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Spectrophotometric determination of uric acid in urine and blood samples

Mousa, hend gamal*and Elgendy, khaleed Mansour
Department of chemistry, science faculty, zagazig university
*hendgamal19935@gmail.com

ABSTRACT **he simple, fast, accurate, and sensitive spectrophotometric method was described for the determination of uric acid in blood, urine, and meat. The method depended on the unreacted Ce^{4+} was determined by using two different dyes indigo carmine and methyl orange at 610 nm and 505 nm respectively. The spectrophotometric method involves the oxidation of uric acid with ceric ammonium nitrate used as a strong oxidant in presence of acetate buffer pH (4) as an acidic medium with indigo carmine dye and the presence of sulfuric acid (pH 0.2) as an acidic medium with methyl orange dye. Optimum conditions affecting the method were studied such as pH and buffer, oxidant volume, dye volume, sequence of addition, time, organic solvent, and temperature. Beer's law was obeyed in the concentration range of (0.016-6.7) mg mL^{-1} with indigo carmine, with molar absorptivity 0.396×102 L mol-1cm-1and (0.084-2.3) mg mL^{-1} with methyl orange, with molar absorptivity 2.08118×10^{2} L $mol^{-1}cm^{-1}$.**

Keywords: **Uric acid, ceric ammonium nitrate, indigo carmine, methyl orange, spectrophotometric method.**

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I. INTRODUCTION

Uric acid (UA) [7, 9-dihydro-1H-purine-2, 6, 8(3H)-trione] is the main antioxidant as well as the final product of catabolization of the purine nucleosides, adenosine, and guanosine. UA is considered to be an important biomarker in urine and serum, and abnormal concentrations of UA in blood and urine are associated with gout, hypertension, cardiovascular diseases, renal disease, and Lesch–Nyhan syndrome, which is related to poor solubility of UA in water, strong interaction between UA and protein molecules and complex redox-dependent pathways in vivo [1-3] . The chemical structure of uric acid is shown in Figure 1.

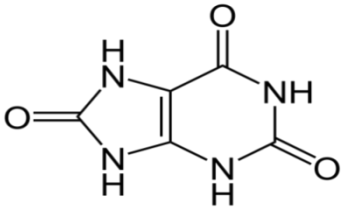

Figure 1. Chemical structure of uric acid

There are numerous methods for the determination of uric acid such as the voltammetric method which is an electrochemical sensor-based multiwalled carbon nanotube (MWCNTs)-poly (4-amino-3-hydroxy naphthalene sulfonic acid) modified glassy carbon electrode (MWCNTs/poly (AHNSA)/GCE) was developed for the determination of uric acid [4]. The fluorescence method was involved in oxidizing uric acid to allaintoin and hydrogen peroxide. The hydrogen peroxide produced was able to quench the QDs fluorescence, which was proportional to Uric acid concentration [5]. The electrochemical method was based on the electrochemical behaviors of UA at various electrodes [6]. The Colorimetric method for detection of uric acid was achieved by using the MoS2 nanoflakes-catalyzed 3,3′,5,5′-tetramethylbenzidine (TMB)-H2O2 system [7]. Spectrophotometric determination of uric acid was done by using diazotized 4-nitroaniline reagent in an alkaline solution to form a stable brown-colored azo dye [8]. Extraction-Spectrophotometric determination of uric acid was based on the

coupled redox and complexation reactions [9]. Spectrophotometric determination of uric acid in urine was based on the enzymatic method of uricase with 4-aminodiphenylamine diazonium sulfate **10**.

Oxidation with Ce^{4+}

Ce (IV) has been used as an effective oxidizing agent for the determination of drugs such as Naltrexone[11], 1,4- Dihydrpyridine[12], Clindamycin phosphate[13]and third generation cephalosporins[14].

Ce4+ also has been used for determining some metals such as Neptunium[15], Iron (II) [16], and Plutonium[17].

