

Folic-Acid-Conjugated Thermoresponsive Polymeric Particles for Targeted Delivery of 5-Fluorouracil to CRC Cells

Sylvia Milewska ^{1,2,†}, Gabriela Siemiaszko ^{3,†}, Agnieszka Zofia Wilczewska ³, Iwona Misztalewska-Turkowicz ³, Karolina Halina Markiewicz ³, Dawid Szymczuk ^{3,4}, Diana Sawicka ¹, Halina Car ¹, Ryszard Lazny ³ and Katarzyna Niemirowicz-Laskowska ^{1,*}

¹ Department of Experimental Pharmacology, Medical University of Bialystok, Szpitalna 37, 15-361 Bialystok, Poland

² Doctoral School, Medical University of Bialystok, Kilinskiego 1, 15-089 Bialystok, Poland

³ Faculty of Chemistry, University of Bialystok, Ciolkowskiego 1K, 15-245 Bialystok, Poland

⁴ Doctoral School of Exact and Natural Science, University of Bialystok, Ciolkowskiego 1K, 15-245 Bialystok, Poland

* Correspondence: katarzyna.niemirowicz@umb.edu.pl

† These authors contributed equally to this work.

Table of content

Table S1. Details of P-(1-3) polymers synthesis.	2
Table S2. Details of PT-(1-3) polymers synthesis.	2
Table S3. Details of PTF-(1-3) polymers synthesis.	2
Table S4. Thermal properties of the polymers.	3
Table S5. Calculations of content of folic acid	3
Figure S1. ¹ H NMR spectrum of exemplary PHEA polymer, P-1, in DMSO- <i>d</i> ₆	4
Figure S2. ¹ H NMR spectrum of exemplary PHEA- <i>b</i> -NIPAAm polymer, PT-1, in DMSO- <i>d</i> ₆	5
Figure S3. ¹ H NMR spectrum of exemplary P[HEA-FA)- <i>ran</i> -(HEA)]- <i>b</i> -P(NIPAAm) polymer, PTF-1, in DMSO- <i>d</i> ₆	5
Figure S4. The juxtaposition of ATR-FT IR, and folic acid (magnification of the region below 1900 cm ⁻¹) – left, and FT IR (in CHCl ₃) – right – spectra of PT-2, PTF-2, PT-3, PTF-3 polymers.	5
Figure S5. UV-Vis spectra of PHEA- <i>b</i> -PNIPAAm polymers, PT-(1-3), in conc. 0.2 mg·mL ⁻¹ in deionized water at 25 °C.	6
Figure S6. UV-Vis spectra of PTF-1, PTF-2, and PTF-3 polymers in conc. 0.2 mg·mL ⁻¹ in deionized water at 25 °C.	6
Figure S7. Normalized SEC-RI traces (A, C) and SEC-UV traces (B, D) of PHEA, PHEA- <i>b</i> -PNIPAAm, and P[(HEA-FA)- <i>ran</i> -(HEA)]- <i>b</i> -PNIPAAm polymers.	6
Figure S8-10. TG curves (top panel) and DTG curves (bottom panel) of P-3, PT-3, and PTF-3.	7-8
Figure S11. MADLS (size by number) measurements data of prepared polymers, PT-(1-3) and PTF-(1-3), with or without presence of 5-FU.	9
Figure S12. TEM images of polymers modified with folic acid.	9

Table S1. Details of **P-(1-3)** polymers synthesis.

Product	CTA			HEA			AIBN			THF (mL)	Conv ^a (%)	M _n , NMR ^a (g·mol ⁻¹)	M _n , th (g·mol ⁻¹)
	Eq	n (mmol)	m (mg)	Eq	n (mmol)	V (mL)	Eq	n (mmol)	m (mg)				
P-1	1.0	2.0	416.6	22.4	44.8	5.15	0.1	0.2	32.8	10.0	96.0	3500.0	2707.5
P-2	1.0	1.0	208.3	44.8	44.8	5.15	0.1	0.1	16.4	10.0	98.0	5800.0	5218.6
P-3	1.0	0.5	104.2	89.6	44.8	5.15	0.1	0.0	8.2	10.0	97.0	8700.0	10209.2

^aDetermined by ¹H NMR.**Table S2.** Details of **PT-(1-3)** polymers synthesis.

