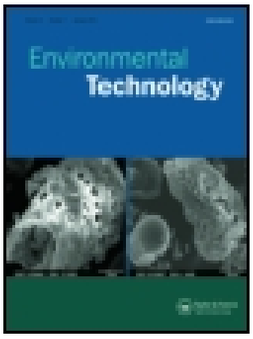

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Title: Carbon quantum dots functionalized agarose gel matrix for in solution detection of nonylphenol

Authors: Sanju Singh[1], Preeti Nigam[2], Aisha Pednekar[1], Souvik Mukherjee[1] and Abhishek Mishra[1]*

[1]Department of Biotechnology, Goa University, Taleigao Plateau, Goa- 403206, India.

[2]Combi Chem Bioresource Centre, Organic Chemistry Division, CSIR- National Chemical Laboratory, Dr. Homi Bhabha Road, Pune- 411008, India

*Corresponding author: Abhishek Mishra E-mail: ab.mish@gmail.com Tel: +91- 832-6519279, Cell: +91-7798025124

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Abstract:

Nonylphenol, an endocrine disrupter, is widely released to the aquatic environment which is accumulative and extremely toxic to aquatic organisms. Here, we report a simple and cost effective large scale green synthesis of water soluble highly fluorescent Carbon quantum dots (CQDs) from marine biowaste (off-shore washed of algae) which gets quenched strongly in presence of nonylphenol. These CQDs were around 20-40 nm in size and exhibit fluorescence constantly at 360nm. XRD profile depicted the interlayer spacing in the particle as 0.284nm with a humped peak around 14 degrees showing the amorphous nature of the CQDs. FT-IR spectra showed the stretching vibration of the hydroxyl groups (OH), asymmetric and symmetric stretching vibrations of the carboxylate anions (COO-). The fluorescence quantum yield of the CQDs is upto 68% and nonyl phenol detection limit is less than 0.1μM. Furthermore, the CQDs were found stable at wide pH range which makes it a suitable nonylphenol sensor for variety of environmental samples. We functionalized these CQDs on a agarose gel matrix to develop a convenient sensor for rapid detection of nonylphenol.

Keywords: carbon quantum dots; nonylphenol; endocrine distrupter; pollutant; marine algae

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Introduction

Carbon Quantum Dots (CQDs) are the new members of the carbon nanoparticles family since their accidental discovery during the purification of single-walled carbon nanotubes through preparative electrophoresis [1]. They form great interest in researchers due to their excellent photo-stability, biocompatibility, low cytotoxicity, outstanding water solubility, high sensitivity and selectivity toward target analytes, tunable fluorescence emission and excitation, high quantum yield and large stokes's shift [2]. The reason for such properties lie in the presence of surface functional groups can be manipulated via a series of controllable chemical treatments and satisfied the demands in the photocatalytic, biochemical and chemical sensing, bioimaging and drug carrier [3]. Several top down approaches such as laser ablation method from graphite powder, electrochemical synthesis, arc discharge from carbon soot have been proposed to produce fluorescent CQDs [4,5]. These methods basically involve a non- selective exfoliation process, and hence may require toxic reagents and special equipment. On the other hand, bottom up approaches like carbonization of glucose, sucrose, glycol, ascorbic acid, citric acids etc. have achieved significant attention for the production of fluorescent CQCs [2,6]. Both these approaches are meticulously documented in recent reviews [6,7,8]. However most of these synthesis methods need several steps, high temperature, strong acids and post treatment with surface passivating agents are essential in order to improve their water solubility and luminescence property. Here, we report one step green synthesis of CQDs from offshore washed off marine algae *Ulva lactuca* by hydrothermal carbonization in an alkaline environment.

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Usually *Ulva* is found washed out on beaches forming a great amount of biomass, imparting high amount of nutrients to soil. Also the rotting algae on the beaches produce toxic gases like hydrogen sulphide leading to harmful effects on environment. CQDs can be used as biosensors to detect the presence of heavy metals like mercury, iron, copper, lead [9]. The CQDs-based biosensors can also be used for visual monitoring of glucose, pH, and nucleic acid [8].

