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PREPARATION OF AMINO ACID-FUNCTIONALIZED CARBON QUANTUM DOTS USING CLEMENTINE PEEL – Potential Application in Biomedical Analysis and as Fluorescent Probe for Fe^{3+} Detection

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INTRODUCTION

Luminiscent carbon quantum dots (CQDs) are widely known as zero-dimensional nanomaterials which have attracted extensive attention, especially in green chemistry and biomedicine. Due to their excellent biocompatibility and low toxicity, water solubility, stability in high ionic media and great optical properties, CQDs have been widely used as functional optical materials in fluorescence sensing. In this study, preparation and modification of CQDs using clementine peel as carbon precursor and amino acids with different chemical complexity (glycine and arginine - nitrogen dopants) has been presented. It has been demonstrated that increasing nitrogen content in CQDs samples has increased the quantum yield percentage of prepared CQDs. Some differences in sample properties were observed regarding structural and chemical diversity, biological and antioxidant activity. The antiproliferative effect of CQD@Gly against pancreatic cancer cell lines (CFPAC-1) was demonstrated. Based on the DPPH assay results, the CQD@Arg demonstrated the highest antiradical activity 81.39 ± 0.39%, and EC_{50} was determined to be EC_{50} = 53.78 ± 0.97 μg/mL (R^2 = 0.9357). Furthermore, due to the highest determined quantum yield, CQD@Arg sample was further used for the ion sensing and cellular imaging of cancer cells. The CQD@Arg was applied as a fluorescent nanoprobe for Fe^{3+} detection, with a good linear correlation in the concentration range from 7.0 μmol dm^{-3} to 50.0 μmol dm^{-3} with R^2 = 0.9931 and limit of detection (LOD) of 4.57 ± 0.27 μmol dm^{-3}. In order to investigate the applicability of prepared CQDs in cell imaging, MCF-7 cells were incubated with CQD@Arg and imaged by confocal microscopy. This study implies the potential application of the prepared CQDs in bioimaging and ion sensing, and also as a fluorescent probe with diverse biological and pharmacological activities in general.

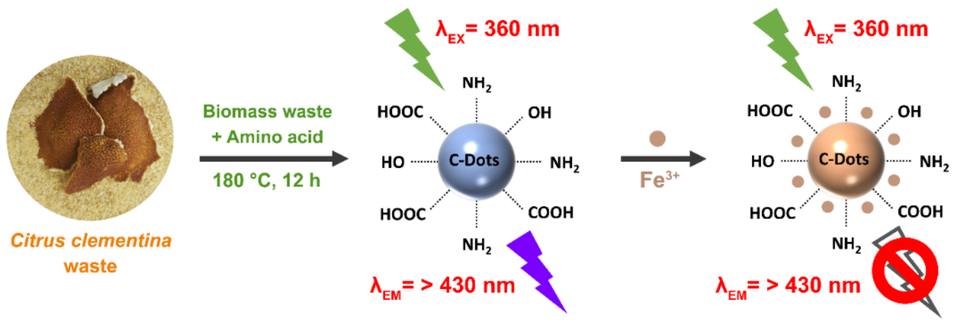
MATERIALS AND METHODS

Citrus clementina peel CARBON PRECURSOR

Amino Acids Gly and Arg NITROGEN DOPANTS

→ HYDROTHERMAL SYNTHESIS (180°C, 12 h) → *BLANK (without amino acids), CQD@Gly and CQD@Arg are obtained* → PURIFICATION (dialysis, MCWO 1kDa); STORAGE AT 4°C; CHARACTERIZATION AND ANALYSIS

The calculated quantum yields of all prepared samples were as follows: 1.17 ± 0.12% for blank CQD, 1.53 ± 0.23%, and 2.17 ± 0.11% for CQD@Gly and CQD@Arg – hence, CQD@Arg was used for further investigations.



