

# Carbon NanoDots for Selective Optical Cation Detection

Raluca Buiculescu, Maria Fouskaki, Nikos A. Chaniotakis

Department of Chemistry, University of Crete, Iraklion, 70013 Crete, GREECE,

E-mail: [raluca@chemistry.uoc.gr](mailto:raluca@chemistry.uoc.gr)

## ABSTRACT

In the present work, custom designed and synthesized carbon nanodots (C-dots) with cation selective functional organic ligands (carboxyl- or amino-functionalized C-dots) are used for the optical detection of metal ions. These C-dots are highly uniform spherical shaped with a narrow size distribution, and high fluorescent efficiency. The ligands on the surface are able to provide the capping for the C-dots, while at the same time interact selectively with positively charged ions. Controlled experiments with carboxylated and aminated C-dots suggest that the fluorescence of these materials is highly dependent on the electrical field generated on their surface from the selective interaction of the charge ions with the functional sites of the C-dots. This charge can be developed selectively using different functionalities with different binding constants to monovalent and divalent metal ions. This allows for the selective optical detection using functionalized C-dots.

**Keywords:** carbon dots, selective recognition, pH, cation, fluorescence

## 1 INTRODUCTION

The continuous development of novel nanostructures provides the scientific community with new possibilities for ultrasensitive, direct, selective and *in-vivo* detection schemes. Quantum nanostructures have played a decisive role in the development of novel highly sensitive detection technologies during the last decades. Ten years ago, a new class of carbon based materials, namely carbon nanodots (C-dots), has been discovered [1-4]. They are fascinating carbon structures which possess high fluorescence that is size and capping dependent [1,5-8] and it is expected that it can also be influenced by the surrounding environment. This can be resolved by grafting chemical recognition functionalities to their surface. In this case, the peripheral functionalities could be custom tailored for selective chemical recognition.

Grafting specific chemically sensitive functionalities onto these materials can be achieved either by covalent binding to surface functionalities or by embedding them within the C-dots during synthesis [9-10]. Controlling the chemical recognition characteristics of the functionalities will determine the observed chemical selectivity, while influencing the solvent compatibility and solubility. Such functionalized C-dots can provide the scientific community with a new and highly promising optical recognition

platform and can be envisaged as novel probes and transducers in a wide area of applications. Due to their biocompatibility, they are emerging as viable alternatives to current nano- and quantum materials such as semiconductor quantum dots, gold and silver nanocrystals, fullerenes, carbon nanotubes and nanofibers [11-14]. For this reason C-dot technology holds a great potential for *in vivo* bioanalysis and bio-sensing. For these applications to be realized, relating the physicochemical activity of the functional surface agents to the optical properties of the C-dots is of paramount importance.

In this work we describe the use of C-dots as selective cation probes. C-dots with H<sup>+</sup> and divalent metal ion sensitive moieties are interrogated for their ion recognition capabilities and set the grounds for the future applications of C-dots as fluorescent probes in chemical sensors and biosensors.

## 2 EXPERIMENTAL

### 2.1 C-dots synthesis

C-dots were prepared according to literature reports [9], by microwave assisted pyrolysis of glycerol in a Speedwave (Berghof) microwave oven, at 70% power. In more detail, the mixture containing glycerol, phosphate buffer and the capping agent (either 4,7,10-trioxa-1,13-tridecanediamine (TTDDA) or ethylenediamine) at a ratio of 5:2:1, was subjected to pyrolysis for 7 min at 110 °C and the obtained solutions of carbon nanodots were cleaned by dialysis on 3500 MWCO membranes (Fisher Scientific). This procedure is expected to result in C-dots with amino functionalities on their surface. Carboxylated C-dots were also obtained by microwave assisted pyrolysis of glycerol in the presence of phosphate buffer, at a ration of 5:2, and purified by dialysis on a 3500 MWCO membrane.

