MODESTUM
OPEN ACCESS

Eurasian Journal of Analytical Chemistry, 2018, 13(5), em47
ISSN:1306-3057
Research Paper https://doi.org/10.29333/ejac/94451

Correlation Assessment of Zeta Potential and Catalytic Activity of Graphene Nano Sheets as Nanozyme

Maziar Bahreini [1], Monireh Movahedi [1*], Maryam Peyvandi [2], Fereshteh Nematollahi [3], Hessam Sepasi Tehrani [4]

[1] Department of Cellular and Molecular, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, IRAN
[2] Department of Biology, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, IRAN
[3] Department of Chemistry, East Tehran Branch, Islamic Azad University, Tehran, IRAN
[4] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, IRAN

Received 5 March 2018 ▪ Revised 11 July 2018 ▪ Accepted 7 August 2018

ABSTRACT
Knowledge of the zeta potential (ζ) gives an insight to the nanoparticles surfaces and helps predict their stability in the solution. In this work Graphene nano sheets were characterized by Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDS), Dynamic Light Scattering (DLS) and UV-Visible Spectroscopy. enzyme activity of Graphene nano sheets as peroxidase mimic were evaluated under different conditions of H_2O_2 - 3,3′,5,5′-tetramethylbenzidine (TMB). Results showed that zeta potential as function of pH conformed to a 6th order polynomial and Point-of-Zero Charge (PZC) of Graphene Nano sheets was between pH = 3-4 and enzyme activity of Nano Carbons obeyed Michaelis–Menten kinetics. Optimum enzyme activity occurred below the PZC agglomeration range.

Keywords: Nanozyme, zeta potential (ζ), Graphene Nano sheets, point of zero charges, polynomial

INTRODUCTION

The past decade has witnessed the advent of nanotechnology and its link with the biosciences that led to the birth of enzyme mimics nano scale materials which are called nanzoymes. These artificial enzymes can efficiently catalyze the conversion of substrates to products and they usually follow the same kinetics and mechanism of their natural counterparts such as the Ping-Pong mechanism observed in peroxidase mimetic activity. Literature has shown that properties and activity of enzyme mimetic-based nanostructures are often dependent on size and catalytic activity. It could be fine-tuned by structure, dopant, charge, morphology and surface modifications [1]. Inspired by the investigation of intrinsic enzyme-like behavior of nanomaterial, new applications have emerged such as biosensors for glucose detection, environmental treatment, Immunological assay, tumor diagnosis and other therapeutics methods [2,3,4]. Various nanoscale materials were investigated to mimic peroxidase. Among them, peroxidase-mimic graphene and its derivatives can be classified in two main categories. The first one are none functionalized graphene and related derivatives whereas the second group consists of functionalized nanomaterial. Nano Fabrication of graphene derivatives to subtypes such as reduced graphene oxide (rGO) graphene oxide (GO) with Nano structures range have formed. Appreciate opportunities in expanding highly efficient functional Nanozymes with catalytic performances similar to or even better than that of natural enzymes. These advances have led to novel nanozymes that contend with and even surpass the natural ones in terms of catalytic performance [5,6,7] to evaluate peroxidase-like activity of nanozymes in a biochemical reaction, the consumption of H_2O_2 as substrate and the formation of oxidized chromogenic products such as,3,3′,5,5′-tetramethylbenzidine (TMB), 2,2′-azino-*bis*-(3-ethylbenzothiozoline-6-sulfonic acid) (ABTS) and 4-aminoatipyrene (4-AAP) could be followed spectroscopically [8]. As definition, the zeta potential is the potential that exists at the

✉ Maziyar.com@gmail.com ✉ mon_movahedi@yahoo.com **(*Correspondence)** ✉ m_peyvandi@iau-tnb-ac.ir ✉ fnematollahi@yahoo.com ✉ sepasi@srbiau.ac.ir

solution boundary layer and it is used as the criterion electrostatic interaction magnitude between colloidal particles. It is a measure of the colloidal stability of the solution. The difference in the zeta potential values of Nanostructures are obviously caused by different ionic micro environment characteristics such as ionic type and strength as well as pH. Knowledge of the controlling parameters of dispersion state of bio inspired Nano scale materials and their Stability in solution will have significant implications on interpretation of biological response, such as intrinsic enzymatic activity [9].

The aim of this study is the evaluation of correlation between Zeta potential variations and kinetic behavior of Nano Carbon structures as Peroxidase mimic Nanozyme.

EXPERIMENTAL

Preparation of Carbon Nano Structures

Graphene nano sheets powders were prepared from US nanomaterial Co. and samples were dispersed in double distilled water. Carbon Nano sheets stocks of various pH for zeta titration analysis were prepared by 0.5N NaOH and 0.5 N HCl [10].

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS)

Aqueous Solutions of nano carbons were obtained and dried at room temperature. Scanning electron microscopy (SEM, TESCAN Vega 3 Mode) was performed for Morphology and Size analysis. SEM HV was adjusted on 20.0 kV and System was operated by SE detector. EDS analysis was done with 133 eV resolution.

