Electronic Supplementary Information (ESI)

Design of Multi-responsive and Actuating Microgel toward On-demand Drug Release

Priyanshi Agnihotri,[a]† Divya Dheer,[a,b]† Anvi Sangwan,[c] Vysakh C. Chandran,[a] Nimisha A. Mavlankar,[a] Gunjan Hooda,[a] Debabrata Patra[c]* and Asish Pal[a]*

[a]*Chemical Biology Unit, Institute of Nano Science and Technology, Knowledge City, Sector 81, Mohali 140306, Punjab, India. E-mail: apal@inst.ac.in*

[b]*School of Pharmacy, Chitkara University, Baddi 174103, Himachal Pradesh, India*

[c]*Energy and Environment Unit, Institute of Nano Science and Technology, Knowledge City, Sector 81, Mohali 140306, Punjab, India. E-mail: patra@inst.ac.in*

[†]*Priyanshi and Divya contributed equally.*

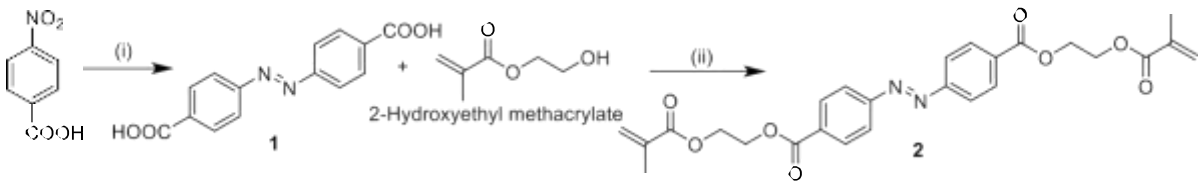
Experimental Section

1. Materials and Methods

The chemicals 4-Nitrobenzoic acid, sodium hydroxide (NaOH), glucose, 2-Hydroxyethylmethacrylate (HEMA), 1-(3-(Dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDCI), dimethylaminopyridine (DMAP), Dimethylformamide (DMF) were purchased from TCI. Solvents used in synthesis were reagent grade. CH_2Cl_2, and $CHCl_3$ were distilled from CaH_2. Silica gel (60−200 μm) was purchased from LOBA CHEMIE. Hydrochloric acid was purchased from Fisher. Deuterated chloroform was purchased from Sigma. 1H NMR spectra were recorded on a Bruker-Neo-400 Instrument (400 MHz). UV-vis, were measured on a Shimadzu UV-3101PC spectrophotometer. DSC analyses were performed with a Perkin Elmer Differential Scanning Calorimeter DSC 8000. The TEM images were recorded using JEOL JEM 2010 high-resolution electron microscope at an accelerating voltage of 200 kV.

2. Synthesis and characterization

The monomer, HEMA coupled with diacid azobenzene was synthesized as depicted in Scheme S1.



Scheme S1: (i) 4-Nitrobenzoic acid, NaOH, glucose, water, 70°C, 12 h, 40%; (ii) **1**, HEMA, EDC, DMAP, CH_2Cl_2, RT, 12 h, 60%.

Azobenzene-4,4'-dicarboxylic acid (1). 4-Nitrobenzoic acid (2.0 g, 12 mmol) and NaOH, (5.75 g, 144 mmol) were mixed in 30 mL of water and heated until the solid dissolved. Aqueous solution of glucose, (20 mL, 72 mmol,) was added dropwise at 70 °C, initially yielding a yellow precipitate and subsequently a brown solution upon further addition of glucose. A stream of air was bubbled into the mixture overnight, resulting in a light brown precipitate, and then the precipitate was filtered. The precipitate was dissolved into 10 mL of water followed by adjustment of the pH by acetic acid to be ~ 5-6. Then the precipitate was filtered followed by washing with 25 mL DI water. Finally, the product was dried in an oven at 80 °C to furnish **1**.[1]

^{1}H NMR (400 MHz, DMSO-d_6): δ = (*s*, 1H), 8.24-8.22 (*d*, 4H), 8.09-8.06 (*d*, 4H).