II.MATERIALS AND METHODS

Reagents and chemicals

All of the chemicals and solvents utilized were analytical grade reagents that were not purified further. Double distilled water was used in all experiments. Uric acid (purity 99.90%) was obtained from Sigma-Aldrich. Ceric ammonium nitrate was obtained from BDH Company, methyl orange and indigo carmine were obtained from Sigma-Aldrich. Methanol, Ethanol, Acetone, Ethylene glycol, Glycerin, and Formaldehyde were obtained from Elnaser Company (Egypt).

Instrumentation

UV/ Vis double beam spectrophotometer (T80 Spectrophotometer, PG instruments Ltd. Company, United Kingdom) was used to measure absorbance by using quartz cells of 10 mm path length and 2 mm bandwidth. Change in pH was evaluated by using an Adwa pH-meter, model AD 1030, from Romania

Solutions

A stock solution of 1.00×10^{-2} M of uric acid was prepared by dissolving 0.168 g in 20.0 mL of NaOH (0.1 M) and completed with distilled water to 100 mL in measuring flask. Working solutions with lower concentrations were obtained by dilution from the stock solutions.

A 1.00×10-3 M solution of Indigo carmine (IC) was formed by dissolving 0.046 g in a standard flask and adding distilled water to 100 mL. A solution of 1.00×10-3 M of methyl orange (MO) could be produced by dissolving 0.032 g of methyl orange in a 100 mL a measuring flask and adding distilled water to the mark. 1.00×10^{-3} M ceric ammonium nitrate solution was made by dissolving 0.054 g in the standard flask and diluting with distilled water to 100 mL.

Buffers

A series of universal buffer solutions ranging in pH from (2.0-10.0) 18, borate buffer solutions (7.0-9.0) 19, acetate buffer solutions (3.0 to 6.0), and phosphate buffer solutions with pH values ranging from 7 to 10 were prepared according to standard methods 20.

General procedure

In a 10 mL standard flask, 1.00 mL of uric acid was added and followed by 3.00 mL of ceric ammonium nitrate, and 1.50 mL of acetate buffer pH (4) then it was heated in a water bath for 10 min at 70 ºC after cooling 1.25 mL (1×10^{-3} M) of indigo carmine dye was added and completed with distilled water to the mark, then absorbance was measured at 610 nm. In another 10 mL standard flask, 1.00 mL of uric acid was added and followed by 1.50 mL of ceric ammonium nitrate and 2.00 mL of sulfuric acid (H_2SO_4), after 10 minutes 0.8 mL of MO (1×10^{-3} M) was added and completed with distilled water to the mark, then absorbance was measured at 505 nm. The shift in absorbance of the dye was measured at the appropriate wavelengths for indigo carmine or methyl orange dyes to determine the unreacted oxidant.

Factors affecting the procedure

Different factors were studied such as (pH and buffer, oxidant volume, dye concentration, time of reaction, organic solvent and sequence of addition).

Beer's law

Appropriate aliquots of (0.016-6.7) mg mL^{-1} of uric acid with indigo carmine, and (0.084-2.3) mg mL^{-1} with methyl orange were transferred into a series of 10 mL standard flasks. To these 1.50 mL of acetate buffer (pH_4) and 3.00 mL of ceric ammonium nitrate were added then it was heated in the water bath for 10 minutes at 70 ºC after cooling 0.5 mL (1×10^{-3} M) of indigo carmine dye was added but with methyl orange dye, 2.00 mL of H_2SO_4, 1.5 mL of ceric ammonium nitrate was added and followed by 1.00 mL of methyl orange after 10 minutes. Volumes were diluted up to the mark with distilled water and shaken well. The absorbance of each solution was measured at 505 and 610 nm against the corresponding reagent blank.