CHEMICAL/STRUCTURAL/OPTICAL CHARACTERIZATION OF CQD@Arg

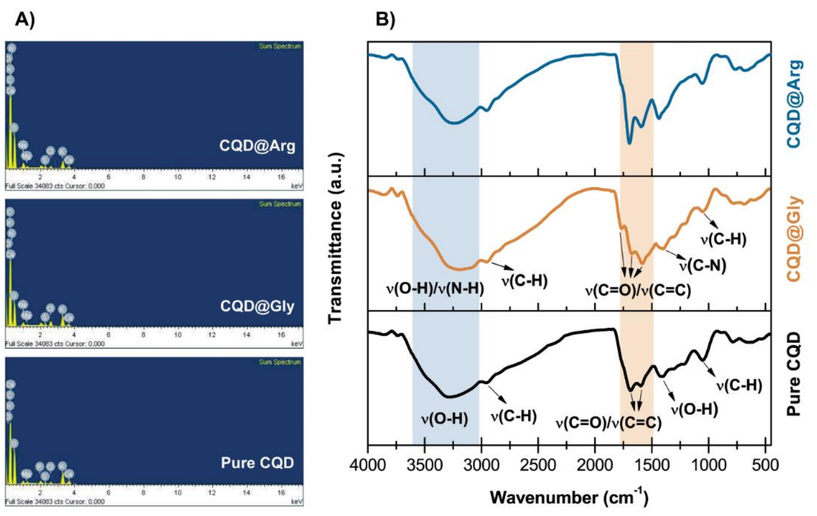


Figure 1. EDS spectrum (**A**) and FTIR spectra (**B**) of prepared CQDs and N-CQDs.

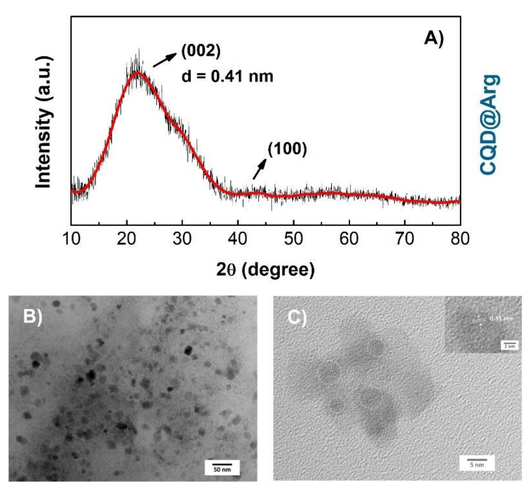


Figure 2. A) XRD pattern of CQD@Arg, **B**) TEM images showing CQD@Arg nanoparticles, and **C**) HR-TEM image showing lattice spacing of *d* = 0.31 nm.

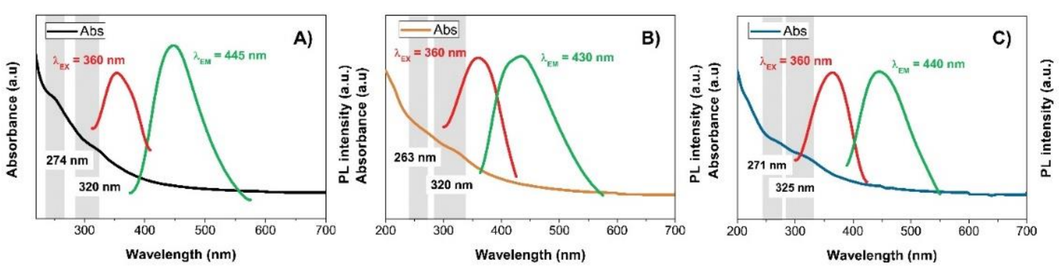
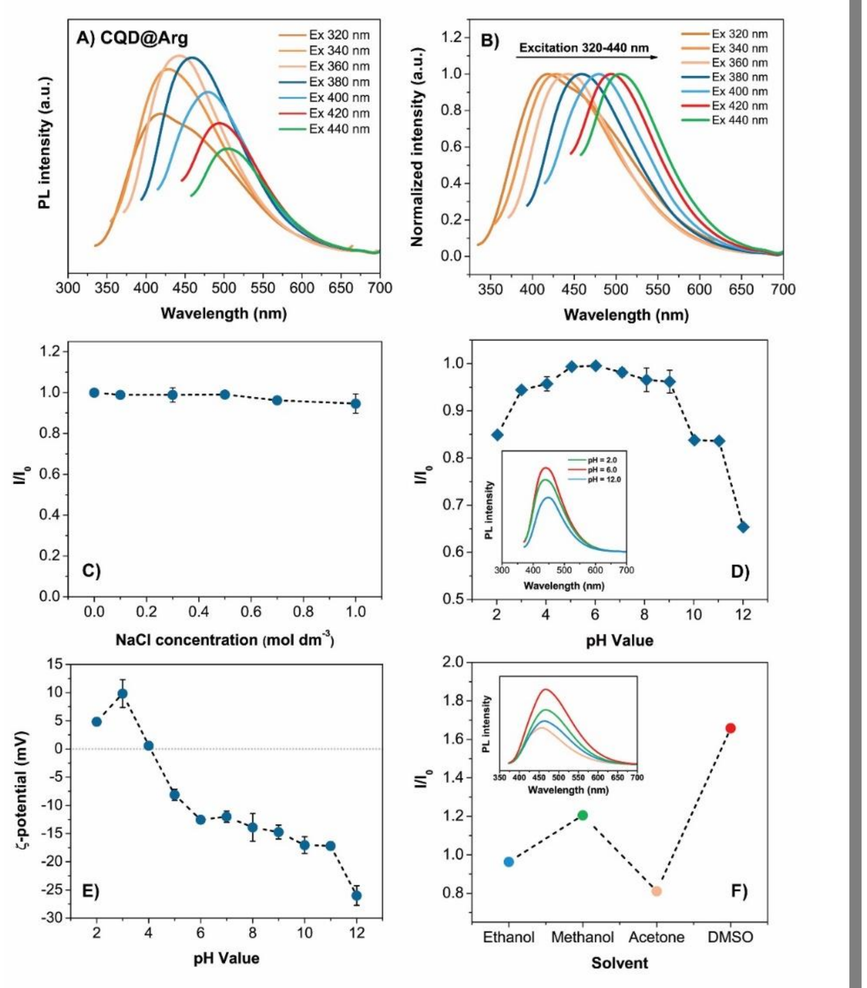


