Determination of Copper (II) in Fungal Drugs, Vehicle Exhaust Particulates and Water Samples with a Novel Kinetic Spectrophotometric Method Based on Its Inhibitory Effect on Redox Reaction between Giemsa Stain and Ascorbic Acid

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Abstract

A new kinetic method is described for determination of trace levels of Cu (II) based on its inhibitory effect on the redox reaction between l-ascorbic acid (LAA) and giemsa stain. The decolorization of giemsa stain by the LAA was used to monitor the reaction spectrophotometrically at 662 nm. The fixed time method used for different time intervals from the initiation of the reaction. The method allows the determination of Cu (II) in the range of 0.1-8.0 µg ml^{-1} (r^2=0.9911) for 0.5-5 min. The relative standard deviation was 2.8 % for 10 determination of 2.0 µg ml^{-1} of Cu (II) and the limit of detection ($3S_b/m$) was 0.188 µg ml^{-1}. The proposed method was applied successfully to determination of Cu (II) in fungal preparation, tap water and NIES CRM No: 8 vehicle exhaust particulates. The results of the method were satisfactory compared to with AAS results.

Keywords: Copper determination; Giemsa stain; Inhibition; Ascorbic acid

1. Introduction

Copper (II) is considered an essential element for living organisms at only limited levels and can be toxic at higher levels. Copper at 40 ng L^{-1} is required for normal metabolism of most of animals and plants. Excessive amount of copper cause damages in the body such as Wilson's disease (Prasad & Halafihi, 2003; Jahan et al., 2008; Pourreza & Behpaur, 1998; Safavi & Tavallali, 1995; Prasad, 2005). Determination of copper at trace levels is very important. There are many methods for determination of copper but the majority of them suffers from poor selectivity (Bjiirklung & Morrison, 1997; Ohno et al., 2003).

Kinetic methods are widely used for analyzing industrial and natural samples because of their high selectivity, and versatility, and, in some cases, extremely high specificity (Bagda, 2010). The best advantage of kinetic determinations is the combination of very low determination limits with a simple and available experimental technique, especially with photometric monitoring of the reaction rate. The relative cheapness of catalymetry and its reliability in analyses for trace determinations informed the reason for the intense studies of catalytic reactions during the last century. Catalymetric methods are based on chemical reactions where the rate of the indicator system influenced by the reaction conditions (Stayanova & Alexiev, 2005)Among the most commonly used methods; kinetic methods are simple, selective and low cost (Chand &Prasad, 2009; Pyrzynska &Wierzbicki, 2004; Madrikan et al., 2011; Przynska, 2005; Zhai et al., 2008). The instruments used for kinetic methods are less complex than the other spectrometric instruments. This property makes kinetic methods more attractive for especially small laboratories of minimum budget (Khan & Sarwava, 2001; Mori et al., 1995; Oliveira et al., 1996; Crespo et al., 2005; Tomiyasu et. al., 2005).

In the present paper we describe a new kinetic methods for determination of copper (II) in µg ml^{-1} levels in fungal formulations. The method is based on inhibitory effect of copper (II) on redox reaction between giemsa stain (GS) and l-ascorbic acid (LAA). Different variables that effect the indicator reaction as well as description of procedures are presented below. The redox reaction of giemsa stain and ascorbic acid is highly selective and simple for the determination of trace Se (IV)) without extraction procedures.

2. Experimental

2.1. Reagents

All chemicals used were of analytical grade purchased from Carlo Erba Reagenti SpA, Merck Company (Darmstadt, Germany) and Sigma (Steinheim, Germany).

The cationic solutions used for interference study were prepared from their nitrate salt, and anionic solutions were from potassium or sodium salt. Double distilled water was used throughout the experiment. 1000 µg mL^{-1} Cu(II) solution of AAS standard was used as stock solution and it was diluted to desired level. 0.010 M L-ascorbic acid solution was prepared by dissolving appropriate amount of LAA in a flask and diluted to 50.0 mL with double distilled water. LAA solution was prepared daily. Stock Giemsa stain solution was prepared by dissolving appropriate amount of GS in 250 ml of double distilled water. At 4^oC, stock solution of GS was stable up to two weeks. If necessary, the stock solutions were diluted to desired concentration level before use. All glassware used for handling solutions was cleaned with detergent solution, rinsed with tap water, soaked in 10% nitric acid overnight, rinsed with double distilled water and dried.

