO_4 \cdot 2H_2O$, 0.067 M and another of Na_2HPO_4, 0.08 M have been prepared.

From the first solution 196 ml were taken and from the second 804 ml were taken and mixed. From this mixture 901 ml were taken and made up to 1 L with deionized water.

5.4. STUDY OF BETACAROTENE (E160)

5.4.1. Beta-carotene solubility study

Three vials with 10 mg of beta-carotene each have been prepared. In one of them 10 ml of double deionized water were added, in another one ethanol and in the last one dichloromethane.

Tests were made with different proportions of the three solvents to obtain a single phase.

The dichloromethane solution with beta carotene was diluted 1/10 to obtain a 100 mg/L solution. From this solution 2.5 ml were taken and added to a vial with 2.5 ml of double deionized water and 5 ml of ethanol.

To prepare more diluted solutions, the dichloromethane solution has been diluted one more time 1/10.

5.5. LINEARITY INTERVALS STUDY OF THE SEVERAL COLORANTS

Approximately 25 solutions with equidistant concentration intervals between 0 and 30 mg/L have been prepared for each dye.

In the case of curcumin and erioglaucine a 70:30 EtOH/H2O mixture was used as solvent. The absorbance at 431 nm and 628 nm was measured for each solution.

In the case of carminic acid the solvent was a phosphate buffer solution at pH 7.5. The spectra of each solution have been recorded, and all results at wavelengths 553, 513 and 335 nm were used.

In the case of beta-carotene, higher concentration solutions were prepared in dichloromethane. To obtain concentrations in the desired range in the desired matrix, 2.5 ml of each of the concentred solutions in dichloromethane were taken and diluted with 2.5 ml of coubly deionized water and 5 ml of ethanol. The spectra of each solution were measured, and all results at wavelengths 484, 458 and 278 nm were used.

5.6. EXTRACTION AND DETERMINATION OF COLORANTS

20 samples of each colour have been weighed in 20 numbered vials and measured with a caliper.

For the yellow, blue and green *m&*m's, 10 ml of EtOH/H2O 70:30 mixture was added to the vial with the sample in it. It waited for the dye to dissolve and for the sample to turn white. Because some of the titanium dioxide present in the sample has also become part of the solution, the solution is filtered with a 10 ml syringe and a 45 µm filter and deposited in the previously weighed empty vial. The candy was removed from the vial and 10 ml more of the mixture was added. This was filtered again and deposited with the previously filtered solution into the empty weighed vial.

For red *m&m's* 10 ml of phosphate buffer solution at pH 7.5 was added to the vial with the sample, and for the orange ones 10 ml of an EtOH/H_2O/CH_2Cl_2 50:25:25 mixture.

Once the filtered solution was in the previously tared vial, this vial was weighed again.

The absorbance was measured at the corresponding wavelength. At 431 nm for the yellow *m&m's*, at 628 nm for the blue ones, and at both wavelengths for the green *m&m's*. In the case of the red and orange ones, the spectra were measured. Absorbance used were 553, 523 and 335 nm for red ones, and 484, 458 and 278 nm for orange ones.

6. FOOD COLORANTS IN M&M'S

The first step of this project has been the research of the dyes present in the *m&m's*. In a sachet there are yellow, blue, green, red, orange and brown candies. On the label of this sachet the colorants E100, E120, E133, E160a, E160e and E171 appear.

The colorant E100 corresponds to the yellow colour candy and it is called Curcumin. It is a natural colorant that belongs to the curcuminoid family [13], polyphenolic pigments present in the ground rithomes of strains of *Curcuma Longa L*. It is obtained by solvent extraction and purified by crystallization [14]. (Figure 5)

Its applications include giving flavour and colour to foods, dyeing yellow or orange textiles, and as a remedy to alleviate various health disorders [13].

Curcumin is highly hydrophobic [15] and it can exist in tautomeric diketo and keto-enol forms [16].

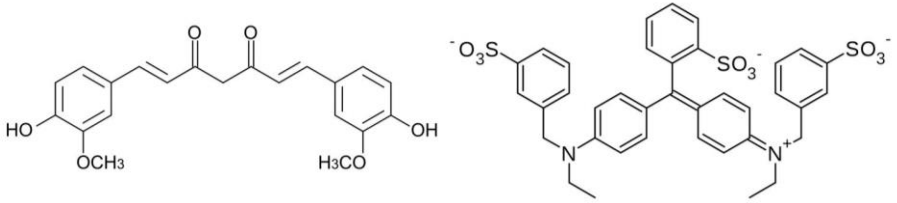

Figure 5. Chemical structure of curcumin (E100) (on the left) and erioglaucine (E133) (on the right)

The blue colour is given by Erioglaucine (E133) is also called Brilliant Blue FCF. It is a triarylmethane synthetic food colorant (Figure 5). Used for soft drinks, confectionery, ice cream, etc. It is also used for cosmetics and body care products. It provides an intense blue colour. Because it is an artificial colorant, it is highly soluble and stable.

There is no green colorant specified on the label, because the colour of the green candy is due to the mixture of E100 (Curcumin) and E133 (Eriogleucine). This is a common procedure due to the absence of stable and intense green dyes.

The E120 colorant corresponds to red candies and it is called Carminic acid. It is a natural colorant and it is obtained from aqueous, hidroalcoholic or alcoholic extracts from Cochineal, which consists of the dried bodies of the female insect *Dactylopius coccus Costa* [14]. (Figure 6)

Carminic acid is soluble in water and is a trivalent acid with pK_1=2.91, pK_2=5.62 and pK_3=8.3 [17]. It has different colours depending on the pH of the solution. Below pK_2 the solution presents orange colour, above pK_2 red colour, and above pK_3 purple colour.

This dye is also used for both food and cosmetics, providing a red colour to the substance where it is applied.

