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PORTO

DEVELOPMENT OF A SEQUENTIAL INJECTION SYSTEM FOR THE ASSESSMENT OF POTASSIUM AND COLOR IN ALCOHOLIC BEVERAGES

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Thesis presented to Escola Superior de Biotecnologia of the Universidade Católica Portuguesa to fulfill the requirements of Master of Science degree in Biotechnology and Innovation

Maria João Morais Nunes (361417005)

Supervision: Dr. Raquel Mesquita and Prof. António Rangel

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Resumo

Nos últimos séculos, as bebidas derivadas da fermentação alcoólica, nomeadamente a cerveja e o vinho, tiveram grande importância, tendo em conta a crescente taxa de consumo, resultando numa necessidade de produção em larga escala. Deste modo, torna-se essencial desenvolver metodologias capazes de medir mais do que um parâmetro em simultâneo, para facilitar o trabalho de monitorização da qualidade na produção em linha.

Neste trabalho foi desenvolvido um sistema biparamétrico para a determinação do teor de potássio e da cor em vinhos e cervejas. Deste modo, ao longo do estudo foram realizados vários processos de desenvolvimento e otimização dos métodos.

A montagem final do sistema de análise sequencial (SIA) é composta por uma válvula de seleção de 10 portas ligada às diferentes soluções e detetores utilizados. Numa das portas colocou-se a ligação à amostra/padrão, noutra porta a ligação ao potenciómetro, para a determinação potenciométrica de potássio, e noutra porta a ligação ao espetrofotómetro, para a determinação da cor. Para facilitar a mudança de amostra/padrão havia também uma porta com ligação a um esgoto, onde se descartava o excesso. O método desenvolvido permitiu a determinação de potássio entre as concentrações de 1.08×10^{-1} M e 1.08×10^{-3} M, com limite de deteção e quantificação de 3.90×10^{-5} M e 1.62×10^{-4} M, respetivamente. Permitiu também a determinação da cor das variadas amostras.

Para a determinação de potássio, os valores obtidos pelo sistema potenciométrico foram comparados com os valores obtidos por fotometria de emissão de chama, e não se verificou diferença estatística significativa (<10%). A curva de calibração referente à determinação de potássio mostrou-se estável ao longo do tempo (mensal), já que as respetivas curvas de calibração foram consideradas idênticas dentro da variação estatística. A metodologia para determinar a cor foi validada por comparação direta dos valores obtidos com os referentes ao método de referência. Conclui-se que o sistema pode ser aplicado a amostras de cerveja e vinho em contexto industrial com o objetivo de facilitar as medições simultâneas dos dois parâmetros, gastando um menor volume e com menos trabalho do operador, facilitando a execução de outras tarefas.

Palavras – chave: sistema de análise por injeção sequencial (SIA); potássio; cor; vinho; cerveja

Abstract

In the last centuries, beverages derived from alcoholic fermentation, namely beer and wine, have been of great importance, given the increasing rate of consumption, resulting in a need for large-scale production. Thus, it becomes essential to develop methodologies capable of measuring more than one parameter simultaneously, to facilitate the work of quality monitoring in line production.

In this work, a biparametric system was developed for the determination of potassium content and color in wines and beers. Thus, several method development and optimization processes were carried out throughout the study.

The final assembly of the sequential analysis system (SIA) consists of a 10-port selection valve connected to the different solutions and detectors used. In one port was placed the connection to the sample/standard, in another port the connection to the potentiometer, for the potentiometric determination of potassium, and in another port the connection to the spectrophotometer, for the determination of color. To facilitate the switch of sample/standard there was also a port with a connection to a waste, where the excess was discarded. The developed method allowed the determination of potassium between the concentrations of 1.08×10^{-1} M and 1.08×10^{-3} M, with detection and quantification limits of 3.90×10^{-5} M and 1.62×10^{-4} M, respectively. This system also allowed the color determination for each sample.

For the potassium determination, the values obtained by the potentiometric system were compared with the values obtained by flame emission photometry, and there was no statistically significant difference (<10%). The potassium calibration curve proved to be stable over time (monthly), as the respective calibration curves were found to be identical within statistical variation. The methodology for determining color was validated by comparing the results with the reference method. It is concluded that the system can be applied to beer and wine samples in an industrial context with the aim of facilitating simultaneous measurements of the two parameters, using less volume and with less operator work, making it easier to perform other tasks.

Keywords: sequential analysis system (SIA); potassium; color; wine; beer

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Contents

1. Introduction

Alcoholic beverages are classified as fermented drinks, such as beer and wine, that contain ethanol and normally are produced in a large scale. These drinks are the result from sugar fermentation, present in grains, berries, and other ingredients (1), and are mostly composed by water and ethanol. Both beer and wine are beverages studied all over the globe in different parameters, namely chemical, physiochemical, and sensorial evaluation.

According to the Portuguese legislation (2), beer is a drink obtained by alcoholic fermentation, conducted by yeasts (*Saccharomyces* spp.), of a wort prepared from cereal malt, mainly barley and other starchy or sugary raw materials, to which hop flowers and potable water have been added. This alcoholic drink, as claimed by Food and Agriculture Organization, 'is an ancient beverage' (3), that is dated back more than 5 thousand years, in Mesopotamia. Although it is an old drink, the methods of production were improved for decades, but the ingredients never change. The main difference is their concentration or when they are added in the process of beer production, leading to different flavors, after tastes and colors (4). Beer can also vary depending on if occurs a top or bottom fermentation, leading to a different type of beer and resulting in a higher or lower percentage of alcohol.

Wine, as well as the beer, results from an alcoholic fermentation, from grapes or its must (5). It can have different composition, that result in colors from white to dark red, and can also vary according to their age. Studies show that wine is dated about 4 thousand years ago and since then, the methods for wine production have been improved, as well as the specific regions and strict current legislation (6). It is known that the grapes are an important part of the process, so they must be grown under strict climatic conditions to achieve the perfect sugar concentration, leading to a complete fermentation rate.

Concerning both alcoholic drinks, researchers have been working towards the development of methods/devices that measures simultaneously some parameters like CO_2, total acidity, and pH, but no studies were found that prove the existence of an apparatus that can measure potassium and color at the same time. The industry looks for equipment to facilitate some procedures namely to reduce the number of workers and the time they spend doing the measurements, leading to a quicker, less expensive, and easy way to do it. This way, the systems based on Sequential Injection Analysis can potentially measure two parameters simultaneously, using less volume of sample, less reagent and less manual labor. Also, as a benefit, the worker could leave the system running while performing other tasks. Therefore, this work proposed to contribute to achieve that objective.

1.1 The importance of potassium on beer and wine

Potassium is a chemical element essential for life and, according to Encyclopedia Britannica (7), it "is the seventh most abundant element in Earth's crust". As a cation, K^+, is very important for enzyme activation, for example on the regular dairy diet of a person, it is necessary to ingest 3.3 g of potassium, namely on a wide range of food like vegetables, fruit, meat, and some drinks (beer or wine). It can also affect the body balance and is used on intravenous fluids to rehydration, nutrition, or replacement of electrolytes.

1.1.1. Potassium in beer

It is known that beer is one of the most consumed alcoholic drinks in the world and, as potassium is present on water, it will be as well in beer. The concentration of this cation in beer can vary according to the region where it is produced, or depending on the malt used, since it also contributes to a large percentage of the total potassium (8). The cation can be measured by two different standard methods, namely flame emission photometry and atomic absorption (9). The latter can detect very low concentrations, which is not the case, since studies have shown that beer can present a large amount of potassium, with approximately 52 mg per 100 mL of drink (corresponding to 13.3 mM) (8,10), and therefore, flame emission photometry will be the standard method used in this work.

1.1.2. Potassium in wine

In wine, potassium has an important role for the determination of pH and can consequently alter the beverage stability. It reacts with tartaric acid and forms potassium tartrate (precipitate), that is responsible to the drop of acidity and pH rise, making the juice acidification difficult (11). Higher pH results in less color and less stability, causing a premature browning over time, that is directly related to the chromatic characteristics. According to OIV - Compendium of International Methods of Wine and Must Analysis (5) – potassium in wines is measured with flame emission photometry as standard method, by establishing a calibration curve with known potassium concentrations. The potassium concentration can vary, depending on if the wine is sweet, dried or sparkling, displaying an amount between 51 mg to 136 mg per 100 ml of drink (13.0 mM to 34.8 mM) (10).

1.2. The importance of color on beer and wine

Color is the most important sensory property in food and beverages industry because it gives the consumer a first impression about freshness, quality, and flavor of determined product. Food's color makes the consumer create a perception about the taste, leading them to buy (or not) the product. Also, every color is related to its composition (12).

1.2.1. Color of beer

The beer color is an important parameter because it is the first characteristic that the consumer observes. The final color is obtained by the combination of all the ingredients used during the brewing process, as well as the oxidation of some existent compounds (13,14). The color is measured frequently according the specific EBC (European Brewery Convention) scale (Figure 1), using a spectrophotometer as standard method that measures the absorbance at 430 nm through a 1 cm cuvette full of beer (9). Depending on the type of beer, the EBC scale reaches different values, resulting in a higher EBC for dark beers, and the main formula to obtain the right value is the following.

$$EBC = A\ (\lambda=430nm) \times Dilution \times 25$$

According to EBC (9), depending on the absorbance values, that must be read until 0.8, the sample can be previously diluted to be read on the spectrophotometer, using a cuvette with an optical path (b) equal to 1 cm. The dilution is always used to calculate the final EBC scale, as the formula above shows.

Figure 1 – EBC scale for color determination on beer, according to its intensity (adapted from (9,15)).

