# Neuroactive carbon dots: perspective neurotheranostic agents and harmful environmental pollutant

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## ABSTRACT

Carbon dots are a newly discovered class of fluorescent carbon nanosized particles. We revealed recently the neuroactive properties of carbon dots synthesized from β-alanine. We investigate whether or not this effect is inherent to β-alanine-derived carbon dots only? Herein, we found that carbon dots synthesized from different starting materials possessed unique stable spectroscopic features, and also neuroactive properties. Combination of fluorescent and neuroactive features of carbon dots (and their nanocomposites) makes them useful for visualization of key transport mechanisms and pathways in the nerve terminals in neurotheranostics. In contrarily, uncontrolled presence of carbon-containing substances in the air and human environment poses a toxicity risk to the central nervous system.

***Keywords***: carbon dots, glutamate, GABA, nerve terminals

## 1 INTRODUCTION

Carbon dots are a newly discovered class of fluorescent carbon nanosized particles that can be photoluminescent (fluorescent) [1]. This means that being illuminated by UV or visible light, they can emit light of different color, usually within the blue-green range of spectrum [2]. This gave the researchers simple and efficient tools to study their behavior in different biological systems using very sensitive analytical tools, such as flow cytometry and cell imaging [3].

The results obtained in different laboratories have shown that such carbon nanoparticles can be made in an easy way by burning any organic materials, such as grass, fruits, paper, etc. [4]. On laboratory scale they can be synthesized from carbohydrates, organic acids and amino acids by heating under special conditions ("green chemistry"). Their use was suggested as the labels and contrast agents and also as the components of nanocomposites possessing the ability of multimodal imaging and drug delivery [5].

Beside their potential use in nanomedicine and nanotechnology, carbon dots may serve a model in studying the toxic effects of carbon-containing particles producing industrial pollution of the environment. Such particles appear in coal mining industry and in different coal-consuming technologies, on operation of diesel engines and in other areas of human activities. Their essential source is forest fires. Being of very small size (1-100 nm) they can form aerosols that can be spread by wind to great distances [6]. In the form of aerosols, possessing highly adhesive surface, they can uptake different molecules from the surrounding so that their surface can become charged and oxidized. Since they become water soluble, they can contaminate water and interfere into ecosystems. Different pathways can exist for their entry into human body, such as uptake by the mucosa of the airways from polluted air, through skin, and in the gastrointestinal system through contaminated food [7]. The recently accumulating evidences suggest that the carbon particles of outdoor air pollution may produce a significant impact on the central nervous system of mammals. This was underlined by the National Institute of Environmental Health Sciences/NIH panel of research scientists [8]. Translocation of inhaled ultrafine particles to the brain was also shown [9].

Despite the presence of carbon nanoparticles in human environment and a great potential of their use in nanotechnologies, their physiological effects are almost unknown. Reported results on model experiments with cultured cells and small animals do not allow providing definite estimates of their safety or toxicity. If the toxicity is concerned, it is not clear, what will be the targets for inducing the changes on the level of biochemistry. Impact of these issues increases in modern society, and the proper level of their understanding is highly needed.

Recently, we revealed neuroactive properties of carbon dots synthesized from β-alanine [10], and it was unclear whether this effect is inherent to these carbon dots only or other types of carbon dots also possess this property. Therefore, the aim of this study was to analyze spectroscopic and neuroactive properties of carbon dots synthesized from different starting materials.

## 2 METHODS AND MATHERIALS

### 2.1 Synthesis of carbon dots

Carbon dots for biological experiments were synthesized as described in [10].

### 2.2 Biological experiments. Ethics statement

Wistar rats (males, 100-120 g of body weight) were kept in animal facilities of the Palladin Institute of Biochemistry NAS of Ukraine, housed in a quiet, temperature-controlled room (22–23°C), and provided with water and dry food pellets *ad libitum*. Rats were decapitated before brain removing. All experimental procedures were conducted according to the Helsinki Declaration “Scientific Requirements and Research Protocols” and “Research Ethics Committees”. The Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol from 19/09-2014) approved the experimental protocols.

