

Sensitive Colorimetric Determination of Hydrogen Peroxide and Glucose using Ammonium Metavanadate as Chromogen: Applications in Human Urine Samples

C.B. PRAVEEN KUMAR¹, N.A. CHAMARAJA^{1,*}, B. MAHESH¹, N. KUMARA SWAMY² and H.V. KAVYA³

¹Department of Chemistry, JSS Academy of Technical Education (Affiliated to Visvesvaraya technological University), Bengaluru-560060, India

²Department of Chemistry, Sri Jayachamarajendra College of Engineering, JSS Science and Technology University, Mysuru-570006, India

³Department of PG Chemistry, JSS College of Arts Commerce and Science (Autonomous), Mysuru-570025, India

*Corresponding author: E-mail: chamarajchem@gmail.com

Received: 2 June 2026

Accepted: 21 September 2026

Published online: 9 October 2026

AJC-22502

A rapid and precise enzymatic method was developed for the determination of hydrogen peroxide (H_2O_2) and glucose using acidified ammonium metavanadate (NH_4VO_3) as a chromogenic reagent. In this method, H_2O_2 is generated *in situ* through the glucose oxidase (GOD)-catalyzed oxidation of glucose and subsequently reacts with vanadate to produce a red-orange coloured complex exhibiting maximum absorbance at 450 nm. The method showed a linear response for H_2O_2 over the concentration range of 0.67-86 $\mu\text{mol L}^{-1}$. For glucose determination, linear ranges of 20-1040 $\mu\text{mol L}^{-1}$ and 5-925 $\mu\text{mol L}^{-1}$ were obtained using rate-based and single-point measurement methods, respectively. The Michaelis-Menten kinetic parameters (K_m and $V_{\max}$) were determined to be 3453 $\mu\text{mol L}^{-1}$ and 0.9582 $\mu\text{mol min}^{-1}$, respectively. The within-day precision ranged from 0.25-0.76% ($n = 10$), whereas the between-day precision was 0.47-1.98% ($n = 20$). Glucose recoveries of 98.9-100.4% were obtained in the analyzed samples, with no appreciable influence from commonly encountered urinary constituents. The measured accuracy fell within the range of 90-102%. The method provided limits of detection and quantification of 0.268 and 0.894 $\mu\text{mol L}^{-1}$, respectively, for glucose. The proposed scheme is a quick and effective for quantifying glucose in human urine samples with a single enzyme, hence the method is possibly intended for use by clinical laboratories.

Keywords: Glucose, Glucose oxidase, Peroxovanadium.

INTRODUCTION

Glucose is an important energy source and a clinically essential biomarker. The constant regulation of biochemical and physiological pathways is a critical task for the human body [1]. Disturbances in the glucose regulation can result in either elevated or reduced glucose levels, characteristic of hyperglycemia and hypoglycemia, respectively [2,3]. According to a World Health Organization (WHO) assessment, diabetes is undiagnosed in every adult in low- and middle-income nations [4]. Diabetes is one of the top ten eradicators of human health, such as severe cardiac disease, nephropathy and blindness [5]. Other renal complications are due to the excretion of glucose through urine [6]. The determination of glucose has received considerable attention in the clinical and analytical research. Urine offers a readily obtainable biological matrix for glucose analysis and can be collected through a non-invasive proce-

dure without the discomfort associated with blood sampling. Urinary glucose measurement may consequently be useful as a simple screening test for identifying elevated glucose concentrations in individuals with hyperglycemia. Urinary glucose analysis is complicated by the presence of diverse inorganic and organic constituents, blood cells and microorganisms that can alter the composition of the sample. These matrix components may interfere with glucose detection and affect analytical results. Regular assessment of glucose levels remains important for appropriate diabetes management. Many analytical methods like electrochemical methods [7-9], biosensors [10], fluorimetric [11,12] and chemiluminescence [13,14], are available for determining glucose in a biological specimen. Contrarily, fluorescence bio-probes such as organic dyes and quantum dots possess flaws like (i) interference from biological auto fluorescence, (ii) low photochemical stability and (iii) high toxicity [15-17]. As a result, those inquiries are not complete

for applications in determining glucose in human serum or urine samples. Several colorimetric methods have been reported for glucose determination in blood using glucose oxidase (GOD) and horseradish peroxidase (HRP) enzyme systems [18-20]. Gold and silver nanoparticles have likewise been explored for glucose detection [21]. The catalytic activity of these nanoparticles is strongly influenced by particle size, with GOD-like activity reported predominantly for particles smaller than 10 nm. Their application may require additional characterization to confirm particle size and catalytic properties, while the sensitivity toward glucose can remain lower than that achieved with conventional enzymatic systems under certain conditions.

Although GOD-based colorimetric methods for glucose determination are well established, conventional assays generally employ a coupled GOD-HRP system in which glucose oxidase generates H_2O_2 , followed by HRP-mediated oxidation of chromogenic substrates. Such methods require multiple reaction components and their performance may depend on the stability and activity of the auxiliary enzyme, chromogenic substrate and reaction conditions. The present study investigates acidified ammonium metavanadate as a direct chromogenic system for detecting H_2O_2 generated during GOD-catalyzed glucose oxidation. The vanadate- H_2O_2 reaction produces a measurable optical response at 450 nm without requiring HRP or conventional chromogenic substrates such as 3,3',5,5'-tetramethylbenzidine (TMB) and *o*-dianisidine, thereby simplifying the signal-generation step. The study evaluates both direct H_2O_2 determination and glucose determination through *in situ* enzymatic H_2O_2 generation using one-time and rate-based measurements. The proposed system is further applied to glucose analysis in human urine to assess its suitability in a biological matrix. The main objective is to determine whether the GOD-vanadate system can provide a simpler and reliable alternative to conventional multi-component colorimetric assays for urinary glucose determination.

