



Research Networking Programmes

Short Visit Grant or Exchange Visit Grant

(please tick the relevant box)

Scientific Report

The scientific report (WORD or PDF file – maximum of eight A4 pages) should be submitted online within one month of the event. It will be published on the ESF website.

Proposal Title: Coupling Silver Nanoparticles with Carbon Dots at the Tip of a Fiber Optic for Biosensing Applications

Application Reference N°: 4932

1) Purpose of the visit

To build a simple device based in carbon dots, fluorescence nanoparticles, to be immobilized in a simple fiber optic

2) Description of the work carried out during the visit

Continuing the line of research fixed previously, in Department of Chemistry and Biochemistry of University of Porto, under the supervision of Prof. Esteves da Silva, was carried out the proposal of an analytical method to determinate pyridine by means Carbon dots (CDs).

CDs and its nitrogen doped (N-CDs) nanoparticles were synthesized from lactose as precursor using a bottom-up hydrothermal methodology. The synthesized nanoparticles have been characterized by elemental analysis, FTIR, Raman, TEM, DLS, XPS and steady-state and life-time fluorescence. The synthesized carbon nanoparticles, CDs and N-CDs, have respectively a size about 5 and 15 nm and quantum yields about 8 and 11%. These techniques demonstrated the effectiveness of the synthesis procedure to functionalize the surface of CDs with amine and amide groups, by the presence of diluted NH₃ in aqueous media. The effect of excitation wavelength and pH on the luminescent properties was analyzed. The nitrogen doped nanoparticles, increased the population of the excited states and can be used as pyridine sensor in aqueous media because they show an enhancement of its fluorescence with a good linear relationship.

3) Description of the main results obtained

TEM images of raw CDs revealed that these spherical nanoparticles were well dispersed from each other, showing a regular mean size of CDs (~ 5 nm). To obtain N-CDs, we selected the direct presence of NH₃ in the raw aqueous media, instead of release it from precursors, to incorporate N in the carbon matrix. These N-CDs shows similar spherical morphology as the CDs but with high dimensions (average diameters of 15 nm). Also, a local nano-environment was observed around each nanoparticle, probably ascribed to the inhomogeneous thermal process.

The zeta potential of CDs and N-CDs were -7.22 and -7.58 mV, respectively, which did not change significantly after the doped effect with N, revealing the presence at pH = 7 of carboxylate as prominent organic groups, as showed in the FTIR spectra (Fig. 1C). The elemental analysis demonstrated the incorporation of N, with a ratio N-CDs / CDs = 3.9.

The FTIR spectrum of CDs compared with the obtained for N-CDs. These spectra shows that CDs exhibit prominent bands at 1632 and 3362 cm⁻¹ due to the stretching of C=O and -OH, respectively, corresponding to carboxylic acids. For N-CDs the most prominent bands appeared at 1070, 1632 and 3249 cm⁻¹, which can be assigned to the stretching of C-N/C-O-C/C-O, C=O and N-H groups, respectively, which corresponds to carboxylic and amide functionalities - the peak at 1431 cm⁻¹ is assigned to the N-H bending. Earlier studies have shown that, emission properties of CDs are strongly dependent on surface passivation of CDs. The surface functionalization of CDs with carboxylic groups is responsible for inception of luminescent of CDs, moreover the presence of these functional groups imparts excellent solubility in water without further chemical modification.

The Raman spectra of N-CDs, compared with CDs, shows over the high fluorescence background upon excitation with the 532 nm laser wavelength, revealing a clear strong feature at 1647 cm⁻¹, together the stretching at 3300 cm⁻¹ associated with the stretching modes of N-H groups. Typical of the carbon graphitic materials is the presence of the G (1600 cm⁻¹) and D (1300-1350 cm⁻¹) bands. We do not observe any Raman signal at 1600 cm⁻¹ (G modes). However at the position of the D modes, at 1350 cm⁻¹ and with small intensity signal ascribable to the D band is detected. These spectroscopic fingerprints suggest that the hydrothermal treatment reduces an important fraction of the oxygen groups of the lactose precursor but does not lead to a full reduction to sp² species

The surface of the samples CDs and N-CDs were characterized by XPS. The C 1s core level spectrum of CDs can be decomposed in three contributions at 284.8 eV (75%), 287.5 eV (18%) and 289.3 eV (7%). The contribution at low binding energy (284.8 eV) is the more intense and is assigned to the graphitic carbon and to adventitious carbon. The contribution at 287.5 eV is mainly derived from the presence of carbonyl groups and finally, the weak contribution at 289.3 eV is due to carboxylic and carboxylate groups. The C 1s core level spectrum of N-CDs can be also decomposed in three contributions at 284.8 eV(41%), 286.1 eV (47%) and 287.8 eV (12%), but shows marked differences with respect to that of CDs. Now the contribution due to the presence of carboxylic or carboxylate groups disappears and a new contribution at 286.1 eV is the more intense. This contribution is due to the presence of C-OH and C-N groups. The N 1s core level spectrum is asymmetric due to the existence of two contributions at 399.5 eV (86%) and

401.2 eV (14%). The more intense is due to the presence of amino groups while the contribution at high binding energy is due to the presence of NH_4^+ or alkyl ammonium groups. In summary, XPS data indicate that the nature of the functional groups at the surface of QDs and N-QDs are markedly different.

