

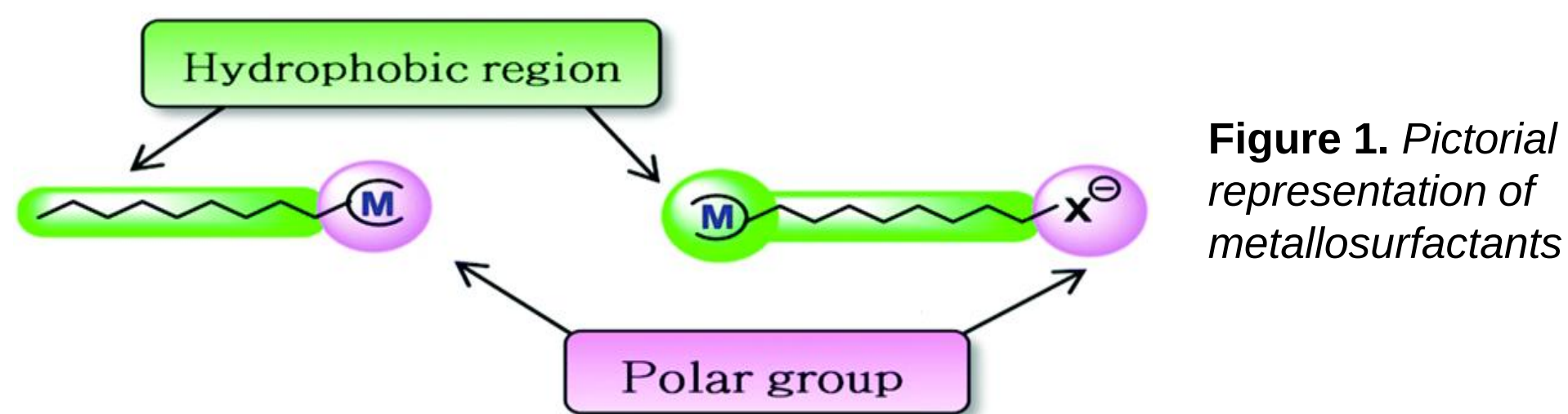
Metal-surfactant mediated metallocatanionic vesicles for antibacterial and anticancer photodynamic therapy

Bunty Sharma^a, Lluïsa Perez-Garcia^b, Ganga Ram Chaudhary^a, and Gurpreet Kaur^a

^a Department of Chemistry, Panjab University, Chandigarh, 160014; ^b Institut de Nanociència i Nanotecnologia UB (IN2UB), Universitat de Barcelona, Avda. Joan XXIII 27-31, 08028 Barcelona, Spain . buntysarmahp1065@gmail.com

Metallosurfactant

Metallosurfactants (MS) are a class of materials that benefit from surface active and delivering properties of both metal and surfactant.¹

