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REGULAR ARTICLE

Spectrophotometric nanomolar determination of glucose by using C-dots/Fe_3O_4 magnetic nanozyme

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Abstract. C-dots/Fe_3O_4 magnetic nanocomposite was known as peroxidase-like nanozyme. In this work, C-dots/Fe_3O_4 magnetic nanocomposite was used to determine glucose in the presence of glucose oxidase (GOx). The color change of 3,3′,5,5′-tetramethylbenzidine (TMB) was used as an indication of glucose in a colorimetric assay. The nanozymatic activity of C-dots/Fe_3O_4 magnetic nanocomposite to detect glucose was depended on TMB and C-dots/Fe_3O_4 nanozyme concentration and pH. The linear range and detection limit to determine glucose using C-dots/Fe_3O_4 magnetic nanocomposite were obtained as 5×10^{-8} to 1×10^{-3} M and 50 nM, respectively. Also, the sensing system shows high selectivity towards the detection of glucose in the presence of fructose, lactose and maltose as interferences. The results indicate that this assay is simple, cheap, and highly sensitive and selective for glucose determination.

Keywords. Glucose; C-dots/Fe_3O_4 nanocomposite; nanozyme.

1. Introduction

Determination of glucose is one of the most important analytical concerns in biochemistry and biotechnology especially in blood tests and in the production and processing of various kinds of feed and food.[1] Also, the frequent monitoring of glucose is an essential part of diabetes management.[2,3] In this way, several kinds of electrochemical[4–6] and optical[1,7] methods were implemented for glucose monitoring. But among them, the colorimetric methods are preferred due to its simplification, convenience, continuous monitoring, practically and rapid determinations.[7,8] Usually colorimetric techniques are employed enzymatic reactions to measure glucose concentration.[9] In this way, glucose oxidase (GOx) and horseradish peroxidase (HRP) as essential enzymes were implemented extensively in the literature.[10] In a typical cascade reaction as illustrated in Scheme 1, glucose in the presence of GOx enzyme reacts with oxygen to produce gluconic acid and H_2O_2. Then, in the second step, H_2O_2 in the presence of HRP converts a substrate such as TMB (3,3′,5,5′-tetramethylbenzidine) to oxTMB which causes the solution to turn blue.[11] But natural enzymes have limited applications due to low stability, catalytic dependency on the environmental conditions, high cost and their difficult recyclability.[12] Therefore, the replacement of nanozyme instead of at least one of the natural enzyme in the above-mentioned colorimetric test for glucose is highly interesting.[13–17]

The enzyme-like activity of nanomaterials was investigated recently[12,18,19] because of the diverse applications of nanozymes/nano enzymes in sensing, imaging, and therapeutics, logic gates, pollutant removal, water treatment, etc.[20,21] Consequently, carbon,[8,11,14,22,23] noble metal[15,16,24,25] and metal oxide-based[26–32] nanomaterials prove to be the promising ones. In this way, the C-dots/Fe_3O_4 magnetic nanocomposite was synthesized previously.[33,34] This nanocomposite showed intrinsic peroxidase-like activity and was used to determine H_2O_2.[33] In the present work, the ability of C-dots/Fe_3O_4 magnetic nanocomposite as peroxidase-like nanozyme was used for colorimetric determination of glucose.

2. Experimental

2.1 *Materials and synthesis*

Carbon soot was obtained by burning a candle. Glucose was purchased from Merck. All other chemicals with analytical grade were obtained from Sigma-Aldrich. C-dots/Fe_3O_4 magnetic nanocomposite was prepared by using the previously reported procedure.[33] Briefly, carbon soot (25 mg)

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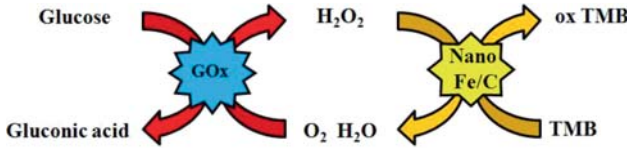

Schematic 1. Schematic illustration of colorimetric detection of glucose using GOx and HRP.

was refluxed in nitric acid (5M) and after centrifugation, the light brownish-yellow supernatant was used as C-dots. Also, magnetic iron oxide nanoparticles were prepared in alkali media based on the previously reported procedure.[35] Briefly, ammonia was rapidly injected to the solution of Na_2SO_3 (0.16 mol L^{-1}) and $FeCl_3$ (0.05 mol L^{-1}) and heated at 60°C for 30 min. After 2 h, the magnetic Fe_3O_4 powder was isolated by using a permanent magnate (1.4 T). Then solutions of Fe_3O_4 nanoparticles and C-dots were mixed together by the ratio of 1:4 in pH2 to prepare nanocomposite. And after 30 min, the as-prepared nanocomposite was separated by a permanent magnet. Finally, the obtained nanocomposite was washed two times with distilled water.

