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Spectrophotometric Determination of Uric Acid in Samples of Urine

Abbas. H. Al-Shukry*[1], Zain E. Mansor[2], Nada. A. Abd Al -Hussein[3]

[1&2&3] *College of Health and Medical Technologies, Uruk University, Baghdad, Iraq.*

a_alshukry@yahoo.com

Abstract A fade of the hexadecyltrimethylammonium-o-hydroxyhydroquinonephthalein-palladium (II) complex was used to quantify uric acid (UA), a spectrophotometric technique that is both straightforward and very sensitive. The effective molar absorbance at 635 nm complies with Beer's law over a range of concentration of 0.01 g ml^{-1}, **with a relative standard deviation of 6.50 × 10^5 dm^3/mol.cm (<1.5%).** Overall, UA was recovered in the range of 98-100% across all samples, with values **were of relative standard deviation (RSD) < 3.0%.** This review paper is based on the elaborate work [1], giving rise to very accurate for uric acid assay using **UV-VIS spectrometry system**, which is about 20 times more sensitive than other methods reported elsewhere. Human urine uric acid assays using this technique were of high accuracy.

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Keywords: *Uric acid, UV- spectrometry, Atomic absorption, UA- analysis, UA-determination, extraction.*

1. INTRODUCTION

Uric acid is found in human serum in concentration range 0.21 to 0.42 mmol/L in men and 0.16 to 0.36 mmol/L in women. Uric acid (UA) consists of a heterocyclic organic compound as seen in figure 1, with the chemical formula ($C_5H_4N_4O_3$) and a molecular weight of 168 g [2].

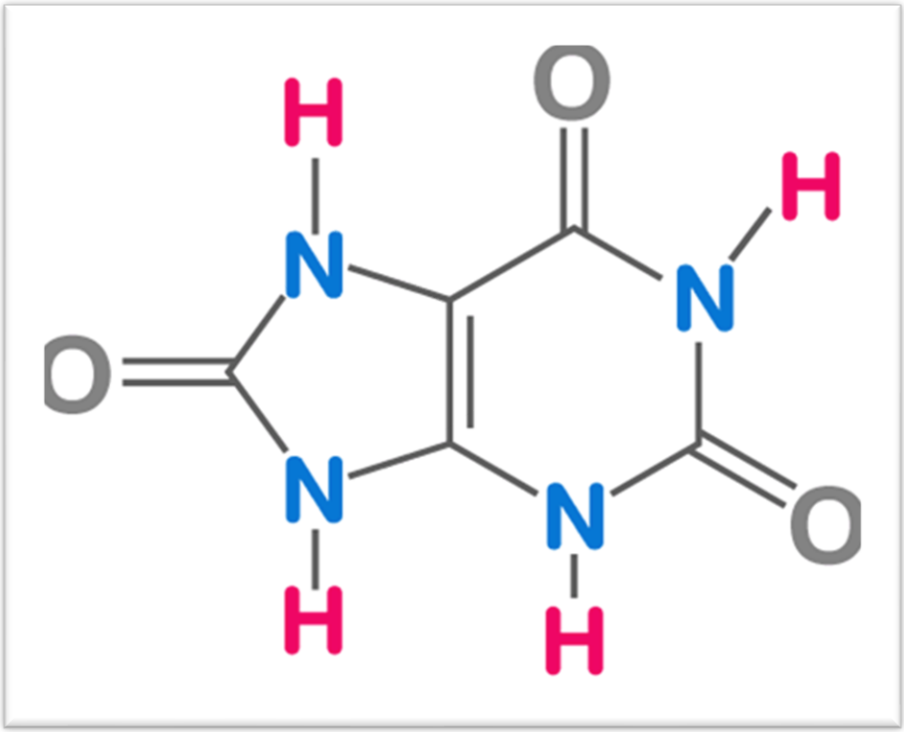

***Fig. 1** – Chemical Structure of uric acid.*

After the purine nucleosides' catabolism, the final product is uric acid (UA). There are several enzymes have roles in alteration of guanine monophosphate (GMP) and adenine monophosphate (AMP), which are purine nucleic

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acids, into uric acid as seen by figure 2. In human, the absence of these enzymes that can breakdown UA into its soluble form resulting in different problems. In contrast, UA has a protection function, as auxiliary antioxidants, whereas UA is involved in different pathological alteration and/or development of damage [3].