Applications

Determination of uric acid in meat

7.28 g of fresh meat was digested by digestion mixture (10.0 mL of HNO_3, 1.00 mL H_2SO_4, 2.00 mL of $HClO_4$) for 5 minutes, and then it was filtered and diluted by 10.0 mL of distilled water. In a 10 mL standard flask, 1.00 mL of filtrate, 1.50 mL of acetate buffer (pH_4), and 3.00 mL from Ce^{4+} (1.00×10^{-3} M) were added, and after 10 minutes 0.50 mL of indigo carmine was added and completed with distilled water to the mark, and absorbance was measured against the blank. The same steps were done with methyl orange but 1.5 mL Ce^{4+}

(1.00×10^{-3} M) and 2.00 mL H_2SO_4 were added to the volume from the meat sample, and absorbance was measured against blank at 610 nm and 505 nm.

Determination of uric acid in urine

A urine sample was obtained from two patients, i.e., an adult man (32 years old) and a woman (24 years old), who did not suffer from kidney problems. 100 mL of urine sample was concentrated by gentle heating to half the sample then it was filtrated. In a 10 mL standard flask, 1.00 and 2.00 mL from filtrate, 1.50 mL acetate buffer (pH $_4$), 3.00 mL from Ce^{4+} (1.00×10^{-3} M) were added, after 10 minutes 0.50 mL of indigo carmine was added and completed with distilled water to the mark and absorbance was measured against blank, the same steps were done with methyl orange but 1.5 mL of Ce^{4+} (1.00×10^{-3} M) and 2.00 mL of H_2SO_4 with the same volume from a urine sample and absorbance was measured against blank.

Determination of uric acid in the blood

A certain volume of blood (5 mL) was extracted from an adult human body. This sample was centered in a centrifuge using a tube that contained EDTA as an anticoagulant to separate plasma where EDTA was added to the blood sample by percent (1.2 to 2.0 mg/mL blood)21 and in a 10 mL standard flask 0.20 mL of plasma, 1.50 mL acetate buffer (pH $_4$) and 3.00 mL from Ce^{+} (1.00×10^{-3} M) were added then after 10 minutes 0.50 mL of indigo carmine was added and completed with distilled water to the mark and absorbance was measured against the blank. The same steps were done with methyl orange but 1.5 mL Ce^{4+} (1.00×10^{-3} M) and 2.00 mL H_2SO_4 were added and absorbance was measured in the same manner.

III.RESULTS AND DISCUSSION

Indigo carmine dye recorded high absorbance at 610 nm and methyl orange dye recorded high absorbance at 505 nm by scanning wavelengths from 200 to 700 nm as it showed in Figure 2.

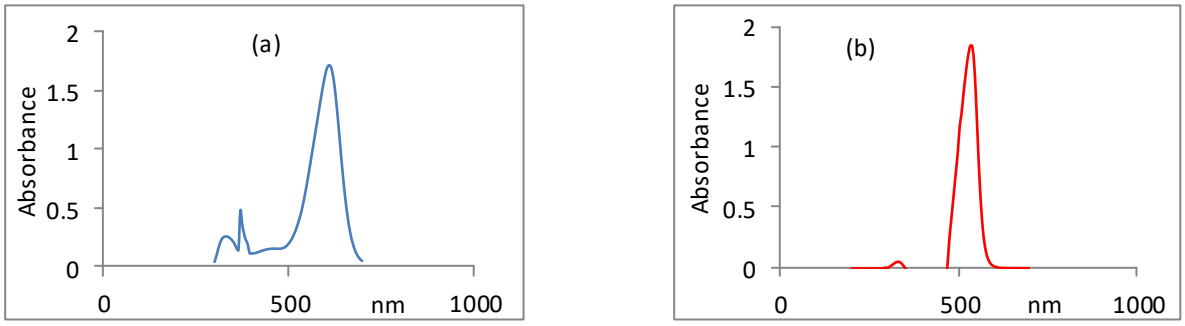

Figure 2. Absorption curve of (a) indigo carmine dye, (b) methyl orange dye

Factors affecting oxidation of uric acid with Ce $^{4+}$

The reaction of ceric ammonium nitrate with uric acid led to the oxidation of uric acid as ceric (IV) is a strong oxidizing agent. Indigo carmine and methyl orange dyes were oxidized by the unreacted ceric ammonium nitrate and these dyes were used to determine residual ceric (IV). The residual Ce^{4+} was calculated spectrophotometrically, where the amount of unreacted Ce^{4+} related to or equaled the change in absorption spectra of IC or MO.