EXPERIMENTAL

Glucose oxidase stock solution (147 units/mg, Sigma Aldrich) was prepared by dissolving 20 mg of enzyme in 10 mL of 100 mM phosphate buffers at pH 6.0. The glucose (E-Merck, India) and was desiccated for 2 h at 80 °C in a hot air oven. A stock glucose solution was prepared in double distilled water and diluted as required using the same solvent. Commercial H_2O_2 (E. Merck, India) was standardized against a standard KMnO_4 solution. NH_4VO_3 (Merck, Germany) was prepared at 1 mg mL^{-1} in distilled water and diluted as required. A 0.5 M phosphate buffer (pH 7.0) was prepared by dissolving appropriate amounts of KH_2PO_4 and K_2HPO_4 in distilled water. An acetate buffer (pH 4.6) was prepared by mixing 50 mL of 0.1 M sodium acetate with 25 mL of 0.2 M CH_3COOH . A Tris buffer (pH 10) was prepared by dissolving tris(hydroxymethyl)aminomethane in distilled water and adjusting the pH with NaOH. Double-distilled water was used throughout for reagent preparation and dilution.

Apparatus: Absorbance was recorded by a JASCO UVIDEK-610 UV-Visible spectrophotometer with a matched quartz cell. Equiptronics 4-Digit LED Display Digital pH Meter (EQ-610) was used to measure and adjust the pH.

Human urine sample collection and preservation for assay of glucose: The urine sample was collected from a healthy and diabetic volunteer and subjected to centrifugation at 14,500 rpm for 15 min to remove any cells and other waste. The centrifuged solution was stored in a polythene sample bottle and refrigerated until further manipulation. The human urine sample was obtained with the permission of Mysore University's Ethical Committee.

Assessment of glucose: Glucose was assessed in a 3 mL reaction mixture containing 2.28 mM NH_4VO_3 and 8.2 EU of GOD in 100 mM $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ buffer at pH 4.0. The reaction mixture was incubated with 100 μL of glucose solutions of different concentrations and maintained at 30 °C with continuous agitation. The absorbance was continuously recorded at 450 nm. Linear responses were obtained over the glucose concentration ranges of 20-1040 $\mu\text{mol L}^{-1}$ and 5-925 $\mu\text{mol L}^{-1}$ using the rate and one-time methods, respectively.

Assessment of GOD: GOD activity was determined using a reaction mixture containing 2.28 mM NH_4VO_3 and 1040 $\mu\text{mol L}^{-1}$ glucose in 100 mM $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ buffer at pH 4.0, with the final volume adjusted to 3.0 mL. The reaction was initiated by adding 100 μL of GOD solutions of different concentrations. The change in absorbance was recorded against a reagent blank at 450 nm and 30 °C. The initial reaction velocity was obtained from the absorbance-time curves and the GOD concentration in the test solutions was determined.

Assessment of H_2O_2 : H_2O_2 was determined using 2.28 mM NH_4VO_3 in 100 mM $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ buffer at pH 4.0. The reaction was initiated by adding 100 μL of H_2O_2 solutions of different concentrations and the final volume was adjusted to 3.0 mL with distilled water. The reaction mixtures were incubated at 30 °C for 10 min, after which the absorbance was measured at 450 nm against an appropriate reagent blank.

RESULTS AND DISCUSSION

UV-spectra of peroxovanadium complex: The proposed method was based on the formation of an orange-red coloured complex. The absorption spectrum of the chromogenic product was recorded over 300-700 nm using different concentrations of H_2O_2 in the presence of fixed amounts of GOD and glucose. The maximum absorbance was observed at 450 nm, whereas the reagent blank showed negligible absorbance. The absorption spectra of the peroxovanadium complex formed at different H_2O_2 concentrations are shown in Fig. 1.

Test of influencing factors: The reaction conditions were optimized to identify the minimum concentration of vanadate required for adequate colour development. The effects of substrate and co-substrate concentrations, temperature, pH and incubation time on the glucose and GOD assays were investigated.

Effect of ammonium metavanadate (NH_4VO_3): The effect of NH_4VO_3 concentration on colour development was investigated and the results are shown in Fig. 2. The colour intensity increased with increasing NH_4VO_3 concentration from 0.28 to 2.28 mM. No appreciable increase in colour intensity was observed above this concentration. Therefore, 2.28 mM NH_4VO_3 was selected for subsequent experiments.