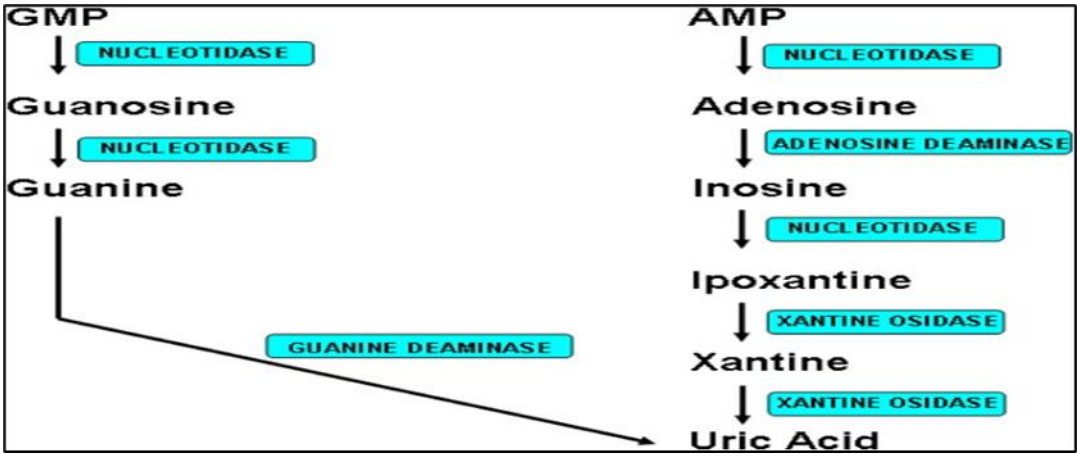

Fig. 2 *Enzymatic degradation of purines in humans.*

Due to its necessary in diagnostic examination and treatment of patients with different diseases like Lesch–Nyhan syndrome, renal insufficiency, gout, diabetes, hemolytic anemia, malignant lymphoma, hyperuricemia and gout, UA must be determined in urine and serum of human. Recently, hyperuricemia has been recognized as an excellent indicator of metabolic syndrome. For this purpose, different methods including chemiluminescence method, fluorometry, high performance liquid chromatography (HPLC), electroanalysis and astrophotometry, are used. Although the **colorimetric assays** for the UA estimation are diverse, majority of them are unacceptable, due to their time-consuming procedure, low reproducibility and low sensitivity, along with foreign substances' interference like ascorbic acid. Also, several donor atoms of UA able to form complex and attaching with **copper (II), zinc (II), nickel (II), iron (II),** and others. A method involving a metal ion and a dye was a significantly greater approach with respect to selectivity, sensitivity, and simplicity when analyzing a molecule with complex-forming capabilities[4-9]. Propranolol, ethylenediaminetetraacetic acid and hydrogen peroxide, biologically active thiols can all be detected with relative ease and sensitivity using the appropriate procedures. Small amounts of UA significantly inhibited the HTA-QP-palladium (II) complex's color development in a hexadecyltrimethylammonium (HTA) surfactant medium, and the reduce in HTA-QP-palladium (II) absorbance was directly proportional to the UA concentrations. This study describes a novel, straightforward spectrophotometrically method for determining UA, and it applies this technique to the UA detection in urine of human. The aim of this work is to develop the traditional uric acid determination method that is based on the formation of more stable colored complex. The existing methods could be conveniently divided into two groups: reductive and enzymatic. The reductive methods are non-specific and involve the **oxidation of uric acid with phosphotungstate reagent to allantoin with resultant blue coloring of tungstate solution.** The enzymatic methods are specific. They involve the catalytic oxidation of uric acid with the enzyme uricase e.g. oxidative coupling reaction between the N-methyl-N-(4-aminophenyl)-3-methoxyaniline (NCP) reagent and the hydrogen – donor reagent, N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS), in the system involving three enzymes: uricase, peroxidase and ascorbate oxidase to determine uric acid in concentrations up to 1.428 mmol/L, with a relative standard deviation of up to 1.8 %. With **limit of detection (LOD) = 0.0035 mmol/L and the limit of quantification was LOQ = 0.015 mmol/L of uric acid [4].**

2. EXPERIMENTAL

2.1 Apparatus and Reagents:

Uric acid sample was analyzed by FTIR system (figure 3) to verify its chemical structure.