Dynamic Light Scattering (DLS) and Phase Analysis Light Scattering (PALS) Measurements

DLS and PALS were performed on a Malvern Zetasizer Nano ZS system based on He–Ne laser with a wavelength of 632.8 nm. A applied DC bias voltage of across cell was 80 V for the electrophoretic light Scattering (ELS) measurements analysis. Water was used as dispersant and Count Rate and temperature of the system were set at 67.9 kcps and 25^{o} C respectively [11]. The Zeta potential can be evaluated directly using the Smoluchowski equation and DLS provides information for length, Volume and Size distribution, whereas ELS generates the Zeta potential, which provides information on the degree of dispersion. Furthermore Stokes-Einstein equation defines the relationship between the size of a particle and its speed due to Brownian motion. Moreover, DLS also measures the oscillating scattered light through the time due to the Brownian motion of particles [12]. The Zetasizer calculates the zeta potential by determining the Electrophoretic Mobility based on Henry equation (1) via links the zeta potential to the electrophoretic mobility.

$$U_E = \frac{2\varepsilon z f(\kappa a)}{3\eta} \tag{1}$$

Where U_E is the electrophoretic mobility, η is the viscosity, ϵ is the dielectric constant, z is the zeta potential and f (ka) is the Henry function. The Electrophoretic mobility is obtained by performing an electrophoresis procedure on the sample and measuring particles velocity using Laser Doppler Velocimetry (LDV). According to DLS theory a laser beam passes from a sample under electrophoresis, and scattered light from particles with stochastic movements is subject to frequency shifts which led to measure zeta potential and other parameters [13].

Catalytic Assay of Graphene Nano Structures as Peroxidase Mimic

Enzymatic activity assay of the peroxidase mimic was carried out based on TMB-H_2O_2 colorimetric. The data were recorded via the kinetics Mode of Varian Cary 100 Bio Spectrophotometer. Two- electron oxidation of TMB with H_2O_2 in mildly acidified, yields diimine (λ_{max}= 450 nm) as the yellow oxidation product In the first series of the experiments [14], the concentration of TMB was set at 0.8 mM and the H_2O_2 concentration was varied. Whereas in the other, the concentration of H_2O_2 was 10 mM and the TMB concentration was varied. Based on the method proposed by Guo, Y 5µg/ml Graphene Nano Sheets was used as Nanozyme [14].

RESULTS AND DISCUSSION

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS)

Graphene nano sheets has been analyzed and Typical SEM images are shown with various magnifications which shows the planed and stacked structures of Graphene sheets. EDS spectrum can be seen in **Figure 1**. CKα