### 2.3 Isolation of nerve terminals (synaptosomes) from rat brain

The synaptosomes were prepared by differential and Ficoll-400 density gradient centrifugation of the homogenate according to [11].

### 2.4 Measurements of L-[$^{14}$C]glutamate uptake by the synaptosomes

The uptake of L-[$^{14}$C]glutamate by the synaptosomes was measured as described in [12, 13].

### 2.5 Measurements of [$^{3}$H]GABA uptake by the synaptosomes

The uptake of [$^{3}$H]GABA by the synaptosomes was measured as described in [14].

### 2.6 Assessment of the ambient level of L-[$^{14}$C]glutamate and [$^{3}$H]GABA in the preparations of synaptosomes

The ambient level of L-[$^{14}$C]glutamate and [$^{3}$H]GABA in the preparations of synaptosomes was assessed according to [15,16].

### 2.7 Measurement of the synaptosomal plasma membrane potential and synaptic vesicle acidification

The measurements of $E_m$ and synaptic vesicle acidification were performed according to [17].

### 2.8 Statistical analysis

The results were expressed as mean ± S.E.M. of *n* independent experiments. Statistical difference within and between groups was verified by One-way Analysis of Variance (ANOVA). The differences were considered significant, when $P \leq 0.05$.

## 3 RESULTS AND DISCUSSION

Carbon dots are strongly fluorescent, non-blinking, and their emission color can be tuned by varying the excitation wavelength, which justifies their use in biological sensing and imaging. Our own experiments were started with the aim to understand the mechanisms of formation and fluorescence emission of these new nanomaterials [18]. We selected the "green chemistry" route for their synthesis using as precursors the well-defined organic compounds with or without different minor additives. As the starting materials, β-alanine, glutamate, γ-aminobutyric acid, glycine, citric acid, urea, and thiourea were used. The method of synthesis was the one-step hydrothermal treatment that can be easily realized in laboratory conditions. The obtained optical parameters are shown in Table 1.

| Precursor/source | Synthesis conditions | Fluorescent properties, max excitation/emission nm |
|---|---|---|
| β-alanine | Microwave heating 600W, 1.5 min | 355/410 |
| Glutamate | 190°C, 60 min | 360/445 |
| γ-aminobutyric acid (GABA) | 190°C, 60 min | 345/375 |
| Glycine | 190°C, 60 min | 365/440 |
| Citric acid +urea | 190°C, 90 min | 405/530 |
| Citric acid +thiourea | 190°C, 90 min | 365/445 |

Table 1. General information on synthesis conditions and fluorescence properties of carbon dots.

Being synthesized from different types of carbon-containing precursors, carbon dots possess core formed of inorganic carbon and expose nitrogen, sulfur and oxygen atoms at their surface in different composition. The surface formed by polar chemical groups allows their solubility in water and other polar solvents. It was found that in all these cases the carbon dots possess distinguished and stable spectroscopic properties. Despite this variety of fluorescent properties, they exhibit similar effects on interacting with live objects such as cell lines [19]. According to our results supported by literature data, carbon dots are strongly fluorescent, and their emission color can be tuned by varying the excitation wavelength. Other researchers suggested their use in optoelectronics as the substitutes of expensive rare earth nanocrystals [20]. Their great potential for use in nanomedicine was recognized, and many suggestions appeared in the literature for their application not only as contrast agents but also as gene and drug carriers into the cells [21].

Possessing the possibility of recording the fluorescent characteristics of carbon dots, our study focused on comparison of their neuroactive properties. Assessment of neuroactivity was conducted based on their effects on glutamatergic and GABA neurotransmission in isolated rat brain nerve terminals, as recommended US EPA, 1998 in the Guidelines for Neurotoxicity Risk Assessment. It was observed that the majority of synthesized carbon dots in the concentration range from 0.5 to 1.0 mg/ml attenuated the initial rate of $Na^+$-dependent transporter-mediated uptake and the accumulation of L-[$^{14}$C]glutamate and [$^3$H]GABA by the nerve terminals in a dose-dependent manner and increased the ambient level of these neurotransmitters.