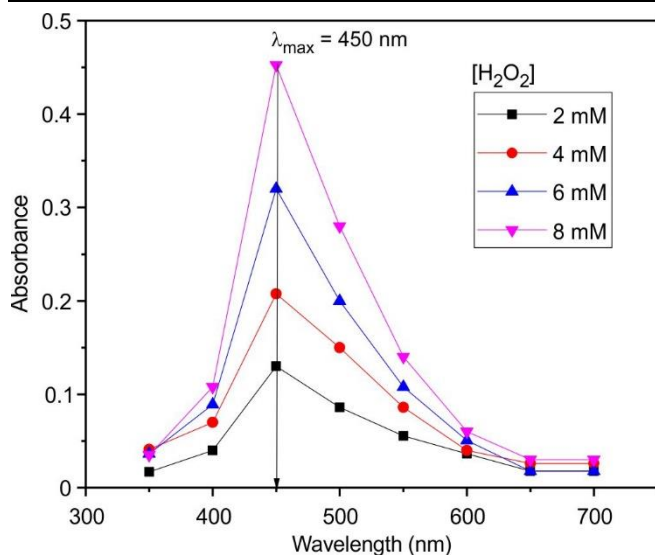


Fig. 1. UV-spectra of peroxovanadium complex of different concentration of H_2O_2

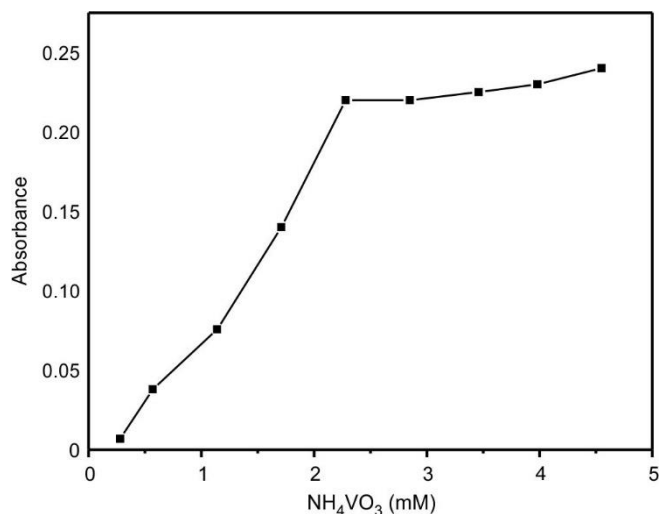


Fig. 2. Effect of ammonium metavanadate on the rate of the reaction

Effect of temperature and stability of the coloured complex: Temperature significantly affected the enzymatic reaction. A reaction mixture containing 2.28 mM NH_4VO_3 , $1040 \mu\text{mol L}^{-1}$ glucose and 8.2 EU GOD in 100 mM $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ buffer (pH 4.0) was prepared and made up to 3.0 mL with double-distilled water. The mixture was incubated for 5 min at temperatures ranging from 0 to 80 °C and the catalytic activity was evaluated by measuring the absorbance of the coloured product. The activity increased with temperature up to 30 °C and decreased at higher temperatures (Fig. 3), which was attributed to loss of enzymatic activity at elevated temperatures. The coloured peroxovanadium complex remained stable at 30 °C for approximately 120 h. Accordingly, 30 °C was selected for subsequent experiments.

Effect of pH on the reaction rate: The effect of pH on enzyme activity was investigated using $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$, $\text{NaOH}/\text{KH}_2\text{PO}_2$ and Tris buffer systems over the pH range of 3.6–10.0. The highest activity was obtained in 100 mM $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ buffer at pH 4.0. No appreciable colour development was observed in the

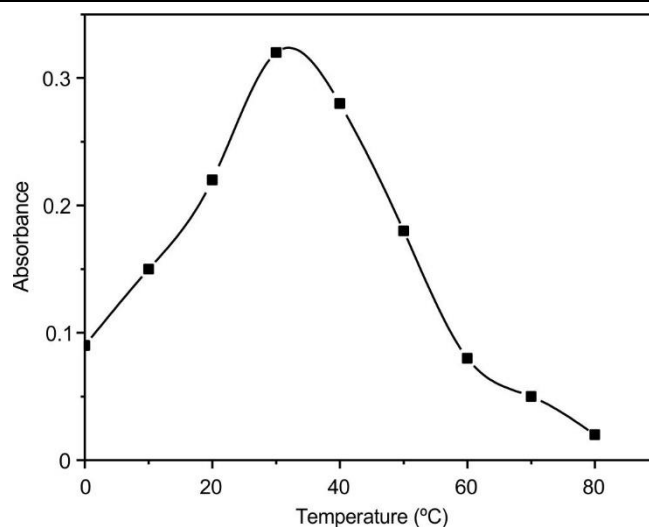


Fig. 3. Impact of temperature on the rate of reaction

$\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, $\text{NaOH}/\text{KH}_2\text{PO}_4$ or Tris buffer systems. Therefore, $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ buffer at pH 4.0 was selected for subsequent studies. The effect of pH on the reaction rate is shown in Fig. 4.