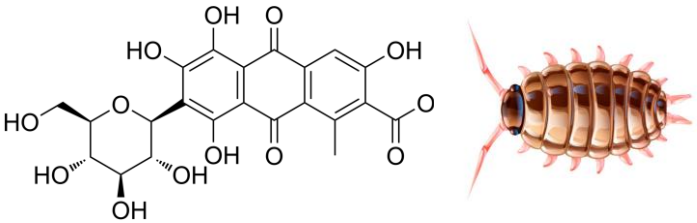

Figure 6. Chemical structure of carminic acid (E120) and an ilustration of a cochineal female. Credit: Shonyjade

The colour of the orange *m&m's* is given by the additives E160a and E160b. The first is beta-carotene (Figure 7) and the second is called beta-apo-8'-carotenal (Figure 8). The second is a degradation product of the first. They belong to carotenoids family. Beta-carotene is mixture of carotene isomers, extracted from palm oil where it is removed as one of the final stages of palm oil manufacture as a decolourisation step [18]. Beta-carotene is a precursor from Vitamin A, this means that once ingested is converted into it.

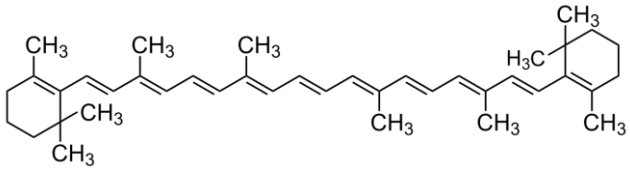

Figure 7. Chemical structure of beta-apo-8'-carotenal (E160e)

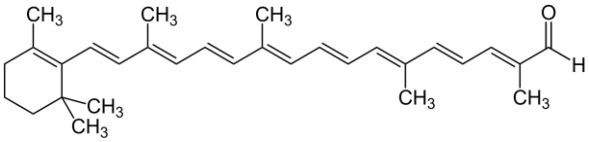

Figure 8. Chemical structure of beta-carotene

The number E171 corresponds to titanium dioxide, which provides the white colour. In the case of *m&m's* it is found in a layer between the chocolate and the colorant layer, and in the letters. It is insoluble in water and in organic solvents. This colorant is not the object of study in this work. There is no colorant corresponding to the brown colour, since it is the colour of chocolate. For that reason, the brown coloured *m&m's* are not the object of study either.

6.1. OPTIMIZATION OF THE SAMPLE EXTRACTION METHOD

Before starting the study of the solubility of the dye standards, a preliminary study of the extraction of the dye present in the samples was carried out. It has been tested to extract all the dyes in water, ethanol and acetone.

The extraction consisted of adding the solvent in a vial with a sample of each colour in it.

The extraction of the samples has been simple, since the dye is in a superficial layer and it is easily detached from it in an aqueous solution. It is observed that in water the extraction is very fast and at most in two minutes all the colorant is dissolved. On the other hand, in ethanol

after 20 minutes the solution is still colourless the colorant remains in the *m&m's* and the chocolate starts to crack. The same was observed in the case of acetone as solvent. This behaviour is observed for all the samples, with independence of the dye present.

Therefore, for all *m&m's* to be analysed, the solvent with which the sample has to be extracted must contain water. Is surprising because of the structure of some of the dyes. One hypothesis is that the dye is mixed with glucose and therefore dissolves only in water.

7. SOLUBILITY, DEGRADATION AND PH INFLUENCE STUDIES

Next step was the solubility study due to differences in the chemical structure of the dyes. It was performed in different groups.

7.1. CURCUMIN AND ERIOGLAUCINE

As mentioned above, the green samples contain a mixture of curcumin and erioglaucine. Therefore, the study of both is done jointly because they will share the medium when analysing green samples.

7.1.1. Solubility study

First, solubility of curcumin in different solvents (water, EtOH/H_2O mixtures, ethanol, acetone, and NaOH 0.1M) was studied. It can be observed that for this concentration (1000mg/L) in water, practically nothing dissolves and that in the vials containing mixtures of ethanol and water very little dissolves after hours of agitation. On the other hand, in acetone, ethanol and sodium hydroxide it dissolves completely and practically immediately without stirring. As shown in the Figure 9 , the result is a yellow colour for the acetone solution, a more orange colour for the ethanol solution and a reddish colour for the basic solution.

Figure 9. E100 1000 mg/L in acetone, EtOH, and NaOH 0.1 M

Next, a study is made at a lower concentration (100 mg/L) using mixtures of different proportions of ethanol and water as solvent, since, as discussed above, the presence of water to make the extraction of the sample is needed.

As Figure 10 shows, curcumin is not dissolved in water or in low proportions of ethanol. In the range of proportions from EtOH/H_2O 40:60 to 100% EtOH the curcumin is completely dissolved.

Figure 10. E100 100 mg/L in different mixtures of ethanol and water

Also, it can be observed a difference of colour in the solutions. An hypothesis is that there is degradation or pH influence. Therefore, a study of degradation and pH variation was performed.

The solubility study has also been done for eriogleucine. It has dissolved in all solvents except acetone, where it is partially soluble. The colour of the resulting solutions has been the same blue in the case of all solvents except in NaOH 0.1 M, which has obtained a more purple colour. An hypothesis is that there is pH influence.

Due to the solubility and stability of eriogleucine in various solvents, as being an artificial colorant, the dye that determines the medium to be used in the determinations will be the curcumin. For curcumin solutions, the stock solution has been dissolved in ethanol and the dilutions in the appropriate hydroalcoholic medium.

7.1.2. Degradation study

These studies are carried out in water as solvent as well as in ethanol and in a mixture of EtOH/H_2O 70:30, this mixture has selected according to the bibliography.

The following graphs (Figure 11) show spectrum measurements of three curcumin solutions over several days. The first one in water (a), where a decrease of absorbance with the time is observed and therefore there is degradation. The second in EtOH (b) and the third in EtOH/H_2O

70:30 mixture (c), where no decrease in absorbance with time is observed, neither of the two indicates degradation.

