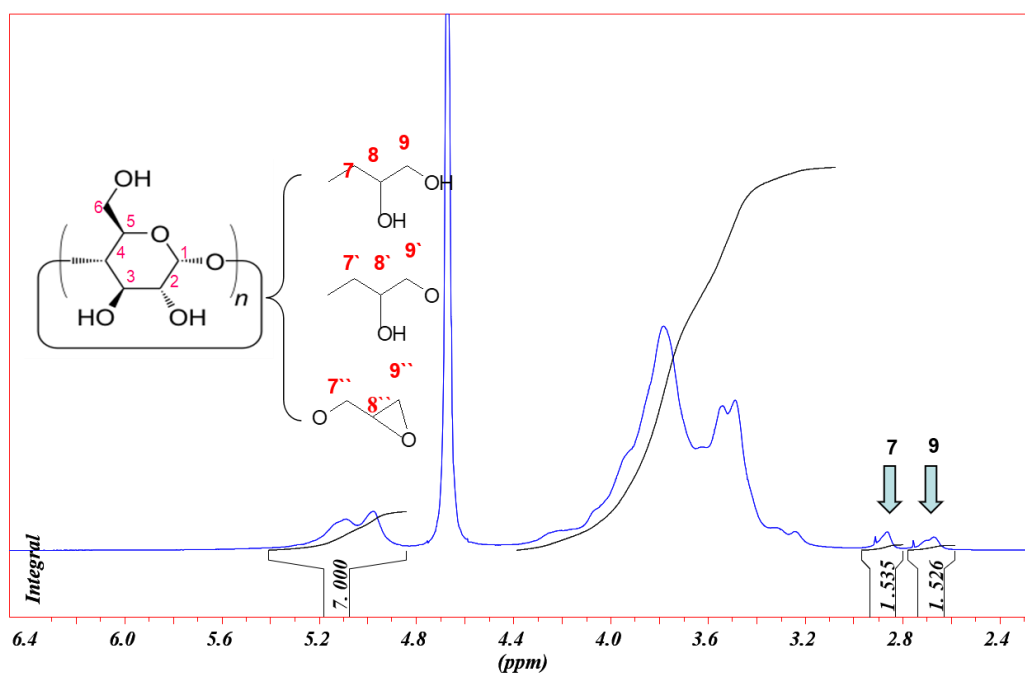


Polymerized β -cyclodextrin-based injectable hydrogel for sustained release of 5-fluorouracil/methotrexate mixture in breast cancer management. *In vitro* and *In vivo* Analytical Validations

1. SI_1_Figure S1 (A and B)

A)



B)

