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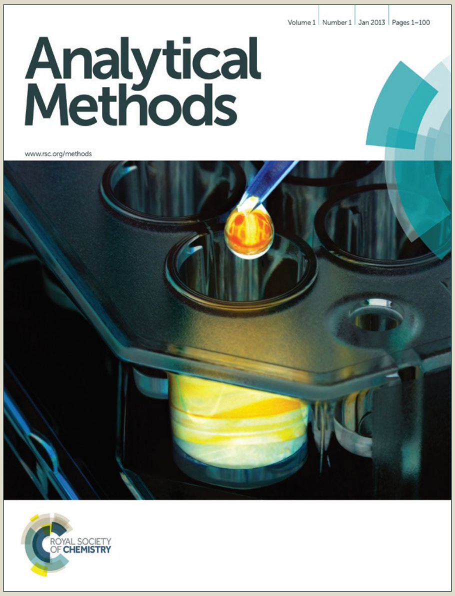

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A simple and green analytical method for determination of copper(II) in whisky and sugarcane spirit by diffuse reflectance spectroscopy

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Abstract: In this paper we propose a new, fast, simple, economical, portable and environmentally friendly analytical methodology for the determination of copper(II) ions in whisky and sugarcane spirit, using the combination spot-test and diffuse reflectance spectroscopy. The method is successfully accomplished by means of the diffuse reflectance measurements at 559 nm, the reaction product formed between the copper(II) ions with the chromogenic agent 1-(2-pyridylazo)-2-naphthol (PAN), using filter paper as the solid support. The best conditions for the reaction occurred with the addition of 30 uL of a PAN solution at a concentration rate of 0.12% m/v in ethanol and 30 uL of an alcoholic solution of copper(II) ions at 40.0% v/v with pH 5.00 and 5 minutes of reaction time. The following elements were found to interfere in the reaction process - iron(III), nickel(II), cobalt(II), cadmium(II), manganese(II), zinc(II) and lead(II) ions. Malonic Acid and Sodium Triphosphate at a concentration of 0.25% m/v was therefore used as a masking agent, which contributed towards the reduction of the magnitude of the interference effect from around 20.3% to approximately 2.0%. Under optimized conditions for the determination of copper(II), the analytical curve obtained could be described by: $\Delta AR = 0.0121\ C_{Cu(II)} + 0.0038$ ($r^2 = 0.9982$). Linearity was obtained up to a concentration of 10.0 mg L^{-1} of copper(II), and limits of detection and quantification (calculated using the standard deviation of the blank) were 0.12 and 0.47 mg L^{-1} of copper(II), respectively. The proposed method was applied for the determination of copper(II) ions in samples of whisky and sugarcane spirit and the results were compared with those obtained by the official method with a 95% level of confidence.

1. Introduction

Whisky (Whiskey) is one of the most popular drinks consumed in the world. In Brazil, the consumption of the beverage has seen a dramatic hike significantly in recent years, with a market growth of about 34% per year. According to the Scotch Whisky Association (SWA), Brazil clinched the eighth position in the world market in sales volume and ninth in the value of whisky import.[1]

Whisky is distinctively defined depending on the region. In Brazil, whisky is defined in Normative Instruction n° 15 of 31/03/2011, of the Ministry of Agriculture, Livestock and Supply (MAPA), as a distilled beverage with an alcohol content of between 38-54% v/v at 20.0 °C, obtained from simple alcoholic distillation of aged grains, partially or fully malted.[2]

Concomitantly, the production and consumption of sugarcane spirit is expanding in Brazil. It is worth pointing out that it is now the second most consumed beverage in the country and the third in the world[3,4]. According to the Brazilian legislation of the Ministry of Agriculture, Livestock and Supply (MAPA), sugarcane spirit is defined by the Normative Instruction nº. 13 of 29/06/2005, as a distilled beverage with an alcohol content of 38-54% v/v at 20.0 °C, obtained from simple alcohol distilled from sugarcane or by the distillation of fermented mash of sugarcane.[5,6]

The increased consumption of whisky and sugarcane spirit, coupled with the increasing imports of whisky and sugarcane spirit exports, require that the manufacturing process of these drinks are based on some carefully enforcing underlying practices so as to obtain a standardized product and proven quality in their physicochemical and sensory aspects[7]. General aspects of quality of these drinks require the performance of physical-chemical analysis for the designation of a good quality drink. Among these tests, the copper content is of fundamental importance in the final quality of the product, a maximum of 5.00 mg L^{-1} in whiskys and spirits is allowed, in accordance with the national legislation[2,6]. In countries like the United States and the European community, the law is even stricter, and the maximum permitted level for copper is 2.00 mg L^{-1}.[8-10]

Both sugarcane spirit as well as whisky are usually produced in copper stills, which gives better sensory quality, functioning as a kind of catalyst for the drink distillation process in order to reduce the acidity and levels of aldehydes and sulfur compounds. Such compounds, confer to the drink flavor and unpleasant odor, yielding a low organoleptic quality beverage when compared to stills made of other materials such as stainless steel, aluminum and porcelain. However, the product may be contaminated with copper when being managed, especially when the still of the cleaning process is inadequate[10,11]. When the constituent metal of the internal walls of the still (copper) is exposed to moist air containing carbon dioxide, it slowly oxidizes being covered by a green layer - copper(II) dihydroxycarbonate ($[CuCO_3Cu(OH)_2]$), commonly called "verdigris".