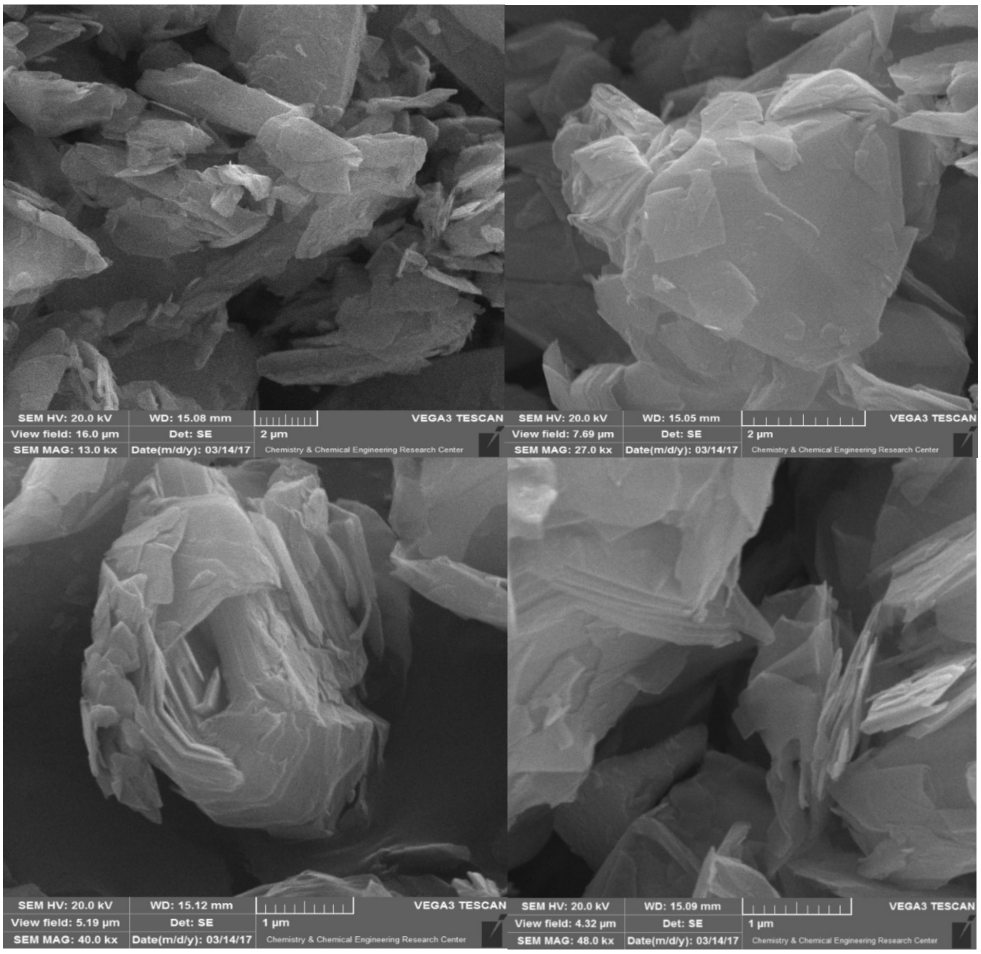


Figure 1. SEM Images of Graphene Nano Sheets with 13.3 kx, 27.0 kx, 40.0 kx and 48kx Magnifications

exited signal belongs to carbon as the major spectal element. Area under the Curve (AUC) indicates the main constituent of the graphene Nano sheets us carbon. Other spectral signals were Ca Lα,O kα, Au Mβ,Au and Mα These belong to residual support catalysts that have been used during synthesis procedure by chemical vapor deposition (CVD).

Dynamic Light Scattering (DLS) Analysis

The Zetasizer Nano system evaluate the rate of the intensity fluctuation and then uses this to calculate intensity variations. The size distribution can be seen in **Figure 3**. This curve represent variations of size versus intensity percentage which were 1780-3091 (d.nm). Moreover Z-score (Size Cumulant), number and volume distributions are shown in **Figure 4** and **Figure 5** respectively. The intensity weighted mean hydrodynamic Dispersity of the ensemble collection of Graphene Nano Structures was estimated to be 2865 (d.nm). The Poly dispersity Index (PDI) was estimated to be 0.262 which indicates acceptable dispersity of the samples. It had indicated that the dispersion surface charge (zeta potential) and consequently the hydrodynamic size of nano structures can be altered by changing the solution pH. The zeta-potential distribution of the Graphene nano sheets can be seen in **Figure 6**, Mean, Area and Width of distribution is displayed in **Table 1**. Zeta potential of samples were estimated to be -13.6mV which was in agreement with prior studies. Furthermore the Zetasizer-Nano™ also measured the electrophoresis mobility using the Doppler Effect. The results can be seen in **Figure 7** and the calculated physical parameters can be found in **Table 2**.

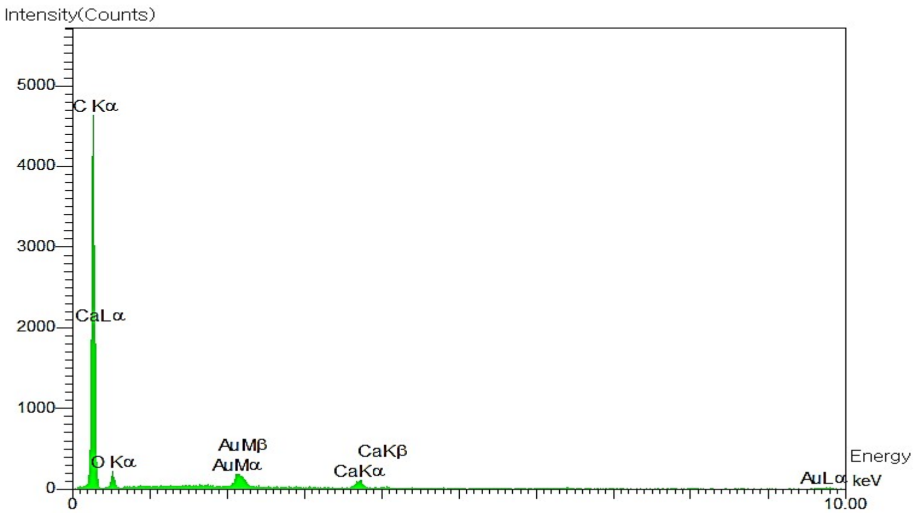


Figure 2. EDS Curve of Graphene Nano Sheets Which Shows C Kα as Major Signal of Spectra Near to 5000 Intenisty

