processes

MDPI

Supplementary Materials

Improved pilot-plant scale synthesis of Chlorin e6 and its efficacy as a photosensitizer for photodynamic therapy and photoacoustic contrast agent

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SI 1. General information

^{1}H NMR spectra was recorded on DPX Bruker (600 MHz) spectrometers in deuterated DMSO using the solvent chemical shift of 2.5 ppm and water peak at 3.35 ppm. HPLC was analyzed in Waters Alliance separation module e2695 (Waters, Milford, MA), coupled with 2998 PDA detector (software: Empower 3). For chromatographic analysis, a Capcell C18 (4.6 mm × 250 mm, 5 μm) column was used in a linear gradient of 45-100% B (acetonitrile) in 0.1% TFA water (A) over 20 min at a flow rate of 1 mL/min. Column temperature was set at 23±2°C and UV detection at 407 nm. Mass analysis (m/z 200–800) was performed by online HPLC–MS using a Waters ZQ2000 mass detector (Waters, Milford, MA) with ESI positive ionization mode. Fourier transform infrared (FT-IR) spectra were recorded on PerkinElmer FT-IR spectrometer Spectrum Two™. Absorbance spectra of the tested compound was recorded at room temperature (298 K) using UV/Vis spectrophotometer (Thermo-scientific, Skanlt software 5.0). The sample was prepared in 95% ethanol solvent (Duksan, HPLC grade pure) at a concentration of 10 μM. The data was corrected for solvent background by the instrument's calibration using the 95% ethanol as a blank. The absorption spectra of sample in solution was obtained in the range of 300-800 nm at 1 nm interval in 3 determination using three trial samples. The fluorescence (Tecan-Spark) measurement was carried out at room temperature at 405 nm (excitation wavelength).

SI 2. Quantification of Chlorophyll a

The chlorophyll quantification was done according to the previously reported method. After the accomplished of desined experiments for the extraction of chlorophyll a in specific solvent (1g spirulina in 10 mL solvent), the spirulina suspension was centrifuged at 3000 rpm for 10 min. The 1 mL supernatant was diluted to 100 mL. Later, the UV absorbance of the diluted sample solution was acquired at a wavelength ranging from 300 to 800 nm in a quartz cuvette having a path length of 1 cm (Thermo Scientific, Multiskan GO). The chlorophyll a quantification was done according to the the following equations:

$$Ch_a \text{mg/g (Acetone)} = \frac{(11.24A_{661.6} - 2.04A_{644.8})\ x\ DF\ x\ S}{1000} \quad (1)$$

$$Ch_a \text{mg/g (MeOH)} = \frac{(16.72A_{665.2} - 9.16A_{652.4})\ x\ DF\ x\ S}{1000} \quad (2)$$

$$Ch_a \text{mg/g (EtOH)} = \frac{(13.70A_{665} - 5.76A_{649})\ x\ DF\ x\ S}{1000} \quad (3)$$

$$Ch_a \text{mg/g (95\% EtOH)} = \frac{(13.36A_{664.2} - 5.19A_{648.6})\ x\ DF\ x\ S}{1000} \quad (4)$$

Where,
Chl_a mg/g = Chlorophyll content,
A = absorbance at specified wavelength,
DF = dilution factor,
S = amount of solvent [mL].