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This compound can be dissolved in the alcoholic beverage of acid vapors, thus contaminating the beverage with copper.[12,13]

The excess copper from the body causes interference in the catalytic activity of some enzymes causing a number of diseases such as aceruplasminemia, sclerosis, rheumatoid arthritis and melanoma. Furthermore, it is suspected that the excess copper is also related to the onset and the attenuation of various neurodegenarative diseases such as Wilson disease, Menkes disease and Alzheimer's disease. Thus, it is of great importance and of essential need to develop reliable analytical methods for the determination of copper ions in these drinks.[12,14-19]

Currently, the development of analytical methods, quantity, toxicity of the reagents or solvents used and the waste generated are quite important as any other analytical feature. Most analytical methods described for the determination of copper ions require laborious and lengthy procedures, either in sample preparation or during the analysis, and some use large amounts of solvents, thereby generating large amounts of waste, which falls outside the standards proposed by the Green Chemistry.[20,21]

Nonetheless, a very simple technique for the quantitative determination of metal ions with chromogenic agents and which provides the cleanest methods needed is the use of spot tests, which have been studied in spectroscopy determinations by diffuse reflectance in the visible region, using filter paper as the solid support.[22-24]

The combination of spot test and diffuse reflectance spectroscopy offers many advantages, among them including the ease of implementation, low reagent consumption and small production waste. However, the most attractive merit of this technique lies in the fact that the diffuse reflectance measurements can be made "in situ" using portable spectrometers available on the market, which are small, lightweight, cheap, and can be above all battery- operated. Features that largely facilitate the analyses of the system under investigation, thus reducing the time for obtaining the results and almost nullifying the problems related to sampling.[25]

Considering the importance of quality control in whiskys and sugarcane spirits relative to the amount of copper(II), and the advantages in using the combination spot test-diffuse reflectance spectroscopy for the quantitative determination of metal ions with chromogenic agents, this work aims to create a cleaner, and therefore more environmentally friendly alternative for the determination of copper(II) ions in whisky and sugarcane spirit using the reaction with the chromogenic agent 1-(2-pyridylazo)-2-naphthol with filter paper as the solid support.

2. Experimental

2.1. Instruments

The diffuse reflectance measurements were collected using a hand-held integrating sphere (ISP-REF, Ocean Optics, Dunedin, USA) connected to a fiber optic to a portable spectrometer (USB2000, Ocean Optics, Dunedin, USA). The portable spectrometer USB2000 is equipped with a 2048 pixels Sony ILX511 CCD array detector. For the acquisition and storage of spectra, the software Spectra Suite (Ocean Optics) was also used.

2.2. Reagents and solutions

Whatman 42 qualitative filter paper was used as solid support. To prepare the solution of the PAN reagent 0.12% m/v in ethanol, the reagent 1-(2-pyridylazo)-2-naphthol (Merck®, purity 99.0%) and ethanol, HPLC grade (JT Baker®, purity 99.9%) were used. The stock standard solution of copper(II) ions was prepared from trihydrate copper nitrate(II) salt (Synth®, purity 98.0%) in the concentration of 1000 mg L^{-1} in water and stored under refrigeration. Working standard solutions were obtained through the appropriate dilution of the stock solution of copper(II) ions. All solutions were daily prepared.

2.3. Optimization of the reaction conditions

2.3.1. Order of addition of reactants

The order of addition of the reagents into the filter paper can directly affect the quality of the stain obtained, thus interfering in the accuracy and precision of the spot test measurements. Hence, in order to obtain a more uniform staining pattern, we assessed the influence of the order of addition of the reagents in the spot tests. For the spot test reaction, the solutions were added at the center of 2.25 cm^2 qualitative filter paper (Whatman 42), using a micropipette fixed in a holder according to the procedure described by Tubino et al.[24]. The orders of addition of the reagents studied included the following:

- 30 uL of chromogenic agent PAN solution at 0.10% m/v ethanol and 30 uL of alcohol solution 40% v/v of copper(II) ions with 5.00 mg L^{-1} at pH 5.00;

- 30 uL of alcohol solution 40% v/v of copper(II) ions with 5.00 mg L^{-1} at pH 5.00 and 30 uL of chromogenic agent PAN solution at 0.10% m/v in ethanol;

- 30 uL of chromogenic agent PAN solution at 0.10% m/v in ethanol, waiting to dry, and 30 uL of alcoholic solution 40% v/v of copper(II) ions with 5.00 mg L^{-1} at pH 5, 00;

- 30 uL of alcohol solution 40% v/v of copper(II) ions with 5.00 mg L^{-1} at pH 5.00, waiting to dry, and 30 uL of chromogenic agent PAN solution at 0.10% m/v ethanol.

