Supporting Information

Fabrication by Laser Irradiation in a Continuous Flow Jet of Carbon Quantum Dots for Fluorescence Imaging

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1. Experimental setup, materials and methods

**Materials:** The flow jet experimental setup consists of simple cost-effective elements, a silicon tube, two pipette tips and a funnel to make the liquid flow in a closed loop boosted by a peristaltic pump (Watson Marlow 313S). A picture showing the distribution of the elements can be seen in Figure S1. One of the pipette tips is cut and inserted in the other to avoid the splash of the liquid in the irradiation zone. The irradiated colloid is composed of 40 mg of carbon glassy dispersed in 100 ml of polyethylene glycol 200. The original size of carbon solid particles is 2-12µm. A ball mill is used during 5 hours at 300 rpm to reduce the particle size.
Figure S1. Passage reactor experimental setup based on two pipette tips to circulate the liquid flow. A funnel to collect the falling liquid. A silicon tube to take the liquid from the funnel to the pipette tip and a pump to generate the liquid flow in closed loop.

The size distribution of the particles measured by Dynamic Light Scattering (DLS, Malvern Instruments Ltd., UK) is shown in Figure S2. Particle size was determined from the velocity of the particles due to their Brownian motion by means of the Einstein–Stokes equation. Measurements were carried out at 25° C and a total number of three tests were run for each sample.
Figure S2. Size distribution of the measured nanoparticles with DLS a) before milling and b) after milling for 5 hours.

The live operation of the batch and the flow jet system are shown in video V1.

2. TGA analysis

Thermogravimetric analysis of the samples was performed with a TG-SDTA Mettler Toledo system, model TGA/SDTA851e/LF/1600. Samples of PEG200 and centrifuged CQDs from flow jet and batch were taken and three measurements were carried out for each one. Each measurement consisted on heating the sample from 100º C to 1000ºC with a heating velocity of 10º/min on a N₂ atmosphere in order to avoid the problems caused by the low autoignition
temperature of PEG200 in air. To obtain the mass percentage of CQDs in the experiment, the average of the three measurements taken for each sample was done. Then, to avoid possible errors in the measurement, the residual average mass of PEG200 was subtracted from the values obtained for batch processing and flow jet samples. Finally, at 1000ºC the remaining mass in the batch and flow jet measurements is the CQDs mass percentage and was compared to the carbon mass percentage added in the initial dilution. The uncertainties shown on each result are calculated by obtaining the standard deviation from the three measurements taken on each case. The calculation of the error in percentage of carbon mass compared to the initial sample was performed by propagation of errors.

3. Theoretical calculation

Particle heating-melting-evaporation model\(^1\,^2\) was applied to estimate the necessary level of laser fluence. The model assumes that all the energy absorbed by a particle from each pulse is generating the particle heating-melting–evaporation process and so the heat loss due to the interaction with the liquid can be neglected. Besides, it is also assumed that the time between two consecutive laser pulses is high enough for the particle to completely cool down by surrounding liquid media before next pulse interacts.\(^2\) Because there is no enough reliable thermodynamic data and absorption characteristics for the black carbon material, all estimation were made for graphite particle of same size distribution. The values of refractive index and absorption coefficient for graphite needed for calculating the absorption cross section of the graphite particles were obtained from the reference book,\(^3\) and thermodynamics characteristics of graphite from reference book.\(^4\)
Graphite does not melt at any temperature, instead, at high temperature values it sublimates by the reaction:

\[ \text{C(s)} \rightarrow \text{C(g)} \quad (1) \]

Using the known thermodynamics characteristics of C(s) and C(g) it is possible to calculate the values of saturated pressure as a function of temperature. Then, only two assumptions/simplifications need to be done to obtain the final model where critical laser fluence values are plotted as a function of particle diameter for two processes, the beginning of sublimation and when sublimation is complete.

1. The intense sublimation process starts when the pressure of saturated vapors calculated for the reaction reaches the atmospheric pressure (p(C) = 1 atm). The same assumption is made for the particle heating-melting-evaporation processes.\(^1\,^2\) This is the so called “sublimation start” condition.