Nonylphenol is produced in large volumes as industrial waste which leads to its widespread release to the aquatic environment which leads to its high detection in high concentration level in sediments, soils, water and sludge [10]. Nonylphenol is persistent in the environment, moderately bioaccumulative and extremely toxic to aquatic organisms. Nonylphenol is a potent endocrine disrupter and can severely affect the developmental stages of fishes and other marine organisms. Despite of its ubiquitous presence in various environments most common method for nonylphenol detection is Soxhlet extraction, solid phase extraction, super critical liquid extraction, capillary/ anion exchange column, reverse phase high performance liquid chromatography or gas chromatography separation followed by mass spectrometry [11, 12, 13, 14, 15, 16, 17]. In this report, we show that CQDs prepared from algal source and its immense potential as nanosensor for nonylphenol detection. These nanosensors will allow simple, cost effective and rapid detection of nonylphenol in aquatic environments without usage of any sophisticated instrument.

Materials and Methods

Materials

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All chemicals were of analytical reagent grade and used without further purification was purchased from Sisco Research Laboratory (SRL). Washed of sample of green marine algae *Ulva lactuca* was sampled in the month of October from the coast of Anjuna Beach, Goa, India. In laboratory the samples were washed with distilled water and stored at 4°C. Alkaline medium for hydrothermal carbonization was prepared in 1M Potassium Hydroxide solution in MilliQ water from Merck Millipore Unit. MilliQ water was used for all other process.

Synthesis of CQDs

In a typical procedure 1g of algae was dissolved in 25 ml of 1M KOH solution, cotton plugged in a conical flask and was kept for constant heating at 90°C in a hot air oven for 12 hours. After the treatment the mixture was cooled down and filtered to remove algae debris and further centrifuged (Mikro200R Hettich zentrifuge and Thermo Scientific Lynx4000) to purify CQDs. The resulted solution was lyophilized to obtain fluorescent CQDs.

Characterization

The UV-Visible absorbance of the CQDs was checked at a wavelength range of 200-450nm using a spectrphotometer (SHIMADZU). The excitation and emission spectra was determination by fluorescence spectrophotometer (Shimadzu RF 5301PC). Transmission Electron microscopy (TEM, Tecnai, T-20, FEI, Netherlands) analysis was performed by casting drop on copper grids and dried at room temperature properly for observation. X-Ray Diffraction was done using Rigatu - Ultima IV, X-Ray Diffractometer. Data was collected at 2θ from ~10º -80º. Fourier Transform Infra-Red spectroscopy (FTIR) was performed using a FTIR spectrometer (400-Shimadzu, IRPrestige-21) with passed KBr pellet at room temperature.

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Sensitivity of CQDs towards nonylphenol and H^+ ions concentrations

CQDs solution was subjected to solution of nonylphenol with a range of concentration from 0 to 10μM. The change in intensity was recorded by fluorescence spectrometer. The effect of Different H^+ and OH^- ion concentrations from 0.01 mM to 10 mM on fluorescence intensity of C-dots was tested. Dilutions were made using analytical grade HCl and NaOH.

Antibacterial activity

Antibacterial activity of the C-dots solution was checked against Gram positive and Gram negative bacteria by Well Diffusion Assay. The zone of inhibition around the well was measured and tabulated.

Quantum Yield Calculation

The Quantum yield of the C-dots was measured by using Tryptophan as the reference standard. For calculation of the Quantum yield five standard solution of tryptophan with increasing concentrations distilled water Blank, 0.02M, 0.04M, 0.06M, 0.08M, 0.1M were taken. The absorbance of the standards was measured at the absorption wavelength of tryptophan (280nm). Absorbance of diluted C-dots solution was also measured at 280 nm wavelength using Uv-Vis Spectrophotometer. A graph between fluorescence intensity and absorbance was plotted and gradient and intercept was calculated. The gradient of the graphs is directly propotional to Quantum yield of different samples. Absolute quantum yield values were calculated according to the following equation using the standard reference samples with fixed and known fluorescence quantum yield values:

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$$\Phi x = \Phi ST\ (mx / mST)\ (\eta 2x / \eta 2ST)$$

Where Φ is the quantum yield, m is slope, η is the refractive index of the solvent, ST is the standard and X is the sample.