By dissolving pure UA into 0.1 M sodium hydroxide solution, then adding water to prepare a stock solution (10^{-2} M) UA. Working solutions were made by diluting the UA stock solution with distilled water to the appropriate concentrations. From a standard stock solution, a palladium (II) solution (5.0×10^{-4} M) was diluted with water.

To prepare a solution of synthesized o-hydroxyhydroquinonephthalein (QP), 1 drop of hydrochloric acid was added to 1.0×10^{3} M methanol. Purified hexadecyltrimethylammonium bromide (HTAB) was placed in sterile water to create a 1.0×10^{2} M aqueous solution. Combining a 0.1 M hydrochloric acid solution with a solution of 0.05 M borax yielded a buffer solution with a pH of 8.2. There was no additional purification required for any of the

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other components or reagents because they were analytical grade of reagent. Deionized water was purified using a Milli-Q Labo device right before usage to make pure water. The absorbance was measured using a spectrophotometer of Shimadzu UV-160 with matching cells of silica of 1 cm in diameter. A calomel electrode of glass and pH meter of Horiba (F-11) were utilized to obtain the pH readings.

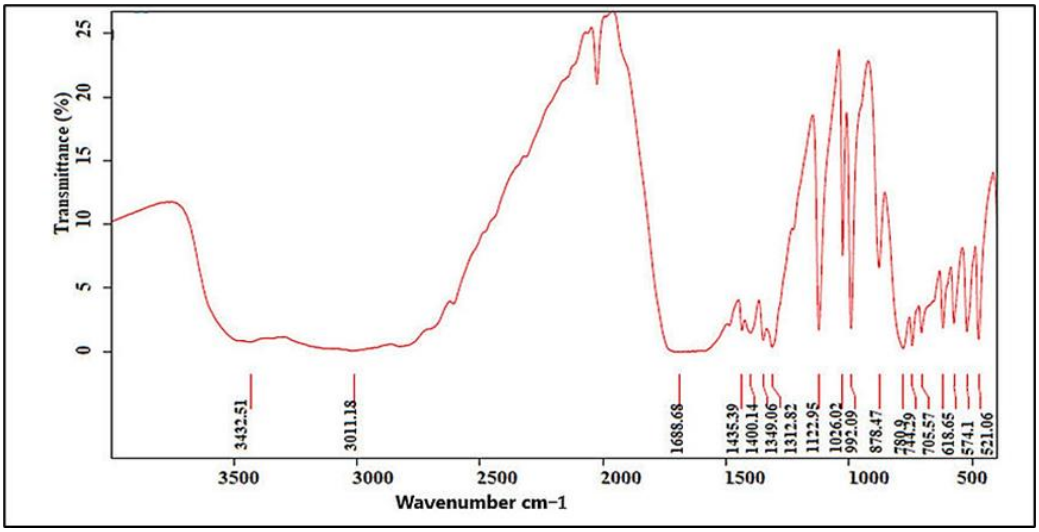

Figure 3 *FTIR analytical chart for uric acid sample showing its bonds interactions by IR wave lengths.*

2.2 The UA Determination Using Standard Procedure:

In a flask (10-ml volume), the buffer solution (3.0 ml), HTAB solution (1.0 × 10-2 M; 0.4 ml), and Pd (II) solution (5.0 × 10-4 M; 0.4 ml) with the solution of QP and UA (5.0 × 10-4 M; 1.0 ml) were mixed. The mixture was transferred to a test tube, diluted with water into a 10 ml volumetric flask, stirred thoroughly, then heated to 60°C for 30 minutes. **The absorbance (ΔA) at 635 nm was measured against a water reference after the solution was cooled using water for five minutes, and the results were compared to those of a solution of reagent blank made in the similar conditions.**

3. RESULTS AND DISCUSSION:

3.1. Selection of stains and metal ions:

The reactions of color between several metal ions and dyes were compared in the UA presence to evaluate the efficacy of a technique for the UA measurement depend on fading of a colored complex of metal-dye. The initial step was to examine the reaction system's sensitivity to metal ions. Table 1 demonstrates how palladium (II) fared better than molybdenum (VI), cobalt (II), manganese (II), iron (II), copper (II), zinc (II), nickel (II), and other elements in a weakly basic solution.

