HIGHLY-LUMINESCENT CARBON NANOPARTICLES AS SENSORS FOR MONITORING OF HEAVY METALS

Polina Mladenova, Alexandre Loukanov, Anatoliy Angelov

Laboratory of Engineering NanoBiotechnology, Department of Eng. Geoecology, University of Mining and Geology "St. Ivan Rilski" – Sofia, Bulgaria

ABSTRACT. Here we develope highly-luminescent carbon nanoparticles as sensor for monitoring of heavy metals in aqueous solutions in microscopic scale objects. The sensor systems possess selectivity and sensitivity towards the detection of some biologically important metal ions as Fe^{3+}, Zn^{2+}, Ni^{2+} and etc. To achieve this goal the nanoparticles were synthesized by microwave assisted pyrolysis. Their quantum yield is over 50 % and with blue photoluminescence peak at 450 nm wavelength. The nanoparticles at fixed concentration were tested on various soluble metal ions. For first time the observed chemically induced fluorescence was detected by fluorescence microscope and CCD camera. Thus it is enables to measure the generated signal in microscopic objects by software ImageJ. The results revealed that the surface of carbon nanoparticles exhibit high sensor affinity to pH of sample solution and some dissolved ions.

Keywords: carbon nanoparticles, sensor systems, fluorescence imaging

ВИСОКО ЛУМИНИСЦЕНТНИ ВЪГЛЕРОДНИ НАНОЧАСТИЦИ КАТО БИОСЕНЗОРИ ЗА МОНИТОРИНГ НА ТЕЖКИ МЕТАЛИ

Полина Младенова, Александър Луканов, Анатолий Ангелов

Лаборатория инж. нанобиотехнология, Катедра „Инженерна геоекология", Минно-геоложки университет „Св.Иван Рилски" – София, България

РЕЗЮМЕ. В този доклад са разработени високо луминисцентни въглеродни наночастици, като сензори за мониторинг на тежки метали във водни разтвори в микроскопични по размер обекти. Сензорните системи притежават селективност и чувствителност при дететкцията на някои биологично важни метални йони, като Fe^{3+}, Zn^{2+}, Ni^{2+} и др. За да се постигне тази цел наночастиците бяха синтезирани чрез микровълнова пиролиза. Техният квантов добив е над 50 % и пика на тяхната синя луминисценция е с дължина на вълната 450 nm. Наночастиците при фиксирана концентрация бяха тествани с различни разтворими метални йони. За първи път наблюдаваната химическа индуцирана флуоресценция беше детектирана чрез флуоресцентен микроскоп и CCD камера. По този начин се дава възможност за измерване на генерирания сигнал в микроскопични обекти чрез софтуера ImageJ. Резултатите показват, че повърхността на наночастиците проявява висок сензорен афинитет към pH на пробата и някои разтворени йони

Ключови думи: въглеродни наночастици, сензорни системи, флуоресцентно изобразяване

Introduction

Among several methods for biosensing of heavy metals, fluorescence is a broadly used characterization technique, because of its characteristics such as high sensitivity, simple and fast response. Fluorescence is a form of luminescence which is the light emitted by a substance that absorbed lighjt or electromagnetic radiation. The observed fluorescence of the studied carbon nanomaterial is a form of electronic phenomenon of molecules which contain π-electrons. When the molecules having conjugated π-electrons absorb light radiation, the π-electrons firstly jump from the ground state to gigher energy state. To be capable of fluorescing, the majority of electrons go from the higher vibrational levels to the lowest one and are ready for radiative emission back to the ground state which resulting the emitted fluorescence. The photoluminescence of the C-dots may be the result of the optical selection of various nanoparticle size and the emissive traps on the C-dots surface.

in the past few years the carbon nanodots, so called C-dots a kind of novel nanomaterial, that are inspiring increasing attention among researchers. C-dots are defined as a form of nanoparticles with features of discrete, quasi-spherical and size usually below 10 nm. C-dots will be one of the ideal eco-friendly nanosensors due to their unique advantage of low cytotoxicity, thus imparting them with favorable property of biocompatibility. Microwave route has been demonstrated to be a green and effective synthesis route for C-dots production and highly studied in the past few years [1,7]. In addition to the excellent fluorescent activity, the as-prepared carbon nanodots are also envisioned to be of great sensitivity and selectivity in tracking and detectiong some metal ions in water [6,8]. In this study, we established the C-dots fluorescence based nanosensor, which sense metal ions [2-5,9] in fixed plant cells. Carboxylate and amine functionalized C-dots are acted as fluorescence probes for detection of iron ions.