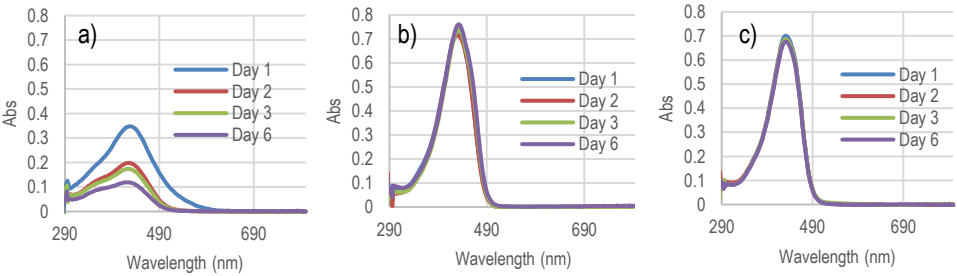

Figure 11. Curcumin degradation in H_2O (a), EtOH (b) and a mixture EtOH/H_2O 70:30 (c)

This non-existent degradation in the presence of ethanol is due to the fact that in its presence, the predominant form of curcumin is the enolic one, which hardly shows degradation by the solvent [16]. According to Gosh S, the curcumin molecule shows maximum stability in ethanol. In water or in a basic solution as a solvent, hydroxyl ions catalyse the degradation by hydrolysing the molecule (Appendix 1).

Next, the photodegradation was studied. Two solutions of curcumin in ethanol 5 mg/L have been prepared from the same stock solution. One of them was exposed to solar radiation and the other was left in the dark. The top graph (a) of figure 12 shows the spectra of the solution left in the dark and the bottom graph (b) shows the spectra of the one exposed to light.

In this study, it can be seen how the vial exposed to solar radiation after 14 days is completely degraded, as it does not show absorbance (b). The one that is left on the dark shows the same absorbance as the first day (a), so it does not degrade.

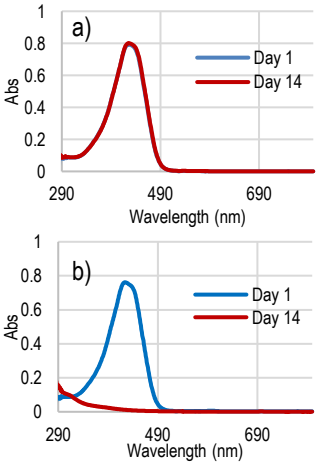

Figure 12. Photodegradation study of E100 in the dark (a) and light exposition (b)

Therefore, when performing this part of the determination, students should avoid exposure to light by leaving the solution to be analysed the next day.

As for erioglaucine, it has not shown degradation in any of the solvents.

Since neither of the two dyes degrades in EtOH/H_2O 70:30 mixture with the time if it is not exposed to light for many days, it was decided to continue studying the behaviour of curcumin in this solvent. Fist of all, a study was done with small variations of the mixture proportions, and secondly a pH variation study.

7.1.3. Study of the absorbance in small variations in the matrix of an EtOH/H_2O 70:30 mixture

This study consisted of making small variations in the proportions of the mixture and observing if they influence the absorbance of curcumin and erioglaucine.

The graphs in Figure 13, show the absorbance of curcumin (a) and erioglaucine (b) in small variations of the EtOH/H_2O 70:30 mixture.

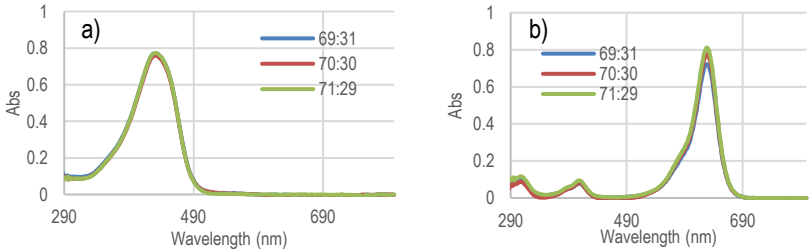

Figure 13. Spectra of E100 (a) and E133 (b) in EtOH/H_2O 69:31, 70:30, 71:29

As can be seen there is no significant variation in absorbance when changing the mixture proportions. Therefore, it is robust medium for the students works with.

7.1.4. Variation of pH study

Next the pH study was performed using EtOH/H_2O 70:30 mixture.

The graphs in Figure 14 show the absorbance of the solutions at different pH of curcumin (on the left) and erioglaucine (on the right).

It can be stated that there is no variation of the absorbance between pH 1 and pH 9 for both colorants. On the other hand, the band at pH 13 are displaced for curcumin, and for erioglaucine it has lost intensity. An hypothesis for curcumin is that, as seen above, hydroxyl ions catalyse its degradation (Appendix 1). Therefore, in a basic solution, this displaced band may be the signal

given by the degradation products. As the intention is to work at neutral pH, it has not been investigated further what happens with erioglaucine.

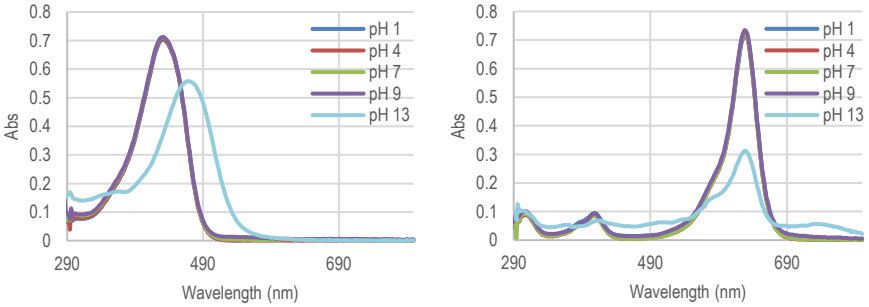

Figure 14. pH study for E100 (on the left) and E133 (on the right) in a mixture of EtOH/H_2O 70:30

A small degradation study with time has been carried out for each solution measured and for both curcumin and erioglaucin the only solution that showed degradation was the one at pH 13.

This pH study was also done using water and ethanol as the only solvents and their results were similar. In the case of the solutions with water all showed degradation.

After these studies, it was decided to extract and determine the yellow, blue and green *m&m's* with a mixture of EtOH/H_2O 70:30. Before taking this decision, it was tried to extract the samples with this mixture, and the colorant was extracted without problem.

7.2. CARMINIC ACID

Next carminic acid was studied. Its solubility, the influence of pH and its stability have been studied.

7.2.1. Solubility study

First, the solubility in water was tested, as it has been stated that the solvent of the extraction must contain water.

Carminic acid was dissolved in water in less than five minutes with agitation, so no other solvents were considered and water was selected as the matrix for the standards and samples extraction.