### 2.2 C-dots characterization

High-resolution transmission electron microscopy (HR-TEM) images of carbon nano-dots were obtained using a JEOL JEM-2100 Electron Microscope, operating at 80 kV. The emission quantum yield of the C-dots was determined from the analysis of the integrated emission intensities of the dots using anthracene as fluorescence standard (quantum yield 0.27 [15]). The ATR-FT-IR spectra were recorded on a Thermo-Electron Nicolet 6700 FT-IR optical spectrometer with a DTGS KBr detector at a resolution of 4 cm<sup>-1</sup>.

## 2.3 Fluorescence measurements

Fluorescence spectra have been recorded on an Aminco-Bowman series 2 fluorescence spectrometer equipped with a continuous high power xenon lamp. All samples were analyzed at room temperature with excitation and emission slits set at 4 nm band-pass and a scan rate of 5 nm/s.

For the assay, C-dots were mixed with solutions of different and precisely controlled pH values in the range from 2.46 to 10.73 and left to incubate for 15 min before recording their fluorescence intensities. The pH solutions were prepared by adding various amounts of KOH (1.0 M) or HCl (1.0 M) to a MES-buffered solution (1.0 mM, pH 4.50).

For the metal selectivity assay, an appropriate amount of the C-dots solution was mixed with MES buffered solutions (pH 10.0) and solutions of different metal concentrations, in the range from  $10^{-8}$  to  $10^{-1}$  M, and left to incubate for 15 min. before recording their fluorescence.

## 3 RESULTS AND DISCUSSION

The common capping functionalities employed for the protection of the C-dots are based on amine moieties [1-4]. Considering the fact that amines can be reversibly charged when the pH changes, there is a large charge build up generated on the surface of the C-dots. It is thus important to examine the effect of this potential on the observed C-dot fluorescence, and relate it to direct optical chemical detection. For this reason, C-dots decorated with carboxyl (carboxylated C-dots) and amine groups (ethylene diamine and TTDDA) were synthesized and examined as cation optodes (Figure 1).

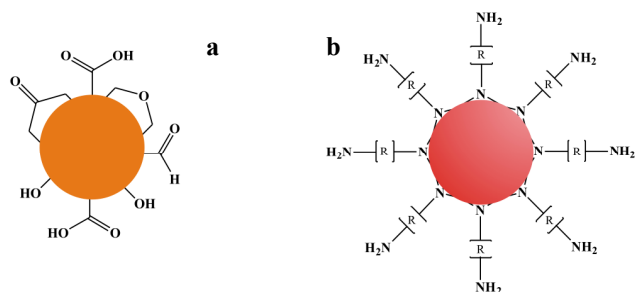


Figure 1 Schematic representation of the synthesized C-dots: carboxylated (a) and amino-functionalized (b) (ethylene diamine  $R = C_2H_4$ ; TTDDA  $R = C_{10}H_{20}O_3$ ) in their non-charged state

Analysis of the synthesized C-dots with the use of HR-TEM imaging reveals populations of spherical shaped and monodisperse particles of different sizes, depending on their capping. Their emission features were evaluated in solutions of different pH values and it was observed that the spectral features do not change as the pH changes, the

maximum of the emission peak remaining at the same wavelength without any observable red or blue shifts (data not presented).

In order to probe the functionalities of the C-dots, the FT-IR spectra of synthesized dots were obtained. As shown in Figure 2, the C-dots without amino groups on their surface present a characteristic strong peak at  $1640\text{ cm}^{-1}$  which is attributed to C=O stretch of the COOH group (dashed line), and thus establish the existence of these groups on the surface of the C-dots. The spectrum of the TTDDA-covered C-dots (solid line) also presents the characteristic peak of the COOH groups, to a smaller extent though, the appearance of carboxyl groups on the surface of the C-dots being unavoidable during pyrolysis. In the same spectrum we can observe the presence of a very strong peak at  $1572\text{ cm}^{-1}$  which is attributed to in plane N-H bending, thus suggesting the formation of a large number of amide bonds on the surface of the TTDDA-covered C-dots. The same was observed in the case of the diamine C-dots.

