Supplementary Information

Design and Evaluation of Dual Release from Anacardic Acid-Based Polyurea Nanocapsules Components

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Profile of anacardic acid

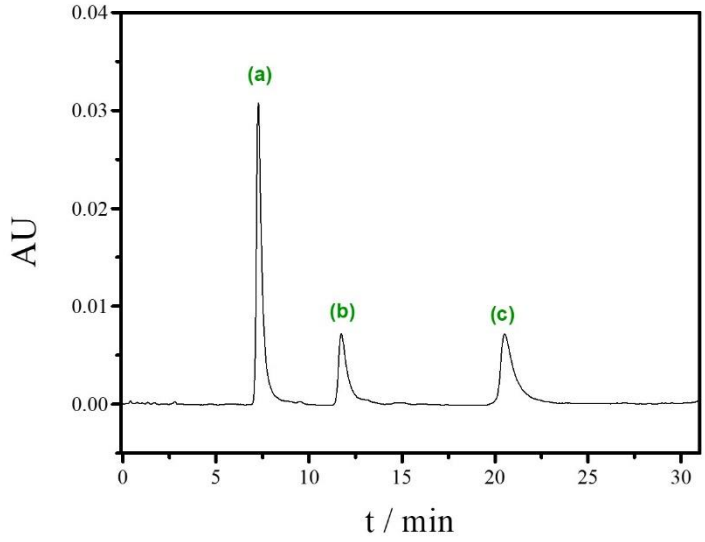


Figure S1. HPLC profile of anacardic acid obtained from cashew nutshell liquid (CNSL): (a) triene, (b) diene and (c) monoene, monitored at 280 nm, using Supelcosil, LC-18-T and acetonitrile/water/acetic acid (80:20:1) as mobile phase.

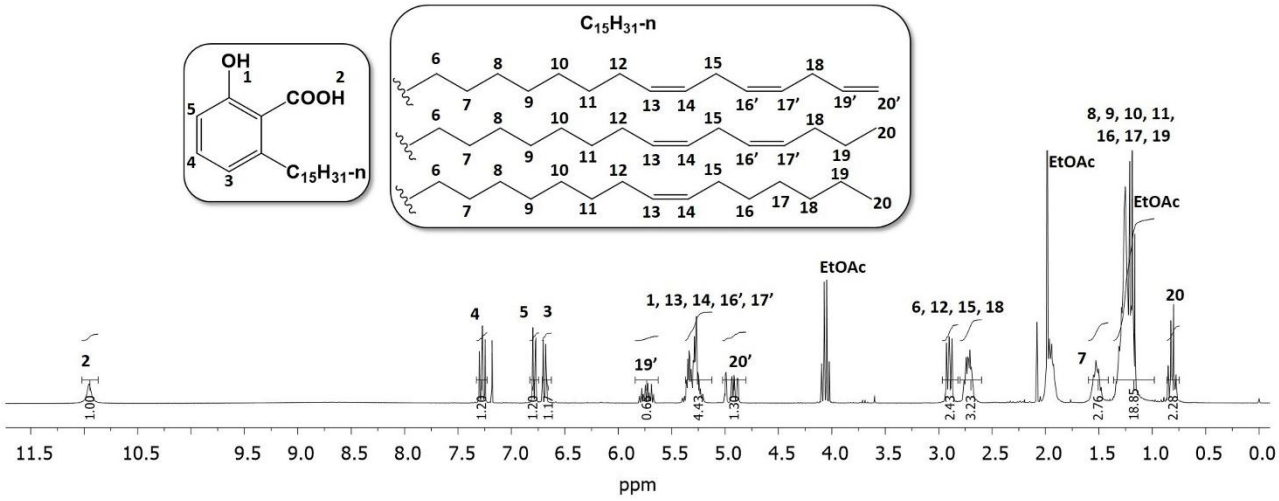
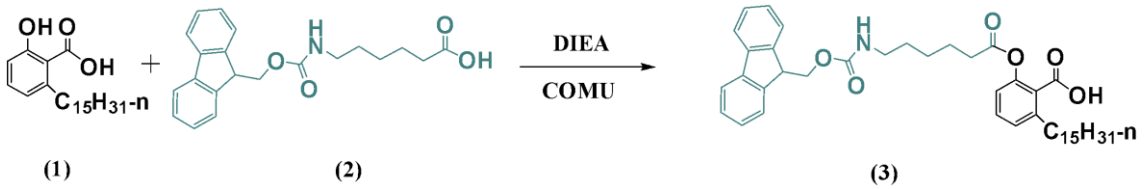


Figure S2. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of anacardic acid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.95 (s, 1H), 7.28 (dd, *J* 14.0, 5.8 Hz, 1H), 6.77 (d, *J* 1.1 Hz, 1H), 6.69 (dd, *J* 7.5, 1.1 Hz, 1H), 5.79-5.63 (m, 1H), 5.37-5.17 (m, 5H), 4.97-4.86 (m, 1H), 2.95-2.87 (m, 2H), 2.76-2.67 (m, 3H), 1.52 (dd, *J* 14.8, 7.2 Hz, 3H), 1.31-1.10 (m, 19H), 0.90-0.75 (m, 3H).

