Electronic Supplementary Material (ESI) for Polymer Chemistry.

Electronic Supplementary Information

Multi-responsive poly(oligo(ethylene glycol)methyl methacrylate)-co-poly(2-(diisopropylamino)ethyl methacrylate) hyperbranched copolymers via reversible addition fragmentation chain transfer polymerization

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TABLE S1: I_1/I_3 values of P(OEGMA-co-DIPAEMA) hyperbranched copolymers at different pHs

Sample	pH	I_1/I_3
HB1	3	1.75
	7.4	1.67
	10	1.59
HB2	3	1.6
	7.4	1.49
	10	1.39
HB3	3	1.35
	7.4	1.22
	10	1.15

From the results of Table S1, it is worth noting that in the copolymers with higher content of DIPAEMA the I_1/I_3 ratio decreases to lower values by increasing pH. This is to be expected as there are more hydrophobic groups of DIPAEMA in the polymer chain resulting in a stronger tendency for aggregation, and thus creating a strong hydrophobic microenvironment suitable for entrapping pyrene more efficiently.

TABLE S2: Light scattering results for the P(OEGMA-co-DIPAEMA) hyperbranched copolymers in aqueous media at different pHs and temperatures.

Sample	pH	Intensity (a.u)		R_h [a] (nm)		PDI [a]		ζ_p [b] (mV)	
		25 °C	60 °C	25 °C	60 °C	25 °C	60 °C	25 °C	60 °C
HB1	3	28	-	2/95	-	0.6	-	5.5	-
	7	110	477	3/97	4/152	0.5	0.4	-15.4	-23.4
	10	57	-	2-90	-	0.5	-	-38.6	-
	7.4/10	205	154	87	82	0.3	0.35	-	-
HB2	3	36	-	44	-	0.43	-	10.2	-
	7	32	279	4	7/220	0.5	0.53	-19.6	-28.2
	10	42	-	5	-	0.5	-	-32.8	-
	7.4/10	52	376	4/105	8/201	0.45	0.43	-	-
HB3	3	77	-	51	-	0.29	-	14.3	-
	7	42	422	11	5/55	0.39	0.45	-27.3	-35
	10	141	-	7	-	0.3	-	-43.2	-
	7.4/PBS	378	17000	10	241	0.5	0.1	-	-

[a] Determined by DLS at 90° angle. (c = 1 x 10^{-3} g/mL)

[b] Determined by ELS.

ATR-FTIR spectroscopy

ATR-FTIR spectra of the hyperbranched copolymers were collected in the solid state and in the infrared range 550-4000 cm^{-1} using an Equinox 55 Fourier transform spectrometer (from Bruker Optics) equipped with a single-reflector ATR diamond (Dura-Samp1IR II by SensIR Technologies). Typically, 100 scans with 4cm^{-1} resolution were performed for each spectrum.

FTIR spectral peaks of P(OEGMA-co-DIPAEMA) hyperbranched copolymer: *v (cm^{-1})*: 2876(s) (CH_2), 2799(s) and 2715(s) (-$N(C_3H_7)_2$), 1725(s) (C=O), 1246(s) (C-O), 1140(s) (O=C-O-C), 1100(s) (C-O-C), 1030(s) (C-N).

FTIR spectroscopy was utilized for the qualitative characterization of the copolymer chemical structure using the vibrational peaks attributed to the chemical groups present in the hyperbranched copolymers. In Figure S1, a typical spectrum of the HB2 copolymer is illustrated. The band at 2875 cm^{-1} corresponds to C-H bond vibrations of the -CH_2 groups of DIPAEMA and OEGMA monomers. The peaks at 2799 and 2715 cm^{-1} belong to bond vibrations of the tertiary amine group of -$N(C_3H_7)_2$ of DIPAEMA.[1] The peak at 1725 corresponds to C=O stretching. The strong peak at 1100 cm^{-1} corresponds to the vibration of the C-O-C bonds of the side groups of OEGMA[2] constituents, and at 1030

cm^{-1} a characteristic vibration of C-N bond of the PDIPAEMA diisopropylamino groups is observed.[1]

