

# Characterization of carbon quantum dots by capillary electrophoresis with laser-induced fluorescence detections

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Interestingly, even though the absorption maximum of prepared capped carbon quantum dots (CQDs) is 210 nm and the emission maximum is 392 nm, using capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) with excitation wavelength of 470 nm and long pass emission filter (510 nm) a signal was observed. Application of separation technique revealed presence of two different species, which corresponded to two well-resolved peaks present in the electropherogram. This fact is probably caused by presence of particles of different sizes.

**Keywords:** carbon quantum dots; fluorescence; capillary electrophoresis

## 1. Introduction

Recently, semiconductor quantum dots (QDs) have been established as a valuable tool for labeling and sensing [1-3]. However, semiconductor quantum dots possess certain limitations such as high toxicity due to the use of heavy metals in their production. It is known that heavy metals are highly toxic even at relatively low levels, which may prove prohibitive to any clinical studies. This prompted the creation of carbon-based fluorescent nanoparticles (CQDs) to replace semiconductor QDs due to their low toxicity, biocompatibility, low cost and chemical inertness in addition to having similar fluorescence properties [4]. Similarly to semiconductor QD, fluorescent carbon nanoparticles can be employed for chemical sensing applications - monitoring of metal ion content [5,6], pH sensing [7], biosensing [8] and/or in vivo imaging [9,10].

Even though the capillary electrophoresis (CE)

is an extremely valuable separation analytical method and in combination with laser-induced fluorescence detection provides exceptionally low limits of detection, its application to analysis of fluorescent carbon nanomaterials is relatively limited [11]. In contrast to stationary fluorescence spectrometry, CE is capable to reveal the presence of various species in the sample due to their different electrophoretic mobility.

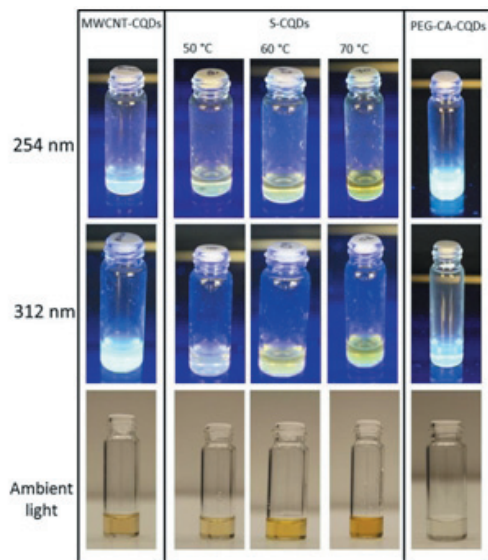
In this work, preparation of CQDs from various precursors such as citric acid, sucrose and multiwall carbon nanotubes were synthesized, optically characterized by fluorescence spectrometry and investigated by capillary electrophoresis with laser-induced fluorescence detection.

## 2. Results and Discussion

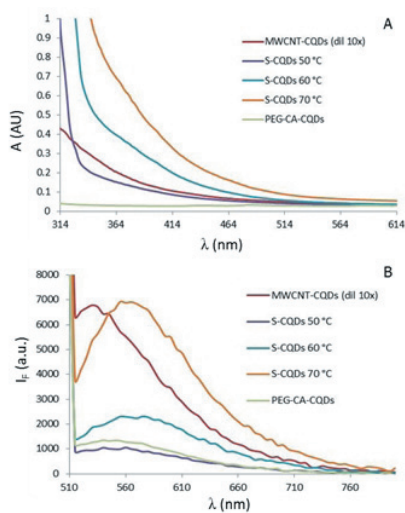
### 2.1 Spectrometric characterization

The solutions of CQDs were prepared from different carbon precursors (citric acid, sucrose

and multiwall carbon nanotubes) according to the procedures described above. Photographs of obtained solutions under illumination by UV light (254 and 312 nm) and ambient light are shown in Fig. 1.



**Figure 1:** Photographs of various types of CQDs solutions (MWCNT-CQDs, S-CQDs, PEG-CA-CQDs) under UV (254 and 312 nm) and ambient light illumination.



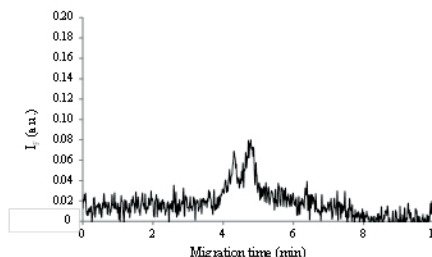
**Figure 2:** (A) Absorbance spectra of synthesized CQDs. (B) Emission spectra of synthesized CQDs after excitation by 488 nm.