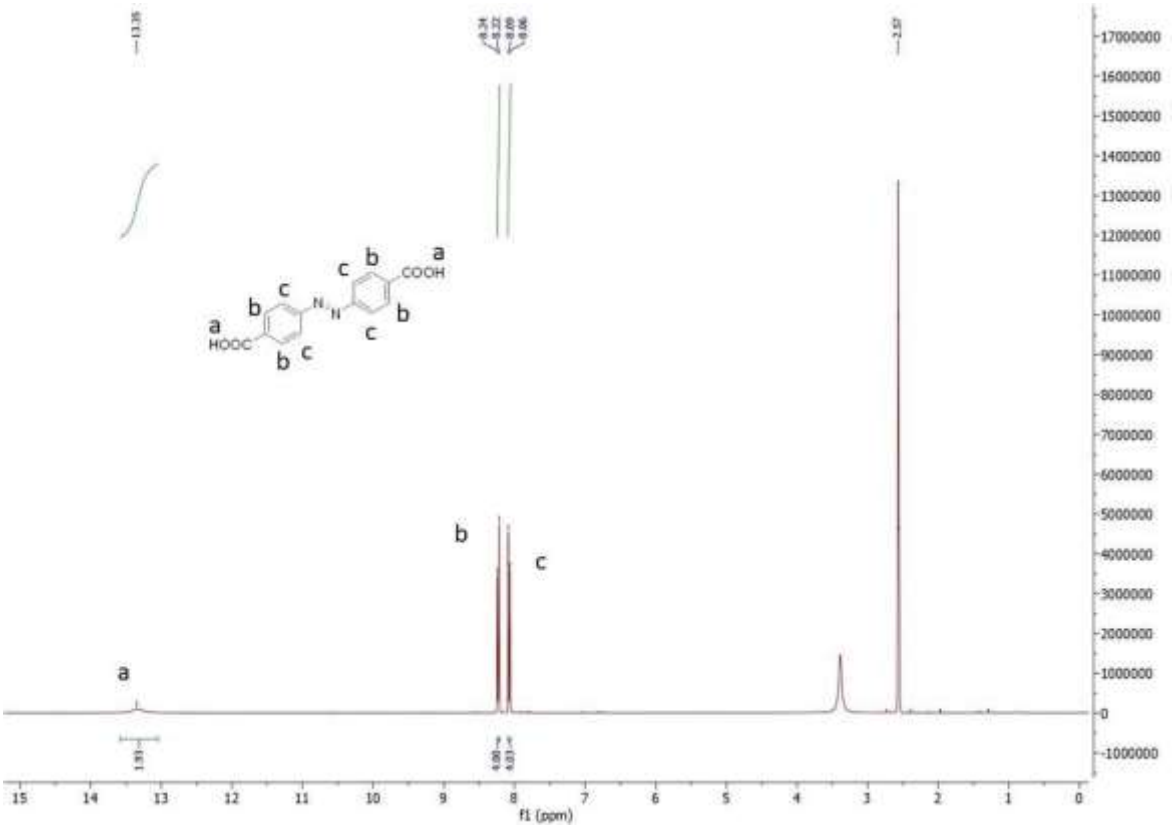


Fig. S1: ^{1}H NMR spectrum of **1**

Bis(2-(methacryloyloxy)ethyl) 4,4'-(diazene-1,2-diyl)(E)-dibenzoate (2). In a 50 mL two-neck round-bottom flask azobenzene (200 mg, 0.74 mmol), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (252 mg, 1.62 mmol) and dimethylaminopyridine (200 mg, 1.62 mmol) were taken and stirred in 5 mL of dry dichloromethane under nitrogen for 20 min. To the resulting solution 2-hydroxyethylmethacrylate (212 mg, 1.62 mmol) was added followed by overnight stirring. Purification of the product using column chromatography (silica gel, CH_2Cl_2/methanol 9:0.1 v/v) yielded 212 mg (60%) of orange solid compound.[2]

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14 (*d*, *J* = 8.5 Hz, 4H), 7.92 (*d*, *J* = 8.5 Hz, 4H), 6.09 (*s*, 2H), 5.54 (*s*, 2H), 4.58 – 4.52 (*m*, 4H), 4.48 – 4.43 (*m*, 4H), 1.90 (*s*, 6H).

