

Supplementary material

Photodynamic Inactivation of Methicillin-Resistant *Staphylococcus aureus* by a Natural Food Colorant (E-141ii)

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Academic Editor: M. Amparo F. Faustino

Received: 27 July 2020; Accepted: 28 August 2020; Published: date

S1. ¹O₂ generation by Methylene blue

2.2 mL of DPBF at 90 μM in DMSO was mixed with 0.4 mL of methylene blue at 30 μM. This solution was placed under 625-nm illumination at 3.5 mW. UV-Vis absorption spectrum was collected every 30 s in a LAMBDA 265 UV/VIS spectrophotometer (Perkin Elmer). The ¹O₂ generation was evaluated by monitoring the DPBF degradation caused by its interaction with ¹O₂ [1].

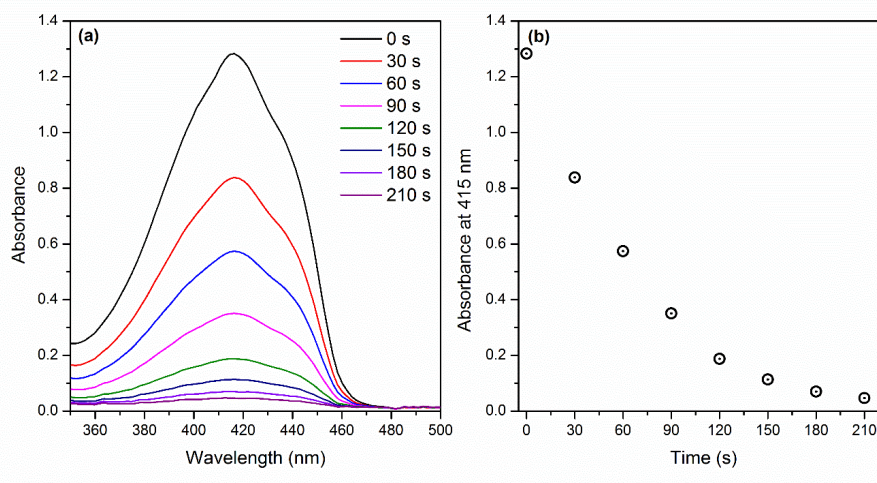


Figure S1. (a) UV-Vis absorption spectra and (b) absorbance at 415 nm of DPBF in the presence of methylene blue as a function of the red-light illumination time.

S2. ROS production kinetic analysis

The ROS production rate constant was determined by analyzing the kinetic of the fluorescent products generated by the interaction between ROS and DHE. A saturating concentration of DHE was used during the measurements, and it was assumed that the formation rate of new fluorescent products [F] was equal to the one for ROS produced by E-141ii under red light illumination, as follows [2]:



Consequently, the rate of ROS generation can be written as:

$$-\frac{d[ROS]}{dt} = k_{ROS} [DHE][ROS] \quad \text{Eq. (S2)}$$

where k_{ROS} is the rate constant of ROS production, with $[ROS] \propto F$. Eq. S2 can be expressed as:

$$-\frac{dF}{dt} = k_f F \quad \text{Eq. (S3)}$$

where $k_f = k_{ROS}[DHE]$. From Eq. S3, the following equation can be obtained.

$$F = a(1 - e^{-k_f t}) \quad \text{Eq. (S4)}$$

Where F is the fluorescence intensity originated from the reaction of DHE with the ROS produced under red light illumination, and t is the illumination time, and k_f and a are constants that can be determined by fitting the experimental data presented in Figure S3 by using Eq. S4.

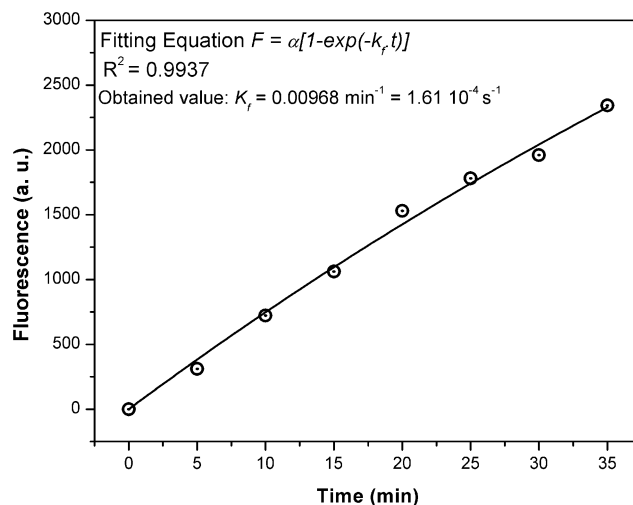


Figure S2. Fluorescence intensity at 610 nm as a function of the illumination time. The gray line represents the fitting curve obtained by using Eq. S4.

Finally, the value of k_{ROS} as $80.5 \text{ M}^{-1} \text{ s}^{-1}$ was determined by knowing that $[DHE] = 2 \text{ }\mu\text{M}$ and $k_f = 1.61 \cdot 10^{-4} \text{ s}^{-1}$.

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