PREPARAÇÃO DE UM NOVO COMPOSTO AZO (HAZM) UTILIZADO PARA DETERMINAÇÃO ESPECTROFOTOMÉTRICA ANALÍTICA DE GLICOSE NO SANGUE E NA SALIVA

PREPARATION OF A NEW AZO COMPOUND (HAZM) USED FOR ANALYTICAL SPECTROPHOTOMETRIC DETERMINATION OF GLUCOSE IN BLOOD AND SALIVA

تحضير مركب آزو جديد (حازم) يستعمل للتقدير التحليلي للجلوكوز في الدم واللحاح

HALBOOS, Mohanad Hazim^{1*}; HUSSEIN, Bayan Jabr²; SAYHOOD, Aayad Ammar³

¹ University of Kufa, Faculty of Science, Department of Ecology. Iraq.

² University of Kufa, Faculty of Dentistry, Department of Oral Histology. Iraq.

³ University of Kufa, Faculty of Dentistry, Department of Basic Sciences. Iraq.

* Corresponding author

e-mail: muhaned.halbus@uokufa.edu.iq

Received 26 May 2020; received in revised form 12 June 2020; accepted 16 June 2020

RESUMO

Como os recursos de saúde são limitados, condições de baixa renda exigem desenvolvimento tecnológico acessível. O projeto e a análise são um método colorimétrico de baixo custo para monitoramento diagnóstico diabético não invasivo por detecção de glicose da saliva e do sangue discutido, onde um novo composto (HAZM) foi preparado por meio de uma reação 4-aminoantipirina com 1-fenil-2-metil-5-pirazolon; este composto sintético foi analisado pelas técnicas de FTIR, ¹HNMR e ¹³CNMR. As amostras foram processadas e analisadas utilizando o equipamento de produção usando a reação das enzimas glicose oxidase (GO) e peroxidase (PO) com (HAZM); dê ao método a capacidade de calcular o nível de glicose em menos de 15 segundos com precisão. A inclinação da curva de calibração é igual a 0,0026, $\epsilon = 468,26$ e sensível a Sandel é igual a 0,384 mg/dL, para quantificar as concentrações salivares de glicose e o sangue (5-500 mg / dL) a 423 nm. Atingir um limite de detecção de 0,421 mg/dL, onde 5 % CV a (n = 3). Porque os testes HAZM foram realizados de acordo com um método de kit, que reflete os valores médios do kit (101,35 $\pm$ 3,647 mg/dL) e os resultados do HAZM (104,41 $\pm$ 4,951 mg / dL) com viés positivo de 3,1 mg / dL no sangue. Comparando os níveis médios de kit e glicose HAZM na saliva, 99,48 $\pm$ 1,26 e 100,97 $\pm$ 2,38 mg/dL, respectivamente, mostram viés positivo de 1,5 mg/dL para o processo do kit. Os resultados do uso desse método para medir glicose em amostras de sangue e saliva indicam que ele é eficaz, pois oferece resultados precisos e baratos, em comparação com outras técnicas comerciais.

Palavras-chave: *Composto Azo, Espectrofotométrico, Glicose, Sangue e Saliva.*

ABSTRACT

Because health resources are limited, low-income conditions demand affordable technological development. The design and analysis is a low-cost colorimetric method for non-invasive diabetic diagnostic monitoring by glucose detection of the saliva and blood discussed, where a new compound (HAZM) was prepared through a reaction 4-aminoantipyrine with 1-phenyl-2-methyl-5-pyrazolon; FTIR, ¹HNMR, and ¹³CNMR diagnosed for this synthetic compound. Samples have been processed and analyzed using the production equipment using glucose oxidase (GO) and peroxidase (PO) enzymes reaction with (HAZM); give the method the ability to calculate glucose level in less than 15 seconds accurately. The slope of the calibration curve is equal to 0.0026, $\epsilon = 468.26$, and Sandel sensitive equals 0.384 mg/dL, to quantify the glucose salivary concentrations and blood (5-500 mg/dL) at 423 nm. Achieve a detection limit of 0.421 mg/dL where 5 % CV to (n = 3). Because HAZM tests performed according to a kit method, which reflects the mean kit values (101.35 $\pm$ 3.647 mg/dL) and the results of HAZM (104.41 $\pm$ 4.951 mg/dL) with 3.1 mg/dL positive bias in blood. Comparing the mean kit and HAZM glucose levels in saliva, 99.48 $\pm$ 1.26, and 100.97 $\pm$ 2.38 mg/dL, respectively, shows 1.5 mg/dL positive bias for the kit process. Results from the use of this method for measuring glucose in blood and saliva samples indicate that it is effective, as it offers accurate, and its cheap results compared to other