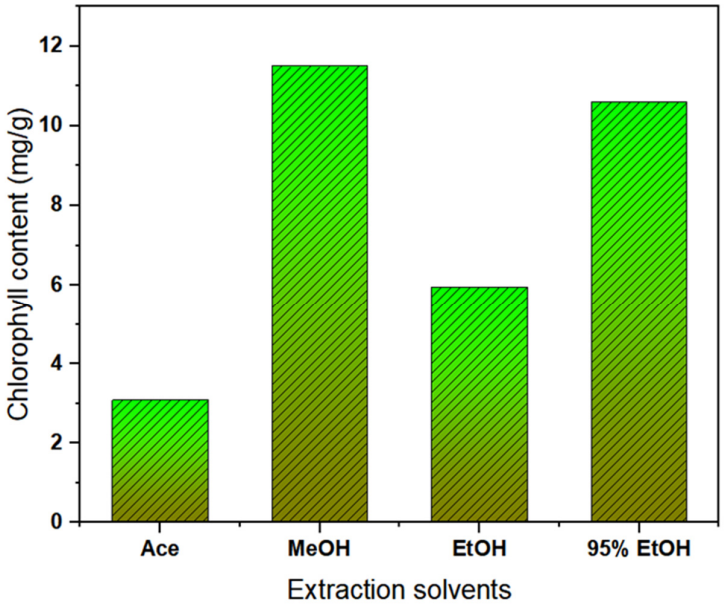

Figure S1. Screening of various solvents for chlorophyll a extraction by using 1 g Spirulina at 25 ° C for 2 h shaking (300 rpm) in incubated shaker.

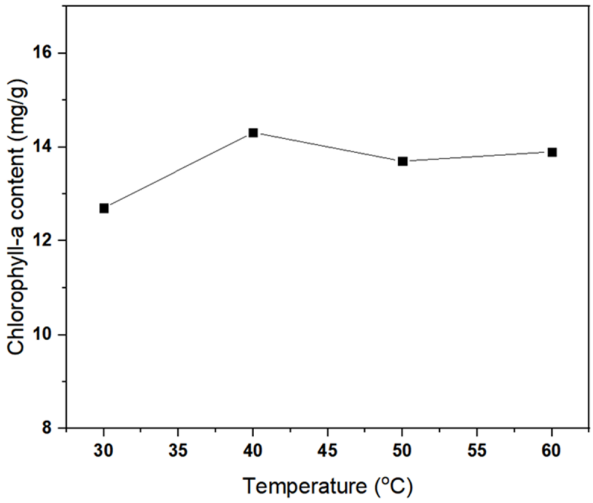

Figure S2. Screening of various temperatures for chlorophyll a extraction by using 1 g Spirulina in 95% ethanol for 2 h shaking (300 rpm) in incubated shaker.

Table S1. Real and coded values of the optimization process expressed by the yields of chlorophyll extracted from Spirulina maxima by CCRD 2^2 using 95% ethanol as solvent.

Run	Design Matrix		Experimental conditions		Chlorophyll content (mg/g)
	Time	Solid-liquid ratio	Time	SLR (g biomass/ solvent)	
1	-1	-1	30	0.05	9.84
2	0	0	90	0.085	13.6
3	-1.4	0	5.147186	0.085	5.57
4	1	-1	150	0.05	13.23
5	1.43	0	174.8528	0.085	13.1
6	0	0	90	0.085	13.7
7	0	-1.43	90	0.035502525	13.4
8	0	0	90	0.085	13.6
9	0	0	90	0.085	13.5
10	1	1	150	0.12	13.2
11	-1	1	30	0.12	9.61
12	0	0	90	0.085	13.5
13	0	1.43	90	0.134497475	13.31

Table S2. ANOVA for the optimization of chlorophyll a extraction.

Response Surface Regression: Chlorophyll content versus Time, SLR

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	69.5593	13.9119	55.83	0.000
Linear	2	38.8666	19.4333	77.99	0.000
Time	1	38.8478	38.8478	155.91	0.000
SLR	1	0.0187	0.0187	0.08	0.792
Square	2	30.6827	15.3414	61.57	0.000
Time*Time	1	30.4231	30.4231	122.10	0.000
SLR*SLR	1	0.0459	0.0459	0.18	0.681
2-Way Interaction	1	0.0100	0.0100	0.04	0.847
Time*SLR	1	0.0100	0.0100	0.04	0.847
Error	7	1.7442	0.2492		
Lack-of-fit	3	1.7162	0.5721	81.72	0.000
Pure Error	4	0.0280	0.0070		
Total	12	71.3035			

