SYNTHESIS AND CHARACTERIZATION OF POLYMER MODIFIED CARBON QUANTUM DOTS

MILOSAVLJEVIC Vedran [1,2], MOULICK Amitava [1,2], SKALICKOVA Sylvie [1,2], KOPEL Pavel [1,2]

[1] *Mendel University in Brno, Department of Chemistry and Biochemistry, Faculty of Agronomy, Brno, Czech Republic EU; paulko@centrum.cz*
[2] *Brno University of Technology, Central European Institute of Technology, Brno, Czech Republic, EU*

Abstract

Nanoparticles are promising scaffolds for applications such as imaging, chemical sensors and biosensors, diagnostics, drug delivery, catalysis, energy, photonics, medicine and more. Surface functionalization of nanoparticles introduces an additional dimension in controlling nanoparticle interfacial properties and provides an effective bridge to connect nanoparticles to biological systems. With fascinating photoluminescence properties, carbon dots (C-dots), carbon-containing nanoparticles that are attracting considerable attention as a new type of quantum dot, are becoming both an important class of imaging probes and a versatile platform for engineering multifunctional nanosensors. In this study we focused on CQDs synthesis, characterization and stability study by spectrophotometric methods.

Keywords: Carbon quantum dots, bioimaging, biosensing, polyvinylpyrrolidone

1. INTRODUCTION

A quantum dot (QD) is a nanocrystal small enough to exhibit quantum mechanical properties. Nowadays, researchers have studied applications for quantum dots in optical physics and for *in vivo* imaging in the field of biomedicine [1, 2]. In this point of view, quantum dots have quickly filled in the role, being found to be superior to traditional organic dyes on several counts, one of the most immediately obvious being brightness as well as their stability [3]. It has been estimated that quantum dots are 20 times brighter and 100 times more stable than traditional fluorescent dyes. Also, their surface modification by various chemical compounds predestine their specific usage and could give them the unique behaviour properties [4]. Carbon-based quantum dots are a new class of carbon nanomaterials with sizes below 10 nm which are characterized by high (aqueous) solubility, robust chemical inertness, facile modification, low toxicity and good biocompatibility, entrust them with potential applications in bioimaging, biosensor and biomolecule/drug delivery [5]. There are many different ways to synthesize various functionalized and non-functionalized CQDs. Their properties depend on the different methods of CQDs preparation such as pyrolysis, electrochemical exfoliation, acidic oxidation, hydrothermal treatments, microwave passivation, laser ablation, thermal oxidation, and emulsion-assisted methods [5]. Commonly used are carbon materials with different sizes such as graphite, carbon nanotubes, carbon soot, activated carbon, graphite oxide or different molecular precursors such as citric acid or glucose [6, 7]. Carbon nanoparticles are being explored widely for use in cancer treatment. Studies reveal that cancer treatment using radio waves can heat and destroy a tumour, lymphoma, or metastasized cancer [8, 9]. Excellent optical properties of CQDs originate from carboxyl or hydroxyl groups passivated by a polymer. One of the promising polymeric compound is water-soluble polyvinylpyrrolidone (PVP) used as a binder in many pharmaceutical tablets [10, 11]. PVP is amphiphilic, non-ionic, soluble in water and in many organic solvents, biocompatible, stable etc. It is a polymer with a large range of applications and is certainly first choice polymer in the biomaterials area. Due to its non-toxicity and water-solubility, PVP is a very attractive polymeric carrier of compound functionalities of interest as those of the scheme, which are multifunctional side chain conjugates analogous for instance to the conjugates PHPMA-dox already in advanced clinical trial in the treatment of cancer [12]. It was proven that PVP led to an efficient composite that showed highly catalytic

activity towards the formation of C-C bonds. In this study, fluorescent CQDs were synthesized using citric acid covered with PVP as the source of carbon precursors and characterized afterwards.

2. EXPERIMENTAL PART

2.1. Chemicals

Chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, USA) in ACS purity unless noted otherwise. The deionized water was prepared using reverse osmosis equipment Aqual 25 (Czech Republic). The deionized water was further purified by using apparatus MilliQ Direct QUV equipped with the UV lamp. The resistance was 18 MΩ. The pH was measured using pH meter WTW inoLab (Weilheim, Germany).