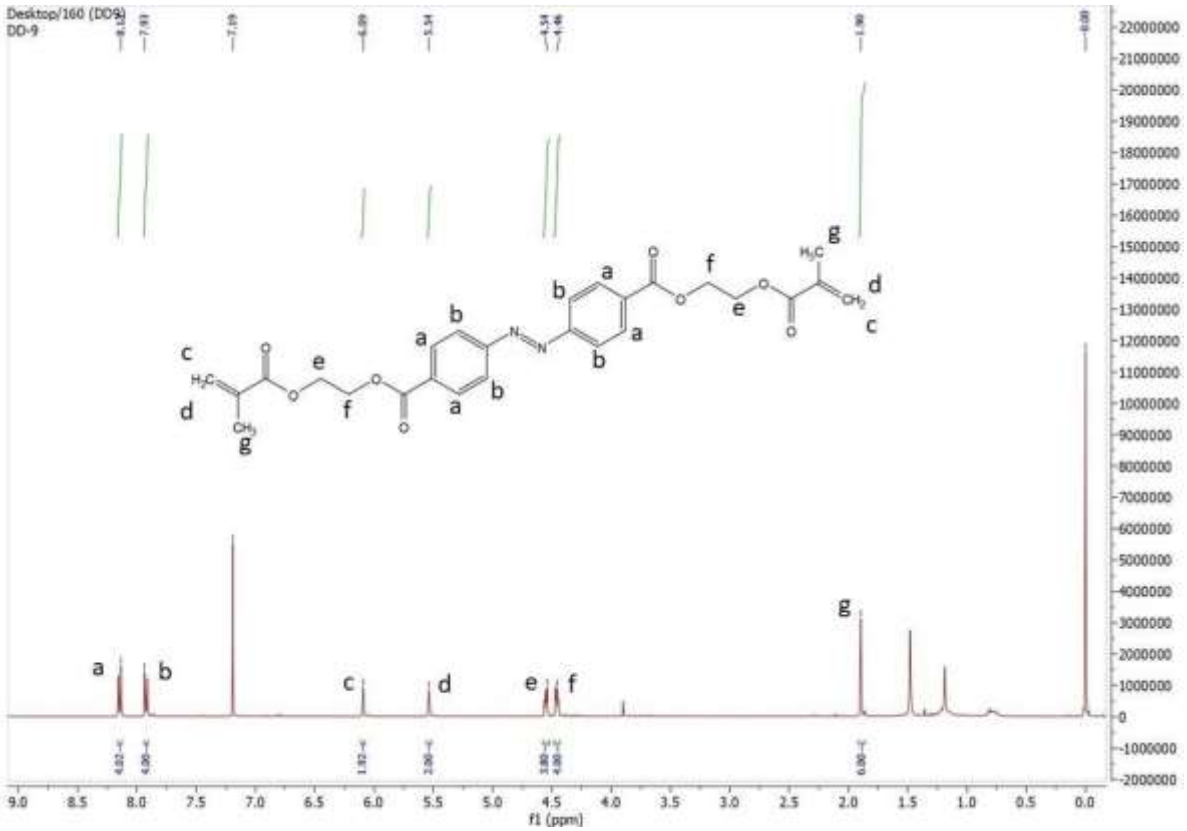


Fig. S2: ^{1}H NMR spectrum of **2**

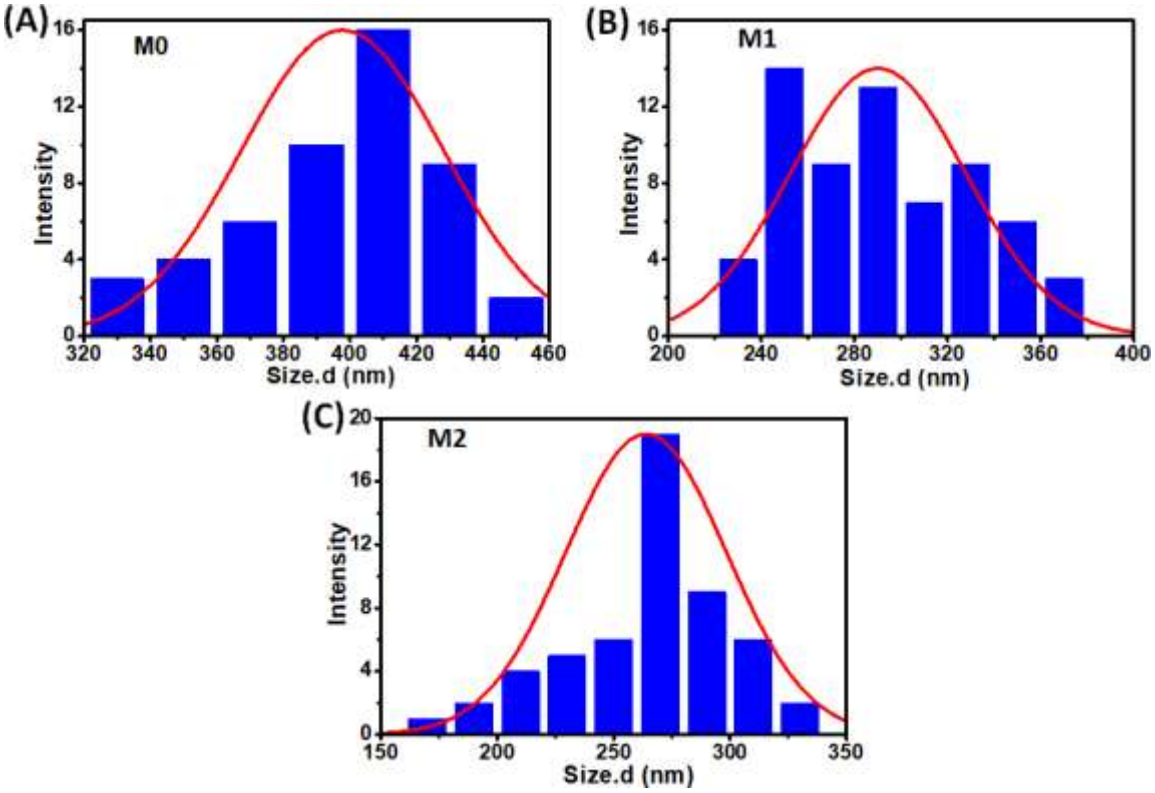


Fig. S3. Histogram showing size distribution as obtained from TEM images for (A) **M0** (B) **M1** and (C) **M2** microgels.

