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01–30 NOVEMBER 2022 | ONLINE

Evaluation of the role of different bottom-up synthesis procedures for Carbon dots in their potential as candidates as drug carriers

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pharmaceuticals MDPI

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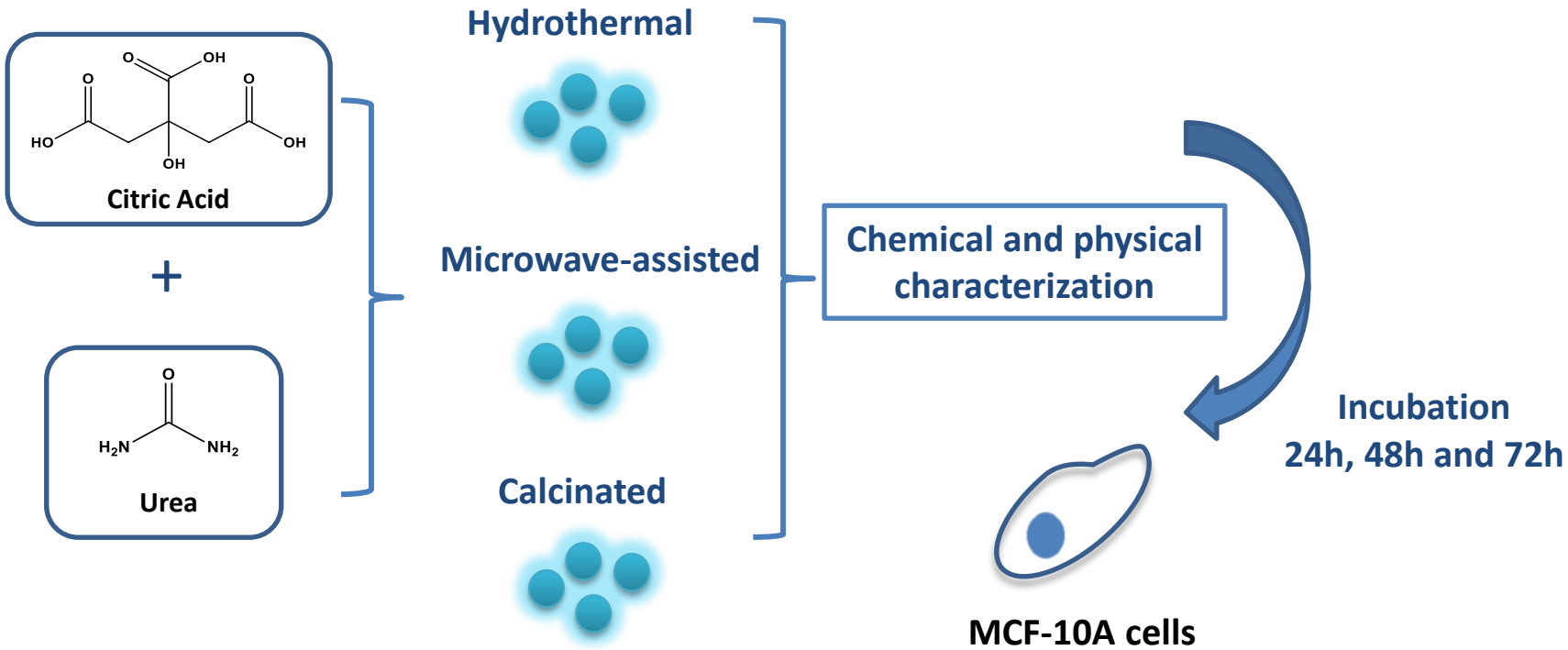
Evaluation of the role of different bottom-up synthesis procedures for Carbon dots in their potential as candidates as drug carriers

Graphical Abstract



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Abstract:

Carbon Dots (CDs) application in biomedicine has been increasing, due to their properties of high photoluminescence, biosafety and low cost, which allows for possible applications in bioimaging and as a drug carrier. However, their synthesis strategies are quite flexible, as tuning reaction precursors and synthesis procedures can lead to an endless number of CDs with distinct properties and applications, which difficult their rational development.