Starting from concentration of 0.2 mg/ml, the majority of synthesized carbon dots evoked partial gradual dose-dependent depolarization of the plasma membrane of the nerve terminals measured with cationic potentiometric dye rhodamine 6G (Fig.1). With this study we demonstrated that the carbon dots produce the influence on cell membranes and they are able to change the membrane permeability.

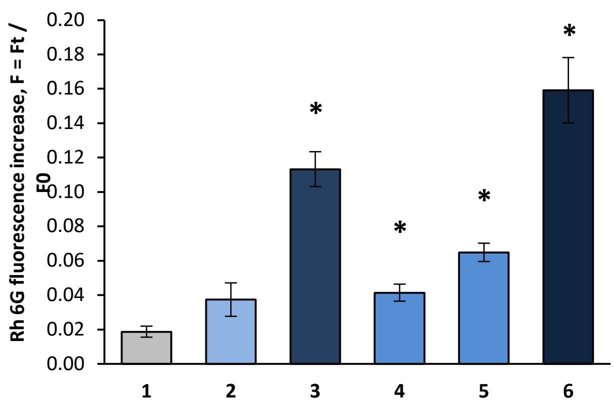


**Fig.1** An increase in the rhodamine 6G fluorescence signal in the synaptosomes in the control after the application of the aliquots of the standard saline solution (**1**) and in response to the application of carbon dots (1.0 mg/ml) synthesized from β-alanine (**2**), glutamate (**3**), GABA (**4**), glycine (**5**) and citric acid and thiourea (**6**). The suspension of synaptosomes was equilibrated with rhodamine 6G (0.5 μM); when the steady level of the dye fluorescence had been reached, the carbon dots were added to synaptosomes. Data is mean ± SEM. *, $p<0.05$ as compared to the column #**1**.

Within the concentration range of 0.1 - 0.5 mg/ml, most synthesized carbon dots caused an "unphysiological" effect. An increased fluorescence intensity of the pH-sensitive dye acridine orange was observed in the synaptosomal preparations (Fig.2). The neuroactive effects of the carbon dots made from different materials with different surface properties and fluorescent features are analogous. However, they are displayed on a different level of efficiency.

The presence of abandoned carbon-containing particles in the aerosol particulate matter and in the food chain poses a toxicity risk for the central nervous system, especially in the case of their increased amount in natural disasters. Fine particles of outdoor air pollution may produce a significant impact on the functioning of central nervous system [8].

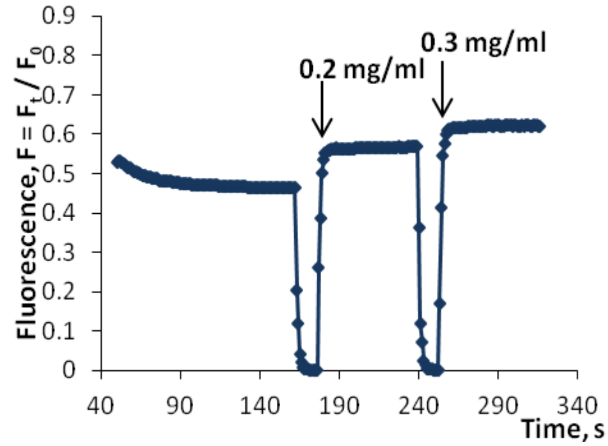


**Fig. 2** Acidification of the synaptosomes in the presence of carbon dots synthesized from citric acid and thiourea. The nerve terminals were equilibrated with acridine orange (5 μM); when the steady level of the dye fluorescence had been reached, the carbon dots (arrows) were added. Trace represents six experiments performed with different preparations.

