

# Spectrometric Method for Copper (II) Determination at Trace Level

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A rapid, reliable and inexpensive method for the direct determination of Cu (II) with the organic reagent Solochrome Yellow 2GS (disodium salt of 2-hydroxy-5-[(4-sulfophenyl)azo]-benzoic acid), **R** is reported. In a 4-fold excess over Cu (II) concentration the organic reagent, **R** form with Cu (II) within pH range 6.30–9.43 an 1:2 (Cu:**R**) stable complex. The value of the total stability constant  $\beta_3 = 1.58 \cdot 10^6 \pm 0.4 \cdot 10^6 \text{ L}^2 \cdot \text{mol}^{-2}$  was determined, which indicates the formation of a rather stable complex. On this basis a spectrometric method for the quantitative determination of copper (II) at traces level was proposed. The method is sensitive, accurate and reproducible, being characterized by a detection limit of  $0.10 \text{ mg} \cdot \text{L}^{-1}$ , a linearity range between  $0.12 - 5.12 \text{ } \mu\text{g} \cdot \text{mL}^{-1}$ , a recovery between 93.27% and 95.49% as well as a good repeatability and reproducibility, characterized by relative standard deviations of 3.333% and 1.392%, respectively. The method was applied in order to determine the copper contents in samples of drinking water and wine.

**Keywords:** copper, organic reagent, determination, spectrometric study

Copper is an essential element having a complex role in hemoglobin synthesis, connective tissue development, normal function of the central nervous system, and oxidative phosphorylation. Excessive intake of copper would lead to its accumulation in liver cells and hemolytic crisis and neurological disturbances [1]. This microelement is present in water, food and in biological samples at trace level concentrations [2]. With the aim of preserving public health, the Commission of European Communities has established the list, the concentration limits, and labeling requirements for the constituents of natural mineral water [3]. The maximum concentration admitted for copper in drinking water is 1.0 mg/L, but 2.0 mg/L is also accepted in water samples analyzed if the distribution is made of copper components [4–6].

Thus, the determination of trace amounts of copper is becoming increasingly important because of the interest in environmental pollution. As a consequence, there are many analytical methods for copper determination in water, food or environmental samples: flame and graphite furnace atomic absorption spectrometry [7–10], inductively coupled plasma optical emission spectrometry [11–13], voltametry [14, 15], potentiometry [16]. Despite of the instrumental improvements of the last decades, the UV-VIS spectrometric methods are widely used because they are cheap, sensitive and simple to implement in laboratories [17]. They involve lesser expensive instrumentation and provide high sensitivity when appropriate chromogenic reagents are available. In view to obtain stable complex combination of copper many types of ligands were used, i.e.: dithizone, dithiocarbamate, cuproine, cuprozone [18–20], 5-bromo salicylaldehyde thiosemicarbazone [21], chloro(phenyl) glyoxime [22], acetophenone-*p*-chlorophenylthiosemicarbazone [23], sodium(I)diethyldithiocarbamate [24], 1,5-diphenylcarbazone [25]; disodium-6-hydroxy-5-[(4-sulphophenyl)azo]-2-naphtalenesulphonic acid (Sunset Yellow FCF) [26], pyrocatechol violet [27], 5-(4-sulphophenylazo)-8-aminoquinoline [28], N,N'-bis(Salicylidene)-methinmethyl-diamine [29].

The organic reagent disodium salt of 2-hydroxy-5-[(4-sulfophenyl)azo]-benzoic acid, noted hereinafter **R**, is known under the commercial name of Solochrome Yellow 2GS whose formula is presented in figure 1. It can be considered a Salicylic Acid derivative. The stability constants for complexes of some substituted salicylic acids formed with different cations have been reported in literature [30–34].

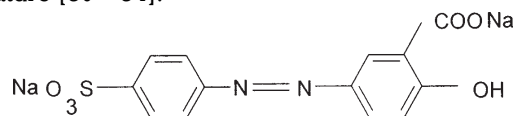


Fig. 1. The formula of the organic reagent **R**.

This paper reports the results of a spectrometric study on the copper behaviour in aqueous solutions of 2-hydroxy-5-[(4-sulfophenyl)azo]-benzoic acid disodium salt. Based on the experimental data a new spectrometric method for copper concentration determination at trace level was established. The proposed method was validated and applied on samples of drinking water and wine.