1.2.2. Color of wine

Like in beer, color is the first thing the consumer sees when enjoying a glass of wine. The color depends mostly on the berry and by the color, producers can determine stability and longevity of wine. According to a study reported on International Journal of Wine Research (16), color is a very important attribute that requires constant care from viticulturists and winemakers. Ageing also has a big impact on chromatic characteristics, composed by luminosity and chromaticity, and these parameters can be measured by spectrophotometry, which is the standard method used on OIV (5). For rose and red wines, absorbance is measured at three different wavelengths, namely λ = 420 nm, λ = 520 nm and λ = 620 nm. The chromatic characteristics are described by the intensity of color, that can be obtained with the sum of absorbance values at these three wavelengths.

$$\text{Color Intensity (I)} = \frac{Abs\ (\lambda=420nm)}{b} + \frac{Abs\ (\lambda=520nm)}{b} + \frac{Abs\ (\lambda=620nm)}{b}$$

Depending on the absorbance values, that must be read until 0.8, glass cells with different optical paths (b = 0.1 cm or b = 1 cm) can be used, so that the wine is not diluted. The final absorbance of all red, white, and rose wine's value must be divided by the b value, according to the method described on OIV. Some studies show that the chromatic characteristics from white wines can be measured simply by an individual wavelength, λ = 420 nm, that is used as brown indicator (5,17). White wines, as time passes, becomes browner due to oxidation, that can happen to opened or corked bottles.

1.3. Flow analysis

Flow analysis is classified as a method that performs analytical chemistry in flowing streams, that has a lot of benefits, namely the reproducibility, the close environment where the solution flows, and the low reagent consumption, as well as improved precision, less waste and a better selectivity (18,19) These analytical techniques date around the 1950s, starting with segmented flow analysis (18), followed by flow injection analysis (20) and sequential injection analysis (19), among others.

In flow analysis techniques, the solution is propelled by a piston or peristaltic pump, and with innovation, it is now possible to control the flow rate, acceleration, suction, or discharge pumping. This process allows the mixing of both reagent and standard or sample towards the flow-through detector, evolving a wide variety of procedures that include the addiction of multiple reagents (21,22).

1.3.1. Flow injection analysis

Flow Injection Analysis (23) is a method for automating a chemical analysis (24) in which a sample is injected into a flowing carrier solution. This solution mixes with the reagent (if it is the case) and reaches the detector, that can vary according to the use. According to J. Ruzicka and E. H. Hansen (25), this method “is based on injection of a liquid sample into a moving unsegmented continuous stream of suitable liquid. The injected sample forms a zone, which is then transported towards a detector that continuously records the absorbance, electrode potential, or any other physical parameter”. This system has a wide vary of uses, especially on water samples and environmental monitoring, resulting in some advantages, namely rapid response and simple standardization (26). This can also have a simple configuration, controlled dispersion, and a fast response time, due to the simplicity of the equipment, as well as being flexible and low cost.

The system is composed by propulsion unit, with a peristaltic pump that has two different channels propelling solutions, namely the carrier and the reagent. The injection valve is the next unit and allows the injection of the sample/standard through the continuously flowing carrier. Finally, the detection unit, that can be composed by a spectrophotometer, a potentiometer, or another specific detection system, depending on the parameter to measure. The Figure 2 above, shows the system configuration, and the Figure 3 shows the representation of the confluence zone.

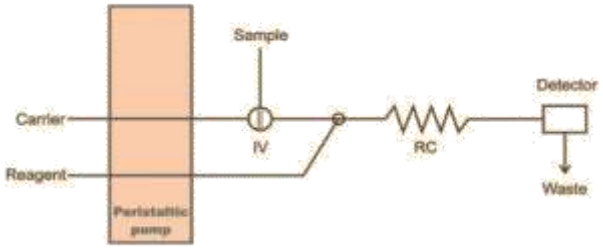


Figure 2 – Flow Injection Analysis System schematics (adapted from (26)).

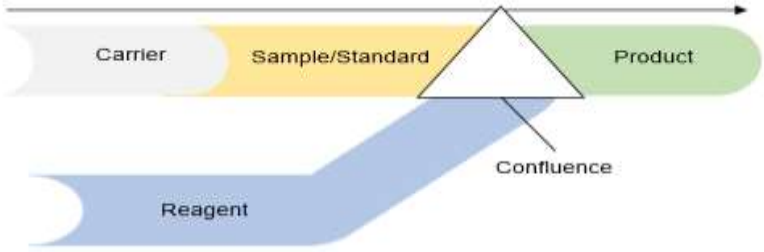


Figure 3 – Flow Injection Analysis schematic representation of the confluence zone.

The following figure (Figure 4) shows the injection, composed by two positions, namely load, where the worker fills the intended volume on the loop, and “inject”, where the valve turns right and changes its inside configuration, so the sample/standard that previously was in the loop, can now be transported by the carrier until the detector.

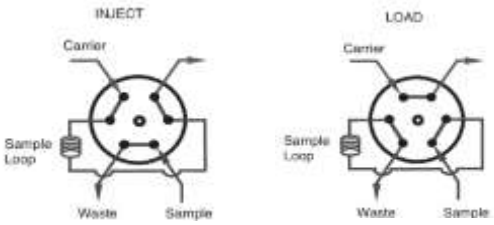


Figure 4 – Rotation of the injection valve employed on FIA system (adapted from (27)).

1.3.2. Sequential Injection analysis

SIA, Sequential Injection Analysis, is also a method for automating a chemical analysis method that comes from an evolution of FIA, with some alterations. The basic principles are the same, namely a “injection of well-defined segments of samples and reagents, which disperse and penetrate, and consequently, the reaction products are formed in well-defined areas of concentration gradient and the transitory signals generated provide reproducible analytical results” (28). The FIA system requires a different manifold for each analytical method, which is a big disadvantage when there is the urge to analyze different parameters. Also, the peristaltic pump need a relatively high maintenance, leading to a challenge to develop a better system, simpler and automated, SIA. According to Lenehan, “SIA has rapidly established itself as a powerful and versatile flow-based sample-handling method as the instrumental setup facilitates the use of different chemistries without the need to reconfigure the manifold”. This system can incorporate a variety of detection methods and can has many different analytical applications, due to the capacity to pump the flow both forward and reverse (29).

The system was also composed by a propulsion unit with a peristaltic pump, a multi-position selection valve, that selects the sample/reagent to be sequentially aspirated, generating zones along the holding coil. As the computer predefined time passes through, the flow is reversed and the valve switches, leading the solution towards the detector (Figure 5). These zones are composed by the reagent and/or sample aspirated, as well as the carrier, that mix along the reaction coil (RC), allowing the monitoring of the solution. The final unit is composed by a detection system, and the final Figure (Figure 6) shows the schematics of the SIA system.

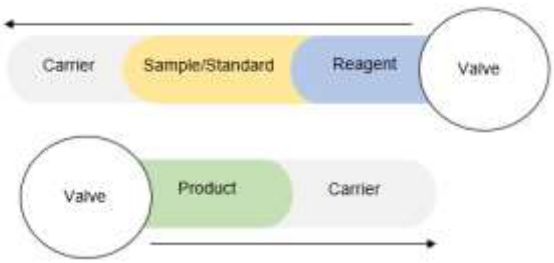


Figure 5 – SIA schematic representation of the aspiration and send of the system components.

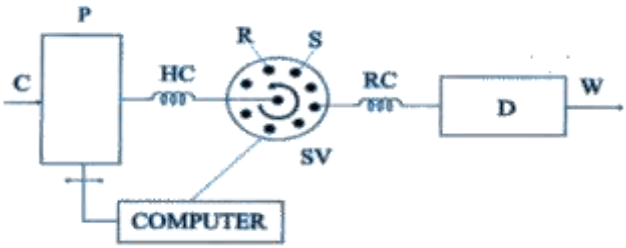


Figure 6 – Sequential Injection Analysis System schematics (adapted from (29)).

1.4. Objectives

The focus of this work was to develop a new SIA system that would be capable of quantifying both potassium and color intensity in the same manifold, in craft beer and wines. The main idea was for this system to be potentially used in the beer and wine industry to facilitate the process of measuring these three parameters.

2. Materials and methods

2.1 Reagents and Solutions

The solutions used in this work were all prepared with analytical grade chemicals and boiled deionized water (resistivity < 0.1 mS/cm).

The standard stock solution of potassium chloride (Merck) was prepared monthly by dissolving 0.804 g of the solid in 100 mL of water. The following standards were prepared by diluting the previously mentioned solution, obtaining concentrations between 5.40 x 10^{-4} M to 5.40 x 10^{-2} M. It was also necessary to prepare an ISA solution of sodium chloride every 3 weeks by adding 29.2 g of the solid in 1 L of water and 1 mL of the 1.08x10^{-3} M solution, obtaining a final concentration of NaCl 0.5 M.

2.2 Sequential injection system manifold

The manifold of the developed sequential injection method is depicted in Figure 7.

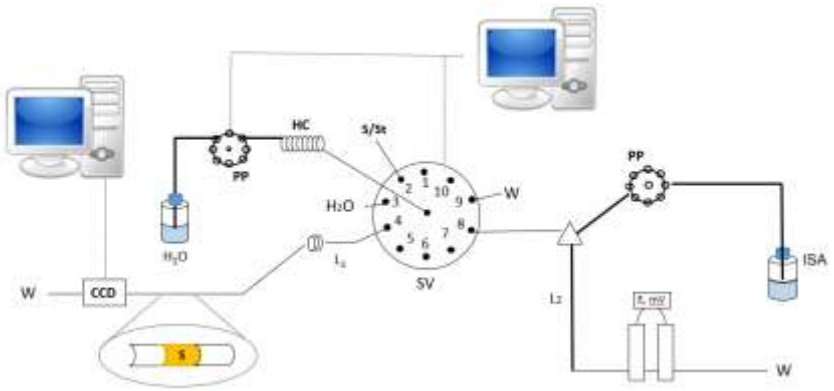


Figure 7 – Sequential injection manifold for the determination of potassium and color; SV, selection valve; PP, peristaltic pump; HC, holding coil, 200 cm; S/St, sample/potassium standard; L_1, reaction coil 22 cm; L2, reaction coil 9 cm; CCD, detector (λ= 430 nm for beer and 420, 520, 620 nm for wines); ISA, ionic solution adjustor, NaCl 0.5 M; Δ, confluence; E, mV, selective electrode for potassium determination; W, waste.