Also, air pollution-mediated changes in the organism, e.g. the cardiovascular, pulmonary and immune systems, simultaneously may alter the brain functioning due to the production of circulating proinflammatory mediators [8]. Novel literature data revealed that the magnetite nanoparticles can be found in the human brain, and exposure to such airborne particulate matter-derived magnetite nanoparticles might need to be examined as a possible hazard to human health [22]. In a mammalian organism, the nanoparticles can be efficiently uptake by the mucosa cells in nasal, tracheobronchial, and alveolar regions and then transported along sensory axons of the olfactory nerve to the central nervous system [9,23]. Intra-nasally installed nanoparticles can target the central nervous system and circumvent the blood-brain barrier [9]. The titanium oxide nanoparticles were found in the brain of exposed mice [24]. Chronic particle inhalation can trigger the release of soluble mediators into circulation and lead to adverse health effects [25]. Epidemiological and clinical studies showed that Alzheimer's and Parkinson's disease, high blood pressure and stroke are presumably associated with ambient air pollution [26].

Therefore, our data on neuroactive features of carbon dots are not only in line with recent literature data but also can explain observations of neurotoxicity of carbon-containing aerosol particulates.

## 4 CONCLUSIONS

The results of the present study demonstrate that despite the difference in surface properties and fluorescent features of carbon dots made from different materials, their neuroactive effects are mainly similar, however displayed on a different level of efficiency.

On the one side, combination of fluorescent and neuroactive features of carbon dots (and of their nanocomposites) makes them useful for visualization of key transport mechanisms and pathways in the nerve terminals, that is elementary for neurotheranostic applications. On the other side, the uncontrolled presence of carbon-containing substances in the air is potential risk of toxicity for the central nervous system. These effects may be enhanced during natural disasters with increased concentration of carbon dots in the air.