Keywords: Azo compound, Spectrophotometric, Glucose, Blood and Saliva.

الخلاصة

لأن الموارد الصحية محدودة ، تتطلب الظروف منخفضة الدخل تطوراً تكنولوجياً ميسور التكلفة. التصميم والتحليل هو طريقة قياس الألوان منخفضة التكلفة لرصد تشخيص السكري عن طريق الكشف عن الجلوكوز في اللعاب والدم الذي تمت مناقشته ، حيث تم تحضير مركب جديد (حازم) من خلال تفاعل 4-امينو انتي بايرين مع 2-1-فينيل ميثيل 5 بيرازولون. تم تشخيص هذا المركب الاصطناعي بواسطة FTIR و ^1H NMR و ^{13}C NMR. تمت معالجة العينات وتحليلها باستخدام معدات الإنتاج باستخدام تفاعل إنزيمات الجلوكوز أوكسيداز (GO) وإنزيم البيروكسيداز (PO) مع (HAZM) ؛ امنح الطريقة القدرة على حساب مستوى الجلوكوز في أقل من 15 ثانية بدقة. يساوي انحدار منحنى المعايرة 0.0026 ، $\varepsilon = 468.26$ ، وحساسية ساندل تساوي 0.384 مج / ديسيلتر ، لتحديد تركيزات الجلوكوز اللعابية والدم ($500\text{--}5$ مج / ديسيلتر) عند 423 نانومتر. تحقيق حد كشف 0.421 مج / ديسيلتر حيث تكون 5% من السيرة الذاتية على ($n = 3$). لأن اختبارات (حازم) تم إجراؤها وفقاً لطريقة عدة ، والتي تعكس متوسط قيم المجموعة (101.35 ± 3.647 مج / ديسيلتر) ونتائج (حازم) (104.41 ± 4.951 مج / ديسيلتر) مع 3.1 ملجم / ديسيلتر تحيز إيجابي في الدم. بمقارنة متوسط المجموعة ومستويات الجلوكوز (حازم) في اللعاب ، 1.26 ± 99.48 ، و 2.38 ± 100.97 مج / ديسيلتر ، على التوالي ، يظهر انحياز إيجابي 1.5 مج / ديسيلتر لعملية المجموعة. تشير نتائج استخدام هذه الطريقة لقياس الجلوكوز في عينات الدم واللعاب إلى أنها فعالة ، لأنها تقدم نتائج دقيقة ورخيصة مقارنة بالتقنيات التجارية الأخرى.

الكلمات المفتاحية: مركب آزو ، طريقة طيفية ، الجلوكوز ، الدم واللعاب.

1. INTRODUCTION:

Diabetes was the leading cause of impairment and death due to higher blood glucose (Deshpande, Harris-Hayes, and Schootman, 2008). The accepted standard level of the blood glucose range is 87.88 to 123.78 mg/dL (Owens, Dada, Cyrus, Adedoyin, and Adunlin, 2020). Blood glucose control levels are essential in the prevention of severe chronic disease complications (Mian, Hermayer, and Jenkins, 2019). In general, coupling enzymatic reactions in glucose oxidase (GO) and peroxidase (PO) because of the advantages of variables such as substrate specificity, reaction rate, pH, products inhibition, quality, and cost of enzymes have been taken into account amongst the various detection techniques (Bankar, Bule, Singhal, and Ananthanarayan, 2009). Glucose coupling enzymatisation begins with GO oxidation, producing by-products of H_2O_2 where used by PO to oxidize the aromatic organic component by electron donor substrate (EDS) (Galant, Kaufman, and Wilson, 2015). It is possible to estimate glucose levels in the blood or saliva by changing the absorbance intensity of the unique interaction with the responsible enzymes by varying the response speed according to each concentration (Villamena, 2017); such as Trinder's method (Abrera, Sützl, and Haltrich, 2020). Azo compounds may interact with the PO enzyme to indicate that the blood sugar level is measured (Sarkar *et al.*, 2020).

