# Carbon nanodots as nanocarrier for Squaraines: an in vitro evaluation of their Photodynamic activity

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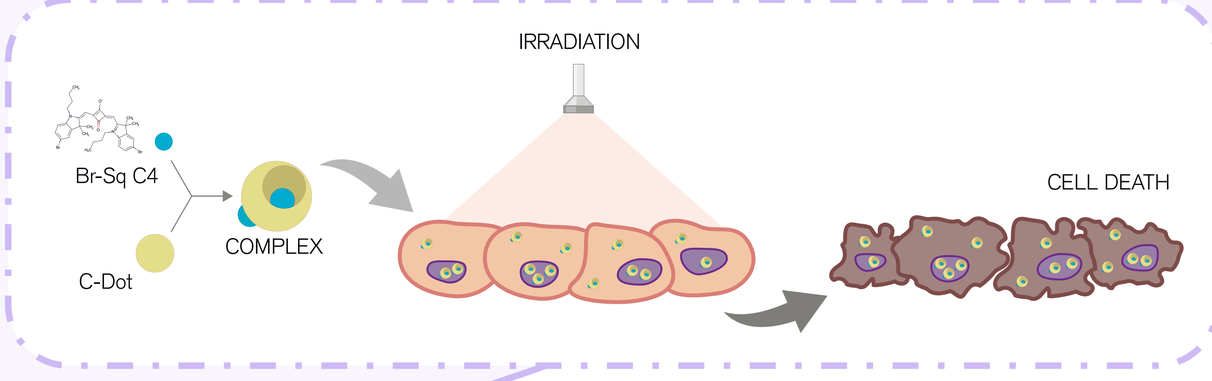
## INTRODUCTION

Carbon dots (CDs) are a new class of fluorescent carbon-based nanoparticles with excellent properties such as strong emission, high chemical stability, aqueous solubility, excellent biocompatibility and low toxicity [1]. As well, an impressive quality lies in the possibility to synthesize CDs from green precursors, making them an eco-friendly choice. Furthermore, CDs are considered outstanding materials for several fields, including biological applications, highlighting their potential as carriers in drug delivery, and in photodynamic therapy (PDT).
PDT is a promising technique for the treatment of cancer conditions, involving systemic or topical administration of a photosensitizer (PS) excited at a specific wavelength with the aim to produce reactive oxygen species (ROS) and lead to cancer cell death [2]. Over the recent years, extensive efforts have been devoted to the development of near-infrared (NIR) PS for PDT. In particular, Squaraines can be considered as innovative photosensitizers due to the easy and low-cost synthesis along with remarkable absorption property in the far-red NIR region, perfectly matching the biological tissues' transparency window (600-900 nm) [3]. Despite their excellent photochemical properties, their chemical instability and self-aggregation when in contact with biological media still limit their effective clinical application. To overcome these drawbacks, the conjugation of these dyes on nanoparticles (NPs) can be considered a promising strategy able to prevent the dye aggregation in an aqueous environment and protect their photophysical characteristics.

## AIM

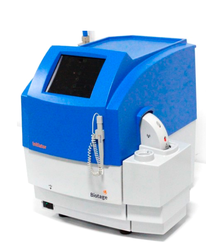
The present contribution deals with the iimprovement of the aqueous solubility of a photodynamic active squaraine, Br-Sq-C4, through complexation with carbon nanodots (CDs) without altering its properties. The photodynamic activity of the obtained complexes has been assessed through in-vitro PDT tests.