7.2.2. Influence of pH

Next, due to fact that carminic acid is a triprotic acid which its pK's are pK_1=2.91, pK_2=5.62 and Pk_3=8.3, solutions have been prepared at different pH between their pK's. In order to have the different protonated species.

After preparing the solutions at different pH, a significant colour change is observed between them (Figure 15). In the acidic solutions an orange colour results, in the neural one a reddish colour and in the basic ones a purple colour.

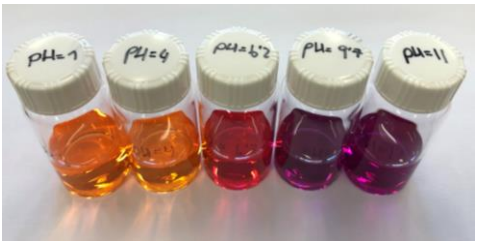

Figure 15. Carminic acid solutions at different pH

When measuring the spectra of the solutions at different pH, as shown in the Figure 16, a large change is observed with respect to the pH variation due to the presence of the different protonated and deprotonated species. It is for this reason that it is considered to use a buffer solution to carry out the determination.

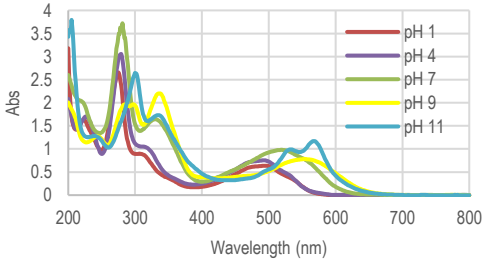

Figure 16. Spectra of carmínic solutions at diferent pH

The buffer solution was a phosphate solution at pH 7.5. This one was choosed because at this pH the carminic acid provides a red solution, and it is more representative of the sample to be analysed, a red *m&m's*.

7.2.3. Degradation study of carminic acid in a phosphate buffer solution at pH 7.5

Then, to study if there is degradation of the carminic acid over several days in the phosphate buffer solution, at pH 7.5, the spectrum of the same solution is recorded for four days.

As no degradation occurs showed by the absence of changes in the absorbance spectra (Figure 17), this buffer solution is considered as the solvent for the carminic acid.

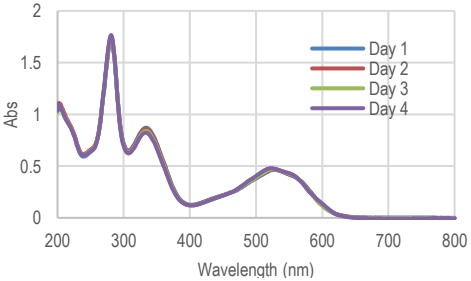

Figure 17. Degradation of carminic acid in a phosphate buffer solution at pH 7.5

7.3. BETA-CAROTENE

The final dye studied was the orange one. The solubility in water, ethanol and dichloromethane of beta-carotene is studied.

An attempt was made to dissolve beta-carotene in water bud, as expected, it did not dissolve.

Due to its chemical structure, beta-carotene is not soluble in water and in ethanol is very poorly soluble. Since it is completely soluble in dichloromethane, the extraction of an orange coloured *m&m's* has been attempted with this solvent. After 20 minutes dichloromethane was still colourless, the orange colorant remained in the *m&m's* and the chocolate started to crack.

Because water is needed for the extraction, the possibility of creating a medium with water and dichloromethane was studied. Because mixing water with dichloromethane results in two phases, various proportions of these solvents with ethanol have been tested in order to achieve a single phase. The appropriate proportions have been found so that the three solvents are mixed and one phase is left: $EtOH/H_2O/CH_2CL_2$ 50:25:25.

The solubility limits have also been studied. It has been concluded that below concentration of 17 mg/L of beta carotene in this medium, the dye does not precipitate.

In the experimental course the students will be able to prepare the intermediate solutions of their dilution trees with the mixture of solvents as long as they do not exceed 17 mg/L. If they do, they will have to make the intermediate dilutions in dichloromethane and the last ones in the mixture. All intermediate dilutions will be made from a stock solution in dichloromethane.

8. LINEARITY INTERVALS

Next, the linearity intervals are stablished. Solutions of each colorant were prepared in each solvent from 0 to 30 mg/L.

After preparing the solutions for each dye, and adquiring the corresponding data (Appendix 2), the following linearity intervals are observed for the dyes analysed.

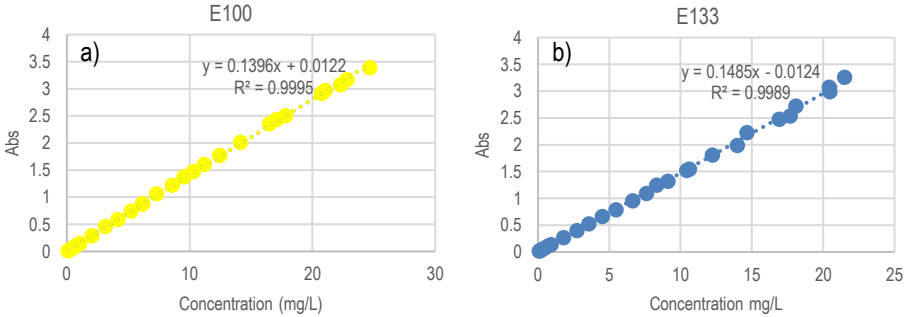

Figure 18. Linearity intervals of curcumin (a) and erioglaucine (b) in a mixture of EtOH/H2O 70:30 as solvent. Curcumin measured at 431 nm and erioglaucine at 628 nm.

It can be stated that there is a linear dependence between the concentration and absorbance of curcumin and erioglaucine between concentrations from 0 to 20 mg/L (Figure 18).