Results and Discussion

A facile low temperature one-step alkaline hydrothermal carbonization was employed for the formation of fluorescent CQDs. The fluorescent carbon dots were prepared by carbonization of *Ulva Lactuca* collected from offshores of Anjuna beach, Goa, India. To obtain high-quality CQDs, the synthesis conditions, such as the mass ratio of dried algae to water, hydrothermal temperature and hydrothermal time, were optimized. The UV-Vis spectra for the CQDs were shown in Fig 1(a), which demonstrates that the absorbance maxima obtained for CQD was at 405nm. A strong absorption in the UV region and peak around 400 nm corresponds to non-uniform size distribution CQDs and typically attributed to π -π* transition of C=O and n- π* transition of C=C, respectively [7,18,19]. The fluorescence spectra of CQDs showed a maximum emission at about 435nm when excited at a wavelength of 360nm. However, to further investigate the optical properties, the fluorescence emission spectrum of the CQDs was recorded at progressively increasing excitation wavelengths (Fig 1b). It can be observed that a red shift toward longer wavelength was attributed in the emission spectra of CQDs from 462 to 511 with increasing excitation wavelengths from 360nm to 460nm, accompanied by a decrease of the fluorescence intensity, revealing that the fluorescence of CQD is strongly dependent on the excitation wavelength. Further, more the unique phenomena of excitation dependent

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fluorescence could arise due to differently sized particles quantum effect, emissive traps, aromatic conjugate structures or presence of surface states [8,9,18,20,21]. A high quantum yield was obtained for the CQDs that is 0.68 with reference to tryptophan [22].

FTIR was done to investigate the surface functional groups and bonds present in the assembly of CQDs. The spectrum (Fig 2a) for CQD have a very strong absorption band spectra at 3600 and 1055cm^{-1} are ascribed to the stretching vibration of the hydroxyl groups (-OH). The high-intensity peaks at 1590 and 1400 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of the carboxylate anions (–COO^{-}), respectively. The peaks at 2930 and 860 cm^{-1} can be assigned to the C–H stretching and out-of-plane bending modes. 1628 cm^{-1} peak correspond to C=C bonds. The spectra shows that synthesized CQDs have multiple functional groups (Carboxylic and Hydroxyl) like –COOH, -C=C, -OH,-CH, which can be attributed to degradation of diverse carbohydrates present in algae during hydrothermal treatment. The availability of various functional groups provides excellent solubility in water without any further requirement of surface modification procedures. The presence of hetero-atoms like oxygen creates more defect sites in the sp^{2} carbon clusters, thus further improving the fluorescence property [14]. XRD profiles of CQDs (Fig 2b) shows a wider peak centered at around 14 degrees indicates the amorphous nature of the powder. The particles have an interlayer lattice spacing (d) of 0.284 nm which is less than that of graphite (0.34 nm). The presence of oxygen containing groups in CQDs is possibly responsible for enhanced interlayer distance and amorphous nature. TEM images revealed spherical shape CQDs in range of 20-40nm (Fig 2c). The crystallite size of the particle was found to be 32.38nm with lattice strain of 0.0041 calculated using Scherrer's formula. These results are in concordance with TEM images.

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However, the size of CQDs is larger than the earlier reported structures from various other starting materials. This may be due to lower temperature, 90°C, used in present study which is significantly lower than the previous studies by other groups. In addition, this method takes longer time for synthesis which implies to a kinetically slower process.