Step 1: synthesis of 2-(6-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)hexanoyloxy)-6-((8*E*,11*E*)-pentadeca-8,11,14-trienyl)benzoic acid-compound **3**



In an flask equipped with a magnetic stir bar, Fmoc-6-Ahx-OH (3.09 g, 8.76 mmol), COMU (3.75 g, 8.76 mmol) and *N*,*N*-diisopropyllethylamine (DIEA) (2.26 g, 17.52 mmol) were dissolved in anhydrous *N*,*N*-dimethylformamide (DMF, 12 mL) and the resulting red solution was stirred at room temperature (rt) for 10 min under a nitrogen atmosphere. Anacardic acid (3 g, 8.76 mmol) in DMF (5 mL) was then injected into the reaction mixture via syringe and vigorous stirring at rt was continued until thin layer chromatography (TLC) confirmed the completion of the reaction (5 h). The reaction mixture was diluted with CH_2Cl_2 (10 mL) and the resulting mixture was washed with 6 M HCl (2 × 10 mL), 5% $NaHCO_3$ (3 × 10 mL) and water (2 × 10 mL). The organic layer was collected, dried (Na_2SO_4), filtered and concentrated. After removal of solvent under reduced pressure, the residue was purified by chromatographic column using silica as stationary phase and hexane/EtOAc as eluent to afford 4.94 g (yield 83%) of the compound **3**.

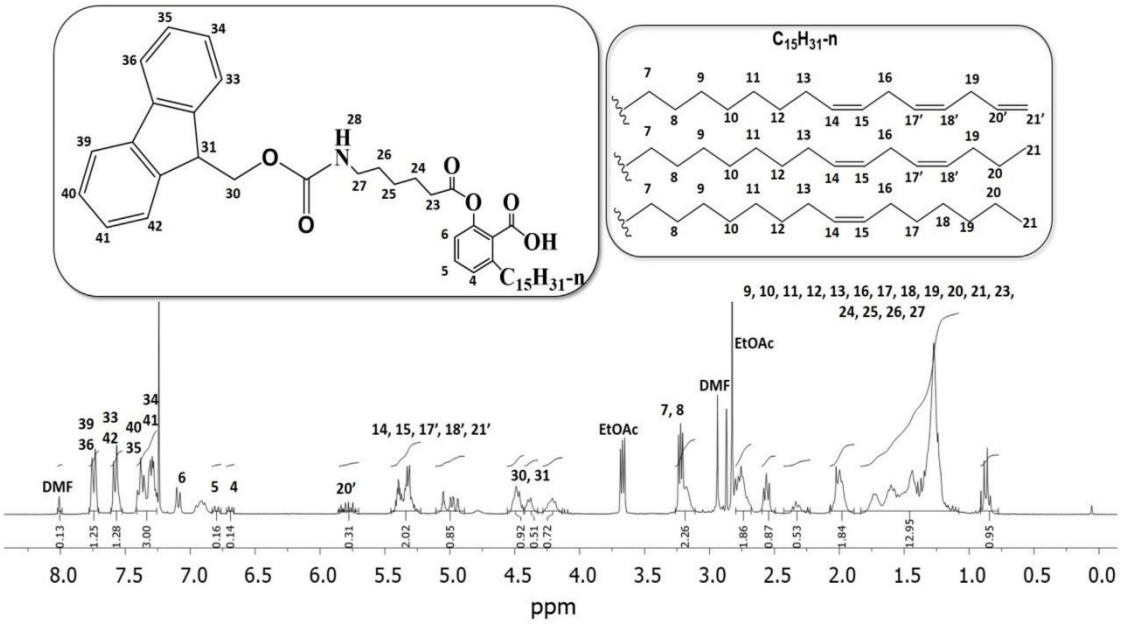
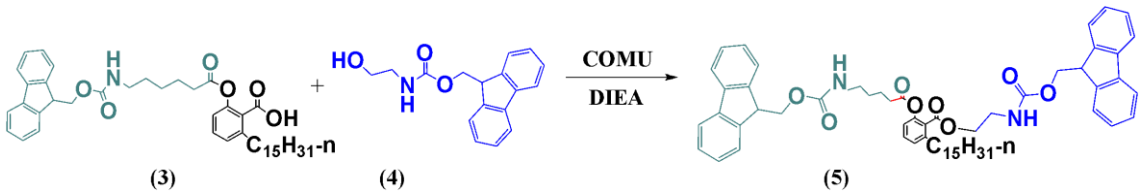