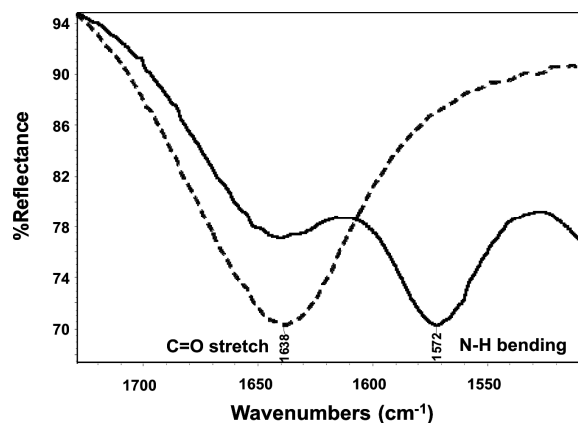


Figure 2 ATR-FT-IR spectra of the carboxylated (Dashed line) and TTDDA-covered C-dots (Solid line)

The fact that the C-dots present different functional groups on their surface provides the grounds for selective chemical recognition and optical transduction via changes in fluorescence. They are nanoparticles with hydrophilic surfaces, a fact that renders them water soluble and selective to different cations. The chemical selectivity was evaluated with different concentrations of  $H^+$ ,  $Cu^{2+}$ ,  $Co^{2+}$ ,  $Ba^{2+}$ ,  $Mn^{2+}$  ions.

Figure 3 shows the changes in the fluorescence intensity of the different C-dots upon changes over a wide range of pH values. The pH changes will have different affects on the functional groups of the C-dots. In addition, the different  $pK_a$  values of the two amines will result in a different protonation pH curve. It is thus expected that the observed pH fluorescence curves of the three different C-dots will be considerably different.

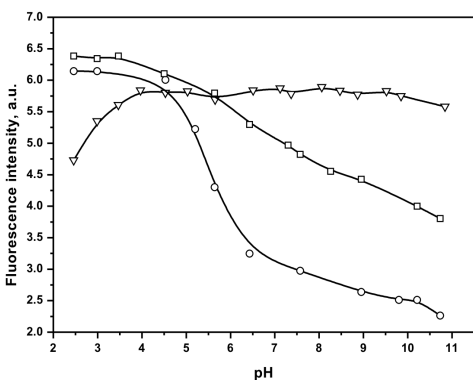


Figure 3 pH influence on the fluorescence intensity of the carboxylated (Triangles), TTDDA (Squares) and diamine (Circles) covered C-dots

It is seen that the signal from the carboxylated C-dots increases as the pH increases. At the initial pH the carboxylated C-dots are protonated. As the pH increases, deprotonation occurs, the negative charge increases, with a concurrent increase in the fluorescence, up to pH values of 4.5. At this point, all carboxyl functionalities are deprotonated, and no further increase in the fluorescence is seen up to pH 11.

On the other hand, the fluorescence of the diamine-coated C-dots presents a sigmoid dependence to pH. At pH 4, where the amino functionalities are still protonated, the signal is at its maximum value, decreasing following a sigmoidal shaped curve up until pH 11, at which point all amino groups are expected to be neutral. The total decrease in the fluorescence intensity of the C-dots in the range between 4 and 11 is approximately 50%.

The TTDDA covered C-dots show a much more smooth fluorescence decrease upon increase in the pH from 4 to 11. The slope is almost constant within the whole pH range, with a small plateau at pH values below 4. In this case, the overall decrease in the fluorescence is smaller as compared to the diamine-covered C-dots, approximately 40% from the pH change between 4 and 11.

The fact that the pH fluorescence curves of the C-dots presents different shapes depending on their surface coverage is a strong indication that there is indeed a drastic but selective effect of the surface change on the fluorescence intensity. Based on this, the fluorescence of the different C-dots was monitored upon changes in the concentration of divalent metal ions. The metals chosen to be studied are  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Mn}^{2+}$  in the order of decreasing binding constants to tetraacetic acids when deprotonated [5].

Figure 4 shows the emission values of the carboxylated C-dots upon addition of the metal ions. It is interesting to note that while the fluorescence of the carboxylated C-dots did not change upon addition of the weak binding  $\text{Mn}^{2+}$  ion, the fluorescence decreased considerably when the stronger binding metal ions  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$  were added. Based on the effect that both metal ions as well as pH have on the carboxylated C-dots it is suggested that the electrical field generated by the negative charge accumulation on their surface results in a higher fluorescence emission signal, as shown schematically in figure 5a.

