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# Carbon dots with media-independent fluorescence

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ABSTRACT Since their discovery, carbon dots have been of great scientific interest due to their unique properties, including strong fluorescence and biocompatibility, which determine their potential application in biosensorics, bioimaging, drug delivery, and many other fields. This paper presents a new approach for the synthesis of high quantum yield carbon dots with media-independent fluorescence developed in the process of searching for solutions to the problem of carbon dot's application in immunochromatographic analysis.

KEYWORDS carbon dots, nanoparticles, immunoassay

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## 1. Introduction

In immunochemical methods of analysis as labels stand for forming an analytical signal depending on the analyte concentration colloidal gold nanoparticles are typically used [1–4]. Such systems are expensive, have high detection limits and low efficiency. Quantum dots are much more advanced labels allowing one to achieve a high level of sensitivity in immunochemical analysis. However, they are still very expensive and have several disadvantages, such as high cost, biotoxicity, and the need for surface modification preceding their conjugation with biomolecules [4–6]. Carbon dots (CDs) can serve as an alternative to quantum dots. CDs have found wide application in sensorics, microelectronics and other fields. CD-based systems sensitivity can be comparable with systems based on the quantum dots, while the CDs themselves are biocompatible, easy to synthesize and cheap to produce [7–9]. The implementation of CDs as analytical labels in immunoassay is complicated by three main disadvantages in comparison with quantum dots: solvatochromism, excitation-dependent emission shift and aggregation-dependent emission shift [9]. Such phenomena can be useful in the development of sensors based on carbon dots [10–12]. However, they lead to unpredictable behavior of optical properties of nanoparticles when conjugated with biomolecules, which significantly complicates the testing process. In this study, a new approach for the synthesis of CDs designed to fix some of the common problems is presented.

## 2. Materials and methods

Reagents were used for the work: Citric Acid anhydrous (Reag. USP) for analysis, ACS, 99.5 % PanReac-AppliChem; Tris(hydroxymethyl)aminomethane; 99.7 % Sigma Aldrich; N,N-Dimethylformamide, 99.8 % for synthesis, PanReac-AppliChem; Dimethylsulfoxide, 99.5 % PanReac-AppliChem; Ethanol, 99.9 % LiChrosolv, Supelco; Bidistilled water.

Consumables were used to clean the obtained materials: Omicron Syringe Filter 0.22 $\mu$m and 0.45 $\mu$m, PES 33 mm, non-sterile; Dialysis Tubing MWCO 0.5 kDa.

Obtaining and study of the physical and chemical properties of the derived samples was carried out with the use of the following equipment: Microwave oven Sineo MDS-10; Spectrofluorometer Solar CM 2203; Spectrophotometer SPEX SCP 715; Fourier transform infrared spectrometer Shimadzu IRAffinity-1S + MIRacle 10.

The calculations of quantum yield of luminescence samples are based on formula (1): [13]

$$\varphi_x = \frac{I_x \cdot \varphi_{st} \cdot \left(1 - T_{st(\lambda_{exst})}\right) \cdot n_x^2}{I_{st} \cdot \left(1 - T_{x(\lambda_{exx})}\right) \cdot n_{st}^2}, \tag{1}$$

where $\varphi_x$ is the fluorescence quantum yield of the analyzed sample, $\varphi_{st}$ is the fluorescence quantum yield of a standard fluorescent dye, $I_x$ is the integral fluorescence intensity of the analyzed sample, $I_{st}$ is the integral fluorescence intensity of a standard fluorescent dye, $T_x$ is the transmission coefficient of the analyzed sample at the excitation wavelength, $T_{st}$ is the transmittance coefficient of a standard fluorescent dye at the excitation wavelength. A 0.5 M quinine sulfate solution in sulfuric acid was employed as a standard, exhibiting a fluorescence maximum at 440 nm and a quantum yield of 54.5 % when excited at 345 nm. The optical properties of the carbon dot sample were studied in a dilute aqueous solution. The fluorescence spectrum ordinates for both the standard and the sample were converted from cps to energy units ($W/m^2 \cdot nm$) to calculate the integral fluorescence intensities.