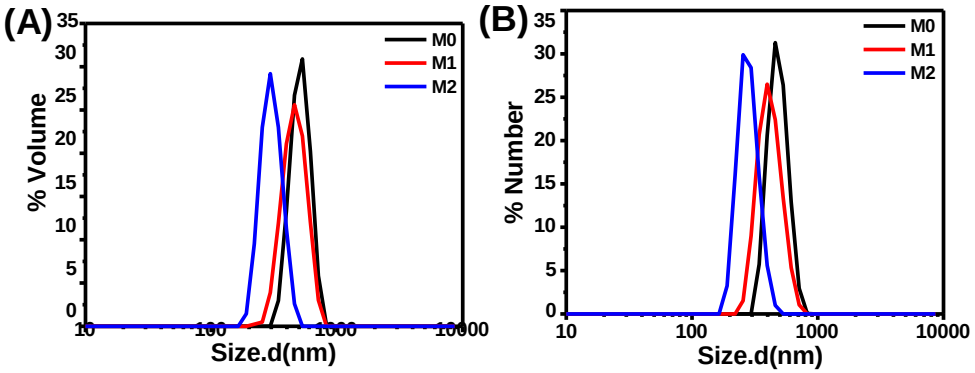


Fig. S4 (A-B) DLS volume and number data for **M0**, **M1** and **M2** microgels.

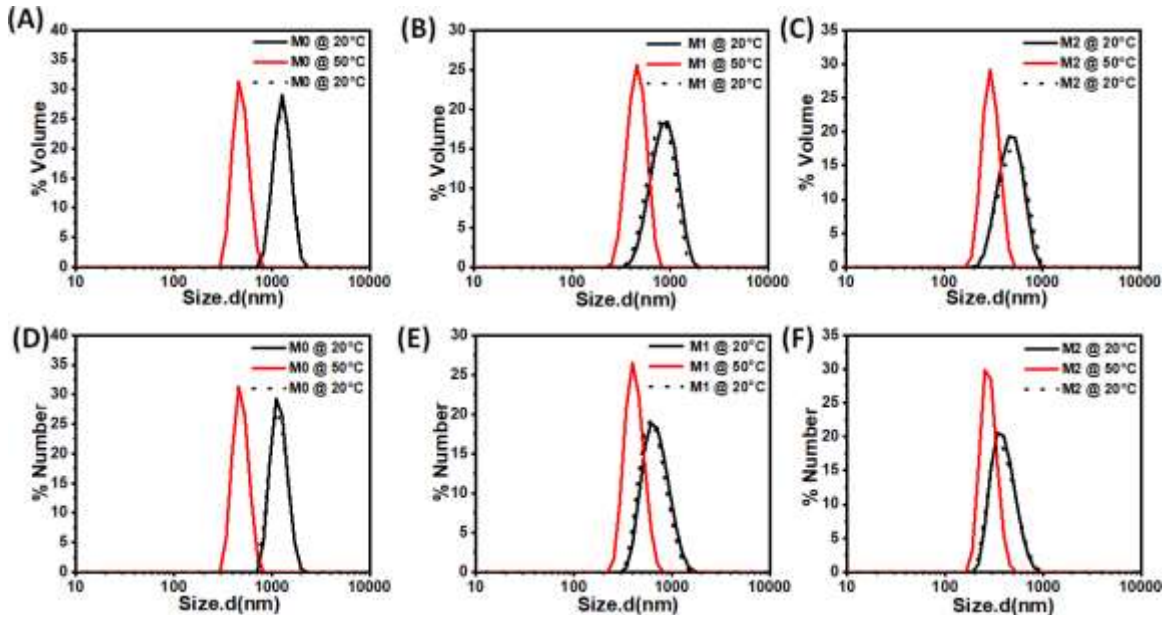


Fig. S5. Temperature-induced reversible swelling/deswelling for **M0**, **M1** and **M2** microgels as monitored by (A-C) DLS volume and (D-F) number distribution data.