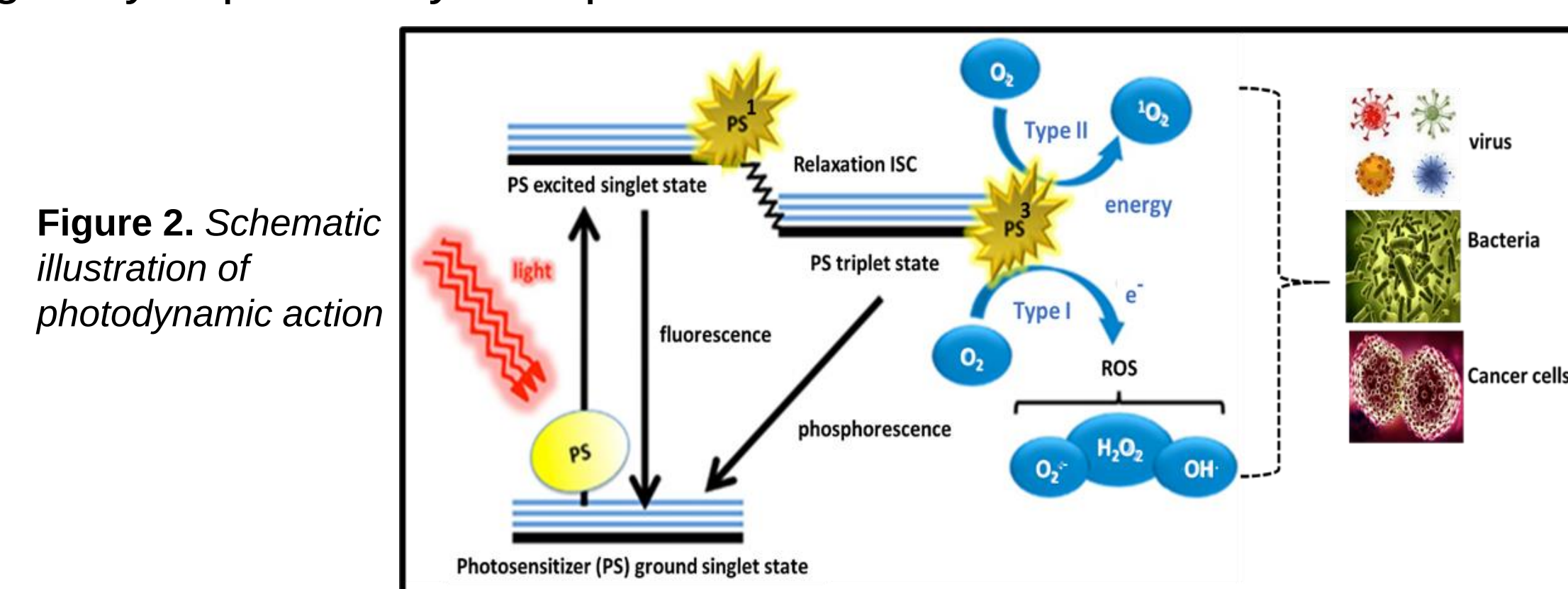


Catanionic Vesicles

Catanionic vesicles, synthesized by mixing the non-stoichiometric ratios of cationic and anionic surfactants, exhibit unique properties,² such as a) Entrapment of both hydrophilic and hydrophobic drugs and controlled release, b) Temperature, light and pH responsivity, c) Variability of surface charge, size and permeability by changing cationic/anionic ratios.

Introduction

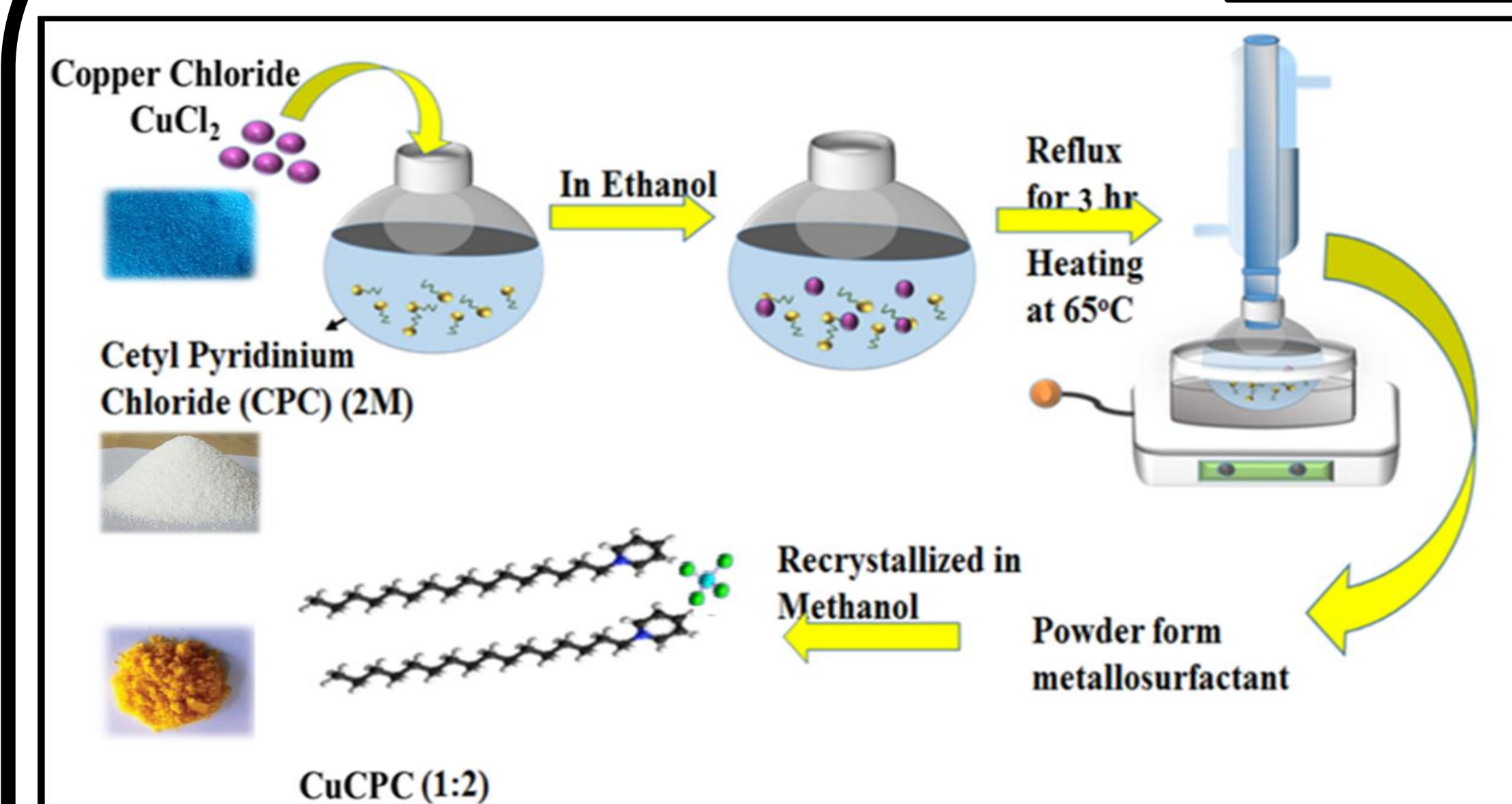
Photodynamic therapy results from combination of photosensitizers (PSs), light of specific wavelength, and molecular oxygen; conversion of molecular oxygen into phototoxic singlet oxygen is greatly improved by incorporation of PSs into nanomaterials.³