Figure S3. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of compound **3**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.74 (d, *J* 7.4 Hz, 1H), 7.57 (s, 1H), 7.33 (ddd, *J* 11.5, 10.9, 5.6 Hz, 3H), 6.80 (d, *J* 8.0 Hz, 1H), 6.69 (d, *J* 7.3 Hz, 1H), 5.79 (ddt, *J* 16.3, 10.1, 6.2 Hz, 1H), 5.41-5.26 (m, 2H), 5.07-4.92 (m, 1H), 4.55-4.43 (m, 1H), 4.38 (s, 1H), 4.20 (d, *J* 6.4 Hz, 1H), 3.21 (dd, *J* 11.3, 6.7 Hz, 2H), 2.74-2.66 (m, 2H), 2.61-2.50 (m, 1H), 2.31 (s, 1H), 2.04-1.94 (m, 2H), 1.75-1.12 (m, 12H), 0.86 (t, *J* 7.2 Hz, 1H).

Step 2: synthesis of 2-(((9*H*-fluoren-9-yl) methoxy)carbonylamino) ethyl 2-(6-(((9*H*-fluoren-9-yl) methoxy) carbonylamino) hexanoyloxy)-6-((8*E*,11*E*)-pentadeca-8,11,14-trienyl)benzoate-compound **5**



In an flask equipped with a magnetic stir bar, compound **3** (4.94 g, 7.29 mmol), COMU (3.12 g, 7.29 mmol), *N*,*N*-diisopropyllethylamine (DIEA) (2.04 g, 15.8 mmol) were dissolved in anhydrous DMF (12 mL) and the resulting red solution was stirred at rt for 10 min under a nitrogen atmosphere. 2-(Fmoc-amino)ethanol (2.06 g, 7.29 mmol) in DMF (5 mL) was then injected into the reaction mixture via syringe and vigorous stirring at rt was continued until TLC confirmed the completion of the reaction (5 h). The reaction mixture was diluted with CH_2Cl_2 (10 mL) and the resulting mixture was washed with 6 M HCl (2 × 10 mL), 5% $NaHCO_3$ (3 × 10 mL) and water (2 × 10 mL). The organic layer was collected, dried (Na_2SO_4), filtered and concentrated. After removal of solvent under reduced pressure, the residue was purified by chromatographic column using silica as stationary phase and hexane/EtOAc as eluent to afford 6.33 g (yield 92%) of the compound **5**.

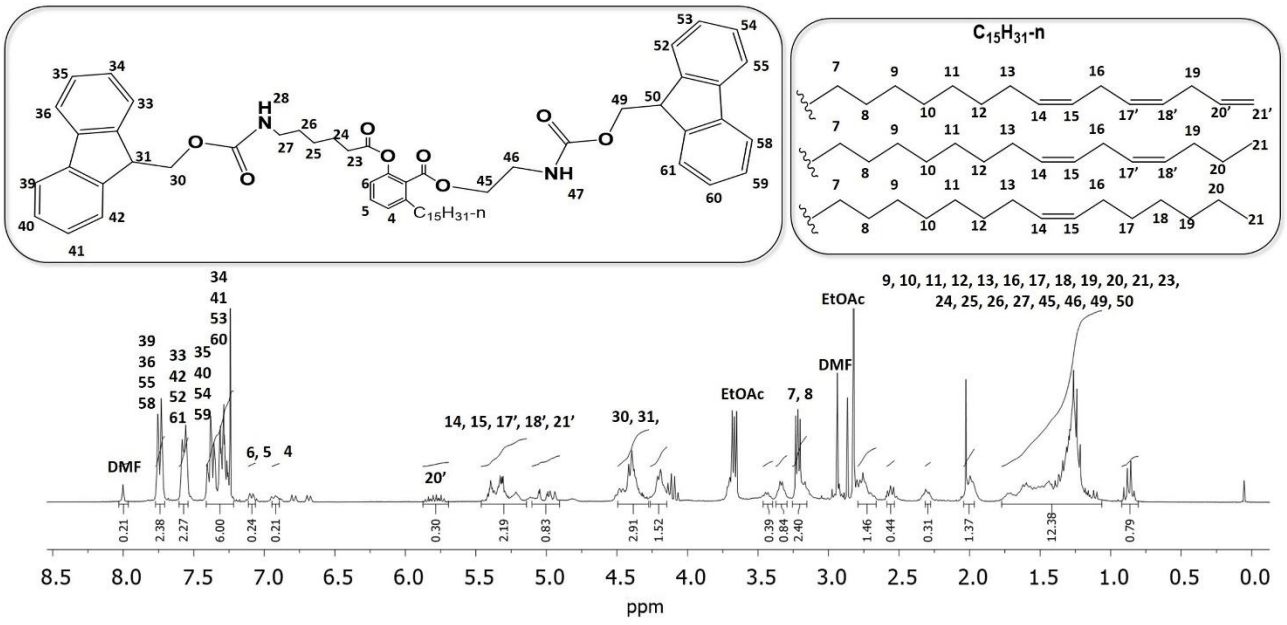
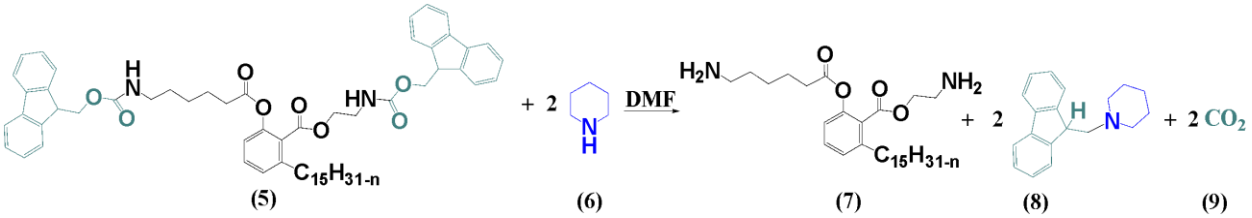