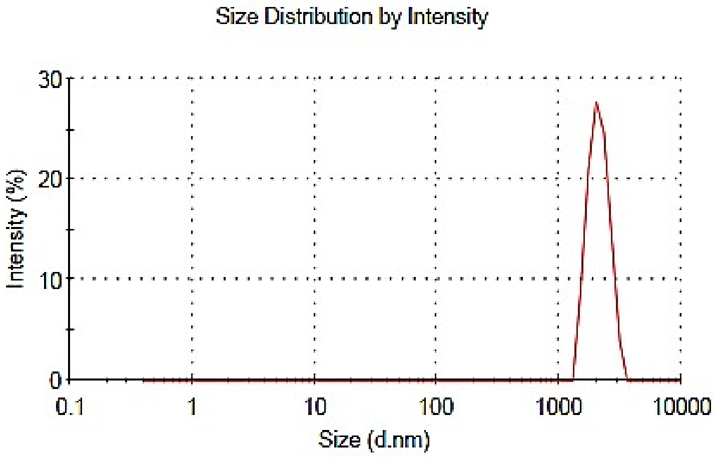


Figure 3. Size Distribution Curve of Graphene Nano Sheets by DLS

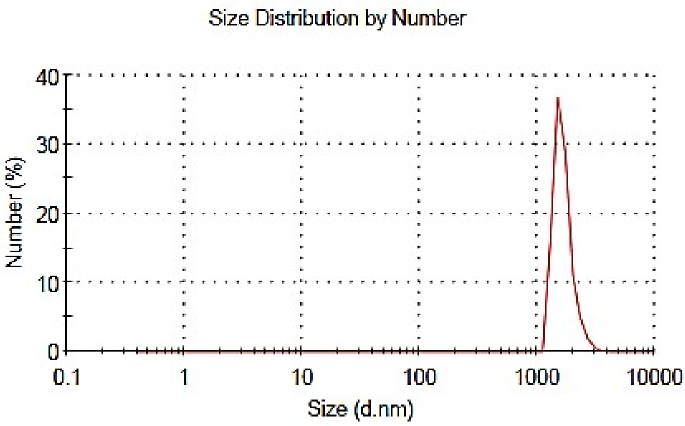


Figure 4. Number Distribution Curve of Graphene Nano Sheets by DLS

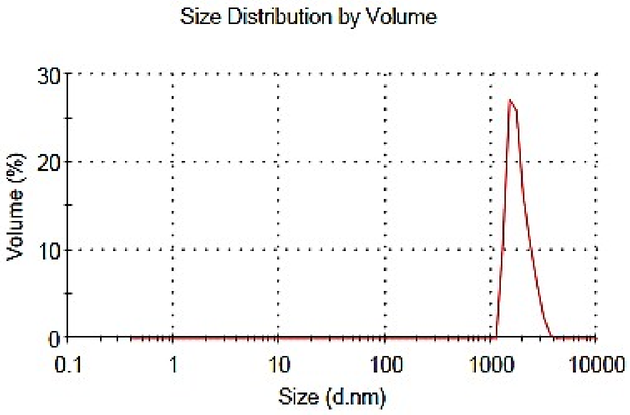


Figure 5. Volume Distribution Curve of Graphene Nano Sheets by DLS

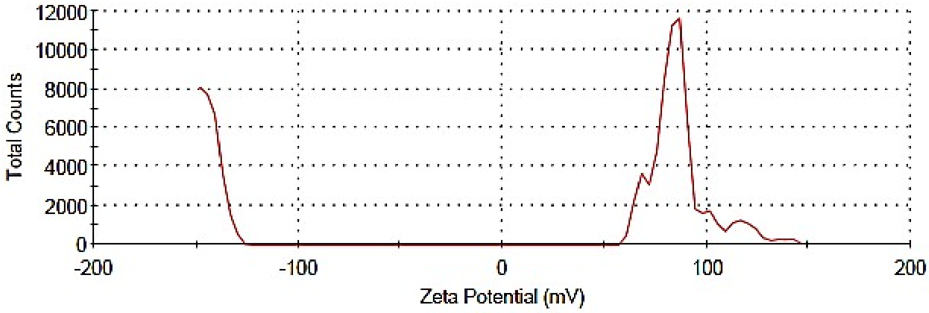


Figure 6. Zeta Potential Curve of Graphene Nano Sheets by DLS

Table 1. Properties of Zeta Potential Curve of the Nano Carbons as Peroxidase Mimic

Conductivity (mS/cm)	ζ-Deviation(mV)	ζ-Potential(mV)	Mean(mv)	Area (%)	Width(mv)
0.0299	116	-13.6	83.7	49.5	6.15
			-143	28.6	4.80
			68	9.5	3.25

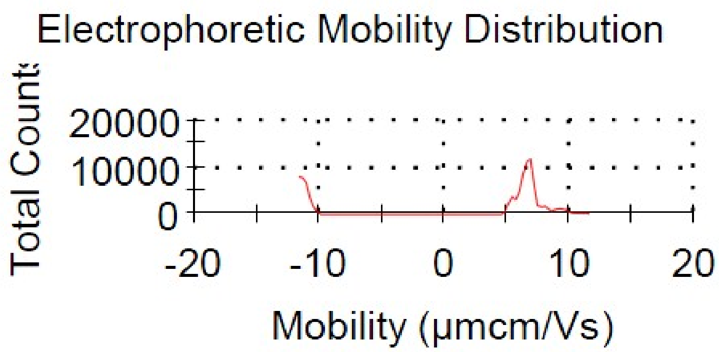


Figure 7. Electrophoretic Mobility Distribution Curve by DLS

Table 2. Physical Parameters of Electrophoretic Mobility

Electrophoretic Mobility Properties				
Mobility (μmcm/Vs)	Mobility SD (μmcm/Vs)	Wall ζ –potential (mV)	Effective Voltage (V)	Conductivity(mS/cm)
-1.067	9.117	-12.0	149	0.0299