The solutions were propelled with a peristaltic pump (PP) Gilson Minipuls 3 equipped with a PVC pumping tube (1.02 mm Gilson PhthalateFree PVC white/white Lot IW018183) connected to the central channel of a ten-port electrically selection valve (SV – Valco VICI Cheminert C25-3180EUHB). The selection valve (SV) position and the peristaltic pump direction and speed were computer controlled (HP Pavilion zt3000 equipped with a National Instruments DAQcard-DI0 interface card) running a homemade software. An Ocean Optics USB2000 device (CCD) was used as the detection system for color measurement, and the flow cell (Hellma, 178.711 – QS) has an inner volume of 30 µL and a light path of 10 mm. Data acquisition signal was obtained at 430 nm to beer's color determination and 420 nm, 520 nm and 620 nm for wine's color determination and it was performed through the OceanOptics – Spectrasuite software running in a university's computer (HP L1706).

For potassium determination, a Thermo Scientific reference electrode, along with a potassium selective electrode (Ref. 9661K) were used, equipped with a Crison pH-meter GPL21, where the results were directly read by the signal shown on the screen.

All tubing connecting the different components of the flow system were made of PTFE (Omnifit) with 0.8 mm i.d.

2.3 Sequential Injection procedure

After, in preliminary studies, setting both potassium and color system separately and optimizing all the parameters, the overall system was assembled to display a biparametric system that includes potassium and color measurement in the same program. This way. it takes less time to analyze the sample, it is only necessary to control the selection salve position and the peristaltic pump direction and speed (Figure 5), leading to a lower percentage of associated errors.

Table 1 – Protocol sequence for the biparametric determination of color and potassium in beer and wine.

Analyte	Step	SV position	Time (s)	Flow rate	Volume (µL)	Description
Potassium	A	2	4	40	226	Aspiration of sample/standard
	B	8	40	40	2277	Propelling to detector, signal registration
Color	C	2	14/4[a]	40/10[a]	796/57[a]	Aspiration of light beer, white and rose wine/ Aspiration of dark beer and red wine
	D	3	13[b] /10[c]	40	739[b] /568[c]	Aspiration of water after aspirating dark beer/ Aspiration of water, after aspirating red wine
	E	4	40	40	2277	Propelling to detector, signal registration

[a] Combination used for the aspiration of dark beer and red wine

[b] After the step C, it was necessary to aspirate water after the dark beer aspiration to obtain a more accurate peak

[c] After the step C, it was necessary to aspirate water after the red wine aspiration to obtain a more accurate peak

The determination of potassium began with the sequential aspiration of sample/standard (step A) and then it was sent to the detector (step B). The color determination started with the aspiration of the sample, depending on if the color had an absorbance lower than 0,8 (step C) or higher than 0.8, marked with "*", followed by the water aspiration for both dark beer and red wine, respectively (step D), and it was sent to the detector (step E).

2.4 Beverage Samples

The work conducted in the present thesis had beer and wine as samples.

2.4.1 Beer samples

The beer samples, mainly craft beers, used in this work were bought in a supermarket and chosen to obtain different brands (Nortada, SuperBock craft, Cristal, Trappe, Delirium and Sovina). All the samples were used directly, as fresh samples, or stored in the fridge at 4ºC (cold samples), closed with parafilm to prevent spoilage. For the reference methods, the pale beers were used directly, and the darkest ones were previously diluted.

2.4.2 Wine Samples

The wine samples were table and sparkling wines. All the samples were used directly, as fresh samples, or stored in the fridge at 4ºC (cold samples) and then allowed to attained room temperature before using. For the reference methods, all the samples were used directly.

2.5 Reference Procedures

In order to check the accuracy of the SIA measurements and to validate the developed methods for potassium and color determination, a comparison was made between the measurements obtained in the SIA system and flame emission photometry (for potassium determination) and the spectrophotometer (for color determination), described in both Compendium of International Methods of Wine and Must Analysis - OIV and EBC Analytica (5,9).

3. Results and discussion

3.1 Preliminary studies

The preliminary studies began with the potassium determination on beer. For this, some optimizations needed to be made. Then, the beer treatment for the color determination was optimized.

3.1.1 Choice of the electrode with better characteristics

Initially, six different available electrode units were available in the laboratory: one was commercial, and the others were laboratory-made. To check which one was in better condition, the different standards were run in the FIA system and six calibration curves were obtained. if the electrode behaves according to the modified Nernst equation, the following equation (T = 25ºC) applies:

$$E\ (mV) = k + \frac{59,1}{z}\log\ [K^+], \text{ if the ionic strength is kept constant}$$

The ionic strength was adjusted with sodium chloride (ISA), as detailed in section 2.1. As we can see in Figure 8, there were two electrodes where the slope was within the expectable range of the electrode, namely between 54 and 60 mV (30). Also, it is important to consider the theorical slope (in mV), given by the Nernst equation, where the slope is $\frac{59,1}{z}$, and, as z corresponds to the ion's charge (+1), the final slope must be approximately 59.1 (31).

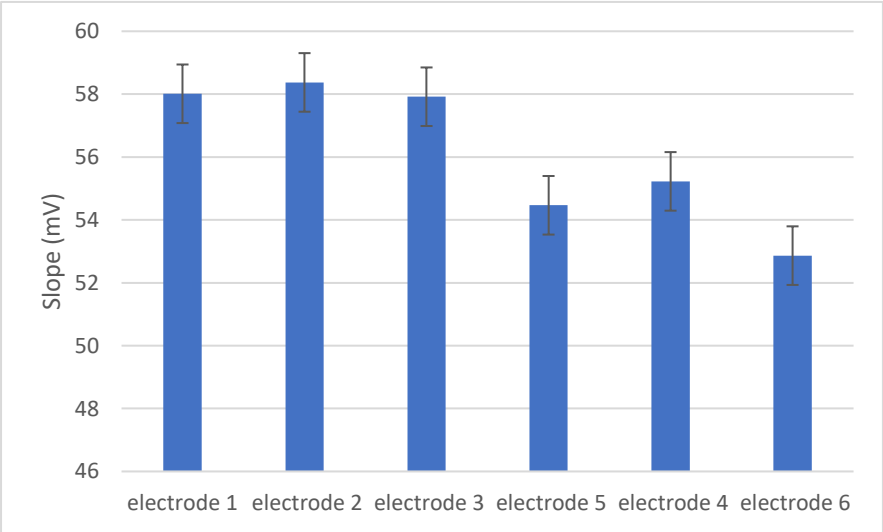


Figure 8 – Different calibration curves for potassium determination obtained with the six electrodes (the error bars correspond to the standard error).

After analyzing the Figure 8, the conclusion was that the three better electrodes are numbers 1 to 3, because they had a slope very similar to the desired one. Regarding the characteristics of these electrodes, it was possible to tell that the electrode 2 was selected as the one that will be used for the rest of the potassium measurements.

3.1.2 Determination of the flow rates for the SIA system

It was necessary to calibrate the pump tubes. For this, the program was set up to send water at different times and velocities, and the final flow rate for each velocity was calculated according to the different obtained slopes. The next table (Table 2) shows the practical flow rates, which will be used to calculate the wanted volumes.

Table 2 – Sequential injection flow rates for the different velocities.

Rotation (arbitrary units)	Flow rate (µL/s)	Intercept
10	57.0	-2
20	44.7	-5
30	30.2	-6
40	14.4	+0

3.1.3 Choice of the pretreatment of beers

The pretreatment normally done in beer industry before the analysis is adding silica dioxide (SiO_2) to a filter and filtering the sample, with the objective of reducing the amount of CO_2, that could cause interferences on the detector (spectrophotometer) due to bubble formation. A wide variety of analyses were made, so it became necessary to look for a new procedure that could reduce the CO_2 amount on freshly open bottles without spending SiO_2. This study was conducted when the SIA system for color determination was being optimized.

In the first place, a polyester filter (Cromafil Pet 45/25) with a pore size of 0.45 µm was used to substitute the treatment used. For this, the sample was inserted in a 6 mL syringe, that pumps it through the filter. As it was noted that only one filtration was not enough, two filtrations were made, to reduce the amount of CO_2 so it doesn't interfere with the detector's readings. As the Table 3 shows, the results, comparing with the reference method, had a similar value of absorbance. When the EBC values were compared, a curve was obtained with the following equation, proving that the values obtained with the SIA system and with the reference method (RM) are not statistically different.

$$EBC_{SIA} = 0.876\ (+/-\ 0.128)\ EBC_{RM} + 0.810\ (+/-\ 1.693)$$

For the following studies, it was necessary to highlight the main formula used, that were mentioned on the introduction, namely the EBC value for beers (9).

$$EBC = A\ (\lambda=430nm) \times Dilution \times 25$$

Table 3 – Comparison of the European Brewery Convention value (EBC) obtained with the conventional spectrophotometer (RM) and with the SIA system (SIA) by calculating the relative deviation percentage (RD); the absorbance value (A) used for the EBC calculation was register at $\lambda = 430\ nm$).