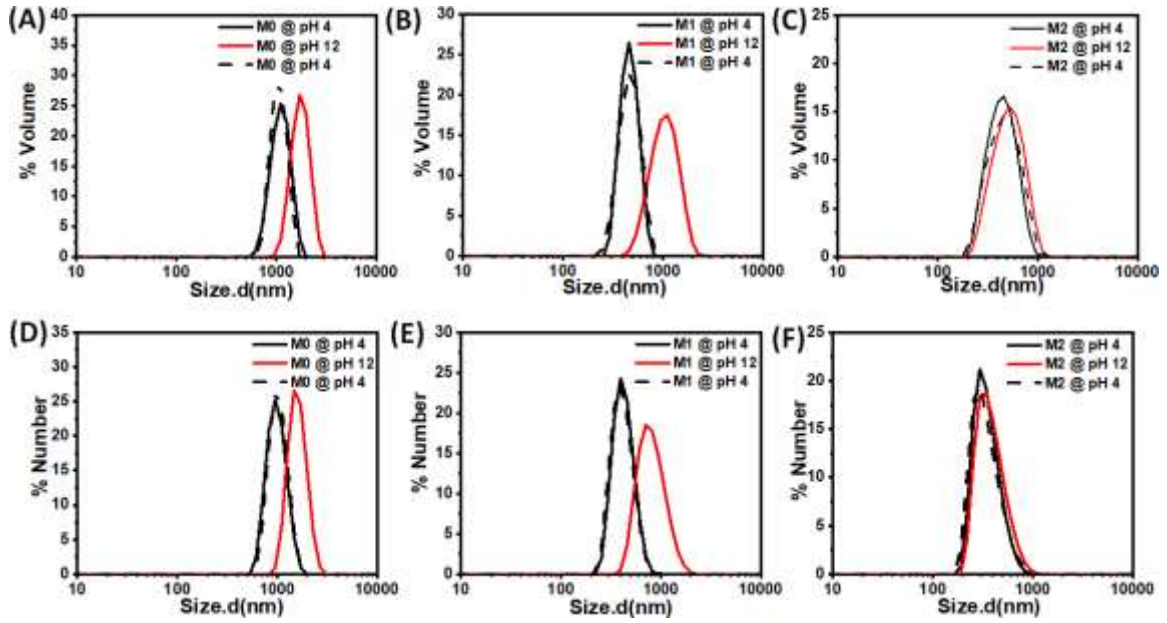


Fig. S6. pH-induced reversible swelling/deswelling for **M0**, **M1** and **M2** microgels as monitored by (A-C) DLS volume and (D-F) number distribution data.

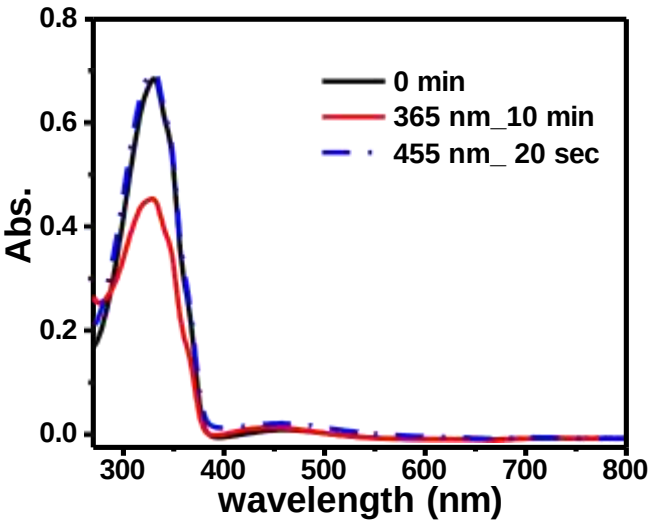


Fig. S7. Reversible photoisomerization of **HEMA-Az** upon irradiation with UV (365 nm) and visible (455 nm) light.