In order to explore the luminescent properties of N-CDs, the detailed fluorescence spectra were studied under different excitation wavelengths. The as-prepared N-CDs also exhibit the distinctive excitation dependent fluorescent behavior. When the products were excited from 300 to 350 nm, the corresponding emission peaks shifted from 440 nm to 460 nm. Moreover, the intensity of the emission peaks becomes strong and then weak with the increasing excitation lengths, showing a showing a full width at half maximum (FWHM) of 139 nm, relative high value, indicating their size distribution dependence.

Due to high solubility in aqueous solution, the size distribution was evaluated for N-CDs by HPLC. The chromatogram obtained of as-prepared N-CDs monitored with fluorescence detection and it is observable five/six different kind of nanoparticles, which demonstrated that the synthesis procedure produce a small range of size distribution of doped carbon nanoparticles with N. This can be explained that with this kind of analysis is possible to affirm that we obtained different nanoparticles with different surface functionalization in terms of ionizable groups, producing different density charges.

The highest blue emission intensity with a quantum yield (QY) as high as ca. 10.75% was obtained when excited at 350 nm – somewhat higher compared with the raw CDs (8.10 %). The observed distinctive luminescence emissions should be closely related to the N doping. The excitation-dependent fluorescent behavior of CDs would not only origin from the N-CDs with different sizes but also from the inhomogeneous distribution of emissive sites due to the N doping.

The influence of the pH on the fluorescence intensity of N-CDs was also studied showing a varying trend as the pH ranges from 4 to 10. The presence of ionizable groups, such N-H and COOH at the surface of the N-CDs is responsible for this property and shows that the surface composition of the nanoparticles directly affects the fluorescence quantum yield. The maximum fluorescence intensity is achieved at pH = 5. Strongly acid (pH lower than 5) and alkaline medium will provoke the ionization of these groups with a decreasing of the fluorescence intensity.

The measured fluorescent lifetimes for CDs and N-CDs were calculated with a two component decay time model in a good fit (χ^2). Although the fluorescence lifetimes are expected to be sensitive to the two types of treatment only small differences were observed for the two CDs samples.

The dilution of the as prepared N-CDs affects its fluorescence intensity. Five dilutions of N-CDs in proportions 1:25, 1:33, 1:50, 1:100, 1:200 and 1:500 with ultrapure water and PBS solution were investigated. In both cases, optimized dilutions of 50-fold for N-CDs were observed and considered as stock solutions for their higher fluorescence intensity. The study of the interaction of different chemical species with the surface of N-CDs is widely reported in the literature. It has revealed that the luminescence properties of these nanomaterials strongly depend on their surface atoms and their environment.

Pyridine detection

In order to exploit the potential applications of the produced N-CDs, the N-CDs were directly applied in the detection of pyridine without any further functionalization. Different concentrations of the aqueous solution of pyridine were injected into the

N-CDs solution and the mixture was treated under ultrasounds bath for 30 min before fluorescence spectra measurement. The presence of pyridine provokes an enhanced on the fluorescence of N-CDs, the emission spectra are broadened and a blue-shift is observed with increasing the concentration of pyridine. These modifications on the photophysical properties of the CDs result from modifications of the surface due to chemical/physical sorption of pyridine. We speculated that there are two important factors attributed to the enhancement of the fluorescence when adding pyridine into the N-CDs solution. One is the electrostatic attraction/repulsion between the pyridine adsorbed on the surface of the N-CDs, which would increase the distance between the N-CDs. The aggregation of fluorescence nanoparticles would decrease the PL quantum yields due to the energy transfer between them. The pyridine molecules would effectively separate the N-CDs from each other, and then decrease the non-radiative transition. Besides that, the pyridine as the ligand would eliminate the surface defects of the N-CDs, and finally enhance the fluorescence of the N-CDs. And the mechanism still needs to be investigated for the further research. From the results, we could observe that the enhancement effect is not increased over time.

The dependence of the measured luminescence intensity signal with increasing concentrations of pyridine was linear. By employing the optimum values of the variables, a calibration graph obtained by the least-squares treatment was:

$$IF = 252873 + 2865.2 [\text{Pyridine}]$$

with a good linear relationship ($r = 0.9997$), and where IF is the fluorescence intensity and the concentration of pyridine in mgL^{-1} . Values of limit of detection (LOD) and limit of quantification (LOQ) were 2.4 and 8 mg L^{-1} and the precision expressed in terms of relative standard deviation (RSD) was 0.25 % ($n = 10$). Previously, the behavior of raw CDs versus pyridine was checked, and low changes were found in the fluorescence emission.

Preliminary experiments were carried out to immobilize the CDs in the tip of a fiber optic, but the time of the grant will not give opportunity to continue the work.

4) Future collaboration with host institution (if applicable)

Using devices devised in the host laboratory to improve analytical methods and in relation with people in this laboratory in future projects.

5) Projected publications / articles resulting or to result from the grant (*ESF must be acknowledged in publications resulting from the grantee's work in relation with the grant*)

The present work, the preliminary results has been submitted to be published in Journal Colloids and Interface Science (Ed. Elsevier) according with experiment has to be finished in University of Málaga, due to the short time for this grant.

Due to interest of this kind of Nanosystems will be presented, as a poster in the VII Workshop en Nanociencia y Nanotecnología Analíticas (<http://nyna2015.usal.es/es>).

6) Other comments (if any)

The present work has serious importance in several aspects:

1. Contact with other laboratory where field of interest are commonly and all facilities have been offered materials, apparatus and human dedication without problems
2. In the future will contribute in my background formation and intent apply, with experience adquired with the present exchange grant, futures projects in the frame of some future related program of your organisation.
3. May be if the grant will be extended a month, the work will be finished at all.