Effect of pH and buffer

To determine the best acidic medium where oxidation occurred, sulfuric acid (pH 0.2) and acetate buffer (pH $_4$ and 5) were used. The results showed that acetate buffer (pH $_4$) was a suitable acidic medium with IC and sulfuric acid was a suitable acidic medium with MO as in Figure 3.

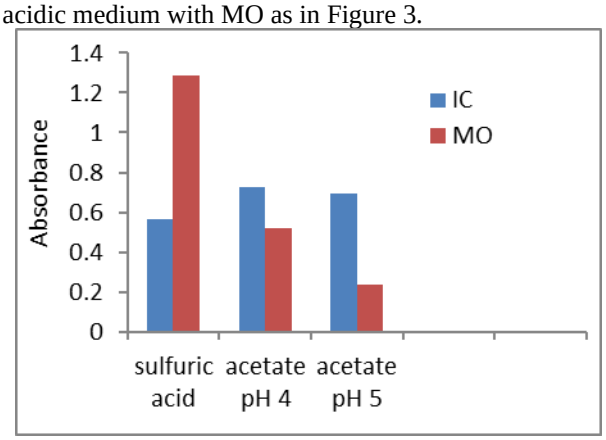

Figure 3. Effect of pH and buffer on the determination of uric acid

Effect of acid volume

To investigate the optimum volume of the acidic medium, different volumes of the acidic medium were used. The results showed that 1.50 mL of acetate buffer was a suitable volume with IC; 2.00 mL of H_2SO_4 was the convenient volume with MO as shown in Figure 4.

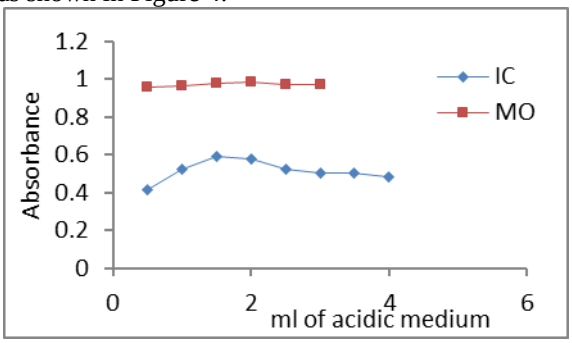

Figure 4. Effect of acidic medium volume on the determination of uric acid

Effect of oxidant volume

For the determination of the ideal volume of (1.00×10^{-3} M) ceric ammonium nitrate, different volumes of Ce^{4+} were used to oxidize uric acid. The results showed that the appropriate volume with using indigo carmine was 3.00 mL but with using methyl orange was 1.5 mL.

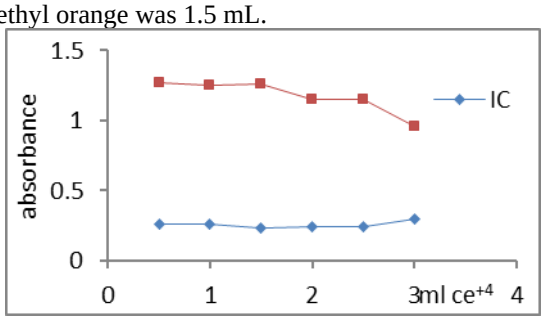

Figure 5. Effect of oxidant volume on the determination of uric acid

Effect of dye volume

Different volumes of IC and MO were used to estimate the optimal amount of (1.00×10^{-3} M) indigo carmine or methyl orange. The results in (Figure 6) showed that the appropriate volume was 0.50 mL for IC and 1.00 mL for MO which gave a maximum absorbance value.