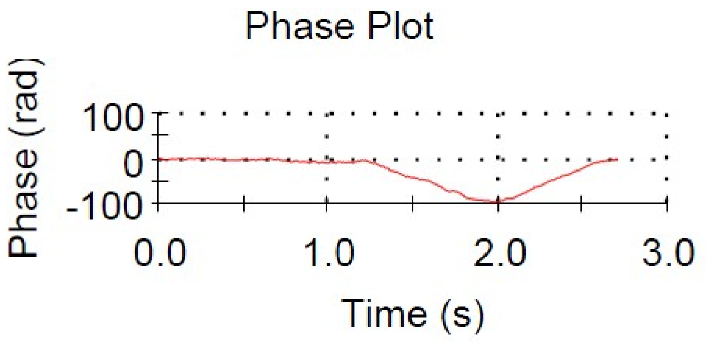


Figure 8. Phase –Time Plot of Assay by DLS

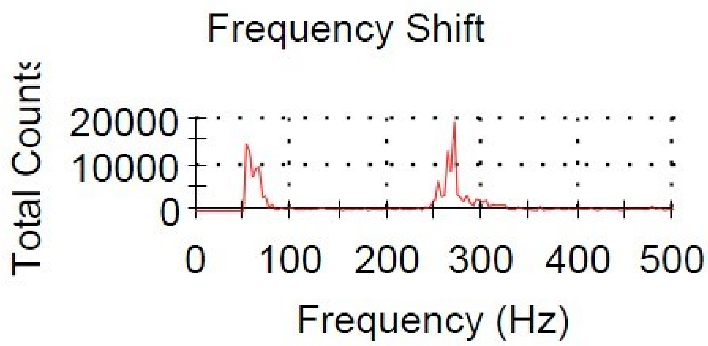


Figure 9. Frequency shift curve by DLS

Phase Analysis Light Scattering (PALS)

The concept of phase is formed by frequency multiplication in time. Phase Analysis Light Scattering (PALS) shows the difference in phase between the measured reference frequency and the beat frequency as a function of time. In other words the measured Phase change is proportional to the mobility of the nanoparticles. Mobility of Nano materials in an applied field produces a frequency shift away from that of the modular frequency. Finally giving an unequivocal measure of Beat frequency and Reference Frequency results in zeta potential value. Evaluation of **Figure 8** as Phase Plot shows significant Signal to Noise Ratio and phase difference between the measured beat frequency and a reference frequency was in range of 1.5 -2.5 s. Frequency shift curve can be seen in **Figure 9** which includes two signals in the (50-100) Hz and (250-300) Hz width range in which changes of frequency (Δf) refers to scattered light of moving of disperse graphene sheets that have passed through the samples undergoing electrophoresis. These were fit with velocity of nanostructures, laser wavelength and scattering angle via trigonometric function. Furthermore **Figure 10** shows the Zeta potential Titration curve which demonstrates the variations of zeta-potential versus pH.

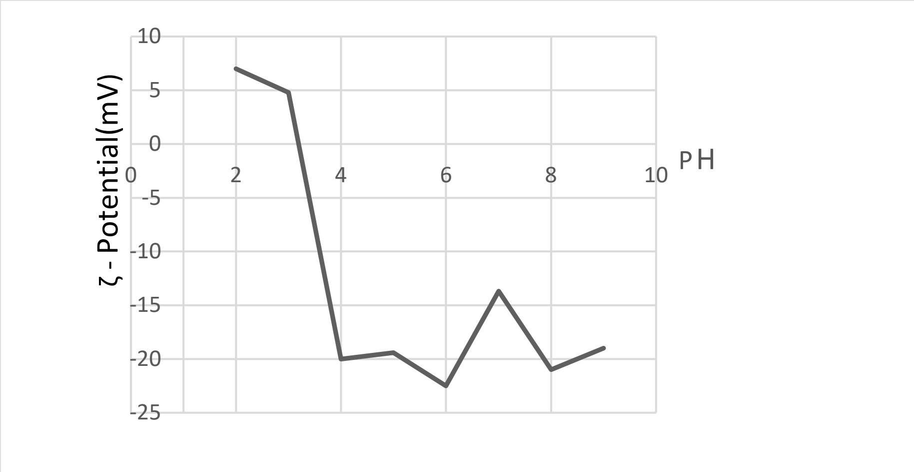
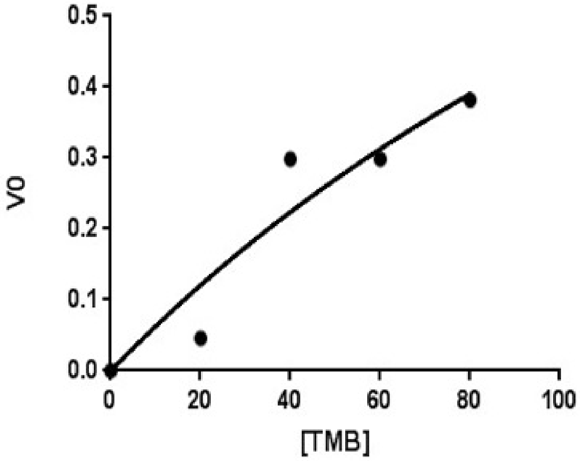