2.2 *Instrumentations*

The morphology of the samples was determined by using a Zeiss, EM10C electron transmission microscope (80 kV). TESCAN VEGA3 was used as energy dispersive spectroscopy (EDS). A D8 ADVANCE type (BRUKER-Germany) X-ray diffractometer (XRD) with Cu-K α radiation was used to identify the structure ($\lambda = 0.1542$ nm). UV–Vis spectra were collected by using Hach DR 5000 spectrophotometer. Also, the pH measurements were performed using a Metrohm pH meter (model 780).

2.3 *Procedure for colorimetric determination of glucose*

For glucose detection, at first solutions of different concentration of glucose and 50 μL GOx 100 U mL^{-1} were incubated for 30 min. Then 1 mg nanocomposite, 2.1 mL acetate buffer pH 2.4 and 500 μL TMB 1.2 mg mL^{-1} were added into the solutions. Then visible spectra were collected after 15 min.

3. Results and Discussion

3.1 *Structural characterization*

Structural analysis of C-dots/Fe_3O_4 magnetic nanocomposite was performed by using TEM, EDS and XRD (Figure 1). TEM image depicts the presence of both C-dots and Fe_3O_4 nanoparticles with different sizes in

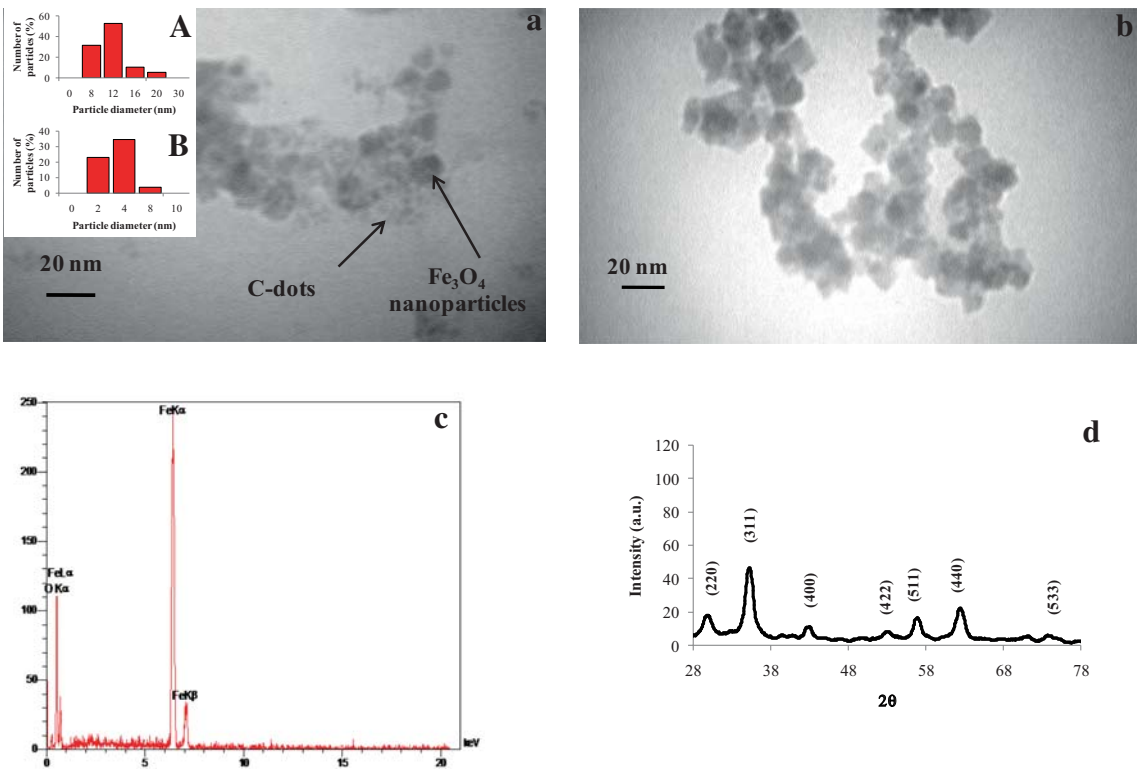

Figure 1. TEM images of (a) C-dots/Fe_3O_4 and (b) Fe_3O_4 nanocomposite (Inset: particle size distribution of (A) Fe_3O_4 NPs and (B) C-dots), (c) EDS pattern and (d) XRD pattern of C-dots/Fe_3O_4 magnetic nanocomposite.

