Supporting Information

of

Stimuli-responsive, biocompatible hydrogel derived from glycogen and poly (N-isopropylacrylamide) for colon targeted delivery of ornidazole and 5-amino salicylic acid

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EXPERIMENTAL:

Characterization:

FTIR spectra of glycogen, pNIPAm, dried cl-Gly/pNIPAm hydrogel, ornidazole, 5-amino salicylic acid, and the drugs loaded hydrogel were performed in the scan range from 400-4000 cm^{-1} using FTIR spectrophotometer (Cary 600 series, Agilent Technologies).

^{1}H NMR spectral analyses of glycogen, NIPAm and cl-Gly/pNIPAm hydrogel were carried out at 400 MHz on Bruker spectrophotometer and the solid state ^{13}C NMR spectral analyses of glycogen and synthesized hydrogel (cl-Gly/pNIPAm) were executed using Bruker Advance II-500 NMR spectrophotometer.

The thermogravimetric analyses (TGA) of glycogen, pNIPAm and cl-Gly/pNIPAm hydrogel were performed in N_2 atmosphere at three different heating rates (5, 10, 15 °C/min) using a TGA analyser (Model: Perkin Elmer Pyris Diamond TG-DTA).

The surface morphology of glycogen and cl-Gly/pNIPAm hydrogel were studied by field emission scanning electron microscope (FESEM, Supra 55, Zeiss, Germany).

The DLS analysis was performed using particle size analyser (Model: SZ-100, Make: Horiba Scientific, Japan).

Swelling study:

The dried pre weighted glycogen and different grades of cl-Gly/pNIPAm hydrogel were taken in a tea-bag and imbibed in buffer solution at prementioned conditions (pH/temperature) for 10 h. The swelled hydrogel was withdrawn after every 1 h and the water present on the surface of hydrogel was carefully blotted off and reweighted.

% ESR was determined using eq. (1)[1]

$$\% \, ESR = \frac{W_{eq} - W_d}{W_d} \qquad (1)$$

Where W_{eq} and W_d are the weight of hydrogel at equilibrium and at dried condition.

Biodegradation test:

The pre-weighted dried c-Gly/pNIPAm 4 hydrogel films (10 × 10 × 0.1 mm) were put into 2 mL of lysozyme solution (pH 7) having a natural concentration as occurred in human body (1.5 μg/mL). The experimental temperature was maintained at 37 ± 0.2 °C. The enzyme solution was regularly (after 24 h) changed to maintain continuous enzyme activity. The films

were withdrawn from the media after regular intervals (3, 7, 14 and 21 days), washed thoroughly with distilled water and kept in the vacuum oven (72 h) for complete dry. Thereafter, the films were reweighted. The extent of *in vitro* degradation was measured as % weight loss with time. A parallel *in vitro* blank degradation experiment was also performed at pH 7 without using lysozyme to confirm the degradation characteristics of the hydrogel.

Drug release kinetics and mechanism models:

The zero order kinetic model (eqn. 2) signifies the drug delivery from the transdermal systems as well as matrix tablets of low soluble drugs, osmotic systems, and coated forms etc.[2]

$$Q_t = Q_0 + K_0 t \quad (2)$$

Where Q_t and Q_0 are the amount of drug release at time t and initially. K_0 is the zero order rate constant of drug release kinetics expressed in the unit of concentration/time and t is the time.

The first order kinetic model (eqn. 3) explains the drug release from porous matrix, containing the water soluble drugs where the drug release is proportional to the amount of drug remains in the interior of the mtrix.[3]

$$log\ Q_t = log\ Q_0 - \frac{K_1 t}{2.303} \quad (3)$$

Where Q_t and Q_0 are amount of drug released at time t and amount of drug present in the loaded hydrogel initially. K_1 is the first order rate constant.

The Korsemeyer-Peppas model (eq. 4), [4] Higuchi model (eq. 5), [5] are given below:

$$\frac{M_t}{M_\infty} = Kt^n \quad (4)$$

Where $Mt/M\infty$ is the fractional release of drug in time t, 'k' is the constant characteristic of drug-polymer system and 'n' is the diffusion exponent. The value of 'n' is used to characterize different release mechanisms, where $n \leq 0.45$ indicates Fickian diffusion, 'n' in the range of $0.45<n<0.89$ indicates the mechanism is non-Fickian diffusion or anomalous diffusion, and when $n > 0.89$, the major mechanism of drug release is Case II diffusion.

$$Q_t = Q_0 + K_H t^{\frac{1}{2}} \quad (5)$$

Where the Q_t is the amount of drug released in time t and Q_0 is the amount of drug present in the solution. K_H is the Higuchi dissolution constant.

RESULTS AND DISCUSSIONS:

Table S1. Synthesis details and % cross-linking and % ESR of the c-Gly/pNIPAm hydrogels, with 0.0031 mol of glycogen

Hydrogel	Amount of Initiator (mol×10^{-5})	Amount of monomer (mol×10^{-2})	Amount of cross linker (mol×10^{-3})	% CR	% ESR		
					pH 1.2, 37 °C	pH 7.4, 37 °C	pH 7.4, 25 °C
cl-Gly/pNIPAm 1	2.77	1.55	1.55	63.70	305 ± 15	631 ± 31	1217 ± 61
cl-Gly/pNIPAm 2	3.69	1.55	1.55	69.89	240 ± 12	517 ± 26	1041 ± 52
cl-Gly/pNIPAm 3	4.62	1.55	1.55	61.70	331 ± 16	671 ± 33	1270 ± 63
cl-Gly/pNIPAm 4	3.69	3.10	1.55	84.16	180 ± 9	363 ± 18	748 ± 37
cl-Gly/pNIPAm 5	3.69	4.65	1.55	63.70	272 ± 13	574 ± 29	1131 ± 56
cl-Gly/pNIPAm 6	3.69	3.10	0.77	79.20	215 ± 11	455 ± 23	963 ± 48
cl-Gly/pNIPAm 7	3.69	3.10	2.32	83.36	198 ± 10	407 ± 20	828 ± 41

