

Scientific paper

Sensitive Kinetic Spectrophotometric Determination of Copper (II) by Partial Least Squares and Fixed Time Method

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Abstract

A very sensitive and selective kinetic catalytic spectrophotometric method has been developed for the determination of Cu (II). The method is based on the catalytic effect of Cu (II) on the reaction of sulfanilic acid with hydrogen peroxide at pH 6 and 25 °C in aqueous solution. The increase in the absorbance of the reaction mixture at 330nm at a fixed time is proportional to the concentration of Cu (II) over the range of 0.05–6 µg ml⁻¹. Partial least squares modeling as a powerful multivariate statistical tool is also applied and compared for determination of Cu(II) in aqueous solution. The experimental matrix for partial least squares calibration (PLS) method was designed with 14 samples. The cross-validation method was used for selecting the number of factors. The root mean squares error prediction (RMSEP) and the relative error prediction REP(%) were 0.11 and 3.78% respectively. The effects of various cations and anions on Cu (II) determination have been investigated. The capability of the method for the analysis of real sample was evaluated by determination of Cu (II) in serum samples of persons with Wilson disease.

Keywords: Copper (II), catalytic kinetic method, fixed time method, partial least squares, hydrogen peroxide, sulfanilic acid, spectrophotometric.

1. Introduction

At present, a lot of analytical methods have been proposed for the determination of copper (II), including, spectrophotometry,^{1–3} atomic absorption spectrometry (AAS)⁴ and spectrofluorometry.⁵ Compared with these methods, Catalytic kinetic spectrophotometric methods show advantages such as, high sensitivity, relative simplicity, and low experimental cost.⁶ Their sensitivity is at least 2–3 orders of magnitude higher than that of the ordinary spectrophotometric methods. Catalytic kinetic methods have been widely used in determining many analytes at nanogram levels.^{7–8}

Copper is an essential heavy metal required for many enzymes to be active, including enzymes involved in cellular respiration (cytochrome c oxidase), iron oxidation (ceruloplasmin), pigment formation (tyrosinase), neurotransmitter biosynthesis (monoamine oxidase), antioxidant defense (superoxide dismutase) and connective tissue formation (lysyl oxidase).⁹ Copper plays many physiological roles, and it is important to carry out the proper functioning by combining with proteins, enzymes,

hormones and vitamins, and so on. Copper distributes widely in animal tissues and is also one of the essential elements in human body. Therefore, the lack of copper in the body will result in health problems, such as anemia, leukopenia and atherosclerosis. Abnormality of copper metabolism in the body will cause some hereditary diseases, (e.g., Wilson diseases).¹⁰ Copper at only very low levels is an essential element and is toxic at higher levels in plants. As far as most living organisms are concerned, copper at about 40 ng mL⁻¹ is needed for their normal metabolism¹¹. Hence, it is necessary to seek highly sensitive, accurate, and selective analytical methods for quantitative determination of copper at trace levels.

2. Theory

The theory and application of partial least squares calibration (PLS) in kinetic method have been discussed by several papers and books.^{12–17} A brief theory is given here. PLS is a quantitative spectral decomposition technique that is closely related to principal component regres-

sion (PCR). However, in PLS the decomposition is performed in a slightly different fashion. Instead of first decomposing the spectral matrix into a set of eigenvectors and scores, and regressing them against the concentrations as a separate step (as done in PCR), PLS actually uses the concentration information during the decomposition process. Thus, the eigenvectors and scores calculated using PLS is quite different from those of PCR. The main idea of PLS is to get as much concentration information as possible in to the first few loading vectors. There are actually two version of the PLS algorithm; PLS1 and PLS2. The differences between these methods are subtle but have very important effects on the results. In PLS1, a separate set of scores and loading vectors is calculated for each constituent of interest. In this case, the separate set of scores and loading vectors are specifically tuned for each constituent, and therefore, should give more accurate predictions than PCR and PLS2.

In this study we proposed a sensitive, selective and simple method for determination of copper, by application a fixed time as a univariate method and PLS using full kinetic curve information as a multivariate method, based on its catalytic effect on the addition reaction of sulfanilic acid – hydrogen peroxide in aqueous solutions.

