Microwave preparation of carbon quantum dots with different surface modification
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Carbon quantum dots (CQDs) have great potential to be utilized as an optical sensing probe due to its unique photoluminescence and less toxic properties. This work reports a simple and novel synthesis method of carbon dots via direct acid hydrolysis in presence of polyethylene glycol (PEG), polyvinylpyrroldione (PVP) and bovine serum albumin protein (BSA). In this study, fluorescent CQDs were synthesized by using citric acid and ascorbic acid as the source of carbon precursors, which was covered with polyethylene glycol (PEG), polyvinylpyrroldione (PVP) and with bovine serum albumin (BSA), by microwave irradiation. Furthermore, the synthesis parameters as power, reaction time and temperature were studied and quality of prepared CQDs were investigated by spectral methods. Short reaction time (20 min) and temperature from 120 °C to 140 °C under microwave irradiation are sufficient to prepare luminescence carbon quantum dots. Absorption spectra and photoluminescence spectra were measured to characterize prepared dots in water solution. The photoluminescence spectra of CQDs doped with different protection compound show the different luminescent and excitation wavelengths starting from 330 nm to 430 nm. Importantly, these CQDs are demonstrated to be excellent bioimaging agents and fluorescent ink due to their stable emission, well dispersibility, low toxicity, long emission life time, and good compatibility with different macromolecules.

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1. Introduction
Carbon quantum dots (CQDs) have received a lot of attention due to their chemical stability, biocompatibility, and low toxicity in comparison with other quantum dots. Unique quantum size effect of quantum dots (QDs) semiconductor nanocrystal stems from dimension smaller than the bulk exaction Bohr radius. This unique effect is connected with QDs properties, such as broad excitation spectrum, excellent photostability, high quantum yield of fluorescence and high photobleaching \textsuperscript{1,2}. Carbon nanoparticles were first discovered accidently by Xu and co-workers \textsuperscript{3}, during the electrophoretic purification of carbon nanotubes and since then, there were search activity in this area. CQDs are not only less toxic, but also we can find in different reports that they have less fluorescent blinking effect. Syntheses of CQDs are much easier compared with other quantum dots, and remarkably cheaper in handling and production costs \textsuperscript{4-3}. However, the main advantage of semiconductor nanocrystal is its high fluorescence. Organic fluorophores usually do not have excellent characteristic like QDs that are associated with high photostability and resistant to photobleaching of QDs. These characteristic changes in size of semiconductor nanocrystal which leads to changes in spectra wavelength \textsuperscript{6}. Application of these semiconductor based QDs such as CdTe \textsuperscript{7}, CdSe \textsuperscript{8,9}, CdSe/ZnSe \textsuperscript{10}, PbS \textsuperscript{11}, and CdS \textsuperscript{12}, is limited through the presence of heavy metals. This serious problem demonstrates the limited ability for their conventional application due to the known toxicity \textsuperscript{13,14}. Compared with these semiconductor nanomaterial’s, CQDs show
many advantages, including chemical stability, biocompatibility and low toxicity. Carbon quantum dots represent a new type of nanomaterial with nanocrystal structure where the crystal size is not generally less than 10 nm in diameter. The importance of CQD is reflected in its electronic, mechanical, chemical and optical properties. All of these properties allow using of CQDs in different fields of research such as catalysis, sensing, bioimaging, tissue engineering, optoelectronic and electronic devices. Carbon nanoparticles have functionalized surface with carboxyl or hydroxyl groups that had a good photo-induced electron transfer properties. Basically, the synthesis of CQDs falls into three main stages; carbonization, passivation, and surface functionalization. Carbonization is the process of converting organic precursor into its basic carbon containing residues via pyrolysis, electrochemical exfoliation, acidic oxidation, hydrothermal treatments, microwave passivation, laser ablation, thermal oxidation and emulsion-assisted methods. Passivation is a next step that is usually required, because pure carbon nanoparticles are usually non-fluorescent and the chemical treatment of the surface with different compounds is important step in production of CQDs. This step introduces an insulating shell on the surface that minimizes the impact of surface-defect, trap sites, and direct quenching from the surrounding, which all will enhance the fluorescence emission. The functionalization is the last step necessary for production of effective carbon dots. Specific chemical or biological reactivity on the surface of the CQDs is important to introduce specific sites that can be used for bio-imaging or sensing purposes. Material for preparation of CQDs depends on the methods which are being used. Commonly used is carbon material with different size such as graphite, carbon nanotubes, carbon soot’s, activated carbon, graphite oxide or different molecular precursors such as citric acid or glucose. Excellent optical properties of CQDs are arising from carboxyl or hydroxyl groups which are passivated by covering materials. These ensure the production of high fluorescent nanomaterial with high quantum yield and excitation wavelength.