In this research, a new chemical azo compound, (Z)-1,5-dimethyl-4-((1-methyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)diazinyl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one, which is

called (HAZM) acronym, it was prepared in a direct synthesis process, through which the concentration of glucose in blood and saliva samples was measured for several volunteers in addition to a number of patients with diabetes and a number of healthy people, depending on enzymatic reactions and a spectral technique that can be relied upon in clinical laboratories in hospitals or routine tests for people with diabetes as they are fast and inexpensive.

2. MATERIALS AND METHODS:

All the chemicals used in this research were highly pure. A number of spectral devices were used, such as FTIR Shimadzu measurements model 8400 Series, ^1H NMR spectra BRUKER AV 400, ^{13}C NMR spectra BRUKER AV 100, and Shimadzu UV-Vis Spectrophotometer Shimadzu UV-1650Pc.

2.1. Preparation of azo compound (HAZM):

2.0325 g from 4-aminoantipyrine (0.01 mol) was diazotized by dissolving it in 50 mL of ethanol; then, 5ml of HCl [5M] was added. The temperature was kept between 0 to 5 °C. It was slowly added a 1% solution NaNO_2 , and then it was left resting for one hour (Sayhood and Mohammed, 2015a, 2015b). Later, an alkaline well-cooled solution of (1.7420 g) 1-phenyl-2-methyl-5-pyrazolon (0.01 mol) was slowly added to the diazonium salt. The mixture formed a precipitated, and it was washed with a 0.5:1 (ethanol: water) to it was recrystallized and dried after that Scheme 1 demonstrates the synthesis of (Z)-1,5-dimethyl-4-((1-methyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)diazinyl)-2-phenyl-

1,2-dihydro-3H-pyrazol-3-one (HAZM); this synthetic compound was analyzed using FTIR, ^1H NMR, and ^{13}C NMR, where these techniques were essential for the exact chemical structure of the synthesis compound to be decided (Balci, 2005; Karabacak *et al.*, 2014).

2.2. FTIR measurement:

The FTIR spectra were recorded in the region (4000-400) cm^{-1} by used 0.1 g of (HAZM), which mixed with 0.1 g KBr and ground together to form a transparent disk about 1 cm in diameter and 1 mm thick after pressing the mixture.

2.3. ^1H NMR and ^{13}C -NMR measurement:

10 mg of (HAZM) dissolved in pure deuterated DMSO as a solvent and tetramethylsilane as an internal standard to the analysis of ^1H NMR and ^{13}C -NMR spectra.

2.4. Preparation of (HAZM) solution with enzymes:

The preparation of the mixture was accompanied by freshly-prepared enzyme solutions. In the phosphate buffer, 0.01 M (2 mL, pH 7.6) of GO (0.25 mg) and PO (0.5 mg) were separately prepared and kept at 4 °C during experimental procedures. (2 mg) of HAZM was dissolved into ethanol with (5 mL, pH 7.6) of 0.01 M phosphate buffer. The solutions have been degaussed as well as the stability of solution changes has been monitored throughout three days with UV / VIS spectroscopy. No change was observed in the HAZM solution absorption spectrum at 423 nm (Figure 1) due to auto-oxidation or precipitation (Banham *et al.*, 2015; Najafi, 2017).

2.5. Preparing blood serum and saliva samples:

For the establishing of the methodologies, the Al-Najaf Center for Diabetes and Endocrine obtained both saliva and blood test. The Sample was taken under a fasting state from the volunteers. Two volunteer groups aged 18-61 years, with 94 diabetics and 22 non-diabetics. Whole blood (5 mL) has been drawn into the non-anti-coagulant vacuum tube. To facilitate coagulation, it remained still for 20 minutes. The serum was suctioned, then maintained at 4 °C after the centrifugation (10 min at 25000 cycles). Collected saliva samples 2 mL of the whole unstimulated saliva has been used to assess the salivary glucose level. The samples were then centrifuged (3 min at 25000 cycles), and glucose tests were performed on the resulting supernatant. In order to determine the connection

between blood glucose and the salivary concentration, the Center carried out simultaneous blood sampling for every volunteer.

