Colorimetric Determination of Uric Acid in Live samples

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Abstract

A simple, sensitive and precise colorimetric method for uric acid determination was described. The method based on the complexion reaction between uric acid, potassium ferricyanide, and ferric chloride in hydrochloric acid medium to form blue colored charge transfer complex measured at maximum wavelength 752 nm. The optimum conditions obtained were volume 0.3 ml for hydrochloric acid, 1.25 ml for both potassium ferricyanide and ferric chloride solutions, 60◦C and 8 minutes as maximum temperature and time of reaction, the order of mixing additives was uric acid, ferric chloride, potassium ferricyanide, and hydrochloric acid. The highly accurate and precise results obtained with RSD%, Recovery%, Ere%, and D.L were (0.755 to 4.376), (99.676 to 97.831), (-0.033 to -2.169), and 0.1569 µg mL^{-1} respectively, the calibration graphs were linear in the concentration range of (2.0-200µg ml^{-1}), the . molar absorptivity (5937.473L mol^{-1} cm^{-1}) The proposed methods were successfully applied for the determination of uric acid in serum and urine samples for twenty persons.

Keywords: *Uric acid, colorimetric, determination, charge transfer complex.*

Introduction

Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula $C_5H_4N_4O_3$. It forms ions and salts known as urates and acid urates, such as ammonium acid urate. Systematic IUPAC name is 7,9-Dihydro-1*H*-purine-2,6,8(3*H*)-trione[(1)].

Uric acid is an end product of the breakdown of compound called purine. Purines (adenine and guanine) are chemical compounds plays a critical role in many body biological processes, by producing some important bioactive molecules; these molecules have a major part in genetic materials synthesis, protein synthesis, energy molecules production, and can act as mediators in nervous system[(2)], The major sources of purines are from diet, body synthesis from non-purine precursors, and turnover of nucleic acid[(3)].

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Many analytical techniques were used for the determination of uric acid including spectrophotometric[(4-9)], flouromatric[(10-13)], enzymatic[(14, 15)], chromatographic[(16-19)], potentiometric[(20-22)], amperometric[(23-27)] , and voltammetric [(28-30)]techniques but these techniques required sophisticated instruments and expensive reagents. Hence, the development of charge transfer complex based on the reaction between uric acid, potassium ferricyanide, and ferric chloride in hydrochloric acid medium was the aim of this study due to its simplicity, accuracy and economy of this method.

Experimental

Apparatus

UV-Visible double beam spectrophotometer (JASCO V-650, Japan), Sensitive balance ± 0.0001 g (Sartorius BL 210 S Scientific balance, Gottingen–Germany), Hot plat (Labtech, Germany), water path (Lab tech, Korea) and centrifuge (Kokusan, Japan) were required in this study.

Reagents and chemical materials

All chemicals used were of analytical reagent

grade including [uric acid ($C_5H_4N_4O_3$) sigma aldrich], potassium ferricyanide($K_3[Fe(CN)_6]$, purity 99%, BDH), ferric chloride ($FeCl_3$, purity 96.8%, BDH) and hydrochloric acid (HCl, 37%, Carolina).

Preparation of solutions

Uric acid stock solution (1000 µmole L^{-1}):- One hundred milligram of uric acid standard material was dissolved by distilled water in a 100 ml volumetric flask.

Potassium ferricyanide solution (0.001 mole L^{-1}):- 0.326 gram of potassium ferricyanide standard material was dissolved in distilled water in 100 ml volumetric flask, 10 ml of this solution was diluted with 100ml distilled water to produce (0.001mole L^{-1}).

Ferric chloride solution (0.002 mole L^{-1}):- 0.162 gram of Ferric chloride standard material was dissolved in distilled water in 100 ml volumetric flask, 20ml of this solution diluted with 100ml distilled water to produce (0.002mole L^{-1}).

Hydrochloric acid solution (0.1mole L^{-1}):- Transfer 0.826 ml from concentrated Hydrochloric acid (37 %, specific gravity 1.184) to 100 ml volumetric flask and diluted to the mark with distilled water.