2.2. Apparatus

Absorption measurements at λ_{max} 662 were performed using a Shimadzu UV 1800 model spectrophotometer with a 1 cm quartz cell. A thermostatic bath from Nüve BM 302 was used to maintain temperature of the reaction mixture. pH measurements were carried out using Sartorious basic model pH meter with an accuracy of ±0.01.

2.3. Procedure

The catalytic reaction was monitored spectrophotometrically by measuring the change in absorbance of the reaction mixture at λ_{max} 662 nm. The fixed time method was used for the 0.5-5 min.

The pH of the working solution was adjusted pH 2.5 with citrate buffer. All the working solution kept at 33 ± 0.2^0C in the thermostatic bath for about one hour. In a 10 mL volumetric flask, appropriate volumes of standard solutions of reagents were taken in sequence of buffer solution, Cu (II), LAA Giemsa stain. The zero time was taken as the moment at which the last drop of Giemsa stain was added, and then the solution was diluted to 10 mL immediately. The reaction mixture was mixed, and transferred to spectrophotometric quartz cell as quickly as possible. The rate of redox reaction was followed spectrophotometrically using fixed time method at 662 nm. Absorbance was measured against water from the initiation of the reaction. The uninhibited reaction was monitored in the same way using blank solution.

3. Results

Giemsa stain is a thiazine (Quinone-imine class) dye and can be reduced by L-AA. Giemsa stain is intensely colored because of delocalization of Π electrons and it allows absorption of light in the visible region. In the presence of Cu (II), LAA-Cu (II) complexation reaction occurs (Lurie, 1975), hence the rate of reduction of Giemsa stain decreases. This decrease is proportional with concentration of Cu (II). Thus trace amounts of Cu (II) can easily be determined via kinetic measurements by monitoring the redox reaction of giemsa stain with LAA at 662 nm in the presence or absence of Cu (II). The effects of concentration of each reagents and temperature on reaction were investigated to find optimum reaction conditions to achieve sensitive and selective results for the determination of trace Cu (II). The reaction condition was optimized by altering each variable in turn while the others were kept constant. The optimum values taken were those giving the maximum net reaction rate (ΔΔA) and under conditions in which small variations in the variable concerned did not greatly affect the reaction rate.

For the optimization of the reaction the influence of reaction variables such as pH, buffer concentration, [LAA], [GS] and temperature were studied. The time for measuring the change in absorbance was 5 minute for optimization of reaction parameters. The optimum conditions used for the final working procedure were chosen as a compromise in order to ensure extended linearity and short measuring time.

3.1. Effect of pH and volume of buffer

Among the studied buffer solutions (acetate, citrate, phosphate, phthalate) citrate buffer solution gave the highest sensitivity, thus citrate buffer was selected to study the effect of pH. The effect of pH on the reaction rate was studied over the range pH: 1.1-4.9 (fig. 1.).The absorbance differences both uninhibited and the reaction inhibited by Cu (II) decreased gradually with pH in the range 1.1-3.0, from pH 3.0 inhibited reactions did not occur anymore. Therefore, uninhibited reaction occurred and rate of reaction decreased until pH 4.0. At pH 2.5, the sensitivity (ΔΔA=ΔAu-ΔAi) had a maximum value. This might be due to formation of Cu (II)-LAA complex ion at pH 2.5.On the other hand, volume of citrate buffer was also studied in the range of 0.5-4.5 mL (fig. 2). ΔAu (absorbance difference for uninhibited reaction) and ΔAi (absorbance difference for inhibited reaction) values increased with citrate buffer amount up to 2.0 mL. Between 2.0-4.5mL ΔAu decreased while ΔAi increased. 2.0 mL citrate buffer selected as optimum for further studies.