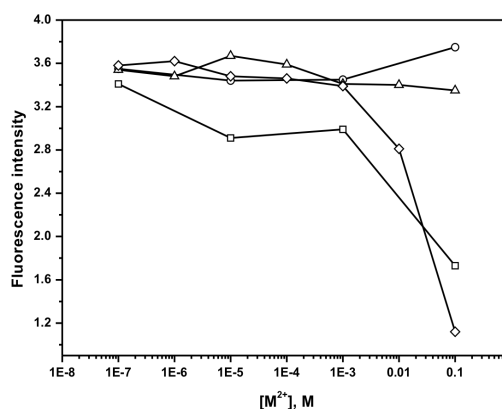


Figure 4 Fluorescence changes of the carboxylated C-dots upon addition of  $\text{Cu}^{2+}$  (Squares),  $\text{Co}^{2+}$  (Diamonds),  $\text{Ba}^{2+}$  (Circles) and  $\text{Mn}^{2+}$  (Triangles)

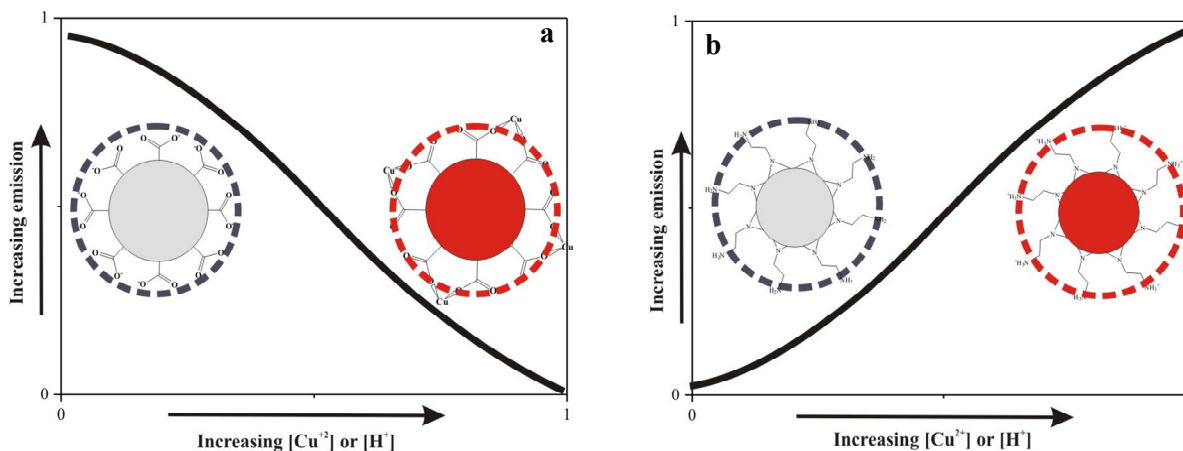


Figure 5 Schematic representations of changes in fluorescence of the carboxylated (a) and aminated (b) C-dots

When the TTDDA C-dots were examined for their response to metal ions as above, it was observed that the addition of the selected metal ions ( $\text{Cu}^{2+}$ ) up to a concentration of 0.1 M, results in an increase of their fluorescence (data not presented). As in the case of pH, the increase of the positive charge on the surface of the C-dots results in higher fluorescence emission signal (figure 5b).

The addition of the copper metal ion to the diamine C-dots resulted in a decrease of the fluorescence signal. This behaviour is similar to that of the carboxylated C-dots, albeit the response is a little smaller. This result together with the fact that in the pH experiment the response of the diamine C-dots is similar to the one of the TTDDA C-dots can be attributed to different functional groups present on their surface, thus two different response modes are expected.

## 4 CONCLUSIONS

In conclusion, C-dots can be functionalized *in situ* during microwave assisted synthesis with ion-selective complexing moieties. Controlled experiments with carboxylated and aminated C-dots suggest that the fluorescence of these materials is highly dependent on the electrical field generated on their surface from the selective interaction of the charge ions with the functional sites of the C-dots. This charge can be developed selectively using different functionalities with different binding constants to monovalent and divalent metal ions. This allows for the selective optical detection using functionalized C-dots. These observations set the grounds for the development of highly selective fluorescent nanoindicators based on C-dots, and their further deployment in the development of novel chemical and bio-sensing biocompatible systems.