Beer sample	RM		SIA		RD
	A	EBC	A	EBC	
1	0.449	11.2	0.406	10.3	-8%
2	0.608	15.2	0.552	13.8	-9%
3	0.718	17.9	0.668	16.7	-7%
4	0.466	11.6	0.434	10.9	-6%
5	0.429	10.7	0.416	10.4	-3%
6	0.441	11.0	0.428	10.7	-3%

Then, it was decided that the double filtration was not an easy procedure, since the final sample volume was small, around 4 mL. Therefore, instead of filtering twice, 10 min of ultrasounds were tried on, followed by a single filtration with the polyester filter, since more volume could be obtained, about 5 mL. As the results show (Table 4), when comparing to the previous double filtration, the deviation was not significant, so it was considered that the method works. When the EBC values were compared, a curve was obtained with the following equation, proving that the values obtained with the SIA system and with the reference method (RM) are not statistically different.

$$EBC_{SIA} = 0.931\ (+/-\ 0.207)\ EBC_{RM} + 1.128\ (+/-\ 2.739)$$

Table 4 – Comparison of the European Brewery Convention value (EBC) obtained with the conventional spectrophotometer (RM) and with the SIA system (SIA) by calculating the relative deviation percentage (RD); the absorbance value (A) used for the EBC calculation was register at $\lambda = 430\ nm$).

Beer sample	RM		SIA		RD
	A	EBC	A	EBC	
1	0.449	11.2	0.458	11.4	2%
2	0.608	15.2	0.643	16.1	6%
3	0.718	17.9	0.691	17.3	-3%
4	0.466	11.6	0.471	11.8	2%
5	0.429	10.7	0.442	11.0	3%
6	0.441	11.0	0.455	11.4	4%

Lately, since this procedure was still long and a small volume was obtained, it was decided to try to agitate, comparing with the method used before. The final objective was to have only one type of

treatment, namely the agitation for a determined time, that will also be studied. For this matter, there was an initial substitution of the ultrasounds for the agitation, (Table 5), and then the retreat of the filtration with polyester filter. When the EBC values were compared, a curve was obtained with the following equation, proving that the values obtained with the SIA system and with the reference method (RM) are not statistically different.

$$EBC_{SIA} = 1.221\ (+/-\ 0.247)\ EBC_{RM} - 2.175\ (+/-\ 2.961)$$

Table 5 – Comparison of the European Brewery Convention value (EBC) obtained with the conventional spectrophotometer (RM) and with the SIA system (SIA) by calculating the relative deviation percentage (RD); the absorbance value (A) used for the EBC calculation was register at $\lambda = 430\ nm$)

Beer sample	**RM**		**SIA**		**RD**
	A	EBC	A	EBC	
1	0.413	10.3	0.446	11.3	9%
2	0.560	14.0	0.611	15.3	9%
3	0.669	16.7	0.723	18.1	8%
4	0.422	10.5	0.435	10.9	4%
5	0.453	11.3	0.424	10.6	-6%
6	0.442	11.0	0.444	11.1	1%
7	0.328	8.20	0.305	7.62	-7%

In a first analysis, the agitation and filtration results show a significant reduction of gas, as already happened with the studies previously made. This way, it was possible to now pass to the next step, to only treat the sample with agitation and see if this method was effective. A freshly open bottle (sample number 7) was also used for this test, namely, to prove if the CO_2 reduction was efficient so it would not interfere with the spectrophotometer, and to test the best agitation time between the times 15 and 30 min (Figure 9).

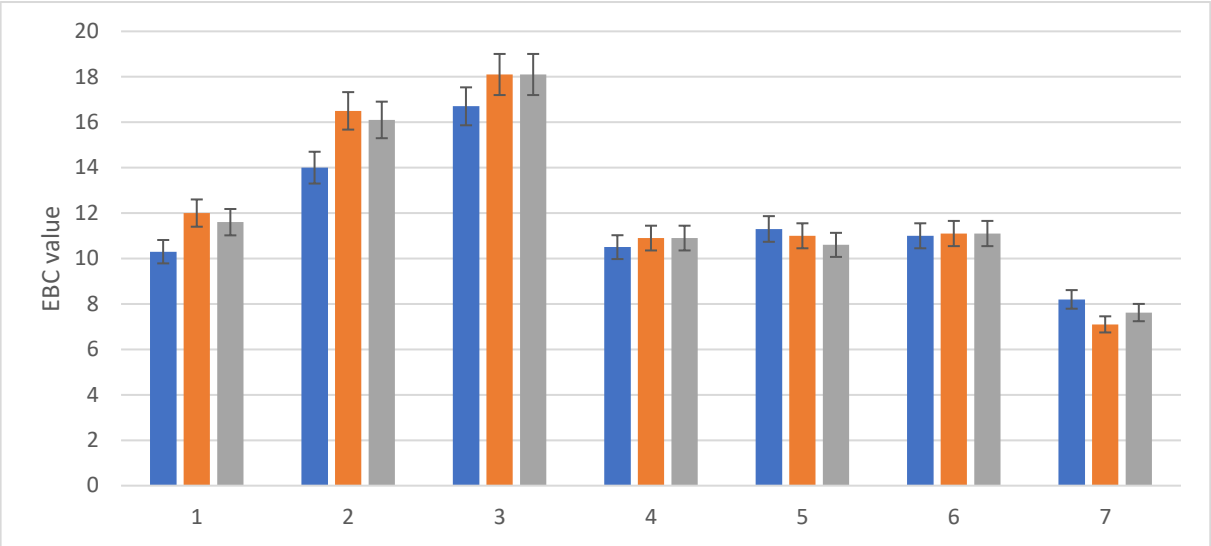


Figure 9 – European Brewery Convention (EBC) value, obtained by the Reference Method (RM – blue bars), the SIA system with 15 min of agitation (orange bars) and with 30 min of agitation (grey bars); the error bars correspond to 5% error.

It was notorious that both agitation times had a significant effect on already opened bottles, probably because the CO_2 percentage was lower, and the gas dissipates as time passes. But the freshly opened bottle (number 7) had a higher CO_2 percentage and needed a higher agitation time, namely the duration of 30 min. Therefore, all the beer samples were agitated for the defined time (30 min) before the propelling trough the SIA system, to obtain better readings and a lower deviation, comparing with the reference method.

3.2 Potassium determination

Potassium determination was the first studied parameter. It began with a conventional use of the pair of electrodes. Then it was improved to use a FIA system with the same pair of electrodes.

3.2.1 FIA set up

For the potassium determination, the FIA system (Figure 10) was composed by an injection valve (IV) and a peristaltic pump that were both manually controlled. As detection system, a Thermo Scientific reference electrode, along with a potassium selective electrode (Ref. 9661K) were used, equipped with a Crison pH-meter GPL21, where the results were directly read by the signal shown on the screen.

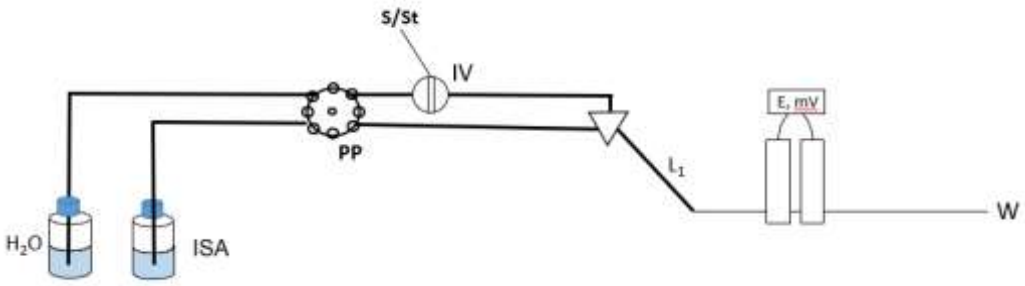


Figure 10 – Flow injection manifold for the determination of potassium; PP, peristaltic pump; S/St, sample/potassium standard; L_1, reaction coil 9 cm; IV, injection valve; ISA, ionic solution adjustor, NaCl 0.5 M; Δ, confluence; E, mV, selective electrode for potassium determination; W, waste.

Both solutions were continuously running with a defined flow rate (Q = 0.9 mL/min) and went through different channels until they reach a confluence point. The sample/standard with a 0.200 mL volume was injected into the carrier tube and flows along with it. After passing the confluence zone, ISA, water, and the sample/standard mix in the 9 cm long reaction coil until the solution reaches the electrode, where a direct reading was performed when the signal reaches its maximum.

The FIA system for potassium determination was the first system used for analyzing this parameter. For this matter, three main studies were conducted, to understand how to maximize the signal and to obtain a better calibration curve.

3.2.1.1 Study of the sample volume and the flow rate influence

The next step was to study the injected volume of sample/standard, using a white/white pump tube. For this, the loop length was measured, and the inner volume was calculated, knowing that 2 meters of loop is equal to 1 mL of solution. In the beginning, the experiments were made with a mono channel, where the carrier was the ISA solution previously prepared, and the flow rate used was 1.4 mL/min, corresponding to a velocity of 15 shown on the pump. The minimum loop tested was 26 cm length, that was equal to a 0.130 mL volume. The second one, was 40 cm length, corresponding to 0.200 mL. For this matter, the two slopes obtained had a slightly different of 6%, which was not considered significative, so more studies were made with these two volumes to determine the better one, when combined with other variables.

For this, the flow rate for each volume mentioned above was also studied, testing between a flow rate of 0.5 mL/min, 0.9 mL/min, 1.4 mL/min and 1.8 mL/min with the different loop lengths mentioned above (Figure 11). Despite the differences between the flow rates, it was still not possible to distinguish for sure between the volumes, and therefore, one more decisive study will be carried. Yet, about this topic, clearly the flow rate of 0.9 mL/min was the better one, due to the better slope, regardless of the injected volume. The flow rate of 0.5 mL/min was studied only with the 0.200 mL volume just to guarantee that effectively there was no flow rate better than the chosen one (0.9 mL/min).