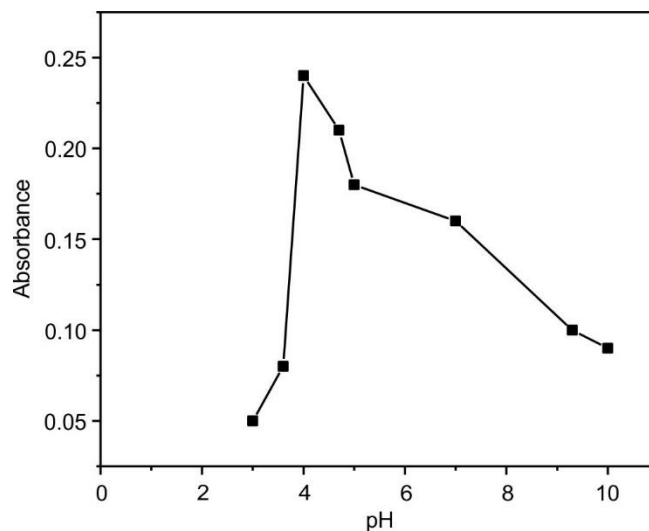


Fig. 4. Effect of pH on the rate of the reaction

Robustness: The robustness of the proposed method was evaluated by introducing small deliberate variations in the optimized experimental conditions. The optimized conditions comprised 100 mM $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ buffer at pH 4.0, 2.28 mM NH_4VO_3 , a reaction temperature of 30 °C, and the optimized reaction time. The effects of minor variations in pH, NH_4VO_3 concentration, reaction time, temperature and buffer concentration on the analytical response were investigated.

Each parameter was varied individually while the remaining conditions were kept constant. The pH was varied from 3.8 to 4.2, corresponding to ± 0.2 pH units around the optimized value. The NH_4VO_3 concentration was varied from 2.18 to 2.38 mM, while the temperature was varied from 28 to 32 °C. The reaction time was varied by ± 1 min from the optimized value, and the $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ buffer concentration

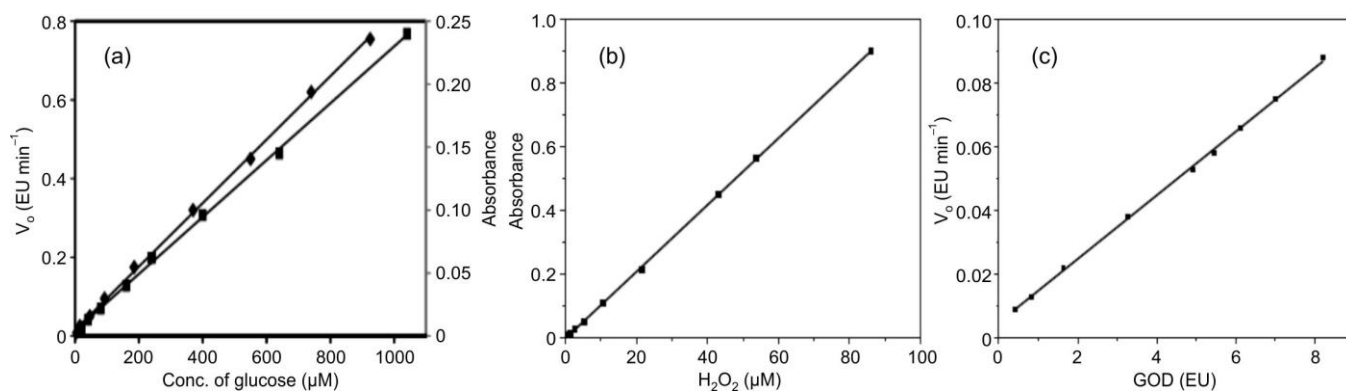


Fig. 5. (a) Calibration curves for the quantification of glucose by ■ rate and ◆ one time method, (b) calibration plot for the assess of glucose oxidase (GOD) by one time method and (c) calibration plot for the quantification of H_2O_2

was varied slightly around 100 mM. For each condition, the absorbance was measured and the corresponding glucose concentration was calculated.

Only minor changes in the analytical response were observed following these deliberate variations. The glucose recoveries remained close to those obtained under the optimized conditions, indicating that the method was not substantially affected by small changes in the investigated parameters. The proposed method therefore showed satisfactory robustness within the tested experimental ranges. The results of the robustness study are shown in Table-1.

TABLE-1 ROBUSTNESS OF THE PROPOSED ANALYTICAL METHOD UNDER DIFFERENT EXPERIMENTAL CONDITIONS			
Parameter	Variation	Absorbance	Glucose found (μmol L ⁻¹)
pH	3.8	0.453	1.9355
	4.0	0.456	1.9483
	4.2	0.452	1.9312
NH_4VO_3	2.18 mM	0.458	1.9569
	2.28 mM	0.457	1.9526
	2.38 mM	0.454	1.9398
Temperature	28 °C	0.455	1.9440
	30 °C	0.458	1.9569
	32 °C	0.454	1.9398
Reaction time	9 min	0.452	1.9312
	10 min	0.456	1.9483
	11 min	0.458	1.9569

Analytical figures of merit and catalytic parameters:

For glucose determination, calibration curves were constructed using both rate and one-time methods. Linear responses were obtained over 20–1040 μmol L⁻¹ and 5–925 μmol L⁻¹ for the rate and one-time methods, respectively. The limits of detection and quantification were 0.268 and 0.894 μmol L⁻¹, respectively. Calibration curves for GOD and H_2O_2 were constructed using the rate method, with linear ranges of 0.42–8.2 EU and 0.67–86 μmol L⁻¹, respectively. The corresponding calibration plots are shown in Fig. 5a–c.