In this work [1,2], we performed a systematic evaluation of the effect of three representative bottom-up strategies (hydrothermal, microwave-assisted, and thermal heating) on the properties of CDs prepared from the same precursors (citric acid and urea). In this way, the CDs were thoroughly evaluated in terms of structure, morphology and photoluminescent properties. To screen their potential as drug carriers, the biosafety of these CDs was tested against the normal breast cell line MCF-10A, as drug carriers need to be compatible with healthy cells to minimize harmful side-effects.

The characterization results demonstrated a similar size range and composition for all CDs. While hydrothermal synthesis generates CDs with lower fluorescence and synthesis yields, and present an emission more dependent on surface states, they have the most promising viability profile of MCF-10A when compared with microwave-assisted and thermal-heating CDs, which present better fluorescence properties and better efficiency towards nitrogen-doping.

Our results suggest these CDs have potential to proceed further investigation in animal models as imaging candidates or biosensing tools as well as drug carriers for a future application in medicine.

Keywords: Bottom-up synthesis; Carbon dots; Drug carriers; MCF-10A

Introduction

Carbon Dots (CDs)

- Carbon dots (CDs) are carbon-based nanoparticles, tipically sized below 10 nm;

- Depending on the synthetic procedure and used precursors we can obtain different functional groups on the CDs` surface, such as carboxylic acids, alcohols and amines;

- CDs have very attractive properties:
  - ✓ High photoluminescence;
  - ✓ Low toxicity;
  - ✓ Good water solubility;
  - ✓ Biocompatibility;
  - ✓ Photochemical stability

Crista, D. M. A.; Esteves da Silva, J. C. G.; Pinto da Silva, L. Evaluation of Different Bottom-up Routes for the Fabrication of Carbon Dots. *Nanomaterials* **2020,** *10* (7), pp. 1316 DOI: 10.3390/nano10071316

Introduction

Carbon Dots (CDs)

- Due to their interesting properties, CDs are good candidates to be used as sensors, in bioimaging, photocatalysis, drug delivery, solar cells and photodynamic therapy;

- CDs can be obtained by two different ways:

Top-down

- ❖ Transformation of bigger graphitic materials into smaller carbon-based materials

Bottom-up

- ❖ Calcination of smaller organic molecules either in powder or solution

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Introduction

CDs as drug carriers candidates

- Breast cancer is the most common type of cancer in women and the second leading cause of cancer death worldwide;

- Despite the recent technological advances, the fields of diagnostic and treatment in breast cancer still have a lot of potential to explore;

- Due to their properties, fluorescent nanomaterials, such as CDs have gained increasing interest to be used in fluorescent imaging, transport drug delivery, medical diagnosis, biosensors, among others.

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Introduction

Carbon Dots (CDs)

- CDs synthesis by bottom-up strategies permits a large degree of tunability;

- Different properties and applications

THREE BOTTOM-UP STRATEGIES

- ✓ Hydrothermal;
- ✓ Microwave-assisted;
- ✓ Calcination.

PRECURSORS: Citric acid and urea

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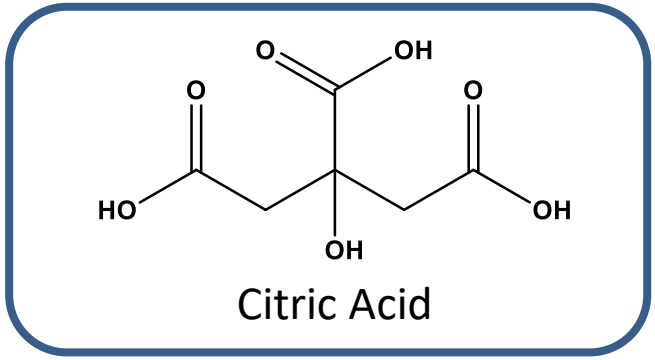
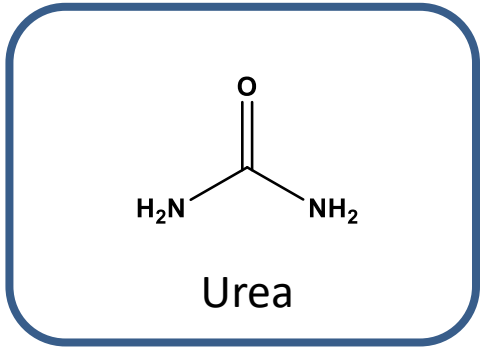
Introduction

Carbon Dots (CDs)



Citric Acid



Urea

Hydrothermal	Microwave	Calcination
CA/Urea (3:1) Deionized water Teflon-lined reactor 200ºC/2 hours in a oven	CA/Urea (3:1) Deionized water Glass beaker 5min/700W (domestic Microwave)	CA/Urea (3:1) Glass petri box 200ºC/2 hours in a oven

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Results and discussion

Carbon Dots (CDs)

Table 1. Synthesis and fluorescence quantum yields (in %) for the three CDs.