Experimental Procedures

Synthesis of carbon nanodots by microwave assisted pyrolysis

Citric acid (1 g) was diluted with 10 ml distilled water and ethylenediamine (0.2 ml, 0.18 g) was injected to the solution under vigorous stirring. The clear transperant solution mixture became a yellowish brown gum after microwave irradiation for 3 minutes at microwave oven (750 W), as shown on Fig. 1. The carbonization proceeded very fast and no inorganic salt or acid was needed. When cooled down to room temperature, the obtained yellowish brown solid was dissolved in Milli-Q and dialyzed against pure water through a dialysis membrane (MWCO of 100 - 500 Da) for 3 days [5]. Finally, 50 ml red-brown aqueous solution containing both, reaction precursor and C-dots was obtained and the nanoparticles were purified by dissolving in acetone (water : aceetone = 2 : 13) and centrifuged (2500 rpm, 10 min). Finally, the obtained precipitate pellets were collected and vacuum-dried at room temperature.

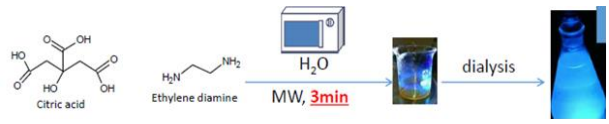

Fig. 1. Synthesis of carbon nanodots by microwave assisted pyrolisys

Preparation of cell culture

Tobacoo cells were cultured in Murashige-Skoog (MS) medium on rotated 110 rpm by rotary shaker. MS medium contain various ions and vitamins, as shown on Table 1:

Table 1.
Components of MS medium

Micro element	**Amount (mg/L)**
$CoCl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.025
FeNaEDTA	36.70
H_3BO_3	6.20
KI	0.83
$MnSO_4.H_2O$	16.90
$Na_2MoO_4.2H_2O$	0.25
$ZnSO_4.7H_2O$	8.60
Macro elements	
$CaCl_2$	332.00
KH_2PO_4	170.00
KNO_3	1900.00
$MgSO_4$	180.54
NH_4NO_3	1650.00
Vitamins	
Glycine	2.00
Myo-Inositol	100.00
Nicotinic acid	0.50
Pyridoxine HCl	0.50
Thiamine HCl	0.10

Staining protocol

First, the cells were incubated for 4 days in MS culture medium. After that the obtained suspension was centrifuged and washed twice with MS medium. Second, such prepared cells were incubated with each C-dots (20 mg/ml) at ambient temperature for several hours. Finally, the labeled cells were fixed glutaraldehyde and washed with Milli-Q to remove the excess of C-dots.

Future, the prepared cells were teated with HEPES buffer solution, which contains iron ions with different concentrations. Fluorescent and transmission images were takken by light optical microscope BX53 (Olympus).

Result and discussion

Optical characterization of carbon nanodots

The absorbance and photoluminescence spectra of C-dots are shown on Fig. 2.

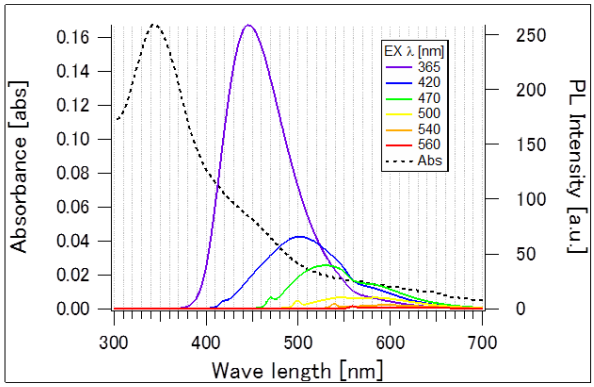

Fig. 2. Absorbance and photoluminescence specta of bare C-dots, prepared by microwave assisted pyrolysis

On the absorbance spectrum there is one main peak at 350 nm with a tail extended to 500 nm. This broad peak is attributed to n $\rightarrow\pi^*$ transition, which correspond to the carbonyl / amine functional groups on the nanoparticle surface. It might shift to longer wavelength depending on the pH of the solution. Fluorescence spectra of C-dots were recorded at different excitation wavelength and the resultant fluorescence spectra obviously represent that C-dots have multi-emission nature and depending on the excitation wavelengths. Upon C-dots excitation varying from 320 to 450 nm, the emitted fluorescence maximum was red shifted from 420 to 520 nm and higher fluorescence intensity was observed at 340 nm excitation [2].

Detection of metal ions

The photoluminescence intensity of the C-dots was significantly quenched when HEPES buffer with Fe^{3+} is applied. The intensity was found to decrease with increasing concentration of Fe^{3+} ions, but peaking at the same position even in the presence of highest iron concentration. The observed fluorescence quenching of the nanoparticles may be due to non-radiative electron transfer from the excited state of the C-dots to the d-orbital of Fe^{3+} ion, as it is shown on Fig. 3. The quenching rate constant reveals that the high efficiency of quenching process is in the excited state and it suggesting that the Fe^{3+} ions plausibly coordinate with -COOH groups on the carbon nanodot surface. When the concentration keeps increasing, the relation Stern-Volmer (S-V plot) begins to deviate from linearity, indicating rhat the observed quenching process may be due to both dynamic and static process.