To study the effect of PH, we prepared blank, 0.001mM, 0.01 mM, 0.1 mM, 1 mM, 10mM concentration of HCl and NaOH separately in pure water. 10µl of CQDs in aqueous solution was added to the HCl and NaOH solutions followed by fluorescence measurement. Data was analyzed in terms of percent relative fluorescence compared to control sample. CQDs were found to be insensitive towards the change in H^+/ OH^- ion concentration (Fig.3a and 3b). Furthermore, on the basis of fluorescence quenching behavior of CQDs a series of cationic, anionic and organic molecules were tested in aqueous solution. Prepared CQDs showed no quenching effect with lead, cadmium, fluoride or phenol. In addition to cations and anions, potential endocrine disrupters like nonylphenol, benzene hexachloride and phthalate were tested for change in fluorescence of CQDs. Benzene hexachloride and phthalate showed no quenching activity with control CQDs. However, upon addition of nonylphenol, the fluorescence intensity of the prepared CQDs decreased significantly (Fig 3c). The reason of quenching could be several interactions with the quencher molecule like electron transfer, collisional quenching, excited state reaction and ground state complex formation [23]. On the basis of record change in the fluorescence of the CQDs by nonylphenol, we believed that they may serve as a potential sensor for the molecule. Sensitivity of the nonylphenol sensing was recorded for the quenching of CQD, with the limit of detection 0.1 µM and a linear range from 0.01 to 1.2µM. The minimum concentration which could significantly decrease the fluorescence intensity is 0.1µM (reduction

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by 20%). Addition of 1μM nonylphenol to CQDs decreased the relative fluorescence intensity by more than 2 log units (Fig. 4a). The linear decrease in log fluorescence intensity of CQDs was proportional to the increasing nonylphenol concentration. These results indicate that CQDs lay the potential to be used as biosensors for the detection of nonylphenol with a sensitivity of 0.1μM (30ng/l). We compared various currently used methods for detection of nonylphenol with our CQDs based method with respect to sample preparation, instrument used, detection limit and sensitivity (Table 1). CQDs based detection nonylphenol appears to be simple, rapid and economical method with reliable sensitivity. However, its sensitivity and detection limit is below par to highly sophisticated method based on ESI-LC-IT-TOF/MS [16]. Nonylphenol is a threat to environment and human health as it is non-biodegradable. According to WHO report 15 mg/Kg/day accumulation of nonylphenol may have adverse effect on human health. These CQDs could serve as a potential facial probe for the detection of the nonylphenol levels in environmental samples. As our results demonstrate a consistent photoluminescence in wide pH range these CQDs may be developed as suitable sensor for diverse environmental samples.

For rapid on-site visual detection of trace of nonylphenol in solution an agarose based matrix has been developed. Functional compatibility of synthesized CQDs was checked on the agarose gel as solid matrix. The agarose gel functionalized with CQDs was immersed in different nonylphenol concentrations to verify its effects on fluorescent intensity. As shown in Fig. 4b, with increasing nonylphenol concentrations (0μM, 1μM, 3μM, 5μM and 10 μM), the fluorescence emission intensity decreased gradually, when the agarose based sensor was observed under a 365 nm UV lamp, which is consistent with the fluorescence emission spectra. In concordance to the results of in solution studies the gel soaked into nonylphenol showed

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marked quenching of fluorescence at different concentartion (Fig. 4b). These results indicate that the portable agarose nanosensor is highly efficient for the rapid and convenient visual detection of nonylphenol in aqueous solution.

The zone of inhibition was tabulated in Table 2. C-dots were found to show antibacterial effect on model gram positive, gram negative and acid fast stained bacterial species. The bioactivities of CQDs are mainly instrumental to the metal composites which have bactericidal properties. Whereas in present study, these CQDs were capable of inhibiting the growth of the test cultures without metal conjugation or dopping which indicate the presence of core bioactive structure/s created from the metabolites of seaweed.

Conclusions

In summary, a novel nanosensor for the *in solution* detection of nonylphenol was developed by one step synthesis of facile, water soluble, highly fluorescent, photostable and polydispersive quantum dots. We have prepared these CQDs at a relatively low temperature of 90°C from offshore washed-off marine algal biomass. Interestingly, these unique structures of CQDs show strong quenching in presence of nonylphenol and can detect upto 1μM concentration in solution. Furthermore, the portable agarose based CQD nanosensor was developed to detect nonylphenol traces in solution. Overall, this work promises an effective method to develop sensitive and portable nanosensor for the visual detection of nonylphenol in different environmental samples. In addition, this approach can be utilized for a variety of applications in sensing a series of pollutants in aquatic environments.