The kinetic behaviour of the GOD-catalyzed oxidation of glucose was investigated over a glucose concentration range of 20–1280 μmol L⁻¹. Initial reaction rates were expressed

in μmol min⁻¹ and fitted to the Michaelis–Menten equation using non-linear regression:

$$v = \frac{V_{\max} [S]}{K_m + [S]}$$

where v is the initial reaction rate, $[S]$ is the glucose concentration, $V_{\max}$ is the maximum reaction rate and K_m is the Michaelis–Menten constant.

The nonlinear Michaelis–Menten analysis yielded a $V_{\max}$ of 0.9582 μmol min⁻¹ and a K_m of 3453 μmol L⁻¹ (3.453 mmol L⁻¹), with an (R^2) value of 0.9929. The calculated reaction rates showed good agreement with the experimentally determined rates. The nonlinear Michaelis–Menten curve is shown in Fig. 6.

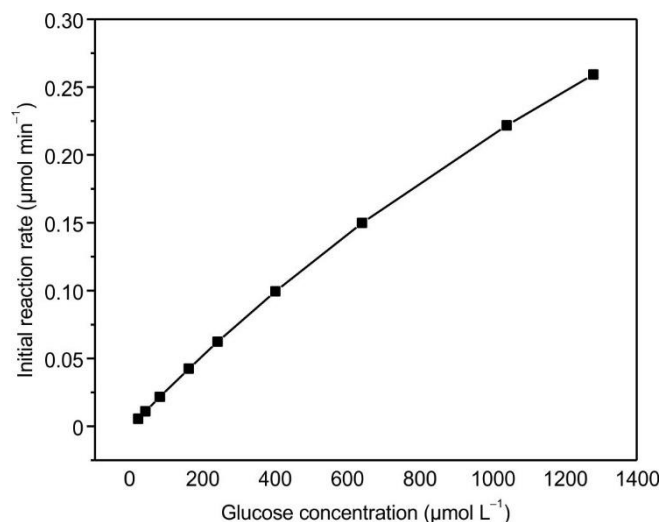
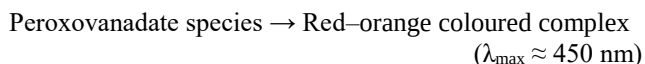
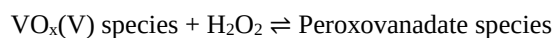
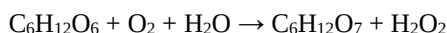


Fig. 6. Michaelis–Menten plot of the initial reaction rate versus glucose concentration for the glucose oxidase-catalyzed reaction

Reaction pathway of the proposed method: Glucose detection was based on the GOD-catalyzed oxidation of glucose to D-gluconic acid with concomitant generation of H_2O_2 . Under the acidic conditions used in the assay, NH_4VO_3 provided vanadate species that reacted with the enzymatically generated H_2O_2 to form peroxide–vanadate species. The formation of these species was accompanied by the development of a characteristic orange-red colour, which provided the basis for spectrophotometric detection at 450 nm.

The vanadate–H₂O₂ system can involve several peroxide–vanadate species depending on the acidity and composition of the reaction species medium. Since the vanadium species and molecular structure of the coloured product were not directly characterized in the present study, the precise reaction pathway could not be established conclusively. The observed colour and absorption response were therefore attributed to the formation of peroxide–vanadate species under the experimental conditions, which is consistent with the reported chemistry of vanadate–H₂O₂ systems [22]. The intensity of the developed colour increased with the amount of H₂O₂ generated during glucose oxidation and was consequently related to the glucose concentration. The proposed reaction pathway is presented in **Scheme-I**.



Scheme-I: Proposed reaction mechanism

The exact composition and oxidation state of the vanadium containing chromogenic species were not directly determined in the present study. Therefore, the reaction scheme represents a proposed interpretation based on the established chemistry of vanadate–peroxide systems.

Method validations

Recovery test: Recovery studies were performed using 6 different urine samples spiked with known amounts of glucose within the linear range of the assay. The results obtained by the proposed method were compared with those obtained using a commercial glucose assay kit (Contour-Link glucometer). Glucose recoveries ranged from 98.95 to 100.42%, with a mean recovery of 100.07% (Table-2). The results indicated good recovery of glucose from the urine matrix. The effects of common urinary constituents were further investigated and no appreciable interference was observed under the tested conditions (Table-2).

The proposed method was further evaluated using 12 different human urine samples, and the glucose concentrations obtained were compared with those measured using the commercial kit. The results showed close agreement between the two methods, with no statistically significant difference under the conditions investigated (Fig. 7). Although the proposed method used GOD and NH₄VO₃ as the enzyme and chromogenic reagent, respectively, without an additional HRP enzyme