3. Experimental

3.1. Reagents

All solutions were prepared from analytical grade chemicals and triply distilled water. A stock copper (II) solution was prepared by dissolving 1.000 g of copper metal (99.99%) into 30 ml nitric acid. The working solutions were made by appropriate dilution of the stock before use. A $5.0 \cdot 10^{-2}$ mol L⁻¹ aqueous solution of (sulfanilic acid), SA (Merck) prepared daily and used. A 1 mol L⁻¹ aqueous solution of hydrogen peroxide was prepared using a commercially 30% hydrogen peroxide (Merck). To keep constant pH of solution at desired values a 0.04 mol L⁻¹ buffer solution was made by mixture of acetic, boric and orthophosphoric acid (all from Merck) and used.

3.2. Apparatus

Electronic absorption measurements were carried out on a CECIL 9000 spectrophotometer using 1.00cm quartz cells. Measurements of pH were made with a Metrohm 692 PH meter using a combined electrode.

3.3. Computer Hardware and Software

All absorption spectra were gathered, digitized and stored from 275 to 600 nm in steps of 1 nm and then transferred (in ASCII format) to a Pentium 200 MHz computer for subsequent manipulation by PLS program. The data treatment was done with MATLAB for windows (Math

works, version 6.5). PLS program (for calibration–prediction and experimental design) of PLS-Toolbox (Eigenvector Company) was used.

3.4. Procedure

In a regular 1ml quartz cell 180 μ L of $5.0 \cdot 10^{-2}$ mol L⁻¹ SA solution, 1.5 ml of 1 mol L⁻¹ hydrogen peroxide solution, and 30 μ L of working solution of Cu(II) 100 ppm were placed and completed to final volume with universal buffer (pH = 6). Then, the quartz cell placed in sample holder. The temperature of the sample compartment was kept constant at 25 °C by circulating water through it and the spectra of the solution were recorded in the visible region. The measuring time of the spectral profile was 1hr.

3.5. Analysis of Serum Samples

Serum samples (0.500 ml) and 5 ml of the mixed acid (HNO₃: HClO₄ ¼ 4 : 1) were accurately transferred into a beaker. The mixture was gently heated carefully until the solution was transparent and nearly to dryness. After cooling the solution was transferred into a 25 ml test tube and analyzed by the recommended method.

4. Results and Discussion

It was found that the reaction between sulfanilic acid and hydrogen peroxide is slow in buffer media. The copper(II) can catalyze the reaction, so that it will proceed much faster with respect to a non catalyzed reaction.(Fig. 1)

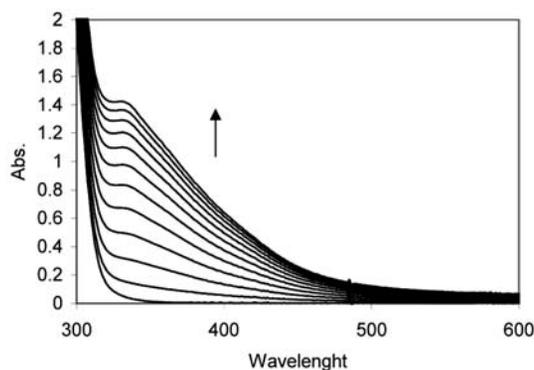


Figure 1. The absorption spectra of the reaction between H₂O₂ and SA, temperature 25 °C, pH = 6, [SA] $3 \cdot 10^{-3}$ mol L⁻¹, [H₂O₂] $4 \cdot 10^{-1}$ mol L⁻¹ and 4 ppm of copper(II) at 5 min intervals.

4.1. Effect of Variables

This part will seek for the optimize values of significant variables. There are two accepted methods to do this, one-at-a-time or sequential and simultaneous or global method which considers all the variables in optimization processes. Each of these methods have advantages and di-

sadvantages and it is not surprising to note that the one-at-a-time is simple to use and so this report we used this method as optimization tool.

The effect of temperature, pH, and concentration of SA and hydrogen peroxide on reaction rate were studied and their optimum values were selected. The influence of temperature on reaction rate between 25–60 °C was studied. The results showed that the changes in absorbance (ΔA) increase with increasing temperature up to 25 °C and then level off for the higher temperature so 25 °C was chosen as an adequate temperature.