Ethical Consent

"Verbal consent was taken from all participants after explaining the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail." (Schroter, Plowman, Hutchings, and Gonzalez, 2006)

2.6. Determination of glucose:

In order to establish the HAZM method, a commercial Glucose Assay Kit was used to examine test samples in which a (1 mL) solution kit was added to ten μL of serum tests. The mixture was 20-minute incubated at 25 °C, and it is measured at 516 nm. The concentration of glucose was determined from the typical vendor curve.

In the HAZM method, a test solution consisting of phosphate buffer (0.01 mM) containing GO (0.025 pM), PO (0.05 pM), and HAZM (0.02 mM), where the final volume was (2 mL), was applied to the blood sample serum (20 μL). After 15 seconds, the optical density change was registered at 423 nm. The concentration of glucose was determined from the resulting calibration curve (Figure 2). In order to determine the HAZM method for evaluating the level of salivary glucose, in the testing solution, 10 μL of a resulting supernatant has been used after drawing 0.5 mL of freshly saliva and centrifugation; and measure at 423 nm.

3. RESULTS AND DISCUSSION:

3.1. Explanation of the process:

Actual blood glucose monitoring systems are based on photometric or electrochemical techniques (Pu *et al.*, 2016). Direct glucose oxidation by GO was used to produce electrochemical sensors for personal optical measuring instruments (Zhang *et al.*, 2019). The replicability of tests, in addition to the sensitivity of electrochemical sensors to specific significant interventions, nevertheless decreases as the system time of life (Aljafery, Sayhood, Abdulridha, and Yousif, 2018). Unlike electrochemical electrode construction, it is simpler and cheaper to create photometric instruments (Al-Shirifi, Dikran, and Halboos,

2018; Halboos, Ammar Sayhood, and Ala'a Hussein, 2019). Even so, the precision of these instruments depends both on the overall performance of the technique and on the technical specifications (Hwang *et al.*, 2017).

Trinder method for measuring glucose in the blood is the first procedure based on the use of H_2O_2 (GO by-product) in clinical laboratories. Still, it has not been developed by researchers to benefit from them in measuring glucose in the miniaturized personal colorimeters (Kengne, Erasmus, Levitt, and Matsha, 2017).

The spectrum of the HAZM method showed one peak at 423 nm Figure 1. HAZM's reaction to varying quantities of PO with a constant H_2O_2 concentration has linear. For the tests, 2 mL of PO 5 nM was chosen, HAZM response was also linear to different amounts of H_2O_2 . Consequently, PO oxidation of HAZM was coupled with GO (2.5 nM) glucose oxidation. The oxidation response of the HAZM procedure to various glucose levels was linear of 5-500 mg/dL glucose; this result is considered good if compared to previous studies (Bruen, Delaney, Florea, and Diamond, 2017; Dominguez, Orozco, Chávez, and Márquez-Lucero, 2017).

3.2. FTIR Analysis

Spectroscopy of the synthetic compound (HAZM) was performed using FTIR, where the analysis showed a number of peaks as shown in (Figure 3) having a wide band of 3485 cm^{-1} (s) attributable for the stretching, vibration of O-H, $\nu\text{C-H}$ aromatic at 3157 cm^{-1} (m). Carbonyl group $\nu(\text{C=O})$ stretching frequency at 1674 cm^{-1} (s). Likewise the frequency at 1537 cm^{-1} (w) and 1491 cm^{-1} (m) corresponding to $\nu(\text{N=N})$.