In the case of carminic acid, when measuring the spectrum of some samples, it is observed that the band in the green region, where the standard solutions have a maximum at 550 nm, is unfolded. For this reason, a linearity study is carried out on the two maxima of the unfolded bands and on another band present in the spectrum

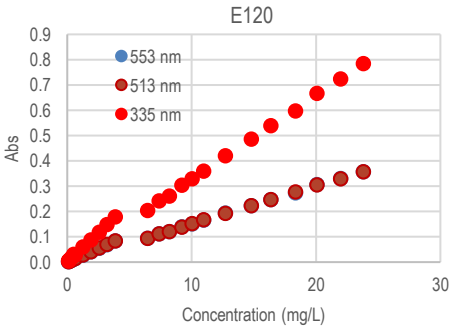

Figure 19. Linearity intervals of carminic acid in a phosphate buffer solution at pH 7.5

(Appendix 2) at 335 nm that do not suffer changes.

As can be seen in Figure 19, there are two linearity intervals, from 0 to 4 mg/L and from 6 to 23 mg/L. it is concluded that data from any of the bands can be used to make the determination, since there is a linear dependence in any of the cases.

Something similar happens in the case of beta-carotene, therefore, the data of all bands are also measured.

It can be stated that between concentrations from 0 to 13 mg/L there is a linear dependence between the concentration and absorbance of beta-carotene (Figure 20).

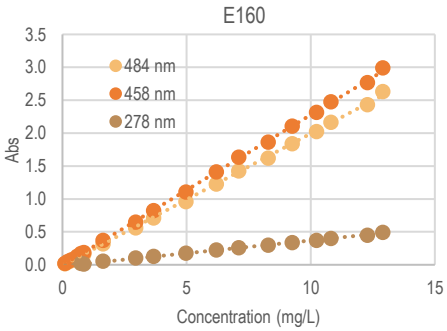

Figure 20. Linearity intervals of beta-carotene in a mixture of EtOH/H_2O/CH_2Cl_2 50:25:25

9. MULTIVARIATE CALIBRATION

In the study of the green samples, where there is a mixture of curcumin and erioglaucine, a multivariate analysis has to be performed. As mentioned in the introduction, the absorbance of this sample at each wavelength is the sum of the absorbance of curcumin plus that of erioglaucine.

As can be seen in the spectrum in Figure 21, there is very little interference between the two signals although erioglaucine absorbs a little in the curcumin region. It will be studied whether it is necessary to do this multivariate analysis or whether the two

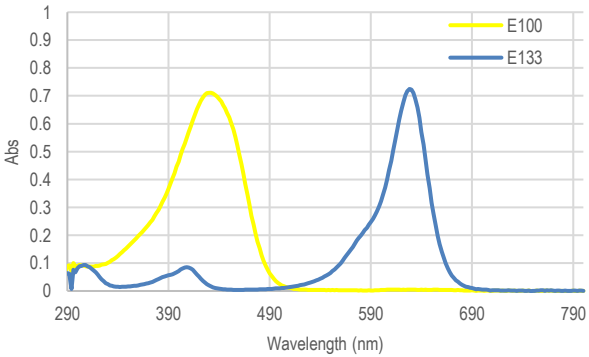

Figure 21. Absorbance spectra of curcumin and erioglaucine in an EtOH/H_2O 70:30 mixture

absorbances could be selectively measured.

Absorbance measures are made at 431 and 628 nm. The maximum of both peaks. In order to solve the following system of equations, first the absorptivity of the two dyes at the two wavelengths has to be found.

$$\begin{cases} A_{431\,nm} = \varepsilon_{E100\,431nm} \cdot b \cdot [E100] + \varepsilon_{E133\,431nm} \cdot b \cdot [E133] \\ A_{628\,nm} = \varepsilon_{E100\,628nm} \cdot b \cdot [E100] + \varepsilon_{E133\,628nm} \cdot b \cdot [E133] \end{cases}$$

To calculate the absorptivity, all the standard solutions of the two dyes at the two wavelengths have been measured. The slope of the obtained linear lines, according to Beer Lambert's law, is the molar absorptivity.

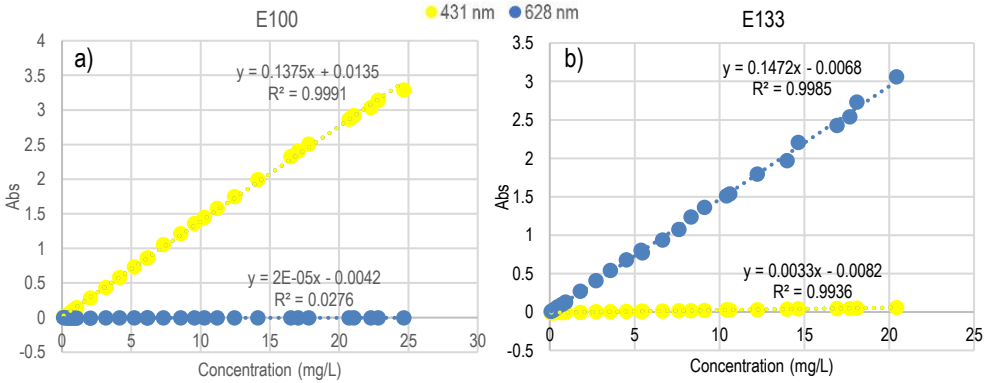

Figure 22. Absorbance of the standard solutions of curcumin and erioglaucine at 431 nm (a) and 628 nm (b)

According to the graphs in the Figure 22, the following absorptivities in Table 1 are obtained:

ε	E100	E133
431 nm	0.1375 L/mg·cm	0.1472 L/mg·cm
628 nm	$2 \cdot 10^{-5}$ L/mg·cm	0.0033 L/mg·cm

Table 1. Absorptivities of circumine and erioglaucine at diferent wavelengths

As expected, the absorptivity of curcumin at 628 nm and that of erioglaucine at 431 nm are practically zero since they do not absorb at these wavelengths. It is therefore questioned whether it would be necessary to do the multivariate analysis, although it is interesting to propose it to the students as it enhances learning and the development of their analytical skills.

To see if there is any difference between using the multivariate calibration or not, the results of the analysis of the green samples of this project will be analysed in both ways and they will be compared in the following section.

10. EXTRACTION AND DETERMINATION OF THE AMOUNT OF COLORANT PRESENT IN THE CANDIES

In this last part, the extraction and determination of the amount of colorant present in the *m&m's* has been done. Twenty *m&m's* of each colour have been analysed. A high number of samples have been determined because there is a variation in the weight and size of the samples, due to the fact that not all candies have the same dimensions.