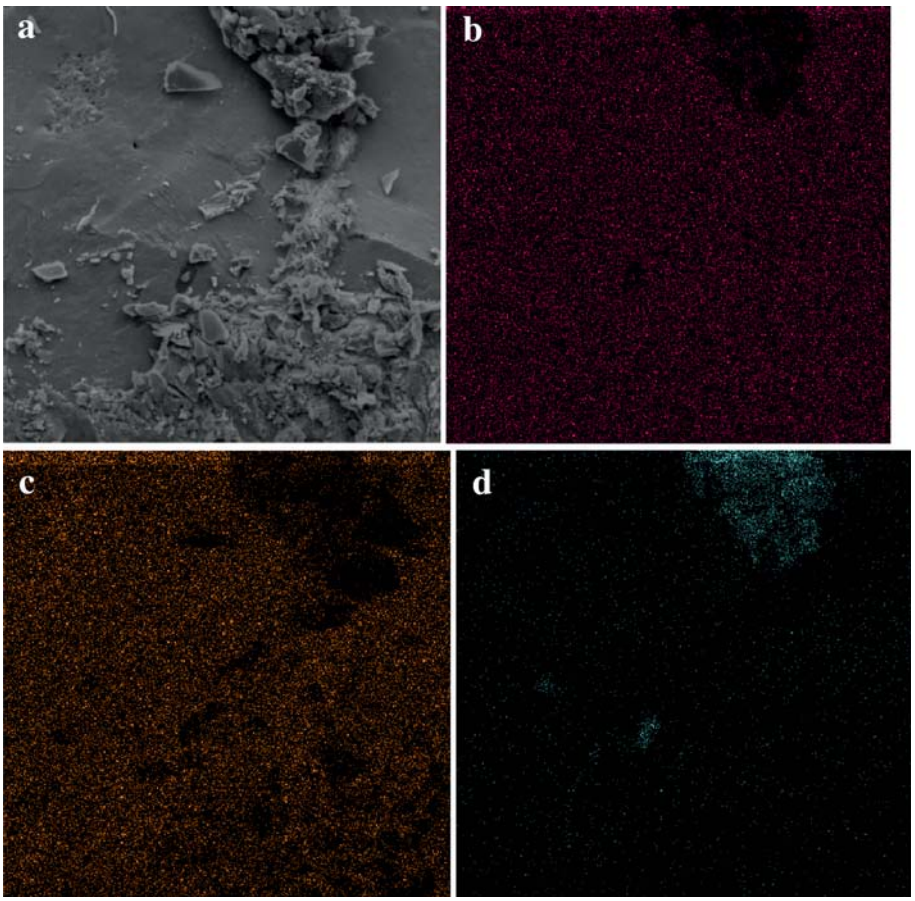

Figure 2. (a) Scanning electron microscopy and elemental mapping of (b) Fe, (c) O and (d) C.

the as-prepared nanocomposite structure (Figure 1a). Clearly, C-dots (average diameter ~4 nm) are smaller than Fe_3O_4 nanoparticles (~12 nm). For better comparison, the TEM image of Fe_3O_4 nanoparticles presents in Figure 1b. Also, X-ray energy dispersive spectroscopy (EDS) analysis shows the presence of carbon, iron and oxygen elements in the nanocomposite (Figure 1c). Elemental mapping of the nanocomposite presents in Figure 2. Clearly, iron and oxygen are homogeneously distributed while carbon is localized in specific areas within the precipitate. At XRD pattern of nanocomposite (Figure 1d), corresponding peaks of Fe_3O_4 nanostructures at 30.1°, 35.7°, 43.1°, 53.5°, 56.9°, 62.6° and 74.2° can be observed which are related to the Miller indices of (220), (311), (400), (422), (511), (440), and (533), respectively. So, the spinel structure of Fe_3O_4 nanoparticles was confirmed by observed indices.[36] So, the XRD pattern of nanocomposite depicts the presence of iron oxide as Fe_3O_4 in the nanostructure. As a result, Fe_3O_4 nanoparticles preserve their structure in the nanocomposite. So, the C-dots/Fe_3O_4 magnetic nanocomposite was synthesized properly based on the previous report.[33]