The synthesis of CDs was performed in a microwave digestion system Sineo MDS-10 with teflon-lined vessels. Suspensions of 3.6 mmol of citric acid and 14.4 mmol of tris-(hydroxymethyl)-aminomethane were dissolved in 5 ml of bidistilled water, then it was placed in a microwave oven for 30 min at a radiation frequency of 2.45 GHz, power 700 W, maximum heating temperature 200 °C. The resulting yellow solution was purified by dialysis (0.5 kDa).

## 3. Results and discussion

Citric acid (CA) is the most common reagent for the fabrication of CDs by the microwave-assisted methods [14]; however, much better results are demonstrated by CDs obtained by a combination of CA and an amino-containing compound such as urea [15]. In this study tris-(hydroxymethyl)-aminomethane (Tris) was proposed as an additional reagent for the production of citric acid-based CDs similar to publications [16–18]. Tris is a polar compound that has a relatively high dielectric loss tangent and is able to react with citric acid by formation of amide and ester bonds [19]. These properties of Tris are commonly used for surface passivation of carbon dots leading to an increase in their quantum yield.

The optical properties of obtained CD's sample were studied by UV/Vis-spectroscopy and fluorimetry. The results are shown in Fig. 1.

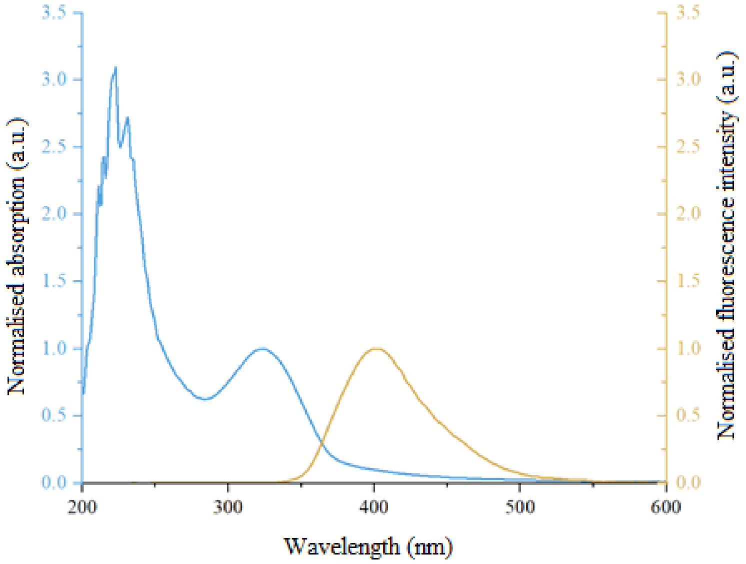

FIG. 1. Absorption and luminescence spectra of CDs derived from citric acid and Tris

The absorption spectrum of CDs derived from citric acid and Tris exhibits two absorption bands with maximums near 225 and 330 nm (Fig. 1). The absorption peak near 225 nm is supposedly due to electronic transitions of organic functional groups in the amorphous part of CDs while the broad band near 330 nm corresponds to absorption of $sp^2$-hybridised carbon fragments of different nature. The fluorescence peak of these CDs at 405 nm can be characterized as rather wide with negligible asymmetry in the long wave region. For the CDs synthesized in this work, the calculated quantum yield of fluorescence was 70 %, which is a great value for this class of fluorescent materials.

The relatively high content of Tris relative to CA distinguishes this study from the others. The excess of the passivation agent leads to bonding with all carboxyl groups conjugated to the graphitic core.

Interruption of conjugation allows to get rid of some media-dependent properties of CDs such as solvatochromism (Fig. 2a, Fig. 2c) and excitation-dependent emission shift (Fig. 2b) [20].

For the CDs dissolved in different solvents, such as water, ethanol, DMF, and DMSO, the fluorescence maximum remains in the range of 400 – 407 nm. Also, the decrease in the quantum yield in alkaline media does not exceed the measurement error. The negligible decrease in the quantum yield in acid media can appear due to the protonation of nitrogen atoms of amide bonds that are still conjugated to the core [12,21–23].

The surface chemistry of obtained CD's sample was studied by IR-spectroscopy. The IR spectrum (TIR mode) of the CDs obtained in this work is shown in Fig. 2.