10.1. EXTRACTION OF THE SAMPLES

First, each dye is extracted in the medium decided above. Since all the solvents contain water, the dyes are easily extracted from the surface layer of the *m&m's*. It is observed that part of the titanium dioxide layer underneath the dye becomes part of the solution and is in suspension. That is why it is decided to filter the solution resulting from the extraction.

When filtering the solutions, the one containing carminic acid was difficult to pass through the filter, due to the viscosity of the phosphate buffer solution. There were no problems with the others. A transparent solution of the colour of the *m&m's* is obtained, as can be seen in Figure 23. As for the red dye, there was a little sample left in the filter.

Figure 23. Colorants extracted and filtered from candies in each solvent

Once all the solutions were prepared, the absorbances were measured and data were analysed.

10.2. DETERMINATION OF THE AMOUNT OF COLORANT

Next, the absorbance was measured for each solution. Those of the yellow *m&m's*, containing curcumin, at a wavelength of 431 nm. Those of the blue ones, with erioglaucine, at

628 nm. Those of the green ones, containing the two previous dyes, were measured at both wavelengths.

For the red and orange samples, the entire spectrum was measured, and the data provided at wavelengths of 553 and 335 nm for the red *m&m's* and 484 and 458 nm for the orange ones were considered.

10.2.1. Yellow, blue, red and orange samples

10.2.1.1. Absorbance at different wavelengths of carminic acid and beta-carotene

As for the results of the red and orange samples, when linearity interval was searched, it was observed that the data from any of the three bands analysed for each colorant could be used, since in no case was linearity lost. But when the samples were analysed, only the information provided from two of the three bands was used.

		Carminic Acid E120			Beta-Carotene E160		
		513 nm	553 nm	335 nm	484 nm	458 nm	278nm
Concentration mg/sample	$\bar{X}$	0.25	0.18	0.16	0.040	0.036	0.19
	s	0.05	0.04	0.03	0.006	0.006	0.04
Concentration mg/g	$\bar{X}$	0.11	0.08	0.07	0.018	0.016	0.09
	s	0.02	0.01	0.01	0.002	0.002	0.02

Table 2. Data obtained from the different absorption bands of the dyes E120 and E160

Because as can be seen in Table 2, in the case of carminic acid the data provided by the band at 513 nm has been omitted, since the result was significantly different from the other two.

In the case of beta-carotene, it has been more evident the need to dispense with the data provided by the band at 278 nm because the spectra showed great irregularities in the absorbance at this wavelength, and it can be seen that the information provided by this band differs greatly from the other two. Therefore, for these two dyes the data provided by the bands not discarded have been averaged for both. The bands at 553 nm for E120 and at 484 nm for E160 are proposed for students because they are the ones that absorb in the characteristic region of the colours.

10.2.1.2. Amount of dye in blue, yellow, red, and orange samples

According to Beer-Lambert's law, the amount of colorant corresponging to the yellow, blue, red and orange *m&m's* has been found. To reach at these results (Table 3), an external standard calibration curve has been used for each colorant.

		Yellow m&m's E100	Blue m&m's E133	Red m&m's E120	Orange m&m's E160
Concentration mg/sample	$\bar{X}$	0.12	0.23	0.17	0.038
	s	0.03	0.05	0.03	0.006
Concentration mg/g	$\bar{X}$	0.05	0.09	0.07	0.017
	s	0.01	0.01	0.01	0.002

Table 3. Amount of colorant expressed in mg/sample and mg/g present in different coloured *m&m's*

Standard deviations of all colorants are higher in the data provided for amount of colorant per sample because not all candies have the same size. In fact there is a great variety in weight and size. A comparative study of the relative standard deviations will be made.

10.2.2. Green samples

As explained above, the green coloured *m&m's* is provided by a mixture of curcumin and erioglaucine. To make the determination of each one, a multivariate calibration has been studied and it has been seen that the absorptivities are very close to zero. For this reason, it is considered whether it is necessary to make this multivariate analysis, or it can be done with the classical method of external standard calibration curve. The amount of dye has been searched in both ways and the following results (Table 4) have been obtained:

		Multivariate calibration		External standard calibration curve	
		E100	**E133**	**E100**	**E133**
Concentration mg/sample	$\bar{X}$	0.05	0.025	0.05	0.023
	s	0.01	0.005	0.01	0.003
Concentration mg/g	$\bar{X}$	0.025	0.013	0.025	0.012
	s	0.003	0.002	0.005	0.002

Table 2. Data obtained using the different calibration methods

As expected and previously deduced, the results for erioglaucine are practically the same using both calibration methods, since curcumin absorbs practically zero at 628 nm due to its absorptivity is $2 \cdot 10^{-5}$ L/mg·cm. On the other hand, the results for curcumine are the same, although erioglaucine does absorb a little at 431 nm because its molar absorptivity is 0.0033 L/mg·cm at this wavelength.

It is concluded that in order to develop the experiment in the subject it would not be necessary to use the multivariate calibration to obtain significative results. Even so, it is interesting to propose it to the students so that they learn how it would be used.

10.3. UNCERTAINTY OF SAMPLES

This proposed activity also explains to students that uncertainty is not only associated with the measurement, but also exists in the samples because they do not all have the same size or the same weight.

Before the extraction, all samples were weighed, and their width and length were measured with a caliper. The following graph shows the relative standard deviation associated with the concentration expressed in terms of dimensions, of unit and of weights.

As Figure 24 shows, the high relative standard deviation is due to the weight of the samples is very varied. What causes this deviation is that the samples do not have the same dimensions and therefore, the amount of dye in each of them is also very different.