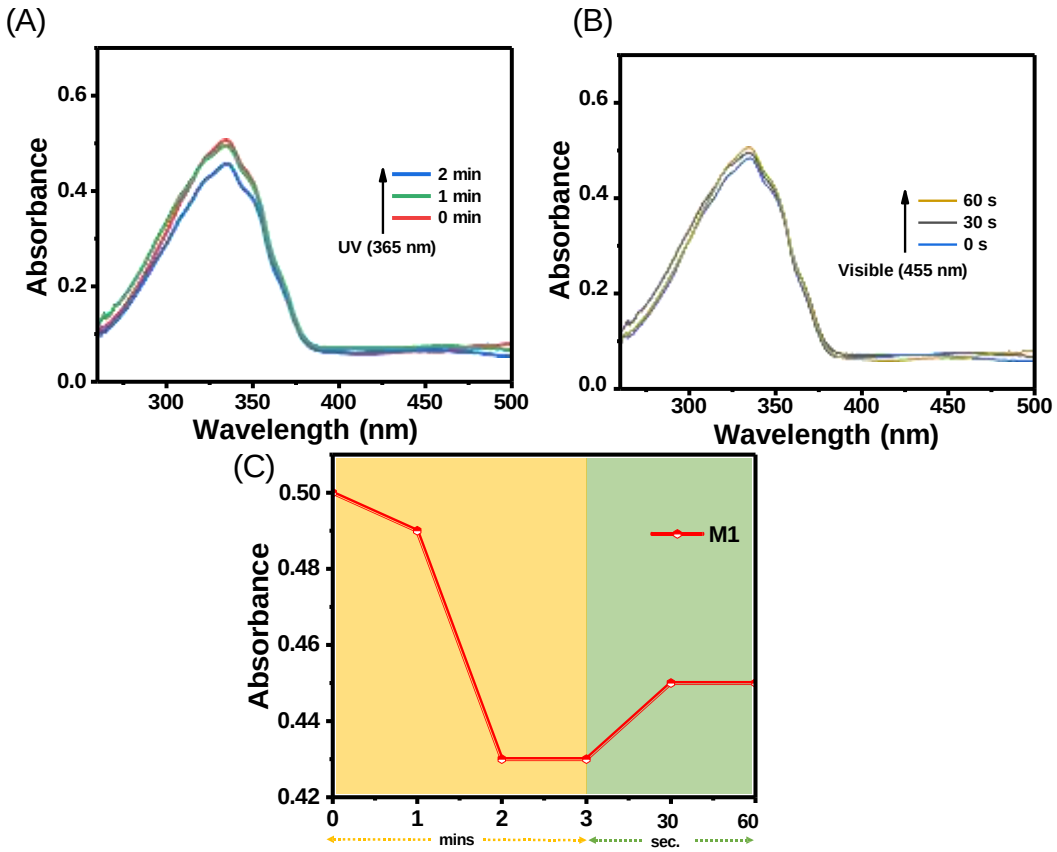


Fig. S8. (A-B) Photoconversion of **M1** microgel from *trans* to *cis* with irradiation of UV light (365 nm) and *cis* to *trans* with visible light (455 nm). (C) Photoswitching kinetics for **M1** microgel.

Stability studies: The stability of **M2** microgels was investigated in four different culture media e.g. Phosphate buffered saline (PBS), Fetal Bovine Serum (FBS), Dulbecco's Modified Eagle Medium (DMEM and Ham's F12 (HF12). Initially, lyophilized microgel particles were dispersed in different media and assesses variation in zeta-potential over the time using the DLS.

Biocompatibity: The biocompatibility of the microgel **M2** was assessed using an MTT assay. In a 96-well plate setup, 10,000 L929 cells per well were exposed to media containing microgel solutions at varied concentrations of 5, 10, 25, 50, 100 and 250 µg/mL for 24 hours at 37 °C. The media used was DMEM (HIMEDIA) supplemented with 10% fetal bovine serum (FBS). The cells were cultured in 25 cm cell culture flasks and passaged when they reached 80% confluency. Control wells without samples were included for comparison. The plates were then incubated for 24 hours in a 37 °C incubator with 5% CO_2. After incubation, 5 mg of MTT dissolved in 1 mL of PBS was filter-sterilized and diluted with DMEM to create a final concentration of 0.5 mg/mL per well. The MTT solution was removed from the wells following a 2-hour incubation with the samples, and DMSO was added to dissolve the formazan crystals in the wells. The absorbance was measured at 570 nm using a TECAN multiplate reader. The assay was performed in triplicate for each sample.

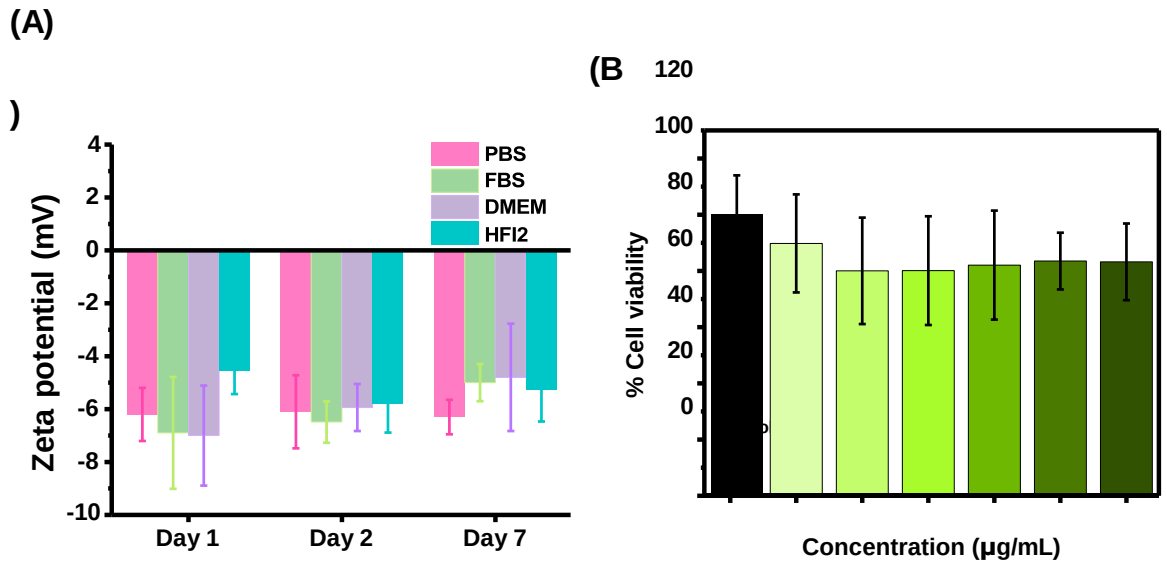


Fig. S9. (A) Stability study of the microgel **M2** in different culture media. (B) MTT Assay for evaluating the biocompatibility of the microgel **M2** at different concentrations with L929 cell lines after 24 h.