Figure 10. Zeta Potential Titration Curve Shows ZPC Between pH=3-4

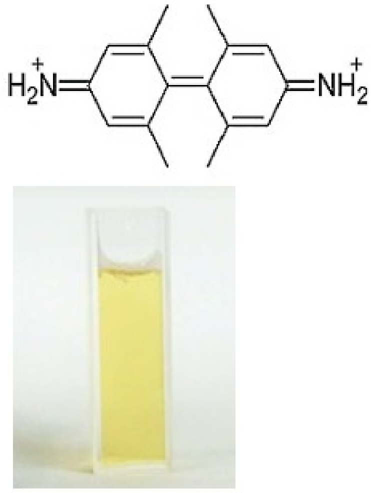
On the Brownian theory of DLS, large particles have slow motions and small particles with fast moving. According to surface chemistry theory, pH is the most important parameter which affects the zeta potential. Note that Zeta's potential regardless of pH, is a nearly meaningless concept. Zeta Potential titration curve have shown that aquatic suspension of carbon nanostructures will be with a negative zeta potential in **Figure 10**. If more alkali is added to the suspension, Nano Carbons will tend to acquire a more negative Charge and when acid was added to the suspension a point would be reached where the negative charge was neutralized and any more addition of acid can cause a built up of positive charge. Therefore a zeta potential versus pH curve will be positive at low pH and lower or negative at high pH. The point where the plot passes through zero zeta potential is called the Zero–point–Charge (ZPC) Where the surface charge density of Nano carbons is very low [15]. This subject is so significant in term of agglomeration behavior and Enzymatic Activity of Carbon Nano structures as Nanozymes. Commonly, the ZPC point is where the nanoscale and Colloidal systems have at least stability and agglomeration was occurred, because of van der Waals force overcomes to electrostatic repulsion. Furthermore surface to volume ratio was diminished. A typical plot of zeta potential versus pH of Nano Carbon suspension is shown in **Figure 10**. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there is no tendency to accumulate. However if the particles have low zeta potential values then there is no force to prevent the particles come together and agglomerate. Also a larger Zeta potential means there have been a better dispersion of particles in the solution. Reported studies have shown that the ζ- potential boundary of the Nano particles stability is between ±30 mV and beyond this range will be unstable [16].

Catalytic Assay of Graphene Nano sheets as Peroxidase Mimic

To demonstrate peroxidase activity of Graphene Nano sheets, catalytic activity was investigated using TMB-H_2O_2 colorimetric system .catalytic activity was studied using TMB-H_2O_2 colorimetric system. Double-reciprocal curves of activity of Graphene nano sheets were generated using a fixed concentration of either of the substrates (H_2O_2 and TMB) and varying the concentration of the second one. Schematic illustration of peroxidase-like activity can be seen in **Figure 11a**, **Figure 11b** and **Figure 12**. So that, Results showed the catalytic activity of Graphene Nano sheets which was based on Steady-state kinetic model and the oxidation of TMB yields the yellow diimine structure as the two- electron oxidation product. kinetic parameters such as Michaelis-Menten constant (K_m) and *kcat* were calculated based on Michaelis–Menten equation and represent in **Table 3** and which indicates low In some reports Formation TMB cation free radical The TMB cation free radical is in equilibrium with a charge-transfer complex, which is led to the yellow color that develops during TMB oxidation. It had previously reported Fluorescein as peroxidase mimic by TMB-H_2O_2 Oxidation system which led to the production of the diimine as yellow two- electron compound [17]. Furthermore peroxidase-like activity of Graphene oxide has been detected for colorimetric detection of Prostate Specific Antigen (PSA) as Cancer biomarker and glucose assay [18]. It has been utilized GO in a TMB-H_2O_2 system for the sensitive voltammetry detection of H_2O_2 [19]. Also it had assayed Nano Carbon based peroxidase by TMB-H_2O_2 colorimetric system with similar results to the present work [20]. Furthermore catalytic activity (optical density as a function of pH) has been evaluated in **Figure 13**. These results imply that most of the catalytic activity of Graphene nano sheets as nanozyme has occurred in around pH = 2 in below of ZPC which has matched with Guo Y and co-workers research results and some prior studies [21,22,23] .



a)



b)

Figure 11. a) Steady-State Curve Of Graphene Nano Sheets Peroxidase Activity in Varied Concentrations of TMB b) TMB-H_2O_2 Oxidation System Led to the Production of the Diimine as Yellow Two- Electron Compound