Model Summary

S	R-sq	R-sq (adj)	R-sq (pred)
0.499167	97.55%	95.81%	82.82%

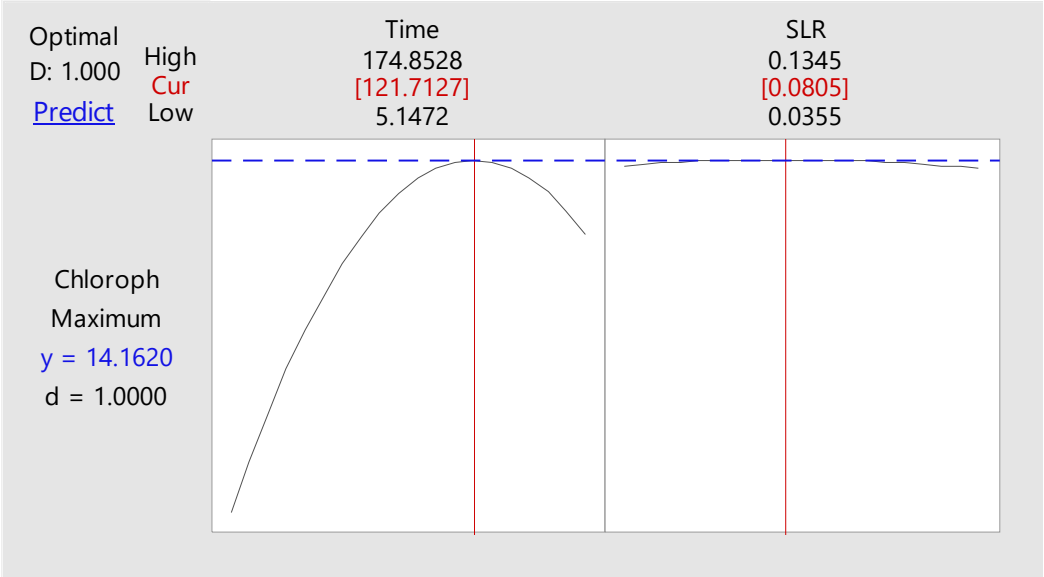

Figure S3. Process optimization curve for the extraction of chlorophyll a.

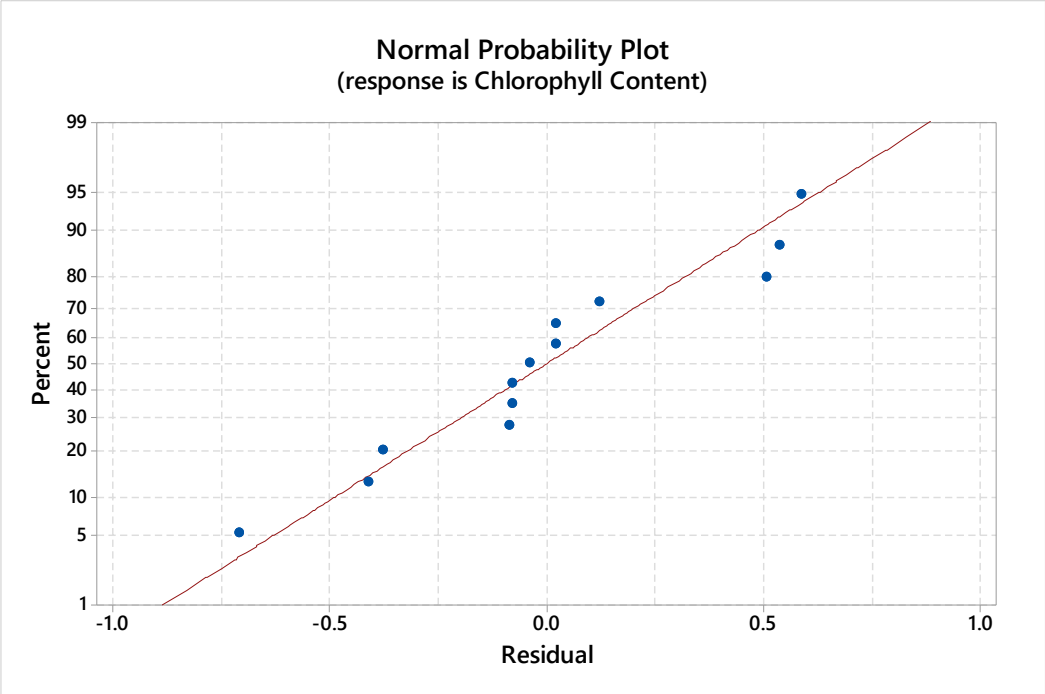

Figure S4. Normal probability plot of the residuals.