3.2. Effect of LAA concentration

Concentration of LAA was investigated in the range $5.0x10^{-4}$-$5.5x10^{-3}$ M. As can be seen from Fig.3, the rate of both uninhibited and inhibited reactions increased with increasing reagent concentration up to $4x10^{-3}$ M range. It is obvious from Fig. 3. reaction is first order with respect to [LAA] in the $5.0x10^{-4}$-$4x10^{-3}$ M range. For further studies $3.5x10^{-3}$ M LAA was selected.

3.3. Effect of GS concentration

The effect of Giemsa stain concentration was studied over the range 1.73 $x10^{-5}$-15.3 $x10^{-5}$ M. (fig. 4.) ΔAu and $\Delta A\imath$ values increased almost linearly with Giemsa stain concentration in the studied range. However, in order to provide high sensitivity $10.20x10^{-5}$ M Giemsa stain was chosen.

3.4. Effect of temperature

A temperature dependence study was carried out in the temperature range 22.8-42.8 to judge whether a rigorous control of temperature is necessary. Fig. 5 shows the temperature dependence of the reaction. As shown from fig. 5, between 22.8-27.8^{o}C sensitivity of the system is almost same, on the other hand between 32.8-42.8 sensitivity is also same but a bit higher than the first range (22.8-27.8^{o}C). Thus 33^{o}C was chosen as the optimum temperature for copper determination.

Analytical parameters

Under the optimum conditions, linear calibration graphs was obtained for different time intervals for Cu(II). The method yields a relative standart deviation of 3.7, 2.8, 3.2 % for ten determinations of 0.4, 2.0, 7.8 μgl^{-1} of Cu(II) relatively for the time interval of 30-300 seconds. The limit of detection was determined as signal to noise ratio (3:1)

3.5. Selectivity studies

In order to assess the potential analytical applications of proposed method, the influence of foreign ions on the determination of Cu (II) was investigated. The tolerated limits for the ions assayed are shown in Table 2 (the tolerance limit was taken as the concentration of the diverse ion causing less than a 6% relative error. As it is seen, almost all studied ions have no considerable effect on the determination of Cu (II).

3.7. Analytical Applications

In order to check the applicability of the proposed method to the real samples, it was applied to the determination of Cu (II) in a fungal preparation, standart reference material and tap water. 0.100 g of fungal preparation was dissolved in 1.0 mL of concentrated HCl and diluted about 50.0 mL and 1 mL of concentrated H_2O_2 added to oxidize all Cu (I) to Cu (II) then, solution is diluted to 500.0 mL with distilled water. The results were compared with data obtained from AAS method.

For dissolution and oxidation of copper to Cu (II) in NIES CRM 8 Vehicle Exhaust, 0.1471 g sample dissolved in 10.0 mL 0f concentrated HCl and 10.0 mL 0f concentrated HNO_3 over night. Then mixture heated to dryness with mild conditions to avoid splashing. The residue was diluted with 0.2 M HNO_3, boiled for two hours, then filtered. The filtrate diluted to 25.0 mL The Cu (II) content of Vehicle Exhaust samples were detected by using standard addition methods.

The tap water sample was collected from Cumhuriyet University. Different amount of Cu (II) was spiked to the sample immediately. For analysis, an aliquot of 500.0 mL of tap water was acidified with HCl and oxidized with H_2O_2 and evaporated to about 200.0 mL, filtered and diluted to 250 mL with distilled water.

The results showed that no significant difference was found between the developed method and standard AAS method. These results demonstrated that the developed method was suitable for rapid determination of Cu (II) in fungal preparations, vehicle exhaust samples, and water.

4. Conclusion

The result of this work show that the inhibition effect of Cu(II) on the indicator reaction between Giemsa stain- L ascorbic acid can be successfully apply for Cu(II) determinations under optimum conditions and adequate real samples. The method is rapid, simple and sensitive with a linear range of 0.1-8.0 μgl^{-1}. This kinetic method proposed for Cu (II) is attractive for especially small laboratories of minimum budget.