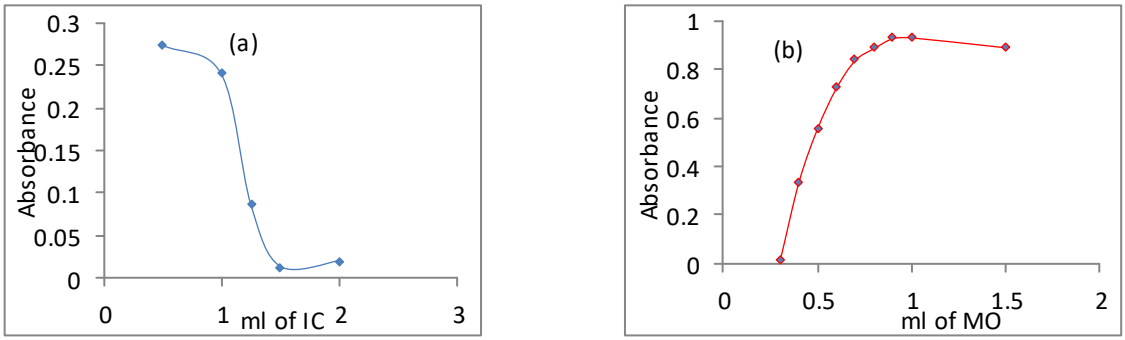

Figure 6. Effect of (a) IC volume on oxidation reaction, (b) MO volume on oxidation reaction of uric acid

Effect of time

The optimum time required to complete oxidation of urea and thiourea was determined by absorbance was measured at different times. The results showed that the change in absorbance was slightly by passing time with the two dyes, as shown in Figure 7.

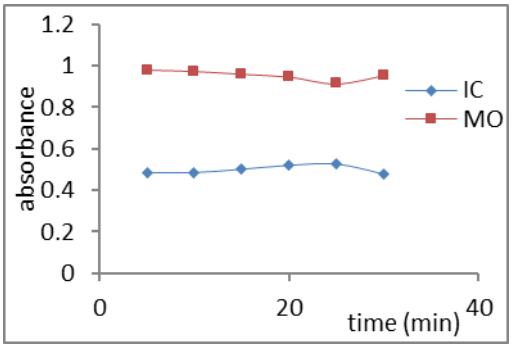

Figure 7. Effect of time on determination of uric acid

Effect of a sequence of addition

For determining the optimum sequence of addition different various absorbance samples were recorded by changing the order of addition of uric acid, Ce4+, and acidic medium (acetate buffer or H_2SO_4). The most proper sequence addition was (acetate buffer- ceric ammonium nitrate - uric acid) with IC and (uric acid- ceric ammonium nitrate- sulfuric acid 1.00 M) with MO as shown in table I.

Table I. Effect of a sequence of addition on the determination of uric acid

Sequence	**Absorbance**	
	IC	MO
Uric acid +Ce^{+4} + acidic medium	0.504	1.029
Ce^{+4} + uric acid + acidic medium	0.399	1.023
acidic medium + uric acid + Ce^{+4}	0.434	1.009
acidic medium + Ce+4 + uric acid	0.657	1.001
Ce^{+4} + acidic medium + uric acid	0.538	0.989

Effect of temperature

Different temperatures were checked to determine the best temperature for uric acid that affect the stability of the formed complex, and the results showed that the best temperature was 70 oC with using indigo carmine and 80 oC with using methyl orange as shown in Figure 8.

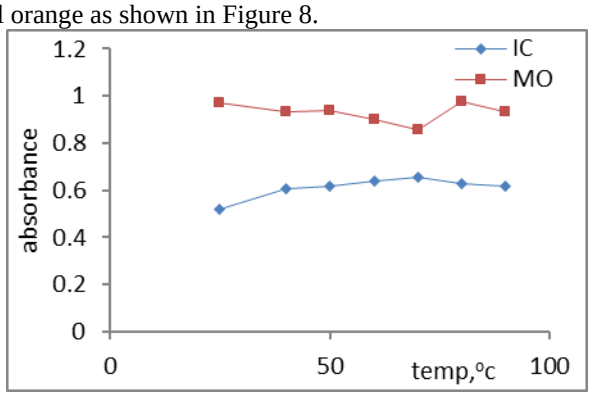

Figure 8. Effect of temperature on the determination of uric acid

Effect of organic solvent

Different organic solvents were used to select the ideal organic solvent on the absorption spectra such as ethanol, methanol, formaldehyde, glycerin, and acetone; the results showed that no definite organic solvent increased the absorbance of uric acid. Using IC ethanol, methanol, formaldehyde, acetone decreased absorbance and glycerin had no effect but with MO organic solvent had no effect, as shown Figure 9.