2.2. Composition of the neutral and acidic cellular simulant fluid

As a neutral cellular simulant fluid was used the mixture of 212 mg/l $MgCl_2 \cdot 6H_2O$, 6415 mg/l NaCl, 318 mg/l $CaCl_2 \cdot 4H_2O$, 179 mg/l $Na_2SO_4 \cdot 10H_2O$, 148 mg/l Na_2HPO_4, 2703 mg/l $NaHCO_3$, 180 mg/l disodium tartrate dihydrate, 144 mg/l trisodium citrate dihydrate, 175 mg/l sodium lactate, 118 mg/l glycine and 172 mg/l sodium pyruvate. As an acidic cellular simulant fluid was used the mixture of 142 mg/l Na_2HPO_4, 6650 mg/l NaCl, 71 mg/l Na_2SO_4, 29 mg/l $CaCl_2 \cdot 4H_2O$, 450 mg/l glycine and 4084.6 mg/l potassium hydrogen phthalate.

2.3. CQDs preparation

CQDs were prepared as follows: into a 100 ml three-neck flask were added 10 ml of ethylene glycol, 1 g of PVP (10 kDa) and 1 g of citric acid. The solution was heated on 180 ºC for 4 h under flow of nitrogen, and then cooled down to room temperature. Into cooled solution Milli-Q water was added and then stirred for few minutes. Solutions were purified 24 h by dialyzing against Milli-Q water with a D-Tube maxi dialyzer. During 24 h of dialysis ethylene glycol was removed from solution.

2.4. Characterization of CQDs size

The average particle size and size distribution were determined by quasielastic laser light scattering with a Malvern Zetasizer (NANO-ZS, Malvern Instruments Ltd., Worcestershire, UK). Nanoparticle water solution of 1.5 ml was put into a polystyrene latex cell and measured at a detector angle of 173°, a wavelength of 633 nm, a refractive index of 0.30, a real refractive index of 1.59, and a temperature 25 °C.

2.5. Scanning electrochemical microscopy of CQDs

Scanning electrochemical microscope model 920C (CH instruments, Austin, TX, USA) consisted of 10 mm measuring platinum disc probe electrode with potential of 0.35 V. Glassy carbon disc electrode with O-ring as conducting substrate used potential of -0.40 V. To immobilizing the CQDs on the substrate GC electrode were allowed to dry at room temperature and washed by electrolyte to remove unbounded dots. Platinum measuring electrode was moving from 20 µm above the surface. The mixture consisted of 5 % ferrocene in methanol mixed in ratio 1:1 with 0.05 % KCl in water (v/v). Measuring was performed in Teflon cell with volume of 1.5 mL according to the following parameters: amperometric mode, vertical scan was carried out in area 800 × 800 µm with rate 500 $\mu m.s^{-1}$. Quiet time was 10 s. Electrochemical measurements were performed in a three-electrode configuration using platinum wire as a counter electrode and Ag/AgCl/ 3 M KCl as a reference electrode.

2.6. Fluorescence measurement

Fluorescence spectra were acquired by a multifunctional microplate reader Tecan Infinite 200 PRO (TECAN, Switzerland). Excitation wavelength was 310 nm. The fluorescence scan was measured within the range from 340 nm to 800 nm per 2-nm steps. The detector gain was set to 100. The samples (50 µl) were placed in

transparent 96 well microplates with flat bottom by Nunc (Thermo Scientific, USA). All measurements were performed at 25 °C controlled by the Tecan Infinite 200 PRO (TECAN, Switzerland).

2.7. Descriptive statistics

Data were processed using MICROSOFT EXCEL® (USA). Results are expressed as mean ± standard deviation (S.D.) unless noted otherwise (EXCEL®).