Recommended procedure

Batch experiments were conducted by the addition of equal volume (1.25ml) of (0.001mole L^{-1}) potassium ferricyanide and (0.002mole L^{-1}) of Ferric chloride solutions to 5ml volumetric flask. Then 0.2 ml (4 drops) of (0.1mole/L) hydrochloric acid solution with 1ml of uric acid was added. The volume of the mixture was completed to the mark and heated to 50◦C for 5 minutes, the absorbance of charge transfer complex formed at range 200-1100 nm was recorded. Various parameters effect experiments were conducted to evaluate the influence of six parameters selected for this study including Hydrochloric acid volume by changing its volume in the range of (0.25 - 2ml), potassium ferricyanide volume by varying its volume in the range of (0.1 - 0.5ml), Ferric chloride volume effect was studied in the range of (0.25-2ml). Also, the effect of temperature on the charge transfer complex formation controlled between (30-90◦C) in water bath was studied. The reaction time between (2-15minutes) was evaluated to reach the maximum absorbance. Finally, the sixteen order of four additives (uric acid, potassium ferricyanide, ferric chloride, and hydrochloric acid) were studied by changing with a different order. Standard calibration curve for uric acid was constructed in the range of (2-260 µg mL^{-1}) concentrations.

Determination of uric acid in live samples:-

Blood serum samples:- After preparation of standard solutions, the absorbance were recorded at optimum conditions controlled previously for plotting calibration curve, the absorbance of twenty serum samples for gout patients separated by centrifuge at (3500 cycle/minute) for 5 minutes was recorded.

Urine samples:- After preparation of standard solutions, the absorbance were recorded at optimum conditions controlled previously for plotting calibration curve, the absorbance of twenty urine samples for gout patients were recorded.

Results and Discussion

Characterization of charge-transfer complex

Potassium ferricyanide and ferric chloride in hydrochloric acid medium (figure 1(a)) forms a charge transfer complex with uric acid which is measurable spectrophotometrically at λmax=752 nm (figure 1(b)).

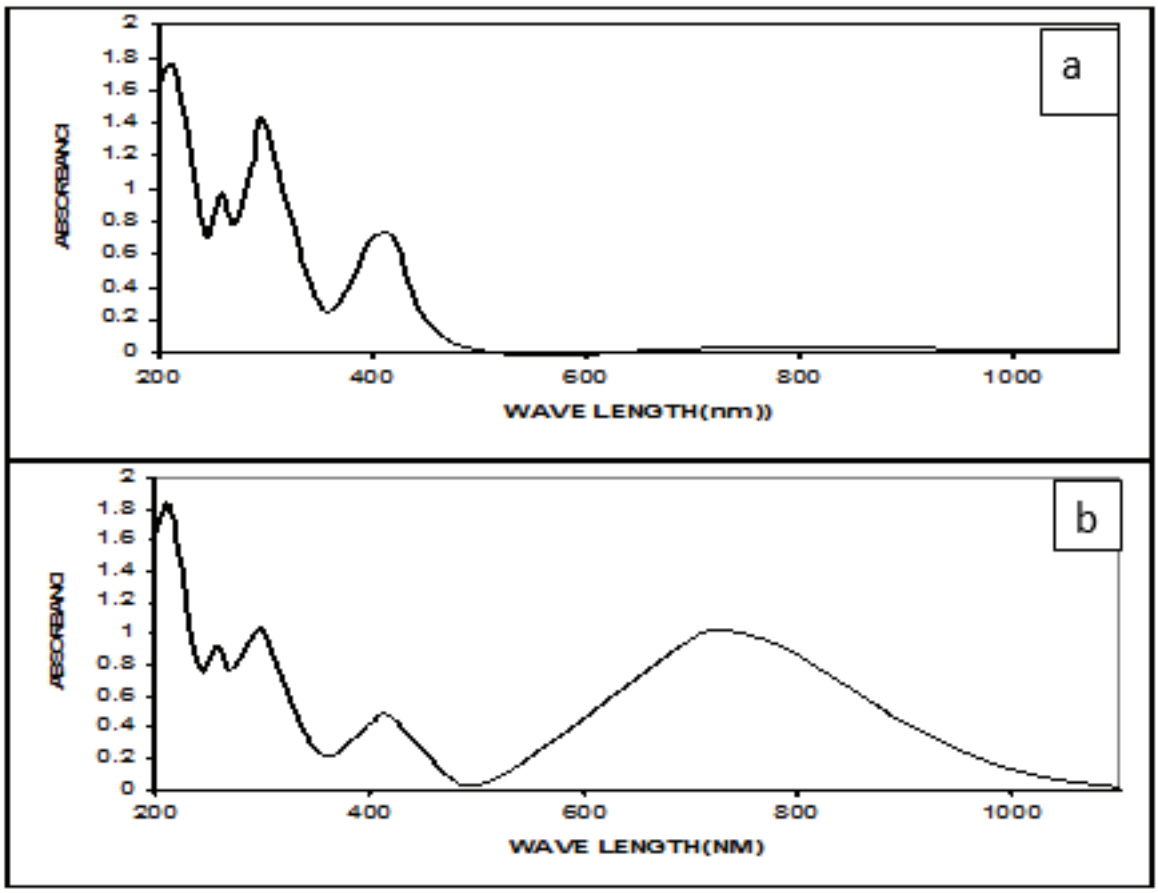

Figure 1. (a) Spectrum of blank solution containing potassium ferricyanide and ferric chloride in hydrochloric acid medium, (b) Spectrum of a charge-transfer complex containing potassium ferricyanide and ferric chloride in hydrochloric acid medium reacting with uric acid.