Objective

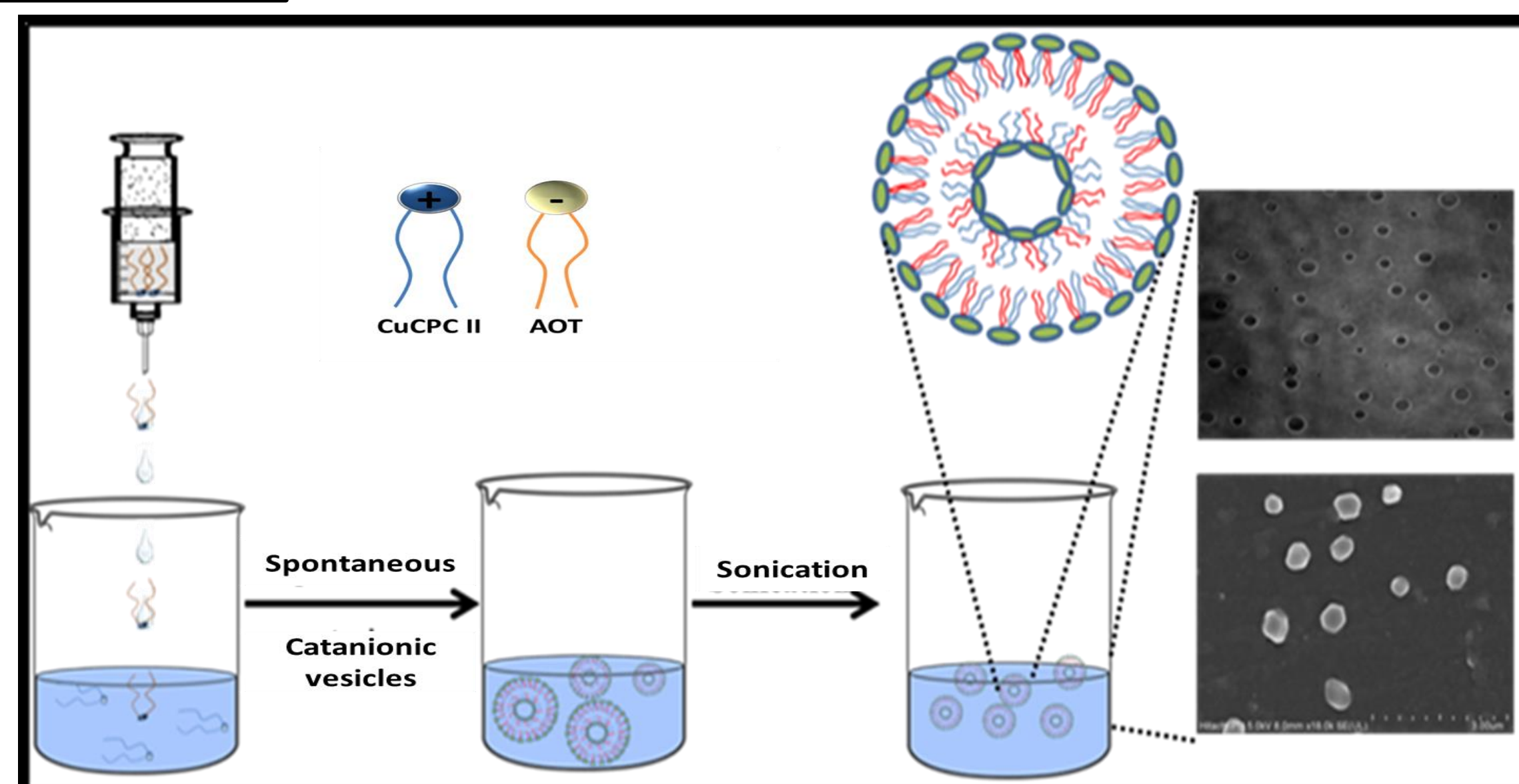
Synthesis and characterization of metallocatanionic vesicles (MCVs), followed by encapsulation of Rose Bengal, and investigation of the phototoxicity both against bacteria and cancer cell lines.