3. RESULT AND DISCUSSION

Here, we have synthetized carbon quantum dots (CQDs) prepared by pyrolysis of the organic precursor (citric acid) and PVP as a capping agent (**Figure 1a**). The polymer provided surface functionality, which leads to the

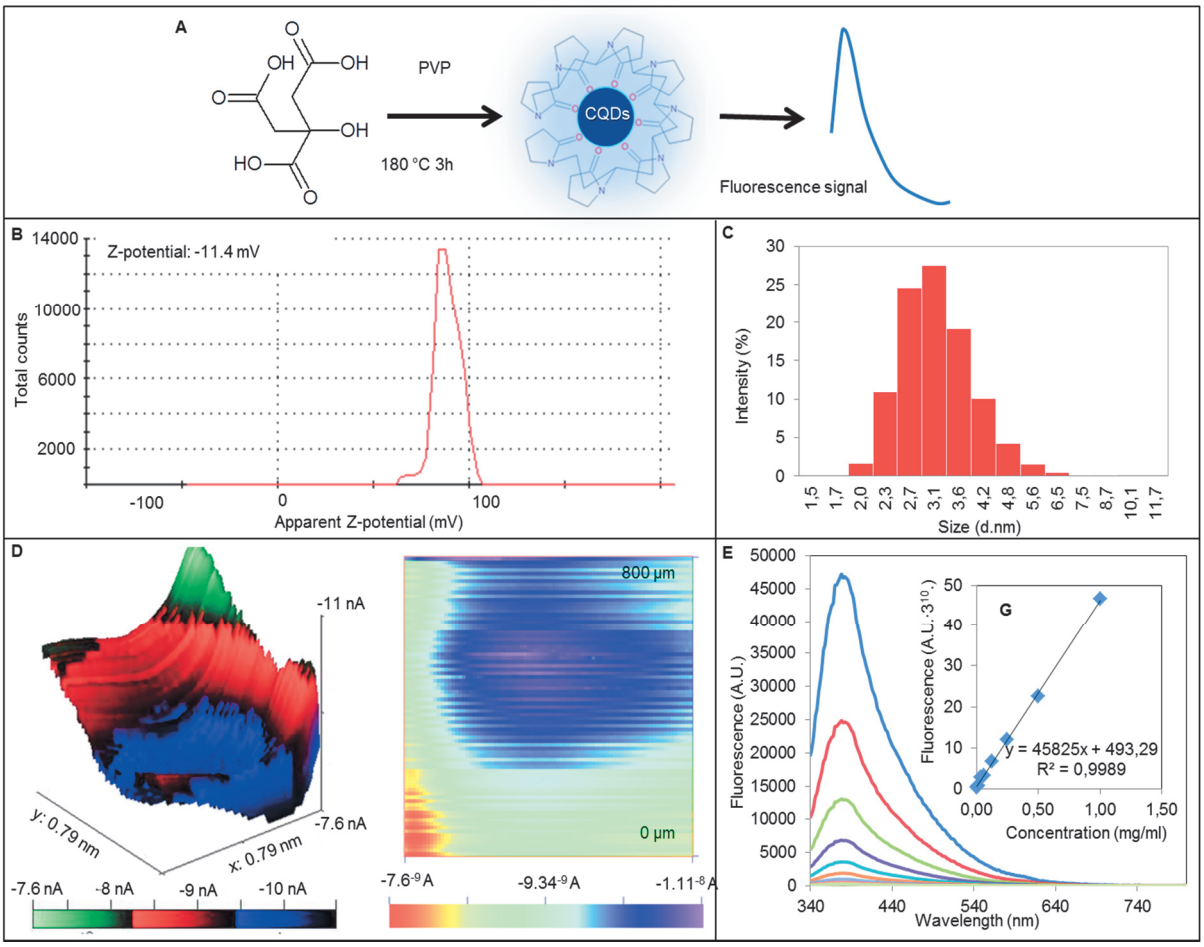

Figure 1 A) Synthesis of PEG-functionalized CQDs using citric acid as the carbon source and PEG as the capping agent. **B**) Z- potential analysis of CQDs and **C**) Size distribution of CQDs. **D**) Scanning electrochemical microscopy of CQDs (right 3D view, left 2D view). **E**) Fluorescence spectra of CQDs in the concentration range 4 - 1000 µg/ml. The excitation wavelength was 310 nm. The fluorescence scan was recorded in the range 340 - 800 nm. **G**) The dependence of CQDs (4 - 1000 µg/ml) on fluorescence intensity

enhanced luminescence and chemical stability as confirmed by fluorescence intensity measurements. The stability behaviour and the particle size were determined using dynamic light scattering measurements used to determine the size distribution profile of nanoparticles in solution (**Figure 1b** and **1c**). From the obtained results the incipient instability of CQDs in aqueous solution and the ability to coagulate or flocculate is obvious.