3.2 *Determination of glucose using GOx and C-dots/Fe_3O_4 magnetic nanocomposite*

The peroxidase-like activity of C-dots/Fe_3O_4 magnetic nanocomposite has been explored previously.[33] By using this nanozyme, nano-molar determination of hydrogen peroxide was possible. The main advantage of the C-dots/Fe_3O_4magnetic nanocomposite is its higher peroxidase-like activity rather than individual Fe_3O_4 magnetic nanoparticles and C-dots.[33] So, in this study, the presence of C-dots/Fe_3O_4 magnetic nanocomposite instead of HRP for determination of glucose was tested. In this way, the effect of the presence of C-dots/Fe_3O_4 magnetic nanozyme and GOx in successive reactions for the determination of glucose was examined. Since GOx would be denatured in acidic pH solution, the glucose detection using peroxidase-like nanozymes and GOx usually were performed in two steps with two different pH values.[11] In fact, at first, glucose reacts with oxygen in the presence of GOx in the mild conditions, i.e., pH = 7.0 and then the produced hydrogen peroxide in other pH condition were detected in the presence of peroxidase-like nanaozymes. In the presence

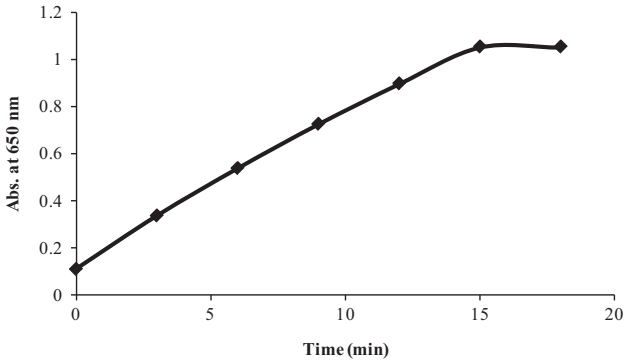

Figure 3. Dependency of absorption at 650 nm on time for glucose detection in the system contains C-dots/Fe_3O_4 magnetic nanocomposite.

of peroxidase-like active nanozyme the solution color change to blue due to the presence of TMB. In this manner, by addition of a solution of glucose and GOx to the solution containing C-dots/Fe_3O_4 magnetic nanocomposite and peroxidase substrate, i.e., TMB, a blue color reaction was produced, indicating that C-dots/Fe_3O_4 magnetic nanocomposite has peroxidase-like catalytic activity (Figure 3). The controlled experiments with C-dots/Fe_3O_4 in the absence of GOx and vice versa do not show any significant peak at 650 nm. The catalytic-reaction was monitored by TMB absorbance change at 650 nm. The time-dependent absorbance changes of TMB are depicted in Figure 3. Clearly, an increase in absorption at 650 nm was observed by increasing the time. After 15 min, the plot reaches the plateau and enzymatic activity reaches a maximum value.

Furthermore, the dependency of C-dots/Fe_3O_4 magnetic nanocomposite activity on TMB and nanozyme concentration and also pH was investigated (Figure 4). The nanozymatic activity of C-dots/Fe_3O_4 magnetic nanocomposite such as natural enzyme depends on the concentration of substrate concentration, i.e., TMB, nanozyme concentration and pH. Also, the maximum relative activity of C-dots/Fe_3O_4 magnetic nanocomposite was observed at $\sim$0.4 mg mL^{-1} TMB and 400 µg mL^{-1} nanocomposite. However, it was found that the C-dots/Fe_3O_4 magnetic nanocomposite exhibited a strong peroxidase-like catalytic activity at a strongly acidic medium, i.e., pH = 2.0 (Figure 4). These observations are well demonstrated that the C-dots/Fe_3O_4 magnetic nanocomposite possess a high degree of activity and stability as peroxidase catalysts in the harsh condition of pH = 2.0.