Metallosurfactant Synthesis



Methods

Metallocatanionic Vesicles (MCVs) Synthesis



MCVs Characterization

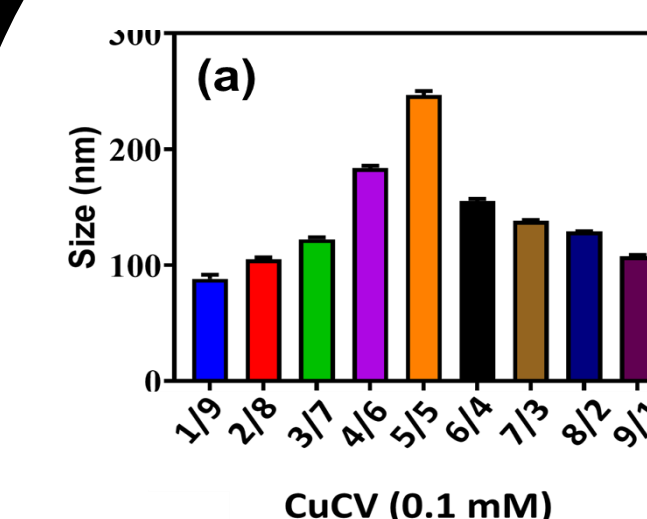
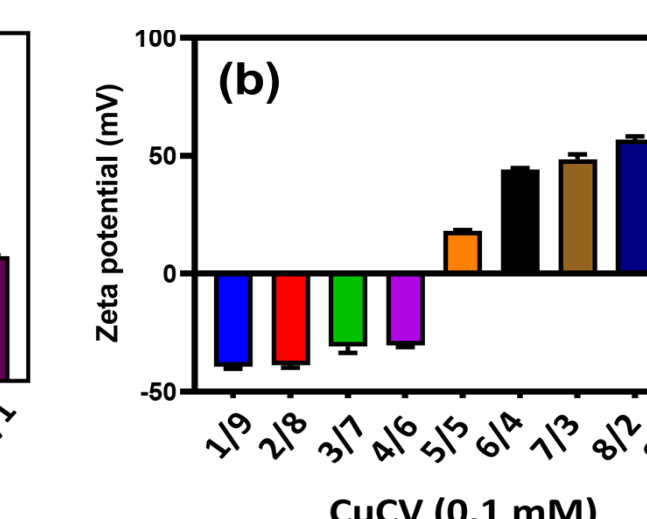


Figure 5. Characterisation of synthesised CuCVs (a) hydrodynamic diameter (b) zeta potential plot at 0.1 mM concentration

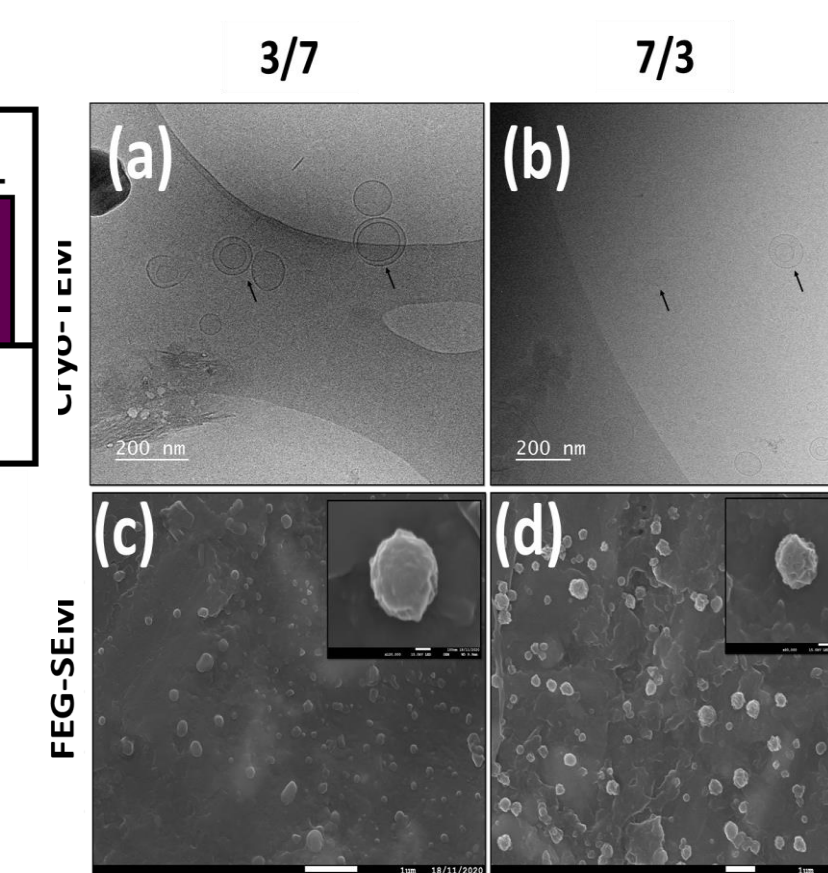


Figure 6. Morphological characterisation of metallocatanionic vesicles. Cryo-TEM images of (a) CuCV3/7, (b) CuCV7/3, FEG-SEM images of (c) CuCV3/7, (d) CuCV7/3.