Product	PHEA				NIPAAm			AIBN			THF (mL)	Conv ^a (%)	M _n , th (g·mol ⁻¹)
	Substrate	Eq	n (mmol)	m (mg)	Eq	n (mmol)	m (g)	Eq	n (mmol)	m (mg)			
PT-1	P-1	1.0	0.14	370.8	64.5	8.84	1.0	0.01	0.001	0.16	4.5	89.0	9206.1
PT-2	P-2	1.0	0.12	600.8	76.8	8.84	1.0	0.01	0.001	0.16	4.5	85.0	12601.9
PT-3	P-3	1.0	0.09	890.8	101.3	8.84	1.0	0.01	0.001	0.16	4.5	82.0	19607.0

^aDetermined by ¹H NMR.**Table S3.** Details of **PTF-(1-3)** polymers synthesis.

Product	PHEA- <i>b</i> -PNIPAAm				FA			DCC			DMAP			DMSO (mL)	Conv th (%)	M _n , th (g·mol ⁻¹)
	Sub- strate	Eq	n (mmol)	m (mg)	Eq	n (mmol)	m (mg)	Eq	n (mmol)	m (mg)	Eq	n (mmol)	m (mg)			
PTF-1	PT-1	1.0	0.033	300.0	10.2	0.331	146.3	11.1	0.362	74.6	1.1	0.036	4.4	6.0	100.0	13695.5
PTF-2	PT-2	1.0	0.024	300.0	11.9	0.283	124.7	13.0	0.311	64.1	1.3	0.031	3.8	6.0	100.0	17840.0
PTF-3	PT-3	1.0	0.015	300.0	15.6	0.239	105.5	17.2	0.263	54.3	1.7	0.026	3.2	6.0	100.0	26502.1

^aDetermined by ¹H NMR.

Table S4. Thermal properties of the polymers.

Sample ID	Maximum of the degradation rate (°C)	Residue at 800 °C (%)	T _g ^a (°C)
P-1	440	5.1	-2.1
P-2	440	4.8	-3.3
P-3	440	5.6	-5.3
PT-1	415	3.7	82.0
PT-2	415	2.8	94.0
PT-3	415	4.2	83.2
PTF-1	405	5.3	119.1
PTF-2	405	7.4	108.2
PTF-3	410	7.1	103.5
PTF-1_5FU	395	7.9	-
5FU	310	5.4	-

^aDetermined by DSC from the second heating run performed with a heating rate of 10 °C·min⁻¹.

Table S5. Calculations of content of folic acid.

	A (y) ^a	c _{polymer} (mg·mL ⁻¹)	c _{FA} (mg·mL ⁻¹) (x) ^b	%wt (%) ^c
PTF-1	1.04	0.2	0.0193	9.65
PTF-2	1	0.2	0.0185	9.23
PTF-3	0.84	0.2	0.0151	7.56

^a Maximum absorbance taken from UV-Vis spectra of polymers in conc. c_{polymer} in deionized water at 25 °C.

^b Concentration of folic acid calculated from calibration curve of folic acid in H₂O (y = 0.048 x + 0.114).