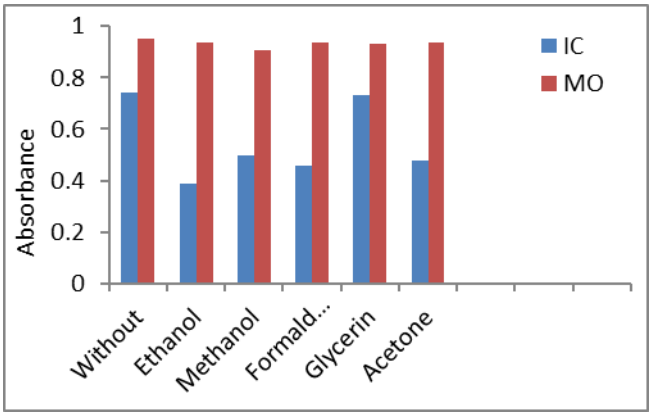

Figure 9. Effect of organic solvent on the determination of uric acid

A Calibration curve (Beer's law)

The calibration curve was created using spectrophotometric data taken after optimizing all of the generated complex's optimal parameters as detailed above. Different concentrations of uric acid with indigo carmine and methyl orange dyes were used to study Beer's law. The results showed that the level of Beer's law was (0.016-6.7) µg mL^{-1} with IC and (0.084-2.3) µg mL^{-1} with MO.

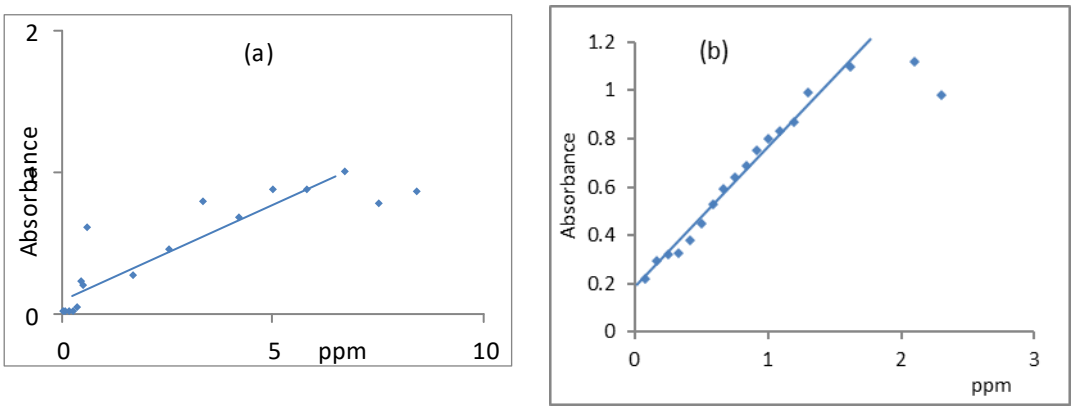

Figure 10. Effect of uric acid concentration with IC (a) and with MO (b)

Effect of interference

The use of interfering materials such as thiourea, ascorbic acid, glucose, hydroxylamine, sodium nitrite, and sodium nitrate of 1×10^{-2} M with 1×10^{-3} M of uric acid was investigated. The results showed that there was no interference with each interfering material with uric acid in the presence of two dyes.

Statistical treatment for determination of uric acid

The method had good reproducibility for a set of seven measurements of 1.68 and 0.75 µg mL^{-1} of uric acid with IC and MO, respectively under optimum conditions. Some different statistical parameters such as standard deviation, molar absorptivity, Sandell's sensitivity, student's t-test, the limit of detection (LOD), and limit of quantification (LOQ) are summarized in Table II. The data indicated the excellent linearity, high sensitivity, good accuracy, and precision of the proposed method.