	HYDROTHERMAL	MICROWAVE	CALCINATION
Particle size (nm)	6.9±2.0	7.3±1.7	6.1±1.7
Synthesis yield (%)	1.8±0.4	28.5±4.5	26.9±1.1
Quantum yield (QY_{FL}) (%)	3.7	25.1	29.3

- The different CDs have similar sizes

- Calcination and Microwave synthesis routes are more suitable for high-yield synthesis (~27-29%) while hydrothermal synthesis present almost negligible yield (~2%)

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Results and discussion

Carbon Dots (CDs)

Table 2. Atomic composition (%) obtained by XPS for the three different synthesis.

	Hydrothermal-CDs	Microwave-CDs	Calcination-CDs
C (%)	62.0	60.0	61.9
N (%)	9.1	13.1	13.5
O (%)	28.8	26.9	24.7

- Microwave and Calcination strategies are more efficient towards nitrogen-doping;
- The studied bottom-up routes can lead to different degrees of N- and O-incorporation in the surface of CDs.

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Results and discussion

Carbon Dots (CDs)

- All CDs present similar blue emission (433-438nm) and UV excitation (320-340nm);
- All samples present an excitation-dependent emission

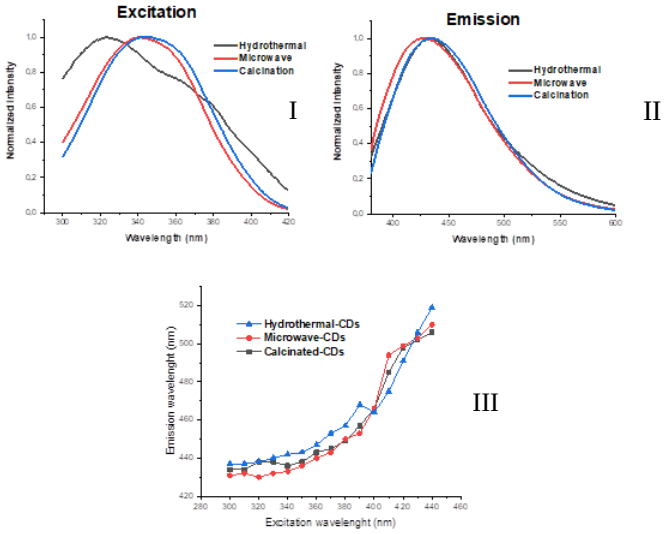

Figure 1. (I) Excitation and (II) emission-spectra in aqueous solution from Hydrothermal-, Microwave-, and calcinated-CDs, and (III) emission wavelength (nm) as function of the excitation wavelength (nm).

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Results and discussion

Carbon Dots (CDs)

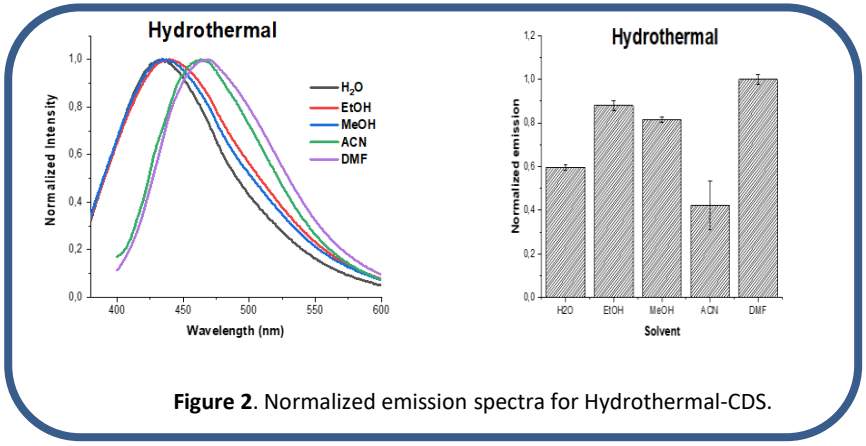

Figure 2. Normalized emission spectra for Hydrothermal-CDS.