References

Bagda, E. (2010). Kinetic/ Spectrophotometric determination of trace amounts of inorganic selenium species at real samples, Ph. Thesis, Cumhuriyet University.

Bjiirklund, L. B., Morrison, G. M., (1997). Determination of copper speciation in freshwater samples through SPE-spectrophotometry. Analytica Chimica Acta, 343, 259-266.

Chand, V., Prasad, S., (2009). Trace determination and chemical speciation of selenium in environmental water samples using catalytic kinetic spectrophotometric method. Journal of Hazardous Materials. 165, 780-788.

Crespo, G. A., Andrade, F.J., Inon, F.A., Tudino, M. B., (2005). Kinetic method for the determination of trace amounts of copper (II) in water matrices by its catalytic effect on the oxidation of 1,5-diphenylcarbazide. Analytica Chimica Acta, 538, 317-325.

Jahan, G., Kiaee, S. H., Azizah, A., Abolfazl, S. (2008). Sensitive Kinetic Spectrophotometric Determination of Copper (II) by Partial Least Squares and Fixed Time Method. Acta Chimica Slovenica, 55, 184-189.

Khan, M. N., Sarwava, A., (2001). Determination of Trace Amounts of Copper(II) by Using Catalytic Redox Reaction between Methylene Blue and Ascorbic Acid. Analytical Science,17, 1195-1197.

Lurie, J., Handbook of Analytical Chemistry, English translation of 1971 Russian edition, Mir publishers, Moscow, 1975.

Madrakian, T., Afkhami, A., Mohammadnejad, M., (2011). Micelle mediated extraction and simultaneous spectrophotometric determination of vanadium(V) and molybdenum(VI) in plant foodstuff samples. Food Chemistry, 127, 769-773.

Magnia, D. M., Olivierib, A. C., Bonivardi, A.L. (2005). Artificial neural networks study of the catalytic reduction of resazurin:stopped-flow injection kinetic-spectrophotometric determinationof Cu(II) and Ni(II). Analytica Chimica Acta, 528, 275–284.

Mori, I., Fujimoto, T., Fujita, Y., Matsuo, T., (1995). Selective and sensitive spectrophotometric determination of copper(II) and benzoylperoxide with N-ethyl-2-naphthylamine. Talanta, 42, 77-81.

Nakano, S., Tanaka, E., Mizutani, Y., (2003). Flow-injection spectrophotometry of vanadium by catalysis of the bromate oxidation of N,N'-bis(2-hydroxyl-3-sulfopropyl)-tolidine, Talanta, 61, 203-210.

Ohno, S., Teshima, N., Zhang, H., Sakai, T., (2003). Utilization of activating and masking effects by ligands forhighly selective catalytic spectrophotometric determination of copper and iron in natural waters. Talanta, 60, 1177-1185

Oliveira, C.C., Sartini, R. P., Reis, B. F., Zagatto, E. A. G., (1996). Multicommutation in flow analysis. Part 4. Computer-assisted splitting for spectrophotometric determination of copper and zinc in plants. Analytica Chimica Acta, 332, 172-178.

Pourreza, N., Behpaur, M. (1998). Catalytic kinetic determination of trace amount of copper (II) based on oxidation of 2,4 dinitrophenylhydrazone-1,2- naphtoquinone-4 sulphonic acid by hydrogen peroxide. Analytical Science, 14, 997-999.

Prasad, S., Halafihi, T. (2003). Development and validation of catalytic Kinetic Spectrophotometric method for determination of Copper (II). Microchimica Acta, 142 237-244.

Prasad, S., (2005). Kinetic method for determination of nanogram amounts of copper(II) by its catalytic effect on hexacynoferrate(III)–citric acid indicator reaction. Analytica Chimica Acta, 540, 173–180.

Pyrzynska, K., Wierzbicki, T., (2004). Determination of vanadium species in environmental samples. Talanta, 64, 823-829.

Pyrzynska,K., (2005). Recent developments in spectrophotometric methods for determination of vanadium. Microchimicia. Acta, 149, 159-164.