Figure S4. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of compound **5**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.74 (d, *J* 7.4 Hz, 2H), 7.56 (d, *J* 3.8 Hz, 2H), 7.41-7.26 (m, 6H), 7.10 (s, 1H), 6.93 (d, *J* 8.2 Hz, 1H), 5.82-5.71 (m, 1H), 5.42-5.14 (m, 2H), 5.09-4.92 (m, 2H), 4.49-4.27 (m, 3H), 4.20 (d, *J* 6.3 Hz, 2H), 3.49-3.40 (m, 2H), 3.34 (d, *J* 4.8 Hz, 2H), 3.24-3.13 (m, 1H), 2.73 (d, *J* 15.3 Hz, 1H), 2.57 (dd, *J* 11.0, 4.8 Hz, 3H), 2.32 (dd, *J* 13.1, 9.4 Hz, 3H), 1.99 (d, *J* 5.4 Hz, 1H), 1.82-1.06 (m, 12H), 0.87 (q, *J* 7.2 Hz, 1H).

Step 3: synthesis of 2-aminoethyl 2-(6-aminohexanoyloxy)-6-((8*E*,11*E*)-pentadeca-8,11,14-trienyl)benzoate- compound **7** - monomer



In an oven-dried round-bottomed flask containing the compound **5** (6.33 g, 21.6 mmol) was added 15 mL of 20% piperidine solution in DMF and the mixture resulting was kept under stirring for 10 h at room temperature. After, removal of solvent under reduced pressure, the products were separated by chromatographic column using silica as stationary phase and hexane/EtOAc as eluent to afford 2.71 g (yield 81%) of the monomer (**7**). A ninhydrin test for the detection of primary amines was processed on the product and turned out to be negative. Thus, we used 1 mL of the ninhydrin solution for steel with 5 mg of sample was added and the mixture heated to boiling.