The obtained solutions were optically characterized using spectrophotometry and fluorescence spectrometry. The best fluorescence properties were detected in case of MWCNT-CQDs followed by S-CQDs and PEG-CA-CQDs (Fig. 2). All synthesized CQDs absorbed the light in the UV range of the spectra as shown in Fig. 2A. Above the wavelength of 500 nm, the absorbance decreased below 0.2 AU in case of all types of CQDs. Therefore, the excitation by the light with the wavelength of 488 nm (excitation light source of CE-LIF instrument) is not optimal; however, as shown in Fig. 2B, some signal was obtained even under such suboptimal conditions. The most intensive signal was obtained in case of S-CQDs (70 °C) and MWCNT-CQDs. MWCNT-CQDs had to be even 10-times diluted to obtain measurable signal.

## 2.2 Capillary electrophoresis with laser-induced fluorescence detection

### 2.2.1 PEG-CA-CQDs

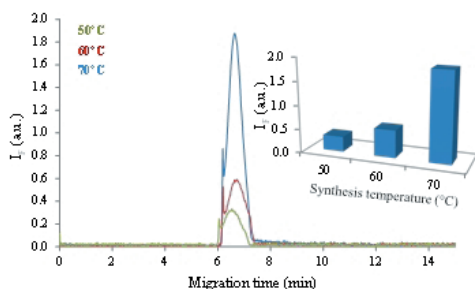
Besides the stationary fluorescence spectrometry, also CE-LIF analysis of CQDs was carried out to investigate the number of species present in the solution. As shown in Fig. 3, in the case of PEG-CA-CQDs, there are two species present. The CE-LIF signal is very low due to the non-ideal setting of the CE-LIF instrument for analysis PEG-CA-CQDs (absorption maximum of PEG-CA-CQDs is 210 nm and the excitation wavelength of the CE-LIF instrument is 488 nm). However, still two species can be recognized in the solution.



**Figure 3:** CE-LIF of PEG-CA-CQDs. Separation conditions: internal diameter - 75  $\mu$ m, length - 54/64.5 cm, separation voltage - 20 kV, hydrodynamic injection - 0.5 psi, 5 s, electrolyte - 20 mM sodium borate, pH 9.

### 2.2.2 S-CQDs

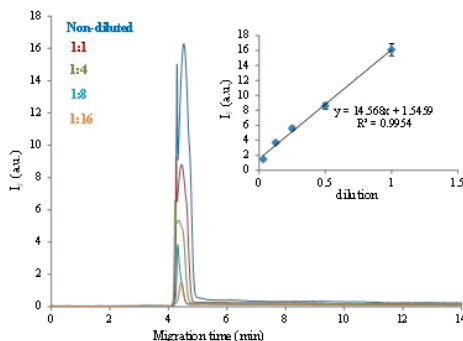
Second precursor tested for CQDs preparation is the sucrose. It was found out that the preparation temperature has a significant influence on the fluorescence properties. In this case, temperatures of 50 °C, 60 °C and 70 °C were tested. Two peaks were recognized in the electropherogram – 6.1 min and 6.6 min. the major peak with migration time of 6.6 min was used for quantification and evaluation. As shown in Fig. 4, the preparation temperature of 70 °C provided nanoparticles of significantly higher fluorescence compared to 50 °C (5.8-times) and 60 °C (1.8-times). In comparison with PEG-CA-CQDs, the CE-LIF signal of S-CQDs (50 °C) was 4-times higher.



**Figure 4:** CE-LIF of S-CQDs prepared by using 50, 60 and 70 °C. Separation conditions: internal diameter - 75 μm, length - 54/64.5 cm, separation voltage - 20 kV, hydrodynamic injection - 0.5 psi, 5 s, electrolyte - 20 mM sodium borate, pH 9. Inset: Dependence of the peak height on the preparation temperature.

### 2. 2. 3. MWCNT-CQDs

MWCNT-CQDs provided the best fluorescence properties from all carbon precursors used. As shown in the Fig. 5, the signal obtained by CE-LIF analysis of MWCNT-CQDs was 8.6-times higher compared to the S-CQDs (70 °C). Similarly to S-CQDs, two peaks are present in the electropherogram of MWCNT-CQDs (4.2 and 4.35 min). As shown in the inset of Fig.4, the fluorescence signal is linearly dependent on concentration with regression equation of  $y = 14.568x + 1.5459$  and determination coefficient  $R^2=0.9954$ .



**Figure 5:** CE-LIF of MWCNT-CQDs. Separation conditions: internal diameter - 75 μm, length - 54/64.5 cm, separation voltage - 20 kV, hydrodynamic injection - 0.5 psi, 5 s, electrolyte - 20 mM sodium borate, pH 9. Inset: Dependence of the peak height on the dilution by separation electrolyte.