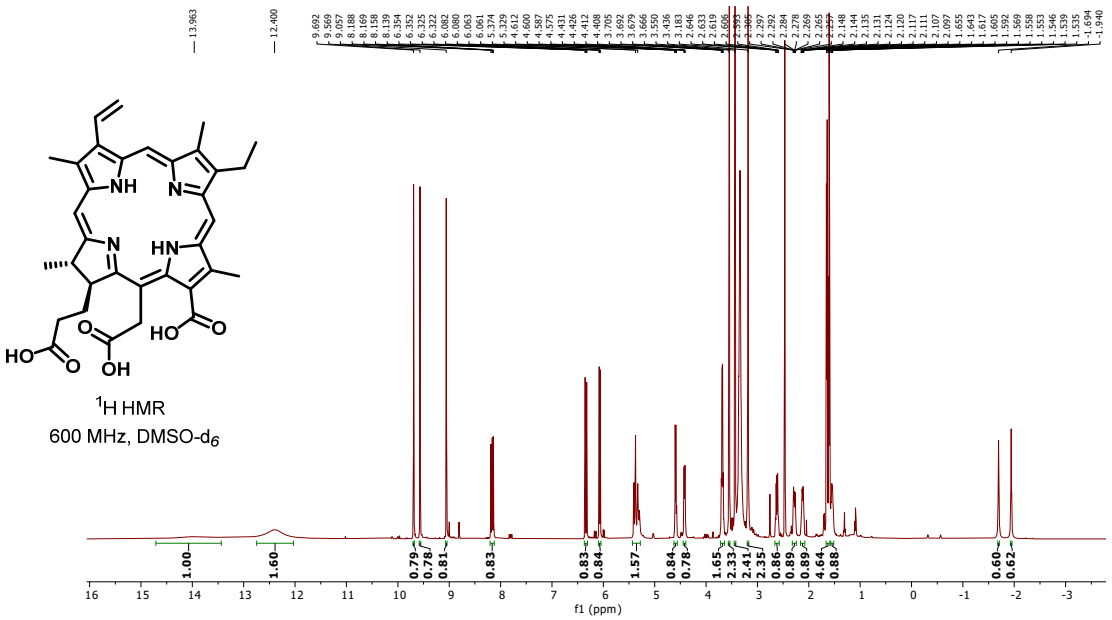

Figure S5. ^{1}H NMR of Ce6 (modified method).

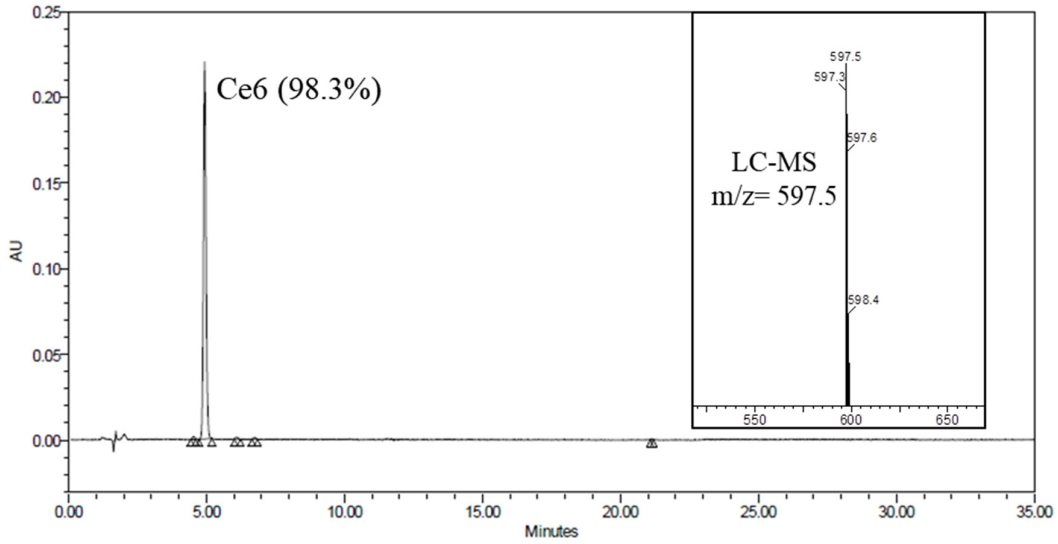

Figure S6. HPLC & ESI-MS chromatogram of Chlorin e6 (modified method).