Table-1 *Metal ions effect*

Metal ion	ΔA at	λ_{max}/nm
Pd(II)	0.400	635
Fe(II)	0.024	605
Zn(II)	0.073	555
Cu(II)	0.035	580
Mn(II)	0.022	575
Co(II)	0	—
Ni(II)	0	—
Mo(VI)	0	—

JA, 0.10 μg ml^{-1}; metal ion, 2.0×10^{-5} M; QP, 5.0×10^{-5} M; HTAE
$.0 \times 10^{-4}$ M; pH 8.2; reference, water.

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Next, using 4-(2-pyridylazo) resorcinol (PAR), phenyl fluorone (PF), Xylenol Orange (XO), Pyrocatechol Violet (PV), Pyrogallol Red (PR), and Methyl thymol Blue (MTB), the dyes were investigated. The results are shown in Table 2.

Table- 2 *Selection of dyes*

Dye	ΔA at	λ_{max}/nm
QP	0.400	635
PF	0.068	625
XO	0.016	600
MTB	0.006	630
PR	0	—
PV	0.008	505
PAR	0.058	530

UA, 0.10 µg ml^{-1}; Pd(II), 2.0×10^{-5} M; dye, 5.0×10^{-5} M; HTAB, 4.0×10^{-4} M; pH 8.2; reference, water.

3.2. Optimization Of Reaction Variables:

Different buffer solutions were used to test the effect of pH on the reaction. These solutions included 0.1 M hydrochloric acid/0.05 M borax, 0.1 M sodium 5,5-diethylbarbiturate/0.1 M hydrochloric acid, 0.1 M hydrochloric acid/M tris-hydroxymethyl aminomethane (0.1), 0.05 M borax/0.1 M potassium dihydrogen phosphate, 0.1 M disodium hydrogen phosphate/0.1 M citric acid, and 0.1 M hydrochloric acid/0.05 M borax solutions. Constant and maximum ΔA was reached over the range of pH 7.7 – 8.5 via utilizing 3.0 ml of hydrochloric acid (0.1 M)/ borax buffer (0.05 M) solution.

For stabilizing development of color and enhancing the sensitivity, several surfactants were tested to examine their effects, including anionic amphoteric (Swanol AM-101, Amphitol 24B), nonionic [polyvinyl alcohol (PVA) and Tween 20, Triton X-405], cationic [stearyl trimethyl ammonium chloride (STAC), Zephiramine (Zep), hexadecyl pyridinium bromide (HPB) and HTAB], and amphoteric (sodium dodecyl sulfate (SDS), sodium di(2-ethylhexyl)sulfosuccinate (Aerosol OT)]. QP-palladium (II) in the cationic surfactant existence displayed a red changing with raising absorbance. The findings were presented in Table-3.

Table-3 *Influence of surfactants*

Surfactant	ΔA at	λ_{max}/nm
HTAB	0.400	635
HPB	0.240	635
STAC	0.202	635
Zep	0.086	—
SDS	0	—
Aerosol OT	0	500
Tween 20	0.012	500
Triton X-405	0.008	500
Brij 35	0.010	500
PVA	0.005	500
Amphitol 24B	0	—
Swanol AM-101	0	—

[A, 0.10 µg ml^{-1}; metal ion, 2.0×10^{-5} M; QP, 5.0×10^{-5} urfactants, 0.1%; pH 8.2; reference, water.

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In the cationic surfactant existence, QP-palladium (II) was also extractable in 1-butanol, while there was no QP-palladium (II) present in the other surfactants. Add more than 0.4 ml of HTAB solution (1.0×10^2 M) as a cationic surfactant for optimal sensitivity. With UA at a fixed concentration of 0.10 g/ml, QP and Pd (II) concentrations affect Δ*A*. All further work regarding the UA determination range and the M QP (5.0×10^{-5}) and Pd (II) (2.0 × 10-5 M) solutions were used to perform QP-Pd (II)-HTAB solution absorbance because a maximum A was attained when the molar ratio of QP to Pd (II) should be more than equimolar. This chemical system took some time at room temperature to produce color. Temperatures and times of incubation were tested by heating for 10–60 minutes at 50–60, and 70°C. after being cooked for 20 to 40 minutes to 60 °C, the maximal and stable A value was reached after 5 minutes of cooling in water. Figure 4 displays the obtained outcomes. After the solution was brought down to room temperature, the Δ*A* value did not change for at least three hours.