Based on the CE-LIF data, the electrophoretic mobilities of all CQDs were calculated (migration times of major peaks were used) according to the equation 1. Migration time of coumarin 334 used as a electroosmotic flow marker was 4.06 min. The obtained values of electrophoretic mobilities are summarized in Tab. 1. It follows from the results that S-CQDs possess the most negative charge, which is caused probably due to the preparation procedure.

$$\mu_{El} = (I_{eff} \times l_{tot} / t_{migCQDs} \times V) - (I_{eff} \times l_{tot} / t_{migEOF} \times V) \quad (1)$$

$\mu_{El}$  – electrophoretic mobility,  
 $l_{eff}$  – effective capillary length,  
 $l_{tot}$  – total capillary length,  
 $t_{migEOF}$  – migration time of the electroosmotic flow,  
 $t_{migCQDs}$  – migration time of CQDs.

CQDs type	Electrophoretic mobility ( $\times 10^9 m^2 V^{-1} s^{-1}$ )
PEG-CA-CQDs	-4.125
S-CQDs	-26.439
MWCNT-CQDs	-5.866

### 3. Experimental Section

#### 3.1 Synthesis of fluorescence carbon nanoparticles (CQDs)

##### 3.1.1 Polyethyleneglycol-capped citric acid-based CQDs (PEG-CA-CQDs)

The solution of ethylene glycol (10 mL), PEG-8000 (1 g) and citric acid (1 g) in a 100 mL three-neck flask was heated at 180 °C for 3 h under nitrogen flow, and then cooled down to ambient temperature. Mili-Q water was then added and the mixture was stirred for a couple of minutes. The obtained solutions were purified for 24 h by dialysis against Mili-Q water with a D-Tube maxi dialyzer to remove ethylene glycol.

##### 3.1.2 Sucrose-based CQDs (S-CQDs)

H<sub>3</sub>PO<sub>4</sub> (70%, 10 mL) was added with stirring to a solution of sucrose (1 g) in water (5 mL). After 30 min stirring, 2 ml of mixture was pipetted to a glass vial and heated in Multiwave 3000 Microwave Reaction System (Anton Paar, Graz, Austria) using rotor 64MG5. Reaction conditions were as follows – power 300 W, time 10 min and temperatures 50, 60 and 70 °C. After cooling, the samples were neutralized with water solution of Na<sub>2</sub>CO<sub>3</sub> (4 g in 15 ml). Neutralized solutions were left overnight and formed dark brown precipitates were removed by centrifugation (10000 rpm, 20 min, 20 °C). Thus, obtained supernatants were used for measurements.

##### 3.1.3 Multiwall carbon nanotubes-based CQDs (MWCNT-CQDs)

MWCNT (0.1 g) were heated in 3:1 mixture of H<sub>2</sub>SO<sub>4</sub> : HNO<sub>3</sub> (10 ml) under reflux at 140 °C for 8 h. After cooling acetone (10 ml) was added. The mixture was left overnight and Na<sub>2</sub>CO<sub>3</sub> (10.6 g) in water (80 ml) was added with stirring. Brown oily viscous liquid was collected on bottom of tube after centrifugation (25000 rpm, 20 min, 20 °C). Water layer was used for fluorescence measurement.

#### 3.2 Fluorescence spectrometry and spectrophotometry

Absorbance and fluorescence spectra were measured using the TECAN microtitration plate reader Infinite 200 PRO (Switzerland) using 50 µL of the sample in the UV transpa-

rent 96-well plate. Each absorbance value is an average of 5 measurements.

##### 3.3 Capillary electrophoresis with laser-induced fluorescence detection (CE-LIF)

The electrophoretic behavior was analyzed by CE-LIF (PACE MDQ, Beckman Coulter, USA) using fused silica capillary with internal diameter of 75 µm was used 54/64.5 cm.

The separation voltage of 20 kV and hydrodynamic injection by 0.5 psi for 5 s was employed. 20 mM sodium borate buffer pH 9 was used as a separation electrolyte. The signal was detected with laser-induced fluorescence after excitation with argon ion laser (488 nm) and emission wavelength in the range 510-530 nm. Coumarin 334 was used as a electroosmotic flow marker.

### 4. Conclusions

It was found out that MWCNT-CQDs provided the best optical properties and were the most suitable for CE-LIF analysis with 488 nm excitation light source. For further labeling purposes the MWCNT-CQDs will be used. On the other hand, the S-CQDs exhibited the most negative electrophoretic mobility suggesting their negative charge compared to the other CQD types, which were very slightly negative or neutral.

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### Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

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