The current research focuses exploring of synthetic routes, more economical synthesis approaches, utilizing greener chemistry, and diversifying possible starting precursors. Covering materials were selected due to its biocompatibility nature, which later will stand a better chance in applications that require low toxicity tolerance. Therefore, we tried to synthesize functionalized CQDs with high fluorescence by using low temperature. The design in CQD synthesis is based on the following considerations: well surface passivation of CQDs plays a key role in quantum dots. However, reaction temperatures are quite high (usually >300 °C) to ensure complete carbonization of molecular precursors, which may destroy the capping reagents and thus lead to bad surface passivation, and loss (or denaturation) of functional groups. Using of microwave irradiation for synthesis of CQDs for both carbonization and passivation would be beneficial to obtain both well passivated and functionalized CQDs. In the case of our research we choose citric and ascorbic acid as the carbon source due to its well-known low carbonization temperature (<200 °C), and use polyethylene glycol, polyvinylpyrrolidone and bovine serum albumin as the capping and functionalizing reagent since they have functionalized and passivation surface states with carboxyl or hydroxyl groups that had a good photo-induced electron transfer properties.

2. Experimental

2.1 Chemicals and materials

Citric acid, ascorbic acid, polyethylene glycol (Mw = 8000), polyvinylpyrrolidone (Mw = 40 000) and bovine serum albumin were purchased from Sigma-Aldrich Co., (St. Louis, Missouri. USA).

2.2 Synthesis of CQDs

For synthesis of water soluble CQDs we prepared six samples were as follows. Sample 1, 2 and 3 have the same procedure of preparation; only for each of the sample were used different capping agent for functionalization and passivation of CQDs surface. In the case of 1,1 g of citric acid was diluted with MiliQ
water (15 ml) and after that was added 1 g of PEG under stirring. Afterwards, sample was stirred for 30 min. The same way of preparation was used for 2 except of capping agent PVP and for 3 capping agent was BSA. Samples 4, 5 and 6 also have the same steps of preparation, but in these cases we used ascorbic acid as carbon source. After 30 min of stirring, from each sample 2 ml of solution was pipetted into glass reaction vessel and heated in Multiwave 3000 Microwave Reaction System (Anton Paar, Graz, Austria) using rotor 64MG5. The reaction conditions were as follows: temperature 60-140 °C, power 300 W and time of heating 20 minutes. Prepared CQDs were stored in dark at 4ºC. Measurements of CQDs fluorescence were conducted by multifunctional microplate reader Tecan Infinite 200 PRO (Tecan group Ltd. Männedorf, Switzerland). The absorbance scans were recorded in the range from 200 to 800 nm each 5 nm. Emission wavelengths were measured in different excitation wavelength on 330 nm, 340 nm, 360 nm, 380 nm, 400 nm 420 and 430 nm, with 100 µl of sample placed in Costar plate, 96 well, with flat bottom. The samples were visualized by UV transilluminator (VilberLourmat, Marne-la-Valle’e Cedex, France) at 312 nm.