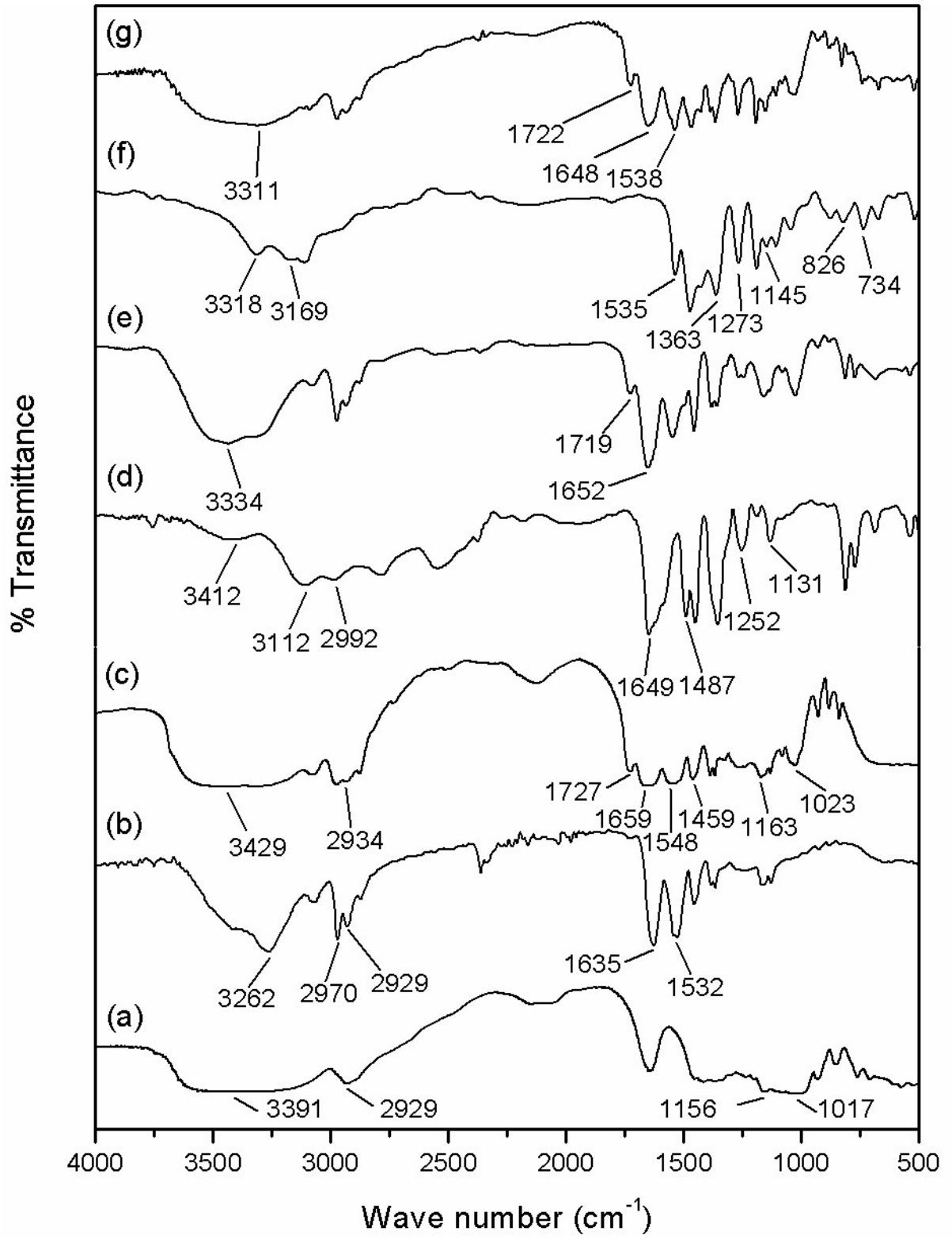

Fig. S1: FTIR spectra of (a) glycogen, (b) pNIPAm) (c) cl-Gly/pNIPAm, (d) 5-ASA, (e) 5-ASA loaded hydrogel, (f) ornidazole, (g) ornidazole loaded hydrogel.

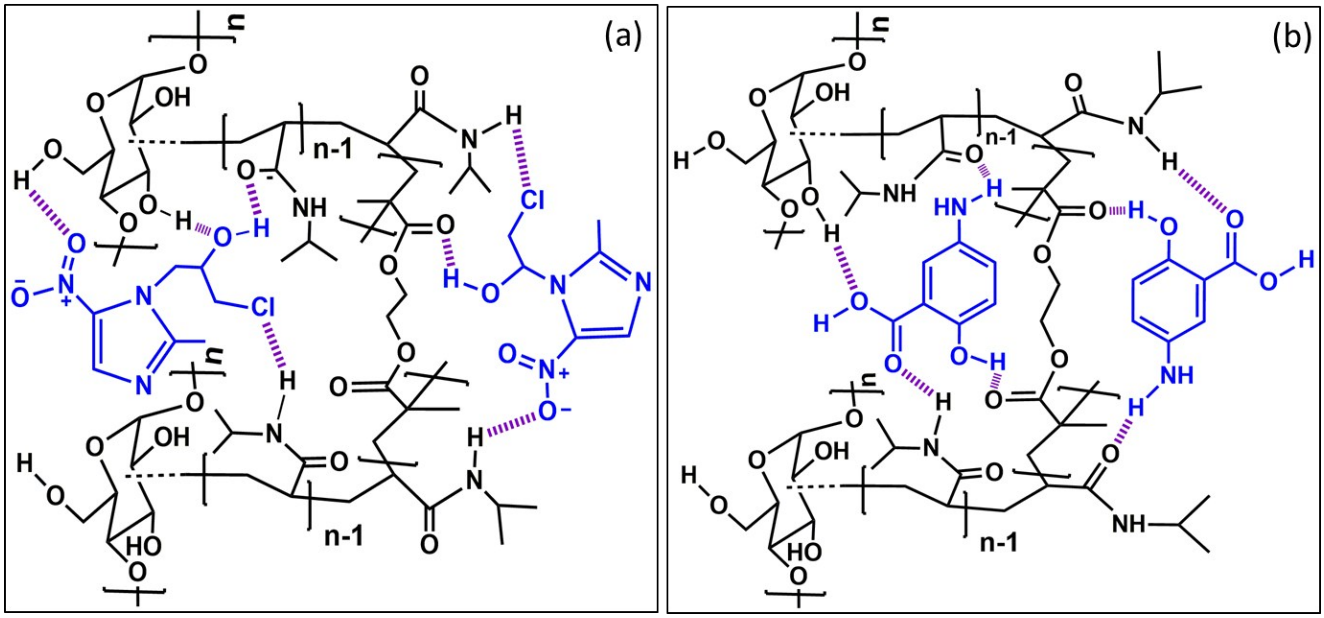

Fig. S2: Probable interaction between cl-Gly/pNIPAm with (a) ornidazole and (b) 5-ASA.

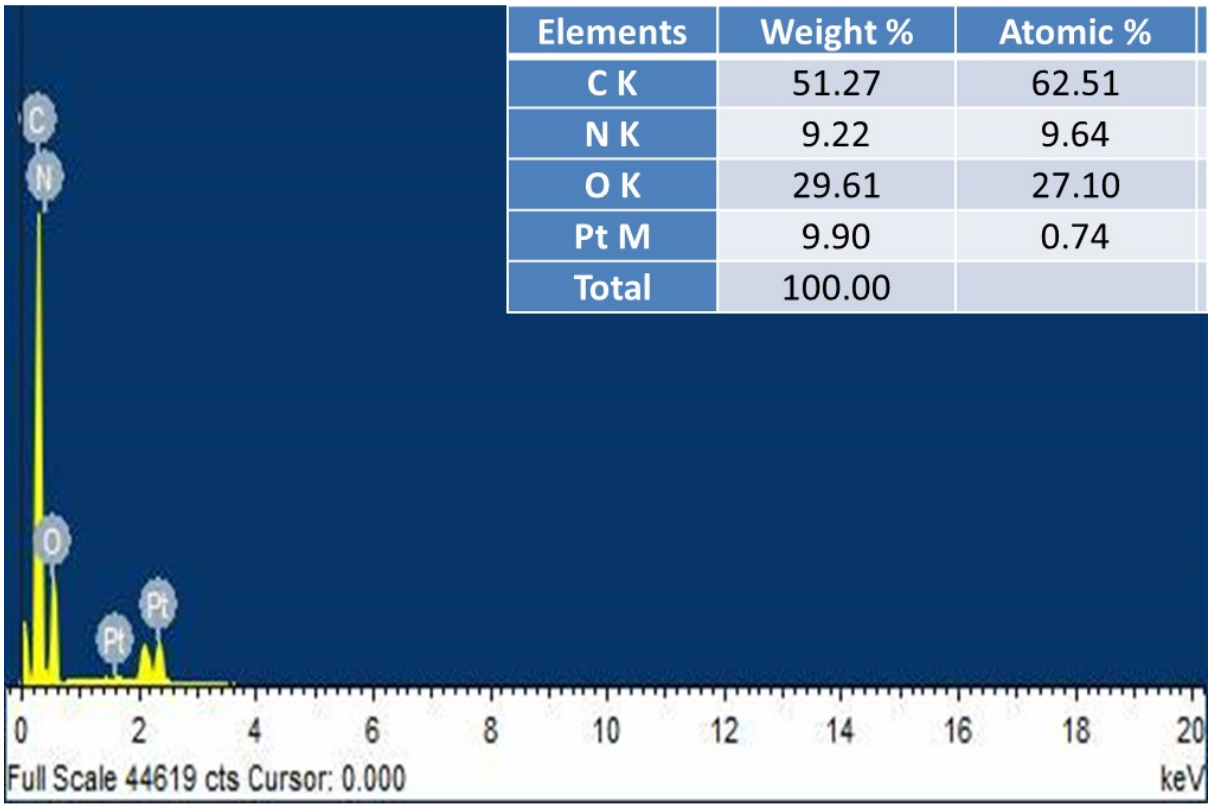

Fig. S3: EDAX analysis of cl-Gly/pNIPAm hydrogel

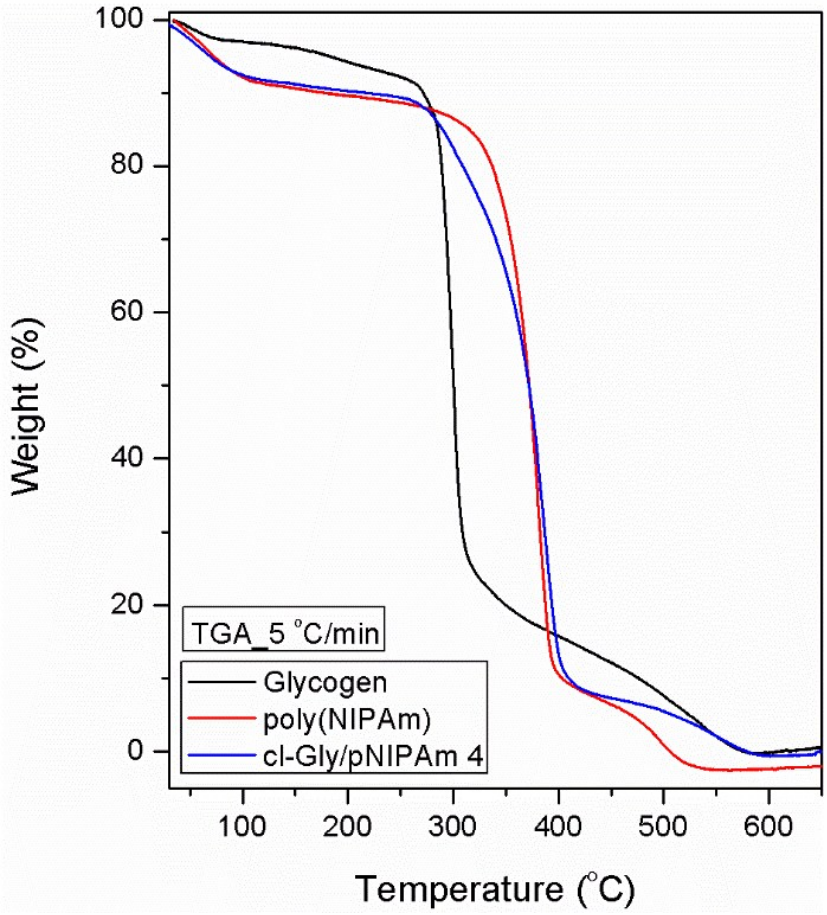

Fig. S4: TGA plots of glycogen, pNIPAm and cl-Gly/pNIPAm with 5 °C/min heating rate.