- ✓ Fluorescent intensity varied widely with the solvent;
- ✓ Emission maxima underwent a ~35 nm red-shift in the two aprotic solvents;
- ✓ The fluorescent moieties should be exposed to the external environment.

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Results and discussion

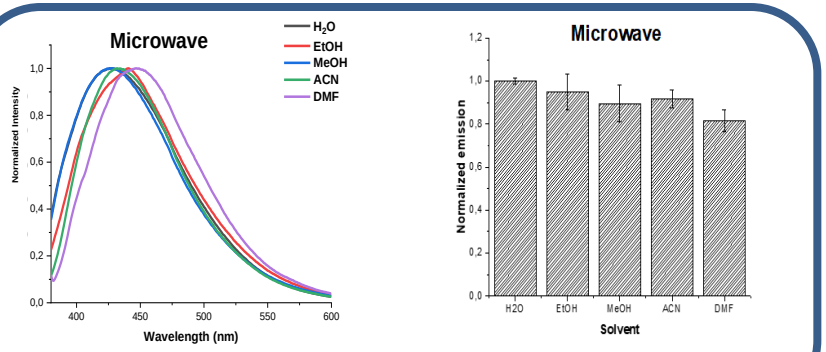

Figure 3. Normalized emission spectra for Microwave-CDS.

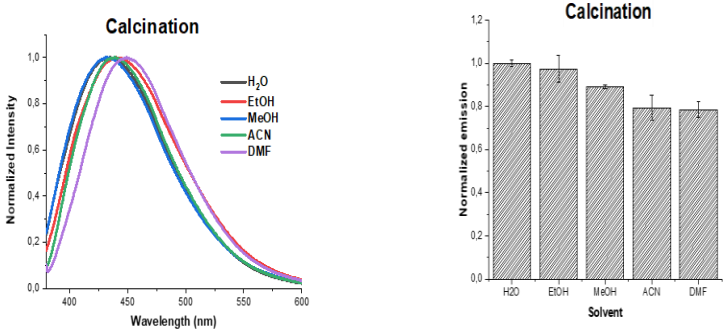

Figure 4. Normalized emission spectra for Calcination-CDS.

- ✓ The solvent has limited influence on the emission intensity;
- ✓ Maximum red-shift of just ~10 nm in DMF;
- ✓ The fluorescent moieties are shielded from the external environment.

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Results and discussion

CDs cytotoxicity against MCF-10A

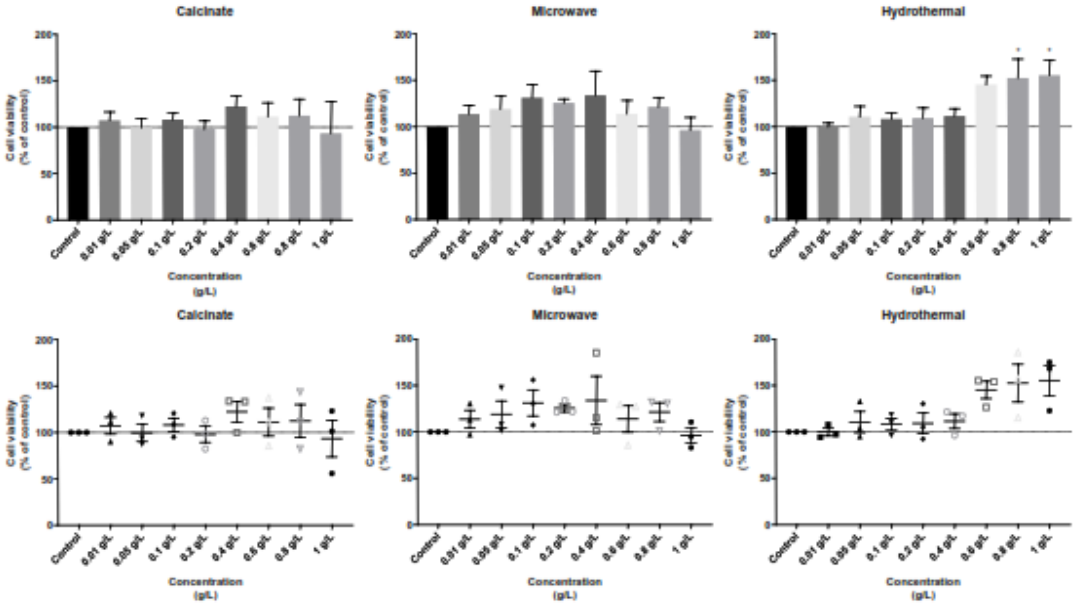

Figure 5. Relative CDs cytotoxicity against MCF-10A after 24 h incubation through MTT assay. The percentagem of cell viavility is represented as relative to negative control. The values represent mean ± SEM (n=3).