Safavi, A., Tavallali, H., (1995). Kinetic spectrophotometric determination of traces of copper. Analytical Science, 11, 453-455.

Tomiyasu, T., Aikou, S., Anazawa, K., Sakamoto, H., (2005), A Kinetic Method for the Determination of Copper(II) by Its Catalytic Effect on the Oxidation of 3-Methyl-2-benzothiazolinone Hydrazone with Hydrogen Peroxide: A Mechanistic Study, Analytical Science, 21, 917-921.

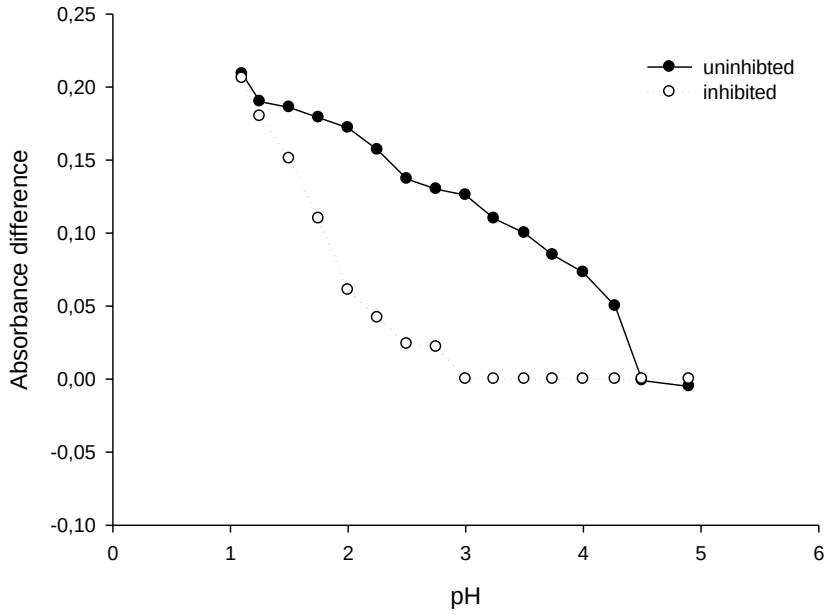

Fig. 1. Effect of pH on the reaction rate (Conditions: 2.0 mL buffer, [LAA]: 3.5x10^{-3}M, [GS]: 1.02x10^{-4} M, 33^{o}C).

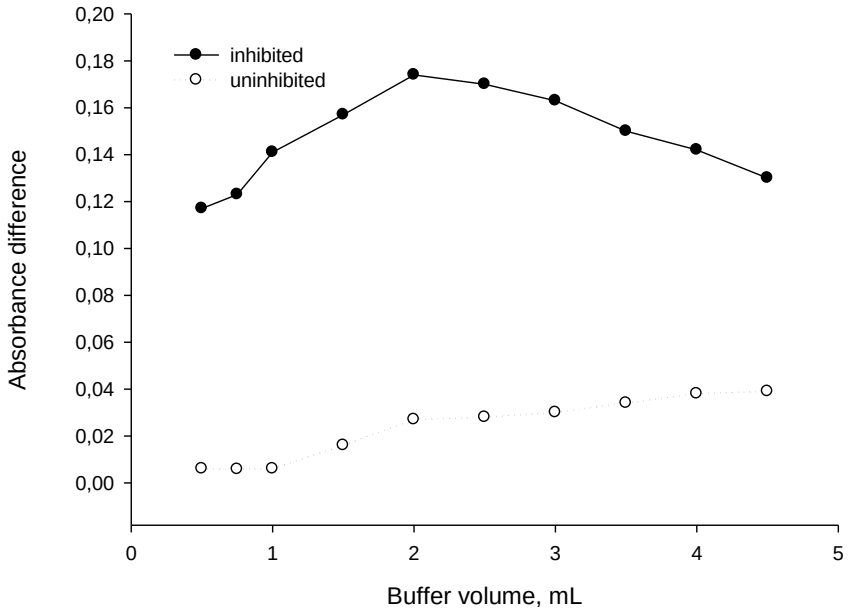

Fig. 2. Effect of buffer amount on the reaction rate (Conditions: pH: 2.5, [LAA]: 3.5x10^{-3}M, [GS]: 1.02x10^{-4} M, 33^{o}C).