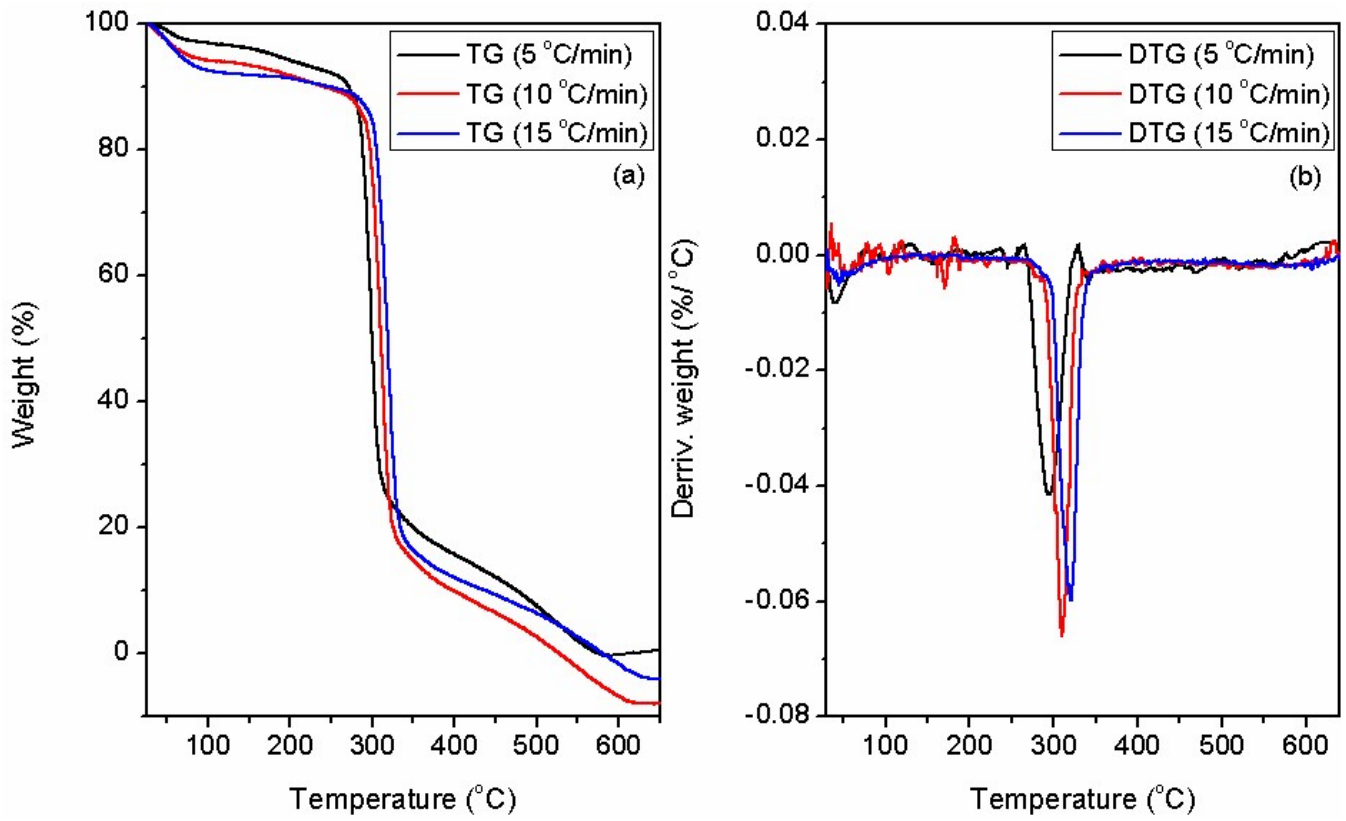

Fig. S5: (a) TG and (b) DTG curve of glycogen at 5 °C/min, 10 °C/min and 15 °C/min heating rate.

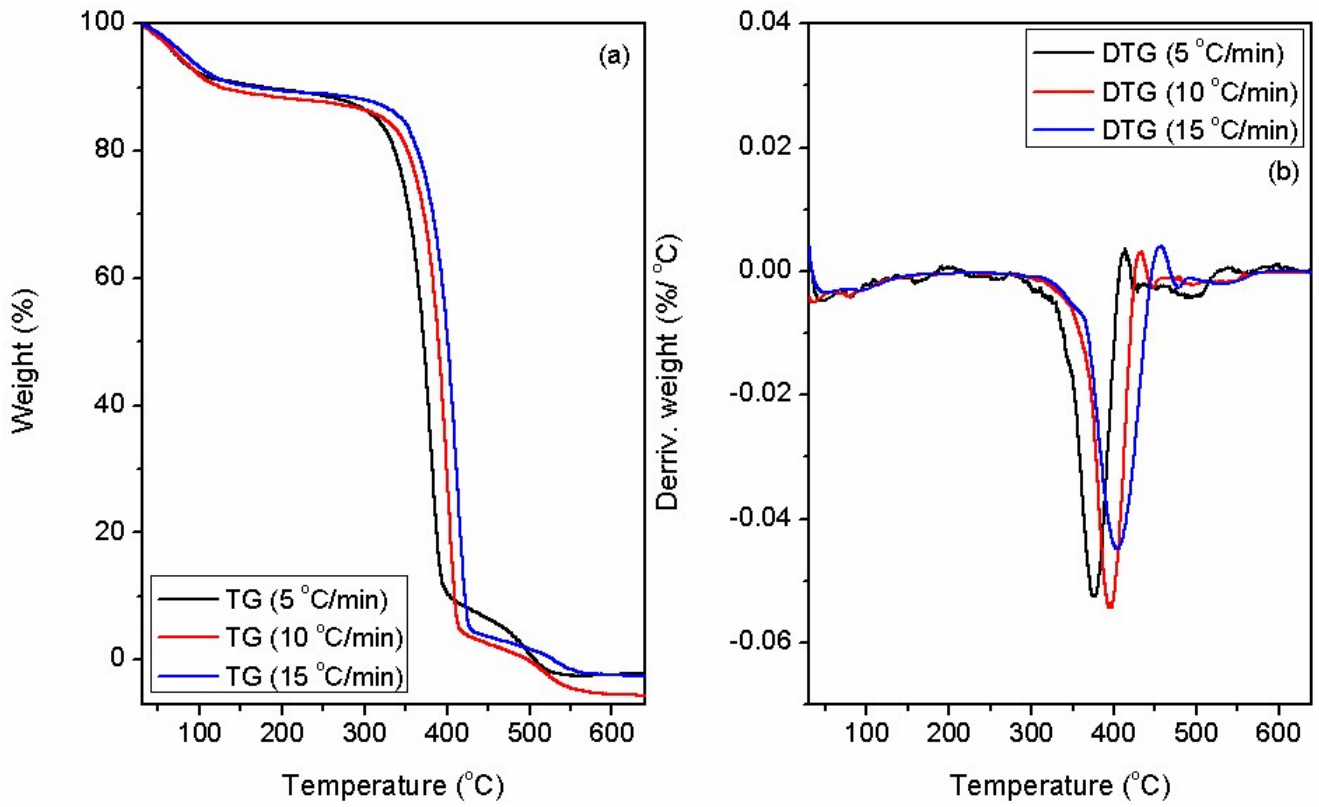

Fig. S6: (a) TG and (b) DTG curve of pNIPAm at 5 °C/min, 10 °C/min and 15 °C/min heating rate.