3.3. ^1H NMR and ^{13}C -NMR Analysis

The following signals are shown in the deuterated DMSO solution with the tetra methylsaline internal standard: ^1H NMR (Figure 4) and ^{13}C -NMR (Figure 5) of the prepared azo compound (HAZM). The ^1H -NMR (HAZM) spectrum has a multiplied spectrum of δ (7.57-7.33) ppm (s, 12H, Ar) due to phenyl groups, -N-CH=(4-Aminoantipyrin) due to δ (3.4) ppm (s, H) and =C-CH₃ due to δ (2.6) ppm, -N-CH= (1-phenyl-2-methyl-5-pyrazolone) due to a multiple δ (3.1) ppm (s, H) and =C-CH₃ due to δ (2.5) ppm. The signals in ^{13}C -NMR show (HAZM) were: (138-126) ppm (12C, Ar-C); 151 ppm (C=O); 31.9 ppm (C-CH₃) (1-phenyl-2-methyl-5-pyrazolone) 39 ppm (N-CH₃); 34.8 ppm (C-CH₃) (4-aminoantipyrine), 40.3 ppm (N-CH₃) 143 ppm (-C-N=N-C-).

3.4. Glucose measurements in blood:

The glucose levels of 116 serum samples were examined by kit and HAZM system in order to verify the relevant HAZM process. A review of results from Pearson showed a strong association ($r > 0.95$) between kit and HAZM tests. The experimental (t-test) of levels of sugar blood there in samples tested using HAZM and kits approaches did not show a significant distinction between these results ($P > 0.05$). Because HAZM tests were performed according to a kit method, more favorable mistakes were reported, which reflect the mean kit values ($101.35 \pm 3.647\text{ mg/dL}$) and the results of HAZM ($104.41 \pm 4.951\text{ mg/dL}$) with 3.1 mg/dL positive bias. This is expected to be caused by delayed glycolysis (Moutasim and Thomas, 2020).

3.5. Glucose measurements in saliva:

Early identification and prevention of many diseases were assisted by salivary diagnosis (Lee, Garon, and Wong, 2009). The reliance on HAZM for non-invasive control of the salivary glucose concentration has been evaluated based on the promising potential saliva application areas in personal dentistry (Olczak *et al.*, 2019), and much more crucially, the limit of detection for the HAZM method (0.421 mg/dL). In this study, both kit and HAZM methods evaluated non-stimulated whole saliva of diabetic and non-diabetic samples. The speedy samples obtained to limit the effects of possible interference substances, particularly $\text{C}_2\text{H}_4\text{OHCOOH}$, where can has a significant effect in saliva pH (Kubáň, Dvořák, and Kubáň, 2019). Analysis of the data of the HAZM and the kit data for salivary glucose concentration indicated insignificant results differences ($P > 0.05$). The results of diabetic and non-diabetic individuals' salivary glucose concentration were also correlated with corresponding fasting glucose level values. Comparing the mean kit and HAZM glucose levels, 99.48 ± 1.26 and $100.97 \pm 2.38\text{ mg/dL}$ respectively, show 1.5 mg/dL positive bias for kit process.

Conflict of Interest:

"The authors declare that they have no conflict of interest."

4. CONCLUSIONS:

The current method for blood sugar calculation and salivary samples using coupled enzyme GO and PO was based primarily on the

spectrophotometric characteristics of HAZM. It is stable and accurately calculates the glucose levels in less than 15 seconds. Taking into consideration that only the first stage in the combined enzyme assay quantification of blood glucose has also been modified in the HAZM method. It will be possible to develop this method in the near future to make it one of the techniques that are characterized by ease of dealing with it.

5. ACKNOWLEDGMENTS:

The authors thank and gratefully appreciate the staff of the Al-Najaf Center for Diabetes and Endocrine for their facilities to accomplish this research.