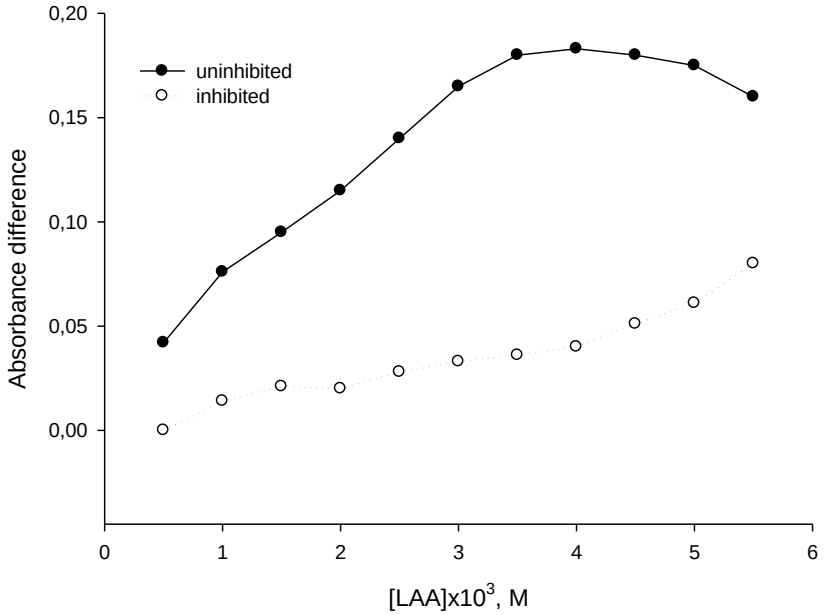

Fig. 3 Effect of concentration of LAA on the reaction rate (Conditions: pH: 2.5, 2.0 mL buffer, [GS]: 1.02x10^{-4} M, 33^{o}C).

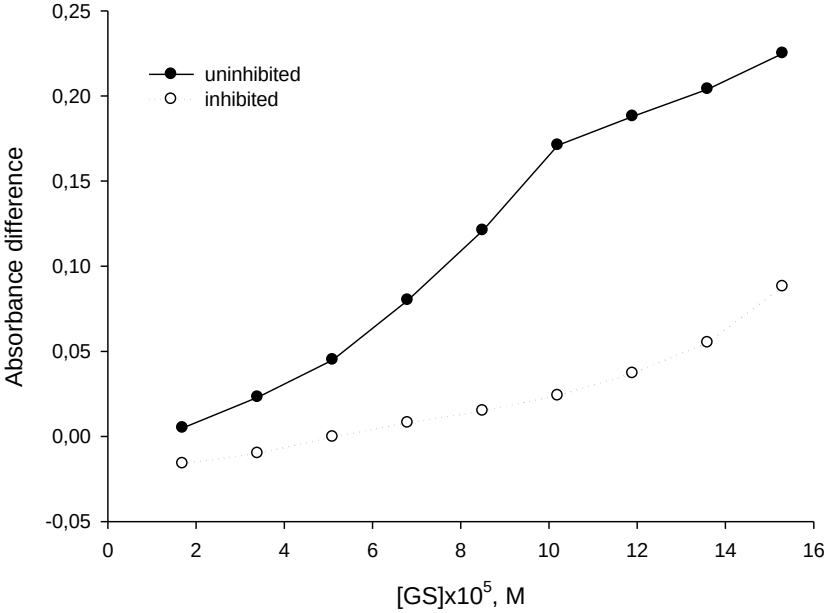

Fig. 4 Effect of concentration of GS on the reaction rate (Conditions: pH: 2.5, 2.0 mL buffer, [LAA]: 3.5x10^{-3}M, 33^{o}C).