2.3.2. Reflectance spectrum

The reflectance measurements were analyzed so as to obtain a spectrum of the complex and of the chromogenic reagent in the visible region (400 to 700 nm). The solutions used in the experiment were: Alcoholic solution 40% v/v of copper(II) ions with 5.00 mg L^{-1} at pH 5.00 and chromogenic reagent solution PAN at 0.10% m/v in ethanol. The substances added included the following: 30 uL chromogenic reagent solution PAN at 0.10% m/v in ethanol, waiting to dry, and 30 uL alcoholic solution 40% v/v of copper(II) ions with 5.00 mg L^{-1} at pH 5.00 to obtain the complex spectrum. For the chromogenic spectrum agent, 30 uL of the chromogenic reagent PAN solution at 0.10% m/v in ethanol was added, waiting to dry, and alcoholic solution of 40% v/v at pH 5.00.

2.3.3. Alcohol content assessment of the copper(II) solution

The alcohol content of the copper(II) solution was evaluated by varying the concentration of ethanol in the solution gradually from 35 to 55% v/v, within the limits of alcoholic percentage of whisky and sugarcane spirit ranging from 38-54% v/v. Therefore, the solutions used were: alcohol solution concentration to be evaluated at pH 5.00

of copper(II) ions with 5.00 mg L^{-1} and chromogenic reagent PAN solution at 0.10% m/v in ethanol, added as follows in the topic 2.3.2. To obtain the blank solid, 30 uL of the chromogenic reagent PAN solution at 0.10% m/v in ethanol was added, waiting for it to dry and, 30 uL alcohol solution with a percentage measured at pH 5.00 and measurement of reflectance at 559 nm.

2.3.4. Concentration of the chromogenic agent in the complex formation

The influence of the concentration of chromogenic agent PAN for the complex formation was assessed by varying the concentration of the chromogenic agent PAN in separate experiments from 0.01 to 0.20%, ranging from 0.02% m/v in ethanol. Thus, for this study, the solutions used included alcoholic solution at a concentration of 40% v/v at pH 5.00 with a concentration of copper(II) ions of 5.00 mg.L^{-1} and PAN chromogenic reagent solution in ethanol at the concentration being studied, which was added as follows in the previous topics and measured reflectance at 559 nm.

2.3.5. Chemometric study of pH and time reaction

To evaluate the influence of the pH of the alcoholic solution of analyte and time in the complex formation reaction in the spot test, we used a chemometric study of response surface analysis[26] with the aid of a computer program (Statistica version 8.0, USA), and the system response for the change in absorbance (ΔAR). The minimum (-) and maximum (+) levels of factorial design 2^2, star type with center point were chosen, the respective values were: 2.00 and 6.00 for the pH of 5 and 20 minutes for the reaction time. An intermediate point was developed in the pH ranges and time. For the purpose of this study, the alcoholic solution was maintained at 40% v/v of copper(II) ions at 5.00 mg L^{-1} and PAN solution of 0.12% m/v in ethanol. The form of addition was the same as in previous experiments done and read after 559 nm, having a reagent blank made the same as reference.

2.3.6. Evaluation of optical stability

To evaluate the stability of the optical chromogenic agent and of the complex in the spot test in relation to the reaction time, a kinetic monitoring of the value (ΔAR) was put in place with readings taken every 5 minutes until completing 120 minutes. For the evaluation of the complex formed, the alcohol solution was maintained at 40% v/v of copper(II) ions at 5.00 mg L^{-1} and PAN solution of 0.12% m/v in ethanol. For the assessment of the chromogenic agent, monitoring of the measurements was evaluated by impregnating the spot test only with the solution of the chromogenic agent PAN 0.12% m/v in ethanol with the reflectance readings made at 480 nm, a maximum wavelength of PAN for 120 minutes and for a week in order to verify the possibility of storing the impregnated spot tests with the chromogenic agent for later use. The form of addition was the same made in previous experiments and readings held at 559 nm.

2.4. Validation of proposal methodology

2.4.1. Interfering study

According to Sandell and Onish[27] the metal ions cadmium(II), cobalt(II), lead(II), iron(III), manganese(II), nickel(II) and zinc(II) react with PAN to form very stable complexes. These ions can be present in sugar cane spirits and have their amounts controlled by law enforced by the National Health Surveillance Agency (ANVISA)[28]. This study was evaluated under two distinct levels, namely the minimum and the maximum concentration for all ions involved and for the total set of ions under the same experimental conditions previously set. For the copper(II) ions, the concentrations studied were 0.50 and 5.00 mg L^{-1}, 0.50 and 5.00 mg L^{-1} for cadmium (II), 0.50 and 5.00 mg L^{-1} for cobalt(II), 0.20 to 2.00 mg L^{-1} for lead (II), iron(III) 0.30 to 3.00 mg L^{-1}, manganese(II) 0.10 and 1.00 mg L^{-1}, nickel(II) 0.30 to 3.00 mg L^{-1} and zinc(II) 0.50 to 5.00 mg L^{-1}. The order of addition of the reagents in the spot test was the same as that of previous experiments, the readings were made at 559 nm, having a reagent blank added in the same pattern as the reference.