3. Results and discussion
In this work we synthesized CQDs and examined the effect of different capping agent on CQDs fluorescence, using citric acid and ascorbic as the source of carbon precursors, which was covered with PEG, PVP and BSA which is illustrated in (Fig. 1).

General method for the preparation of water soluble CQDs was described in the previous part. Heating of solution was used to carbonize the precursor. To produce CQDs, a high temperature (180 ºC) is needed to reach the temperature required for carbonization of precursor. However, higher temperature may cause the destruction of organic capping reagents and this can result in bad surface passivation and functional group deprivation. Using citric acid and ascorbic as the carbon source we prevented the loss of organic matter and we have preserved the good properties of surface passivation. Low carbonization temperature (<140 °C) of citric and ascorbic acids enabled us to synthesize functionalized CQDs by microwave irradiation. For stability of nanomaterial we used surface capping agent PEG, PVP and BSA. The polymer provides surface functionality, which leads to enhanced luminescence and chemical stability that was confirmed by measurement of fluorescent intensities, that show that after two months CQDs have high luminescence and bright blue color.

The influence of various synthesis parameters, including the reaction temperature and time, on stability, quality and photoluminescence (PL) of CQDs were investigated. The spare solutions were colorless and no luminescence was observed. It was also found that after 24 h the solution shows no fluorescence. However, the use of fresh solutions is to prevent formation of any centers for crystallization. Under microwave irradiation, CQDs with blue emission wavelength were produced by varying the reaction temperature and time. It is obvious that higher temperature leads to increasing growth of CQDs. In our experiments we have heated six samples used the same power of microwave oven (300 W) and temperature in the range from 60 ºC to 140 ºC for 20 min. The samples heated in temperature range from 60 ºC to 120 ºC didn't show any emission of fluorescence under transilluminator and after fluorescence spectra measuring, from that we can conclude that lower temperature (<120ºC) is not sufficient for the synthesis of CQDs independently of length of the sample heating. Higher temperature (120 ºC - 140 ºC) led to production of small particles which blue emission luminescence. The same was observed in case when temperature above 120 ºC was used and time of reaction was 20 min. The photoluminescence (PL) spectra of six samples prepared at 300 W, 20 min of heating and different temperatures (120 ºC, 130 ºC and 140 ºC) and pictures of the samples under transilluminator are given in Fig. 2.

Measuring the fluorescence at excitation wavelengths of 280, 330, 340, 360, 380, 400, 420 and 430 nm were used for CQDs. In the case of the best results were obtained after heating of sample for 20 minutes at 130 ºC (red curve).
Fig. 1: Diagram for the synthesis of PEG, PVP and BSA-functionalized CQDs. (A) Synthesis of CQDs using citric acid and ascorbic as carbon source. (B) Capping of CQDs with PEG. (C), Capping of CQDs with PVP. (D), Capping of CQDs with BSA.