## Experimental part

### Reagents

All chemicals used were analytical reagent grade: Solochrome Yellow 2GS (Leeds, U.K.) and aqueous solution  $10^{-3} \text{ M}$ ; copper (II) sulphate (Merck) and aqueous solution  $10^{-3} \text{ M}$ ; iron (III) chloride (Merck) and aqueous solutions  $10^{-3} \text{ M}$  and  $5 \cdot 10^{-4} \text{ M}$ , both in  $\text{HCl } 10^{-1} \text{ M}$  solution; nickel (II) chloride (Merck) and aqueous solution  $10^{-3} \text{ M}$ ; cobalt (II) nitrate (Merck) and aqueous solution  $10^{-3} \text{ M}$ ; uranyl nitrate (Merck) and aqueous solution  $10^{-3} \text{ M}$ ; lead (II) nitrate (Merck) and aqueous solution  $10^{-3} \text{ M}$ ; hydrochloric acid (Merck) 30%,  $\rho = 1.15 \text{ g/cm}^3$  and aqueous solution  $10^{-1} \text{ M}$ ; sodium hydroxide, Titrisol (Merck) 1 M and aqueous solution  $10^{-3} \text{ M}$ ; barium chloride dihydrate (Merck) and aqueous solution  $10^{-3} \text{ M}$ ; calcium carbonate (Merck) and aqueous solution  $10^{-3} \text{ M}$ ; sodium chloride (Merck) and aqueous solution  $10^{-3} \text{ M}$ ; potassium chloride (Merck) and aqueous solution  $10^{-3} \text{ M}$ ; magnesium chloride hexahydrate (Merck) and aqueous solution  $10^{-3} \text{ M}$ .

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### Equipment

The spectrometric study was performed with a spectrophotometer UV-VIS Jasco V-530. Copper concentrations in drinking water and wine samples were also determined with an atomic absorption spectrometer AAnalyst 700 (Perkin Elmer, Germany). The pH was measured using a pH-meter Consort P 901 (Consort, Belgium), provided with a combined pH electrode.

### Procedure

The solutions to be studied were prepared in 25 mL calibrated flasks where exactly measured volumes of  $10^{-3}$  M Cu (II) in  $10^{-1}$  M hydrochloric acid aqueous solution and  $10^{-3}$  M reagent **R** aqueous solution were introduced; a calculated volume of NaOH solution of known concentration was added to obtain a determined pH. The flasks were filled up to the mark with distilled water. Absorbance of each solution was measured against a corresponding Cu (II) free reagent blank, similarly prepared.

### Results and discussions

The organic reagent *Solochrome Yellow 2GS* forms instantaneously, at room temperature, with Cu (II) a complex soluble in water. In order to establish the optimal working conditions for the quantitative determination of Cu (II), the influence of wavelength, pH, amount of organic reagent and concentration of copper (II) on the absorbance was studied.

#### Influence of the wavelength

The absorption spectra of the complex and of the organic reagent **R** solutions are shown in figure 2. The maximum absorbance of the solution containing the complex is at  $\lambda = 435$  nm, while the solution that contains only reagent has a low absorbance. All absorbance measurements were performed at  $\lambda = 435$  nm against a corresponding blank reagent.

#### The effect of pH on the absorbance of the complex

In order to establish the influence of the pH on the spectra of the complex, solutions containing 1 mL  $10^{-3}$  M  $\text{Cu}^{2+}$  and 4 mL  $10^{-3}$  M reagent **R**, having various pHs between 3.86 and 10.05 were prepared in 25 mL calibrated flasks.

The absorbance of each solution was measured at  $\lambda = 435$  nm using a blank containing 4 mL  $10^{-3}$  M solution of **R** having the same pH as the sample. As it shown in figure 3 the complex absorbance increases with pH increase, reaching the maximum value within pH range 6.30 – 9.43

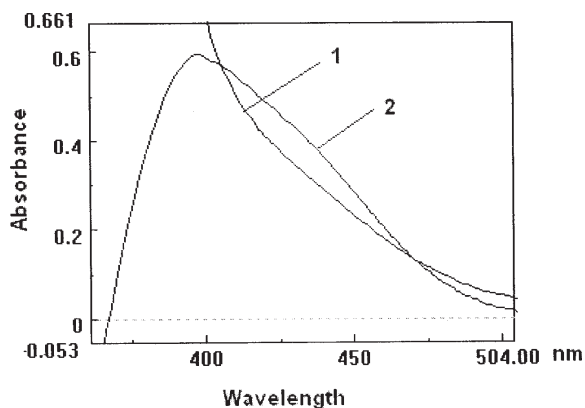


Fig. 2. Absorption spectra:

- 1- Reagent solution against water (4 mL  $10^{-3}$  M **R**; calibrated flask of 25 mL);
- 2- Solution of the complex against blank reagent solution (1 mL  $10^{-3}$  M  $\text{Cu}^{2+}$  + 4 mL  $10^{-3}$  M **R**; 25 mL calibrated flask).

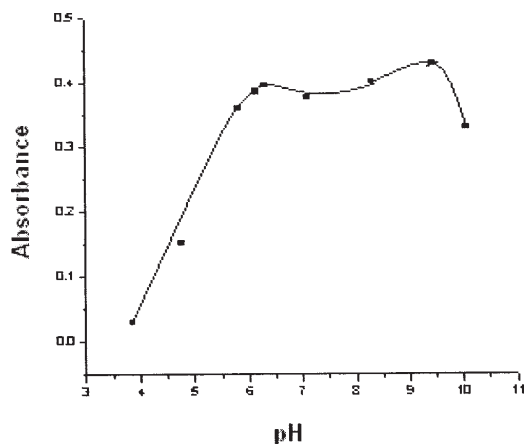


Fig. 3. Effect of pH on the absorbance of the complex ( $\lambda = 435$  nm).

and then decreases. Further on, a  $10^{-3}$  M reagent solution was used within pH range of 6.30 – 7.00.

#### Stoichiometry of the complex

The effect of reagent concentration on the spectra of the complex was examined by measuring the absorbance of the solutions containing a known concentration of Cu (II) and different amounts of organic reagent **R** at a pH value kept between 6.30 – 7.00. The curve obtained (fig. 4) shows a 1: 2 molar ratio Cu (II): **R**; a small excess of reagent over copper is required in order to obtain a maximum constant absorbance. The spectrometric titration using a constant amount of organic reagent **R** and variable concentrations of copper (fig. 5), as well as Job's method (fig. 6) show also a 1: 2 molar ratio Cu (II): **R**.

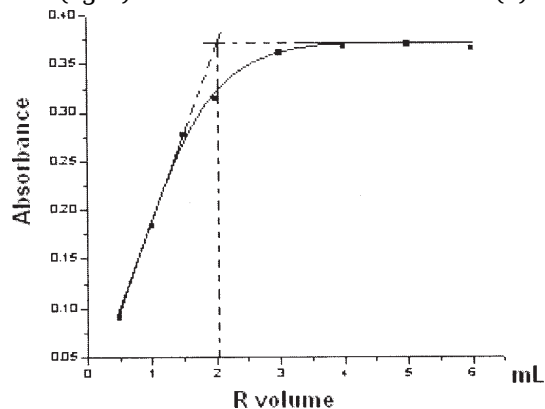


Fig. 4. Spectrometric titration of  $\text{Cu}^{2+}$  by reagent solution (Samples: 1 mL  $10^{-3}$  M  $\text{Cu}^{2+}$  + V mL  $10^{-3}$  M **R**; pH ~ 6.5; 25 mL calibrated flask;  $\lambda = 435$  nm).

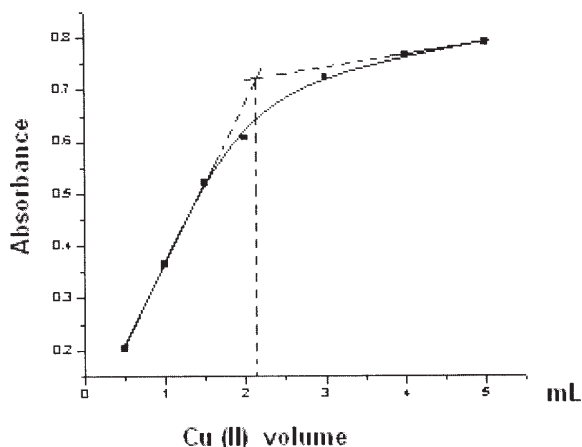


Fig. 5. Spectrometric titration of the reagent by copper (II) solution (Samples: 4 mL  $10^{-3}$  M **R** + V mL  $10^{-3}$  M  $\text{Cu}^{2+}$ ; pH ~ 6.5; 25 mL calibrated flask;  $\lambda = 435$  nm).