With the results of the reflectance measurements, the interference factor (I.F.) was then calculated and based on that we went further to determine whether the ion studied is in fact an interfering element or not. The interference factor is calculated by mathematical reason of the analytical signal variation of copper(II) ion and the ion interference by the analytical signal variation of copper(II) ion alone.[29]

2.4.2. Study of reduction and/or elimination of the interference effect

A proposal to prevent or reduce the interference effects caused by metal ions lies in the use of chemicals that react with these ions and not those of copper(II), and such substances are called masking agents. For cadmium(II) ions, cobalt(II), iron(III) and nickel(II) the use of malonic acid is proposed, while the use of sodium triphosphate (pentasodium tripolyphosphate or sodium tripolyphosphate) is proposed for lead(II) ions, manganese(II) and zinc(II)[30]. Thus for assessing the effect of such masking agents applied to the respective ions, a concentration of 0.20% m/v was set for both masking agents. For the ions, the concentrations studied were the same as the previous experiment. An evaluation was also made using the two together in the proposed masking combination of concentrations of all the ions together. With the results of reflectance measurements, the interference factor was calculated and based on that we were able to determine whether or the masking tape used is indeed or not decreasing the effect of interference caused by the metal ion. All the experiments were performed in optimal conditions as aforementioned.

2.4.3. Optimization of the concentration of masking

The concentration of the masking agents was optimized in the set of ions that are specific to each masking agent and for the total set of ions. The concentration range studied was 0.05 to 0.25% m/v in solution with the other conditions kept according to the previous item. For the total set of interfering ions, the copper(II) concentration was made to remain constant at 0.50 mg L^{-1} and cadmium(II) ions at 5.00, cobalt(II) 5.00, lead(II) 2,00, iron(III) 3.00, manganese(II) 1,00, nickel(II) 3.00 and zinc(II) 5.00 mg L^{-1}. With the results of reflectance measurements, the interference factor was calculated (which aided in helping us draw a conclusion as to whether or not the concentration of the masking agents used is in fact decreasing the effect of interference caused by the metal ion.

2.4.4. Analytical calibration curve

The calibration curve was obtained by varying the concentration of copper(II) ions in the alcoholic solution of 40% v/v pH 5.00 of 1.00 to 10.00 mg L^{-1} with the concentration of malonic acid and sodium triphosphate constant at 0.25% m/v in the solution and keeping the concentration of the chromogenic agent PAN at 0.12% m/v in ethanol and added in the same way as in previous experiments. Absorbance values were obtained at 559 nm and using a reagent blank as a reference.

Tests were then made to verify the repeatability of the method within the same day (intraday) and between different days (interday). To this end, it is noteworthy to point out that three experiments were done repeating three points within the linearity of the calibration curve, 1.00; 5.00, 10.00 mg L^{-1}, two of these experiments performed on the same day and at different times while the third one was done on the following day in order to calculate the relative standard deviation (RSD).

2.4.5. Precision and accuracy of the method

The precision and accuracy of the proposed method was evaluated by analyzing five synthetic samples simulated in the laboratory. These samples were made with an alcohol content of 40% v/v with known concentrations of 0.60; 1.00; 3.00; 5.00; 7.00 mg L^{-1} copper(II) ions. Synthetic samples were analyzed by the proposed method (P.M.) and compared with the reference methodology (R.M.) by Association of Analytical Chemists (AOAC).[31]

For the proposed method, a rate of 20.00 ml of sample was pipetted into a volumetric flask of 25.00 mL, it was in turn added to a volume of 1.25 ml of masking malonic acid and sodium tripolyphosphate, both 5.00% m/v, this enabled the two masking agents to be kept at a concentration of 0.25% m/v solution. After that, 2.00 mL of absolute ethanol was finally added while the remaining space of the volumetric flask was completely filled with deionized water. The solution was subsequently analyzed in the previously optimized conditions and all the calculations of concentration were made.

By the AOAC reference method[31], the synthetic spirits samples were analyzed as described in the guidelines using a Varian® equipment mark model 50 SpectrAA hollow cathode lamp for copper Varian®, wavelength of 324.8 nm, air-acetylene flame.

All analyses were carried out in five replications and compared in analytical concentration curve for both methods.

2.5. Determination of copper(II) ions in samples of whiskey and brandy of commercial sugarcane

Five samples of whiskey and ten samples of spirits distilled from sugarcane brands commonly sold in local supermarkets were analyzed. The alcohol content of the samples were determined with a specific hydrometer for alcohol (alcoholometer).

The copper(II) content was analyzed by the method proposed in this study and compared with the AOAC standard method.[31]

The experiments for the analysis by the standard and propose methodologies, were performed in the same conditions as described above with the synthetic samples.

3. Results and discussion

3.1. Optimization of the reaction conditions

3.1.1. Order of addition of reactants

Analyzing the spots tests, it was observed that by adding the first PAN, without drying, no intense uniform spot was obtained compared to the others. This fact can be explained by the addition of alcoholic solution of the copper(II) portion of the eluted PAN on the edges of the spot test where few chromogenic agents were left to react with the copper(II) ions.