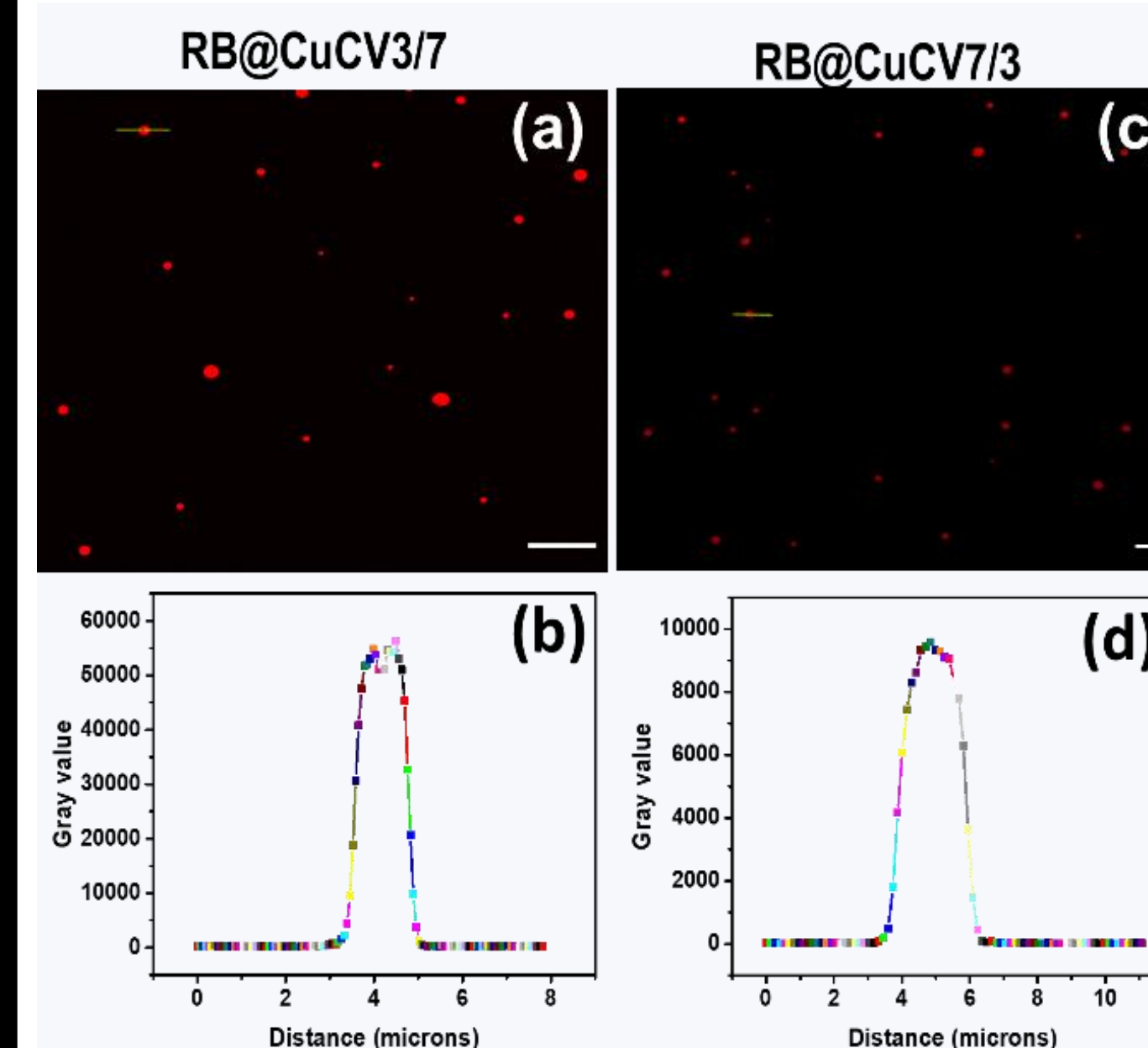


Figure 7. Rose Bengal distribution to CuCVs. Super-resolution confocal microscopic image of RB loaded (a) CuCV3/7 and their (b) Gray value plot (c) CuCV7/3 and their (d) Gray value plot