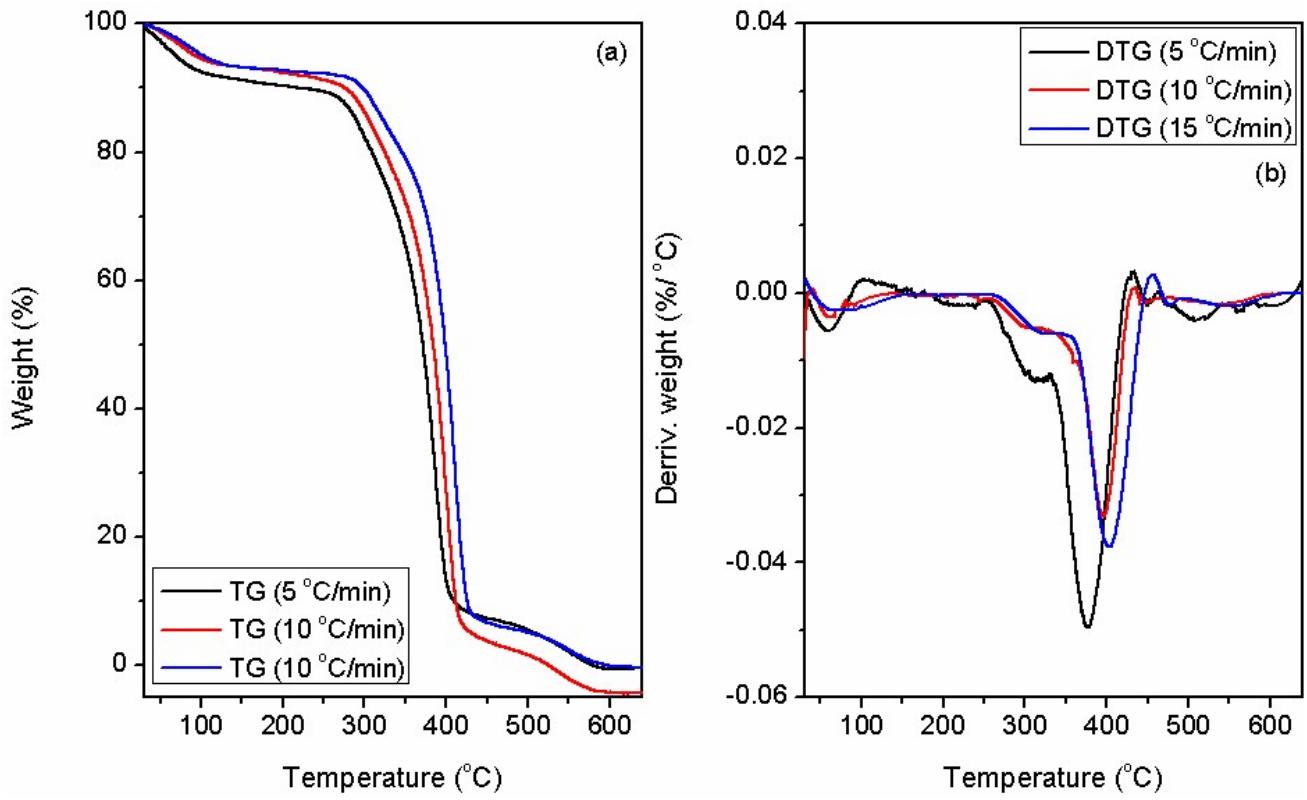

Fig. S7: (a) TG and (b) DTG curve of cl-Gly/pNIPAm hydrogel at 5 °C/min, 10 °C/min and 15 °C/min heating rate

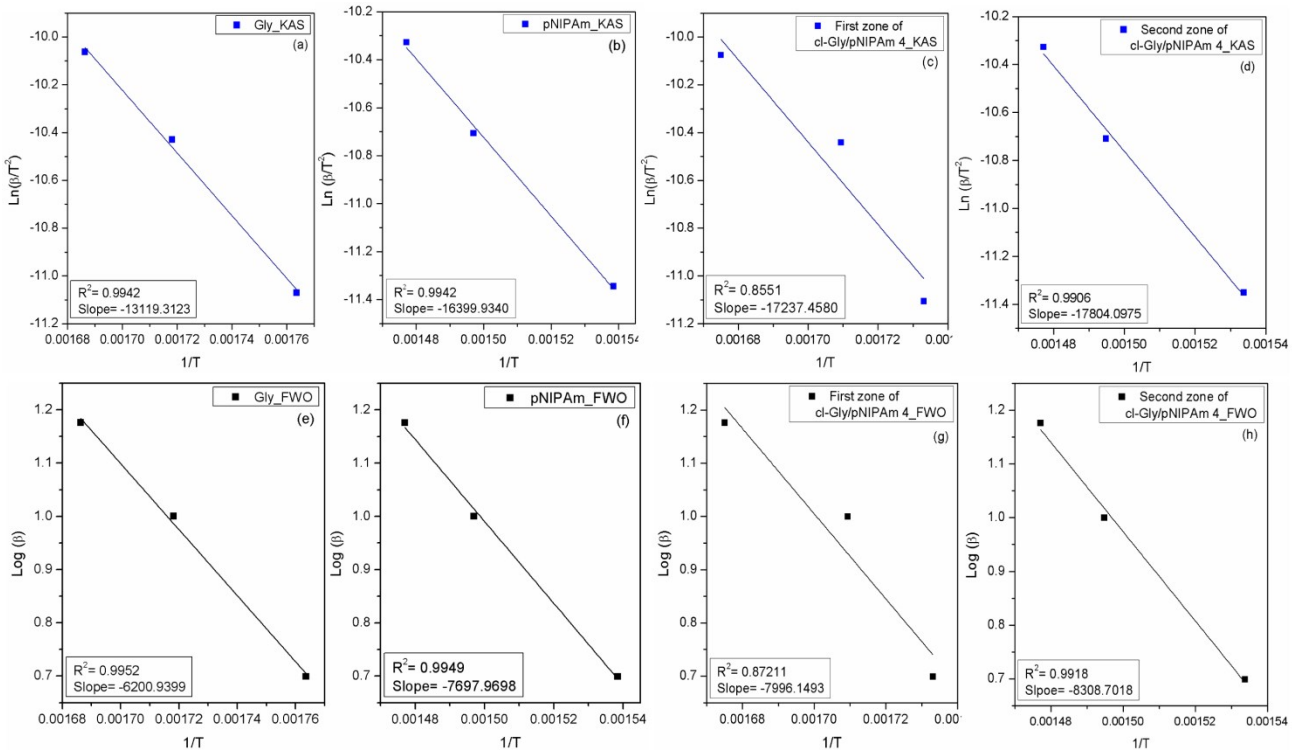

Fig. S8: Fitted graphs for glycogen, pNIPAm and cl-Gly/pNIPAm 4 hydrogel obtained from Kissinger-Akahira-Sunose (KAS) method (a-d) and Flynn-Wall-Ozawa (FWO) method (e-h).

Table S2. The activation energy of glycogen, *poly* (NIPAm), and cl-Gly/pNIPAm 4 hydrogel

Polymers	**Activation energy (E) kJ/mol**	
	Kissinger–Akahira–Sunose (KAS) method	**Flynn–Wall–Ozawa (FWO) method**
Glycogen	109.07	112.88
pNIPAm	136.34	140.13
First zone of hydrogel	143.31	145.56
Second zone of hydrogel	148.02	151.25