The elution effect is found to be nullified upon the addition of the PAN reagent solution of copper though a non-uniformity of the spot is observed. One can see an orange color at the center of the stain (relative to the chromogenic agent PAN) which did not react with the copper(II) ions, indicating incomplete reaction of the PAN with the copper ions.

Lastly, for the purposes of evaluating the drying effect, the PAN was added after the copper solution got dried in the spot test, the stain then presented a more uniform and intense character, thus demonstrating to be the most efficient form of addition. This became, therefore, the reagents addition form that was chosen to run the reaction.

3.1.2. Reflectance spectrum

The spectrum of the compound coordination $Cu(PAN)_2$, shows a maximum wavelength at 559 nm (Figure 1), in contrast with the region of minimum of the chromogenic agent PAN, which has its maximum wavelength at 480 nm. Therefore, this wavelength shows higher variation of the signal reflectance (ΔAR), and was as such adopted for the remaining studies.

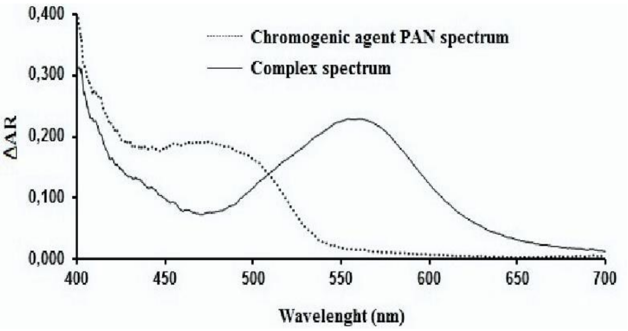

Figure 1. Reflectance spectrum in the range of 350-700 nm, chromogenic agent PAN and complex.

3.1.3. Alcohol content assessment of the copper(II) solution

As the whiskey and brandy of sugarcane are practically a mixture of water and ethanol (38-54% v/v), we studied the influence of the alcohol content of the copper(II) solution. We observed the highest reflectance signals at the alcohol concentrations in the range of 35 to 40% v/v. As the alcohol there increases, a decrease is seen in the signal though not in a larger extent, starting from 45% v/v then remaining practically constant up to the alcohol concentration of 55% v/v. This decrease in the signal observed in relation to the increase in the alcohol content occurs probably owing to a greater elution of the chromogenic PAN elution agent that was previously impregnated in the spot test, as such a smaller amount of the PAN reagent reacted with the copper ions causing a slight drop of the analytical signal.

Given the results obtained, we adopted the alcohol concentration of 40% v/v for further studies once this concentration apart from its good rate of absorption and the fact that it does not present any significant difference compared to the concentration of 35% v/v, it is the average concentration of alcohol of most brands of whiskys and sugarcane spirits found in the market.

3.1.4. Concentration of the chromogenic agent in the complex formation

Knowing that the quantification of copper(II) is carried out by the product of this reaction with the chromogenic PAN agent, the PAN concentration was hence optimized varying its concentration in the ethanol solvent. As the concentration of the PAN increases, a positive change is seen in the reflectance signal to approximately 0.10% m/v concentrations of PAN solution in ethanol, from which a stabilization is observed in the value of the signals, with a slight drop of 0.20% m/v. Given these results, the conclusion that can be drawn is that sensitivity does not increase significantly for PAN concentrations above 0.10% m/v. Thus, the concentration of PAN of 0.12% m/v in ethanol was chosen to conduct the studies, to present an absolute value of appropriate reflectance signal and a considerable gain in sensitivity, in addition to being in the middle of the concentration range of the interval. It is in that regard that any concentration of this small signal variation, either up or down, is bound not to cause any significant difference.

3.1.5. Chemometric Study of pH and reaction time

In order to check the influence of the variables, namely, the pH of the alcoholic solution of copper(II) ions as well as the time for the formation of the reaction product, aiming at obtaining greater variation of analytical signal, that is, maximum sensitivity for the determination of copper(II) ions, a chemometric study was carried out with the factorial design of 2^2, a star type, with center point. Table 1 presents the conditions studied and the results of the reflectance measurements at 559 nm.

Table 1. Factorial design (2^2, star-type with central point) used for the optimization of the variables pH and time of reaction.

Variable				Levels		
				(-)		(+)
pH (UpH),	pH			2		6
Time (min),	t			5		20
Order of execution	**Scaled variables**			**Results**		
	pH	**t**		**Cu(PAN)₂**	**PAN**	**ΔAR**
2	+1	-1		0.197	0.011	0.186
9	0	0		0.200	0.007	0.193
3	-1	+1		0.107	0.008	0.099
6	-1,41	0		0.042	0.006	0.036
7	0	+1,41		0.203	0.007	0.196
8	0	-1,41		0.214	0.008	0.190
1	-1	-1		0.099	0.009	0.090
4	+1	+1		0.201	0.012	0.189
5	+1,41	0		0.220	0.007	0.156

For the experiment, we selected two levels (maximum (+) and minimum (-)) for each variable. The minimum level for the pH was chosen considering a minimum safely measured by the electrode. On the other hand, the maximum level of pH was chosen considering that for the pH values higher than 6.00 there is a destabilization of the complex formed, probably due to the formation of copper(II) hydroxide ($Cu(OH)_2$) in solution.