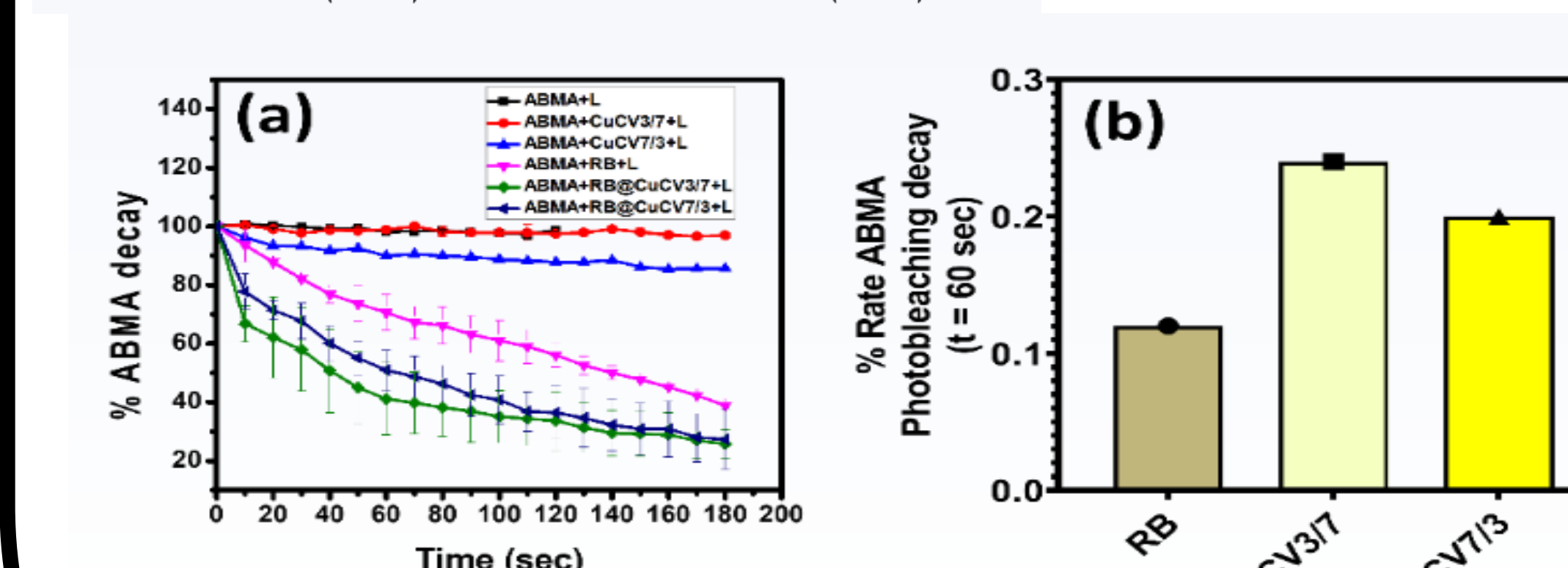


Figure 8. Singlet oxygen generation using green light-mediated RB-loaded CuMCVs. (a) ABMA fluorescence intensity change upon RB@CuCV mediated laser light irradiation (L) (b) Rate of change in ABMA photobleaching for a period of per 60 sec and RB concentration.

Results

Antibacterial Photodynamic Therapy

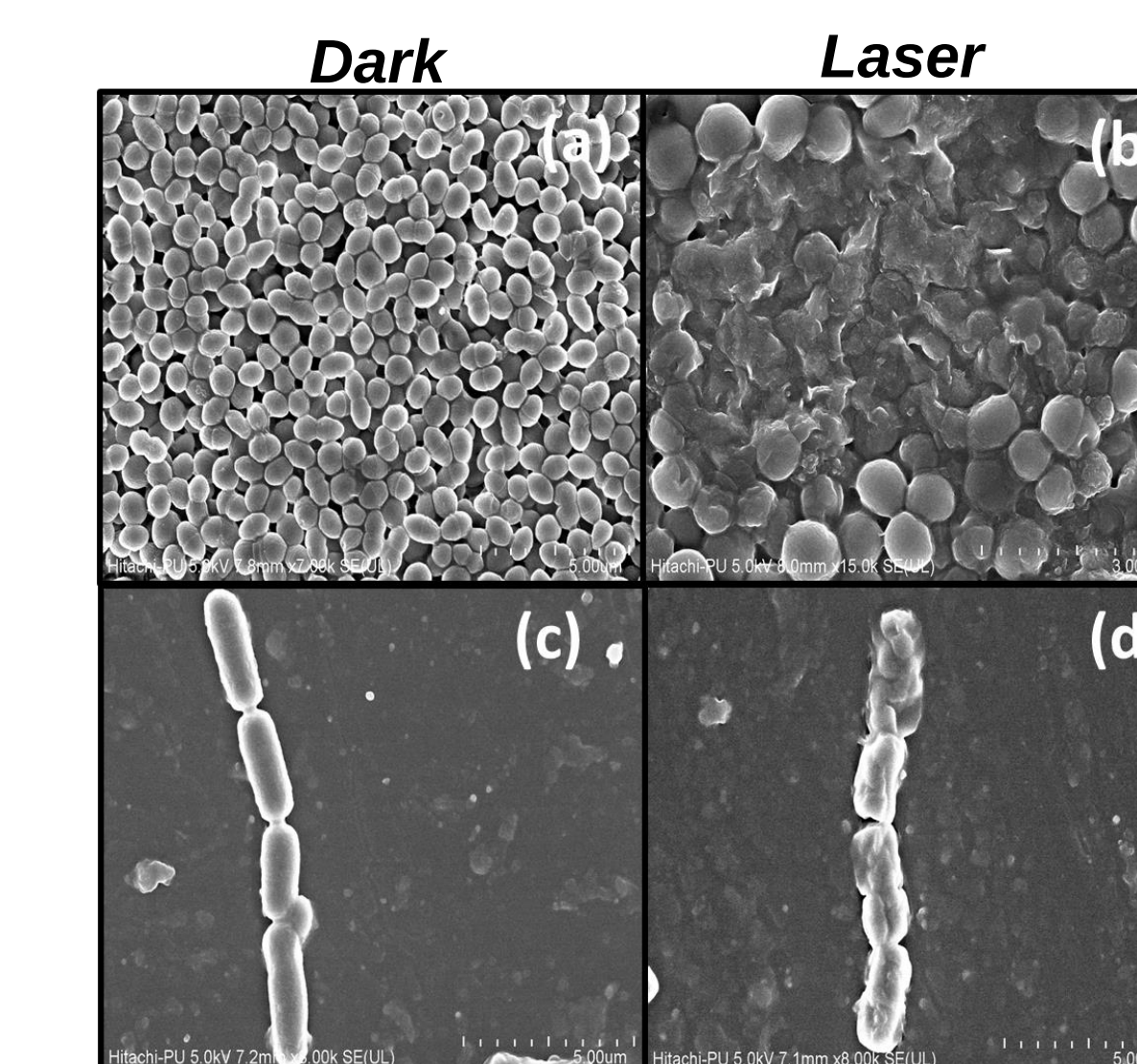


Figure 9. FESEM images *S. aureus* cells (a) Control {dark} and (b) with RB@CuCV7/3 {irradiation 30 min} and *E. Coli* cells (c) Control {dark} (d) RB@CuCV7/3 {irradiation 30 min}

Comet Assay

Used for detection of DNA damage on individual cells. We have found that PDT with RB@CuCV 7:3 shows maximum tail length implying maximum DNA damage.