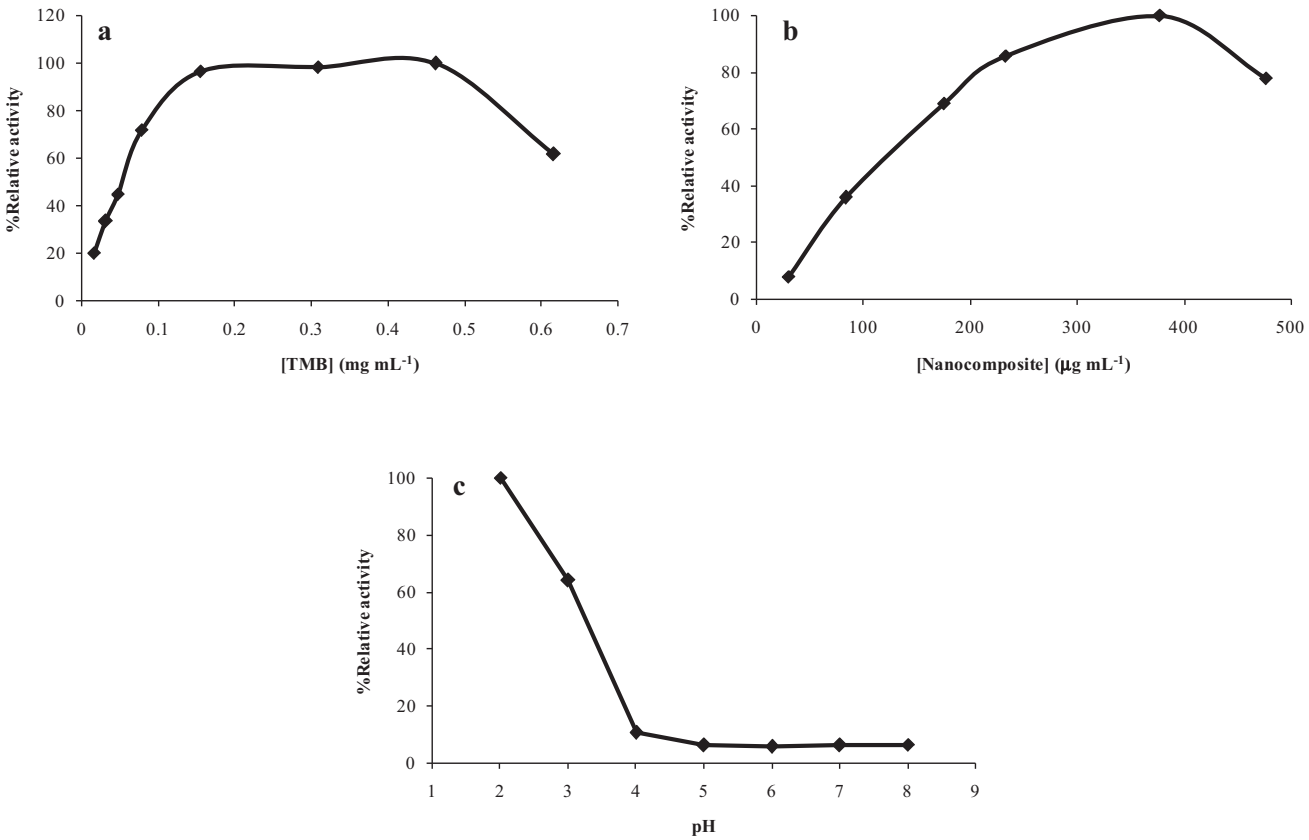

Figure 4. Dependency of absorption spectra on (a) TMB concentration, (b) C-dots/Fe_3O_4 magnetic nanocomposite concentration and (c) pH in presence of GOx and C-dots/Fe_3O_4 magnetic nanocomposite.

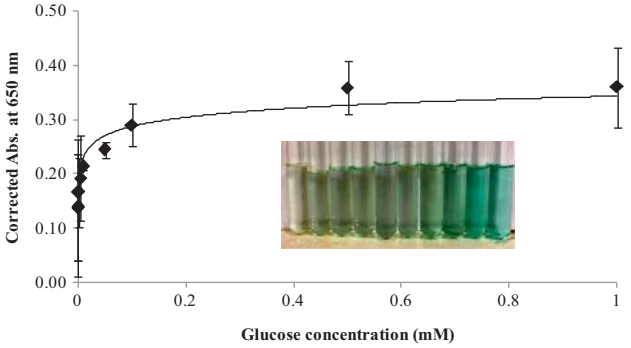

Figure 5. Calibration plots for glucose detection using GOx and C-dots/Fe_3O_4 magnetic nanocomposite (inset: corresponding photograph).