For the time variable, the minimum level was chosen considering a minimum time for drying the spot test while the reaction is being processed. For the maximum level, a reasonable period was chosen for the reaction to be conducted completely, when and if it occurs over time, and to have a reasonable analytical frequency.

Through the association of ΔR values and values of variables spaced pH (pH, UpH) and time (t, min) in Table 1, the mathematical model that gives a clear description of the system was found (Equation 1).

$$\Delta AR = -0.1061 + 0.1227pH + 0.0011t - 0.0124pH^2 - 1.4691.10^{-5}\,t^2 - 0.0001pHt$$

Equation 1

With the mathematical model in hand (Equation 1) the response surface was constructed with the aid of a graphical computer program (Statistica, version 8.0, USA).

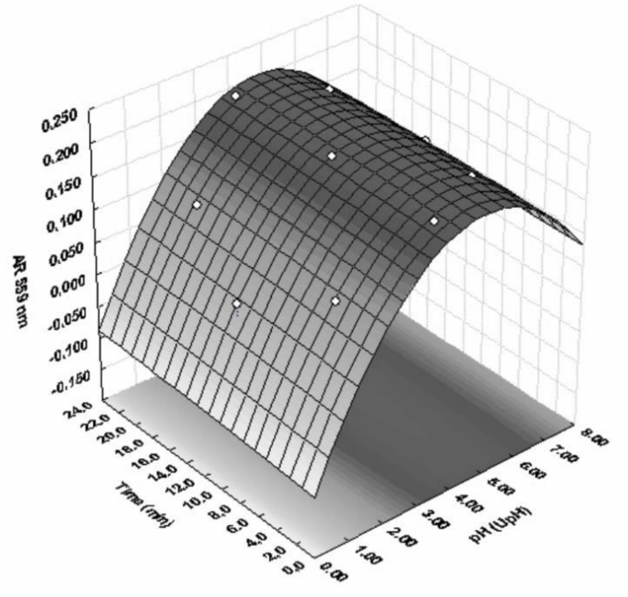

Figure 2. Response surface graph for the influence of the variables pH and time on the formation of the complex.

The mathematical model (Equation 1) through the interaction term between the pH and reaction time (0.0001pHt) shows that both are practically independent. The response surface and its contour lines show that at pH around 5.00, the higher the formation of the complex, the higher the likelihood of the maximum signal undergoing a change (ΔAR). The reaction times do not interfere with the increased sensitivity. Thus, in the pH conditions of 5.00 and the time of 5 minutes, there will be an increased sensitivity in the determination of copper(II) ions and as such these conditions have been set for further studies.

3.1.6. Evaluation of optical stability

In order to evaluate the optical stability of chromogenic agent PAN and PAN formed complex with copper ions in the spot test, over time, there was a kinetic monitoring of the reflectance signal value every 5 minutes to complete 120 minutes.

The results show that the chromogenic agent PAN has no significant variation signal showing optical stability for 120 minutes, losing their color strength characteristics and is not degraded by the time period studied. Therefore, the spot test impregnated with the chromogenic agent PAN can be used up to 120 minutes after the addition.

In monitoring the optical stability of the chromogenic agent PAN during the period of a week, a gradual decrease was observed in the signal over some days, losing their color intensity coupled with the degradation of their characteristics. Thus, it is concluded that no viable storage of spot tests was found impregnated with the chromogenic agent PAN for later use aimed at the reaction with copper(II) ions.

The results for the complex formed demonstrate that the product reaches its optical stability following 5 minutes of the addition of a copper(II) ions solution, showing a constant analytic signal for 120 minutes. Therefore, the reading of the spot test impregnated with the product of the reaction between chromogenic agent PAN and copper(II) ions can be taken up to 120 minutes later after the addition of analytical reagents without signal loss.

3.2. Methodology validation proposal

3.2.1. Interfering Study

By means of the interference factor calculation, we are able to assess whether cadmium(II) ions, cobalt(II), lead(II), iron(III), manganese(II), nickel(II) and zinc(II) as well as the mixture of all these metallic ions are in fact interfering factors and if they cause any effect when it comes to the determination of copper(II) ions by the proposed methodology.

From the values of the calculated interference factors and presented in the tables for each individual ion and for the whole set of these ions, the interference factors showed all the values lying outside 0.95 and over 1.05, ie above the threshold percentages tolerable (5.0%). Thus, it is possible to observe a significant effect of interference, in other words, the combination of them actually exerts any amount of interference. This interference effect is enhanced by increasing the concentration of interfering ions and also when the concentration of copper(II) ions is at its lowest level.