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Conflict of interest

Authors declare that they have no conflict of interest.

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Figure Legends

Fig1 (a) Absorbance and emission spectra of CQDs. Inset shows the photographs of the CQDs in aqueous solutions and under the irradiation of a 365 nm UV light lamp **(b)** Emission spectra showing progressive increase in excitation wavelength and its effect on photoluminescence intensity.

Fig2 (a) FTIR **(b)** XRD and **(c)** TEM of prepared CQDs

Fig3 Influence of the **(a)** HCl **(b)** NaOH (c) cationic, anionic and organic molecules concentration on the fluorescence intensity of fluorescent CQDs

Fig4 (a) linear relationship between log fluorescence intensity of CQDs and nonylphenol concentration (b) nonylphenol concentration in carbon quantum dots agarose gel matrix on UV transilluminator.

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Table. 1

Method	Sample preparation	Instrument requirement	Sensitivity	Detection limit	Reference
Solid phase extraction followed by GC-MS	Cumbersome	Sophisticated	High	2µg/L	12,13
Ultrasonic extraction and fluorescence based detection by HPLC detection	Cumbersome	Sophisticated	Moderate	2ng/g	14,15
ESI-LC-IT-TOF/MS	Cumbersome	Highly Sophisticated	Very high	0.1ng/L	16
Anaerobic /oxic and Biological Aerated Filter (BAF)	Moderate	Advanced	Moderate	10ng/L	17
CQD based detector	Easy	Simple	High	0.1µM	Present study

Table. 2

Bacterial species	Zone of Inhibition (mm)
Mycobacterium smegmatis	10
Escherichia coli	15
Proteus mirabilis	16
Staphylococcus aureus	13
Micrococcus Luteus	11

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Bacillus subtilis	13
Klebsiella pneumoniae	13
Streptococcus pyogenes	16
Pseudomonas aeruginosa	11

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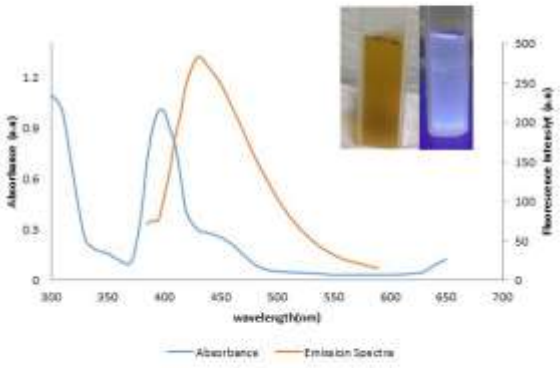