^c %wt = (c_{polymer} : c_{FA}) · 100%

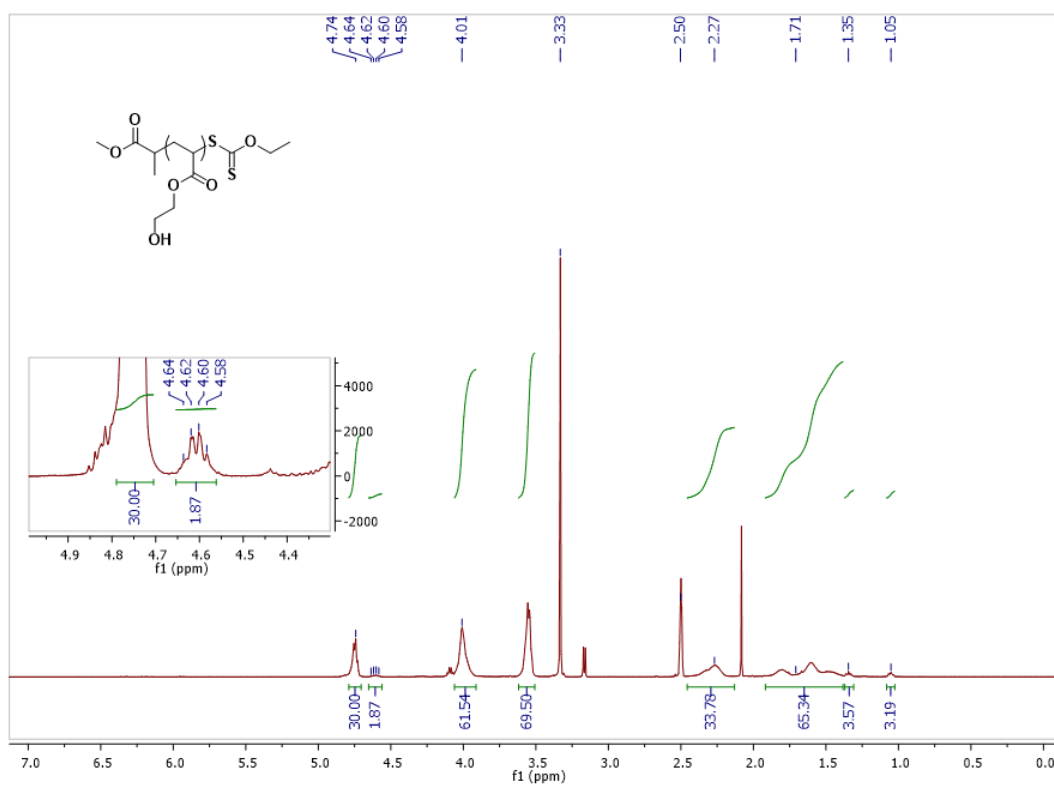


Figure S1. ^1H NMR spectrum of exemplary PHEA polymer, **P-1**, in $\text{DMSO-}d_6$.

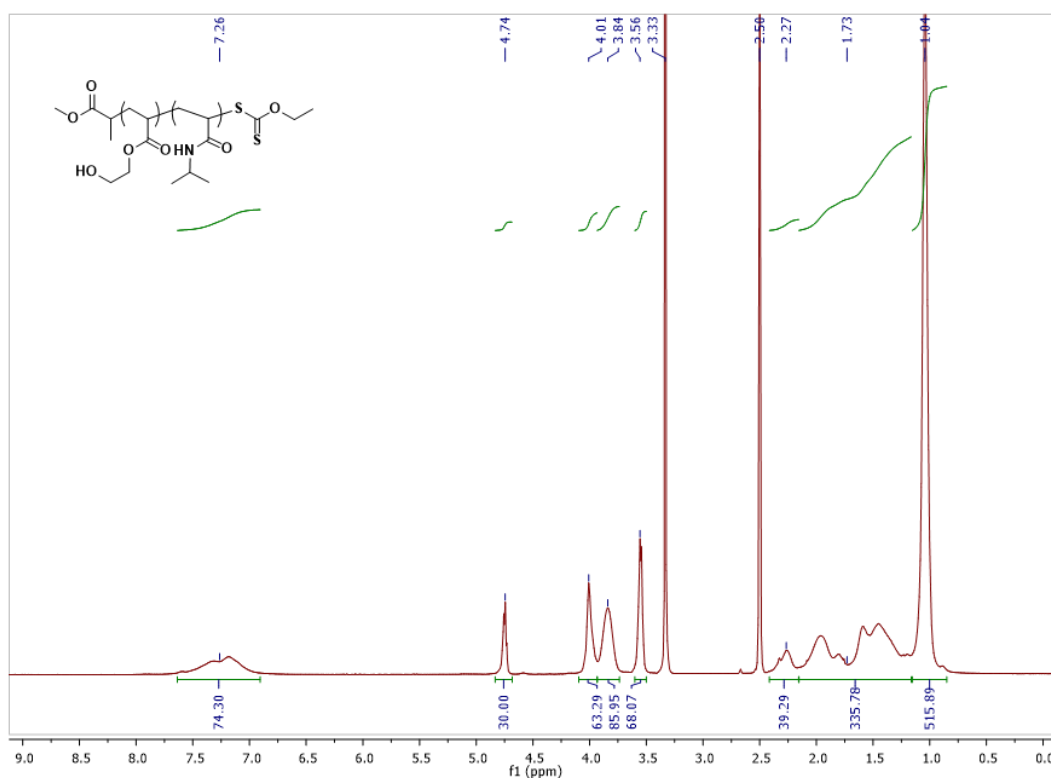


Figure S2. ^1H NMR spectrum of exemplary PHEA-*b*-PNIPAAm polymer, **PT-1**, in $\text{DMSO-}d_6$.