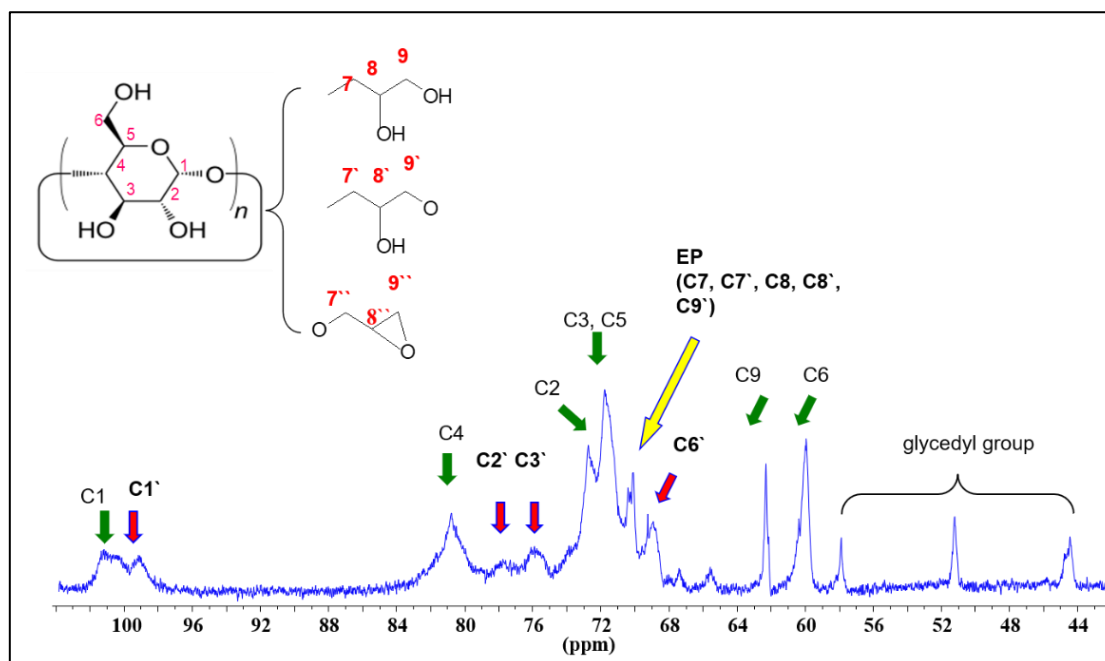


Figure S1. ^1H NMR (600 MHz, D_2O) spectrum of p β -CD, (A). ^{13}C NMR (600 MHz, D_2O) spectrum of p β -CD, (B).

2. SI. 2_Figure S2

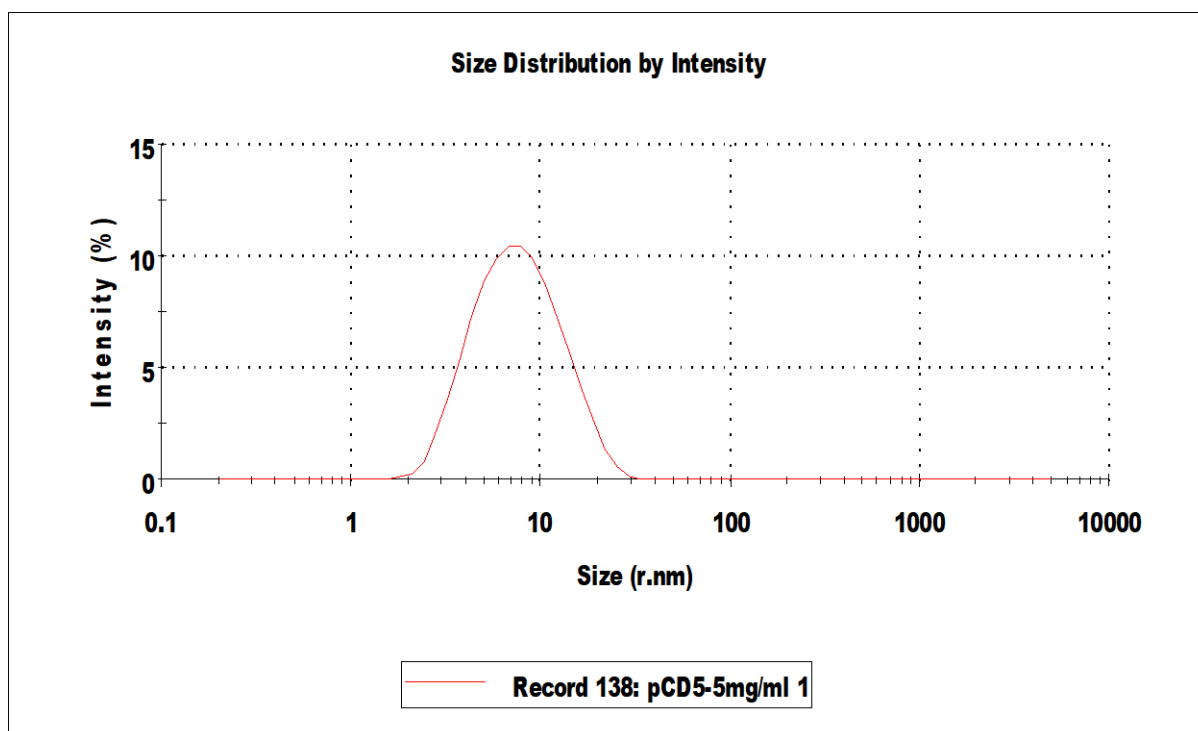


Figure S2. DLS spectrum of linear water soluble p β -CD polymer synthesized from the interaction between 10 g (8.8 mmole) β -CD and 5g (5.4 mole % or 54 mmole) epichlorohydrin in alkaline medium in the presence of toluene to produce linear polymer.

