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Synthesis of Amino Acid-Functionalized Carbon Quantum Dots from *Citrus clementina* Peel: Investigating the Antiradical Activity and Selectivity of Metal Ion Detection

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INTRODUCTION

Carbon quantum dots (CQDs) belong to a group of new and efficient carbon photoluminescent nanomaterials that have attracted the attention of many scientists over the past decade, especially due to their excellent chemical and optical properties. Their chemical stability, biocompatibility/low toxicity, water solubility and optical efficiency represent a huge potential for a wide range of applications in biomedical research and nanotechnology. This study presents a simple, inexpensive, environmentally friendly and green synthesis of N-doped carbon quantum dots from biomass, specifically from freeze-dried *Citrus clementina* peel and five different amino acids (Leu, Trp, Arg, Ala, His). Firstly, the differences between the chemical, structural and biological properties were studied. The incorporation of nitrogen into the CQDs obtained from biomass has led to an increase in quantum yield and in general, improvement in the performance and sensitivity of nanoprobes, compared to the blank system (without the addition of amino acids). The prepared N-doped CQDs showed great stability in aqueous/high ionic media and similar optical properties, while differences in biological activity and selectivity in metal ion detection were observed.

CHEMICAL/STRUCTURAL/OPTICAL CHARACTERIZATION

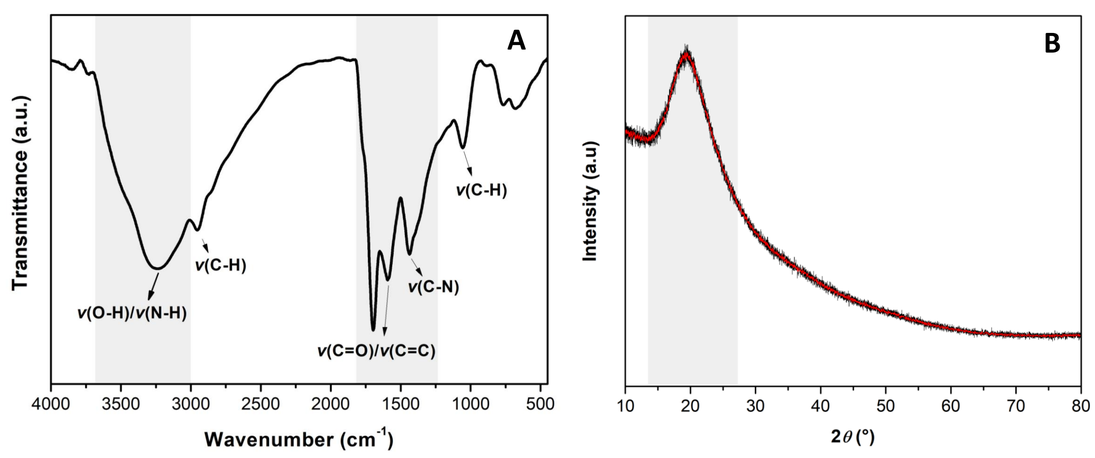

Fig 1. Chemical characterization of CQD@Arg: A) FTIR spectra, and B) PXRD diffractogram of sample.

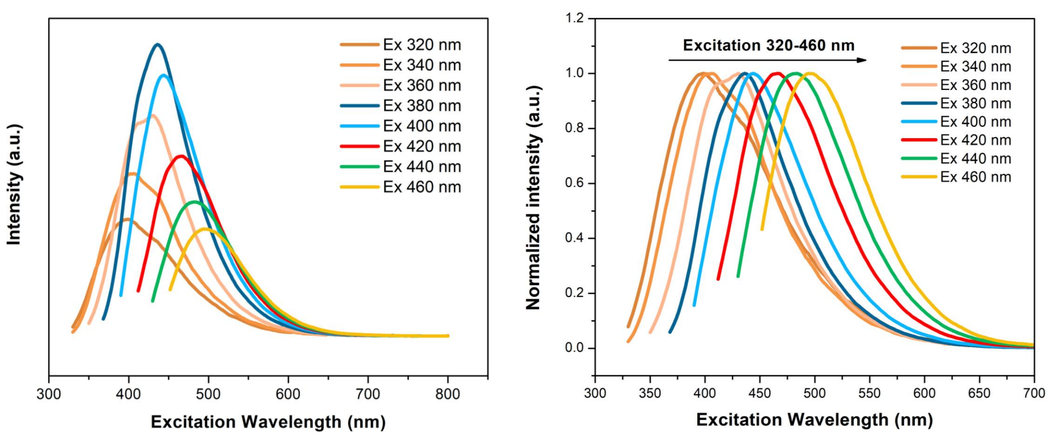

Fig 2. Optical characterization of CQD@Arg: A) Fluorescence emission spectra of CQD@Arg at different excitation wavelengths ranging from 320 nm to 460 nm (increments of 20 nm); (B) normalized fluorescence emission intensity.