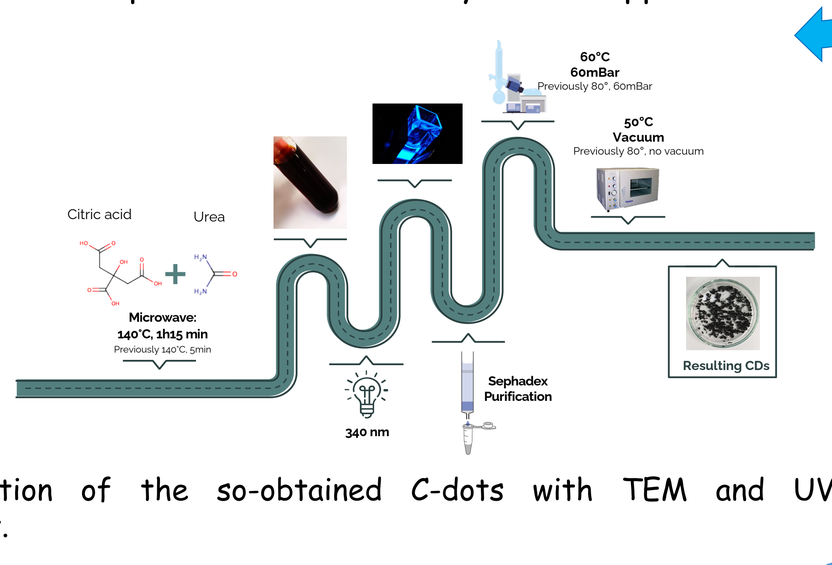

## METHODS

### SYNTHESIS AND CHARACTERIZATION OF A C-DOTS

Using a bottom-up microwave-based synthesis approach



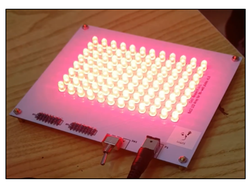
Single-mode Biotage Initiator 2.5 (Uppsala, Sweden)




- Characterization of the so-obtained C-dots with TEM and UV-vis spectroscopy.

### IN VITRO PDT TESTS

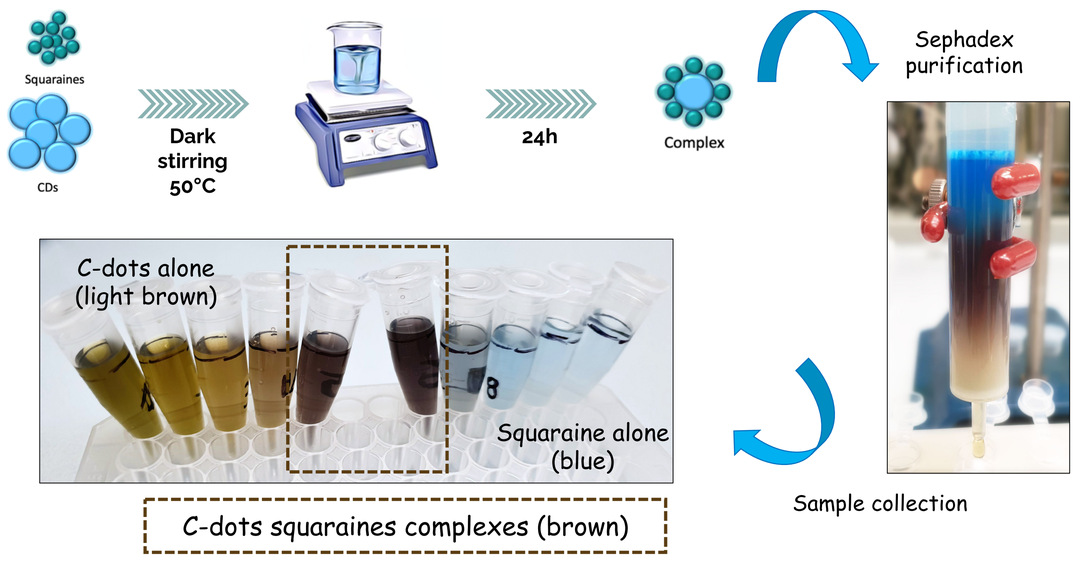
The photodynamic activity of C-dot - squaraines complexes was evaluated in terms of cell viability (MCF-7 cell line) before and after 15 min of irradiation, by using a compact LED array-based illumination system produced by Cicci Research s.r.l.



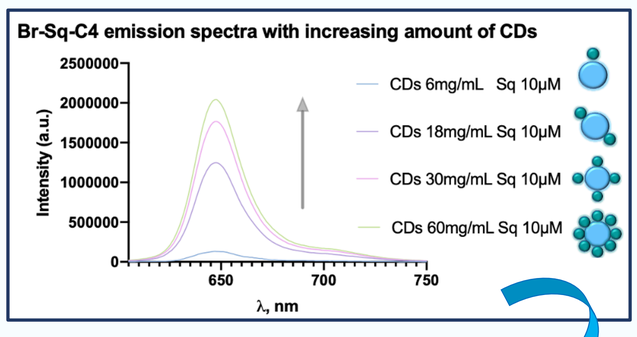
RED-LED array (light source with excitation wavelength: 640 nm, and voltage: 15 volt) composed of 96 LEDs in a 12 × 8 arrangement.