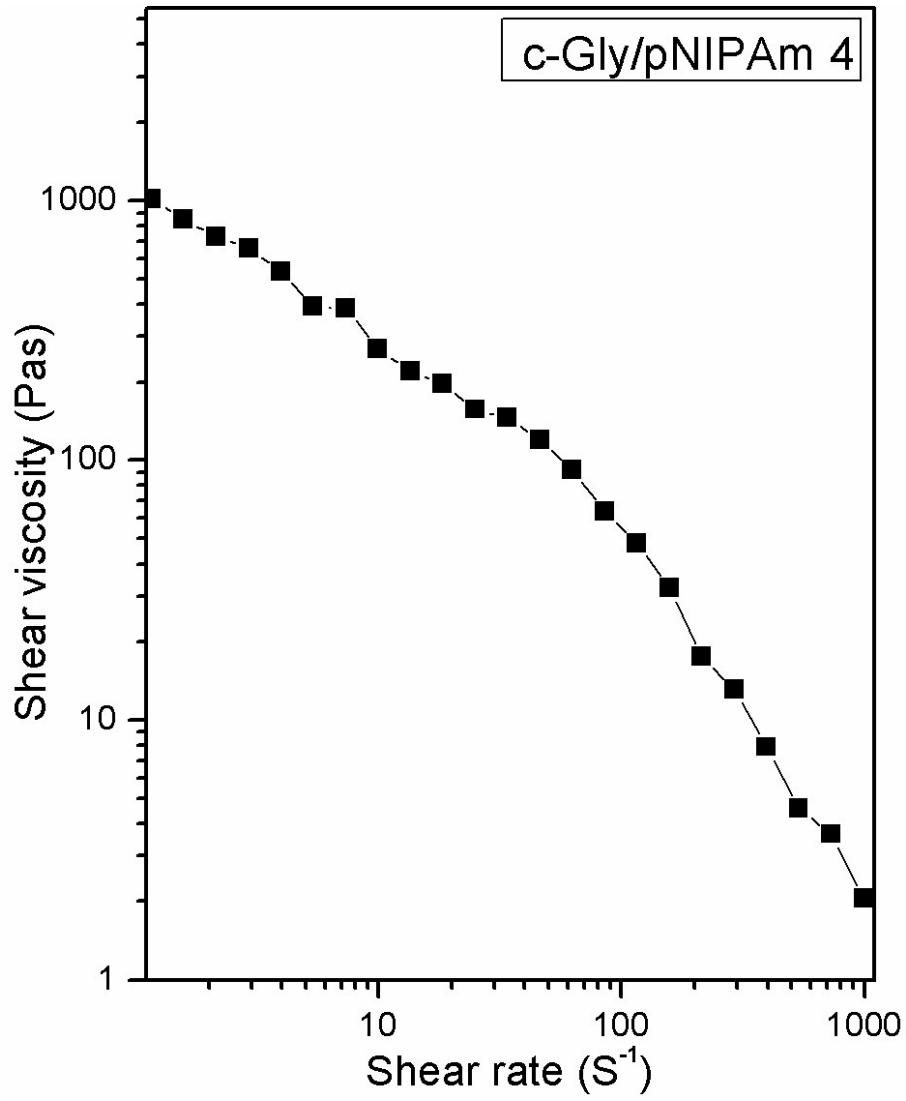

Fig. S9: Plot of shear viscosity vs. shear rate

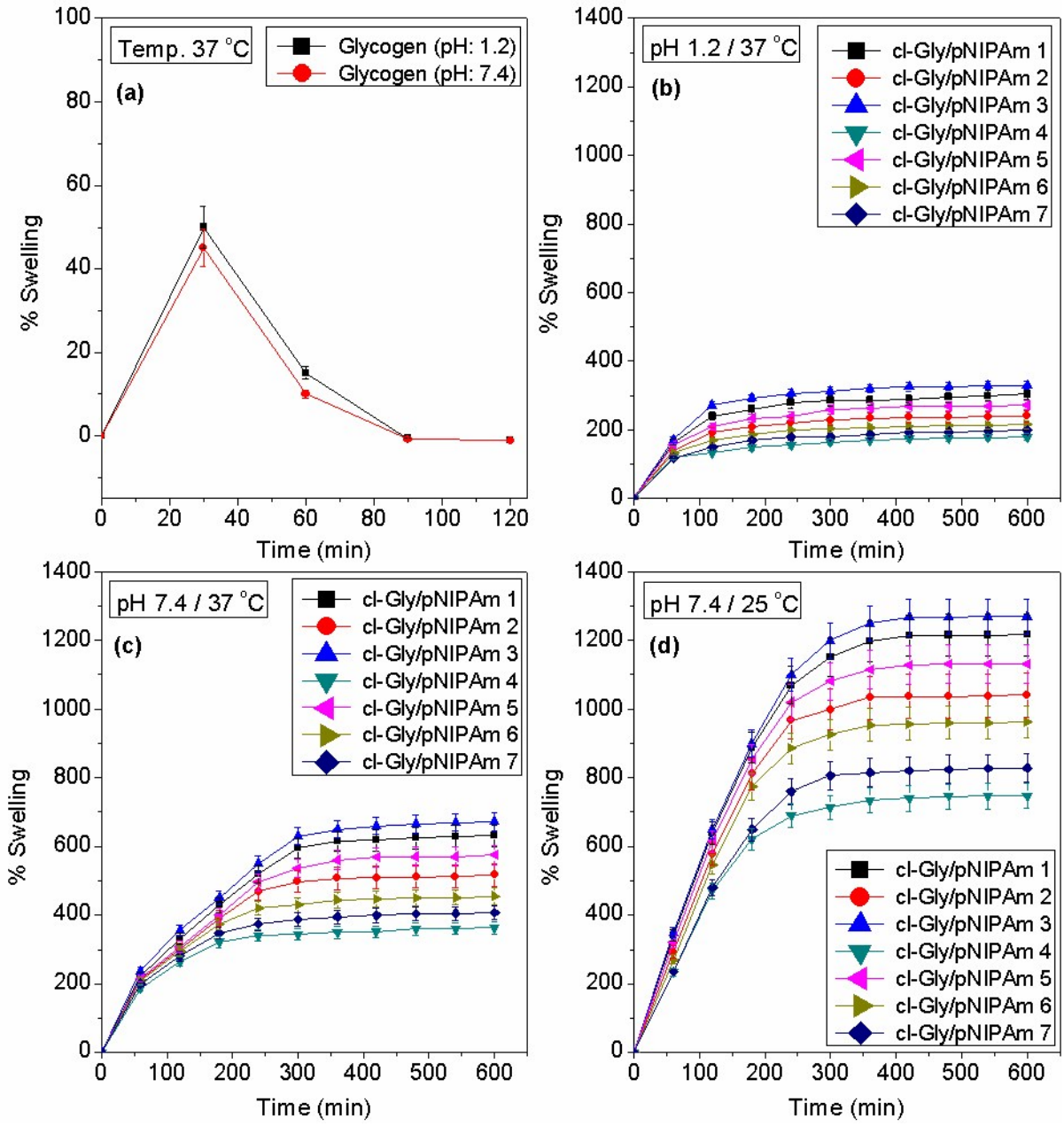

Fig. S10: Swelling characteristics of glycogen (a) at pH 1.2 & 7.4/37 °C and different cl-Gly/pNIPAm hydrogels at (b) pH 1.2/37 °C, (c) pH 7.4/37 °C and (d) pH 7.4/25 °C.

Table S3. Swelling rate parameter of different hydrogels at various buffer solutions and temperatures

Hydrogel	Value of rate parameter (τ)		
	pH: 1.2 and Temp: 37 ˚C	pH: 7.4 and Temp: 37 ˚C	pH: 7.4 and Temp: 25 ˚C
cl-Gly/pNIPAm 1	241	203	152
cl-Gly/pNIPAm 2	261	239	173
cl-Gly/pNIPAm 3	239	201	149
cl-Gly/pNIPAm 4	321	254	189
cl-Gly/pNIPAm 5	254	215	168
cl-Gly/pNIPAm 6	277	250	178
cl-Gly/pNIPAm 7	294	251	182