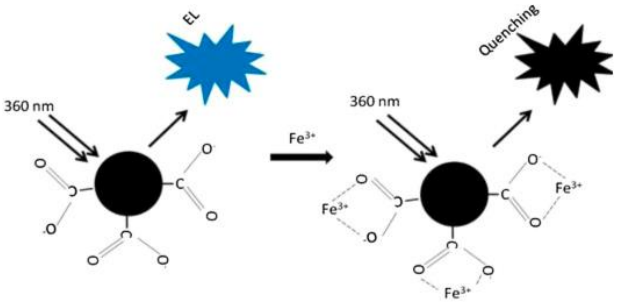

Fig. 3. Schematic representation of the quenching of nanoparticle photoluminescence and the sensing process of metal ions with C-dots

Biosensoring of iron (III) ions in plant cells

On figures 4 and 5 are shown fluorescence microscopic images of labeled with C-dots plant cells.

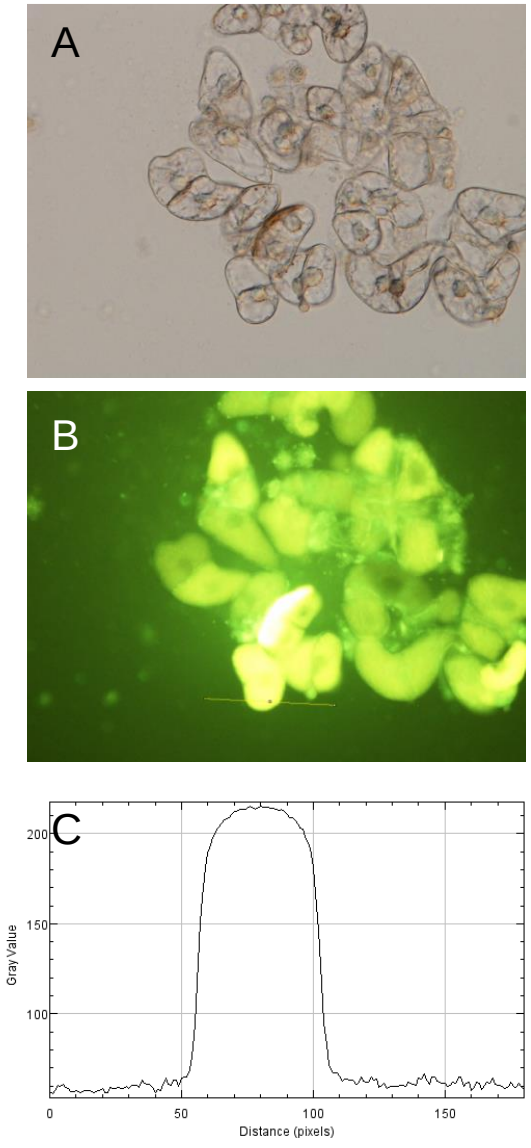

Fig. 4. Control experiment: labeled plant cells with carbon nanodots, fixed and washed with Milli-Q. (A) light microscopic image, (B) fluorescence microscopic image and (C) plot profile of the microscopic image

The cells are first labeled with carbon nanodots and after that fixed with 2 % solution of glutaraldehyde. In the control experiment the labeled cells are future washed with ultra pure water. As it is shown on fig. 4 they have very high photoluminescence under irradiation in light microscope. As it is shown on fig. 4C the plot profil of the image possess very high peak (over 200 units). It correspond to the high quantum yield of the nanoparticles. Nevertheless, if the labeled cells are treated with solution contains iron (III) ions in buffer solution with concentration 10 mg/L there is a strong quenching of the photoluminescence, as it is shown on fig. 5. The intensity of the profil plot on fig. 5C is significantly lower (around 50 units) in comparison with the control experiment. The reason for this quenching effect is the interaction between carboxyl groups on nanoparticle surface with the iron ions and the followed formation of complex compounds as it was shown on fig. 3. The decreasing of the photoluminescence in the labeled cells is linearly depended of the iron concentration. This is an evidence for the biosensing properties of carbon nanodots for detection of heavy metals within individual microorganism.