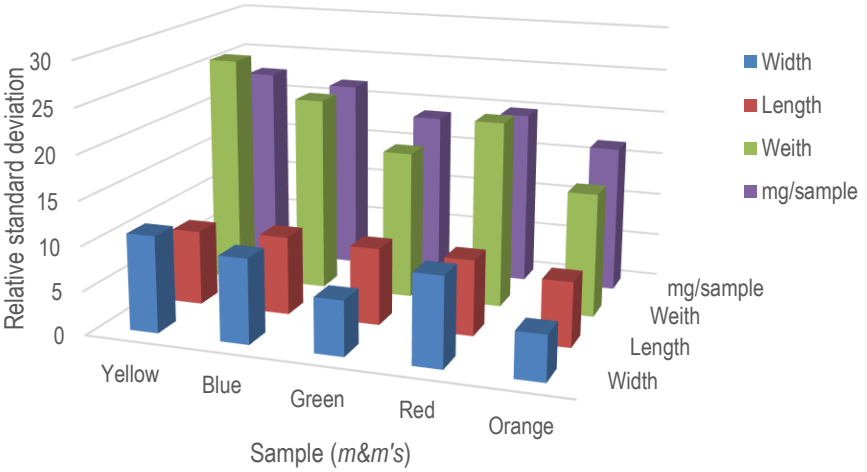

Figure 24. Relative standard deviation of the dimensions, weigth and mg colorant/g sample of the diferent colour *m&m's*

As can be seen, as expected, for all colours, the relative standard deviation is larger for the mg of dye per unit and for the weight, then for the dimensions. This is because even if the samples have similar dimensions, they may have different weights due to their composition.

As mentioned above, this will teach students that uncertainty is not only associated with the measurement, but also exists in the samples.

10.4. ACCEPTABLE DAILY INTAKE (AID)

The Acceptable Daily Intake (AID) determines the amount of colorant that a person can take daily throughout his or her life without harming his or her health. Students will be asked to compare their results with this indicator.

This amount is expressed in mg per kg of body weight per day.

According to European Food Safety Authority (EFSA), the Acceptable Daily Intake for Curcumin is 3 mg/Kg body weight per day [19]. In the case of Brilliant Blue FCF is 6mg/Kg body weight per day [20], and for Carminic Acid is 2.5 mg/Kg bw/day [21]. As for beta-carotene, being a precursor of vitamin A, it does not have an associate AID [22].

A reflection is made on whether the ingestion of the colorants present in the *m&m's* on daily basis is detrimental to health. Considering the results of this experiment in Tables 3 and 4.

For the presence of dye to be harmful to health, a 70 Kg person would have to eat more than 1790 yellow *m&m's*, more than 1826 blue *m&m's*, more than 1029 red ones, and more of 4200 green *m&m's*.

It is concluded that it would be more harmful to ingest so much chocolate and sugar than the presence of the colorants.

Figure 25. Filtered blue and oragne samples

11. CONCLUSIONS

From the degradation studies it can be stated that there will be no significant changes in the absorbance of the standards if the students prepare the solutions one day and do measurements the next day. Although in the case of curcumin it is recommended that they are done in the same day, or that the solutions are stored in a dark place.

The matrix for standards and extractions and determinations of the yellow, blue and green *m&m's* will be made with an EtOH/H_2O 70:30 mixture. Those of the reds with a phosphate buffer solution at pH 7.5, and those of the oranges with a EtOH/H_2O/CH_2Cl_2 50:25:25.

The absorbance of the dyes showed a linear behaviour versus concentration in the following intervals: 0-20 mg/L for curcumin and erioglaucine, 6-23 mg/L for carminic acid, and 0-13 mg/L for beta-carotene.

The measurements have been made at the next wavelengths: 431 nm in the case of curcumin, 628 nm for erioglaucine, 335 and 553 nm for carminic acid, and 458 and 484 nm for beta-carotene.

The amounts of colorant found in each sweet are as follows. 0.05 mg/g in yellow *m&m's*, 0.09 mg/g in the blue ones, 0.07 mg/g in red ones, 0.017 mg/g in orange ones and in green *m&m's* 0.025 mg E100/g and 0.013 mg E133/g. It has been seen that these quantities do not cause health problems.

The determination of the green samples has ben done with multivariate calibration and with an external standard calibration curve. It has been seen that there is practically no difference in the results. Therefore, both could be used.

Throughout the study, it has been kept in mind that a method was being developed for a practical to be carried out in the subject of "*Laboratory of Analytical Chemistry*". Therefore, it has been thought about how to make the extraction and determination of the candies in a simple way for students.

The purpose of developing this practise is that students learn how the UV – Vis Spectrometry technique works. Doing simple analysis and multivariate analysis.

It is remarked that the *m&m's* are motivating samples, and this will cause the students to have a better and more attractive learning.

12. REFERENCES AND NOTES

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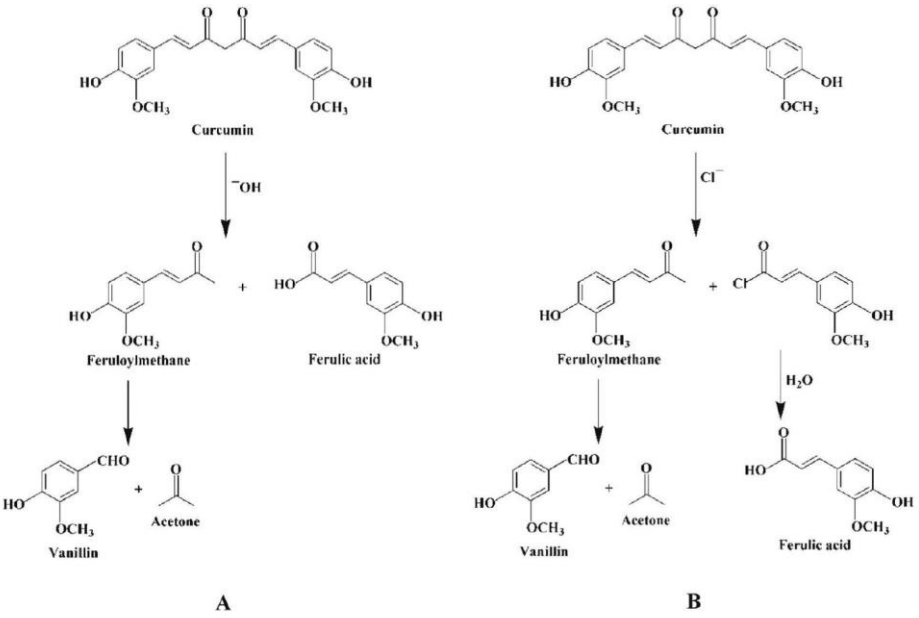
APPENDICES