6. REFERENCES:

1. Abrera, A. T., Sützl, L., and Haltrich, D. (2020). Pyranose oxidase: A versatile sugar oxidoreductase for bioelectrochemical applications. *Bioelectrochemistry*, 132, 107409. <https://doi.org/10.1016/j.bioelechem.2019.107409>
2. Al-Shirifi, A. N. M., Dikran, S. B., and Halboos, M. H. N. (2018). Application of central composite design method to oxidative coupling spectrophotometric determination of metoclopramide hydrochloride in pure form and pharmaceutical preparations. *Journal of Global Pharma Technology*, 10(5), 143–152.
3. Aljafery, A. M. A., Sayhood, A., Abdulridha, W. M., and Yousif, A. M. (2018). Evaluation the tensile strength of cold-cured acrylic resin denture base material by adding silver nanoparticles. *Indian Journal of Public Health Research and Development*, 9(10), 917–922. <https://doi.org/10.5958/0976-5506.2018.01258.5>
4. Balci, M. (2005). *Basic 1H- and 13C-NMR Spectroscopy*. Elsevier. <https://doi.org/10.1016/B978-0-444-51811-8.X5000-1>
5. Banham, D., Ye, S., Knights, S., Stewart, S. M., Wilson, M., and Garzon, F. (2015). UV-visible spectroscopy method for screening the chemical stability of potential antioxidants for proton exchange membrane fuel cells. *Journal of Power Sources*, 281, 238–242. <https://doi.org/10.1016/j.jpowsour.2015.02.002>
6. Bankar, S. B., Bule, M. V., Singhal, R. S., and Ananthanarayan, L. (2009). Glucose oxidase - An overview. *Biotechnology Advances*, 27(4), 489–501. <https://doi.org/10.1016/j.biotechadv.2009.04.003>
7. Bruen, D., Delaney, C., Florea, L., and Diamond, D. (2017). Glucose sensing for diabetes monitoring: Recent developments. *Sensors*, 17(1866), 1–21. <https://doi.org/10.3390/s17081866>
8. Deshpande, A. D., Harris-Hayes, M., and Schootman, M. (2008). Epidemiology of Diabetes and Diabetes-Related Complications. *Physical Therapy*, 88(11), 1254–1264. <https://doi.org/10.2522/ptj.20080020>
9. Dominguez, R. B., Orozco, M. A., Chávez, G., and Márquez-Lucero, A. (2017). The Evaluation of a Low-Cost Colorimeter for Glucose Detection in Salivary Samples. *Sensors*, 17(2495), 1–11. <https://doi.org/10.3390/s17112495>
10. Galant, A. L., Kaufman, R. C., and Wilson, J. D. (2015). Glucose: Detection and analysis. *Food Chemistry*, 188, 149–160. <https://doi.org/10.1016/j.foodchem.2015.04.071>
11. Halboos, M. H., Ammar Sayhood, A., and Ala'a Hussein, T. (2019). Determination celioprolol hydrochloride drug by used zero, first, second and third order derivative and peak area spectrophotometry method in its pure form and in pharmaceutical tablets. *Journal of Physics: Conference Series*, 1294, 52035. <https://doi.org/10.1088/1742-6596/1294/5/052035>
12. Hwang, J. H., Kim, H., Cho, S., Bellemans, T., Lee, W. Do, Choi, K., ... Joh, C. H. (2017). An examination of the accuracy of an activity-based travel simulation against smartcard and navigation device data. *Travel Behaviour and Society*, 7, 34–42. <https://doi.org/10.1016/j.tbs.2017.01.001>
13. Karabacak, M., Kose, E., Atac, A., Sas, E. B., Asiri, A. M., and Kurt, M. (2014). Experimental (FT-IR, FT-Raman, UV-Vis, 1H and 13C NMR) and computational (density functional theory) studies on 3-bromophenylboronic acid. *Journal of Molecular Structure*, 1076, 358–372. <https://doi.org/10.1016/j.molstruc.2014.07.058>
14. Kengne, A. P., Erasmus, R. T., Levitt, N.