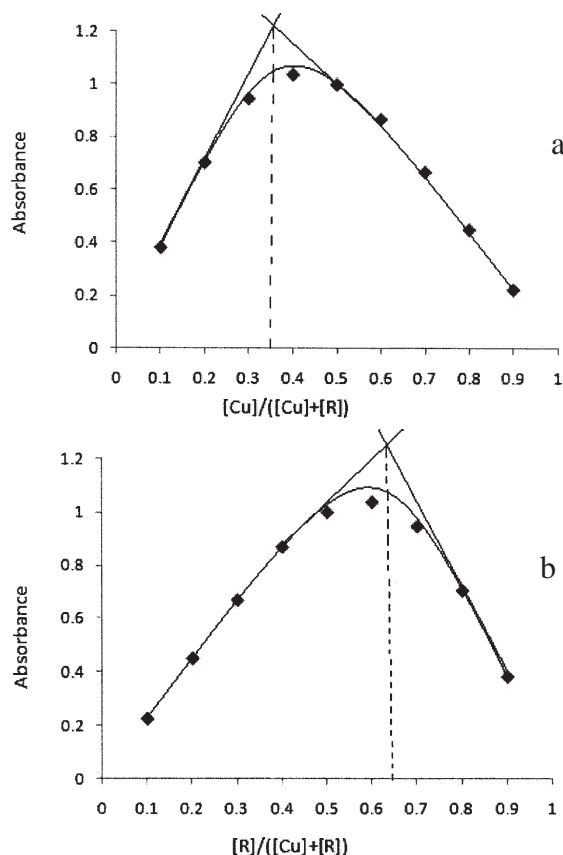


Fig. 6. Determination of the molar ratio by Job's method ( $10^{-3} M$  solutions of  $R$  and  $Cu^{2+}$ ;  $pH \sim 6.5$ ; 25 mL calibrated flask;  $\lambda = 435$  nm). a.  $A = f$  (molar fraction of copper) curve; b.  $A = f$  (molar fraction of the reagent) curve.

#### The stability constant

The stability constant of the complex resulting from the reaction between reagent  $R$  and copper (II) was calculated from data obtained using the Job method. Three series of nonisomolar solutions, containing a fixed total number of moles of  $Cu(II)$  and organic reagent  $R$ , but in which the ratio  $Cu:R$  is systematically varied from large to small were used. The formation of the 1:2,  $Cu:R$  complex in solution of  $pH$  ranging between 6.30–7.00 is complete at the inflexion point corresponding to the stoichiometric ratio (fig. 7). The average value of the stability constant is  $\beta_s = 1.58 \cdot 10^6 \pm 0.4 \cdot 10^6$  (table 1). The value of the stability constant obtained at  $20 \pm 2^\circ C$  shown a very stable complex in low acidic solution ( $pH$  range of 6.30–7.00) for the corresponding stoichiometry; it is in agreement with those determined polarographically [32].

#### Validation of spectrometric method

For the validation of spectrometric method for copper (II) determination, several validation parameters were considered according to the international rules [35, 36].

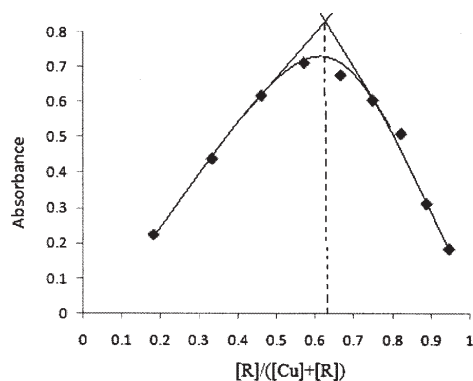


Fig. 7. Experimental data for the determination of instability constant (solutions:  $10^{-3} M$  reagent and  $5 \cdot 10^{-4} M Cu^{2+}$ ;  $pH \sim 6.5$ ; 25 mL calibrated flask;  $\lambda = 435$  nm).