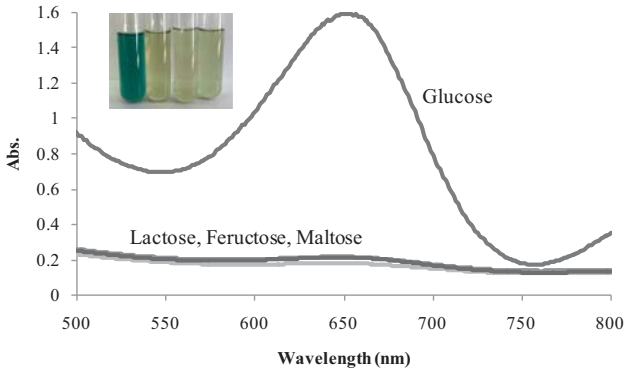

Figure 6. Spectra of 5mM glucose, fructose, maltose and lactose using GOx and C-dots/Fe_3O_4 magnetic nanocomposite (inset: corresponding photograph).

On the basis of the above results, C-dots/Fe_3O_4 magnetic nanocomposite was implemented for the determination of glucose. A calibration curve was attained (Figure 5), which demonstrated a logarithmic relationship between the absorbance and the glucose concentration ranging from 5×10^{-8} to 1×10^{-3} M. The equation of the corresponding curve is *Corrected Abs. = 0.024 ln([glucose](mM)) + 0.344* with $R^2 = 0.927$. The detection limit (DL) for glucose was obtained experimentally as 5×10^{-8} M. Table 1 summarizes the figures of merit for determination of glucose and compares the results with previously reported nanozymes. Clearly, in this case, the determination range was expanded to three orders of magnitude larger, and detection limit was also improved one or three order of magnitude rather than C-dots and Fe_3O_4 magnetic nanoparticles, respectively.

To investigate the selectivity of the proposed method the response of fructose, lactose, and maltose as glucose analogues were recorded. The results (Figure 6) demonstrated that the absorbance of these interferences was negligible compared to that of glucose even at concentrations as high as 5 mM. Therefore, this biosensing system indicates high selectivity for glucose due to the high affinity of glucose oxidase for glucose.

Also, the reproducibility of the proposed method was examined. RSD of 10.2% was obtained for three times determination of 1 μM glucose which is an acceptable value for precision. Moreover, the determination of glucose in human serum was performed to test the wider utility of the nanocomposite. The exact amount of glucose in the real sample was 100 mg dL^{-1}, while the obtained value using the proposed procedure was 109.5 mg dL^{-1}. In this way, a relative deviation of 9.5% was obtained for the determination of glucose.

4. Conclusions

Performance of C-dots/Fe_3O_4 magnetic nanocomposite in the presence of GOx for determination of glucose was investigated. The response of the proposed assay was dependent on pH, TMB and nanocomposite concentrations. Figures of merit for introduced assay were calculated. The obtained linear range was wider than other previous similar reports. Also, the proposed methodology to determine glucose is simple and cheap and most importantly, determination in nanomolar level is possible.

Table 1. Figures of merit for colorimetric determination of glucose by the use of various peroxidase-like nanozymes and GOx in cascade reactions.

Implemented nanozyme	Determination range (M)	DL (M)	References
Fe_3O_4 nanoparticle	$5 \times 10^{-5} - 1 \times 10^{-3}$	3×10^{-5}	37
C-dots	$1 \times 10^{-6} - 5 \times 10^{-4}$	4×10^{-7}	38
Graphene oxide–Fe_3O_4 nanocomposites	$2 \times 10^{-6} - 2 \times 10^{-4}$	7.4×10^{-7}	28
Casein-Fe_3O_4 nanocomposite	$3 \times 10^{-6} - 1 \times 10^{-3}$	1×10^{-6}	39
C-dots-Fe_3O_4 nanocomposite	$5 \times 10^{-8} - 1 \times 10^{-3}$	5×10^{-8}	This work