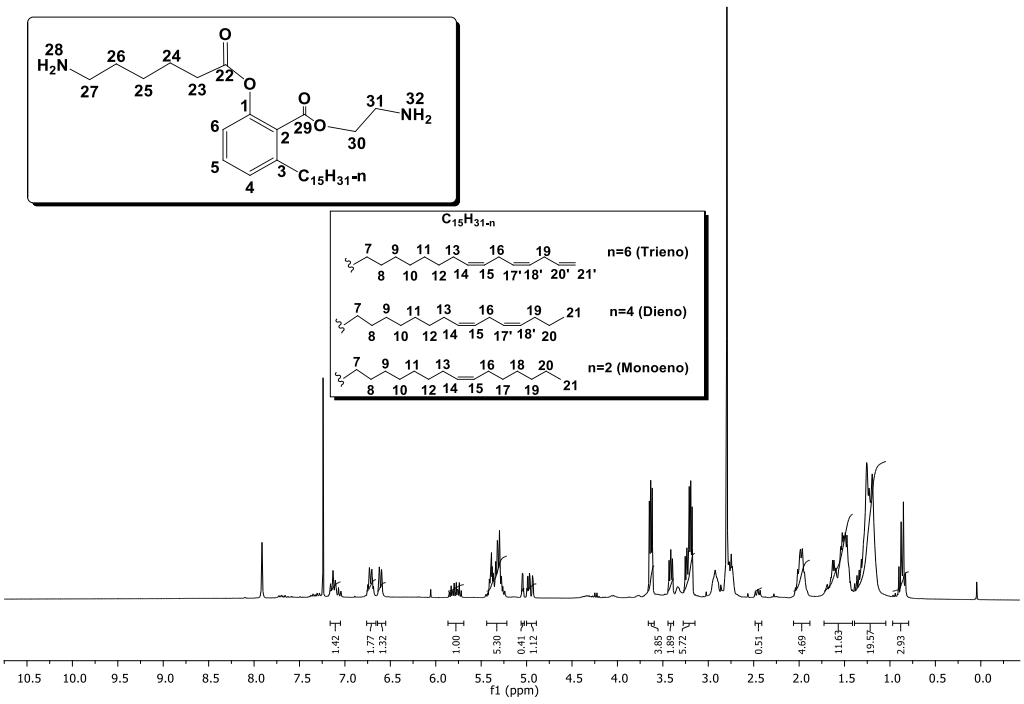


Figure S5. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of monomer.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18-7.01 (m, 1H), 6.72 (dd, *J* 13.2, 5.2 Hz, 1H), 6.65-6.57 (m, 1H), 5.86-5.70 (m, 1H), 5.46-5.22 (m, 3H), 5.07-5.01 (m, 1H), 5.01-4.91 (m, 1H), 3.40 (dd, *J* 12.6, 7.0 Hz, 1H), 3.26-3.17 (m, 3H), 2.68 (s, 1H), 2.43 (dd, *J* 20.7, 12.7 Hz, 1H), 1.98 (dd, *J* 14.1, 7.9 Hz, 2H), 1.71-1.42 (m, 7H), 1.41-1.16 (m, 11H), 0.86 (q, *J* 7.1 Hz, 2H).

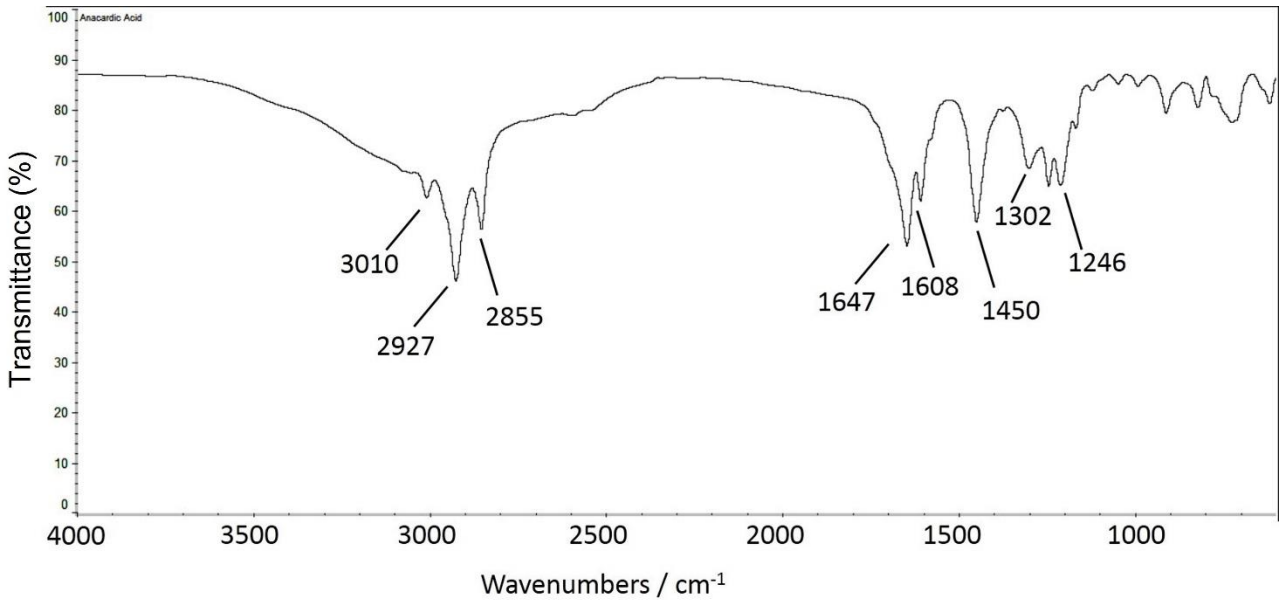


Figure S6. FTIR (KBr) spectrum of anacardic acid.

IR (KBr) ν / cm^{-1} 3010 (Ar-H and vinyl-H), 2927 and 2855 (aliphatic C–H), 1647 (–COOH), 1608 (aromatic C=C), 1450 (aromatic C=C), 1302 (Ar–OH and –COOH), 1246 (phenolic C–O).