Condition of experiment:

Standard: poly(styrene) of RI 1.59, Dispersant: water, Temp: 25, Attenuator: 9

Reading:

Size average (r.nm): 6.59 hydrodynamic diameter (d.nm): **8.22** % intensity : 100

PI: 0,2

Mn (g/mol): on the light of hydrodynamic diameter and regarding the published data, the molecular weight of this polymer is 106 kD.

3. SI. 3._Figure S3

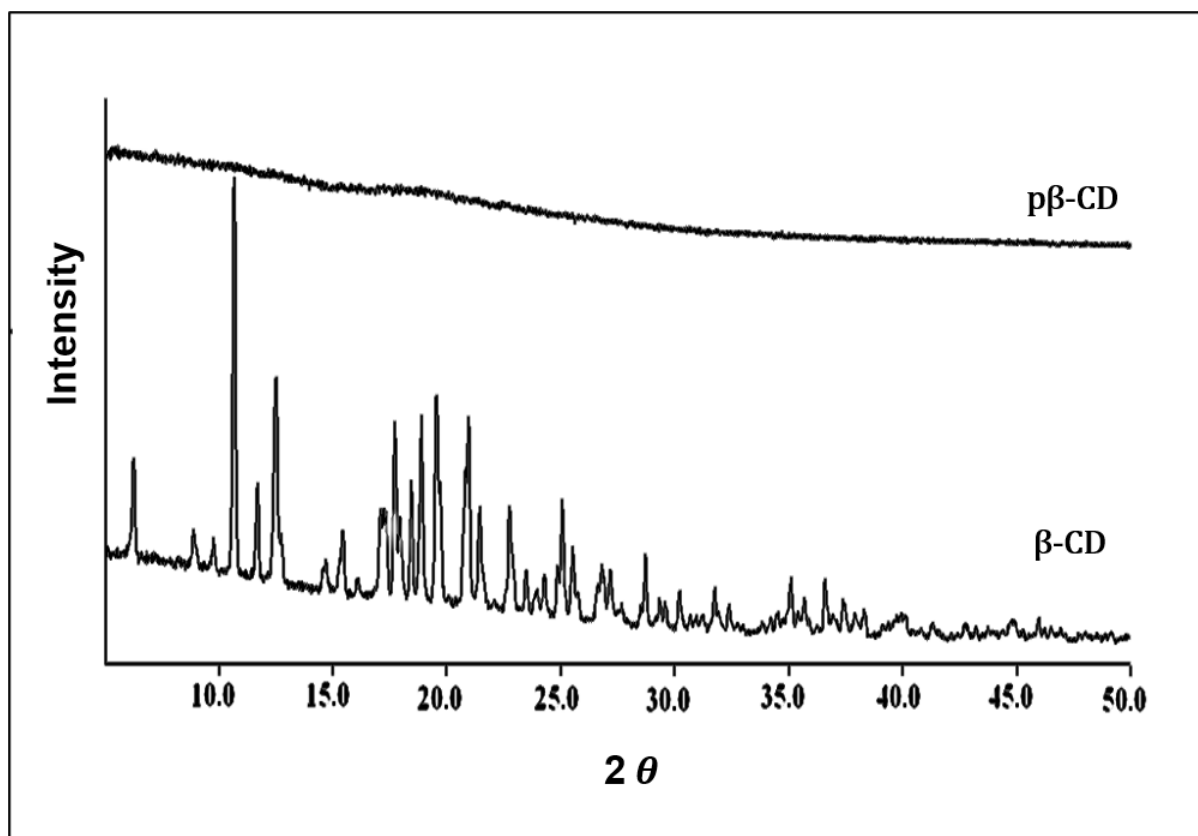


Figure S3. X-ray diffraction patterns of β -CD and $p\beta$ -CD, showing the complete disappearance of crystalline intensity peaks of native cyclodextrin upon polymerization. The x-ray confirmed the conversion of crystalline B-CD from crystalline form to amorphous one.