#### Linearity of the method

All the absorbance measurements were performed at  $\lambda = 435$  nm against a corresponding blank reagent (within  $pH$  range of 6.30–7.00). The calibration curve was obtained by plotting the absorbance value against the copper (II) concentration in the solution. A linear relationship was obtained for copper (II) concentrations between 0.12–5.12  $\mu g \cdot mL^{-1}$ . The equation of absorbance,  $A$  against concentration,  $C$  was:

$$A = 0.017 + 0.123C \text{ with } R^2 = 0.992.$$

The detection limit determined experimentally according to the ICH rules [33, 34] is 0.10  $mg \cdot L^{-1}$ . The molar absorbance coefficient was calculated as being:  $\epsilon = 9197.5 L \cdot mol^{-1} \cdot cm^{-1}$ .

#### Accuracy of the method

In order to establish the accuracy of the method, samples having 0.77  $mg \cdot L^{-1}$  as known copper (II) concentration were analyzed. The copper (II) concentration was initially determined from each sample under analysis. The results obtained are: mean value 0.772  $mg \cdot L^{-1}$  and the standard deviation,  $SD = 0.025 mg \cdot L^{-1}$ . Then, a set of assays having various amounts of copper (II) was prepared through an addition to each sample of a known quantity of copper (II); the copper (II) concentration was determined from each assay being prepared as such. The data for the recovery studies presented in table 2 show that the accuracy of the proposed method is characterized by a recovery between 93.27% and 95.49%.

#### Precision of the method

The precision of the proposed method, expressed by reproducibility and repeatability, was determined through the application of the method on samples with known copper (II) content.

Repeatability was established based on the analysis of 6 samples, the determination of copper concentration in each assay being made by the same analyst during the

Table 1  
EXPERIMENTAL DATA FOR THE CALCULATION OF THE TOTAL STABILITY CONSTANT  $\beta_s$

$C$ $M$	$p$	$X_{max}$	$\beta_s$ $L^2 \cdot mol^{-2}$
$5 \cdot 10^{-4}$	2	0.625	$2.00 \cdot 10^6$
$5 \cdot 10^{-4}$	2	0.630	$1.56 \cdot 10^6$
$5 \cdot 10^{-4}$	2	0.635	$1.20 \cdot 10^6$
			$1.58 \cdot 10^6 \pm 0.4 \cdot 10^6$

**Table 2**  
DETERMINATION OF THE ACCURACY OF THE METHOD

Sample No.	Quantity of Cu present in the sample mg·L <sup>-1</sup>	Quantity of Cu added in the sample mg·L <sup>-1</sup>	Quantity of Cu found in final sample mg·L <sup>-1</sup>	Recovery %
1	0.772	2.560	3.108	93.27
2	0.772	2.816	3.373	94.01
3	0.772	3.072	3.637	94.61
4	0.772	3.328	3.915	95.49

**Table 3**  
COOPER CONCENTRATION DETERMINATION IN REAL SAMPLES

Sample	Concentration of Cu (II) determined by proposed method mg/L	Concentration of Cu (II) determined by FAAS [37]* mg/L
Wine sample	0.52 ± 0.05	0.50 ± 0.08
Tap water	0.11 ± 0.009	0.10 ± 0.02

Notes: Each result is the means of 6 determinations. \* Standard additions method was used.

same day. For Cu (II) concentrations the following values were obtained: 4.230; 4.258; 4.054; 4.090; 3.878 and 4.090 mg·L<sup>-1</sup>. Based on experimental data, average value of 4.100 mg·L<sup>-1</sup>, standard deviation SD = 0.1367 and relative standard deviation RSD = 3.333% were calculated.

Reproducibility was studied based on the determination of copper (II) concentration in two series each made of 6 samples, by two different analysts in different days the following Cu (II) concentrations were obtained: 4.191; 4.212; 4.141; 4.181; 4.136 and 4.163 mg·L<sup>-1</sup>, and respectively 4.127; 4.105; 4.020; 4.131; 4.036 and 4.107 mg·L<sup>-1</sup>. Based on the experimental data, average value of 4.129 mg·L<sup>-1</sup>, with a standard deviation SD = 0.0575 and a relative standard deviation RSD = 1.392% were calculated.

#### Interferences

In order to establish any possible interferences, the UV-VIS spectra were recorded for solutions containing each one of the following ions: Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Ba<sup>2+</sup>, Fe<sup>3+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup>, UO<sub>2</sub><sup>2+</sup> and R in the working conditions established as optimal for copper (II) determination. The spectra are showed in figure 8.