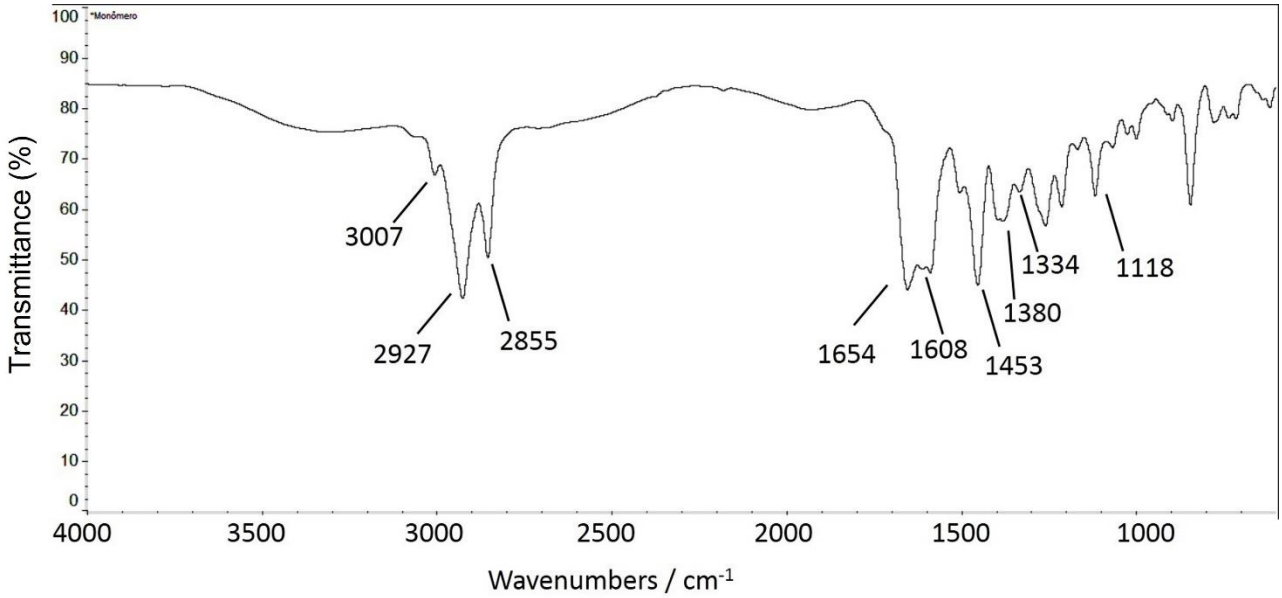


Figure S7. FTIR (KBr) spectrum of monomer.

Thermogravimetry

Thermogravimetric (TG) analyzes were performed for the nanocapsules prepared in two TDI equivalents and redispersed in water in order to examine the thermal properties of the NC polymer wall. The obtained TG curves are shown in Figure S8. In both samples, in the temperature range between 240 and 400 °C, the TG curve shows a gradual weight loss of approximately 75% indicating thermal stability of the synthesized nanocapsules, within the test conditions.

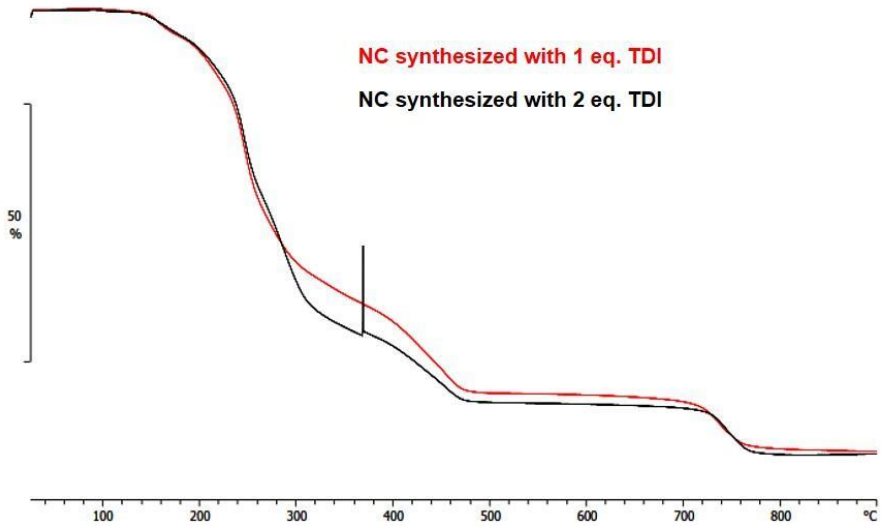


Figure S8. TG curves obtained for the nanocapsules synthesized and dispersed in water.

Encapsulation of the sulforhodamin

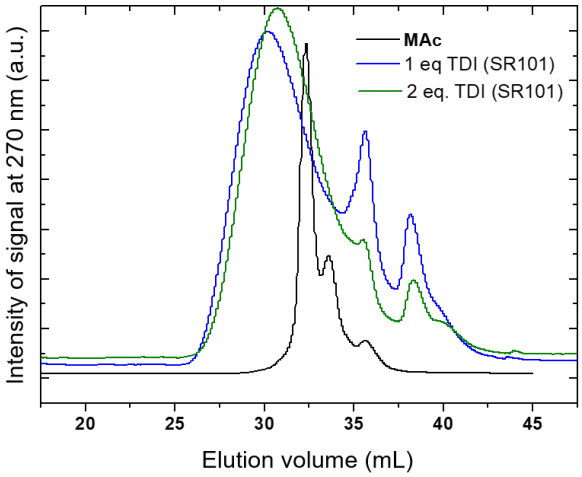


Figure S9. GPC chromatograms obtained for the miniemulsions with dye prepared in the two equivalents of TDI (1 eq. TDI and 2 eq. TDI) in comparison to the monomer.