Fig. 2: Measuring the CQDs fluorescence with excitation wavelength at 280, 330, 340, 360, 380, 400, 420 and 430 nm. Blue curve-120 °C, red curve-130 °C and green curve 140 °C. Maxima of fluorescence intensity, (A) Citric acid capped with PVP, (B) Ascorbic acid capped with PVP, (C) Citric acid capped with PEG, (D) Ascorbic acid capped with PEG, (E) Citric acid capped with BSA, (F) Ascorbic acid capped with BSA.
When we look at the excitation wavelength the highest narrow peak at 340 nm of emission wavelength was found. The fluorescence intensity at 340 nm increases according to temperature of heating; it can be seen from the obtained fluorescence intensity (Fig. 2a). However, second peak was also obtained for fluorescence measurement at 340 nm. This can be explained as aggregation of smaller particles into larger structure according to Goncalves et al.\textsuperscript{29}. Samples heated on 120 °C (blue curve) and 140 °C (green curve) show that they have the same narrow peak at 340 nm, but fluorescence intensity were decreased with changing temperature of heating. From observed results we can see that the fluorescence intensity of CQDs was the best when the sample was heated on 130 °C. The variation in fluorescence intensity at 340 nm can be explained by bonding of PVP by oxygens on the surface of carbon precursor and stabilization of CQDs under different temperature of heating the sample. Also, independently from fluorescence intensity, picture that was taken under UV light does not show bright blue color. Sample 3 shows the same results as can be seen from (Fig. 2c), the fluorescence intensity are lower if we compare with 1 and picture of sample taken under UV light do not show any luminescence. For sample 5 (see Fig. 2e) two peaks were also observed. When the sample was heated at 130 °C (red curve) the highest narrow peak was obtained at 340 nm and second peak was obtained at 400 nm. According to Pyng Yu et al.\textsuperscript{29}, the peak at 340 nm is described to π−π* transition of aromatic C-C bonds, while a shoulder at 400 nm attributes to n−π* transition of C-O bonds. Carboxyl group may originate from the surface of CQDs, while C-C from the core. Sample heated on 140 °C (green curve) and sample heated on 120 °C (blue curve) show highest peak at 360 nm and second peak at 340 nm and this can be explained as an effect of samples heating on different temperature. However, if we compare this figure with another we can see that these CQDs who were heated at 130 °C have the higher fluorescence according to the results and picture taken under UV light.

In the case of samples 2, 4 and 6 carbon precursor was ascorbic acid. Measuring the fluorescence of sample 2 (Fig. 2b) the best results were obtained after heating of sample for 20 minutes at 120 °C (blue curve) were the highest narrow peak was found at 380 nm of emission wavelength with highest fluorescence. At 140 °C the narrow peak was found at 400 nm but fluorescence decreased, and on 130°C the narrow peak was found at 360 nm with very low fluorescence. However, sample 4 shows that sample heated on 130 °C have highest fluorescence with highest narrow peak found at 360 nm (red curve). Samples heated on 120 °C (blue curve) and 140 °C show that they have the same narrow peak at 360 nm, but fluorescence intensity decreased with temperature changing. In the case of sample 6 heated on 130 °C has highest fluorescence with highest narrow peak found at 360 nm (red curve). Sample that was heated on 140 °C (green curve) shows lower fluorescence and narrow peak was found at 340 nm and sample heated on 120 °C (blue curve) shows the lowest fluorescence with narrow peak at 360 nm. However, if we compare results in Fig. 2 b, d and f we can see that for synthesis of CQDs the main role plays the temperature were with changing the temperature narrow peak was found on another excitation wavelength and the capping agent according from the results and picture taken under UV light. Also the highest luminescence with bright blue color shows the sample 6 capped with BSA. The observed fluorescence of samples 5 and 6 seems to be promising for imaging in biological samples. It is also very important to mention that citric and ascorbic acid don’t show any fluorescence after heating at same temperature without capping agent.

4. Conclusion

This work has demonstrated the success in converting citric and ascorbic acid as carbon precursor and PEG, PVP, BSA as capping agent into CQDs via a simple and effective carbonization method. The CQDs were found to be highly fluorescent with good stability. The highest fluorescence was obtained using BSA as capping agent. However, another samples
also show high fluorescence but without high luminescence under UV light. Using a citric acid as carbon source show that higher fluorescence were obtained after heating sample at 130 °C. The resulting fluorescence of the samples was found to decrease at different temperature above 130 °C. But in the case of citric acid used as carbon precursors we can see that main role in increasing of fluorescence has capping agent. Depending on capping agent the temperature necessary for synthesis of highly fluorescent CQDs were constantly changing. This study serves as a novel demonstration of an easy and economical method to produce CQDs that can lead to their potential for optical sensing applications. This is especially with further modifications such as surface capping of the CQDs or doping the CQDs with various dopants are performed that might improve the sensitivity of the detection.

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