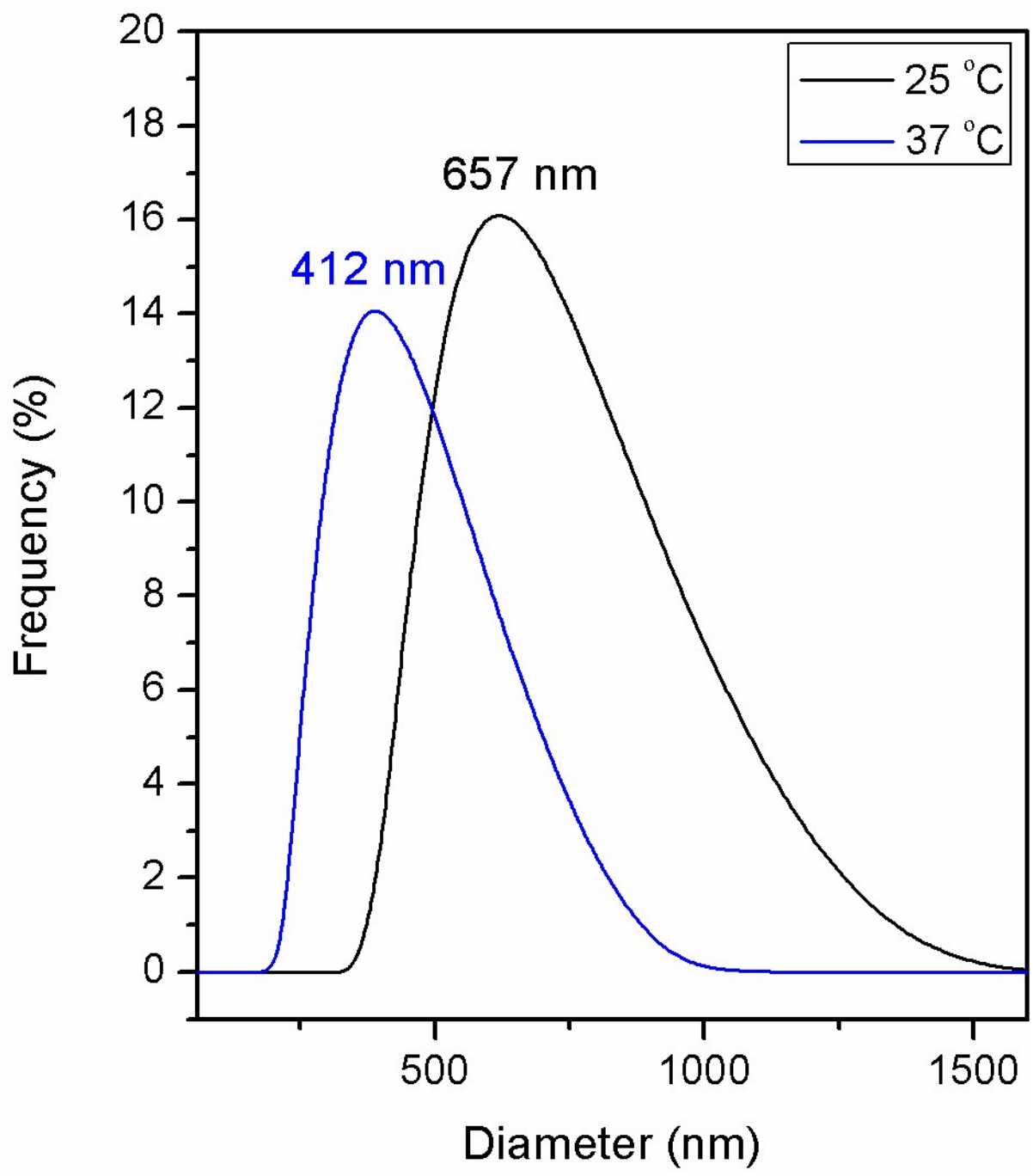

Fig. S11: DLS analysis result of cl-Gly/pNIPAm 4 hydrogel at 25 and 37 °C.

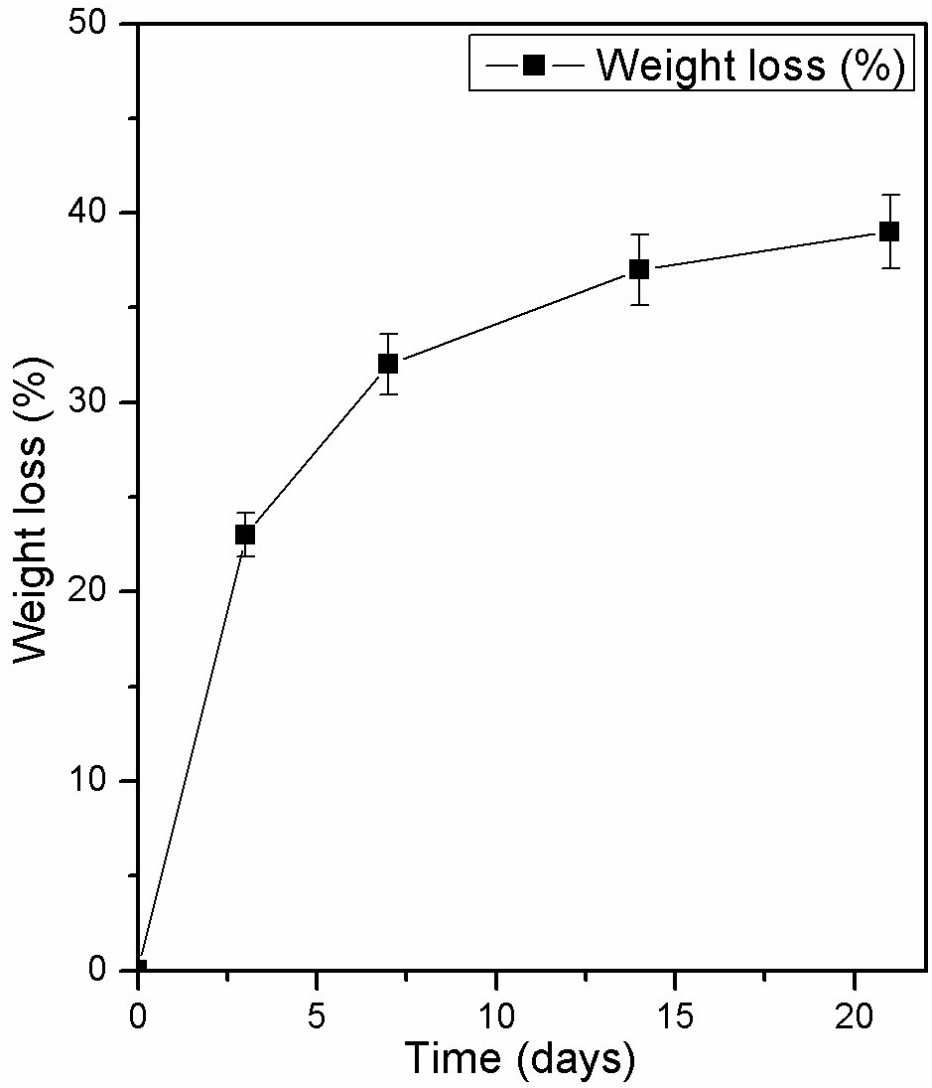

Fig. S12: Progressive mass loss of cl-Gly/pNIPAm hydrogel film in Lysozyme/PBS

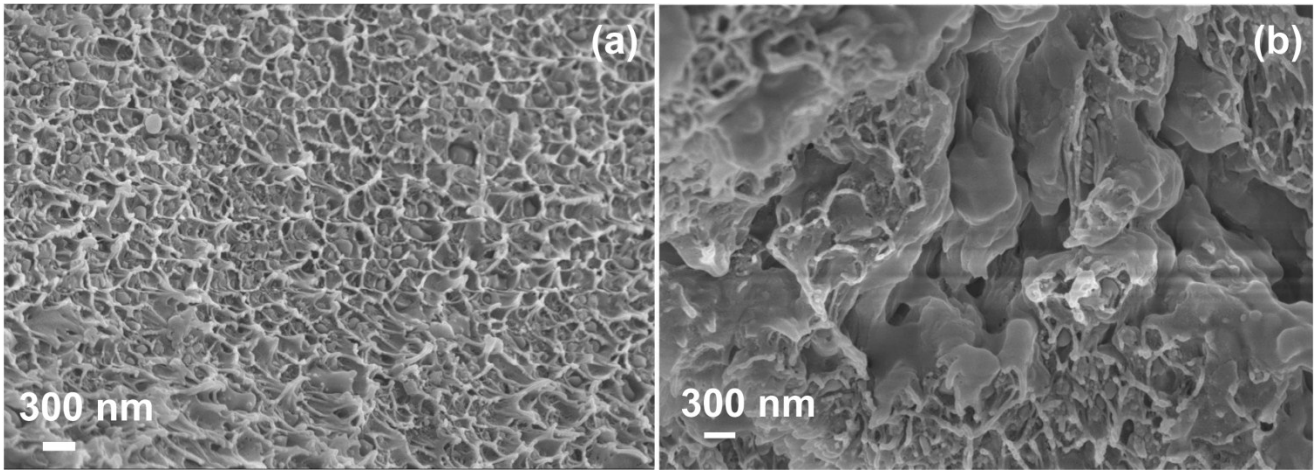

Fig. S13: FESEM morphology of cl-Gly/pNIPAm hydrogel (a) before and (b) after biodegradation.