APPENDIX 1: CURCUMINE DEGRADATION IN AQUEOUS MEDIUM



APPENDIX 2: LINEARITY INTERVAL DATA

Measurements of the absorbance of solutions of different concentrations of curcumin and erioglaucine:

Vial	CURCUMIN		ERIOGLAUCINE	
	Conc (mg/L)	Abs (431 nm)	Conc (mg/L)	Abs (628 nm)
1	0.1112	0.0059	0.0897	0.0128
2	0.2145	0.0255	0.1814	0.0259
3	0.4187	0.0524	0.2750	0.0396
4	0.6792	0.0896	0.4608	0.067
5	0.8503	0.1144	0.7238	0.108
6	1.0786	0.1468	0.9247	0.1338
7	6.1612	0.872	5.4672	0.7831
8	7.3352	1.0633	6.6415	0.9514
9	8.5882	1.2243	7.6193	1.0892
10	9.5718	1.3744	8.3327	1.2427
11	10.2951	1.4693	9.1108	1.3195
12	11.2044	1.6025	10.4294	1.5169
13	12.4656	1.7714	10.6177	1.5453
14	14.1446	2.0141	12.2229	1.8082
15	16.5157	2.3545	13.9930	1.9855
16	17.8221	2.5054	16.9228	2.477
17	20.7385	2.9176	17.6856	2.5352
18	22.3080	3.0772	20.4599	2.9899
19	24.6759	3.3897	21.5057	3.2563
20	2.0685	0.2873	1.8042	0.2652
21	3.1615	0.4614	2.7295	0.3994
22	4.1658	0.5892	3.5750	0.5221
23	5.2361	0.7439	4.5088	0.6593
24	6.2028	0.8812	18.0972	2.7209
25	21.0644	2.9663	20.4308	3.0737
26	22.8224	3.1699	14.6541	2.225
27	17.0622	2.4327		

Table 3

Measurements of the absorbance spectra of solutions of different concentrations of carminic acid and beta carotene:

	CARMINIC ACID				BETA-CAROTENE			
Vial	**Conc (mg/L)**	**Abs (553 nm)**	**Abs (513 nm)**	**Abs (335 nm)**	**Conc (mg/L)**	**Abs (484 nm)**	**Abs (458 nm)**	**Abs (278 nm)**
1	0.1135	0.00166	0.00169	0.00548	0.1021	0.01715	0.02061	-0.02171
2	0.1329	0.00301	0.00298	0.00546	0.2105	0.04222	0.04995	-0.01589
3	0.2635	0.00613	0.00621	0.01290	0.3571	0.06444	0.07592	-0.01252
4	0.3915	0.00869	0.00888	0.01942	0.5851	0.11113	0.13006	-0.00011
5	0.5226	0.01222	0.01270	0.03123	0.8589	0.16125	0.18809	0.01373
6	0.6486	0.01388	0.01383	0.02839	0.7437	0.14724	0.17081	0.02201
7	1.2853	0.02714	0.02776	0.05976	1.6339	0.31804	0.36885	0.05422
8	1.9242	0.04098	0.04146	0.08742	2.9445	0.55941	0.64779	0.10291
9	2.5752	0.05487	0.05582	0.11804	3.6715	0.71196	0.82182	0.12666
10	3.2131	0.06902	0.06967	0.14852	4.9677	0.96113	1.10698	0.17431
11	3.8698	0.08359	0.08457	0.17937	6.2050	1.22727	1.41122	0.22733
12	6.4726	0.09433	0.09476	0.20360	7.1044	1.42504	1.63507	0.26079
13	7.4066	0.11182	0.11201	0.24203	8.2935	1.62251	1.86223	0.29717
14	8.2354	0.12075	0.12026	0.26073	9.2561	1.84004	2.10888	0.33843
15	9.2157	0.13947	0.13878	0.30408	10.8086	2.16288	2.47614	0.40004
16	10.0488	0.15155	0.15232	0.32896	12.2845	2.43036	2.76583	0.45000
17	10.9865	0.16595	0.16699	0.35927	10.2314	2.02228	2.31363	0.37246
18	12.7183	0.19360	0.19326	0.41997	12.9031	2.63177	2.98915	0.49031
19	14.8017	0.22277	0.22272	0.48608				
20	16.3890	0.24679	0.24681	0.53873				
21	18.3519	0.27424	0.27701	0.59728				
22	20.0970	0.30635	0.30567	0.66760				
23	22.0040	0.33056	0.32982	0.72423				
24	23.8306	0.35775	0.35700	0.78467				

Table 4

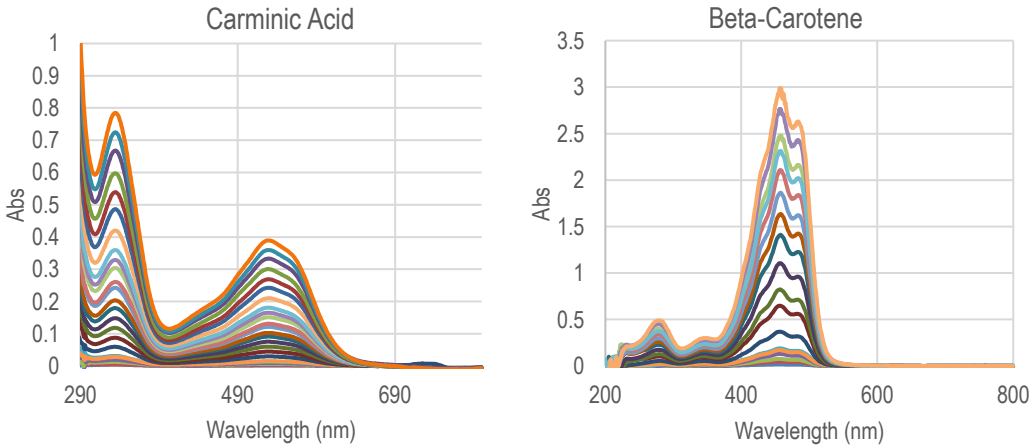


Figure 26. Spectra of solutions at different concentrations to make the linearity intervals