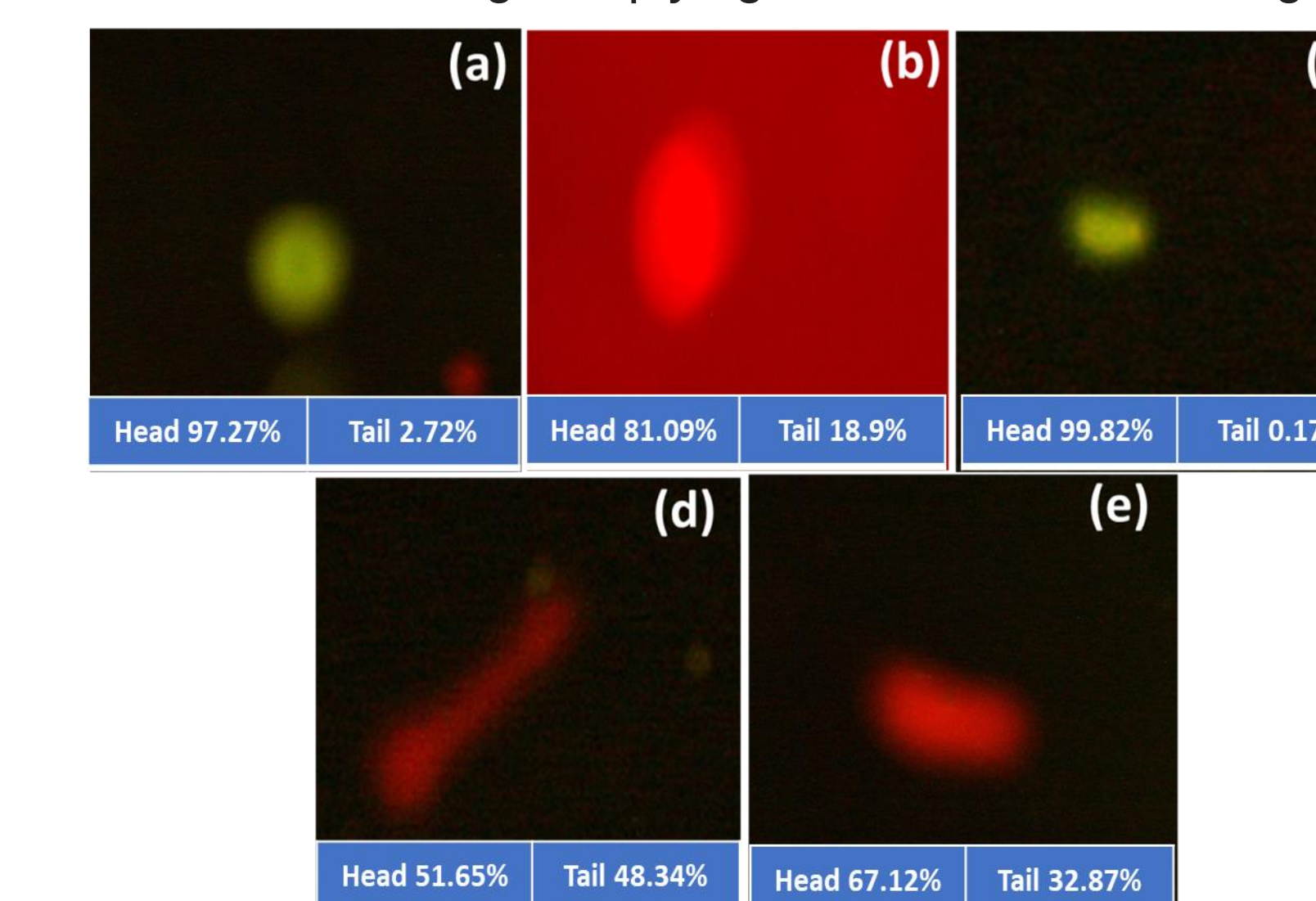


Figure 10. Comet assay images of *S. aureus* cells (a) control undamaged, (b) 7/3 (c) 3/7 (d) RB@CuCV7/3 after irradiation {30 min} and (e) RB@CuCV3/7 after irradiations {30 min} and their respective head and tail percentage calculated from CASP (Comet Assay Software Project) lab software