Figure 3. UV-vis absorption spectrum with maximum fluorescence excitation and emission spectrum of A) Pure CQD, B) CQD@Gly, and c) CQD@Arg.

Figure 4. Optical characterization of CQD@Arg: A) Fluorescence emission spectra of CQD@Arg at different excitation wavelengths ranging from 320 nm to 440 nm (increments of 20 nm); B) normalized fluorescence emission intensity, C) stability of CQD@Arg in the high ionic medium of NaCl (0–1.0 mol dm^{-3}; λ_{Ex} = 360 nm); D) fluorescence intensity vs. pH variation (λ_{Ex} = 360 nm); E) variations of ζ-potential as a function of pH; F) fluorescence spectra of CQD@Arg in ethanol, methanol, acetone, and DMSO (λ_{Ex} = 360 nm).

OPTICAL CHARACTERIZATION OF CQD@Arg



APPLICATION IN IRON(III) DETECTION – CQD@Arg AS NANOPROBE

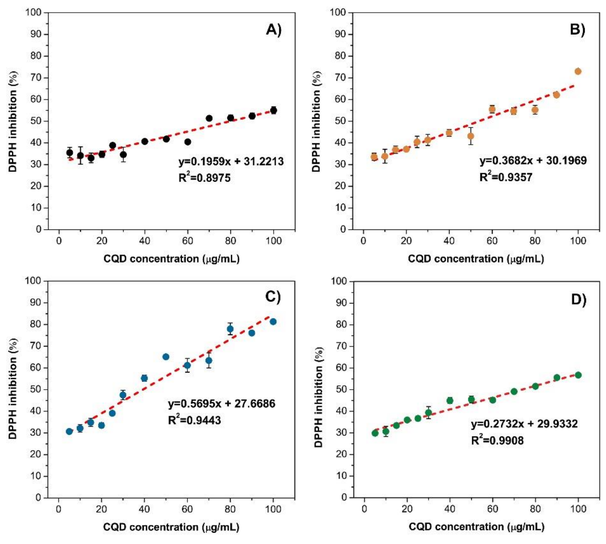


Figure 5. Antioxidant activity of prepared CQDs using DPPH free radical assay for **A**) pure CQD; **B**) CQD@Gly; **C**) CQD@Arg, and **D**) *Citrus clementina* extract.