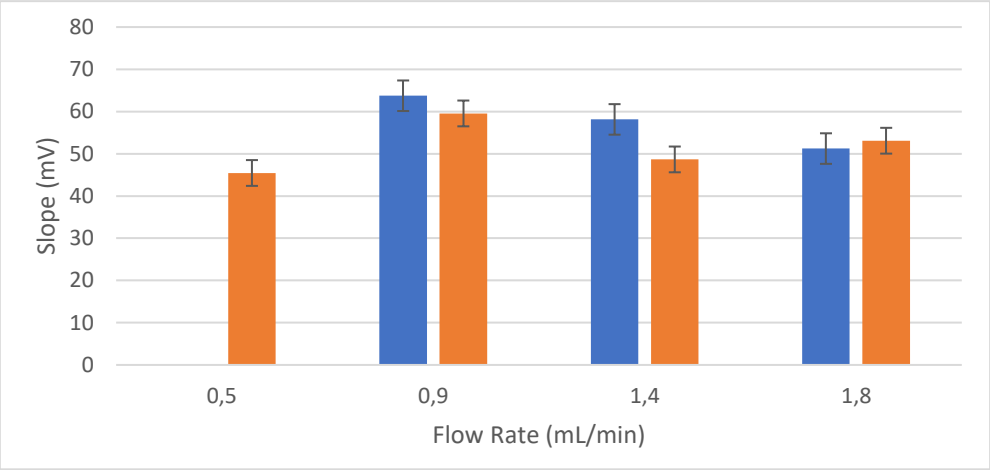


Figure 11 – Slope values obtained for the two injected sample/standard volumes, 130 (blue bars) 200 (orange bars) at the different flow rates; the error bars correspond to standard error.

3.2.1.2 Study of the flow rate influence

After setting the flow rate and, to finally decide which would be volume to be used, the pump tube was study, namely, to check which was better between white/white and black/black (0.78 mm Gilson PhthalateFree PVC black/black, Lot: IW014791). The main difference was that the black/black tube had a lower inner volume.

The obtained slope values shown in Figure 12 led to choose the 0.200 mL volume, and the white/white tube.

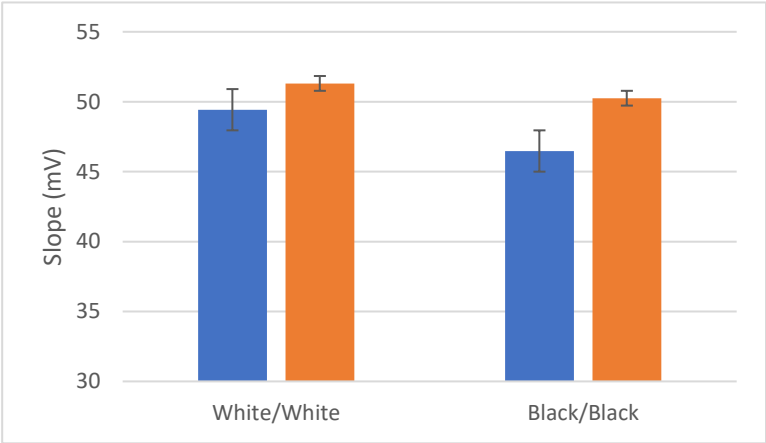


Figure 12 – Slope values of the different pump tube channels; blue bars correspond to 0.130 mL and orange bars correspond to 0.200 mL; the error bars correspond to standard error.

Then, the FIA system configuration was altered, adding another channel (white/white pump tube on position 2), that will now carry the ISA solution, while the first tube will carry water. It became necessary to study flow rate of the second pump tube, the same way it was made previously, and the results had no significant difference (~1%). Regarding the choice made before, it was decided to keep the white/white tube channel in both positions since, generally, the results are better.

3.2.2 SIA set up

After determining the flow rates and choosing the electrode, a SIA system was assembled, with the objective of carrying out both potassium and color determination in the same manifold. Comparing with FIA system, the SIA system has advantages, namely the possibility of incorporating the detectors for the two parameters and the computerization of the volumes, minimizing the errors.

In the potassium SIA system (Figure 13), two different peristaltic pumps were used, in which one of them carried boiled deionized water with a white/white carrier and was controlled by a computer program. It was programed to aspirate 226 µl of the sample/standard on position 2, and then send into the confluence channel on position 8, where it mixes with the ISA solution and reaches the electrode, where the reading is directly done when the maximum is reached. On the other way, ISA was continuously flowing on the second peristatic pump with a white/white channel, to stabilize the electrode (Figure 13).

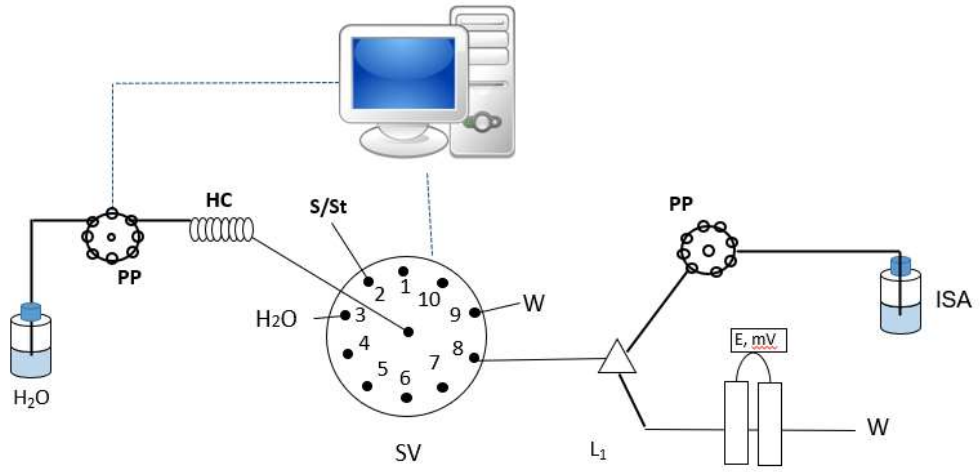


Figure 13 - Sequential injection manifold for the determination of potassium; SV, 10 port selection valve; PP, peristaltic pump; HC, holding coil, 200 cm; S/St, sample/potassium standard; L_1, reaction coil 9 cm; ISA, ionic solution adjustor, NaCl 0.5 M; E, mV, selective electrode for potassium determination; W, waste.

3.2.2.1 Study of the alcohol influence

The determination of the potassium concentration was compared with the reference method, which could be by atomic absorption or flame emission photometry. Atomic absorption, due to its sensitivity, detects very low concentrations of potassium, approximately until 4 mg/L, corresponding to 10^{-4} M. The calibration curve used for the potassium determination had standards between 10^{-1} M and 10^{-3} M, so it is not possible to make a 1000x dilution, due to the associated errors. On the other way, flame emission photometry (FEC) can measure potassium until around 10^{-2} M, which implies only a 10x dilution of the samples and standards.

The next step was the verification if the alcohol percentage interfere with the readings, since the standards had 0% alcohol, and both beer and wine had around 10%. It is known that the matrix of the sample/standard interferes on the FEC readings and makes it important to measure the alcohol percentage. For this, standards with 1% and 5% of alcohol were prepared, additionally to the 0%

previously prepared and run in both SIA system and FEC, to determine the better calibration curves. The following figure (Figure 14) shows that the percentage of alcohol in the standards does not interfere significantly in the slope (1% and 6% deviation comparing to the 0% alcohol data), and so it was decided to maintain the standards with 0% alcohol.

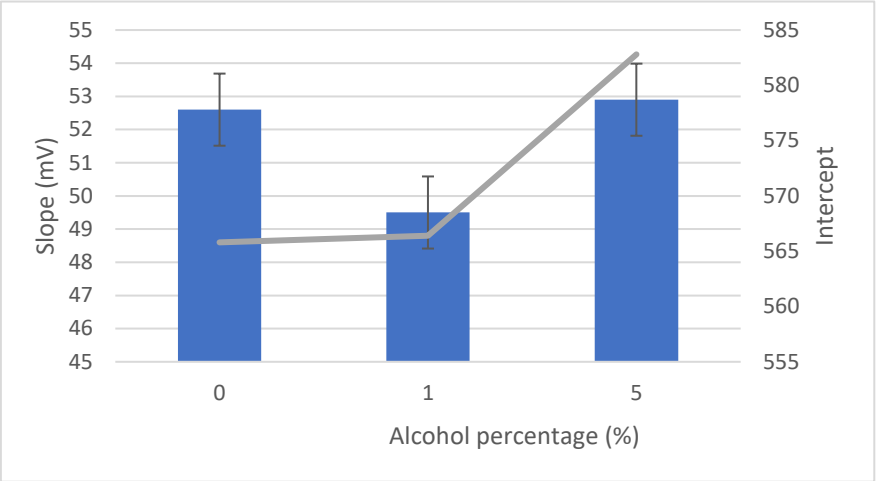


Figure 14 – Slope (bars) and intercept (lines) values obtained for the three different calibration curves in the SIA system (the error bars correspond to standard error).

The stability of the standards over time was also evaluated. For this, standards with two weeks old and freshly prepared were compared, and the results show no significative differences (~2%).

The samples were also diluted according to the alcohol degree (10x) and were measured with the values obtained by the 0% alcohol calibration curve. The Table 6 below show the obtained results and the respective comparation with the reference method.