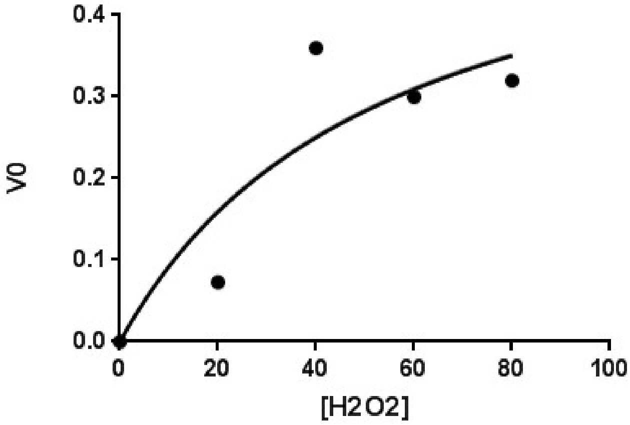


Figure 12. Steady-State Curve of Graphene Nano Sheets Peroxidase Activity in Varied Concentrations of H_2O_2

Table 3. Steady State Kinetic Parameters of Graphene Nano Sheets in Aquatic Solution

Substrate	**V_{max}**	**K_m(mM)**	**k_{cat} (s^{-1})**	**Catalytic Efficiency**
H_2O_2	0.58	0.053	0.116	2.1886
TMB	0.46	0.039	0.092	2.358

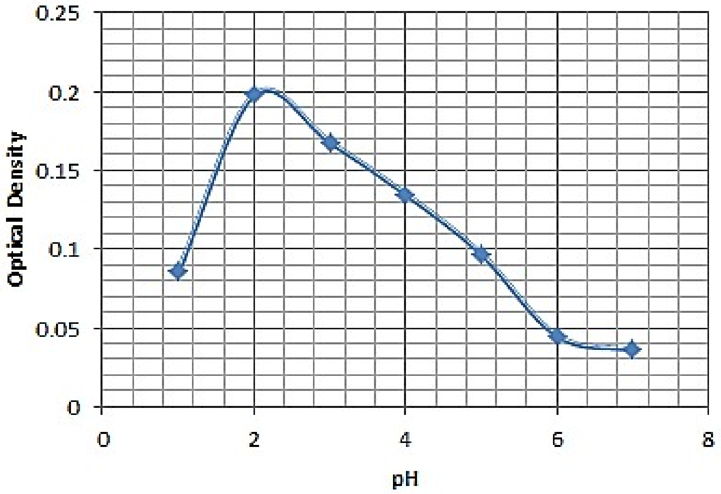


Figure 13. Catalytic activity of Peroxidase Mimic of Graphene Nano Sheets as Function of Optical Density in pH varied Conditions

Results showed that if pH was far from the ZPC, then the value of zeta potential was higher and the electrostatic repulsive force is dominant over the van der Waals force. In this case, agglomeration is suppressed which led to change Surface to Volume (S/V) ratio, decreased hydrodynamic radius and increased peroxidase activity of graphene Nano sheets Structures. Notably, optimum pH for catalytic activity was in acidic range.

CONCLUSION

Catalytic activity of Graphene nano sheets as a function of ζ –potential in various pH was examined and discussed in this study. It was demonstrated that Graphene Nano Sheets as Carbon Based Nanozymes have relatively lower efficiency, specificity and selectivity compared to natural peroxidases. Having lower Limit of Detection (LOD) of substrates. Being catalytic activity and stability in acidic optimum range are susceptibilities of Graphene nanozyme as superiority compared to natural peroxidases. Furthermore, their poor water dispersibility is one of their major weaknesses agents in synthesis and their functionalization is considerable subject which should need to more discuss further. Since the reagents used in their synthesis and functionalization are toxic, there are environmental hazards and human health challenges associated with their application which should be addressed in further researches. Increased thermal and chemical stability, ease of synthesis in terms of cost and labor, however add to their value as biomimetic substitutes of their natural enzymatic counterparts.

ACKNOWLEDGEMENT

We would like to indicate our sincere gratitude to our laboratory colleagues and experts in INST and CCERCI institutions.

REFERENCES

1. Garg B, Bisht T, Ling YC. Graphene-Based Nanomaterials as Efficient Peroxidase Mimetic Catalysts for Biosensing Applications: An Overview. Molecules. 2015 Aug 4;20(8):14155-90. https://doi.org/10.3390/molecules200814155 PMID:26248071
2. Gao L, Zhuang J, Nie L, et al. Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. Nat Nanotechnol. 2007 Sep;2(9):577-83. https://doi.org/10.1038/nnano.2007.260 PMID:18654371
3. Wei H, Wang E. Fe3O4 magnetic nanoparticles as peroxidase mimetics and their applications in H2O2 and glucose detection. Analytical Chemistry. 2008;80:2250–2254. https://doi.org/10.1021/ac702203f PMid:18290671
4. Wei Hui, Wang E. Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes, Journal of chemical society. 2013;reviews, Issue 14.
5. Song Y, Qu K, Zhao C, et al. Graphene oxide: intrinsic peroxidase catalytic activity and its application to glucose detection. Adv Mater. 2010 May 18;22(19):2206-10. https://doi.org/10.1002/adma.200903783 PMID:20564257