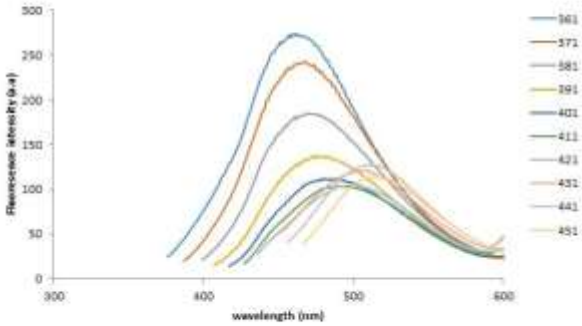

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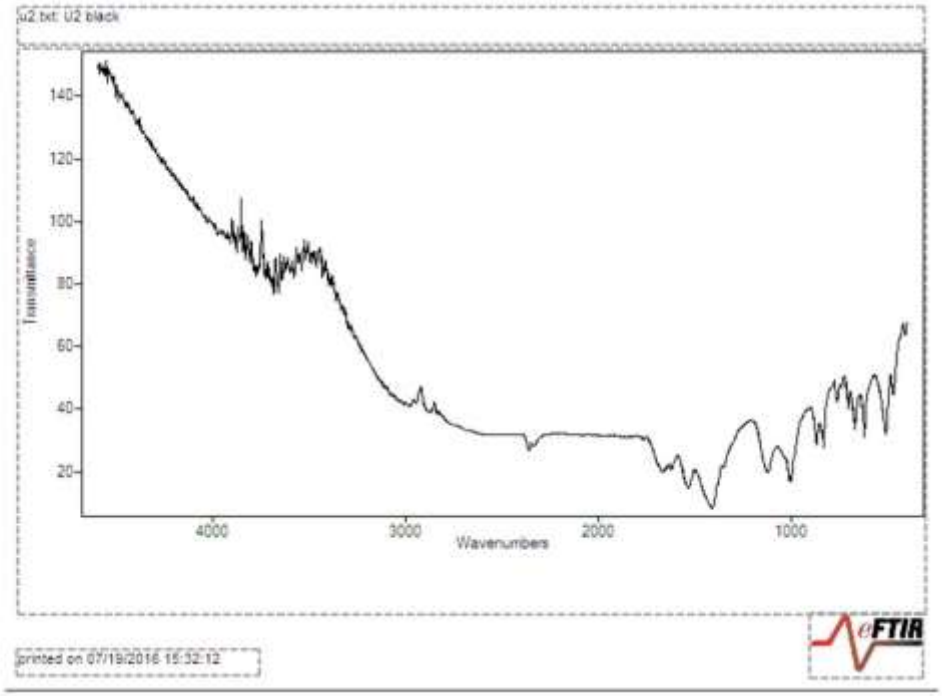

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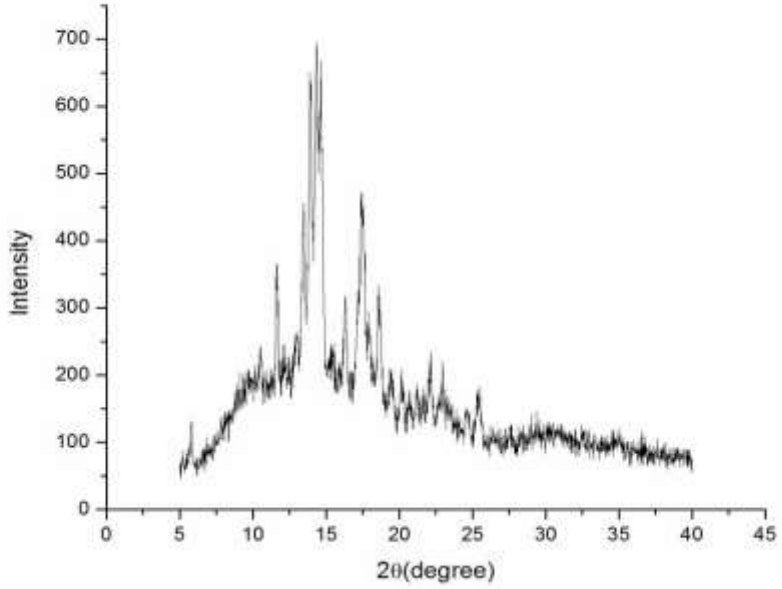

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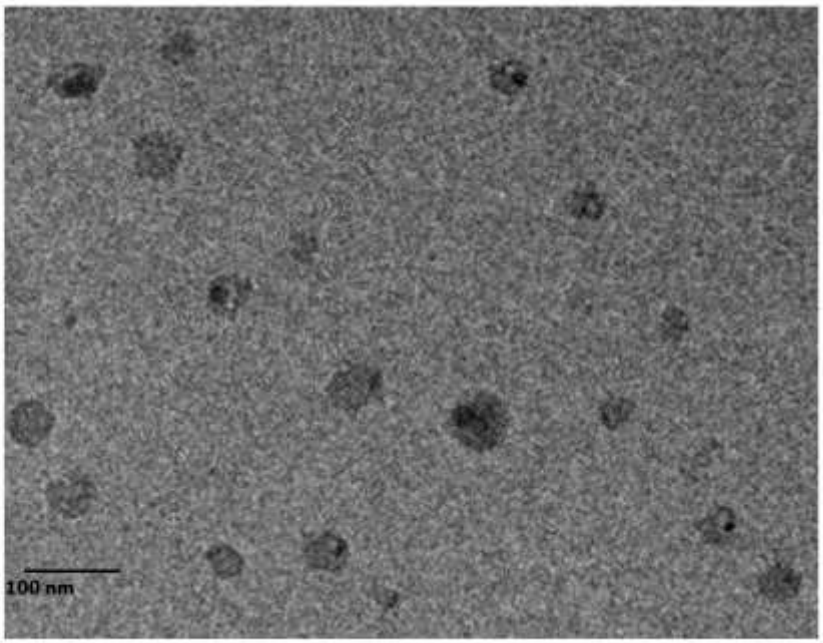