As can be seen from figure 1 (a and b), the absorbance of yielded charge transfer complex shows the best wavelength is located at λmax=752 nm , which shows negligible absorbance at corresponding λmax for the blank.

Optimization conditions

Initial experiments were conducted towards the optimization of various conditions involving the volume of potassium ferricyanide, hydrochloric acid, and ferric chloride reagents, temperature and reaction time to establish the maximum sensitivity for the charge transfer complex required for uric acid determination. The experiments were done by varying one parameters at the same time fixing the other parameters and the absorbance was measured at λmax=752 nm against reagent blank.

As cited in table 1, the addition of for potassium ferricynde solution increased the absorbance up to 1.25 ml, further addition resulted in a decrease in the absorbance. Therefore, 1.25 ml was chosen for further experiments. The other parameter studied was the volume of hydrochloric acid, it was found that 0.3 ml gives the best absorbance. Thus, 0.3 m was adequate for the maximum absorbance. Ferric chloride solution volume absorbance increased and reached to a plateau between (1.5 and 1.25ml). However, 1.25 ml was selected due the minor difference.

Table 1. The study of optimum parameters

Optimum reactant volume study						Optimum temperature		Optimum reaction time	
Potassium ferricyanide		Hydrochloric acid		Ferric chloride					
Volume (ml)	Absorbance	volume(ml)	Absorbance	volume(ml)	Absorbance	◦C	Absor-bance	minute	Absor-bance
0.25	0.526	0.1	0.547	0.25	0.359	30	0.382	2	0.759
0.5	0.830	0.2	0.563	0.5	0.710	40	0.391	4	0.806
0.75	0.884	0.3	0.584	0.75	1.021	50	0.430	6	0.872
1	0.891	0.4	0.504	1	1.090	60	0.477	8	1.022
1.25	0.940	0.5	0.439	1.25	1.403	70	0.448	10	0.985
1.5	0.863			1.5	1.406	80	0.412	12	0.944
1.75	0.730			1.75	1.288	90	0.370	14	0.439
2	0.625			2	1.090			15	0.125

The influence of temperature and reaction time on the charge transfer complex formation was studied within the temperature range 30 to 80 ºC as can be seen in table 2, the absorbance increase with increasing temperature up to 60 º which gave the maximum absorbance. Higher temperature resulted in a decrease in the absorbance. Reaction time is a fundamental parameter for economical uric acid analysis. Consequently, a study over a period of time ranged from 2 to 10 minutes were conducted and can be seen from table 1, 8 minute was sufficient to give the maximum absorbance that was used in all subsequent study. In order to avoid the loss in color intensity, the addition of uric acid followed by the ferric chloride, potassium ferricyanide and hydrochloric acid was followed in all experiments as shown in table 2.

Table 2. The optimum order for additives.

The order mixing additives				Absorbance
K3[Fe(CN)6]	HCl	FeCl3	Uric Acid	1.222
K3[Fe(CN)6]	Uric Acid	HCl	FeCl3	0.980
K3[Fe(CN)6]	FeCl3	Uric Acid	HCl	0.934
K3[Fe(CN)6]	HCl	Uric Acid	FeCl3	0.710
FeCl3	HCl	K3[Fe(CN)6]	Uric Acid	1.183
FeCl3	Uric Acid	HCl	K3[Fe(CN)6]	1.083
FeCl3	K3[Fe(CN)6]	Uric Acid	HCl	1.019
FeCl3	HCl	Uric Acid	K3[Fe(CN)6]	0.269
Uric Acid	HCl	FeCl3	K3[Fe(CN)6]	1.634
Uric Acid	K3[Fe(CN)6]	HCl	FeCl3	1.66
Uric Acid	FeCl3	K3[Fe(CN)6]	HCl	1.960
Uric Acid	HCl	K3[Fe(CN)6]	FeCl3	1.679
HCl	K3[Fe(CN)6]	FeCl3	Uric Acid	1.545
HCl	Uric Acid	K3[Fe(CN)6]	FeCl3	1.030
HCl	FeCl3	Uric Acid	K3[Fe(CN)6]	1.064
HCl	K3[Fe(CN)6]	Uric Acid	FeCl3	0.455

Calibration graph, accuracy and precision

Under optimum conditions, calibration graph was plotted between standard solutions over the range (2-200) µg mL^{-1} and its absorbance at λ_{max} = 752 nm as shown in figure 2. The analytical data for uric acid determination illustrate in table 2, shows the limit of detection of 0.1569 and recovery average of 98.682.