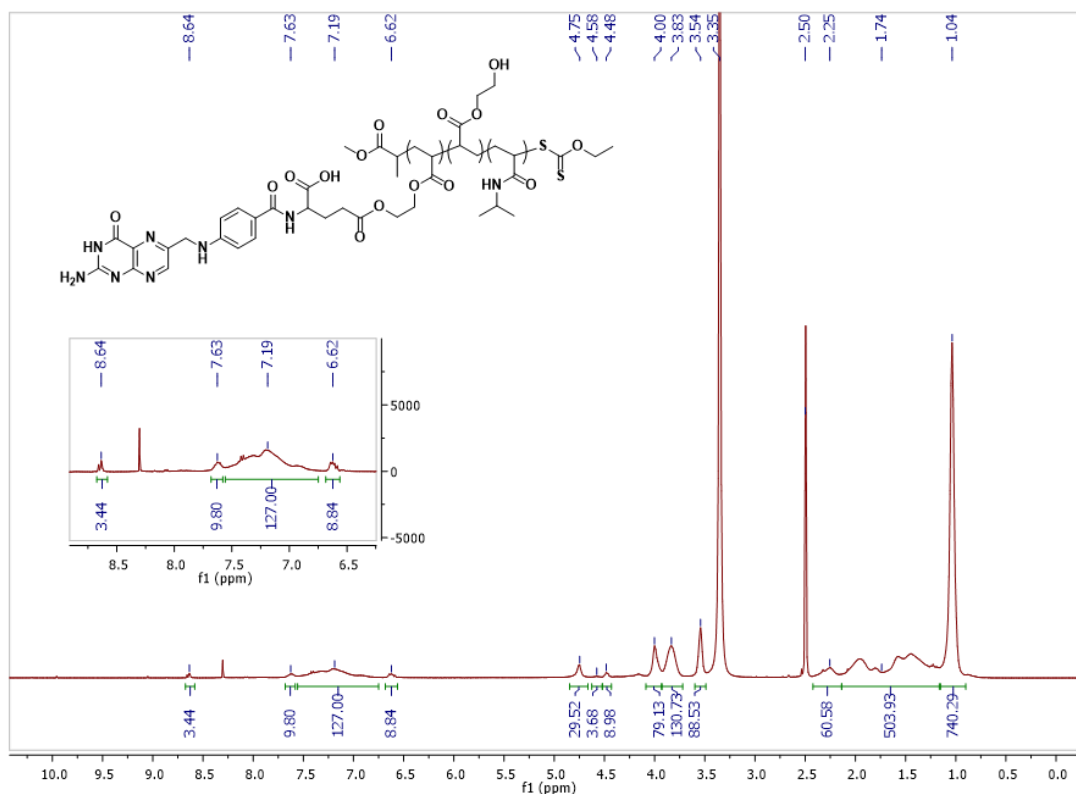


Figure S3. ^1H NMR spectrum of exemplary P[HEA-FA)-*ran*-(HEA))-*b*-P(NIPAAm) polymer, **PTF-1**, in DMSO- d_6 .