Table 6 – Comparison of the potassium concentration obtained with flame emission photometry (RM) and with the SIA system (SIA) by calculating the relative deviation percentage (RD)

Sample type	Sample ID	RM[1]	SIA	RD
		[K+], mM	[K+], mM	
Beer	1	9.86	10.3	4%
	2	8.15	8.8	8%
	3	13.3	12.1	-9%
	4	22.6	21.8	-4%
	5	20.0	18.2	-9%
	6	20.5	18.8	-8%
Wine	1	17.7	16.9	-5%
	2	25.4	25.6	1%
	3	16.8	17.3	3%
	4	11.4	10.9	-4%
	5	19.8	19.5	-1%

It is now possible to conclude that the SIA system for the determination of potassium was similar results to the reference method and the details of this method are defined on 3.6. When the potassium concentrations were compared, a curve was obtained with the following equation, proving that the concentration obtained with the SIA system and with the reference method (RM) are not statistically different.

$$[K^+]_{SIA} = 0.941\ (+/-\ 0.110)\ [K^+]_{RM} + 0.5116\ (+/-\ 1.938)$$

Also, the main conclusion was that both beer and wine have a large amount of potassium, and the concentrations found are in accordance with the theoretical values that were mentioned on 1.1.1. and 1.1.2

3.3 Color determination

After optimizing the determination of potassium in the FIA system, another parameter started to be optimized, namely the color determination, since it had a simple protocol, that consists on sending the sample to the detector (spectrophotometer) with a wavelength equal to 430 nm, according to EBC (9) and there was no need to add reagents or do calibration curves. All the samples were directly injected/aspirated, and some studies were made to maximize the absorbance peaks.

3.3.1 FIA set up

For the color determination on a FIA system, 1 grey/grey pumping tube (1.30 Gilson PhthalateFree PVC grey/grey Lot: IW018936) was introduced on the second position of the peristaltic pump, which carries deionized boiled water. The water was continuously running with a defined flow rate (Q = 0.9

mL/min), and when it reaches the injection valve, the sample with a 0.830 mL volume was injected into the carrier tube and flows along with it.

Data signal on FIA system was performed through the OceanOptics – Spectrasuite software running in a university's computer (Sony Vaio C2D U77600). This system was composed by a peristaltic pump that was manually turned on and controlled. The FIA system was also composed by an injection valve (IV) that was manually controlled, with two positions, namely load and inject.

Both water and beer mix along a 22cm long reaction coil until the solution reaches the detector (λ=430 nm), where the absorbance was maximized with the final objective of reading the beer's color without diluting. The reading was done with the help of Spectrasuite program, that reads the absorbance of the solution that was running through the cell (optical path of 1 cm) and created an absorbance peak, which corresponds to maximum absorbance measured for the injected sample (Figure 15).

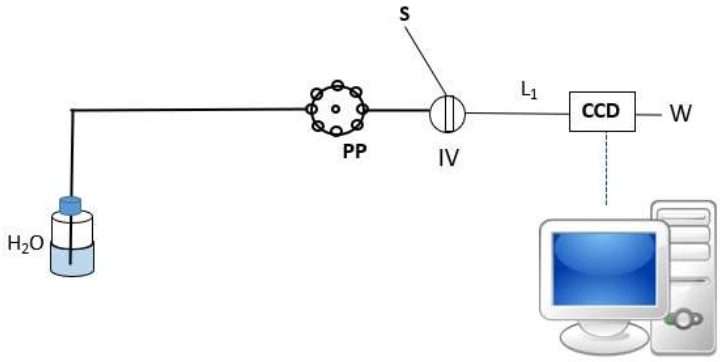


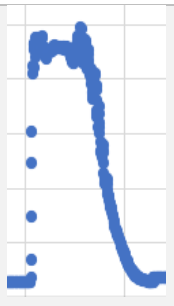
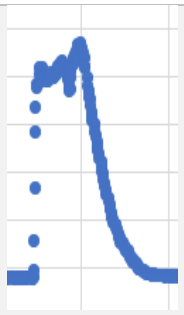
Figure 15 - Flow injection manifold for the determination of color; IV, injection valve; PP, peristaltic pump; S, sample; L_1, reaction coil 9 cm; CCD, detector (λ = 430 nm); W, waste.

3.3.1.1 Study of the flow rate influence

Initially, right after studying the importance of the color determination for both beer and wine, it became necessary to understand which was the volume that could maximize the absorbance peak obtained. The volume is directly related to the loop length and, the bigger the loop was, the higher injected volume. For this, the initial tested volume was 130 µL, followed by volumes of 200 µL, 280 µL, 340 µL, 430 µL, 530 µL, 630 µL, 830 µL, 1150 µL. The samples used for this test were all light beer and were treated with the standard treatment, namely the filtration with silica dioxide.

Although many volumes were tested, the volumes of 130 µL, 200 µL, 280 µL and 340 µL were discarded, since on a first analysis it was noticed that the results were very different from the absorbance obtained with the conventional spectrophotometer. The study was conducted with the remaining volumes (Table 7), with the objective of measuring the absorbance of the sample without any dilution when crossing the flow cell. This way, we also assured that the sample was read on the detector in the same conditions as for the conventional spectrophotometer reading. The maximum absorbance can be easily read, as a "plateau", and that is why it was decided to test the above-mentioned higher volumes.

Table 7 – Absorbance values (λ = 430 nm) and figures for the different loop volumes.

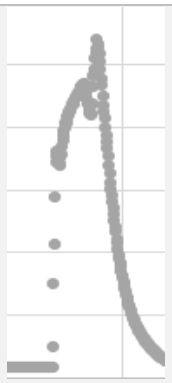
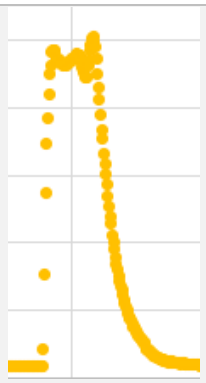
Volume	430 µL	530 µL	630 µL	830 µL	1150 µL
Absorbance's Peak Figure					
Absorbance value	0.401	0.417	0.408	0.574	0.568

The Table 7 above shows the absorbance values obtained and, after analyzing the visual peak, it was possible to say that the saturated peak was only obtained for the volumes of 630 µL, 830 µL and 1150 µL. For the following studies, the chosen volume was the 830 µL, corresponding to a 166 cm length, which ensures that the absorbance was maximized (the values obtained with the conventional spectrophotometer were comparable with SIA's detector) and to spend the less possible volume.

3.3.1.2 Study of the Injected Volume Influence

The next step was to understand the color intensity variation, according to the flow rate and the amount of sample that reaches the detector. In this specific case, the focus was to understand which flow rate allows a better absorbance peak. First, the volume of the injected sample was determined, namely the 830 µL, and, with a maximized absorbance, it was possible to proceed with the study of the flow rate (Table 8). Note that, in FIA system, the flow rate was introduced manually in the peristaltic pump and for this, four different flow rates were measured and tested with the sample injection.

Table 8 – Absorbance values (λ = 430 nm) and figures for the different flow rates (mL/min).

Flow rate	0.62 mL/min	0.88 mL/min	1.40 mL/min	1.70 mL/min
Absorbance's Peak Figure				
Absorbance value	0.472	0.465	0.464	0.466

Looking at the table above (Table 8), it was noticed that the absorbance values have no significant differences, so all of them were a good option for the flow rate. However, there were some details that required attention, namely the time it takes to reach the detector and the absorbance saturation, previously availed. The flow rate of 0.62 mL/min was too slow and requires a longer time to analyze and run the samples. On the other way, the flow rates of 1.40 mL/min and 1.70 mL/min do not have a perceptive “plateau”, due to the fast flow, where the sample goes in and out of the flow cell very quickly, not giving enough time to measure the absorbance, leading to a lack assurance that the absorbance is maximized. Finally, the flow rate of 0.88 mL/min had a perceptive absorbance and was not to slow, and, for this matter, this was the chosen flow rate for the rest of the color determination on FIA system.

3.3.1.3 Study of the absorbance in light and dark beer

All the tests made so far were conducted with light beer. But, to extend the application, dark beers were also tested, to give an easy alternative to the worker who will analyze the beer with this system. Initially, the absorbance was read on the spectrophotometer, and it was higher than the values mentioned by the EBC (>0.8), so it was decided to dilute the beer samples (Table 9).

The darkest samples were diluted between 5 and 20 times so a proper reading could be made, and the lighter ones were diluted 2 times, to compare if the final EBC value was identical to the not diluted samples. The chosen dilution factor depended on the initial absorbance value, for example, the sample 7 on Table 9 had an absorbance of 2.163 and was diluted 5 times to obtain an absorbance around 0.4 and, as the results show, the final absorbance had a value of 0.459, which means the dilution was correctly made. So, all the samples used conventionally, inserted on a 1 cm cuvette, and FIA system were previously diluted and, for the flow system, a volume of 830 µL was directly injected.

Table 9 – Comparison of the European Brewery Convention value (EBC) obtained with the conventional spectrophotometer (RM) and with the FIA system (FIA) by calculating the relative deviation percentage (RD); the absorbance value (A) used for the EBC calculation was register at $\lambda = 430\,nm$); DF corresponds to the dilution factor of each sample.

Sample ID	DF	RM		FIA		RD
		A	EBC	A	EBC	
1	10	0.609	152	0.625	156	3%
	20	0.283	142	0.288	144	1%
2	1	0.422	10.6	0.431	10.8	2%
	2	0.206	10.3	0.205	10.3	0%
3	10	0.501	125	0.518	129	3%
	20	0.255	128	0.249	124	-3%
4	1	0.701	17.5	0.694	17.4	-1%
	2	0.342	17.1	0.345	17.3	1%
5	1	0.557	13.9	0.571	14.3	3%
	2	0.277	13.9	0.288	14.4	4%
6	1	0.415	10.4	0.409	10.2	-2%
	2	0.199	10.0	0.182	9.10	-9%
7	1	2.163	54.1	-	-	-
	5	0.459	57.4	0.430	53.8	-6%
8	1	1.789	44.7	-	-	-
	5	0.335	41.9	0.307	38.4	-8%
9	1	1.645	41.1	-	-	-
	5	0.377	47.1	0.347	43.4	-8%

Finalizing this topic, it is interesting to refer that the results with the developed FIA system were compared with the reference method and the results show that the differences between these two systems are not significant, which means that the combination of flow rate and injected volume were correctly chosen. When the EBC values were compared, a curve was obtained with the following equation, proving that the values obtained with the SIA system and with the reference method (RM) are not statistically different.

$$EBC_{FIA} = 1.015\ (+/-\ 0.027)\ EBC_{RM} - 1.089\ (+/-\ 1.989)$$

3.3.2 SIA set up

The same reason why we chose a SIA system for potassium over a FIA system, led us to make the same choice here, to automatize the system and make it possible to assemble two determinations in the same manifold, controlled uniquely for a computer. It also allows to reduce the wasted volumes of reagent and water (carrier), since the peristaltic pump is not continuously flowing.