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## REFERENCES

[1]Y.-P. Sun, B. Zhou, Y. Lin, W. et al. Quantum-Sized Carbon Dots for Bright and Colorful Photoluminescence, J. Am. Chem. Soc. 128 (2006) 7756–7757.
[2]A.P. Demchenko, M.O. Dekaliuk, Novel fluorescent carbonic nanomaterials for sensing and imaging, Methods Appl. Fluoresc. 1 (2013) 42001.
[3]G. Shen, M. Hu, J. Qi, Progress of Luminescent Carbon Dots in Biomedicine Engineering, Nano Biomed Eng Rev. Nano Biomed. Eng. Nano Biomed. Eng. 8 (2016) 190–202.
[4]J.C.G. Esteves da Silva, H.M.R. Gonçalves, Analytical and bioanalytical applications of carbon dots, TrAC Trends Anal. Chem. 30 (2011) 1327–1336..
[5]N. Durán, M.B. Simões, A.C.M. de Moraes, et al., Nanobiotechnology of Carbon Dots: A Review, J. Biomed. Nanotechnol. 12 (2016) 1323–1347.
[6]D.A. Lack, H. Moosmüller, G.R. McMeeking, R.K. Chakrabarty, D. Baumgardner, Characterizing elemental, equivalent black, and refractory black carbon aerosol particles: a review of techniques, their limitations and uncertainties., Anal. Bioanal. Chem. 406 (2014) 99–122.
[7]M. Pacurari, K. Lowe, P. Tchounwou, R. Kafoury, A Review on the Respiratory System Toxicity of Carbon Nanoparticles, Int. J. Environ. Res. Public Health. 13 (2016) 325.
[8]M.L. Block, A. Elder, R.L. Auten, S.D. et al. The outdoor air pollution and brain health workshop., Neurotoxicology. 33 (2012) 972–84.
[9]G. Oberdörster, Z. Sharp, V. Atudorei, et al., Translocation of Inhaled Ultrafine Particles to the Brain, Inhal. Toxicol. 16 (2004) 437–445.
[10]T. Borisova, A. Nazarova, M. Dekaliuk, et al., Neuromodulatory properties of fluorescent carbon dots: effect on exocytotic release, uptake and ambient level of glutamate and GABA in brain nerve terminals., Int. J. Biochem. Cell Biol. 59 (2015) 203–15.
[11]T.A. Borisova, N.H. Himmelreich, Centrifuge-induced hypergravity: [3H]GABA and l-[14C]glutamate uptake, exocytosis and efflux mediated by high-affinity, sodium-dependent transporters, Adv. Sp. Res. 36 (2005) 1340–1345.
[12]T. Borisova, Cholesterol and Presynaptic Glutamate Transport in the Brain, Springer Science & Business Media, New York, NY, 2013. doi:10.1007/978-1-4614-7759-4.
[13]O. Soldatkin, A. Nazarova, N. Krisanova, et al., Monitoring of the velocity of high-affinity glutamate uptake by isolated brain nerve terminals using amperometric glutamate biosensor., Talanta. 135 (2015) 67–74.
[14]N. Pozdnyakova, M. Dudarenko, L. Yatsenko, et al., Perinatal hypoxia: different effects of the inhibitors of GABA transporters GAT1 and GAT3 on the initial velocity of [3H]GABA uptake by cortical, hippocampal, and thalamic nerve terminals., Croat. Med. J. 55 (2014) 250–8.
[15]T. Borisova, Permanent dynamic transporter-mediated turnover of glutamate across the plasma membrane of presynaptic nerve terminals: arguments in favor and against., Rev. Neurosci. 27 (2016) 71–81.
[16]T. Borisova, A. Borysov, Putative duality of presynaptic events., Rev. Neurosci. 27 (2016) 377–83.
[17]T. Borisova, The neurotoxic effects of heavy metals: Alterations in acidification of synaptic vesicles and glutamate transport in brain nerve terminals, Horizons Neurosci. Res. 14 (2014) 89–112.
[18]M.O. Dekaliuk, O. Viagin, Y. V. Malyukin, A.P. Demchenko, Fluorescent carbon nanomaterials: "quantum dots" or nanoclusters?, Phys. Chem. Chem. Phys. 16 (2014) 16075.
[19]M. Dekaliuk, K. Pyrshev, A. Demchenko, Visualization and detection of live and apoptotic cells with fluorescent carbon nanoparticles, J. Nanobiotechnology. 13 (2015) 86.
[20]F. Yuan, S. Li, Z. Fan, X. Meng, L. Fan, S. Yang, Shining carbon dots: Synthesis and biomedical and optoelectronic applications, Nano Today. 11 (2016) 565–586.
[21]X.T. Zheng, A. Ananthanarayanan, K.Q. Luo, P. Chen, Glowing Graphene Quantum Dots and Carbon Dots: Properties, Syntheses, and Biological Applications, Small. 11 (2015) 1620–1636. doi:10.1002/smll.201402648.
[22]B.A. Maher, I.A.M. Ahmed, V. Karloukovski, et al., Calderon-Garciduenas, Magnetite pollution nanoparticles in the human brain, Proc. Natl. Acad. Sci. 113 (2016) 10797–10801.
[23]Y.-Y. Kao, T.-J. Cheng, D.-M. Yang, et al., Demonstration of an olfactory bulb-brain translocation pathway for ZnO nanoparticles in rodent cells in vitro and in vivo., J. Mol. Neurosci. 48 (2012) 464–71.
[24]K. Takeda, K. Suzuki, A. Ishihara, et al., Nanoparticles Transferred from Pregnant Mice to Their Offspring Can Damage the Genital and Cranial Nerve Systems, J. Heal. Sci. 55 (2009) 95–102.
[25]W.G. Kreyling, M. Semmler-Behnke, S. Takenaka, W. Möller, Differences in the biokinetics of inhaled nano- versus micrometer-sized particles., Acc. Chem. Res. 46 (2013) 714–22.
[26] S. Genc, Z. Zadeoglulari, S.H. Fuss, K. Genc, The adverse effects of air pollution on the nervous system., J. Toxicol. 2012 (2012) 782462.