3.2.2. Study of reduction and/or elimination of the interference effect

An alternative towards the reduction and/or elimination of the interference effects of these metal ions lies in the use of masking. To mask the metal ions cadmium(II), cobalt(II), iron(III) and nickel(II), Perrin proposes the use of malonic acid, while for the lead(II) ions, manganese(II) and zinc(II), he proposed the use of sodium triphosphate.[31]

For the studied concentration of 0.20% m/v of masking malonic acid and sodium triphosphate, a great reduction is seen in the interference effects of all the metal ions though some of the interference effects were found to be within tolerable limits (between 0, 95 and 1.05). It is also observed that some of these interference effects are still above the allowable limit though the potential of the masking reagents used is undeniably seen as capable of reducing the interference levels and acting as real masking agents for the proposed method by virtue of the fact that an increase in concentration of these masking agents in the reaction medium may exert a vital role in reducing the interference levels of the metal ions studied as well as the set of all of them within tolerable limits, around 5.00%.

3.2.3. Optimization of the concentration of masking

Aiming at further reducing the effects of interference posed by the metal ions analyzed and setting them within tolerable limits, the concentration of masking malonic acid and sodium triphosphate was evaluated both separately and together in solution.

Masking malonic acid and sodium triphosphate when employed together also cause a positive change in the measure of the decrease in interference effect when their concentration is increased. Nonetheless, the concentration of 0.25% m/v for each of the masking agents was found to be sufficient enough to reduce the interference factor to 0.98, representing an interference of only 2.0% and being within tolerable interference parameters.

3.2.4. Analytical calibration curve

The analytical curve of calibration has to be linear up to a concentration of 10.00 mg L^{-1} and is described by the equation $\Delta AR = 0.0121\ C_{Cu(II)} + 0.0038$ ($R^2 = 0.9982$).

The method has a detection limit of 0.12 mg L^{-1} and a quantitation limit of 0.47 mg L^{-1}, calculated in relation to the standard deviation of the reagent blank, having a linear working range of 0.47 mg L^{-1} to 10.00 mg L^{-1}.

The relative standard deviation of the calibration curve of intraday calibration was 1.82% and 2.64% interday.

3.2.5. Precision and accuracy of the method

The precision and accuracy of the method was evaluated by doping synthetic samples synthesized in the laboratory. The recovery results, standard deviation, statistic test “F” and "t" are all displayed in Table 2.

Table 2. Recoveries, standard deviations, statistic tests “t” and “F” and relative errors obtained for the measurement of copper(II) ions in synthetic simulated samples of sugarcane spirit.

Synthetic samples	[Cu(II)] (mg L^{-1}) (R.M.)	[Cu(II)] (mg L^{-1}) (P.M.)	Test “F”[a]	Test “t”[b]
0.60	0.57 ± 0.03	0.55 ± 0.05	0.3538	1.2591
1.00	0.96 ± 0.04	0.98 ± 0.07	1.8158	0.8231
3.00	2.95 ± 0.07	2.98 ± 0.08	0.7433	0.1010
5.00	5.01 ± 0.04	5.04 ± 0.12	0.2779	0.3754
7.00	7.04 ± 0.01	6.99 ± 0.03	0.7176	1.8898

[a] Test “F” performed at 5% probability level, n=5
Critical value of “F” at 5% probability level = 5,050.
[b] Test “t” performed at 95% confidence level, n=5;
Critical value of “t” at 95% confidence level = 2,776.

For the concentrations of copper(II) ions evaluated in the synthetic samples of sugarcane spirit, all results were statistically similar falling below the standard value and error within the acceptable range (≤ 5.00%). The recoverable amounts showed good accuracy, precision, thus making it a reliable methodology.

Table 3. Concentrations of copper(II) ions in commercial samples of whisky.

Sample	Alcohol content (% v/v)	Type	[Cu(II)] (mg L^{-1}) (reference method)	[Cu(II)] (mg L^{-1}) (proposed method)	Test "F"[a]	Test "t"[b]
1	40.0	Blend/Scoth	0.42 ± 0.02	0.47 ± 0.06	1.0158	1.1531
2	40.0	Blend/Scoth	0.62 ± 0.01	0.58 ± 0.08	0.7778	1.7925
3	40.0	Blend/Scoth	0.34 ± 0.01	0.36 ± 0.07	1.3333	1.8898
4	40.0	Blend/Scoth	0.24 ± 0.02	0.21 ± 0.02	1.2857	2.2942
5	39.0	Blend/Scoth	0.76 ± 0.03	0.71 ± 0.04	1.7500	2.5925

[a] Test "F" performed at 5% probability level, n=5
Critical value of "F" at 5% probability level = 5,050.
[b] Test "t" performed at 95% confidence level, n=5;
Critical value of "t" at 95% confidence level = 2,776.

Table 4. Concentrations of copper(II) ions in commercial samples of sugarcane spirit.