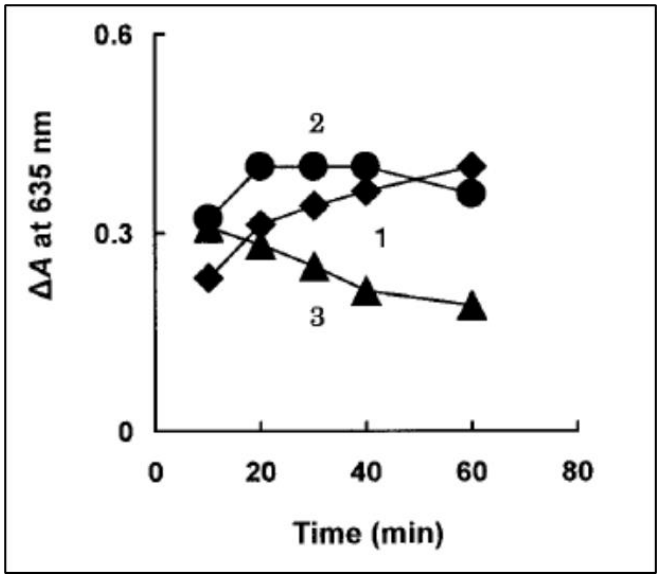

Fig. 4 *Influences of temperature and time (pH 8.2, UA, 0.10 μg ml^{-1}, HTAB, 4.0 × 10^{-4} M, QP, 5.0 × 10^{-5} M, Pd (II), 2.0 × 10^{-5} M, and water as the reference). 1, 50°C; 2, 60°C; 3, 70°C curves.*

Figure 5 displayed the different spectra using the conventional technique. The UA concentration was proportionate to the ΔA635nm.

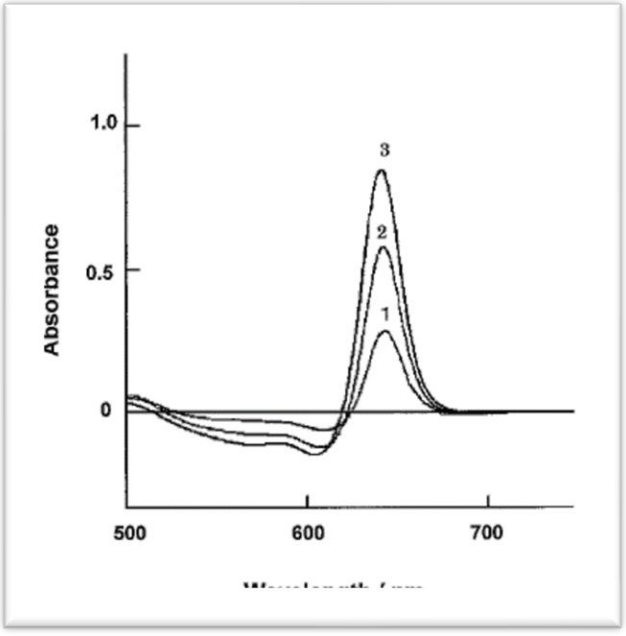

Fig. 5 *Differential spectra of solution of UA-Pd (II)-QP and solution of Pd (II)-QP. 4.0 × 10–4 M; UA: Curve 1, 0.0670 μg ml–1; HTAB, pH 8.20; QP, 5.00× 10–5 M; 2, 0.1300 μg ml–1; Pd (II), 2.00 × 10–5 M; 3, 0.200 μg ml–1.*

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3.3 Calibration curve:

Uric acid calibration curve (Absorbance against UA concentration was drawn using the foregoing technique, as seen in figure 6, is showing a decent linear connection was seen over UA concentration range 0.01–0.20 µg ml^{-1}. The relative standard deviation (RSD) was 1.5% of UA with 0.10 µg $ml^{-1}$for five times. The effective molar absorptivity (ε) was calculated from the calibration graph slope, and the value was 6.5×10^5 dm^3/mol.cm. The sensitivity of this method is almost 20 times higher than that of standard procedures, and it provides great reproducibility.