4. SI. 4_Figure S4 (A and B)

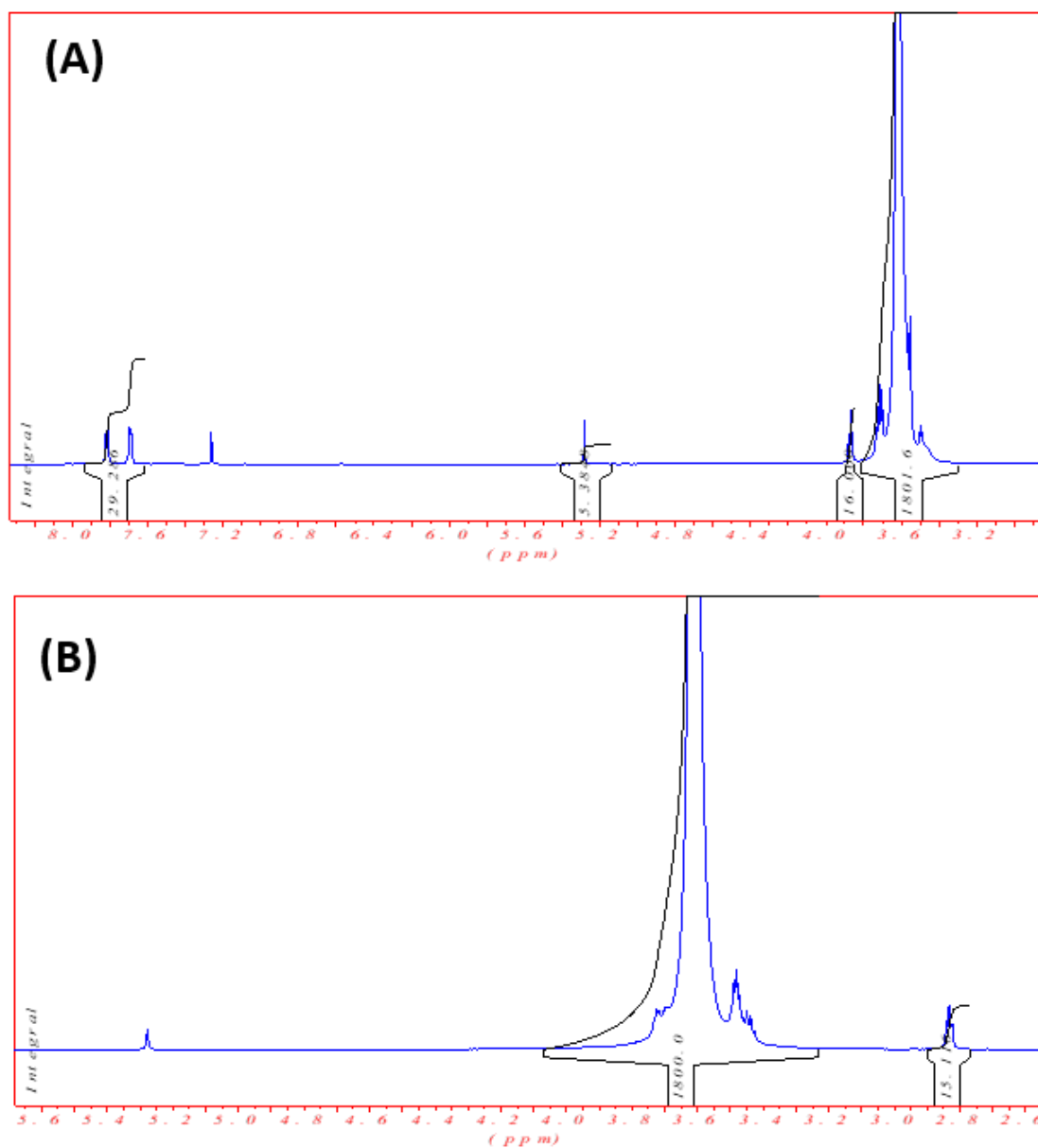


Figure S4. ^1H NMR 600 MHz, 8armPEG20k-Phthalimide (A) and 8armPED20k-Amine (B).