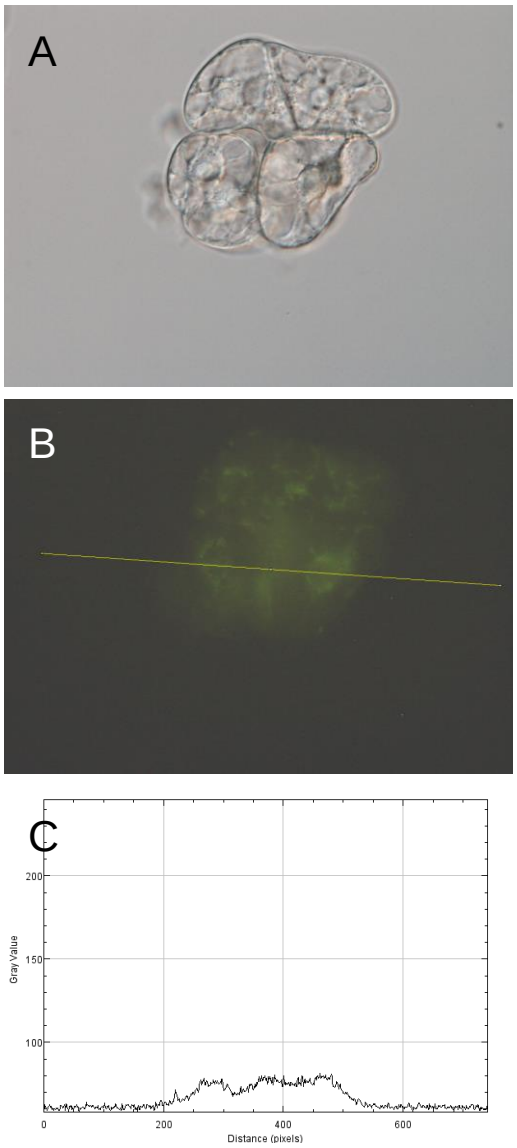

Fig.5. Biosensing experiment: labeled plant cells with carbon nanodots, fixed and treated with iron (III) ions in buffer solution. (A) light microscopic image, (B) fluorescence microscopic image and (C) plot profile of the microscopic image

Conclusion

In this report we developed carbon nanodots as biosensors for detection of heavy metals. Their quantum yield values are linearly depended from the concentration of the iron (III) ions in HEPES buffer. Due to this reason the concentration of Fe^{3+} in individual cells can be detected by the correlation between the obtained fluorescence image and its plot profile produced by the software ImageJ.

Acknowledgment
The authors are thankful to the laboratory of Nakabayashi and Yoshikawa, Saitama University - Japan for the providing of the plant cells used in this project.

Refferences

[1] Baker SN, Baker GA, Luminescent carbon nanodots: emergent nanolights, *Angew Chem Int Ed Engl*. 2010 Sep 10;49(38):6726-44

[2] Clarkson T.W., Magos L., The toxicology of mercury and its chemical, Crit. Rev. Toxicol., 36 (2006), pp. 609–662

[3] Glebov E.M., Pozdnyakov I.P., Grivin V.P., Plyusnin V.F., Zhang X., Wu F., Intermediates in photochemistry of Fe(III) complexes with carboxylic acids in aqueous solutions, *Photochem. Photobiol. Sci.*, 10 (2011), pp. 425–430

[4] Loukanov Alexandre, Sekiya Ryota, Yoshikawa Midori, Kobayashi Naritaka, Moriyasu Yuji, Nakabayashi Seiichiro, Photosensitizer-Conjugated Ultrasmall Carbon Nanodots as Multifunctional Fluorescent Probes for Bioimaging

[5] Lin Y., Tseng W.L., Ultrasensitive sensing of Hg2+ and CH3Hg+ based on the fluorescence quenching of lysozyme type VI-stabilized gold nanoclusters, *Analytical Chemistry*, 82 (2010), pp. 9194–9200

[6] Roy Prathik, Chen Po-Cheng, Periasamy Arun Prakash, Chen Ya-Na and Chang Huan-Tsung, Photolum inescent carbon nanodots: synthesis, physicochemical properties and analytical applications

[7] Shoujun Zhu, Qingnan Meng, Lei Wang, Junhu Zhang, Yubin Song, H an Jin, Kai Zhang, Hongchen Sun, Haiyu Wang, and Bai Yang, Highly Photoluminescent Carbon Dots for Multicolor Patterning, Sensors, and Bioimaging

[8] Xu Wu,a Fei Tian, Wenxue Wang, Jiao Chen, Min Wu, and Julia Xiaojun Zhaoa, Fabrication of highly fluorescent graphene quantum dots using L-glutamic acid for in vitro/in vivo imaging and sensing

[9] Zhi Yang, Zhaohui Li, Minghan Xu, Yujie Ma, Jing Zhang, Yanjie Su, Feng Gao, Hao Wei∗, Liying Zhang, Controllable Synthesis of Fluorescent Carbon Dots and Their Detection Application as Nanoprobes

The article has been recommended for publication by department “Engineering geoecology”.