Anticancer Photodynamic Therapy

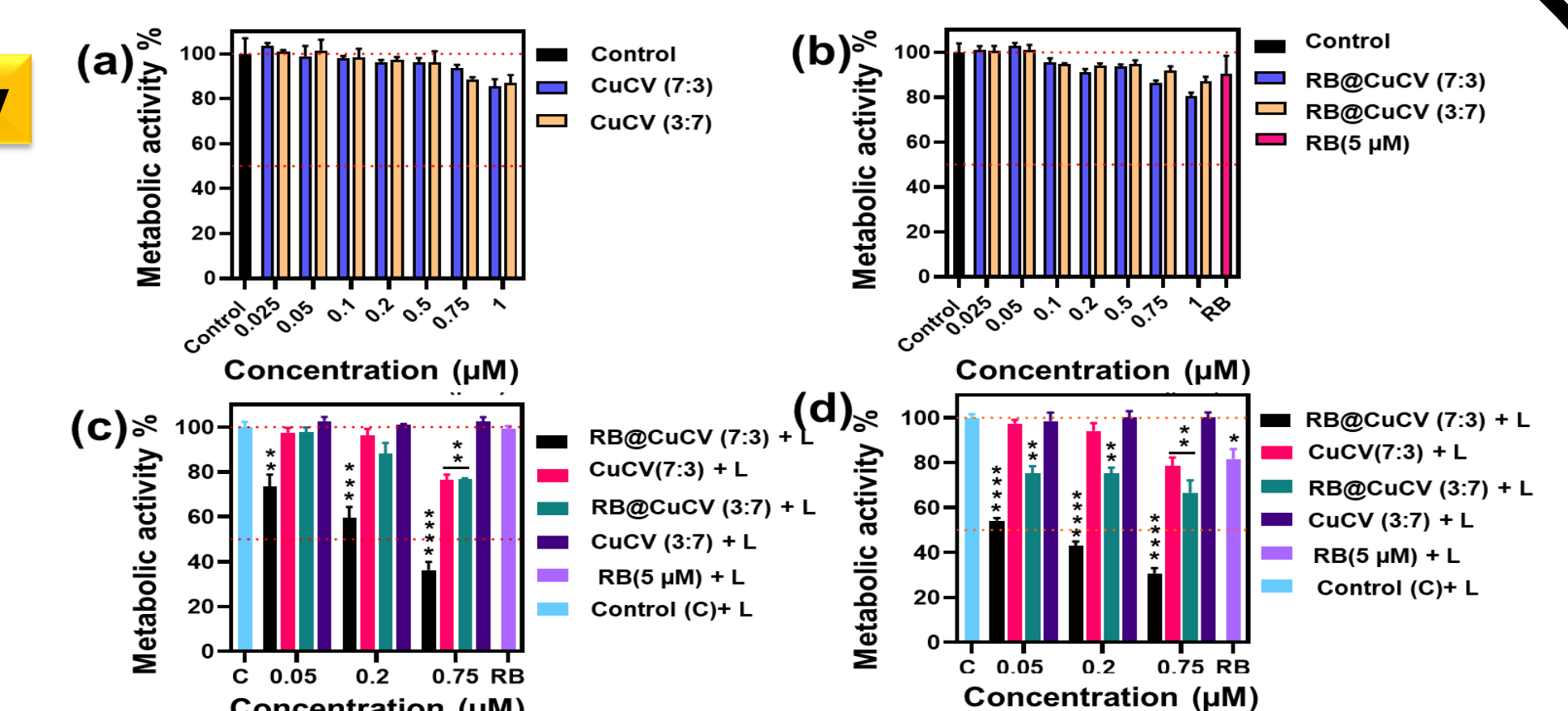


Figure 11. In-vitro dark and phototoxicity of in U251 cell lines. Dark toxicity of (a) CuCV (b) RB@CuCV and their phototoxicity after green laser light at energy fluency (c) 40 Jcm⁻² (d) 80 Jcm⁻².

Cellular uptake study

Figure 12. Cellular uptake of RB@CuCV7/3 and laser irradiation (DAPI (blue) – nucleus, and red - RB-loaded MCVs).

SOSG assay

Figure 13. Intracellular ¹O₂ generation in U251 cells treated with RB@CuCV7/3 vesicles upon treatment with 532 nm laser.

DNS assay

Figure 14. Fluorescence microscopic picture of dead U-251 Glioblastoma cells after treatment of RB@CuCV7/3 with laser treatment at 80 Jcm⁻² laser dose

Caspase 3/7 assay

Figure 15. Cell death pathway analysis using Caspase 3/7 assay. Fluorescence microscopic picture of apoptotic U-251 Glioblastoma cells after treatment of RB@CuCV7/3 with laser treatment at 80 Jcm⁻² laser dose

Results

- MCVs were prepared by a facile and quick solution-solution mixing method using a cationic metallosurfactant in combination with an anionic surfactant.
- These MCVs self-assembled at very low concentration and carry both cationic and anionic surface charge with control size.

- Prepared MCVs can engulf both cationic and anionic photosensitizers with enhancement in singlet oxygen generation effect.
- MCVs loaded photosensitizer effectively employed for antibacterial PDT (Gram+ and Gram- bacteria) and anticancer PDT (U251 cancer cells).

References

- P. C. Griffiths et al., *J. Colloid Interface Sci.*, **2006**, 122, 107-117.
- S. Kaler et al., *Science*, **1989**, 245, 1371-1374.
- M. Samperi et al., *Cell Reports Physical Science*, 2020, 1, 100030.
- G. Kaur et al., *Phys. Chem. Chem. Phys.*, **2016**, 18, 23961-23971.
- B. Sharma et al., *Biomater. Sci.*, **2020**, 8, 2905-2920.

Acronyms

CuCV = CuCPC I:AOT; MCV = Metallocatanionic vesicles; RB = Rose Bengal SOSG = Singlet Oxygen Sensor Green; CAC = Critical aggregation concentrations PS = Photosensitizer; DNS = Differential nuclear staining (INCN-2019-409), and the University of Nottingham.