The effect of pH on reaction rate was investigated using $\text{H}_3\text{PO}_4\text{--CH}_3\text{COOH--H}_3\text{BO}_3$ solution as buffer solution. The changes in absorbance (ΔA) were measured at different pH values. The results showed that the changes in absorbance increases with increasing pH up to pH = 6, whereas higher pH decreases the changing in absorbance. Therefore pH = 6 was selected in subsequent study. (Fig. 2)

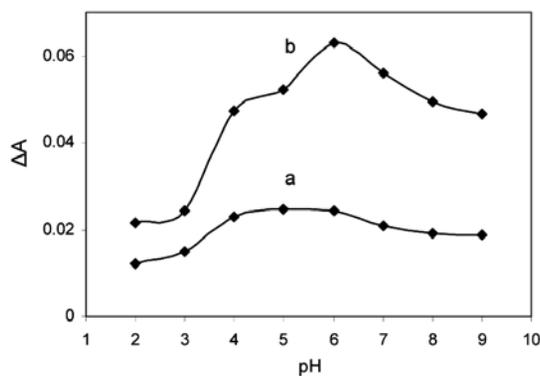


Figure 2. Effect of pH in the absence(a) and the presence (b) of the catalyst 1 ppm Cu(II): $\lambda = 330$ nm, temperature 25 °C, [SA] $8 \cdot 10^{-3}$ mol L $^{-1}$, [H $_2$ O $_2$], $3 \cdot 10^{-1}$ mol L $^{-1}$.

The influence of sulfanilic acid concentration on reaction rate in the range of 0.0005–0.1 mol L $^{-1}$ was studied. The concentration of 0.003 mol L $^{-1}$ was chosen as optimal concentration. (Fig. 3)

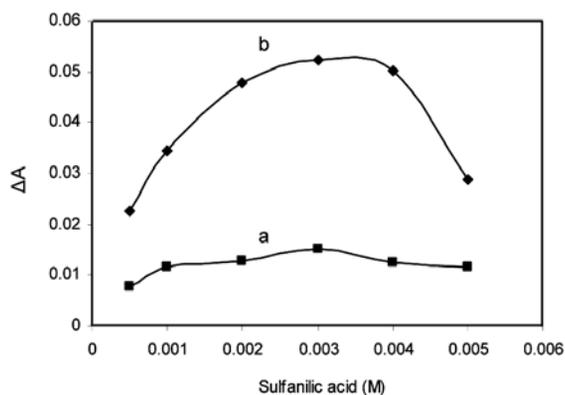


Figure 3. Influence of sulfanilic acid concentration on the reaction of the SA and H $_2$ O $_2$. Condition as in Fig. 2.

Also the influence of hydrogen peroxide concentration on reaction rate in the range of 0.1–0.6 mol L $^{-1}$ was studied. The concentration of 0.4 mol L $^{-1}$ was chosen as optimal concentration. (Fig. 4)

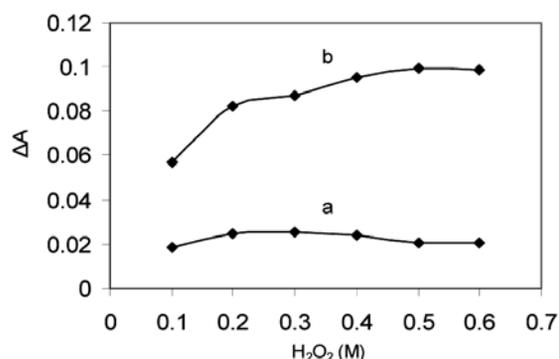


Figure 4. Influence of H $_2$ O $_2$ concentration on the reaction of the SA and H $_2$ O $_2$. Condition as in Fig. 2.

4. 2. Univariate Calibration

The absorbance vs. time was plotted for the reaction of SA with hydrogen peroxide in the absence and presence of the catalyst. The changes in absorbance at 330 nm were recorded. Fig. 1 shows the change in spectra of reaction in presence of copper at first 300sec intervals of the reaction period. Under these optimum working conditions a calibration curve was prepared, using fixed time method. The ΔA vs. concentrations was linear in the range of 0.05–6 $\mu\text{g ml}^{-1}$ for copper (II) with a detection limit of 0.028 $\mu\text{g ml}^{-1}$. Ten replicate analyses of 10 sample solutions (0.02 $\mu\text{g ml}^{-1}$) of Cu (II) gave a R.S.D. of 1.6%.

4. 3. Multivariate Calibration

To perform the determination of the Cu (II), the PLS1 method as a multivariate method that can handle the higher level of the noise in experimental data, was evaluated. For this purpose a synthetic set of 21 solutions of copper (II) were prepared randomly and change in spectral profiles were recorded and saved for subsequent computation. From the series, 14 solutions were chosen for the ca-

Table 1. Concentration data of calibration set for Cu (II).

Solution number	Copper(II) ($\mu\text{g ml}^{-1}$)	Solution number	Copper(II) ($\mu\text{g ml}^{-1}$)
1	0.05	8	3.0
2	0.5	9	4.0
3	0.9	10	4.2
4	1.0	11	5.0
5	1.5	12	5.7
6	1.9	13	6.3
7	2.5	14	6.6

libration (Table 1) and the other seven were used as problem or test set solutions (Table 2). The test solutions were chosen according to their score situation on the graph of scores of first principal component vs. second principal component inside the range of calibration solutions.