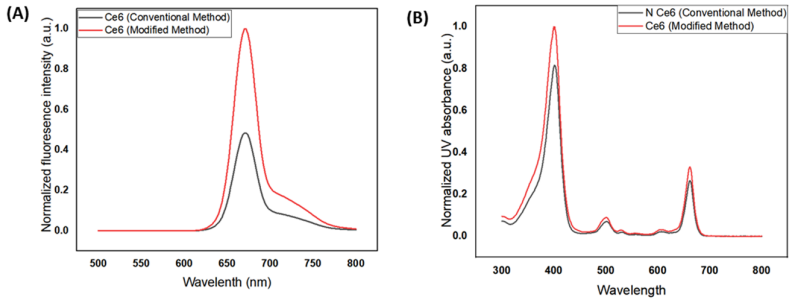

Figure S7. Comparative figures of UV and fluorescence absorbance of Ce6 (conventional and modified method).

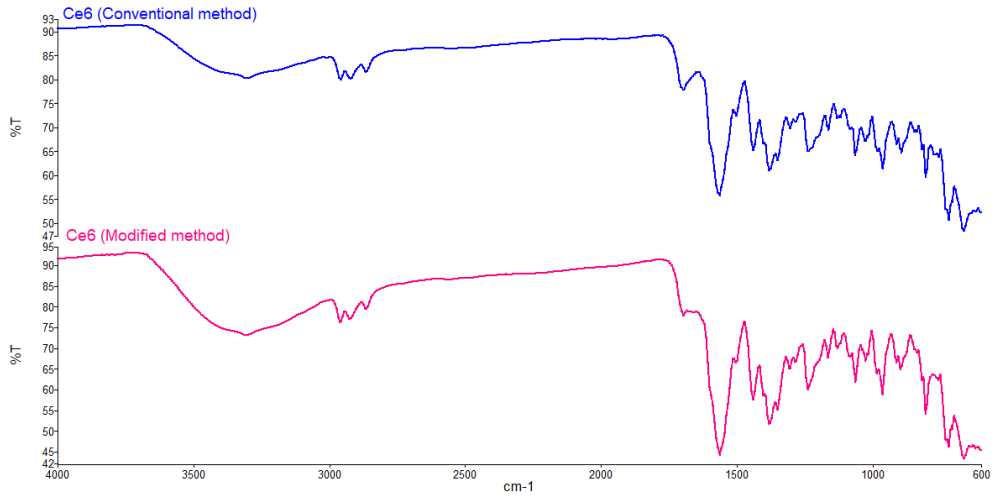

Figure S8. Comparative figure of FT-IR of Ce6 (conventional and modified method).

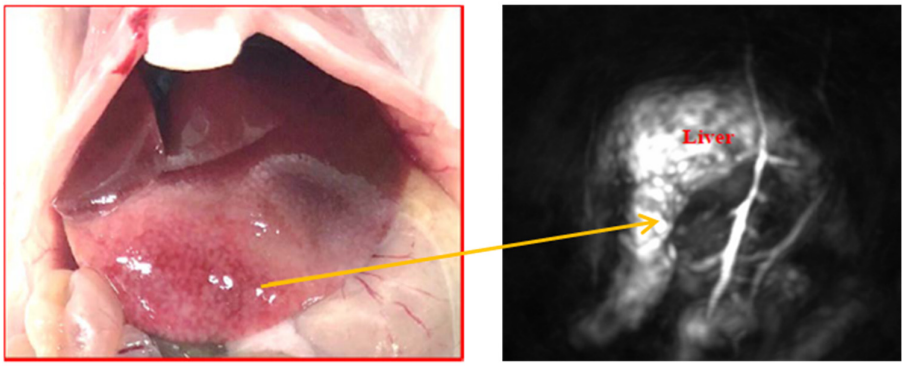

Figure S9. PA image of liver compared with liver autopsy.