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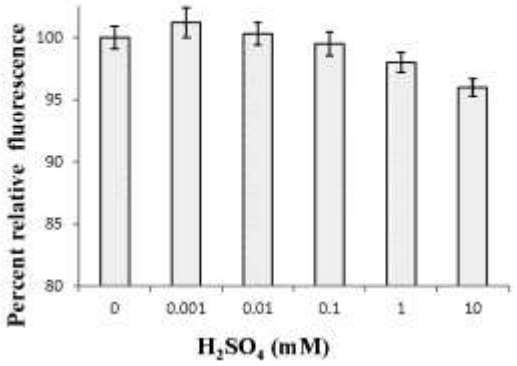
Fig 3(a)



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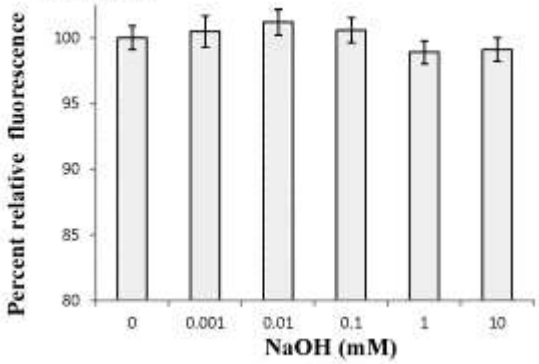

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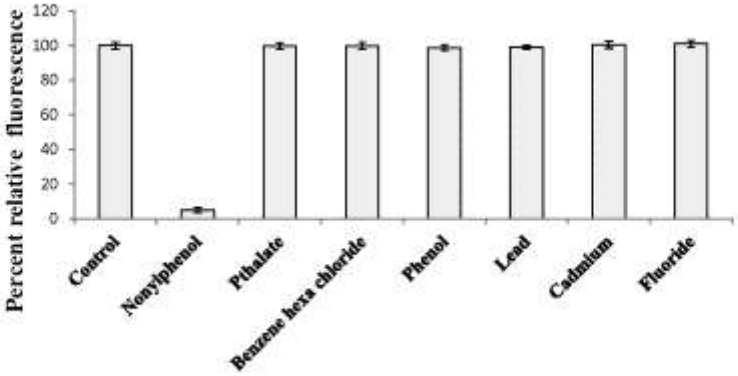

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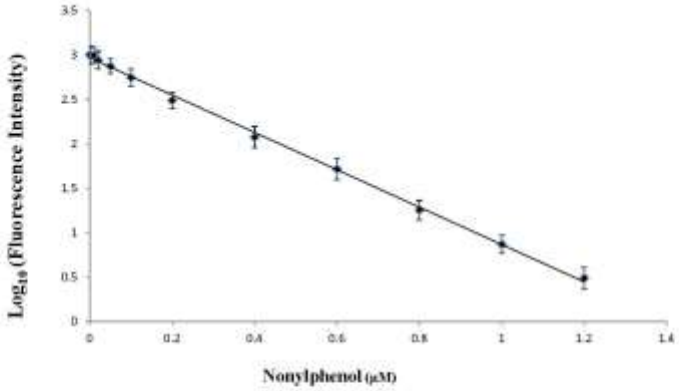
Fig 4 (a)



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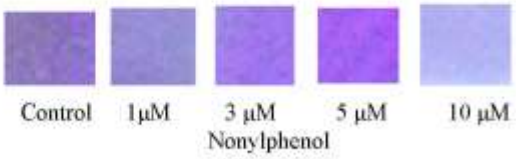

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