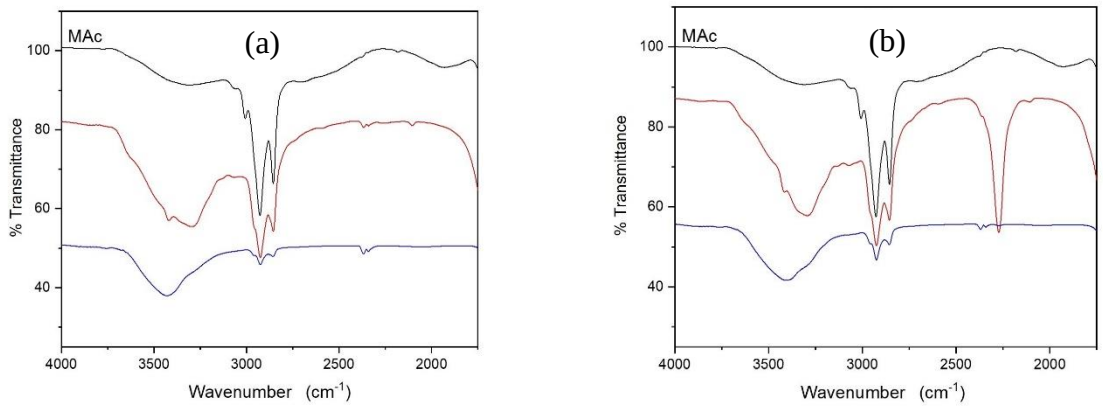


Figure S10. IR spectra obtained for the MAc (black) and for the NCs with encapsulated SR101 dye in cyclohexane (red) and redispersed in water (blue), synthesized using 1 molar eq. of TDI (a) and 2 molar eq. of TDI (b).

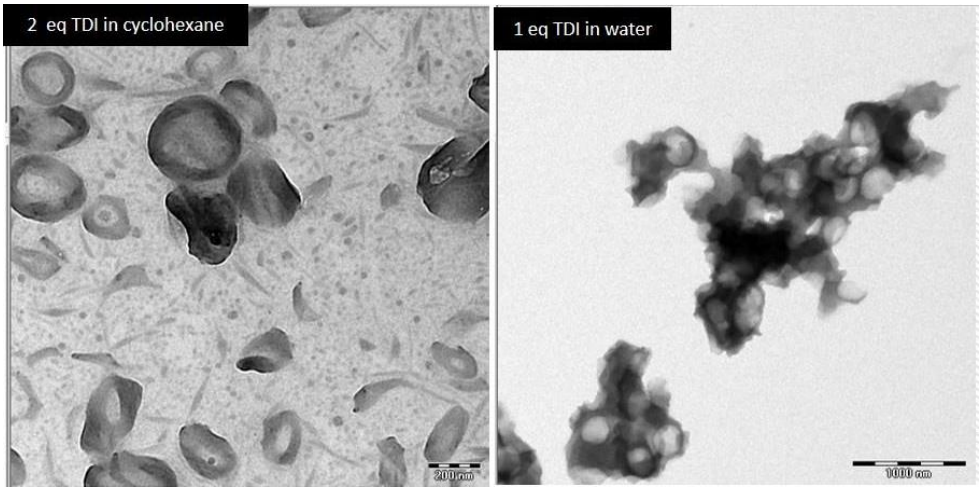


Figure S11. TEM micrographs obtained for the nanocapsules with encapsulated SR101 dye synthesized in cyclohexane and redispersed in water.

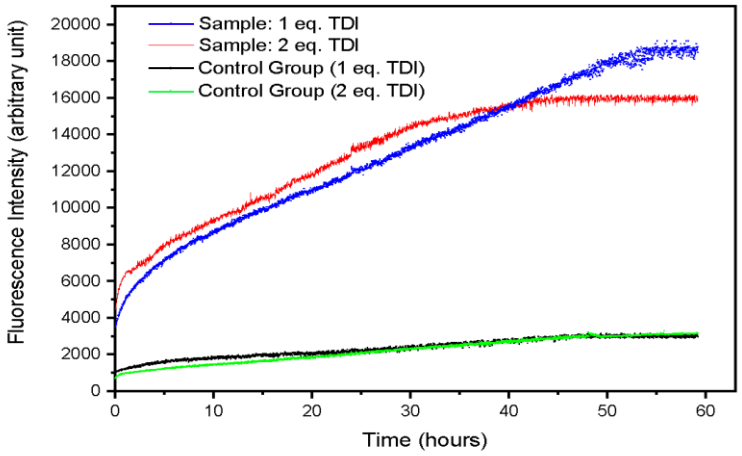


Figure S12. Monitoring by fluorescence of dye release during polymeric enzymatic degradation.