For the 24 h incubations, there were no significant decrease in the viability of MCF-10 A cells.

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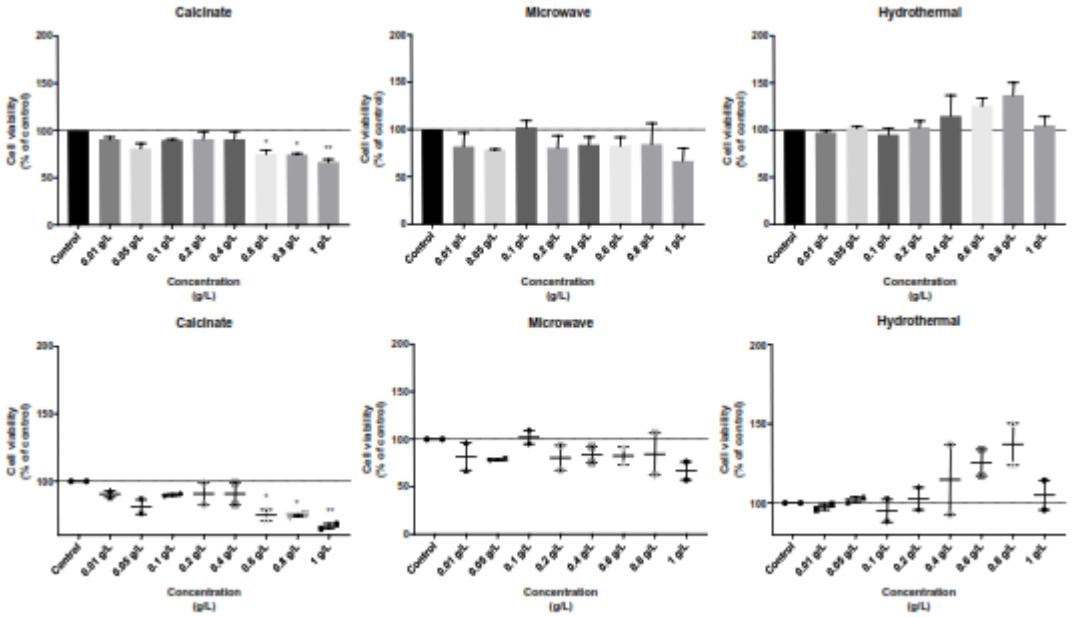
Results and discussion

CDs cytotoxicity against MCF-10A



- ✓ For the 48 h incubations, there was a significant decrease in the viability of the cells treated with calcinated CDs.
- ✓ No significant cellular viability changes for microwave and hydrothermal CDs.

Figure 6. Relative CDs cytotoxicity against MCF-10A after 48 h incubation through MTT assay. The percentagem of cell viavility is represented as relative to negative control. The values represent mean ± SEM (n=2) (*p<0.05).