5. SI_5_Figure S5 (A and B)

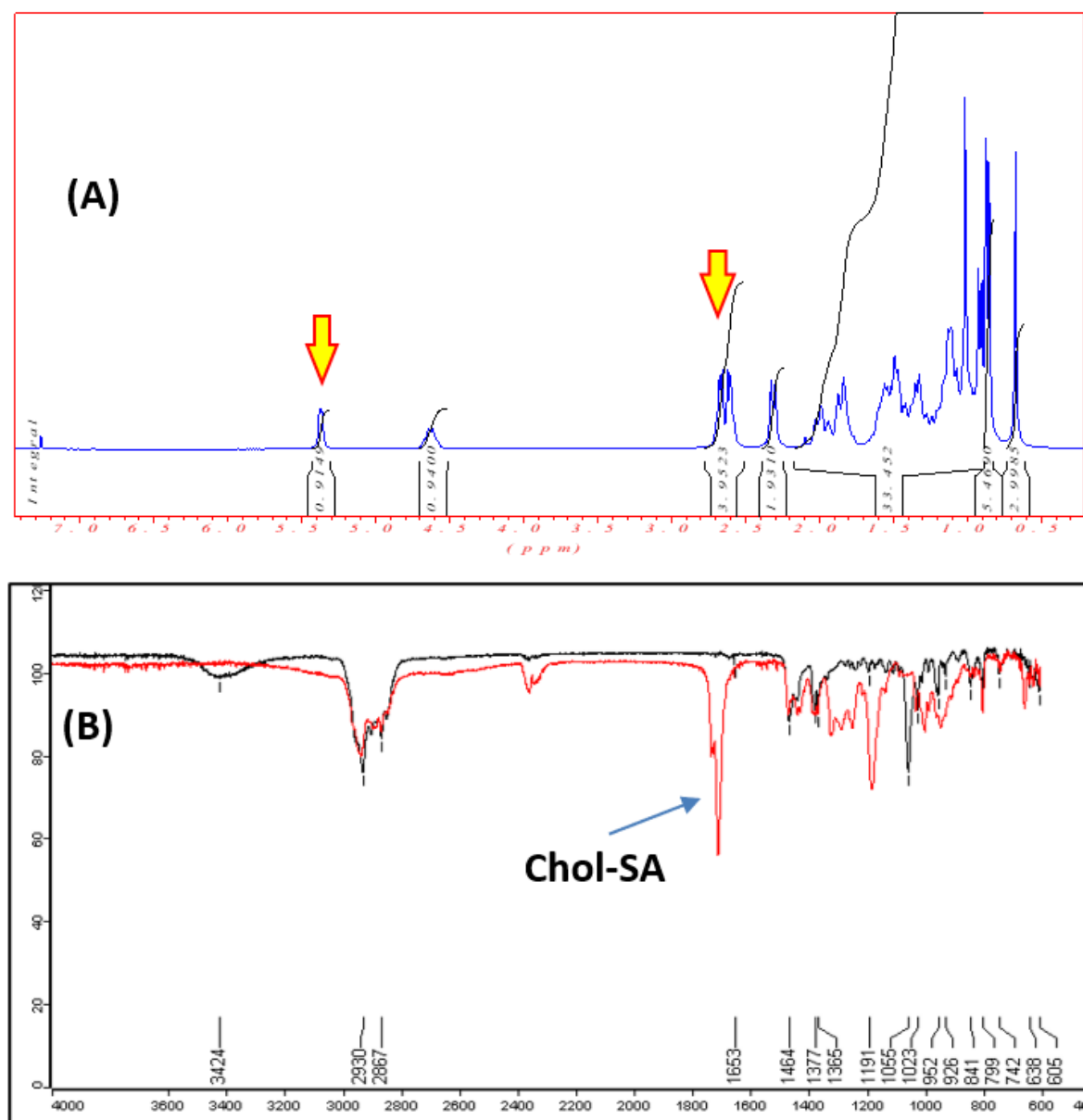


Figure S5. ^1H NMR (CDCl₃, 300 MHz) (A); IR spectroscopy (B) analysis of cholesterol-propionic acid, compared with cholesterol.