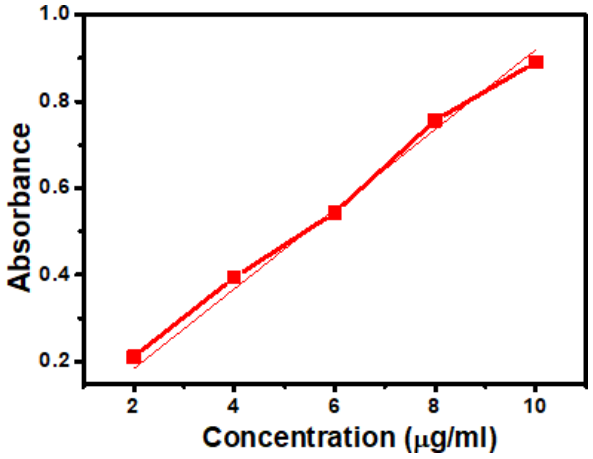


Fig. S10. Standard curve of Ciprofloxacin hydrochloride monohydrate.

Drug release kinetic studies:

The drug release kinetics data were fitted with different pharmacokinetic models including zero, first, Higuchi and Korsmeyer-Peppas model in order to analyze the release pattern of the microgel systems under different conditions. The graphical representation of release kinetics at 25 ^{o}C and 37 ^{o}C are depicted in Fig. S11-S12. All the fitting parameters derived from the kinetic equations are listed in Table S1. The study observed that Korsmeyer-Peppas model fitted well showing better R^2 values in comparison to other models.

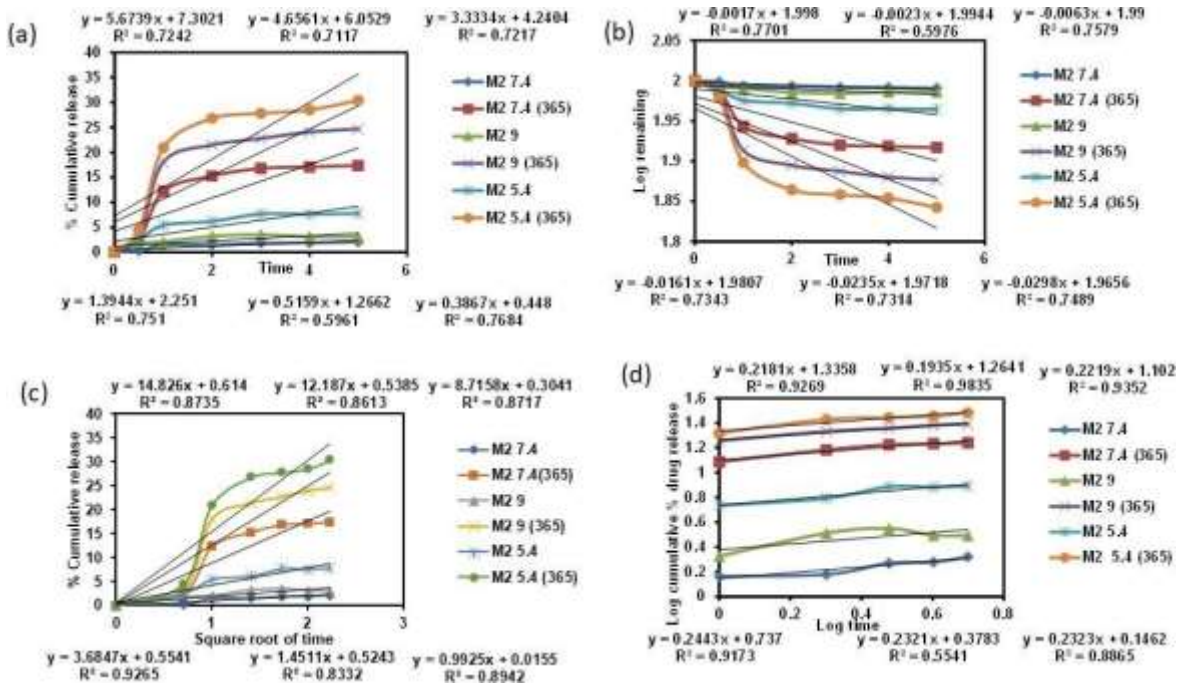


Fig. S11. Drug release kinetics for (a) zero order, (b) first order, (c) Higuchi, and (d) Korsmeyer-Peppas models involving linear regression equations of experimental **Cf** drug release data as obtained from **M2** micorgel at 25 ^{o}C.