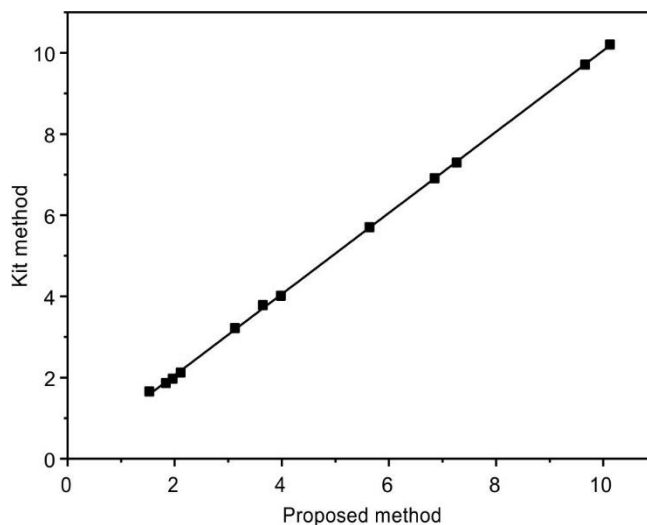


Fig. 7. Plot of glucose concentrations determined by the proposed method versus the enzymatic kit method

or conventional chromogenic substrate, comparison with a standardized laboratory reference method, such as a validated hexokinase or GOD–POD assay, would provide a more rigorous assessment of analytical accuracy. However, further validation using a larger number of clinical samples would be required to establish its applicability to routine clinical analysis.

The analytical characteristics of the proposed method were compared with those of previously reported glucose determination methods (Table-3). Several reported methods require sophisticated instrumentation, multiple reagents or coupled enzyme systems. The proposed procedure provided spectrophotometric detection using GOD-mediated H₂O₂ generation followed by the vanadate-based chromogenic reaction, thereby reducing the number of reaction components.

Interference studies: The selectivity of the proposed method was evaluated in the presence of common urinary constituents, for example, urea, uric acid and creatinine. The effect of these potentially interfering species was investigated at a glucose concentration of 0.240 mmol L⁻¹. An interference level corresponding to a deviation of less than 3% in the analytical response was considered acceptable. No appreciable interference was observed from the tested constituents at the investigated concentrations (Table-4). Maltose, which may interfere with GOD-based glucose assays depending on the enzyme specificity, was also examined. No significant interference was observed up to three times the glucose concentration under the experimental conditions. These results con-

TABLE-2
QUANTIFICATION OF GLUCOSE IN HUMAN URINE SAMPLES USING THE PROPOSED METHOD.

Urine samples	Glucose (mmol L ⁻¹)		Added (mmol L ⁻¹)	Recovery proposed method (mmol L ⁻¹)	Recovery (%)	CV (%)
	Proposed method	Kit method				
Healthy person	1.526	1.510	3.00	4.510	98.95	1.08
	1.835	1.845	3.00	4.840	100.27	1.16
	2.113	2.101	3.00	5.122	100.42	1.12
Diabetic patient	6.855	6.848	3.00	9.860	100.07	1.15
	7.155	7.150	3.00	10.180	100.35	1.06
	7.266	7.310	3.00	10.290	100.33	1.14

TABLE-3
COMPARISON OF ANALYTICAL PARAMETERS OF THE PROPOSED
METHOD WITH REPORTED METHODS FOR GLUCOSE DETERMINATION

Method	Linear range (μM)	LOD (μM)	Ref.
Colorimetric method	500–15 00	450	[23]
Colorimetric method	200–1100	09	[24]
Potentiometric method	$0.32\text{--}1.0 \times 10^3$	0.19	[25]
Voltammetric glucose sensor	$2.0 \times 10^2\text{--}8.0 \times 10^3$	1.0×10^4	[26]
Cyclic voltammetry method	$3 \times 10^3\text{--}9 \times 10^3$	1×10^3	[27]
Fluorimetric method	5–900	1.8	[28]
Electrochemical method	500–3000	–	[29]
Colorimetric assay by nanozyme	5–1000	1.79	[30]
Amperometric method	$1.0 \times 10^3\text{--}32 \times 10^3$	0.1×10^3	[31]
Chemiluminescence	$0.005 \times 10^3\text{--}1.0 \times 10^3$	0.1×10^3	[32]
Proposed method	5–925	0.268	Proposed method

TABLE-4
TOLERANCE OF POTENTIAL INTERFERENTS IN THE
DETERMINATION OF $0.240 \text{ mmol L}^{-1}$ GLUCOSE

Foreign species	[Interferants] (mmol L^{-1})	Tolerance* ratio
Ascorbic acid	0.0012	0.005
Bilirubin	0.0019	0.0079
NO_2^-	0.0048	0.02
Fe(II)	0.0098	0.041
Fe(III), uric acid	0.382	1.592
Maltose, urea	0.510	2.125
KCl, Ca(II), magnesium, serine	0.755	3.146
Glycerin, acetaminophen	1.08	4.50
Citric acid, L-histidine	1.662	6.925
DL-methionine, phenylalanine, EDTA	2.286	9.525
Fructose, oxalic acid	3.126	13.025
Mannose, alanine	4.858	20.242
Creatinine, sodium, sulphate, carbonate	8.278	34.492
Glycine, sucrose, D-glactose	20.26	84.417
Ammonium, L- serine	28.44	118.50

firmly that tested urinary matrix had no appreciable effect on the selective determination of glucose.

Imprecision studies: The precision of the proposed method was evaluated at three glucose concentrations (80, 250 and $450 \mu\text{mol L}^{-1}$) within the established linear range. The reaction mixtures were prepared using the optimized reagent concentrations, and fresh solutions were prepared daily. Within-day precision was assessed from 10 replicate measurements performed at 1 h intervals, whereas day-to-day precision was evaluated from 20 measurements performed over intervals of 24–25 h. The within-day and day-to-day imprecision results are shown in Table-5. The within-day imprecision ranged from

0.25 to 0.76% ($n = 10$), while the day-to-day imprecision ranged from 0.47 to 1.98% ($n = 20$).