Sample	Alcohol content (% v/v)	Region (city/state)	[Cu(II)] (mg L^{-1}) (reference method)	[Cu(II)] (mg L^{-1}) (proposed method)	Test "F"[a]	Test "t"[b]
1	45.0	Brotas/SP	2.90 ± 0.04	2.85 ± 0.06	2.9626	2.2469
2	39.0	Pirassununga/SP	0.52 ± 0.03	0.54 ± 0.07	1.8746	0.9818
3	40.0	Jaú/SP	4.30 ± 0.06	4.22 ± 0,04	0.6587	2.3983
4	49.0	Jaú/SP	4.39 ± 0,01	4.33 ± 0.03	0.8704	2.0591
5	39.0	Santa Rita Passa do Quatro/SP	0.99 ± 0.02	0.97 ± 0.05	1.1200	1.7143
6	39.0	Pirassununga/SP	0.67 ± 0.04	0.68 ± 0.08	4.6908	0.3388
7	39.0	Rio Claro/SP	1.15 ± 0.02	1.19 ± 0.01	2.6133	2.5762
8	38.0	Pirassununga/SP	0.46 ± 0.03	0.48 ± 0.09	3.4814	1.1749
9	38.0	Patrocínio Paulista/SP	n.d.	n.d.	-	-
10	39.0	Jundiaí/SP	n.d.	n.d.	-	-

[a] Test "F" performed at 5% probability level, n=5
Critical value of "F" at 5% probability level = 5,050.
[b] Test "t" performed at 95% confidence level, n=5;
Critical value of "t" at 95% confidence level = 2,776.

4. Determination of copper(II) ions in brandy samples of commercial sugarcane

Five samples of whisky along with ten samples of commercial sugarcane spirit marketed in the supermarkets and specialty stores were used for the application of the proposed method. The results of the concentrations are shown in Tables 3 and 4.

Comparing the two methods, you can see a slight difference in results in the concentration of copper(II) ions in the samples. This difference is within acceptable reliability standards, showing that the proposed method has good accuracy and precision. It is worth noting that taking all the analyzed samples of whisky and sugarcane spirit into consideration, none had levels of copper(II) ions above those permitted by Brazilian law, thus showing a proper care and cleaning of stills used in the production of spirits distilled from sugarcane. However, the samples 1, 3 and 4 of sugarcane spirit via the proposed method presented levels above tolerable limits of copper(II) ions under the legal framework of the United States and the European Union (2.00 mg L^{-1}), being as such unfit for export.

5. Conclusions

This work proposed a new method for the determination of copper(II) ions in whisky and sugarcane spirit, using the diffuse reflectance spectroscopy and applying the spot tests technique. The advantages that this methodology presents include the economy of reagents, low waste generation, use of very low toxicity solvents, rendering the method cleaner, simple, fast, economical and portable. The proposed method has the advantages of relatively low cost of equipment and maintenance when compared to the equipment required for the application of the reference method. In addition, it features low cost of analysis owing to the fact that there is no use of gas as in the reference methodology. A further merit associated to this method has to do with the possibility of carrying out "in situ " measurements once the apparatus for diffuse reflectance measurements can be carried anywhere and connected to mobile computers and alternative energy sources such as batteries, which is impossible for an atomic spectrophotometry equipment that requires a reserved specific location for the analyses, aside presenting great volume and the need for it to be connected to a source of gas for flame power.

We observed good limits of detection and quantification - 0.12 and 0.47 mg L^{-1}, respectively, for the determination of copper(II) ions. The method exhibited good analytical sensitivity, good repeatability and according to statistical tests, good precision and accuracy.

Commercial samples of whisky and sugarcane spirit did not show levels of copper(II) ions above the limit permitted by Brazilian law. However, some sugar cane brandy samples are with copper(II) ions levels above the level allowed by the legislation applicable in the United States and the European community, and thus being considered unfit for export.

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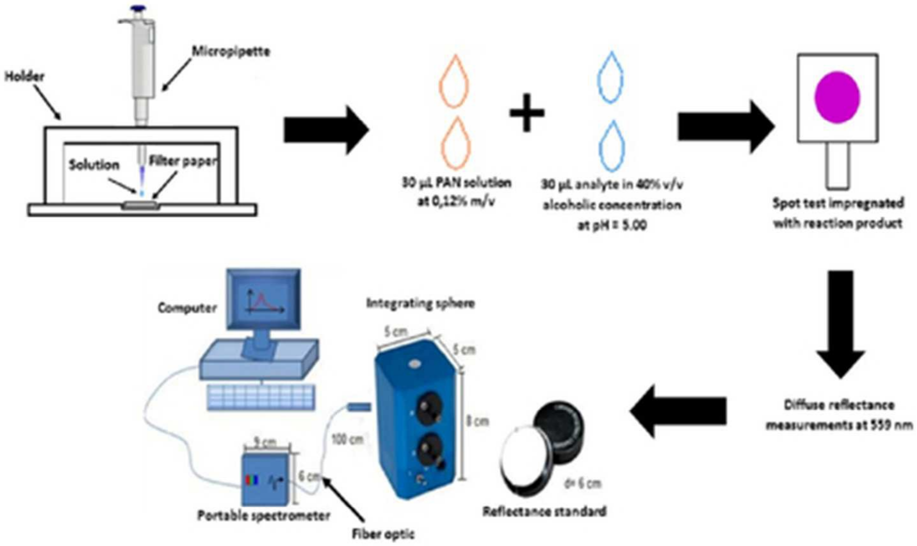

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