- S., and Matsha, T. E. (2017). Alternative indices of glucose homeostasis as biochemical diagnostic tests for abnormal glucose tolerance in an African setting. *Primary Care Diabetes*, 11(2), 119–131. <https://doi.org/10.1016/j.pcd.2017.01.004>
15. Kubáň, P., Dvořák, M., and Kubáň, P. (2019). Capillary electrophoresis of small ions and molecules in less conventional human body fluid samples: A review. *Analytica Chimica Acta*, 1075, 1–26. <https://doi.org/10.1016/j.aca.2019.05.004>
 16. Lee, J. M., Garon, E., and Wong, D. T. (2009). Salivary diagnostics. *Orthodontics and Craniofacial Research*, 12(3), 206–211. <https://doi.org/10.1111/j.1601-6343.2009.01454.x>
 17. Mian, Z., Hermayer, K. L., and Jenkins, A. (2019). Continuous Glucose Monitoring: Review of an Innovation in Diabetes Management. *American Journal of the Medical Sciences*, 358(5), 332–339. <https://doi.org/10.1016/j.amjms.2019.07.003>
 18. Moutasim, K. A., and Thomas, G. J. (2020). Salivary gland tumours: update on molecular diagnostics. *Diagnostic Histopathology*, 26(4), 159–164. <https://doi.org/10.1016/j.mpdhp.2020.01.002>
 19. Najafi, A. (2017). An investigation on dispersion and stability of water-soluble fullerene (C60OH) in water via UV–Visible spectroscopy. *Chemical Physics Letters*, 669, 115–118. <https://doi.org/10.1016/j.cplett.2016.12.030>
 20. Olczak, M., Poniatowski, Ł. A., Niderla-Bielińska, J., Kwiatkowska, M., Chutorański, D., Tarka, S., and Wierzbobrowicz, T. (2019). Concentration of microtubule associated protein tau (MAPT) in urine and saliva as a potential biomarker of traumatic brain injury in relationship with blood–brain barrier disruption in postmortem examination. *Forensic Science International*, 301, 28–36. <https://doi.org/10.1016/j.forsciint.2019.05.010>
 21. Owens, F. S., Dada, O., Cyrus, J. W., Adedoyin, O. O., and Adunlin, G. (2020). The effects of *Moringa oleifera* on blood glucose levels: A scoping review of the literature. *Complementary Therapies in Medicine*, 50, 102362. <https://doi.org/10.1016/j.ctim.2020.102362>
 22. Pu, Z., Wang, R., Wu, J., Yu, H., Xu, K., and Li, D. (2016). A flexible electrochemical glucose sensor with composite nanostructured surface of the working electrode. *Sensors and Actuators, B: Chemical*, 230, 801–809. <https://doi.org/10.1016/j.snb.2016.02.115>
 23. Sarkar, S., Banerjee, A., Chakraborty, N., Soren, K., Chakraborty, P., and Bandopadhyay, R. (2020). Structural-functional analyses of textile dye degrading azoreductase, laccase and peroxidase: A comparative in silico study. *Electronic Journal of Biotechnology*, 43, 48–54. <https://doi.org/10.1016/j.ejbt.2019.12.004>
 24. Sayhood, A. A., and Mohammed, H. J. (2015a). Synthesis of new azo reagent for determination of Pd(II), Ag(I) and applied to enhance the properties of silver nano particles. *International Journal of Chemical Sciences*, 13(3), 1123–1136.
 25. Sayhood, A. A., and Mohammed, H. J. (2015b). Synthesis of novel azo reagents derived from 4-aminoantipyrine and their applications of enhancement of silver nano particles. *Der Pharma Chemica*, 7(8), 50–58.
 26. Schroter, S., Plowman, R., Hutchings, A., and Gonzalez, A. (2006). Reporting ethics committee approval and patient consent by study design in five general medical journals. *Journal of Medical Ethics*, 32(12), 718–723. <https://doi.org/10.1136/jme.2005.015115>
 27. Villamena, F. A. (2017). UV–Vis Absorption and Chemiluminescence Techniques. In *Reactive Species Detection in Biology* (pp. 203–251). Elsevier. <https://doi.org/10.1016/b978-0-12-420017-3.00006-2>
 28. Zhang, D., Chen, X., Ma, W., Yang, T., Li, D., Dai, B., and Zhang, Y. (2019). Direct electrochemistry of glucose oxidase based on one step electrodeposition of reduced graphene oxide incorporating polymerized L-lysine and its application in glucose sensing. *Materials Science and Engineering C*, 104, 109880. <https://doi.org/10.1016/j.msec.2019.109880>

Scheme 1. Steps to synthesize the compound (HAZM)

Figure 1. Absorption spectra of HAZM method at 423 nm

Figure 2. Calibration curve of HAZM method at 423 nm

Figure 3. FTIR spectra of HAZM compound

Figure 4. ¹H NMR spectra of HAZM compound

Figure 5. ^{13}C NMR spectra of HAZM compound