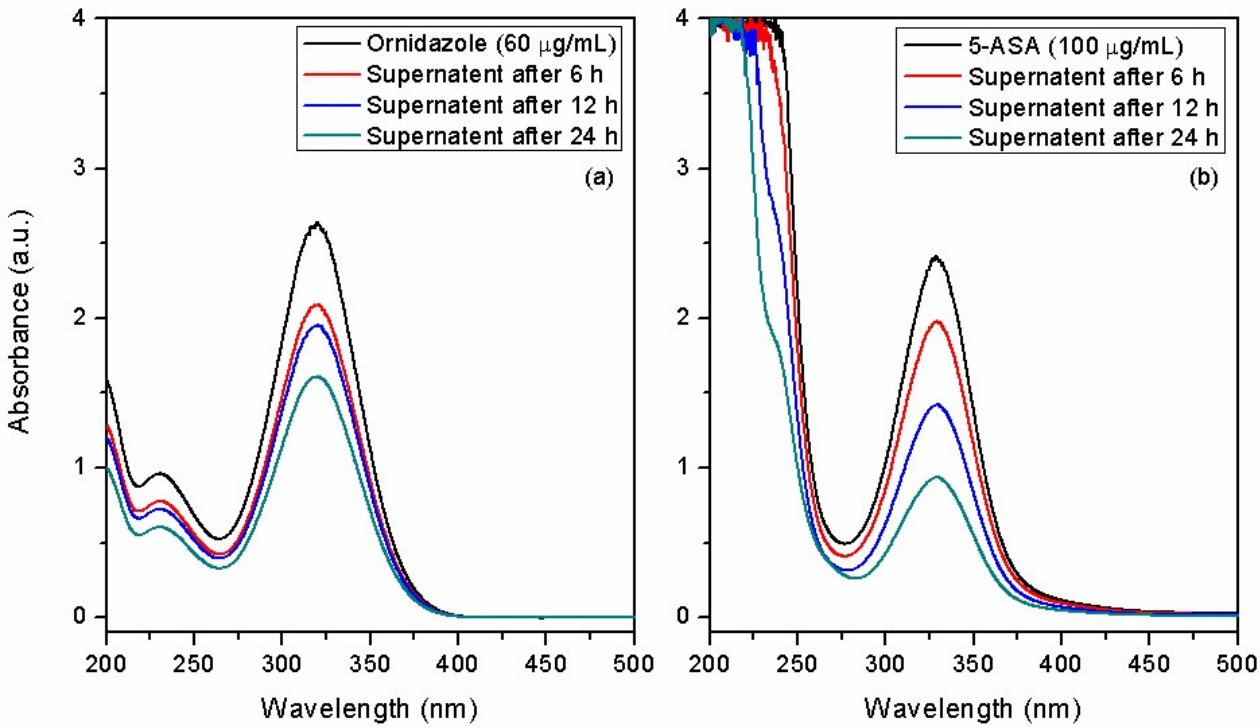

Fig. S14: UV-Vis spectra of standard (a) ornidazole and (b) 5-ASA solutions, and supernatants after drug loading

Table S4. % loading and % encapsulation of ornidazole and 5-ASA

Drugs	Weight of cl-Gly/pNIPAm 4 (mg)	Weight of drugs (mg)	Time (h)	% loading	% encapsulation
Ornidazole	20	6	6	5.97	19.00
			12	7.50	25.00
			24	11.75	31.17
5-ASA	40	10	6	4.60	18.40
			12	10.30	41.20
			24	15.32	61.30

Table S5: Release kinetics and mechanism data

Model drug: Ornidazole					
Drug loaded hydrogel	Zero order model	First order model	Higuchi model	Korsemeyer-Peppas model	
	R^2	R^2	R^2	R^2	n
cl-Gly/pNIPAm 4 (pH: 1.2)	0.7417	0.8559	0.9856	0.9901	0.25
cl-Gly/pNIPAm 4 (pH: 7.4)	0.8395	0.8725	0.9694	0.9864	0.43
Model drug: 5 ASA					
cl-Gly/pNIPAm 4 (pH: 1.2)	0.8787	0.9260	0.9662	0.9709	0.44
cl-Gly/pNIPAm 4 (pH: 7.4)	0.9087	0.9206	0.9760	0.9737	0.30

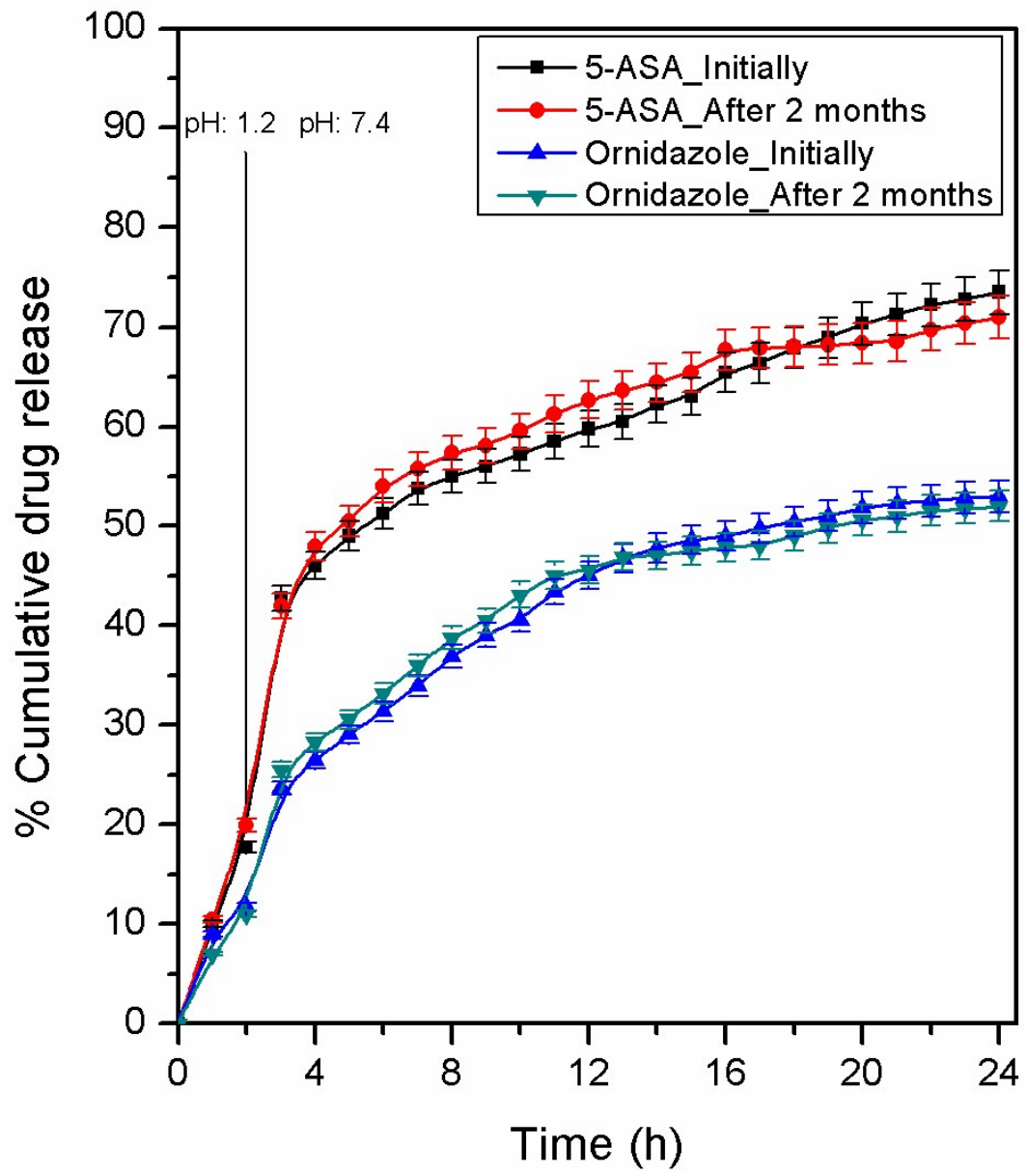

Fig. S15: Release of 5-ASA/ornidazole at initial state and after 2 months of stability study