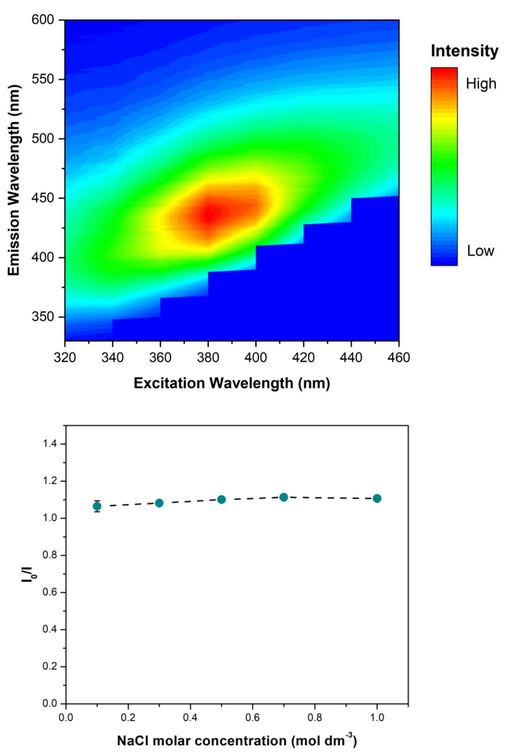

Fig 3. Optical characterization of CQD@Arg: A) Contour map of the three-dimensional fluorescence spectra of CQD@Arg, and B) stability in high ionic media (NaCl solution).

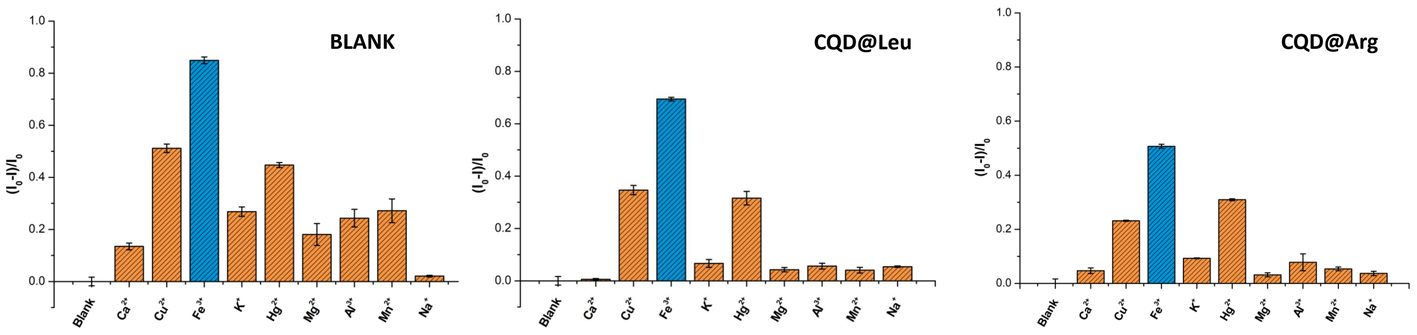

Fig 4. Fluorescence response of different CQDs to different metal ions.

MATERIALS AND METHODS

BIOMASS WASTE – CITRUS CLEMENTINE PEELS
- Freeze-dried peels
- grinded (5000 RPM, t=1min)
- sieved (pore 600 µm)

CITRUS CLEMENTINE PEEL EXTRACT
- 1 g of grinded peels – dispersed in 100 ml of Mill-Q H_2O
- ultrasonicated (t=30min, f=37 Hz, P=50 W)
- Samples filtered using syringe filters (pore size: 0.2 µm)

PREPARED SAMPLES:
RUN 1 – blank
RUN 2 – 175 mg Leu
RUN 3 – 175 mg Trp
RUN 4 – 175 mg Arg
RUN 5 – 175 mg Ala
RUN 6 – 175 mg His

HYDROTHERMAL SYNTHESIS – RUN:
1 – BLANK – Pure CQDs
- 20 ml of the extract – transferred to a Teflon-lined stainless steel autoclave (Parr Instruments, USA)
- Heated up in an air oven
- Conditions: θ=180°C, t = 9h