Table II. Statistical treatment for spectrophotometric determination of uric acid

Parameter	**Uric acid**	
	IC	MO
Molar absorptivity L.mol-1. cm-1	0.396×10^{2}	2.08118×10^{2}
Sandell's sensitivity µg cm-2	4.245	0.8077
Mean value ($\bar{x}$)	1.69	0.7568
Standard deviation (SD)	0.01586	0.002829
(RSD %)	0.93846	0.37381
Standard error of the mean (SE)	0.005994	0.001069
Student t – test	1.668	6.3595
Slope	0.112329	0.42703

Limit of detection (µg / ml)	0.46593	0.0218619
Limit of quantification (µg / ml)	1.411923	0.06624

Applications of uric acid in urine and blood by using IC and MO dyes

The suggested method was used to determination of uric acid in human urine, human blood, and meat by using IC and MO dyes. The results in Table (III-IV) were in good agreement with the recommended method, indicating that the proposed method could be used.

Table III. Applications of uric acid by using IC dye (the recommended method is according to Swislocka 9)

Sample	**IC dye**				
	Taken	Found	Recommended method	%Recovery	%RSD
Man urine	4.23	4.3	4.2	99.999	0.001
Women urine	1.88	1.8	1.86	99.9994	0.0006
Blood	2.44	2.5	2.42	99.9992	0.0008
Meat	4.18	4.2	4.16	99.9996	0.0004

Table IV. Applications of uric acid by using MO dye (the recommended method is according to Swislocka 9)

Sample	**MO dye**				
	Taken	Found	Recommended method	%Recovery	%RSD
Man urine	1.34	1.36	1.32	99.9996	0.0004
Women urine	1.36	1.38	1.35	99.9997	0.0003
Blood	1.22	1.20	1.21	99.9999	0.0001
Meat	1.1	1.13	1.0	99.987	0.013

IV.CONCLUSION

The obtained data confirmed that the method was accurate and sensitive. It did not require expensive chemicals. The method did not also need organic solvent or high temperature and determine a low concentration of material. The method did not require uricase enzyme or deprotientation. Beer's law range showed that the determination of uric acid by MO was better than determination by IC. We proposed this method as a simple and quick procedure for uric acid estimation. The color of the reaction was stable for a long time. Data confirmed that the method determined micro concentration. The simplicity of the developed system and low costs of measurements were the main advantages of the presented method. This method determined uric acid easily in human urine, blood and meat.

REFERENCES

Lu, J. Y.; Xiong Y. H.; Liao, C. J., and Ye, F. G. Colorimetric Detection of Uric Acid in Human Urine and Serum Based on Peroxidase Mimetic Activity of MIL-53(Fe). Anal Methods. 2015, 7(23), 9894–9899

Wu, D.; Lu, H. F.; Xie, H.; Wu, J.; Wang, C. M. and Zhang, Q. L. Uricase-Stimulated Etching of Silver Nanoprisms for Highly Selective and Sensitive Colorimetric Detection of Uric Acid in Human Serum. Sens Actuator B: Chem. 2015, 221, 1433–1440

Sun, Y. P.; Chen, J.; Qi, H. Y. and Shi Y. P. Graphitic Carbon Nitrides Modified Hollow Fiber Solid Phase Microextraction for Extraction and Determination of Uric Acid in Urine and Serum Coupled with Gas Chromatography-Mass Spectrometry. J.Chromatogr B. 2015,1004, 53-59

Tefera, M.; Tessema, M.; Admassie, S. and Wubet, W. Voltammetric Determination of Uric Acid Using Multiwall Carbon Nanotubes Coated-poly (4-amino-3-hydroxy naphthalene sulfonic acid) Modified Glassy Carbon Electrode. Heliyon. 2021, 7(7), e07575.