For the determination of color on the SIA system, the peristaltic pump that carries boiled deionized water was controlled by a computer program. For light beers, white and rosé wines, it was programed to aspirate 796 µL of the sample on position 2, and then send it to the Ocean Optics detector (λ = 430 nm for beer, 420 nm, 520 nm, and 620 nm for wine), where the absorbance was maximized with the final objective of reading the color without diluting. The reading was done with the help of Spectrasuits program, that read the absorbance of the solution and created an absorbance peak, which corresponds to maximum absorbance measured for the aspirated sample. For dark beer and red wine, the program was modified to aspirate 57 µL of sample, followed by aspiration of 739 µL of water for beer and 568 µL of water for wine measurement. The absorbance reading was also the peak shown on Spectrasuits program (Figure 16).

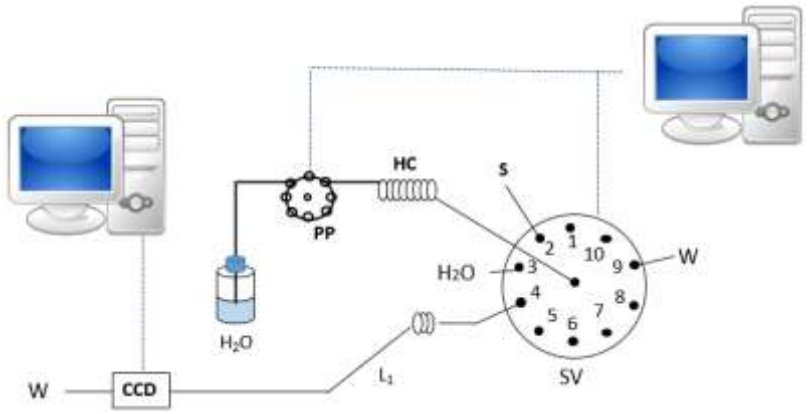


Figure 16 – Sequential injection manifold for the determination of color; SV, selection valve; PP, peristaltic pump; HC, holding coil, 200 cm; S, sample; L_1, reaction coil 22 cm; CCD, detector (λ= 430 nm for beer and 420 nm, 520 nm, 620 nm for wines); W, waste.

3.3.2.1 Differences between dark and light craft beer

Until this point, all studies were conducted on a FIA system. Therefore, when we chose to use a SIA system, it was necessary to check if the conditions tested previously show no significant differences. Since in SIA the volume is not defined by a loop, an aspirated volume similar to the one previously used in FIA was attempted, and the volume found was 796 µL. The values were compared with the reference method, and the results are the following (Table 10).

Table 10 – Comparison of the European Brewery Convention value (EBC) obtained with the conventional spectrophotometer (RM) and with the SIA system (SIA) by calculating the relative deviation percentage (RD); the SD value corresponds to the Standard Deviation of the samples; the RSD value corresponds to the Relative Standard Deviation of the samples; the DF corresponds to the Dilution Factor of the samples.

Sample ID	DF	RM	SIA[a]			RD
		EBC	EBC	SD	RSD	
1	1	11.0	11.4	0.1	1%	4%
2	1	18.0	17.3	0.1	1%	-4%
3	1	15.7	16.1	0.3	2%	3%
4	1	10.4	11.0	0.2	1%	6%
5	1	11.1	11.8	0.1	1%	6%
6	1	11.0	11.4	0.1	1%	4%
7	10	129	125	1	1%	-3%
8	10	163	156	2	1%	-4%
9	10	58.5	61.5	0.7	1%	5%
10	5	45.3	45.3	0.2	0%	0%
11	5	32.1	32.1	0.8	3%	0%

[a] n = 3

Since the beer was previously agitated for 30 min, a dilution procedure could be time consuming and unnecessary. This can be solved by finding a way to dilute the beer in line, inside the tubes, and for this it was essential to determine the volume of sample that will be aspirated. On an initial attempt, the main idea was to use a ten times smaller volume, since all the dilutions were made on this proportions. To fulfill the sample volume that was referenced, the remaining volume to reach the 796 µL will be completed by the aspiration of water. Different volumes were tested namely 29 µL, 58 µL, 72 µL, and 83 µL, and a bars graph was made (Figure 15) comparing the results with the reference method. When the EBC values were compared, a curve was obtained with the following equation, indicating that the values obtained with the SIA system and with the reference method (RM) are similar but statistically different. More determinations should be carried out to further validate the method.

$$EBC_{SIA} = 0.957\ (+/- 0.032)\ EBC_{RM} + 1.403\ (+/- 1.746)$$

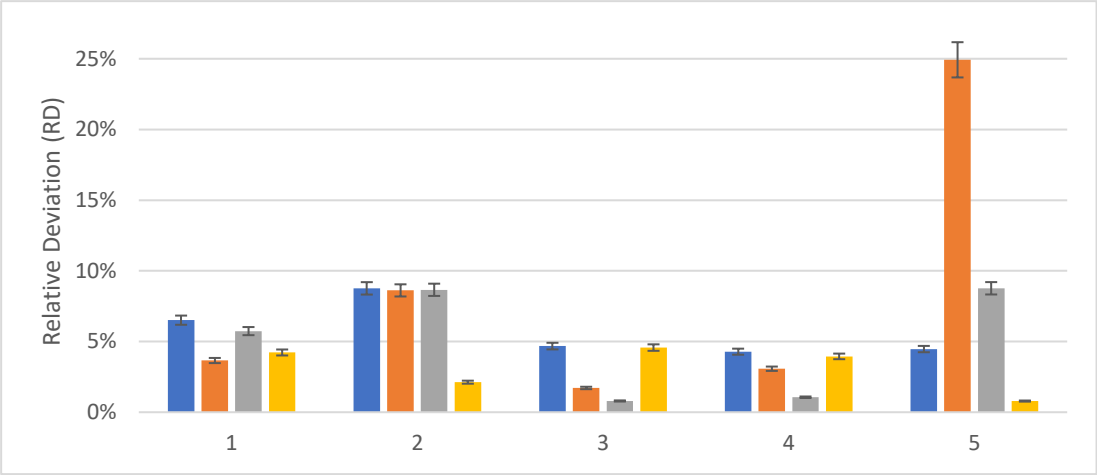


Figure 17 – Comparison of the relative deviation obtained on different samples, for the four tested volumes; blue corresponds to 29 µL; orange corresponds to 83 µL; grey corresponds to 72 µL; yellow corresponds to 58 µL; the error bars correspond to 5% error.

This study was made for five dark samples, named from 1 to 5 on the Figure 17 above, that normally had absorbances higher than recommended (0.8) and had to be diluted. According to each combination of sample and water aspirated, a dilution factor was calculated for, posteriorly, the EBC value could be calculated. As it was shown on the results above, the 83 µL volume varies and had a deviation of -25%, which means that this volume was not the correct one. Then, the 72 µL had a high deviation, namely 9% in most samples, which means that may not be the ideal volume either. The volume of 58 µL, comparing with the last one, 29 µL, was more stable on its variations, and had a lower percentage of deviation. In sum, it was decided that the volume that will be used for now on to dilute in line was the 58 µL of aspiration of sample and 747 µL of aspiration of water, followed by sending to the detector. Finally, all the dark samples were measured and compared with the reference method, as Table 11 shows, and it was correct to affirm that the color determination for beer samples was completed and referenced. When the EBC values were compared, a curve was obtained with the following equation, proving that the values obtained with the SIA system and with the reference method (RM) are not statistically different.

$$EBC_{SIA} = 0.982\ (+/-\ 0.039)\ EBC_{RM} - 3.784\ (+/-\ 4.456)$$

Table 11 – Comparison of the European Brewery Convention value (EBC) obtained with the conventional spectrophotometer (RM) and with the SIA system (SIA) by calculating the relative deviation percentage (RD); the SD value corresponds to the Standard Deviation of the samples; the RSD value corresponds to the Relative Standard Deviation of the samples

Sample ID (Dark beer)	**RM [a]**			**SIA[a]**			**RD**
	EBC	SD	RSD	EBC	SD	RSD	
1	154	10	6%	155	1	1%	1%
2	190	6	3%	190	4	2%	0%
3	57.8	1.0	2%	62.8	2.4	4%	9%
4	48.1	1.0	2%	50.9	0.5	1%	6%
5	35.4	0.6	2%	36.7	0.5	1%	4%

[a] n = 3

3.3.2.2 Study of the influence of sample volume for wines

As the method was previously referenced for beer, the protocol for wine's color determination was identical. Firstly, for white wines, a volume of 796 µL was aspirated and sent to the detector, and the intensity value was compared with the reference method (Table 12). When the color intensity was compared, a curve was obtained with the following equation, proving that the values obtained with the SIA system and with the reference method (RM) are not statistically different.

$$I_{SIA} = 1.006\ (+/-\ 0.092)\ I_{RM} - 0.002\ (+/-\ 0.021)$$

For intensity calculation, since the white wines are only measured by one wavelength, the intensity is equal to the absorbance values times the b value of the cell. The formula used for the color intensity calculation was the following for white wines, where b = 1.

$$\text{Color Intensity (I)} = \frac{Abs\ (\lambda=420nm)}{b} + \frac{Abs\ (\lambda=520nm)}{b} + \frac{Abs\ (\lambda=620nm)}{b} \Leftrightarrow$$

$$\Leftrightarrow \text{Color Intensity (I)} = \frac{Abs\ (\lambda=420nm)}{b}$$

Table 12 – Comparison of the Color Intensity (I) obtained with the conventional spectrophotometer (RM) and with the SIA system (SIA) by calculating the relative deviation percentage (RD); the SD value corresponds to the Standard Deviation of the samples; the RSD value corresponds to the Relative Standard Deviation of the samples.