6. SI. 6._Figure S6 and Table S1

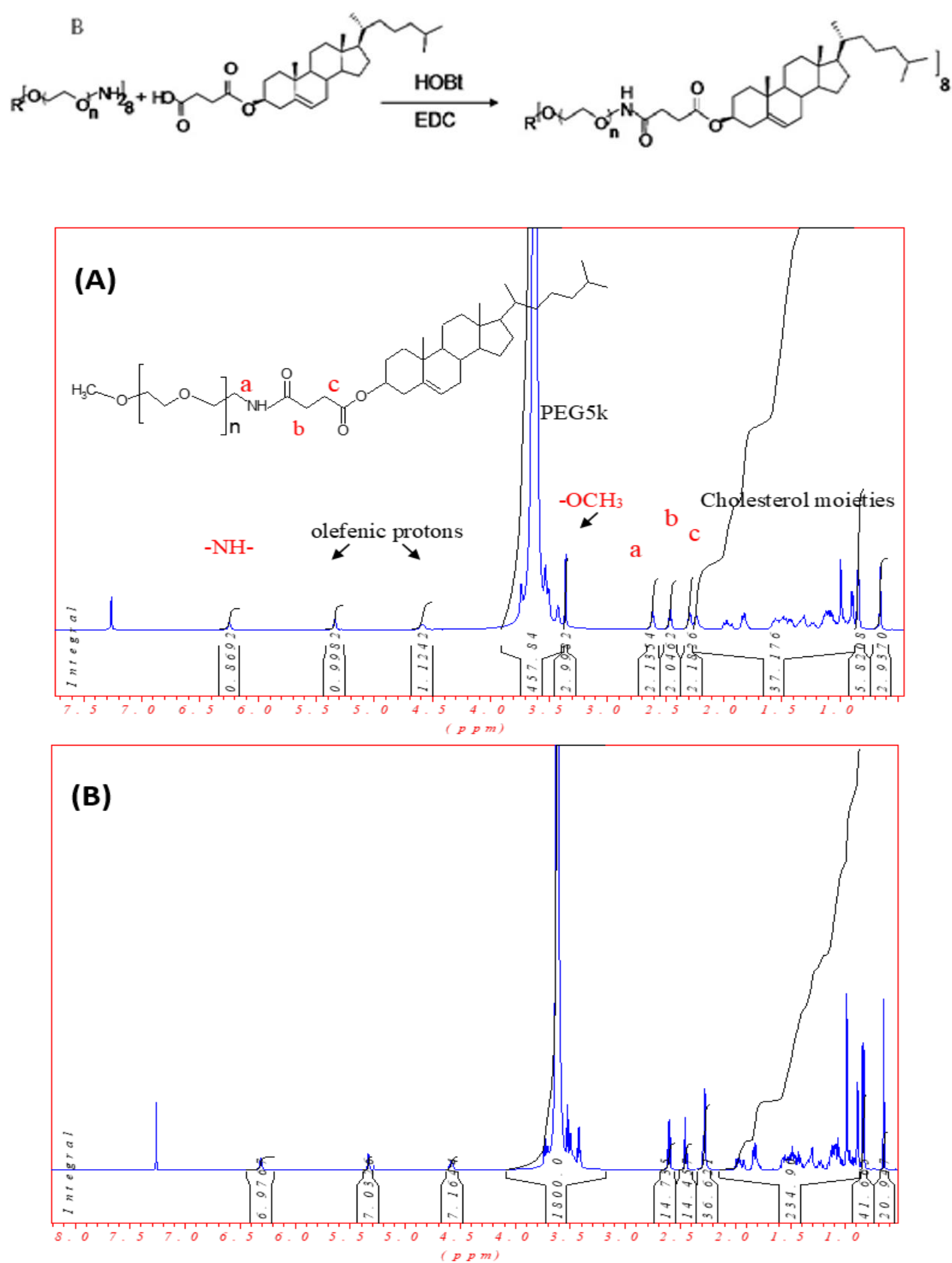


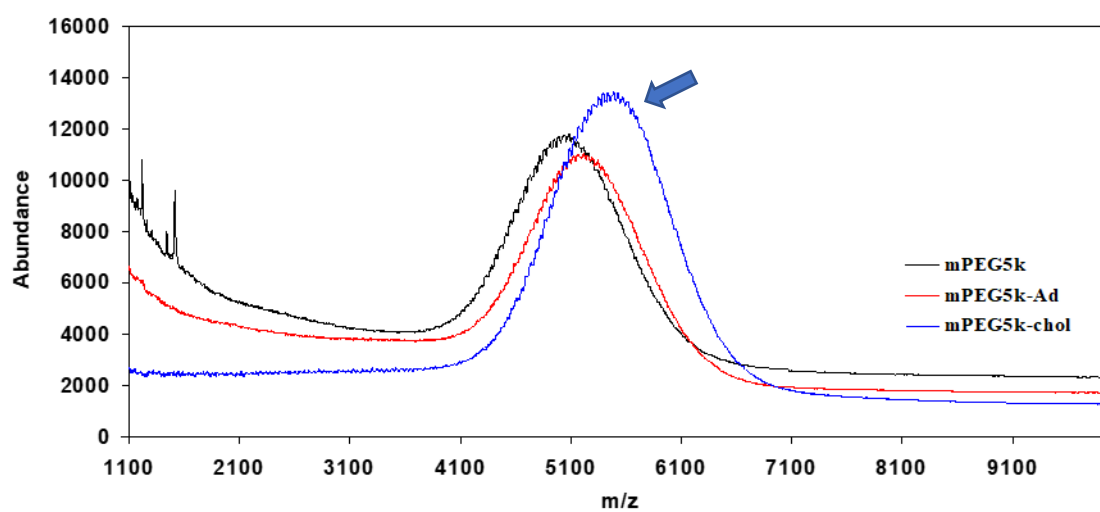
Figure S6. ^1H NMR spectra, 600 MHz of mPEG5k-Chol (A) and 8armPEG20k-Chol (B) in CDCl_3 .

Table S1: Quantification of the NMR signals of the modified PEG-(chol)_n polymers.

Polymer, ppm		2.3-2.5 ppm	2.65 pmm	3.35 pmm	3.4 - 4.9 Pmm Reference peaks	4.6 pmm	5.4 pmm	6.3 pmm	DS	Mwt
mPEG5k-Chol	Calculated	4	2	3	458	1	1	1	100	100
mPEG5k-Chol	Collected	4.1	2.1	2.99	457.8	1.1	0.99	0.86		99
8armPEG20k- (Chol) ₈	Calculated	32	16		1800	8	8	8	100	100
8armPEG20k- (Chol) ₇	Collected	28	14		1800	7	7	7	7	87.5

7. SI. 7_Figure S7 (A and B)

A)



B)