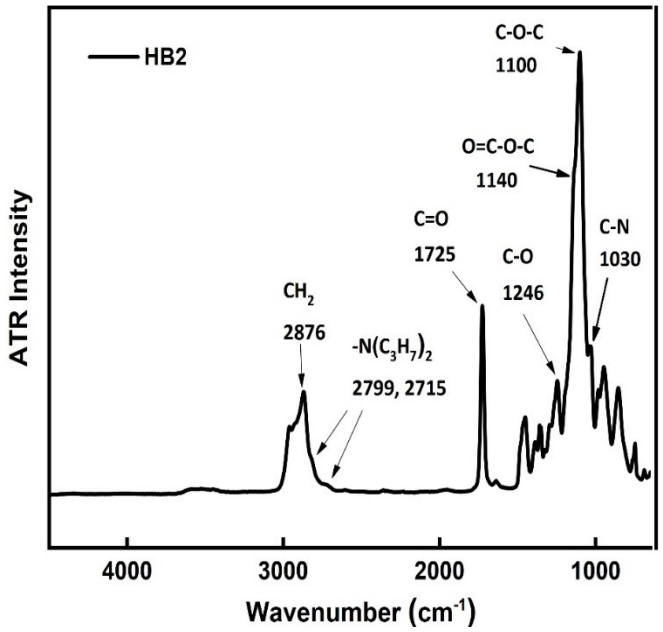

FIGURE S1: ATR-FTIR spectrum of HB2 hyperbranched copolymer.

DLS results on hyperbranched copolymers in FBS/PBS mixtures

DLS was also utilized to determine the colloidal stability of the aggregates in the presence of serum. All hyperbranched copolymers were diluted in PBS (3×10^{-3} g/mL) and two FBS/PBS mixtures (1:9 and 1:1 v/v) were prepared. It should be noted that FBS includes proteins with dimensions traceable by DLS. The mixing process is described below. Initially, 50μL of aggregate solution was added to the respective FBS/PBS solution and then the mixed nanoparticles were left for 1h. Afterwards, light scattering measurements were carried out at 25°C and at 90° angle. The results are presented in Figure S2 in the form of size distributions.

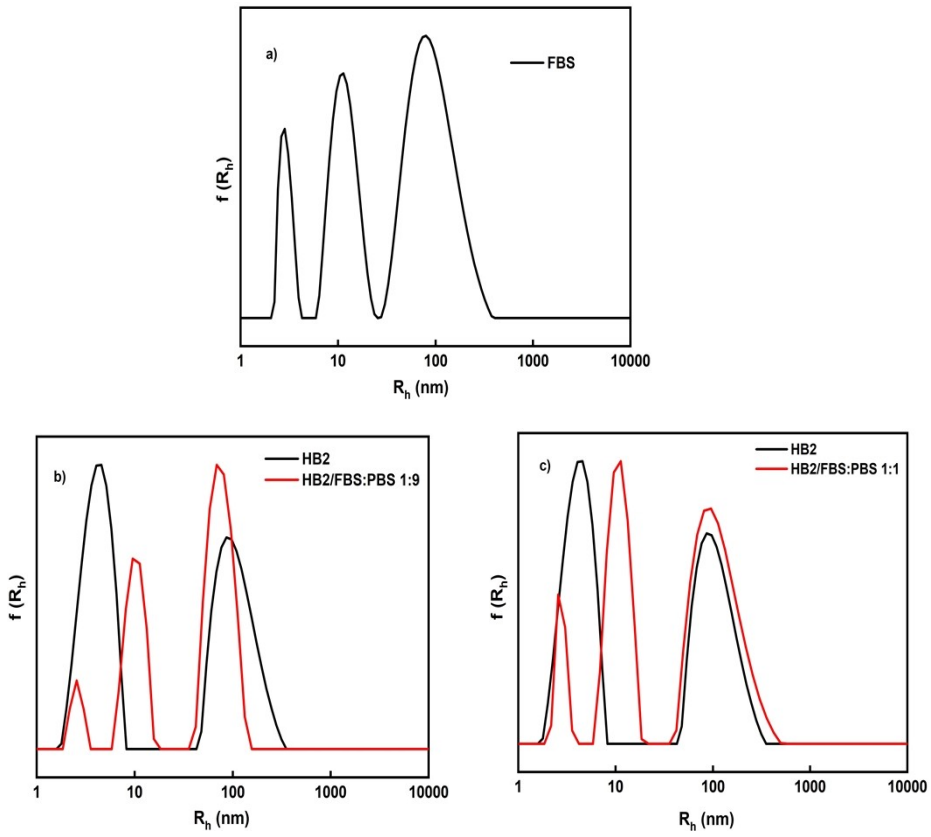

FIGURE S2: Size distributions from DLS for: a) FBS, b) HB2/FBS:PBS(1:9) before and after mixing with aggregates, c) HB2/ FBS:PBS(1:1) before and after mixing.