Sample ID (White wines)	**RM[a]**			**SIA[a]**			**RD**
	I	SD	RSD	I	SD	RSD	
1	0.484	0.001	0%	0.489	0.006	1%	-1%
2	0.791	0.000	0%	0.827	0.027	3%	-5%
3	0.116	0.001	0%	0.126	0.001	1%	-9%
4	0.149	0.000	0%	0.146	0.011	7%	2%
5	0.073	0.000	0%	0.070	0.006	8%	4%
6	0.105	0.001	1%	0.096	0.003	3%	9%
7	0.108	0.001	1%	0.105	0.002	2%	3%
8	0.685	0.001	0%	0.645	0.008	1%	6%
9	0.099	0.001	1%	0.105	0.006	6%	-6%

[a] n = 3

Regarding the white wines, it was important to understand that these wines age, gaining a brown hue, that is also measured by the absorbance at 420 nm, according to some authors that were already mentioned. Unlike beer, the wines with a high absorbance cannot be diluted, so a smaller cell was used, namely with an optical path of 0.1 cm. For comparison, the same volumes as for dark beers were used, the combination between 58 µL of sample and 747 µL of water. Unfortunately, the relative deviation had values around 25%, which led to a new study of the volume of aspirated water. As it was made for beer, it was tried to have a ten times higher volume of water than sample, namely a 58 µL aspiration of sample, followed by a 568 µL aspiration of water, and the results were compiled in the table below (Table 13). The formula used for the color intensity calculation was the following for aged white wines, where b = 0.1.

$$\text{Color Intensity (I)} = \frac{Abs\ (\lambda{=}420nm)}{b} + \frac{Abs\ (\lambda{=}520nm)}{b} + \frac{Abs\ (\lambda{=}620nm)}{b} \Leftrightarrow$$

$$\Leftrightarrow \text{Color Intensity (I)} = \frac{Abs\ (\lambda{=}420nm)}{b}$$

Table 13 – Comparison of the Color Intensity (I) obtained with the conventional spectrophotometer (RM) and with the SIA system (SIA) by calculating the relative deviation percentage (RD); the SD value corresponds to the Standard Deviation of the samples; the RSD value corresponds to the Relative Standard Deviation of the samples.

Sample ID (Aged white wines)	**RM[a]**			**SIA[a]**			**RD**
	I	SD	RSD	I	SD	RSD	
1	1.15	0.00	0%	1.12	0.07	6%	3%
2	1.00	0.01	1%	1.05	0.04	4%	-5%
3	1.75	0.00	0%	1.91	0.00	0%	-9%
4	2.52	0.01	0%	2.73	0.05	2%	-8%

[a] n = 3

The intensity values obtained for these samples (Table 14) for both SIA system and reference method showed no significant differences. This way, it was correct to say that this method works for wines with a high absorbance and, therefore, this would be the right volumes to use for red wines' color determination. The red wines color was measured by three different wavelengths, namely 420 nm, 520 nm and 620 nm, and the color intensity was calculated by the sum of these absorbances, all divided by the optical path of the cell used. Since red wines had a high absorbance value on the spectrophotometer, the optical path was changed to 0.1 cm, and, on SIA system, was maintained as 1 cm. The results for red wines are the following (Table 14). The formula used for the color intensity calculation was the following for red wines, where b = 1 (on SIA system's cell) and b = 0.1 (on spectrophotometer).

$$\text{Color Intensity (I)} = \frac{Abs\ (\lambda{=}420nm)}{b} + \frac{Abs\ (\lambda{=}520nm)}{b} + \frac{Abs\ (\lambda{=}620nm)}{b}$$

Table 14 – Comparison of the Color Intensity (I) obtained with the conventional spectrophotometer (RM) and with the SIA system (SIA) by calculating the relative deviation percentage (RD); the SD value corresponds to the Standard Deviation of the samples; the RSD value corresponds to the Relative Standard Deviation of the samples.

Sample ID (Red wines)	**RM [a]**			**SIA [a]**			**RD**
	I	SD	RSD	I	SD	RSD	
1	7.32	0.02	0%	7.90	0.042	0%	-8%
2	10.2	0.0	0%	10.8	0.120	1%	-6%
3	7.05	0.01	0%	6.74	0.233	3%	4%

[a] n = 3

Finally, and since the results obtained for the red wines were not different from the reference method, it was possible to say that this system is efficient and reliable regarding the measurement of the wines color with high absorbances. The last studied type was the rosé wine, that had a low absorbance and, for that, can be directly measure with an optical path of 1 cm (for both spectrophotometer and SIA

system) and with the aspirated volume of 796 µL, that was measured in the same three wavelengths (Table 15). The formula used for the color intensity calculation was the following, where b = 1.

$$\text{Color Intensity (I)} = \frac{Abs\ (\lambda{=}420nm)}{b} + \frac{Abs\ (\lambda{=}520nm)}{b} + \frac{Abs\ (\lambda{=}620nm)}{b}$$

Table 15 – Comparison of the Color Intensity (I) obtained with the conventional spectrophotometer (RM) and with the SIA system (SIA) by calculating the relative deviation percentage (RD); the SD value corresponds to the Standard Deviation of the samples; the RSD value corresponds to the Relative Standard Deviation of the samples.

Sample ID (Rosé wines)	**RM[a]**			**SIA[a]**			**RD**
	I	SD	RSD	I	SD	RSD	
1	0.575	0.004	1%	0.588	0.017	3%	2%
2	0.586	0.006	1%	0.639	0.021	3%	9%
3	0.603	0.001	0%	0.553	0.004	1%	-8%
4	0.589	0.002	0%	0.549	0.009	2%	-7%

[a] n = 3

In sum, the color determination for both beer and wine show no significative differences between the SIA system and the reference method, and we can now conclude that all the studies were finished and that, depending on the absorbance, the program volumes vary but the results are identical to the pretended ones.

3.4 Analytical Features

The analytical features of potassium determination were then summarized on a table (Table 17), where all the details of the developed system were studied and compiled. For the color determination, since there was no calibration curve, the details are written along the step 3.3. Also, for the final bi parametric system, that gathers the analytes of potassium and color, a determination rate was calculated, with the purpose of understand how long it takes to run a cycle and how many peaks the system can read per hour. Since there are three different combinations for the color determination, depending on the matrix, the average between the values was approximately 36 peaks per hour, or, with the three replicates for each sample/standard, 12 cycles/h.

Table 17 – Analytical features of the SIA system for potassium determination.

Analyte	**Dynamic range**	**Calibration curve**	**LOD**	**LOQ**	**% RSD concentration**
K^+	1.08 x 10^{-1} M to 1.08 x 10^{-3} M	E = 54.04 (+/- 2.07) log [K^+] + 560 (+/- 22) [a]	3.90 x 10^{-5} M	1.62 x 10^{-4} M	14%[b] 14.4 mM

[a] n = 5 [b] n = 6

The limit of detection (LOD), also known as the lower limit of linear response is the value obtained from the smallest measure that can be detected with certainty, and the limit of quantification (LOQ) or practical detection limit, is defined as the “concentration value corresponding to the interception point of the extrapolations of the linear parts of the calibration curve, according to the IUPAC (32,33) recommendations.

The reproducibility of the SIA system was evaluated using the relative standard deviations (RSD), which was calculated by dividing the sample standard deviation by the average of the potential values obtained for the same sample.

3.5 Application of the developed SIA method

After setting the conditions for both potassium and color determination, it was necessary to check if the system could analyze the potassium and the color in the same program, by aspirating the sample and sending to the potentiometer and aspirate again and send it to the spectrophotometer. Since these two systems were already working separately, the following results (Table 18) show that the determination of potassium and color can be made on the same program.

Table 18 – European Brewery Convention (EBC) values and potassium concentration obtained for the samples run in the developed biparametric system.

Sample ID	EBC	$[K^+]$, mM
1	17.3	13.3
2	16.1	22.6
3	11.0	20.1

4. Conclusion and suggestions for future work

The focus of this work was to develop a biparametric system for potassium and color detection and quantification in beverages, with the final objective to be applied to the beer and wine's industry to facilitate the analytical procedure of potassium and color measurement, so the final solution could be adjusted as needed. This way, there is not a necessity to measure these parameters separately on a spectrophotometer and on a potentiometer.

To validate the method, the absorbance and potential measurements of several samples were compared with the measurements obtained from the spectrophotometer and the flame emission photometry, described in both OIV and EBC compendiums (5,9), and there were no statistically significant differences between the values from these two methods.

It was not possible to find any equipment in the market that could simultaneously measure these two parameters, which could be a benefit of the system, as well as the time saving, the less volume required and the double task. Comparing to the reference method, this spectrophotometer requires less work, since it can read multi wavelengths simultaneously, there was no need to change the wavelength after every measurement. However, many more parameters need to be determined, and as future work, it would be interesting to develop a system that could measure acidity and some other ions like magnesium, calcium, or ammonia. Since the valve is composed by ten entrances and only four of them are occupied, it is viable to include some other detectors for these measurements, so this system could be an all-in-one equipment for analysis of beverages.

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