The negative charge is proposed for modification by positively charged ligands which could be specified to various tissues in the *in vivo* imaging approaches. The particle diameter was 3.1 nm as showed on the graph **Figure 1c**. In order to image the reduction and oxidation properties of nanoparticles, scanning electrochemical surface scans were measured at constant height and the resulting maps are depicted in **Figure 1d**. From SECM map it was obvious that the surface was currently in the range from -7.6 to -11 nA which refers to oxidation surface properties of CQDs. Finally, we determined the fluorescence properties of CQDs. We have studied the fluorescence spectra at different excitation energy ranging from 325 nm - 600 nm and found out that the highest fluorescence intensity was achieved at the excitation wavelength of 310 nm, which shows emission maximum at 376 nm. On the **Figure 1e** the fluorescence spectra of CQDs measured at optimized conditions are shown. Subsequently, we determined the dependence of nanoparticle concentration on fluorescence intensity. The calibration curve in the range 0 - 1000 µg/ml showed a linear trend with following equation y = 45825x + 493.29 and R^2 = 0.9989. From obtained results we can conclude, the quenching phenomenon of CQDs has not been observed in higher concentrations.

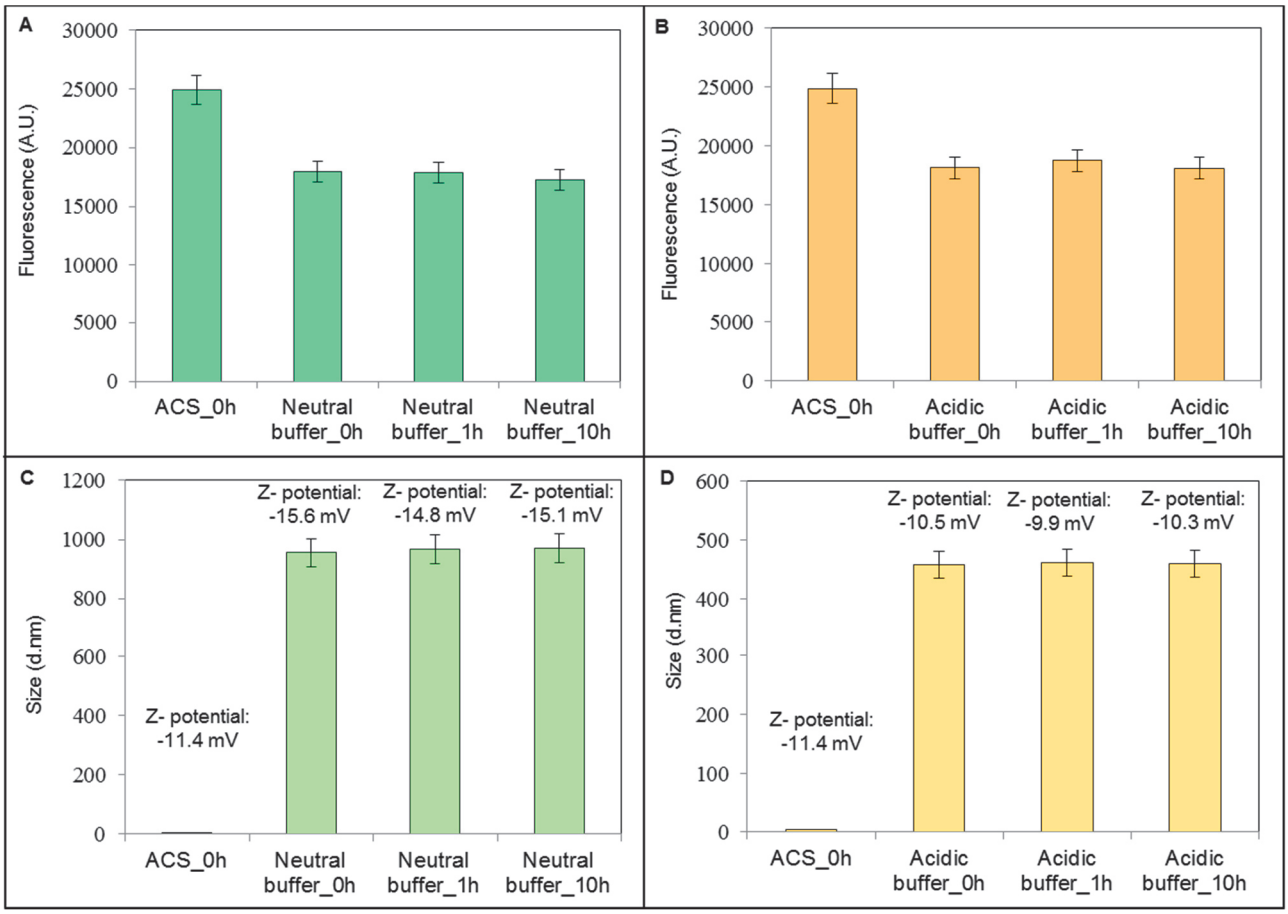

Figure 2 Dependence of CQDs fluorescence on time (0 - 10 hours), **A**) neutral cellular simulant fluid (pH 7.6) and **B**) acidic cellular simulant fluid (pH 4.6). Dependence of CQDs size on time (0 - 10 hours), **C**) neutral cellular simulant fluid (pH 7.6) and **D**) acidic cellular simulant fluid (pH 4.6)

Herein, we also have studied the stability of CQDs in two environments presented by natural and acidic cellular simulant fluid, pH 7.6 and 4.6, respectively. At first, we studied stability of fluorescence 500 µg/ml carbon quantum dots. From obtained results the decrease of fluorescence is obvious, caused probably by change of environment due to its ionic strength. In the case of Neutral buffer it is shown (**Figure 2a**) that the fluorescence of CQDs is stable in time 0 - 10h and the same trend was estimated in the case of acidic environment of buffer solution (**Figure 2b**). The results obtained of light scattering measurement provide an aggregation of CQDs in