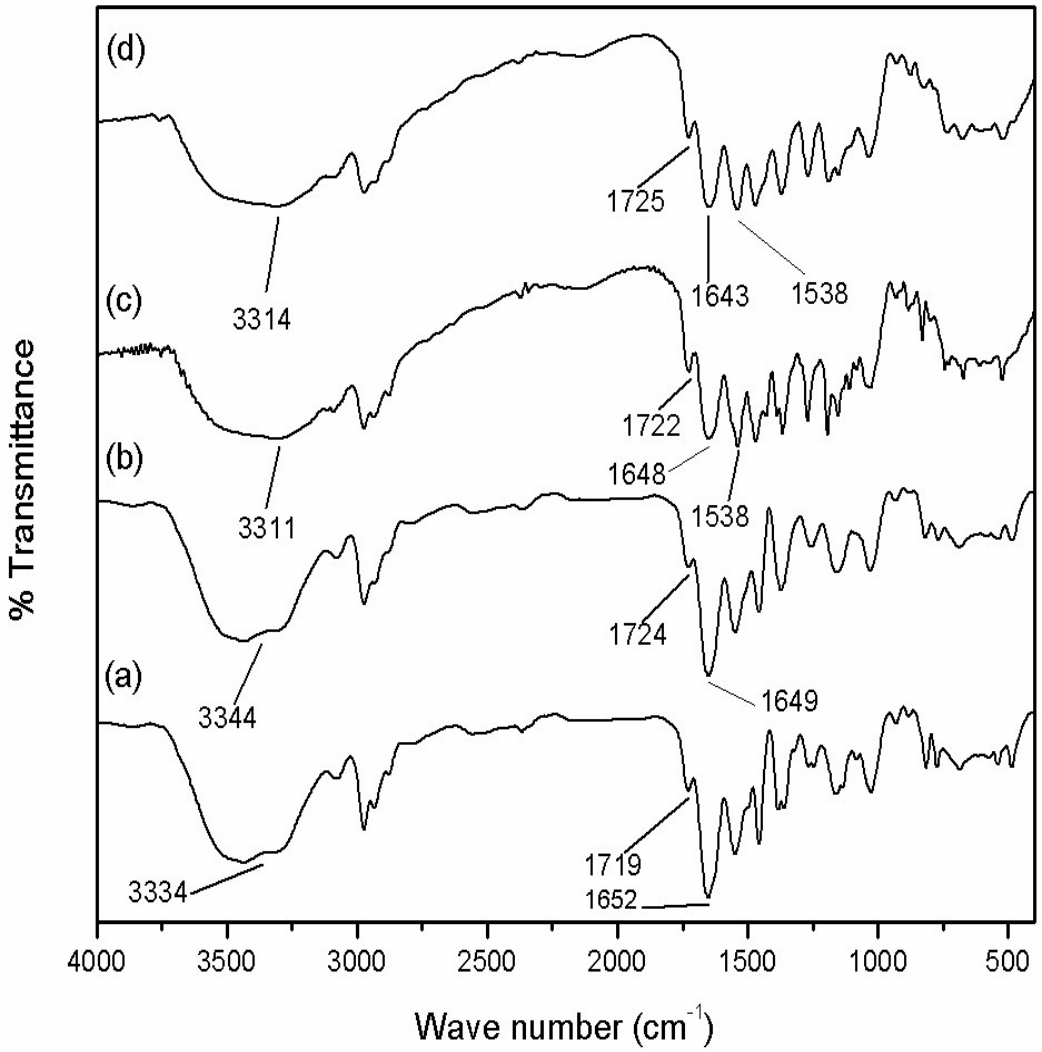

Fig. S16: FTIR spectra of (a) ornidazole loaded hydrogel (initially), (b) ornidazole loaded hydrogel after 2 months (c) 5-ASA loaded hydrogel (initially), and (d) 5-ASA loaded hydrogel after 2 months.

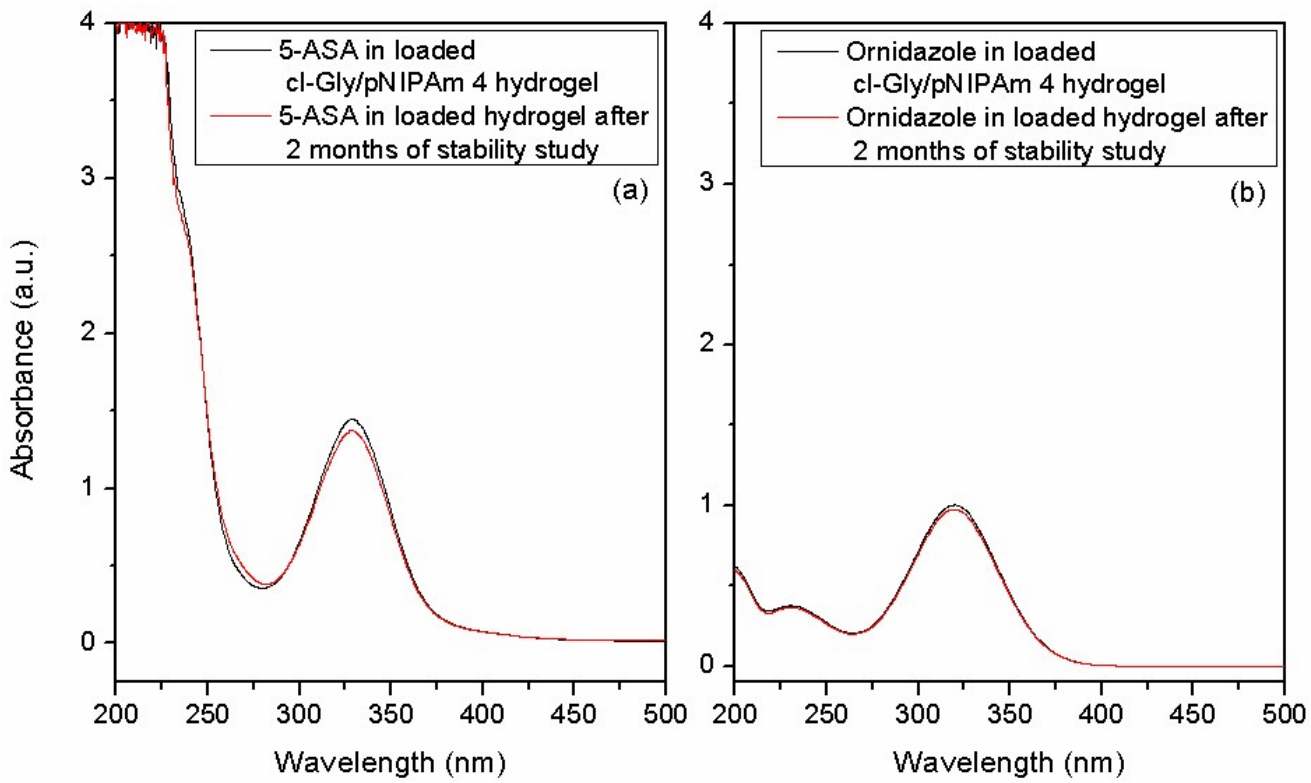

Fig. S17: Drugs present in the hydrogel matrix initially and after 2 months of stability study (a) 5-ASA and (b) Ornidazole.

Table S6: Stability test results

Parameters	5-ASA		Ornidazole	
	Initially	2 month latter	Initially	2 month latter
Average weight (drug loaded gel, mg)	41.57	41.5	20.5	19.8
	In-vitro release in pH 1.2 (% of cumulative drug)			
1 h	10.00 ± 0.30	10.50 ± 0.31	9.00 ± 0.27	7.00 ± 0.21
2 h	17.75 ± 0.53	19.93 ± 0.59	11.75 ± 0.35	11.00 ± 0.33
	In-vitro release in pH 1.2 (% of cumulative drug)			
3 h	42.75 ± 1.28	42.00 ± 1.26	23.66 ± 0.71	25.49 ± 0.76
4 h	46.00 ± 1.38	48.00 ± 1.44	26.46 ± 0.79	28.27 ± 0.84
5 h	49.00 ± 1.47	50.52 ± 1.51	29.05 ± 0.87	30.56 ± 0.91
6 h	51.25 ± 1.53	54.00 ± 1.62	31.37 ± 0.94	33.17 ± 0.99
7 h	53.75 ± 1.61	55.73 ± 1.67	33.97 ± 1.01	35.96 ± 1.07
8 h	55.00 ± 1.65	57.34 ± 1.72	36.92 ± 1.10	38.74 ± 1.16
9 h	56.00 ± 1.68	58.10 ± 1.74	39.04 ± 1.17	40.49 ± 1.21
10 h	57.25 ± 1.71	59.52 ± 1.78	40.57 ± 1.21	43.08 ± 1.29
11 h	58.50 ± 1.75	61.29 ± 1.83	43.37 ± 1.30	45.06 ± 1.35
12 h	59.75 ± 1.79	62.65 ± 1.87	45.06 ± 1.35	45.60 ± 1.36
13 h	60.50 ± 1.81	63.66 ± 1.90	46.75 ± 1.40	46.90 ± 1.40
14 h	62.22 ± 1.86	64.42 ± 1.93	47.81 ± 1.43	47.03 ± 1.41
15 h	63.00 ± 0.53	65.43 ± 1.96	48.60 ± 1.45	47.50 ± 1.42
16 h	65.44 ± 1.96	67.70 ± 2.03	49.00 ± 1.47	47.84 ± 1.43
17 h	66.35 ± 1.99	67.90 ± 2.04	49.75 ± 1.49	48.06 ± 1.44
18 h	67.9 ± 2.03	68.00 ± 2.04	50.44 ± 1.51	49.02 ± 1.47
19 h	68.9 ± 2.06	68.20 ± 2.04	51.01 ± 1.53	49.81 ± 1.49
20 h	70.36 ± 2.11	68.40 ± 2.05	51.85 ± 1.55	50.62 ± 1.51
21 h	71.26 ± 2.13	68.60 ± 2.06	52.30 ± 1.57	50.98 ± 1.52
22 h	72.21 ± 2.16	69.78 ± 2.09	52.50 ± 1.57	51.98 ± 1.54
23 h	72.84 ± 2.18	70.39 ± 2.11	52.85 ± 1.58	51.75 ± 1.55
24 h	73.50 ± 2.20	71.00 ± 2.13	53.00 ± 1.59	51.99 ± 1.56

References:

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