From DLS measurements, after mixing the polymeric nanoparticles with the FBS solution, the appearance of a new peak is not observed which indicates that no appreciable further aggregation of the polymeric aggregates takes place. These results reveal the colloidal stability of the polymeric aggregates in a simulated biological environment and the near absence of interactions with the serum proteins. Similar behavior was demonstrated for the HB1 and HB3 nanoparticles in the presence of serum.

UV-Vis spectroscopy of hyperbranched copolymers/drug formulations

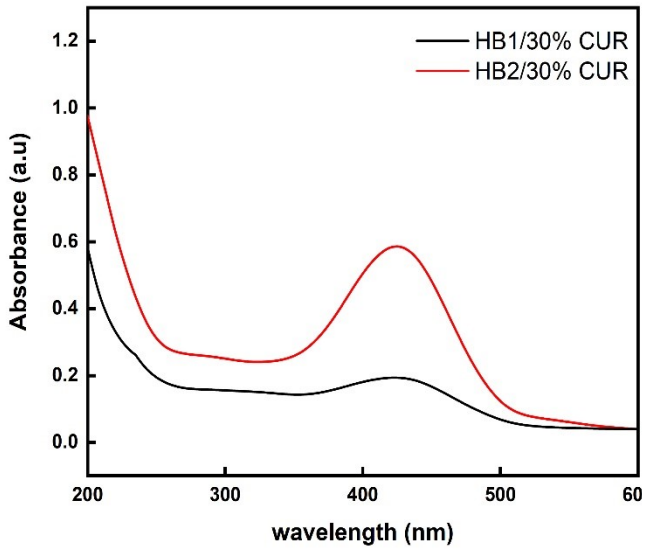

FIGURE S3: UV-Vis spectra from CUR-loaded HB1 and HB2 in aqueous media at pH7.4 (PBS).

The absorbance peak of curcumin at 420nm is well documented in the literature.[3] In Figure S3 the respective UV-Vis spectra of CUR-loaded aggregates for HB1 and HB2 hyperbranched copolymers are presented. The successful encapsulation of curcumin is supported by the presence of the characteristic peak of curcumin in both copolymer aqueous solutions. The drug-loaded HB2 nanostructures contain more hydrophobic DIPAEMA component, and thus, exhibit greater absorption. This is because they have the ability to entrap a higher amount of CUR as shown in Table S2 below.

TABLE S3: Curcumin encapsulation in P(OEGMA-co-DIPAEMA) hyperbranched aggregates.

Sample	Quantity of curcumin used (mg)	Maximum encapsulation (%w/w)	Actual % encapsulation	
			Efficiency	Loading
HB1	0.29	30	10.5	0.6
HB2	0.87	30	13.8	1.3

ATR-FTIR spectroscopy of copolymer/drug formulations

FTIR is an excellent technique for pharmaceutical analysis and can be used to determine the copolymer/drug interactions. For this reason, FTIR measurements were recorded. It is possible to study the copolymer-drug interactions as they each absorb at different frequencies. The measurements were performed in the solid state (Figure S4) after water evaporation at room temperature, under nitrogen flow.