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Results and discussion

CDs cytotoxicity against MCF-10A

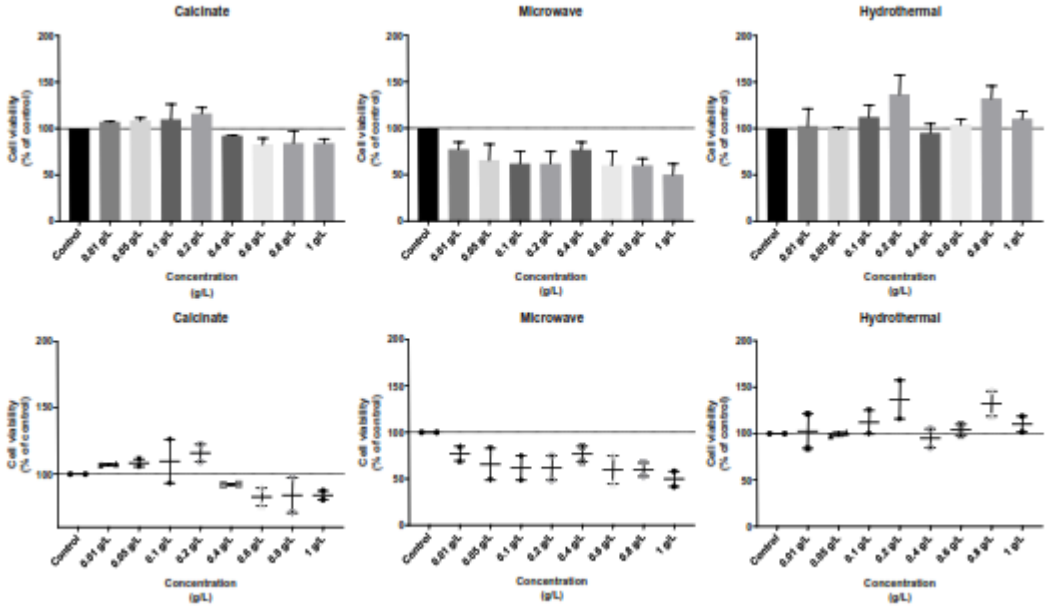

Figure 7. Relative CDs cytotoxicity against MCF-10A after 72 h incubation through MTT assay. The percentagem of cell viavility is represented as relative to negative control. The values represent mean ± SEM (n=2).

For the 72 h cells treated with hydrothermal CDs have the most promising viability profile.

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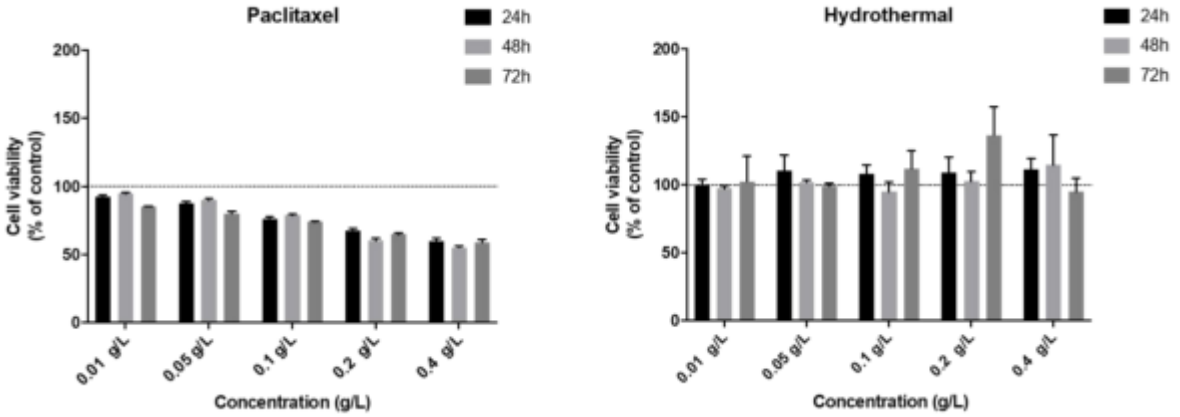
Results and discussion

CDs cytotoxicity against MCF-10A



Comparing with paclitaxel, these CDs have a safety profile, being less toxic than paclitaxel for the same range of concentrations.

Figure 8. Relative viabilities of MCF-10A cells after 24, 48 and 72 h treatments with paclitaxel vs hydrothermal CDs. The percentagem of cell viability is represented as relative to negative control. The values represent mean ± SEM. The data for Paclitaxel is in agreement with literature.

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Conclusions

- Microwave and Calcination synthesis procedures are more suitable for high-yield synthesis and high quantum yields;
- The three synthesis routes led to nanoparticles with similar sizes, identical excitation-dependent blue-to-green emission, and similar types of surface functional groups;
- Both microwave and calcination strategies are more efficient for nitrogen-doping than hydrothermal synthesis;
- Hydrothermal-CDs present emission more susceptible to the external environment. On the contrary, microwave and calcination procedures appear to generate CDs with emission more dependent on core states than on surface states;
- Hydrothermal CDs have the most promising viability profile in the MCF-10 A cells.

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