6. Liu T, Niu X, Shi L, et al. Electrocatalytic analysis of superoxide anion radical using nitrogen-doped graphene supported Prussian Blue as a biomimetic superoxide dismutase. Electrochimica Acta. 2015;176:1280–1287. https://doi.org/10.1016/j.electacta.2015.07.155
7. Wang X, Guo W, Hu Y, Wu J, Wei H. Nanozymes: Next Wave of Artificial Enzymes. Springer Briefs in Molecular Science. 2016. https://doi.org/10.1007/978-3-662-53068-9
8. Biener J, Wittstock A, Zepeda-Ruiz LA, et al. Surface-chemistry-driven actuation in nonporous gold. Nat Mater. 2009 Jan;8(1):47-51. https://doi.org/10.1038/nmat2335 PMID:19043420
9. Sun HJ, Zhao AD, Gao NL, et al. Deciphering a nanocarbon-based artificial peroxidase: Chemical identification of the catalytically active and substrate-binding sites on graphene quantum dots. 2015;Angewandte Chemie-International Edition. 54:7176–7180
10. Van Thien N, Sufian D, Nurlidia SM, et al. Characterization of Carbon Nanofibers Treated with Thermal Nitrogen as a Catalyst Support Using Point-of-Zero Charge Analysis. Journal of Nanomaterials. 2014, Article ID 631069.
11. Guo Y, Deng L, Li J, et al. Hemin-graphene hybrid nanosheets with intrinsic peroxidase-like activity for label-free colorimetric detection of single-nucleotide polymorphism. ACS Nano. 2011 Feb 22;5(2):1282-1290. https://doi.org/10.1021/nn1029586 PMID:21218851
12. Herman IP. Zeta-Potential Measurements of Surfactant-Wrapped Individual Single-Walled Carbon Nanotubes. J. Phys. Chem. C. 2007;111:13684-13690.
13. De la Cruz E, Zheng Yudong F, Torres E, Li W, Song W, Burugapal K. Zeta Potential of Modified Multi-walled Carbon Nanotubes in Presence of poly (vinyl alcohol) Hydrogel. Int. J. Electrochem. Sci. 2012;7(4):3577–3590.
14. Rhee SG, Woo HA, Kil IS, et al. Peroxiredoxin functions as a peroxidase and a regulator and sensor of local peroxides. J Biol Chem. 2012 Feb 10;287(7):4403-10. https://doi.org/10.1074/jbc.R111.283432 PMID:22147704 PMCID:PMC3281607
15. Zuccaro L, Krieg J, Desideri A, et al. Tuning the isoelectric point of graphene by electrochemical functionalization. Sci Rep. 2015 Jul 2; 5:11794. https://doi.org/10.1038/srep11794 PMID:26134956 PMCID:4488746
16. Jiang J, Oberrdorster G, Biswas P Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies, J Nanopart Res. 2009;11:77–89. https://doi.org/10.1007/s11051-008-9446-4
17. Li L, Ying S, Li Yang Y, et al. Fluorescein as an Artificial Enzyme to Mimc Peroxidase. Journal of chemical Communications. 2016;96.
18. Qu F, Li T, Yang M. Colorimetric platform for visual detection of cancer biomarker based on intrinsic peroxidase activity of graphene oxide. Biosens Bioelectron. 2011 May 15;26(9):3927-3931. https://doi.org/10.1016/j.bios.2011.03.013 PMID:21482098
19. Ya Z, Feifan M, Zhiyong Z, Yuting Z, et al. Low-Cost Nanocarbon-Based Peroxidases from Graphite and Carbon Fibers, Appl. Sci. 2017;7:924. https://doi.org/10.3390/app7090924
20. Sun W, Ju X, Zhang Y, Sun X, Li G, Sun Z. Application of carboxyl functionalized graphene oxide as mimetic peroxidase for sensitive voltammetric detection of H2O2 with 3,3′,5,5′-tetramethylbenzidine. Electrochem. Commun. 2013;26:113–116. https://doi.org/10.1016/j.elecom.2012.09.032
21. Guo Y, Li J, Dong S. Hemin functionalized graphene nanosheets-based dual biosensor, Sensors and Actuators B: Chemical. 2011;160(1):295-300. https://doi.org/10.1016/j.snb.2011.07.050
22. Nam HK, Choi JS, Kwon J. Gold nanozyme-based paper chip for colorimetric detection of mercury ions. Scientific Reports. 2017;7:2806. https://doi.org/10.1038/s41598-017-02948-x
23. White B, Banerjee S, O'Brien S, Turro N, Herman IP. Zeta-Potential Measurements of Surfactant-Wrapped Individual Single-Walled Carbon Nanotubes, J. Phys. Chem. C. 2007;111:13684-13690. https://doi.org/10.1021/jp070853e