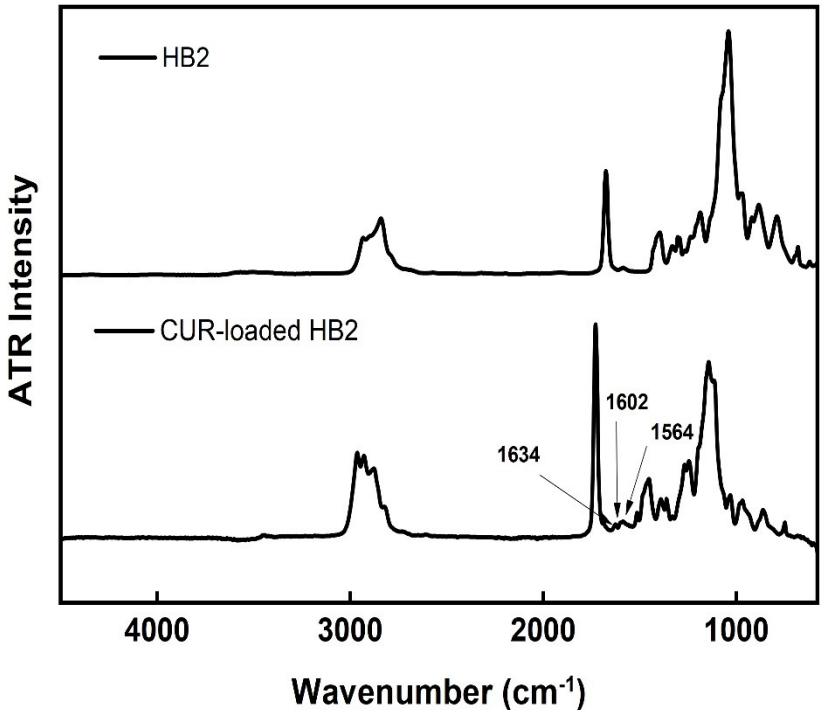

FIGURE S4: FTIR spectra for CUR-loaded HB2 nanostructures at the solid state.

In Figure S4, the FTIR spectra of HB2 hyperbranched copolymer and the CUR-loaded copolymer are illustrated (upper spectrum). After curcumin entrapment three new peaks are observed. Specifically, the absorption peak at 1634cm^{-1} belongs to the aromatic C=C vibrations, the peak at 1602cm^{-1} seems to belong to phenyl ring fingerprint and the absorption peak at 1564cm^{-1} to the C=C and C=O groups. The FTIR spectra confirm the successful encapsulation of curcumin within the copolymer aggregates.[4]

Temporal stability of CUR-loaded hyperbranched copolymer structures

The CUR-loaded polymeric nanoparticles of HB1 and HB2 copolymers were characterized in terms of temporal stability by the DLS technique at 25^{o}C, and at 90^{o}

angle. The results are presented in Figure S5 in the form of plots of the scattering intensity and the hydrodynamic radius vs. time.

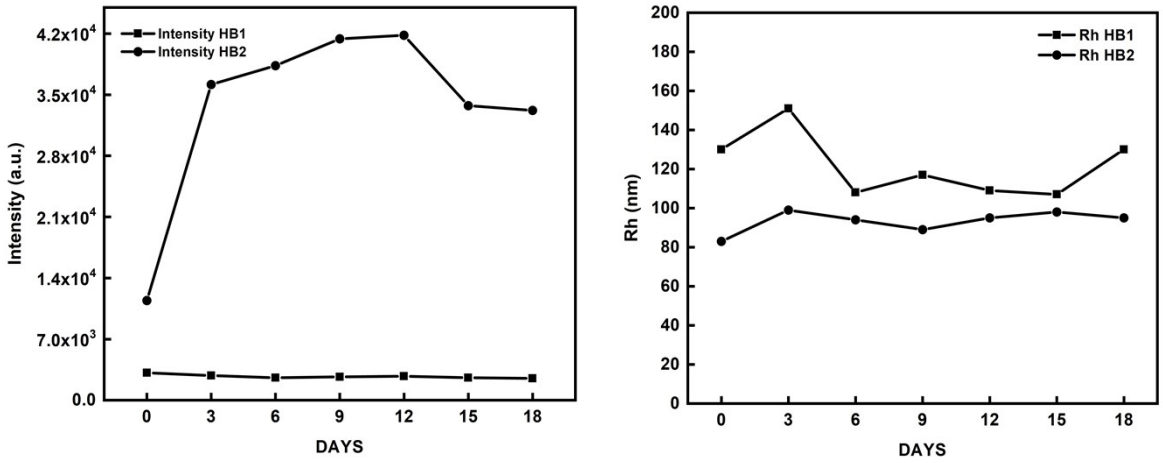

FIGURE S5: DLS data from temporal stability studies for CUR-loaded nanoparticles of HB1 and HB2 copolymers.

It can be observed that the HB1 solution shows greater stability over time based on the measured scattering intensity and hydrodynamic radius. The observed colloidal stability can be attributed to the higher OEGMA content in the HB1 copolymer, which provides better stability and protects the drug loaded nanoparticles from precipitation.

REFERENCES AND NOTES

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