HYDROTHERMAL SYNTHESIS – N-doped CQDs (RUN 2 – RUN 6)
- 175 mg of amino acid dissolved in 5 ml of Mill-Q H_2O + 15 ml of citrus extract - transferred into autoclave and heated up at same conditions

PREPARED CQDs
- centrifuged at 10 000 RPM
- filtered (pore 0.20 µm)
- dialyzed against Milli-Q H_2O (t=48h)

PURIFIED CQDs

stored in the dark at 4 °C for further analysis

ANALYTICAL METHODS
- ✓ Quantum Yield (QY)
- ✓ UV/Vis spectroscopy
- ✓ X-Ray Powder Diffraction (PXRD)
- ✓ Antiradical Activity (DPPH)
- ✓ Selective and sensitive metal detection (Ca^{2+}, Cu^{2+}, Fe^{3+}, K^+, Hg^{2+}, Mg^{2+}, Al^{3+}, Mn^{2+}, and Na^+)

Table 1. The list of all prepared CQDs samples with corresponding Max λ_{EM} / λ_{EX} and determined quantum yield values.

No.	Sample of CQDs	Max λ_{EM} / λ_{EX}	Quantum yield (%) at 360 nm
1.	Blank	465/380 nm	0.91
2.	CQD@Leu	460/380 nm	1.72
3.	CQD@Trp	453/380 nm	3.35
4.	CQD@Arg	436/380 nm	4.59
5.	CQD@Ala	440/380 nm	4.20
6.	CQD@His	437/380 nm	4.19

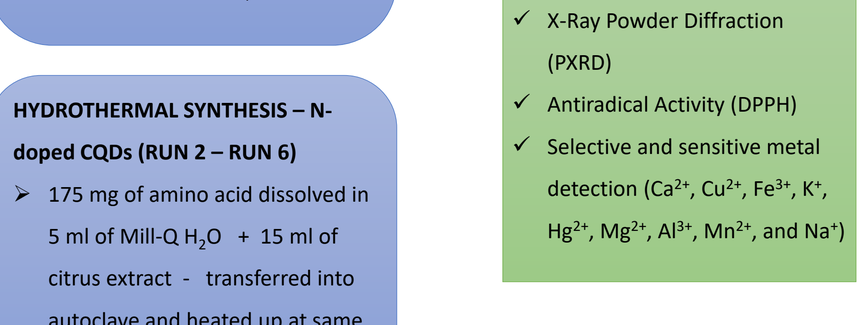

Image 1. CQDs under UV light – 1) Blank, 2)CQD@Leu, 3)CQD@Trp, 4)CQD@Arg, 5)CQD@Ala, 6)CQD@His

Table 2. Results of antiradical activity investigated on all prepared samples of CQDs.

Sample of CQDs	Antiradical activity (%) at 100 µg/mL	
	(Average ± StDev)	EC_{50} (µg/mL)
Blank	97.41±2.59	17.94±1.12
CQD@Leu	99.48±0.13	24.87±0.12
CQD@Trp	99.85±0.26	32.14±1.45
CQD@Arg	99.43±0.46	42.35±2.47
CQD@Ala	95.70±0.11	49.01±0.77
CQD@His	93.22±0.87	42.11±1.13

CONCLUSIONS

<u>This work reports biomass-derived carbon quantum dots (CQDs) modified with different amino acids, resulting in a successful nitrogen doping of CQDs.</u>

1. Prepared amino acid-funtionalized CQDs possessed good biocompatibility, satisfactory optical and chemical properties.
2. The samples of CQDs were prepared at temperature of 180°C during 9 hours.
3. The highest determined quantum yield was achieved with the CQD@Arg (QY=4.59%). The Blank sample demonstrated the lowest quantum yield (QY=0.91%).
4. The maximum emission wavelenght of CQD@Arg eas determined at 436 nm, when excited at 380 nm.
5. The excellent stability in high ionic media (c(NaCl)=1 mol dm^{-3}) and solubility in water demonstrated the great potential application in biological systems.
6. All the prepared samples showed excellent antiradical activity, while the optimal EC_{50} = 17.94±1.12 µg/mL was determined with Blank sample.
7. Overall, the antiradical activity among the prepared samples ranged from 93.22±0.87% to 99.85±0.26% at highest concentration of 100 µg/mL .

The presented study may represent a novel and useful approach for efficient utilization of the waste for practical applications, including those in analytical chemistry, biomedicine and environmental monitoring.

ACKNOWLEDGEMENT

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