The effect of potential interfering ions on the spectrometric determination of copper (II) with the organic

reagent Solochrome Yellow 2GS at  $\lambda = 435$  nm within pH range 6.30 – 7.00 was investigated by adding known concentration of each above ion to a solution containing 1 mg·L<sup>-1</sup> copper (II) and 4 mL of 10<sup>-3</sup> M R solution. The ions: Fe<sup>3+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup> does not interfere when being in equal or lower concentrations than those of copper (II). Only the ion UO<sub>2</sub><sup>2+</sup>, being in the same or larger concentration as Cu<sup>2+</sup>, increases the absorbance of the solutions at  $\lambda = 435$ . It was found that: Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Ba<sup>2+</sup> do not interfere at all.

#### Practical applications

The spectrometric method proposed for the quantitative determination of copper (II) with the organic reagent Solochrome Yellow 2GS was applied on samples of drinking water and wine and the results were compared with those given by the standardized FAAS method [37]. In the case of FAAS analysis, the method of standard additions was used, because the Cu (II) concentration in water is very low.

The results presented in table 3 show a good agreement between the values determined by the proposed method and those given through the application of FAAS method [37].

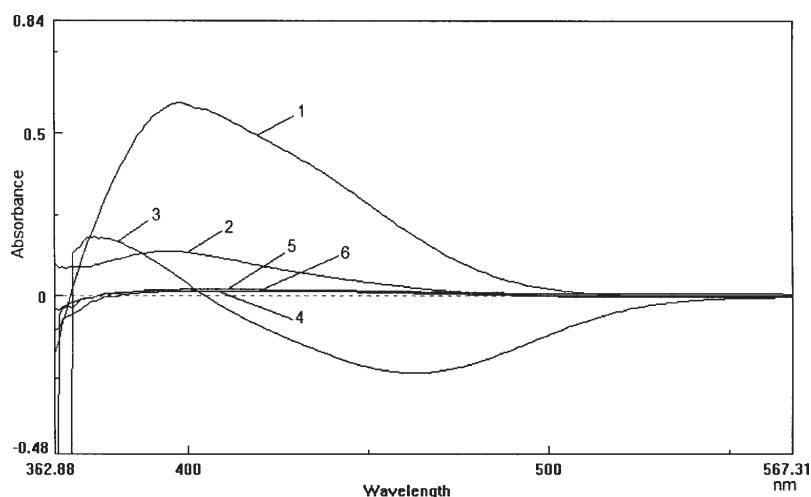


Fig. 8. Absorption spectra of the following ions solutions in the presence of the organic reagent Solochrome Yellow 2GS: 1 - Cu (II); 2 - U (VI); 3 - Fe (III); 4 - Ni (II); 5 - Co (II); 6 - Pb (II); (solutions: 10<sup>-3</sup> M reagent and 10<sup>-3</sup> M of Cu (II), U (VI), Fe (III), Ni (II), Co (II), Pb (II); pH ~ 6.5; 25 mL calibrated flask)

## Conclusions

A rapid, reliable and inexpensive method for the direct spectrometric determination of Cu (II) with the organic reagent Solochrome Yellow 2GS (disodium salt of 2-hydroxy-5-[(4-sulfophenyl)azo]-benzoic) is reported. In a 4-fold excess over Cu (II) concentration, the organic reagent forms with Cu (II), within pH range 6.30 – 7.00, a 1:2 Cu:R stable complex.

The value of the total stability constant  $\beta_s = 1.58 \cdot 10^6 \pm 0.4 \cdot 10^6$  was determined, which indicates the formation of a rather stable complex.

The proposed method is sensitive, accurate and reproducible, being characterized by a detection limit of  $0.11 \text{ mg}\cdot\text{L}^{-1}$ , a linearity range between  $0.76 - 5.12 \text{ }\mu\text{g}\cdot\text{mL}^{-1}$ , a recovery between 93.38% and 95.58%, as well as a good repeatability and reproducibility, characterized by relative standard deviations of 3.333% and 1.392%, respectively.

The spectrometric method proposed for the quantitative determination of copper (II) with the organic reagent Solochrome Yellow 2GS was applied with good results on real samples of wine and water.

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