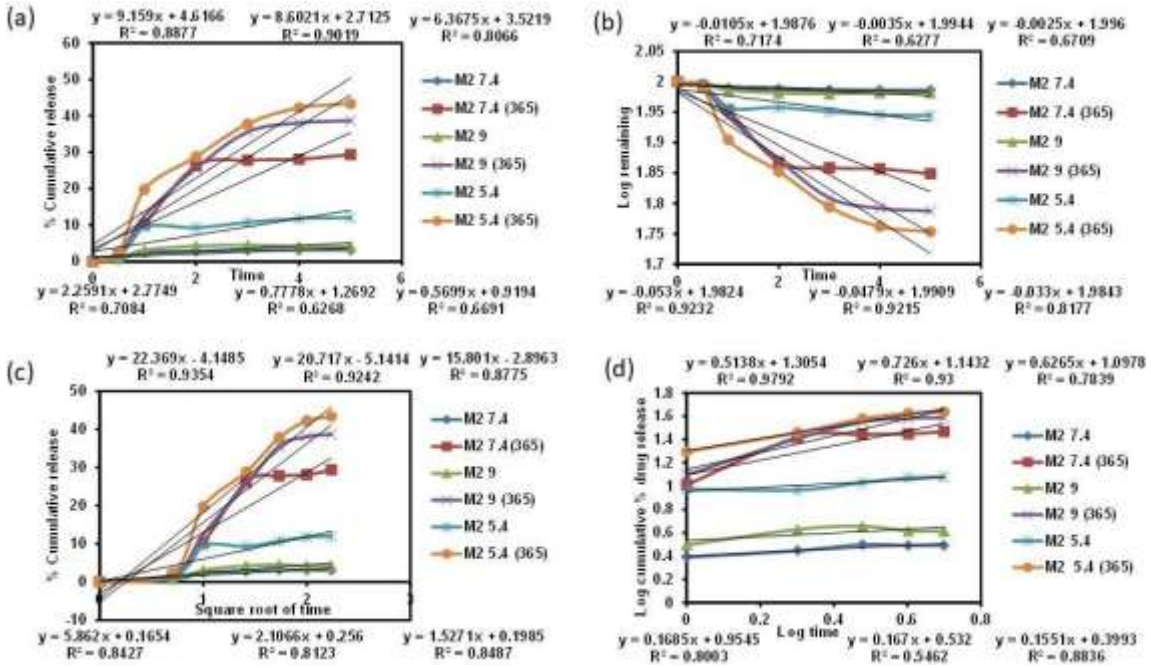


Fig. S12. Drug release kinetics for (a) zero order, (b) first order, (c) Higuchi, and (d) Korsmeyer-Peppas models involving linear regression equations of experimental **Cf** drug release data as obtained from **M2** microgel at 37 ^{o}C.

Table S1: Calculation of **Cf** drug release kinetics using model fit analyses from **M2** microgel

°C	pH	zero-order		first order		Higuchi		Korsmeyer-Peppas		
		k_o	R^2	K_1	R^2	k_H	R^2	k_{KP}	R^2	n
25	5.4	1.394	0.751	-0.006	0.757	3.684	0.926	0.737	0.917	0.244
	7.4	0.386	0.768	-0.001	0.770	0.992	0.015	0.146	0.886	0.232
	9	0.515	0.596	-0.002	0.597	1.451	0.833	0.378	0.554	0.232
	5.4 (365)	5.673	0.724	-0.029	0.748	14.826	0.873	1.335	0.926	0.218
	7.4 (365)	3.333	0.721	-0.016	0.734	8.715	0.871	1.102	0.935	0.221
	9 (365)	4.656	0.711	-0.023	0.731	12.187	0.861	1.264	0.983	0.193
37	5.4	2.259	0.708	-0.010	0.717	5.862	0.842	0.986	0.819	1.150
	7.4	0.569	0.669	-0.002	0.670	1.527	0.848	0.954	0.800	0.168
	9	0.777	0.626	-0.003	0.627	2.106	0.812	0.532	0.546	0.167
	5.4 (365)	9.159	0.887	-0.053	0.923	22.369	0.935	1.305	0.979	0.513
	7.4 (365)	6.367	0.806	-0.033	0.817	15.801	0.877	1.097	0.783	0.626
	9 (365)	8.602	0.901	-0.047	0.921	20.717	0.924	1.143	0.930	0.726

References:

1. L. He, G. Wang, Q. Tang, X. Fu and C. Gong, *J. Mater. Chem. C*, 2014, **2**, 8162–8169.
2. A. Pal, S. Karthikeyan and R. P. Sijbesma, *J. Am. Chem. Soc.*, 2010, **132**, 7842–7843.