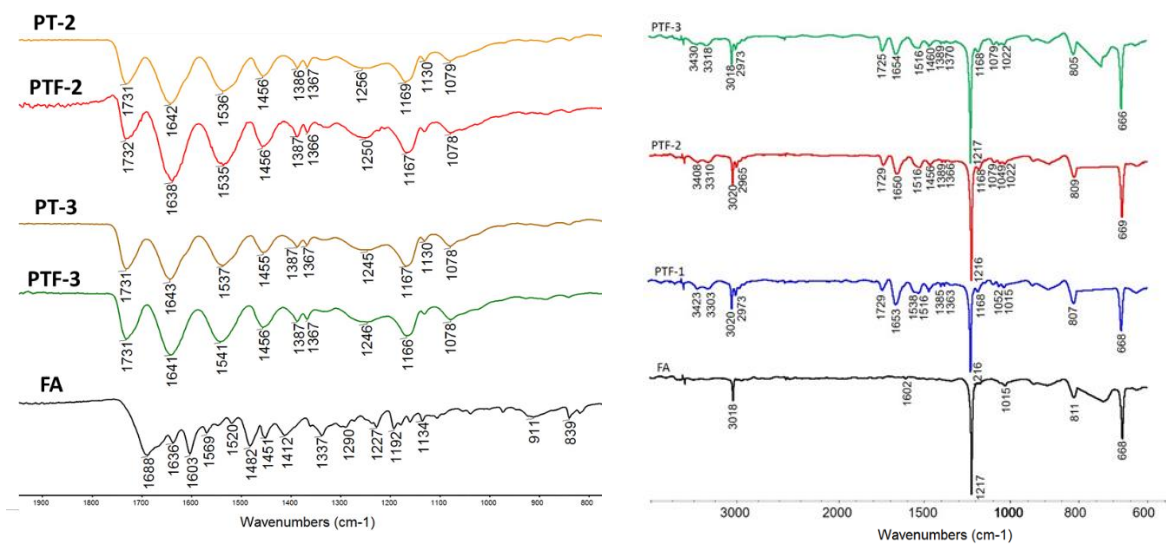


Figure S4. The juxtaposition of ATR-FT IR, and folic acid (magnification of the region below 1900 cm^{-1}) – left, and FT IR (in CHCl_3) – right – spectra of **PT-2**, **PTF-2**, **PT-3**, **PTF-3** polymers.

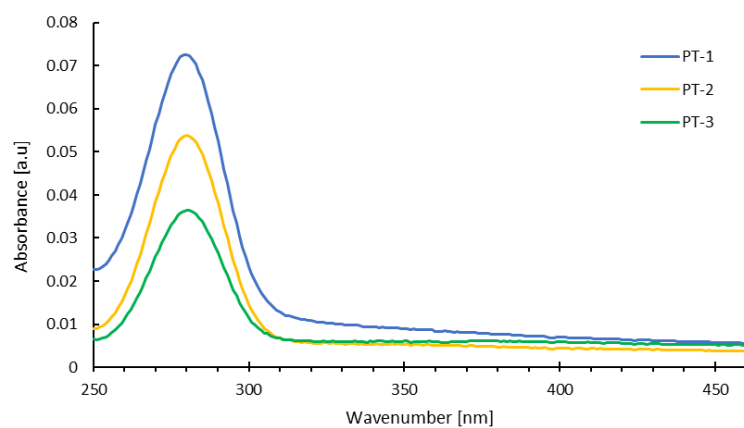


Figure S5. UV-Vis spectra of PHEA-*b*-PNIPAAm polymers, **PT-(1-3)**, in conc. 0.2 mg·mL⁻¹ in deionized water at 25 °C.

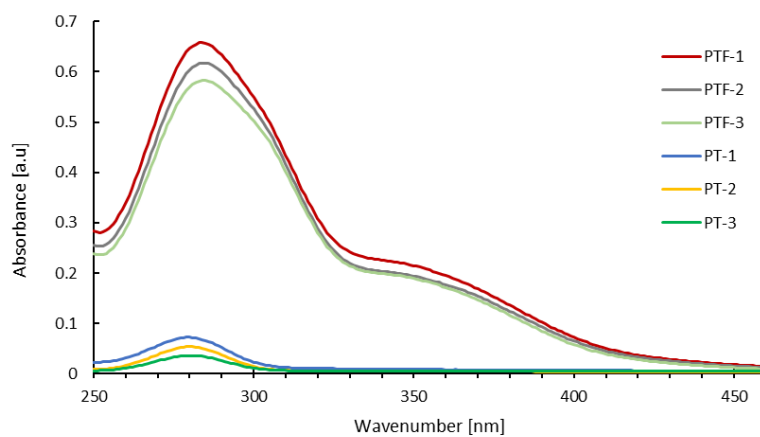


Figure S6. UV-Vis spectra of PT-1, PT-2, PT-3, PTF-1, PTF-2, and PTF-3 polymers in conc. 0.2 mg·mL⁻¹ in deionized water at 25 °C.