2. For complete sublimation of graphite, additional energy equal to the difference in heat formation values of solid graphite and gaseous carbon need to be supplied. It is assumed by analogy with evaporation process, that all further sublimation occurs at the same temperature as sublimation started, therefore the difference in heat formation values for gas and solid could be calculated from the “sublimation start” temperature.
4. Temporal evolution measurements

The suspensions were irradiated for 4 hours. During the process, 6 aliquots were taken for visual inspection at 15min, 30min, 1hr, 2hrs, 3hrs, and 4hrs of irradiation. Pictures of the process evolution are shown in Figure S3, where the brown coloration indicates the presence of CQDs.

Figure S3. Evolution of the carbon glassy-PEG200 dilution within the irradiation time. From the initial mixture to the 4 hours irradiated samples by both systems flow jet, left, and batch, right.

A transmission electron microscope (TEM) JEOL 2100 thermionic gun lanthanum hexaboride (LaB6) which works at a voltage of 200KV maximum acceleration was used for structural and morphological examination of specimens. To prepare the samples, a droplet of the colloidal suspension was dispersed in a carbon-coated copper-based TEM grid. The excess of the liquid
content was removed by an absorbent paper, so the most of solid particles can remain in the grid surface. A sample was prepared for each aliquot taken, obtaining 12 samples for TEM analysis. The change in morphology, size and abundance of CQDs and initial carbon particles from 15 min irradiation to the final result, 4 hours, is summarized in Fig. S4. The images reveal that the flow jet technique allows obtaining smaller nanoparticles with reduced educt.

**Figure S4.** Evolution of the laser fragmentation with increasing laser irradiation time for both flow jet and batch systems. Near field images provide information of the size, morphology and abundance of CQDs. Far field images show the abundance of big particles and its size, showing a bigger amount of micrometric particles for the batch strategy.

### 5. Optical properties

**Fluorescence measurements**

Fluorescence spectra shown in Fig. 2c were recorded using a Cary Eclipse Fluorescence Spectrophotometer (Varian) with excitation wavelengths from 200 to 400 nm and a 10 mm path
length quartz cuvette. The image in Fig. S4 shows the excitation of a PEG200, right, and CQDs sample, left, with a 405 nm laser pointer of 8 mW.

**Figure S5.** Fluorescence excitation of a PEG200 sample, left, and the CQDs generated by the passage reactor method, right.

**Infrared spectroscopy spectrum**

The FTIR spectrum, Fig. 3a, was measured using a FT/IR-6200 (Jasco) Fourier transform infrared spectrometer.

**Absorption spectrum**

UV-Vis absorption spectra, Fig. S4, was recorded in the range from 200 to 800 nm using a Cary (Varian) 500 Scan UV-Vis Spectrometer UV-VIS-NIR spectrophotometer unit. A 10 mm path length quartz cuvette was used. The information from 200 nm to 400 nm is represented
because is the region where the samples are excited, the absorption exhibits its maximum and so the fluorescence signal obtained is maximized.

![Absorption spectrum](image)

**Figure S6.** Absorption spectrum of the passage reactor CDs from 200 nm to 400 nm with the spectra measured from 200 nm to 800 nm as an inset.

6. Internalization of CQDs in human cells

The internalization of the CQDs into samples of OECs taken from 10 different subjects was analysed by fluorescence images acquired with an inversed confocal microscope Leica TCS SP8
using a 405 nm diode as excitation source and a PMT (Photomultiplier Tube) collecting light in the 420-637 nm region as detector.

**Figure S7.** Fluorescence images of oral cells from six of the healthy subjects proving the complete internalization of the CQDs both in the membrane and the nuclei of the cells for all the subjects.

Internalization was observed in all of the OECs samples, even in the nuclei. The central images were obtained as a projection from measurements at several depths, revealing the volumetric distribution of the CQDs inside the cell, while the other are images of only one optical plane. Information of the 3D structure of a cell or a set of cells is also measured and an example is represented in V2.
7. Long term CQDs properties

**Figure S8.** a) CQDs samples generated by flow jet and batch method after being stored for 10 months. Dark color shown in the batch sample indicates the sedimentation process. b) Fluorescence image of an oral epithelial cell using the flow jet CQDs sample stored for 10 months.

**References**

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