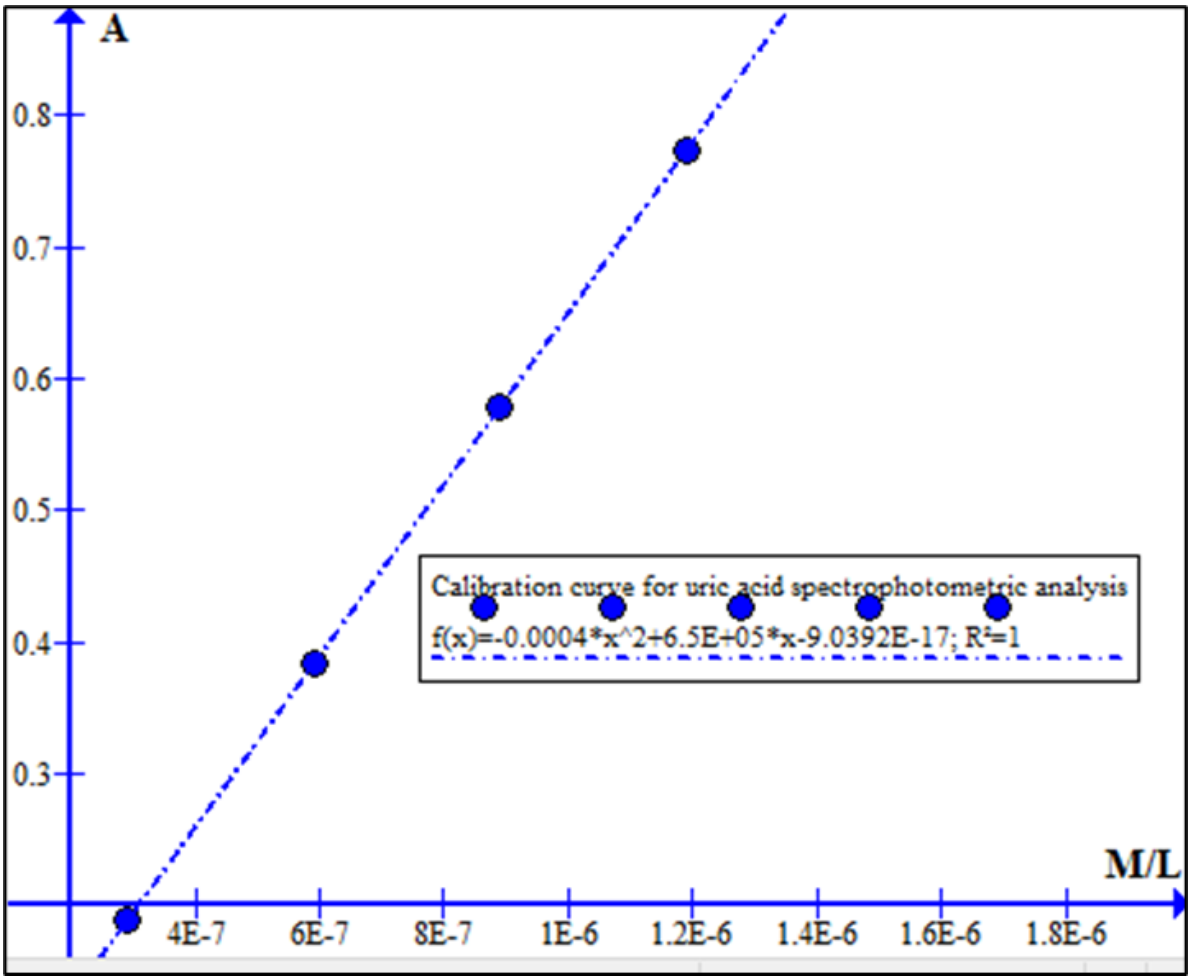

Fig. 6 *The calibration curve of uric acid concentration according to absorbance.*

3.4. Interference of Foreign Substances:

Many compounds were tested under normal settings to see if they caused interference. Even when present in very high excess amounts compared to that of UA, foreign substances like urea, bilirubin, glycine, caffeine, glucose, phosphate, sulfate, nitrate, chloride, magnesium, calcium, potassium, and sodium had no discernible effect on the precision of the UA determination. Reductants such as ascorbic acid and oxalate ions had no effect whatsoever on UA testing even human serum albumin (HSA) is present, creatinine, or iron (III) significantly interfered with the assay. Normal concentrations in the sample solution had little effect on the Δ*A* values at 635 nm. Table 4 provides a summary of the findings. Calculated $t = 0.159$ critical t value (n = 4; p= 0.05) of 2.776 utilizing the **T-test** represented that finding obtained using the results obtained with the current method and the comparator procedure, the uricase/N-(3-sulfopropyl)-3-methoxy-5-methylaniline method, were identical (Wako Pure Chem, L type Wako UAM, a clinical kit). Table 5 displays these findings.