## 5 ACKNOWLEDGMENTS

This study was financially supported by the FP7 IP "NANOTEC" of the European Commission and the Greek "ARISTEIA II" Action of the "OPERATIONAL PROGRAMME EDUCATION AND LIFELONG LEARNING" and is co-funded by the European Social Fund (ESF) and National Resources.

## REFERENCES

1. Sun YP, Zhou B, Lin Y, Wang W, Shiral Fernando KA, Pathak P, Meziani MJ, Harruff BA, Wang X, Wang H, Luo PG, Yang H, Kose ME, Chen B, Veca LM, Xie SY (2006) Quantum-sized carbon dots for bright and colorful photoluminescence. *J Am Chem Soc* 128:7756-7757
2. Zhou J, Booker C, Li R, Zhou X, Sham TK, Sun X, Ding Z (2007) An electrochemical avenue to blue luminescent nanocrystals from multiwalled carbon nanotubes (MWCNTs). *J Am Chem Soc* 129:744-745
3. Zhao QL, Zhang ZL, Huang BH, Peng J, Zhang M, Pang DW (2008) Facile preparation of low cytotoxicity fluorescent carbon nanocrystals by electrooxidation of graphite. *Chem Commun* 41:5116-5118
4. Baker S, Baker GA (2010) Luminescent carbon nanodots: emergent nanolights. *Angew Chem Int Ed* 49:6726 – 6744
5. Li H, He X, Kang Z, Huang H, Liu Y, Liu J, Lian S, Tsang CHA, Yang X, Lee ST (2010) Water-soluble fluorescent carbon quantum dots and photocatalyst design. *Angew Chem Intl Ed* 49:4430-4434
6. Krysmann MJ, Kelarakis A, Dallas P, Giannelis EP (2012) Formation mechanism of carbogenic nanoparticles with dual photoluminescence emission. *J Am Chem Soc* 134:747-750
7. Peng J, Gao W, Gupta BK, Liu Z, Romero-Aburto R, Ge L, Song L, Alemany LB, Zhan X, Gao G, Vithayathil SA, Kaiparettu BA, Marti AA, Hayashi T, Zhu JJ, Ajayan PM (2012) Graphene quantum dots derived from carbon fibers. *Nano Lett* 12:844-849
8. Yan X, Li B, Cui X, Wei Q, Tajima K, Li LS (2011) Independent tuning of the band gap and redox potential of graphene quantum dots. *J Phys Chem Lett* 2:1119-1124
9. Liu H, Ye T, Mao C (2007) Fluorescent carbon nanoparticles derived from candle soot. *Angew Chem Intl Ed* 46:6473-6475
10. Liu C, Zhang P, Tian F, Li W, Lib F, Liu W (2011) One-step synthesis of surface passivated carbon nanodots by microwave assisted pyrolysis for enhanced multicolor photoluminescence and bioimaging. *J Mater Chem* 21:13163-13167
11. Sapsford KE, Pons T, Medintz IL, Mattoussi H (2006) Biosensing with luminescent semiconductor quantum dots. *Sensors* 6:925-953
12. Pellegrino T, Kudera S, Liedl T, Javier AM, Manna L, Parak WJ (2005) On the development of colloidal nanoparticles towards multifunctional structures and their possible use for biological applications. *Small* 1:48-63
13. Agüí L, Yáñez-Sedeño P, Pingarrón JM (2008) Role of carbon nanotubes in electroanalytical chemistry A review. *Anal Chim Acta* 622:11-47
14. Yang W, Thordarson P, Gooding JJ, Ringer SP, Braet F (2007) Carbon nanotubes for biological and biomedical applications. *Nanotechnology* 18:412001, 1-12
15. Melhuish WH, (1961) Quantum efficiencies of fluorescence of organic substances. Effect of solvent and concentration of the fluorescent solute. *J Phys Chem* 65:229-235