Table 2. Concentration data for the prediction set and their predicted values for the Cu (II).

Solution number	Copper (II) ($\mu\text{g ml}^{-1}$)		
	Added ($\mu\text{g ml}^{-1}$)	Found ($\mu\text{g ml}^{-1}$)	Error (%)
1	0.70	0.67	4.28
2	1.30	1.28	1.50
3	2.10	2.15	-2.30
4	3.40	3.37	0.88
5	4.60	4.71	-2.39
6	6.00	6.14	-2.30
7	7.00	6.92	1.15

4. 4. Selection of Optimal Number of Factors

To find the optimum number of significant factors (latent variables) in the PLS modeling, to have a model without over fitting the concentration data, a cross-validation method, leaving out one sample at a time was used.^{18–19} As the training set comprised 14 spectra, the PLS calibration was performed on all of them and the concentration of the excluded sample was predicted. The process was repeated 14 times (one for each sample), and using this calibration, the concentrations of the compounds in each sample were compared with the known concentration of the compounds in the reference sample and the prediction error sum of squares (PRESS) was calculated. The PRESS was calculated in the same manner each time a new factor was added to PLS models. One reasonable choice for the optimum number of factor would be the number which yields the minimum PRESS. However, using the number of factors that yields a minimum PRESS, h^* (number which corresponds to the minimum values of the PRESS curve vs. number of factors or principal components) leads to over fitting. A better criterion for selecting the optimum number of factors involves the comparison of PRESS from models with fewer than h^* factors. The F -statistic was used to determine significance. Haaland and Thomas¹⁸ empirically determined that an F -ratio probability of 0.75 is a good choice. So, we also selected as the optimum the number of factors for the PRESS value the F -ratio probability of which drops below 0.75. The PRESS value has minimum value at the number of factors of 3 for copper. Therefore, this number of latent variables was selected as the optimum number of factors for PLS1 model building. The results obtained by applying PLS1 algorithm to the seven prediction samples are listed in Table 2. Table 2 that also show the percentage error for prediction of a series of coppers. The percentage errors were also quite acceptable, as they never exceeded 4%.

4. 5. Statistical Parameters

For the optimized model two parameters were selected to evaluate the prediction ability of the model for determination of copper (II) in the prediction set. The predictive ability of a multivariate calibration model is usually expressed in terms of root mean square error of prediction RMSEP which is determined for an independent test set. Clearly, the adequate validation of a multivariate model requires a certain minimum number of test samples. A reasonable criterion for the number of test samples would be to demand that the resulting estimate of RMSEP is sufficiently precise for the application at hand.²⁰ The root mean square error prediction (RMSEP) (cross validation), which is an indication of the average error in the analysis. The RMSEP values are an estimate of the absolute error of prediction for each component.

$$\text{RMSEP} = \left[\frac{1}{n} \sum_{i=1}^n (\hat{x}_i - x_i)^2 \right]^{0.5}$$

Second parameter was relative error of prediction (REP) that shows the predictive ability of each component, and is calculated as:

$$\text{REP}(\%) = 100/\bar{x} \cdot \left[\frac{1}{n} \sum_{i=1}^n (\hat{x}_i - x_i)^2 \right]^{0.5}$$

where x_i is the true concentration of the analyte in the sample i , \hat{x}_i represents the estimated concentration of the analyte in the sample i , \bar{x} is the mean of true concentrations in the prediction set and n is the total number of samples used in the prediction sets. The value of RMSEP, REP% and PRESS in the optimum number of factors for copper concentration in the prediction set were 0.11, 3.78, and 0.53 respectively.

4. 6. Effect of Foreign Ions

The interference due to several cations and anions was studied in detail. Different amounts of ionic species were added to the reaction solution containing $4 \mu\text{g ml}^{-1}$ of

Table 3. The maximum tolerance value of various cations and anions on the measurement of $4 \mu\text{g ml}^{-1}$ Cu(II) by the suggested method.