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References

1. Steiner M-S, Duerkop A and Wolfbeis O S 2011 Optical methods for sensing glucose *Chem. Soc. Rev.* **40** 4805
2. Vashist S K 2012 Non-invasive glucose monitoring technology in diabetes management: a review *Anal. Chim. Acta* **750** 16
3. Soleymani H, Saboury A A, Moosavi-Movahedi A A, Rahmani F, Maleki J, Yousefinejad S and Maghami P 2016 Vitamin E induces regular structure and stability of human insulin, more intense than vitamin D3 *Int. J. Biol. Macromol.* **93** 868
4. Rahman M M, Ahammad A J S, Jin J H, Ahn S J and Lee J J 2010 A comprehensive review of glucose biosensors based on nanostructured metal-oxides *Sensors* **10** 4855
5. Zhu Z, Garcia-Gancedo L, Flewitt A J, Xie H, Moussy F and Milne W I 2012 A critical review of glucose biosensors based on carbon nanomaterials: carbon nanotubes and graphene *Sensors (Basel)* **12** 5996
6. Rao D, Sheng Q and Zheng J 2016 Novel nanocomposite of chitosan-protected platinum nanoparticles immobilized on nickel hydroxide: facile synthesis and application as glucose electrochemical sensor *J. Chem. Sci.* **128** 1367
7. Wang X, Chen H, Zhou T, Lin Z, Zeng J, Xie Z, Chen X, Wong K, Chen G and Wang X 2009 Optical colorimetric sensor strip for direct readout glucose measurement *Biosens. Bioelectron.* **24** 3702
8. Li R, Zhen M, Guan M, Chen D, Zhang G, Ge J, Gong P, Wang C and Shu C 2013 A novel glucose colorimetric sensor based on intrinsic peroxidase-like activity of C60-carboxyfullerenes *Biosens. Bioelectron.* **47** 502
9. Oliver N S, Toumazou C, Cass A E G and Johnston D G 2009 Glucose sensors: a review of current and emerging technology *Diabet. Med.* **26** 194
10. Ferri S, Kojima K and Sode K 2011 Review of glucose oxidases and glucose dehydrogenases: a bird's eye view of glucose sensing enzymes *J. Diabetes Sci. Technol.* **5** 1068
11. Song Y, Qu K, Zhao C, Ren J and Qu X 2010 Graphene oxide: intrinsic peroxidase catalytic activity and its application to glucose detection *Adv. Mater.* **22** 2206
12. Lin Y, Ren J and Qu X 2014 Catalytically active nanomaterials: a promising candidate for artificial enzymes *Acc. Chem. Res.* **47** 1097
13. Honarasa F, Kamshoori F H, Fathi S and Motamedifar Z 2019 Carbon dots on V_2O_5 nanowires are a viable peroxidase mimic for colorimetric determination of hydrogen peroxide and glucose *Microchim. Acta* **186** 234
14. Chen S, Hai X, Chen X W and Wang J H 2014 In situ growth of silver nanoparticles on graphene quantum dots for ultrasensitive colorimetric detection of H_2O_2 and glucose *Anal. Chem.* **86** 6689
15. Wang Q, Zhang L, Shang C, Zhang Z and Dong S 2016 Triple-enzyme mimetic activity of nickel-palladium hollow nanoparticles and their application in colorimetric biosensing of glucose *Chem. Commun.* **52** 5410
16. Jv Y, Li B and Cao R 2010 Positively-charged gold nanoparticles as peroxidase mimic and their application in hydrogen peroxide and glucose detection *Chem. Commun.* **46** 8017
17. Zeng D, Luo W, Li J, Liu H, Ma H, Huang Q and Fan C 2012 Gold nanoparticles-based nanoconjugates for enhanced enzyme cascade and glucose sensing *Analyst* **137** 4435
18. Wei H and Wang E 2013 Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes *Chem. Soc. Rev.* **42** 6060
19. He W, Wamer W, Xia Q, Yin J and Fu P P 2014 Enzyme-like activity of nanomaterials *J. Environ. Sci. Heal. Part C* **32** 186
20. Wang X, Hu Y and Wei H 2016 Nanozymes in bionanotechnology: from sensing to therapeutics and beyond *Inorg. Chem. Front.* **3** 41
21. Nasir M, Nawaz M H, Latif U, Yaqub M, Hayat A and Rahim A 2017 An overview on enzyme-mimicking nanomaterials for use in electrochemical and optical assays *Microchim. Acta* **184** 323
22. Tian J, Liu Q, Asiri A M, Qusti A H, Al-Youbi A O and Sun X 2013 Ultrathin graphitic carbon nitride nanosheets: a novel peroxidase mimetic, Fe doping-mediated catalytic performance enhancement and application to rapid, highly sensitive optical detection of glucose *Nanoscale* **5** 11604
23. Yang W, Huang T, Zhao M, Luo F, Weng W, Wei Q, Lin Z and Chen G 2017 High peroxidase-like activity of iron and nitrogen co-doped carbon dots and its application in immunosorbent assay *Talanta* **164** 1
24. Hu L, Yuan Y, Zhang L, Zhao J, Majeed S and Xu G 2013 Copper nanoclusters as peroxidase mimetics and their applications to H_2O_2 and glucose detection *Anal. Chim. Acta* **762** 83
25. He W, Han X, Jia H, Cai J, Zhou Y and Zheng Z 2017 AuPt alloy nanostructures with tunable composition and enzyme-like activities for colorimetric detection of bisulfide *Sci. Rep.* **7** 40103
26. Qiao F, Chen L, Li X, Li L and Ai S 2014 Peroxidase-like activity of manganese selenide nanoparticles and its analytical application for visual detection of hydrogen peroxide and glucose *Sens. Actuators B Chem.* **193** 255
27. Ci S, Huang T, Wen Z, Cui S, Mao S, Steeber D A and Chen J 2014 Nickel oxide hollow microsphere for non-enzyme glucose detection *Biosens. Bioelectron.* **54** 251
28. Dong Y, Zhang H, Rahman Z U, Su L, Chen X, Hu J and Chen X 2012 Graphene oxide–Fe_3O_4 magnetic nanocomposites with peroxidase-like activity for colorimetric detection of glucose *Nanoscale* **4** 3969
29. Liang M, Fan K, Pan Y, Jiang H, Wang F, Yang D, Lu D, Feng J, Zhao J, Yang L and Yan X 2013 Fe_3O_4 magnetic nanoparticle peroxidase mimetic-based colorimetric assay for the rapid detection of organophosphorus pesticide and nerve agent *Anal. Chem.* **85** 308
30. Xie J, Cao H, Jiang H, Chen Y, Shi W, Zheng H and Huang Y 2013 Co_3O_4-reduced graphene oxide nanocomposite as an effective peroxidase mimetic and its