Azmi, N. E.; Ramli, N. I.; Abdullah, .J.; Abdul Hamid, M. A.; Sidek, H.; Abd Rahman, S. and Yusof, N. A. A Simple and Sensitive Fluorescence Based Biosensor for the Determination of Uric Acid Using H2O2-Sensitive Quantum Dots/Dual Enzymes. Biosensors and Bioelectronics. 2015 , 67, 129–133

Guo, X. M.; Guo, B. Li .C and Wang, Y. L. Amperometric Highly Sensitive Uric Acid Sensor Based on Manganese (III) porphyrin-Graphene Modified Glassy Carbon Electrode. Journal of Electroanalytical Chemistry. 2016 , 783, 8–14

Wang, X.; Yao, Q.; Tang, X.; Zhong, H.; Qiu, P. and Wang X. A Highly Selective and Sensitive Colorimetric Detection of Uric Acid in Human Serum Based on MoS2-Catalyzed Oxidation TMB. Analytical and Bioanalytical Chemistry. 2018, 411(4), 943–952

Al-Kass, S. Y. and Younis, T. I. Spectrophotometric Determination of Uric Acid by Coupling with Diazotised 4-Nitroaniline Reagent. Raf. Jour. Sci. 2004, 15 (2), 174 - 183

Swislocka, R. and Tomicka, H. S. Extraction Spectrophotometric Determination of Uric Acid. Chem. Anal. (Warsaw). 1996, 41, 793-800

Hamzah, H. H.; Zain, Z. M.; Musa, N. L. W.; Lin, Y. C. and Trimbee, E. Spectrophoto -metric determination of uric acid in urine based-enzymaticmethod uricase with 4- amino-diphenylamine diazonium sulfate (Variamine Blue RT Salt). J. Anal. Bioanal .Tech. 2013, 2-6

El-Didamony, A. M. and Hassan, W. S. Spectrophotometric and Fluorimetric Method for Determination of Naltrexone (NALT) in Urine, Serum and Tablets by Oxidation with Cerium IV. J. Chil. Chem. Soc. 2012, 57(4), 1404-1408

Askal, H. F.; Abdelmegeed, O. H.; Ali, S. M. S. and Abo El-Hamd, M. Spectrophotometric and Spectrofluorimetric Determination of 1,4- dihydropyridine Drugs Using Potassium Permanganate and Cerium IV Ammonium Sulphate, Bull. Pharm. Sci. 2010, 33(2), 201-215

Badi, S. S. and Tuwar, S. M. Oxidation of clindamycin phosphate by cerium (IV) in perchloric acid medium – A kinetic and mechanistic approach. Arabian Journal of Chemistry. 2017, 10, 1469–1476

Zaky, M.; Fadel, S. and Elgendy, K.M. Spectrophotometric method for determination of some third generation cephalosporins antibiotics using sodium molybdate, Eurasian J. Anal. Chem. 2020, 15(1), 59-73

Godbole A. Determination of Neptunium by Redox Titration. Talanta. 1979, 26(4), 330–332

Adamson, M. G.; Dainton, F. S. and Glentworth, P. Kinetics of the oxidation of iron (II) by cerium (IV) in aqueous perchloric acid. Transactions of the Faraday Society. 1965, 61, 689

Dhamodharan, K. and Pius, A. Determination of Plutonium Present in Highly Radioactive Irradiated Fuel Solution by Spectrophotometric Method. Nucl. Eng. Technol.2016, 48(3), 727–732

Britton, H. T. S. Hydrogen Ions, 4th ed., Chapman and Hall, London, 1952

Bower, V. E. and Bates, R. G. pH Values of the Clark and Lubs buffer Solutions at 250C . J.Res.Natl. Bur. Stand. 1995, 55, 197-200

Britton, H. T. K. and Robinson, R. A. Universal Buffer Solutions and the Dissociation Constant of Veronal. J.Chem. Soc. 1931, 1456-1462

Guide I. Terms and definitions used in connection with reference materials, 30, 2nded, 1992