Interfering ions	Tolerance limit ($\mu\text{g ml}^{-1}$)
Al^{3+} , Co^{2+} , Mg^{2+} , K^+ , Na^+ , Li^+ , NH_4^+ , Ni^{2+} , Ca^{2+} , Ba^{2+} , SO_3^{2-}	130
HPO_4^{2-} , $\text{Cr}_2\text{O}_7^{2-}$, $\text{C}_2\text{O}_4^{2-}$	15
Br^- , SO_4^{2-}	2
CO_3^{2-} , Hg^{2+} , NO_2^- , Fe^{3+} , NO_3^- , S^{2-}	Interfered

Note: The interference effect of Fe^{3+} , Hg^{2+} , Al^{3+} , Co^{2+} , Mg^{2+} , Ni^{2+} , Ca^{2+} , Ni^{2+} , Ca^{2+} , Ba^{2+} eliminated by using F^- ($3 \cdot 10^{-2} \text{ mol l}^{-1}$) as masking agent.

copper (II). The tolerance limits are the concentrations of interfering ions that cause a 3–5% change in actual concentration of Cu^{2+} . As can be seen in Table 3, some metal ions including Fe^{3+} , Hg^{2+} can interfere with the determination of copper. The tolerance limits for the different ions studied are listed in Table 3.

4. 7. Application of the Model to Spiked Real Matrix Samples

The optimized PLS1 model was applied to the analysis of three serum samples of Wilson patients. Results are summarized in Table 4. The results in Table 4 show that the PLS1 model is able to predict the concentrations of copper in real samples. A comparison of prediction ability of PLS1 and fixed time methods in the recovery results obtained by application of two methods are depicted in Fig. 5. As shown the prediction ability of PLS1 is superior to the fixed time method. It is not surprising to say, the PLS1 method is considered as a principal component based method which commonly can utilize whole spectrum in the analysis. This property enables it to decrease the effect of the random noise in the different steps of the analysis. As can be seen from Tables 5, the recoveries of copper (II) by PLS1 method were 95–106% and the results obtained by the PLS1 method were 104–113%. The results obtained by the proposed methods (PLS1 and fixed time methods) and the AAS method showed good agreement.

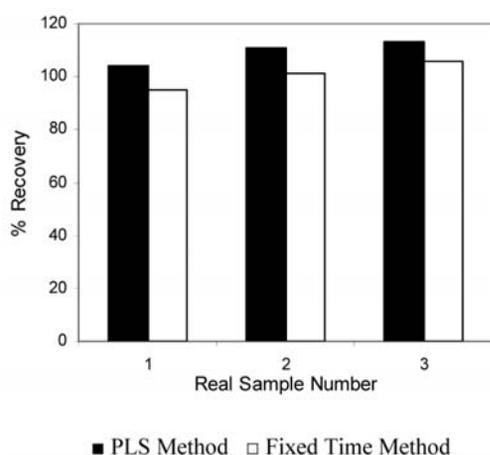


Figure 5. Recovery study of Cu (II) in real samples by PLS and fixed time methods.

Table 4. Recovery study of real samples.

Sample	AAS	Added ($\mu\text{g ml}^{-1}$)	Found ^a ($\mu\text{g ml}^{-1}$)	Recovery ^a (n = 5, %)	Found ^b ($\mu\text{g ml}^{-1}$)	Recovery ^b (n = 5, %)
Serum 1	2.26	2.20	2.29	104	2.1	95
Serum 2	2.82	2.50	2.78	111	2.53	101
Serum 3	3.09	2.70	3.06	113	2.87	106

^aFixed time method. ^bPLS1 method.

5. Conclusion

Determination of copper (II) based on its catalytic effect on the reaction of SA and hydrogen peroxide with the fixed time method and a multivariate calibration method, PLS1 modeling is presented established. The method is very simple and has satisfactory prediction ability for the real samples. The results showed the approximate superiority of the PLS1 method over the fixed time method as a univariate method. The method has acceptable detection limit and the sensitivity and reproducibility are in the margin of these type of studies.

6. References

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Povzetek

Razvili smo občutljivo in selektivno katalitsko spektrofotometrično metodo za določanje Cu(II). Osnovana je na oksidaciji sulfanilne kisline z vodikovim peroksidom v prisotnosti Cu(II) pri pH 6 in pri 25 °C. Povečanje absorbance pri 330 nm pri stalnem času je bilo linerano odvisno od koncentracije Cu(II) v območju 0.05–6 $\mu\text{g ml}^{-1}$. Za določitev Cu(II) v vodnih vzorcih smo uporabili metodo delnih najmanjših kvadratov (PLS) s 14 vzorci in uporabili navzkrižno validacijo. Preučili smo učinke prisotnosti različnih anionov in kationov pri analizi realnih vzorcev krvnega seruma oseb z Wilsonovo boleznijo.