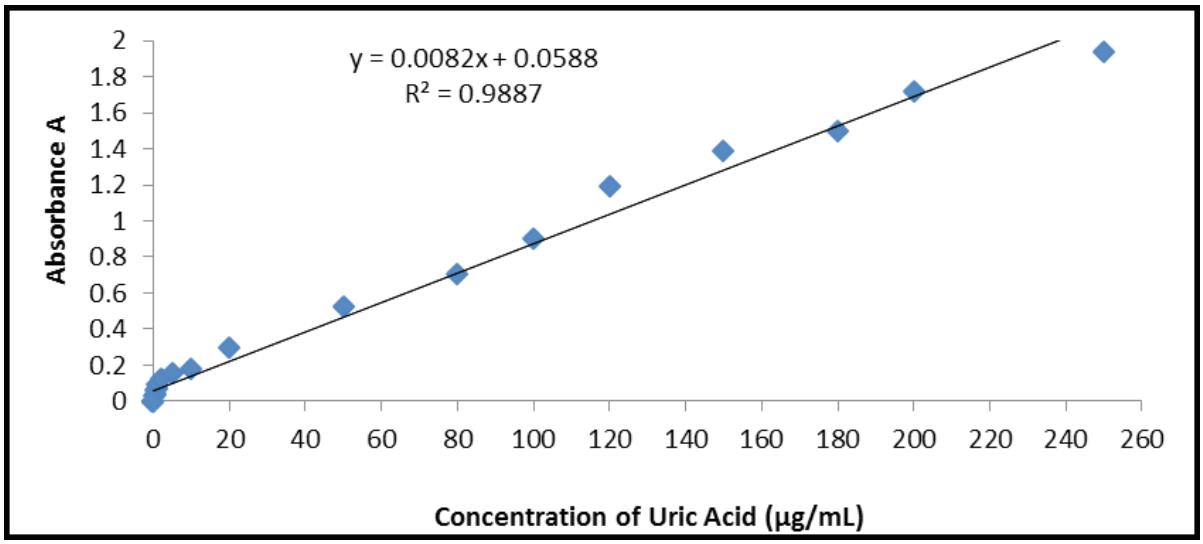

Figure 2. Calibration graph for uric acid determination.

To assess the accuracy and precision of the uric acid determination method, three different standard solutions (10, 50 and 100 µg mL^{-1}) prepared from 1000 µgmL^{-1} stock solution was analyzed according to the proposed method suggest in the paper, the results are shown in table 3.

Table 3. Accuracy and precision for uric acid analysis.

Uric acid Conc. (µg.mL-1)		Relative Error R.E.%	Percentage Recovery Rec.%	Average Recovery %	Standard Deviation S.D.	Relative Standard Deviation % R.S.D.
Taken	Found*					
10	9.967	-.033	99.676	98.682	0.148	0.755
50	49.225	0.775-	98.540		0.251	2.283
100	97.831	- 2.169	97.831		0.362	4.376

***Average of three determinations**.

Application of the adapted method for determination of uric acid in live samples:- The charge transfer complex method was effectively applied for uric acid determination in live sample including twenty men biological samples (serum and urine). The results indicated in table 4.

Table 4. The analysis of uric acid in live samples.

Concentration (µg mL-1)		Sample number	Concentration (µg mL-1)		Sample number
Urine	serum		Urine	serum	
78.195	276.975	11	19.958	390.390	1
100.146	385.512	12	29.414	385.512	2
47.707	444.048	13	31.853	385.512	3
18.439	273.317	14	20.878	352.585	4
17.219	141.609	15	8.682	142.829	5
16.000	139.170	16	9.902	220.878	6
31.853	268.439	17	19.658	168.439	7
39.170	385.512	18	17.951	330.634	8
91.609	347.707	19	4.463	26.975	9
105.024	409.902	20	2.285	86.731	10

Conclusion

The literatures listed a variety analytical techniques for determination of uric acid, this method were simple, accurate, rapid, precise, sensitive, and don't needs additional steps or special working conditions. Moreover, charge transfer complex formed owing good stability in the solution.

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Ethical approval: This study was permitted by the gout patients and all the experiments were done in compliance with the statements on informed consent by all patients.

Conflicts of Interest: There are no conflicts to declare.

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