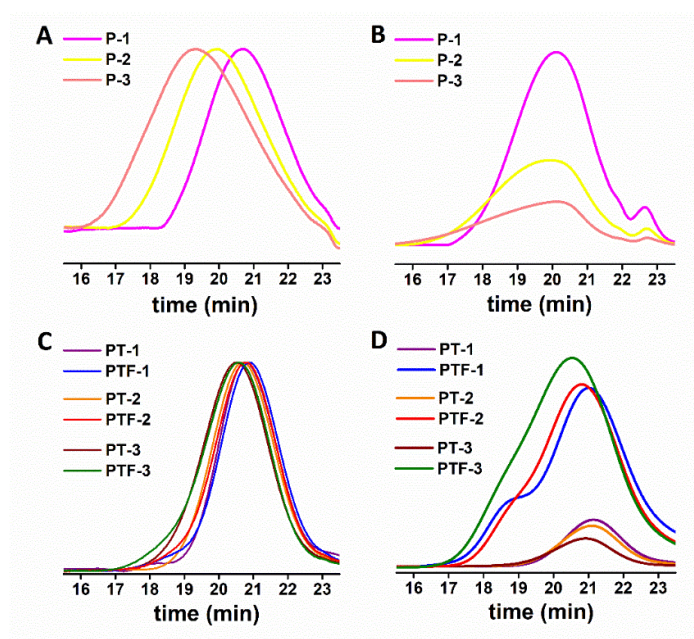


Figure S7. Normalized SEC-RI traces (A, C) and SEC-UV traces (B, D) of PHEA, PHEA-*b*-PNIPAAm, and P[(HEA-FA)-*ran*-(HEA)]-*b*-PNIPAAm polymers.

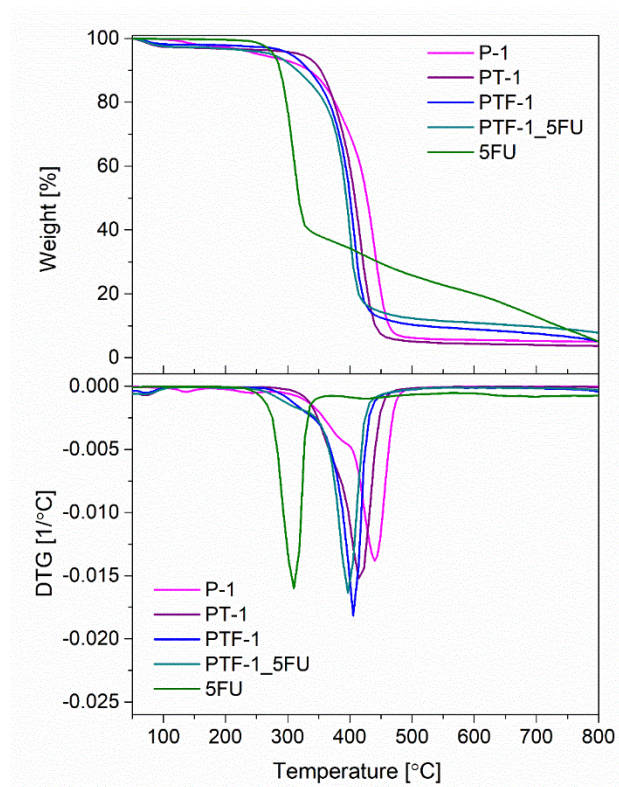


Figure S8. TG curves (top panel) and DTG curves (bottom panel) of **P-1**, **PT-1**, **PTF-1**, **PTF-1** with 5-FU, and 5-FU.