Determination of glucose in human urine samples:

The applicability of the proposed method was evaluated using human urine samples. A 3.0 mL reaction mixture containing 2.28 mM NH_4VO_3 and 8.2 EU GOD in 100 mM $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ buffer at pH 4.0 was mixed with a fixed volume of urine sample. The absorbance was measured against a reagent blank containing all assay components except the urine sample. Glucose concentrations were calculated from the established calibration curve. Recovery and precision were evaluated using urine samples spiked with known concentrations of glucose within the linear range. Satisfactory recoveries and low within-day and day-to-day imprecision confirmed the reproducibility of the proposed method under the tested conditions.

Conclusion

An acidified ammonium metavanadate-based colorimetric method was developed for glucose determination using glucose oxidase (GOD). GOD catalyzed the oxidation of glucose with associated generation of H_2O_2 , which reacted with acidified ammonium metavanadate to produce a coloured peroxide-vanadate species with maximum absorbance at 450 nm. Unlike conventional GOD–HRP assays, the proposed system used GOD as the sole enzyme and ammonium metavanadate as the chromogenic reagent, eliminating the requirement for an additional peroxidase enzyme and conventional chromogenic substrates. The method provided linear responses for glucose over $20\text{--}1040 \mu\text{mol L}^{-1}$ and $5\text{--}925 \mu\text{mol L}^{-1}$ using the rate and one-time methods, respectively. The limits of detection and quantification were 0.268 and $0.894 \mu\text{mol L}^{-1}$, respectively. Michaelis–Menten analysis gave a K_m of $3453 \mu\text{mol L}^{-1}$ and a $V_{\max}$ of $0.9582 \mu\text{mol min}^{-1}$ under the experi-

TABLE-5
DAY TO DAY AND WITHIN-DAY PRECISION

Within day precision*				Day-to-day precision*			
X (μM)	SD	CV	n	X (μM)	SD	CV	n
80	0.602	0.76	10	80	1.586	1.98	20
250	1.025	0.44	10	250	1.891	0.75	20
450	1.162	0.25	10	450	2.127	0.47	20

mental conditions. The recovery, selectivity and precision studies supported the applicability of the method to glucose determination in urine samples. The method used fewer reaction components and eliminated the requirement for an auxiliary peroxidase enzyme, offering a simple spectrophotometric approach for urinary glucose determination.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Late Prof. Dr. P. Nagaraja, Department of Chemistry, University of Mysuru, Mysuru and VVIT Research center Mysore and the authors also grateful to the Management of JSS Mahavidyapeetha, Mysuru, Vision Group on Science and Technology (GRD-326, 2014-15(2015-16)), GOK, India for the financial support under CISEE scheme and Principal, JSSATE, Bengaluru for the facilities and support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