The IR spectrum (TIR mode) of the CDs obtained in this work contains characteristic absorption bands at 1039 $cm^{-1}$ (O–H def.), 1652 $cm^{-1}$ (C=O val. of amide) and wide characteristic band at 3280 $cm^{-1}$ (O–H val.) proving the presence

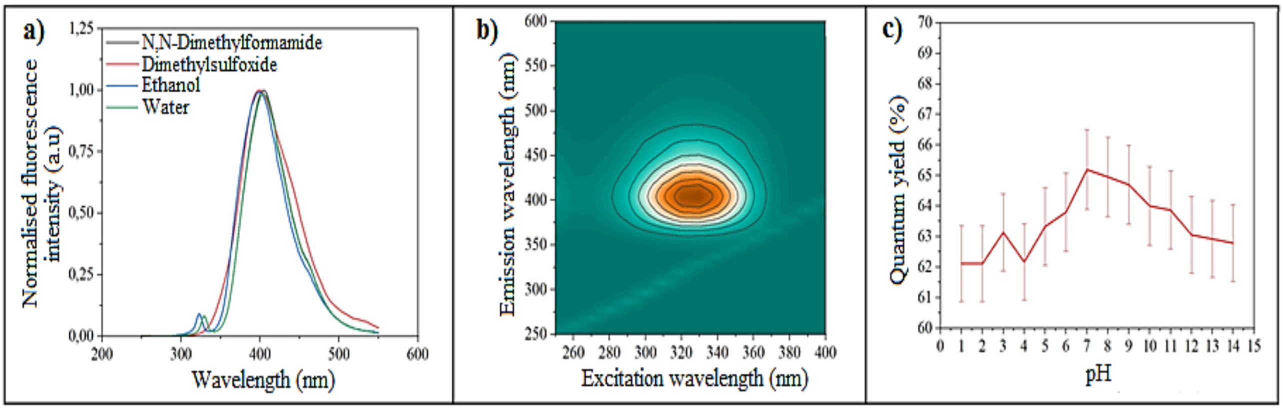

FIG. 2. a) – Fluorescence spectra of CDs derived from CA and Tris in different solvents; b) – Excitation to emission wavelengths diagram of CDs derived from CA and Tris in water; c) – Quantum yield of CDs derived from CA and Tris in aqueous solutions with different pH values

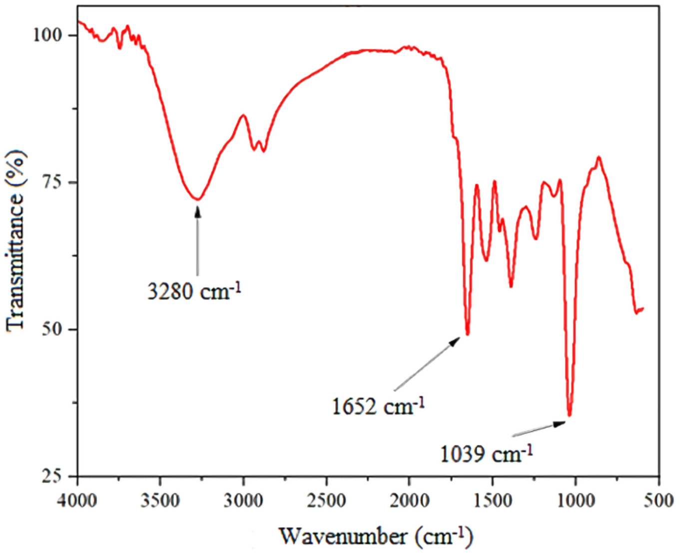

FIG. 3. IR spectrum of CDs derived from CA and Tris

of ester and amide bonds into the CD's surface (Fig. 3.). CDs derived from CA usually possess high quantity of terminal carboxylic groups [20,24]. The complete absence of characteristic bands of free carboxyl groups indicates that the surface passivation was carried out completely.

## 4. Conclusion

CDs derived from citric acid and Tris are characterized by a rather wide fluorescence peak but with a high quantum yield of 70 %. Fluorescence independent of pH and excitation wavelength highlights them among CDs obtained by other methods. This result was achieved due to the interrupted conjugation of surface carboxyl groups with the core of CDs via Tris excess. All these features make citric acid and Tris-based CDs perspective for use as inexpensive, easy-to-produce, and highly fluorescent analytical labels for immunochemical assay.

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