application in visual biosensing of glucose *Anal. Chim. Acta* **796** 92
31. Jia H M, Yang D F, Han X N, Cai J H, Liu H Y and He W W 2016 Peroxidase-like activity of the Co_3O_4 nanoparticles used for biodetection and evaluation of antioxidant behavior *Nanoscale* **8** 5938
32. Xiang Z, Wang Y, Ju P and Zhang D 2016 Optical determination of hydrogen peroxide by exploiting the peroxidase-like activity of $AgVO_3$ nanobelts *Microchim. Acta* **183** 457
33. Yousefinejad S, Rasti H, Hajebi M, Kowsari M, Sadravi S and Honarasa F 2017 Design of C-dots/Fe_3O_4 magnetic nanocomposite as an efficient new nanozyme and its application for determination of H_2O_2 in nanomolar level *Sens. Actuat. B Chem.* **247** 691
34. Masoudi A and Honarasa F 2018 C-dots/Fe_3O_4 magnetic nanocomposite as nanoadsorbent for removal of heavy metal cations *J. Iran Chem. Soc.* **15** 1199
35. Zuo X, Peng C, Huang Q, Song S, Wang L, Li D and Fan C 2009 Design of a carbon nanotube/magnetic nanoparticle-based peroxidase-like nanocomplex and its application for highly efficient catalytic oxidation of phenols *Nano Res.* **2** 617
36. Chang Y-C and Chen D-H 2005 Preparation and adsorption properties of monodisperse chitosan-bound Fe_3O_4 magnetic nanoparticles for removal of Cu(II) ions *J. Colloid Interface Sci.* **283** 446
37. Wei H and Wang E 2008 Fe_3O_4 magnetic nanoparticles as peroxidase mimetics and their applications in H_2O_2 and glucose detection use of the novel properties of Fe_3O_4 MNPs as peroxidase *Anal. Chem.* **80** 2250
38. Shi W, Wang Q, Long Y, Cheng Z, Chen S, Zheng H and Huang Y 2011 Carbon nanodots as peroxidase mimetics and their applications to glucose detection *Chem. Commun.* **47** 6695
39. Liu Y, Yuan M, Qiao L and Guo R 2014 An efficient colorimetric biosensor for glucose based on peroxidase-like protein-Fe_3O_4 and glucose oxidase nanocomposites *Biosens. Bioelectron.* **52** 391