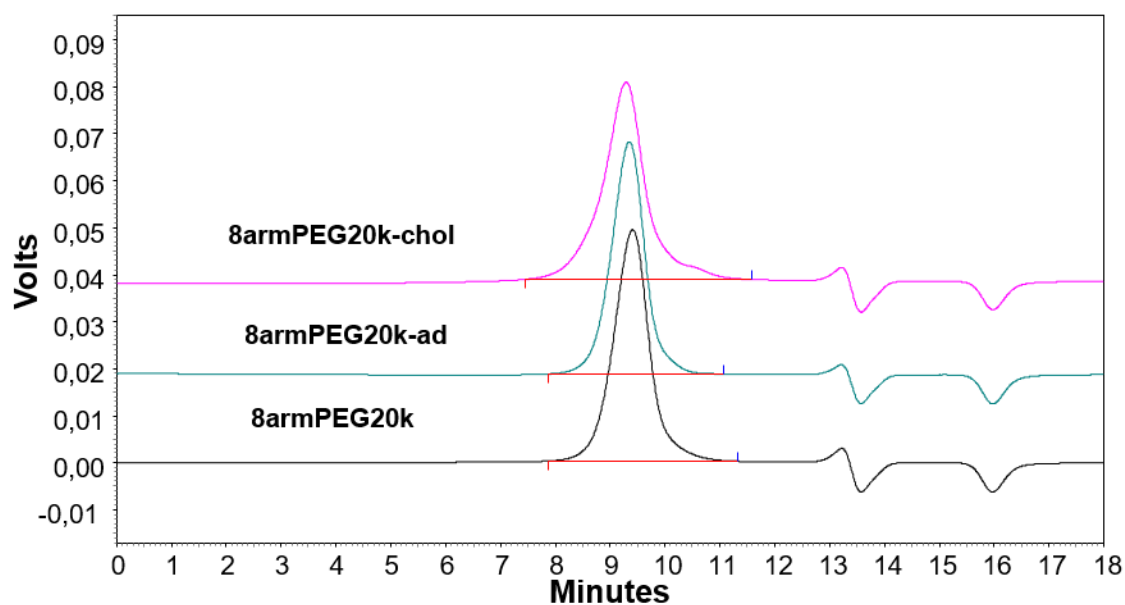


Figure S7. (A) MALDI-ToF mass spectra of unmodified mPEG5k, mPEG5k-Ad and mPEG5k-Chol in THF. The matrix is IAA-THF layer by layer technique. (B) GPC profiles of chromatograms of unmodified and hydrophobically modified 8armPEG20k.

8. SL. 8_Figure S8

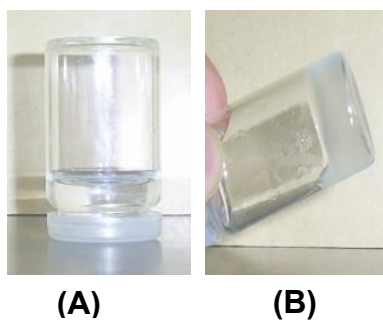


Figure S8. Fotos of the modified hydrogel, composed of 10% w/v 8armPEG20k-cholesterol- β -CD (B) in comparison with viscous liquid composed 10% w/v 8armPEG20k-OH/ β -CD (A).

9. SL 9_Figure S9

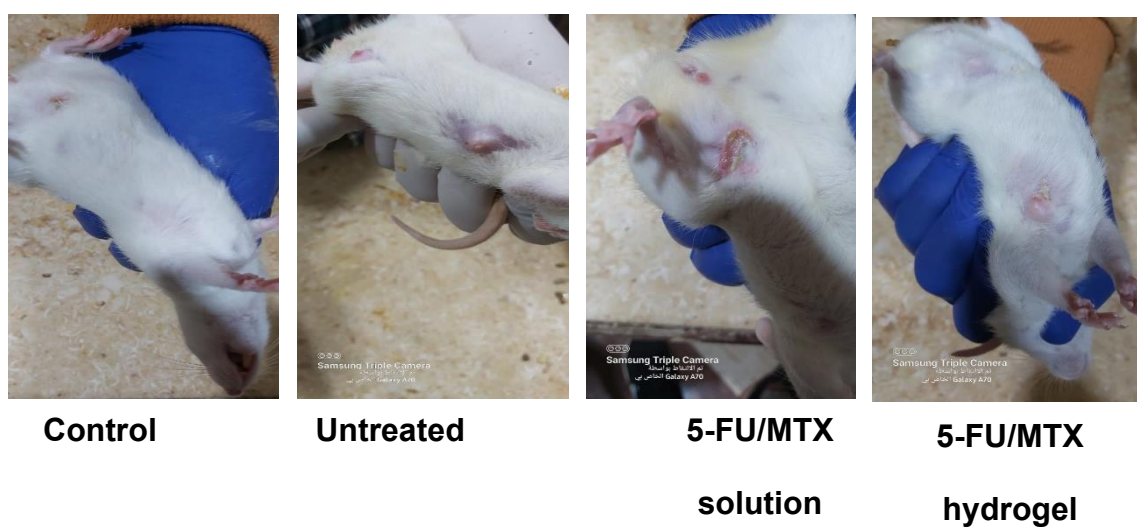


Figure S9. Pictures of the rats bearing breast cancer before and after treatment by 5-FU/MTX either a saline solution or loaded hydrogel.