MTS assay was performed 24h after irradiation in order to evaluate cell viability.

### COMPLEXATION OF C-DOTS AND SQUARAINES

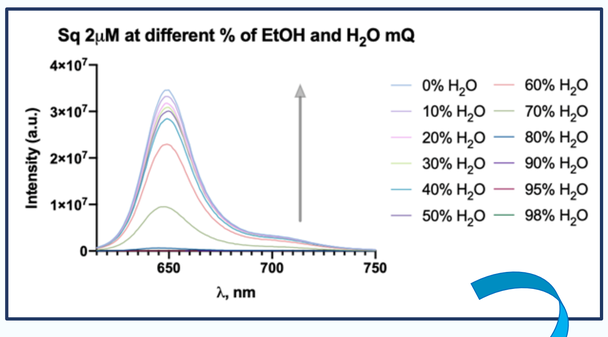



## RESULTS




Squaraine emission spectra with different percentages of CDs

**The intensity of squaraines emission increases as the concentration of carbon dots increases**

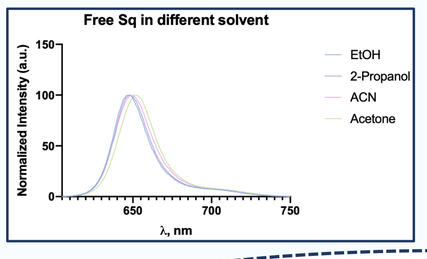


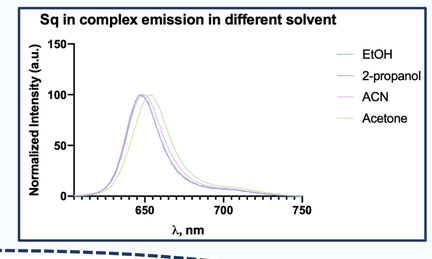

Squaraine emission spectra with different percentages of $H_2O$ and EtOH

**The intensity of squaraines emission increases as the percentage of EtOH increases**

## ENCAPSULATION or PHYSICAL ADSORPTION?

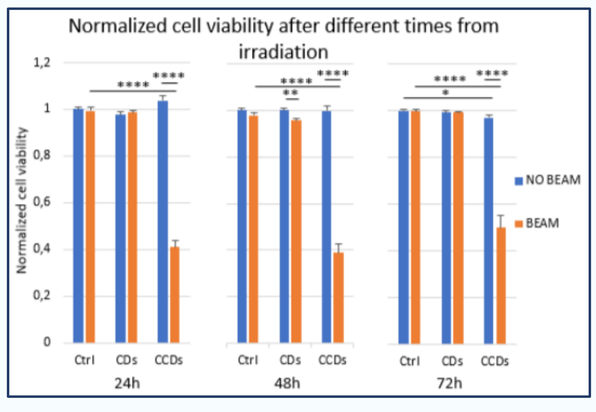
Br-Sq-C4 | CDs-Sq




**PHYSICAL ADSORPTION**

## IN VITRO EVALUATION OF CDs-Sq (CCDs) PHOTODYNAMIC THERAPY




### PRELIMINARY RESULTS

CDs-5min

**CDs don't present citotoxicity as well**

Ongoing CCDs (CDs-Sq) citotoxicity tests

## CONCLUSIONS

In the present contribution, a microwave synthesis of N-doped CDs from citric acid and urea has been reported, leading to the realization of CDs with higher fluorescence emission intensity and QY.
CDs were used as delivery system for a highly hydrophobic squaraine, the Br-Sq-C4, showing the ability to decrease its aggregation state in aqueous environment, to increase its emission fluorescence in water and to promote its use for photodynamic therapy as alternative treatment of cancer.
An *in vitro* study has been conducted to test CDs cytotoxicity on MCF-7 cells, confirming their biocompatibility, while the investigation on CDs-Sq cytotoxicity and photodynamic activity is currently ongoing.

## ACKNOWLEDGEMENTS

The authors acknowledge the financial support from the University of Torino (Ricerca Locale ex-60%, Bando 2020) and from the Fondazione Cassa di Risparmio (CRT) di Torino, Italy.

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