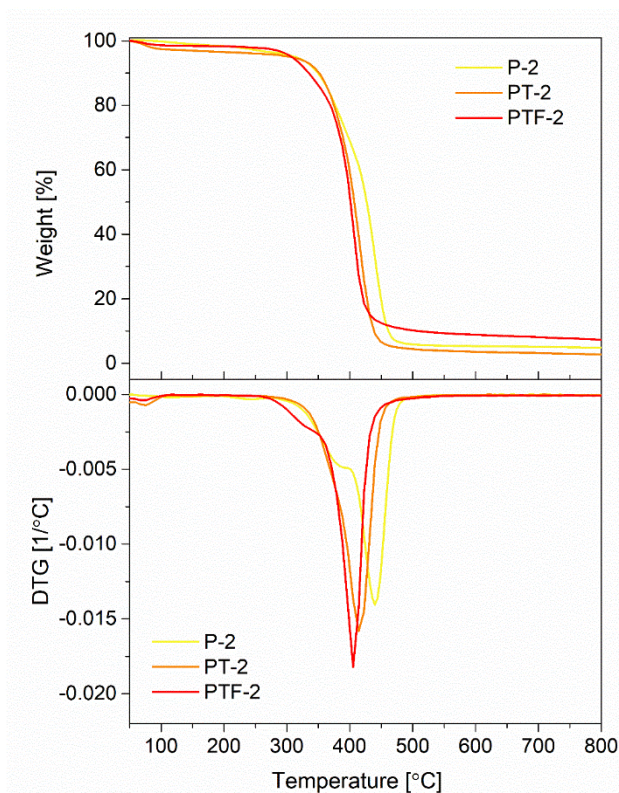


Figure S9. TG curves (top panel) and DTG curves (bottom panel) of **P-2**, **PT-2**, and **PTF-2**.

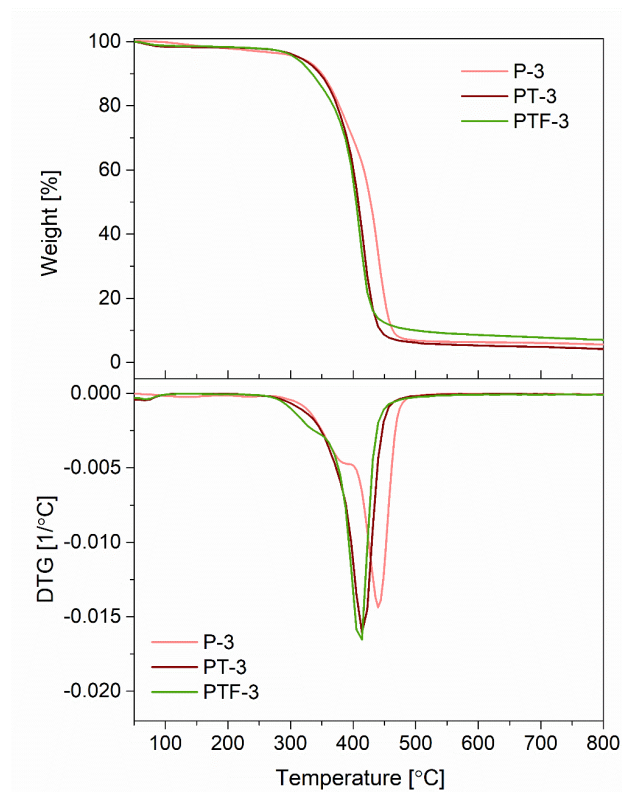


Figure S10. TG curves (top panel) and DTG curves (bottom panel) of **P-3**, **PT-3**, and **PTF-3**.