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Table-4 *Foreign materials' influences*

Substance	Added as	Added		ΔA at 635 nm	Rec., %
		μg ml⁻¹	Mole ratio[a]		
None	—	—	—	0.400	100.0
Fe(III)	Alum	3.3×10^{-2}	1	0.320	80.0
Cu(II)	Sulfate	0.76	20	0.400	100.0
Na(I)	Chloride	6.9	500	0.400	100.0
K(I)	Nitrate	11.7	500	0.400	100.0
Mg(II)	Chloride	7.3	500	0.400	100.0
Ca(II)	Chloride	12.0	500	0.400	100.0
HPO_4^{2-}	Disodium	5.8	100	0.400	100.0
$C_2O_4^{2-}$	Sodium	5.3	100	0.400	100.0
Citrate	Trisodium	7.6	100	0.400	100.0
Glucose	—	53.5	500	0.400	100.0
Glycine	—	22.5	500	0.400	100.0
Urea	—	26.0	500	0.400	100.0
Caffeine	—	58.3	500	0.400	100.0
Ascorbic acid	—	10.6	100	0.400	100.0
Bilirubin	—	35.1	100	0.400	100.0
Creatinine	—	0.68	10	0.515	128.8
HSA	—	10.0	—	0.476	119.0

UA, 0.10 μg ml⁻¹; Pd(II), 2.0×10^{-5} M; QP, 5.0×10^{-5} M; HTAB, 4.0×10^{-4} M; pH 8.2; reference, water.
a. Mole ratio: substance/UA.

Table-5 *UA determination in samples of human urine*

Sample	Found/μg ml⁻¹		RSD,[b] %	Rec.,[c] %
	Present method	Other method[a]		
A	155 ± 4.8[d]	146	3.1	101.5
B	224 ± 5.6	230	2.5	98.2
C	194 ± 5.4	182	2.8	103.6

The calculated $t = 0.159$ was lower than critical t value ($p = 0.05$, $n = 4$) of 2.776.
a. Other method: the uricase/*N*-(3-sulfopropyl)-3-methoxy-5-methylaniline method (a clinical kit, L type Wako UA-M, Wako Pure Chem.).
b. Average of 5 determinations.
c. UA taken: 5.0×10^{-2} μg ml⁻¹.
d. Average ± standard deviation.

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3.5 Application

The urine samples were tested for UA using the proposed approach. Urine was simply diluted by a factor of 100 without any preparatory treatment to eliminate confounding contaminants. Under the same conditions, spotless benchmarks were created.

3.6 Composition of The Complex of Color And Reaction Mechanism:

To gain a better understanding of the reaction mechanism, the composition of the colored complex was examined using the molar ratio methods and Job's method of continuous variation. Palladium (II) to QP had a one-to-one ratio, and the ratio of [palladium (II)/QP-to-HTAB] was also 1:1. Nonetheless, the UA to palladium (II) ratio was precisely 1:1 in both the presence and absence of QP. Experimental data indicate that a color complex [1:1:1] between remaining palladium (II), HTAB, and QP is involved in the reaction pathway after generating the palladium (II)-UA [1:1] complex. In summary, palladium (II)-QP-HTAB is a ternary complex that must be confirmed by future research**.**

4. CONCLUSION

An HTAB micellar medium containing palladium (II) and QP was used to produce a straightforward and precise spectrophotometric technique for UA. The suggested approach showed a considerable improvement in the effects of ascorbic acid, and a marked increase in sensitivity (ε = 6.5 105 dm3/mol.cm) than the conventional spectrophotometric methods [4-8], furthermore, the method is helpful for a highly sensitive and straightforward assessment of UA in field specimens directly because it does not require solvent extraction process which may not ensure a quantitative recovery for the uric acid under analysis. The analytical method in this research is 20 times more sensitive than the previous methods reported elsewhere for urine uric acid assays, therefore using this technique was highly fruitful.

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