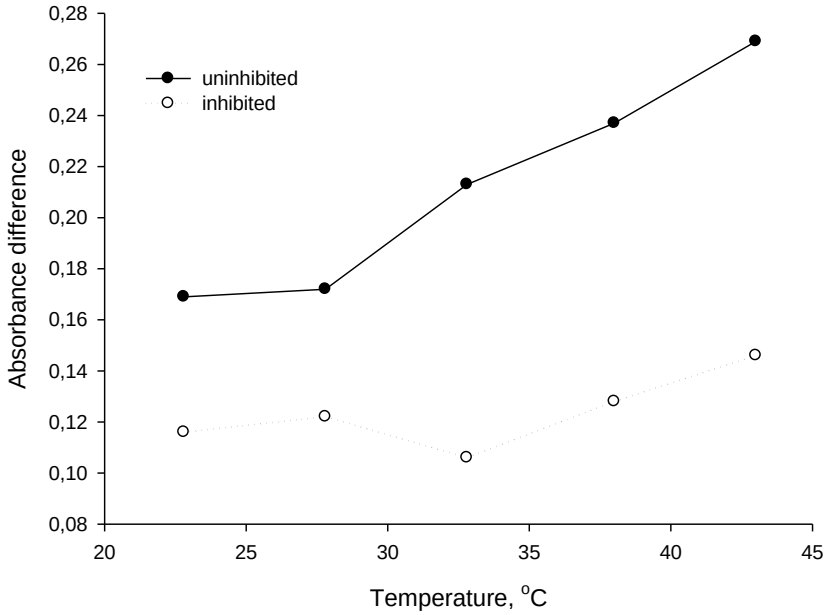

Fig. 5 Effect of temperature on the reaction rate (Conditions: pH: 2.5, 2.0 mL buffer, [LAA]: $3.5x10^{-3}$M, [GS]: $1.02x10^{-4}$ M).

Table 1. Calibration equations with respect to different time intervals.

Time interval (sec.)	Calibration equation, correlation coefficient, (r^2)	LOD	Linear range, µg ml^{-1}
30-120	$\Delta(\Delta A)=0.0132C_{cu} + 0.0176$, (0.9812)	0.293	1.0-8.0
30-180	$\Delta(\Delta A)=0.0195C_{cu} + 0.0658$, (0.9884)	0.200	0.5-8.0
30-240	$\Delta(\Delta A)=0.0286C_{cu} + 0.0965$, (0.9903)	0.188	0.2-4.0
30-300	$\Delta(\Delta A)=0.0372C_{cu} + 0.1119$, (0.9911)	0.131	0.1-8.0

Table 2.Tolerance limits of diverse ions on the determination of 2 µg/mL Cu(II) ion.

Interfering ion	Tolerance Limit
Na^+, K^+, Li^+, Co^{2+}, Ba^{2+}, $H_2PO_4^-$, Br^-, NO_3^-, Cl^-	1000*
Cd^{2+}, Mn^{2+}, Sr^{2+}, SO_4^{2-},	800
Zn^{2+}	600
HPO_4^{2-}, Hg^+	400
F^-, PO_4^{3-}, IO_3^-,SO_3^-	100
Cr^{3+}, Mo^{6+}	50

*Above of which was not tested

Table 3. Determination of Cu (II) in fungal preparation and standard reference material (n: 5).

Sample	Developed method		Relative error%
Fungal preparation	111,52 ± 0,72 (µg/ml)	109,88± 1,22 (µg/ml) (AAS method)	2.4
NIES CRM 8 Vehicle Exhaust	64.24±0,61(µg/g)	67±3 (µg/g) (Reference value)	4.1

Table 4. Determination of Cu (II) in tap water (n:5).

Added (µg/ml)	Found (µg/ml)	Recovery %	AAS method (µg/ml)
0	-	-	-
0.370	0.361±0.011	97.6	0.373±0.016
0.625	0.609±0.07	97.4	0.633±0.012
0.875	0.883±0.018	100.9	0.887±0.027