environment with higher ionic strength in both cases of acidic and neutral buffers. The size of nanoparticles increased up to 900 nm in neutral solution (**Figure 2c**). This could be caused by various ions contained in cellular simulant fluid such as $MgCl_2$, NaCl, $NaHCO_3$, $CaCl_2$ and others that are present in lower concentrations. The exact composition of the buffers is described in materials and methods. The particle size shows stability in time, thus, they do not disintegrate in environment with higher ionic strength. The Z-potential shows similar values in both buffers and the same fluctuation effect as in ACS water was confirmed. As regards the neutral buffer, the Z-potential increases in higher ionic strength up to 15 mV. Similar results were obtained in the case of particle incubated in acidic environment. As shown on **Figure 2d**, the particle size increases to 490 nm, whereas the Z-potential exhibits gentle decrease to ~10 mV in comparison with CQDs in ACS water. As shown previously, the various ions such as NaCl, potassium hydrogen phthalate, glycine and others mentioned in materials and methods, caused the rapid nanoparticle aggregation. These results provide topic for further investigation of CQDs, functionalized by PVP, as its behaviour has to be improved before application in *in vivo* experiments.

4. CONCLUSION

Highly fluorescent CQDs have been prepared by one-step carbonization of citric acid followed by coating with PVP. The CQDs are graphite nanospheres (3.1 nm average diameter) show low negative charge (-11 mV) and oxidation properties of its surface. The CQDs are a promising tool in *in vivo* imaging due their easy preparation and good fluorescence properties. The investigation of CQDs stability behaviour shows, the fluorescence decreases in the environment of neutral and acidic cellular simulant fluid as well as nanoparticles aggregation in the presence of higher ionic strength. Further investigation of CQDs stability in body fluids is still required.

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