REFERENCES

1. D. Sim, M.C. Brothers, J.M. Slocik, A.E. Islam, B. Maruyama, C.C. Grigsby, R.R. Naik and S.S. Kim, *Adv. Sci.*, **9**, 2104426 (2022); <https://doi.org/10.1002/adv.202104426>
2. P. González, P. Lozano, G. Ros and F. Solano, *Int. J. Mol. Sci.*, **24**, 9352 (2023); <https://doi.org/10.3390/ijms24119352>
3. T. Yang, F. Qi, F. Guo, M. Shao, Y. Song, G. Ren, Z. Linlin, G. Qin and Y. Zhao, *Mol. Med.*, **30**, 71 (2024); <https://doi.org/10.1186/s10020-024-00824-9>
4. R.M. Carrillo-Larco, W.C. Guzman-Vilca, X. Xu and A. Bernabe-Ortiz, *Diabet. Med.*, **41**, e15174 (2024); <https://doi.org/10.1111/dme.15174>
5. N.A. Chamaraja, M. Basavaraju and N.K. Swamy, *Anal. Biochem.*, **590**, 113536 (2020); <https://doi.org/10.1016/j.ab.2019.113536>
6. V. Vallon and T. Nakagawa, *Compr. Physiol.*, **12**, 2995 (2022); <https://doi.org/10.1002/j.2040-4603.2022.tb00207.x>
7. H. Sohrabi, F. Maleki, P. Khaaki, M. Kadhon, N. Kudaibergenov and A. Khataee, *Biosensors*, **13**, 347 (2023); <https://doi.org/10.3390/bios13030347>
8. M. Harun-Or-Rashid, M.N. Aktar, V. Preda and N. Nasiri, *Sens. Diagn.*, **3**, 893 (2024); <https://doi.org/10.1039/D4SD00086B>
9. Z. Li, W. Zeng and Y. Li, *Molecules*, **28**, 4891 (2023); <https://doi.org/10.3390/molecules28134891>
10. H. Khosravi Ardakani, M. Gerami, M. Chashmpoosh, N. Omidifar and A. Gholami, *Biochem. Res. Int.*, **2022**, 2964705 (2022); <https://doi.org/10.1155/2022/2964705>
11. S. Fang, S. Li, P. Yin, G. Yao, H. Yu, Y. He, X. Li, M. Yang and W. Tan, *Chemosphere*, **364**, 143171 (2024); <https://doi.org/10.1016/j.chemosphere.2024.143171>
12. Z. Yuan, M. Fu, X. Wang, M. Wang, Y. Wei, Y. Sun, Q. Zhang, Y. Zhang and B. Zhang, *Anal. Methods*, **17**, 320 (2025); <https://doi.org/10.1039/D4AY01878H>
13. W. Yao, X. Zhang and Z. Lin, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **265**, 120401 (2022); <https://doi.org/10.1016/j.saa.2021.120401>
14. I. Rahmawati, Y. Einaga, T.A. Ivandini and A. Fiorani, *ChemElectroChem*, **9**, e202200175 (2022); <https://doi.org/10.1002/celec.202200175>
15. J. Xu, M. Back and S. Tanabe, in eds.: R.-S. Liu and X.-J. Wang, Near-Infrared Phosphors with Persistent Luminescence over 1000 nm for Optical Imaging, In: *Phosphor Handbook: Experimental Methods for Phosphor Evaluation and Characterization*, CRC Press, edn 3, pp. 363-418 (2022).
16. N.R. Jalal, F. Mollarasouli, M.R. Jalali Sarvestani, S. Khalili, S. Asadi, Z. Derakhshan, T. Madrakian, A. Afkhami and M. Ahmadi, in eds.: M. Thompson and Z. Ramezani, *Quantum Dots in Medical Detection/ Diagnosis*, In: *Quantum Dots in Bioanalytical Chemistry and Medicine*, Royal Society of Chemistry: Cambridge, U.K., vol. 22, pp. 75-106 (2023).
17. W. Zheng, P. Huang, D. Tu, E. Ma, H. Zhu and X. Chen, *Chem. Soc. Rev.*, **44**, 1379 (2015); <https://doi.org/10.1039/C4CS00178H>
18. L. Tong, L. Wu, Y. Zai, Y. Zhang, E. Su and N. Gu, *Biosens. Bioelectron.*, **219**, 114787 (2023); <https://doi.org/10.1016/j.bios.2022.114787>
19. P.C. Lee, N.S. Li, Y.P. Hsu, C. Peng and H.W. Yang, *Analyst*, **144**, 3038 (2019); <https://doi.org/10.1039/C8AN02440E>
20. M. Schröder and F.J. Meyer-Almes, *ChemTexts.*, **11**, 14 (2025); <https://doi.org/10.1007/s40828-025-00211-9>
21. S.Ü. Pektaş, M. Keskin, O.C. Bodur and F. Arslan, *J. Food Compos. Anal.*, **129**, 106133 (2024); <https://doi.org/10.1016/j.jfca.2024.106133>
22. M.H. Hadwan and S. Ali, *Anal. Biochem.*, **542**, 29 (2018); <https://doi.org/10.1016/j.ab.2017.11.013>
23. S. Naveen Prasad, P. Weerathunge, M.N. Karim, S. Anderson, S. Hashmi, P.D. Mariathomas, V. Bansal and R. Ramanathan, *Anal. Bioanal. Chem.*, **413**, 1279 (2021); <https://doi.org/10.1007/s00216-020-03090-w>
24. S. Ma, C. Wei, Y. Bao, Y. Liu, H. Jiang, W. Tong, D. Chen and X. Huang, *Mikrochim. Acta*, **191**, 107 (2024); <https://doi.org/10.1007/s00604-023-06150-x>
25. D.-M. Kim, J.-M. Moon, W.-C. Lee, J.-H. Yoon, C.S. Choi and Y.-B. Shim, *Biosens. Bioelectron.*, **91**, 276 (2017); <https://doi.org/10.1016/j.bios.2016.12.046>
26. Y. Yang, C. Yi, J. Luo, R. Liu, J. Liu, J. Jiang and X. Liu, *Biosens. Bioelectron.*, **26**, 2607 (2011); <https://doi.org/10.1016/j.bios.2010.11.015>
27. Riyanto and M.S. Hakim, *IOP Conf. Ser.: Mater. Sci. Eng.*, **299**, 012001 (2018); <https://doi.org/10.1088/1757-899X/299/1/012001>
28. M. Liang, Y. Ren, H. Zhang, Y. Ma, X. Niu and X. Chen, *Luminescence*, **32**, 1031 (2017); <https://doi.org/10.1002/bio.3287>
29. M. Chen, X. Cao, K. Chang, H. Xiang and R. Wang, *Electrochim. Acta*, **368**, 137603 (2021); <https://doi.org/10.1016/j.electacta.2020.137603>
30. L. Dong, R. Li, L. Wang, X. Lan, H. Sun, Y. Zhao and L. Wang, *Int. J. Biol. Macromol.*, **172**, 289 (2021); <https://doi.org/10.1016/j.ijbiomac.2021.01.049>
31. D. Zheng, S.K. Vashist, K. Al-Rubeaan, E. Lam, S. Hrapovic, J.H. Luong and F.S. Sheu, *J. Nanopharm. Drug Deliv.*, **1**, 64 (2013); <https://doi.org/10.1166/jnd.2013.1017>
32. Z. Wang, F. Liu and C. Lu, *Biosens. Bioelectron.*, **38**, 284 (2012); <https://doi.org/10.1016/j.bios.2012.06.003>