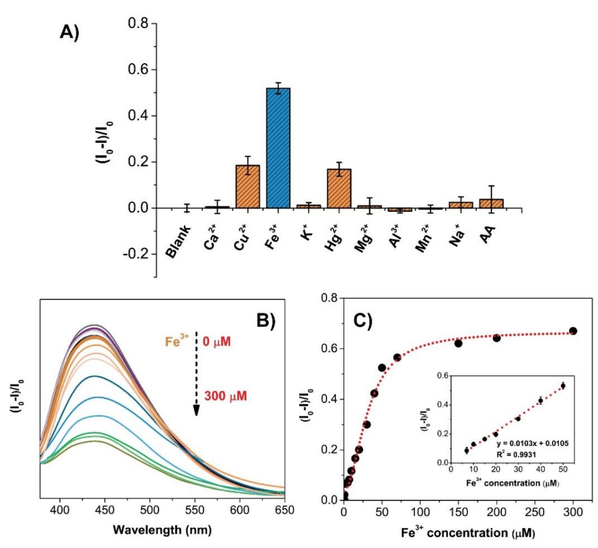
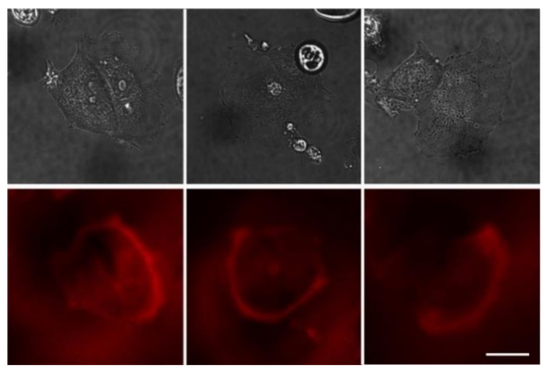


Figure 6. A) Fluorescence response of CQD@Arg to different metal ions and ascorbic acid, **B**) the fluorescence spectral quenching upon the addition of different Fe^{3+} concentrations (0.5–300 μmol dm^{-3}); and **C**) the relative fluorescence response $(I_0-I)/I_0$ of CQD@Arg with the Fe^{3+} addition confirming exponential behaviour (inset: linear response ranging from 7.0 to 50.0 μmol dm^{-3}).

Sample	Cell line IC_{50} (μg/mL)[1]					
	HepG2	CFPAC-1	MCF-7	HCT-116	HFF-1	
Pure CQD	>100	>100	>100	>100	1st experiment	20.59 ± 0.02
					2nd experiment	1.50 ± 0.02
					3rd experiment	>100
CQD@Arg	>100	>100	>100	>100	1st experiment	7.85 ± 0.02
					2nd experiment	Proliferative effect
					3rd experiment	>100
CQD@Gly	>100	6.91 ± 0.81	>100	>100	1st experiment	0.46 ± 0.01
					2nd experiment	Proliferative effect
					3rd experiment	>100

Table 1. Antiproliferative activity in vitro for samples of pure CQD, CQD@Arg, and CQD@Gly presented as IC_{50} values (μg/mL) ± standard deviation. Results from experiments carried out on human fibroblasts HFF-1 are presented for each biological replicate separately, while results for tumour cell lines are presented as means from three consecutive experiments.

Figure 7. A) Microspectrofluorimetry of CQD@Arg in the visible light range. The double-lambda plot of CQD@Arg adhered to the glass surface was obtained using excitation between 470–650 nm and detecting emission of fluorescence between 490–770 nm. The maximum emission was detected using excitation at 610 nm, and those conditions were used for cell imaging. B) Living MCF-7 cells labelled with CQD@Arg imaged by confocal microscopy in transmission (upper row) and fluorescence (lower row) channels (λexc = 610 nm; λem = 620–690 nm). Average fluores-cence intensity projections of 3D stacks covering the cell thickness are shown in the fluorescence channel: scale bar, 20 μm.



CONCLUSIONS

<u>This work reports carbon quantum dots (CQDs) from citrus peel modified with amino acids Gly and Arg, resulting in a successful nitrogen doping of CQDs.</u>

1. The prepared samples containing nanosized particles (of the average size=8.52±1.72 nm) possessed good biocompatibility, satisfactory optical and chemical properties, and interesting biological activities.
2. The prepared CQD@Gly exhibited the specific antiproliferative effect against pancreatic cancer cell line CFPAC-1, and CQD@Arg showed strong antioxidant activity (81.39 ± 0.39%) at a low concentration of 100 μg/mL.
3. The highest quantum yield was determined with CQD@Arg, which was further investigated for the Fe^{3+} ion sensing and bioimaging.
4. The developed model was described by an exponential function with a suitable coefficient of determination of R^2=0.9891, while the linear range was determined in the concentration range from 7 μmol dm^{-3} to 50 μmol dm^{-3} with a determined limit of detection of LOD=4.57±0.27 μmol dm^{-3} and limit of quantification of LOQ=15.24±0.89 μmol dm^{-3}.

These findings could demonstrate the potential application of the prepared CQDs in bioimaging and ion sensing as a fluorescent probe with antioxidative or specific antitumor effects. The presented study may represent a novel and useful approach for efficient utilization of the waste for practical applications, including those in biomedicine and analytical chemistry.

ACKNOWLEDGEMENT

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