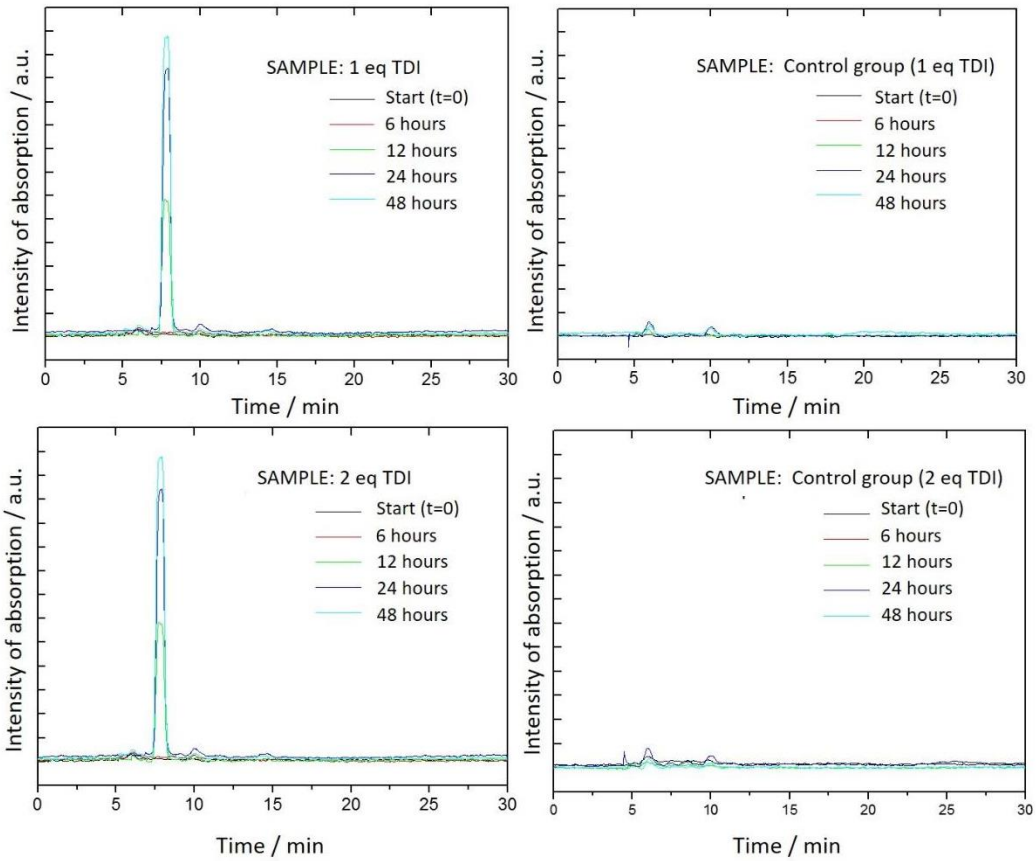


Figure S13. HPLC of AA release during the polymeric enzymatic degradation.

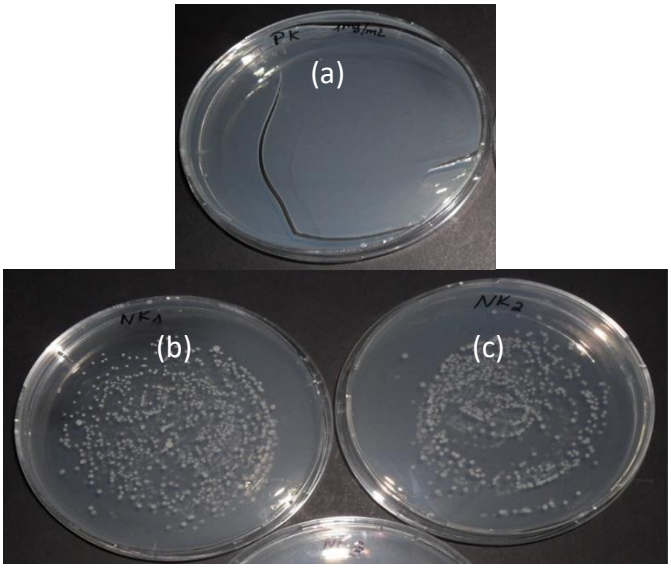


Figure S14. Control groups for bacterial tests: (a) with 200 µg anacardic acid in 2.5% DMSO, (b) with only water and (c) 200 µL of water with 2.5% DMSO.

Table S1. HPLC quantification of the free anacardic acid released (mean and 95% confidence intervals derived from n = 3) by enzymatic degradation assays of the nanocapsules

time / h	Concentration of free AA / (mg mL^{-1})	
	1 eq. TDI	2 eq. TDI
Start (time = 0)	0.006	0.006
6	0.013	0.021
12	0.083	0.110
24	0.159	0.183
48	0.184	0.199

TDI: 2,4-toluene diisocyanate; eq.: equivalent; AA: anacardic acid.