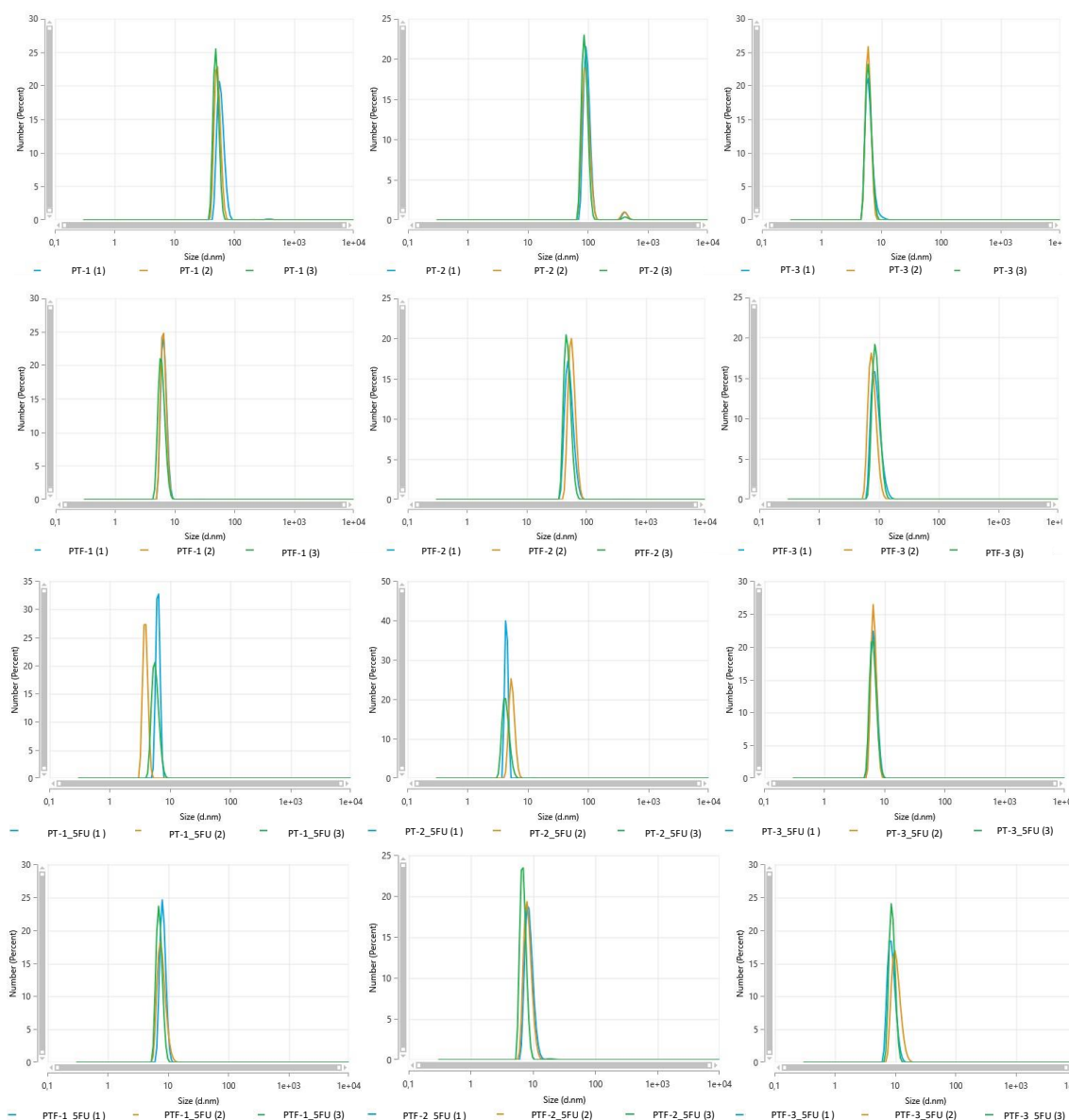


Figure S11. MADLS (size by number) measurements data of prepared polymers, **PT-(1-3)** and **PTF-(1-3)**, with or without presence of 5-FU.

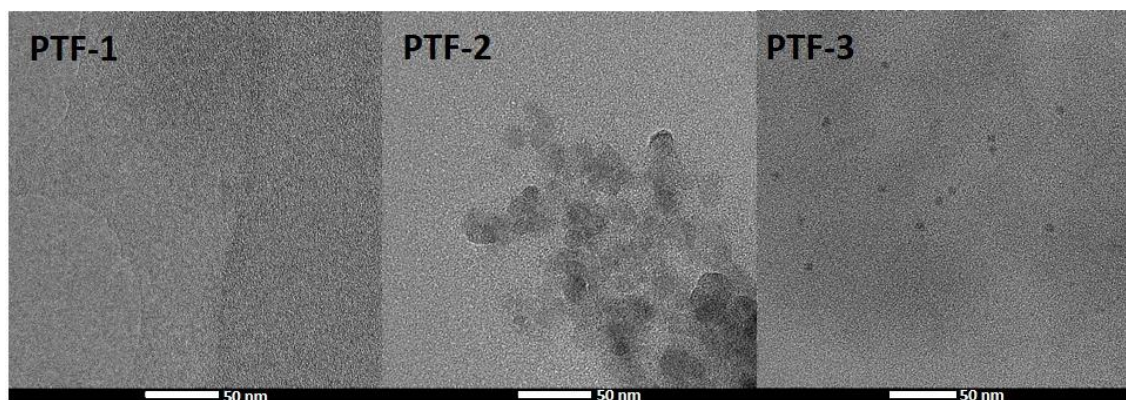


Figure S12. TEM images of polymers modified with folic acid.