

Supporting information

For

**UCST Phase Transition of Dextran Based Copolymer in Aqueous Media with Tunable
Thermoresponsive Behavior**

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EXPERIMENTAL SECTION

Materials. The polysaccharide dextran (Dxt) was obtained from TCI, India. Both the monomers i.e., methacrylamide (MAM, >98%) and acrylic acid (AA, >99%) were purchased from TCI, India. Potassium persulfate (KPS, 98%) was procured from Qualigens Thermofisher, UK. Tetramethylethylenediamine (TEMED, 99%) was purchased from Sigma-Aldrich, USA. All the materials were used as obtained. Ultrapure water was used for the experimental work.

Synthesis of the graft copolymer. The graft copolymer Dxt-g-(PMAM-co-PAA) was prepared by conventional free radical polymerization through grafting of MAM and AA simultaneously on to the backbone of Dxt. Firstly, 0.5 g of Dxt was taken in a two necked round-bottom (RB) flask and dissolved in 30 mL ultrapure water at ambient temperature. After that, the RB was transferred to a silicon oil bath and the temperature of the bath was raised to 80°C with constant stirring at a speed of 400 rpm. Then to generate inert atmosphere in the RB, the RB was sealed and N₂ gas was purged for 5 min. After 10 min, 3.69×10^{-5} mole KPS dissolved in 2 mL ultrapure water was poured to the prior mixture. After 10 min, 1.17×10^{-2} mole of MAM dissolved in 7 mL H₂O was added to the reaction mixture followed by the addition of 6.66×10^{-4} mole of TEMED. Then after 5 min, 4.37×10^{-2} mole of AA was mixed with the reaction mixture and kept it for 3 h under the same condition. 3 h later, the white colored reaction mixture was kept to the open atmosphere in room temperature to cool down. After that, the product was immersed in large volume of acetone for 5 h. Then the product was centrifuged and dried under vacuum. Another three grades of Dxt-g-(PMAM-co-PAA) were prepared by taking 5.87×10^{-3} , 1.76×10^{-2} and 2.35×10^{-2} moles MAM keeping the feed amount of AA constant (Scheme S1, ESI).

Polymer characterization. ¹H NMR spectra of Dxt and the four graft copolymers were recorded by a 400 MHz NMR spectrophotometer (Model: Ascend 400, Bruker, USA) using DMSO-d₆

solvent. Solid state ^{13}C NMR spectra of Dxt and the four synthesized graft copolymers were examined by a 400 MHz FT-NMR spectrophotometer (Model: JNM-ECA/ECX series, Delta V4.3, JEOL, Singapore). The solid state ^{13}C NMR spectra were acquired by using the 1024 scan, 6KHz rotation frequency and 5 sec relaxation delay. To determine the percentage of C, H, N of Dxt and the copolymers, Elemental Analyzer (Elementar Analysensysteme GmbH, Germany) was used. FTIR spectra of Dxt and the four synthesized copolymers were recorded by a FTIR spectrophotometer (Perkin Elemer, Model: Spectrum 2000). MALS/GPC (Model: BI-RI/620; Serial No. 15 105; Power: 90-260 V 47–63 Hz, USA) analysis was performed to determine the average molecular weight of the Dxt and all the grades of copolymers. The % transmittance of aqueous solutions of the polymers was recorded in a UV-visible spectrophotometer (Shimadzu, Japan: Model: UV 1800) as a function of temperature between 549-551 nm wavelength ranges. The % transmittance of the polymers were recorded with a heating rate of $1^\circ\text{C}/\text{min}$ and with a stirring rate of 60 rpm. The DLS (Zetasizer Nano ZS, Malvern, UK) technique was employed to measure the hydrodynamic diameter of aqueous polymer solutions at different pH. During DLS measurement, 10% solution of Dxt-g-(PMAM-co-PAA)-2 was prepared in different pH. The pH of the solution was changed by adding hydrochloric acid (HCl) and sodium hydroxide (NaOH) to the solutions. Then the DLS study was carried out below and above UCST. The DLS cuvette used here was a four openings disposable cell. The surface morphologies below and beyond UCST of the copolymer were evaluated using Supra55, Zeiss field emission scanning electron microscope (Germany).

Cytocompatibility Study. Solutions of four grades of copolymers were prepared in ultrapure water and TCP was employed for MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. The copolymers were sterilized using 70% ethanol for 2 h and then washed by

Phosphate buffer saline (PBS) for three times. The samples were incubated for overnight in complete MEM media (Himedia). The cytotoxicity study was carried out using MCF-7 cells, obtained from NCCS Pune, cultured in MEM media in 5% CO₂ atmosphere at 37 °C (CellXpert C170, Eppendorf, Germany). After that, the cells were collected using 0.25% trypsin from the T75 flask and added equal volume on control as well as four grades of copolymers. Cell density was calculated by Automated Cell Counter (Thermo Fisher Countess II FL) and the cells were seeded in 96 well plate. MTT assay of the MCF-7 cells of the samples and control were examined after 1, and 3 days of experiment. The culture medium was discarded from 96 well plate followed by washing with PBS and incubated with 5 mg/mL MTT solution (SRL) at 37 °C for 4 h. 100 µL DMSO (SRL) was added to each well to dissolve the insoluble purple formazan crystals formed by the reduction of MTT by the mitochondrial dehydrogenase enzyme that present in active cells. The culture plate was placed on a horizontal shaker for 60 min. Absorbance was recorded with the help of a microplate reader (Thermoscientific, Multiskan FC) at 405 nm. The % of cell viability was plotted against day. For cytotoxicity experiment, the concentration of the copolymers were 30 mg/mL. Tissue culture plate containing only cells was considered as control whereas wells without cells were considered as blank. Percent cell viability for each sample was calculated using equation (S1):

$$\% \text{ Cell viability} = \frac{OD_{405} [\text{Sample}] - OD_{405} [\text{Blank}]}{OD_{405} [\text{Control}] - OD_{405} [\text{Blank}]} \times 100 \quad (S1)$$

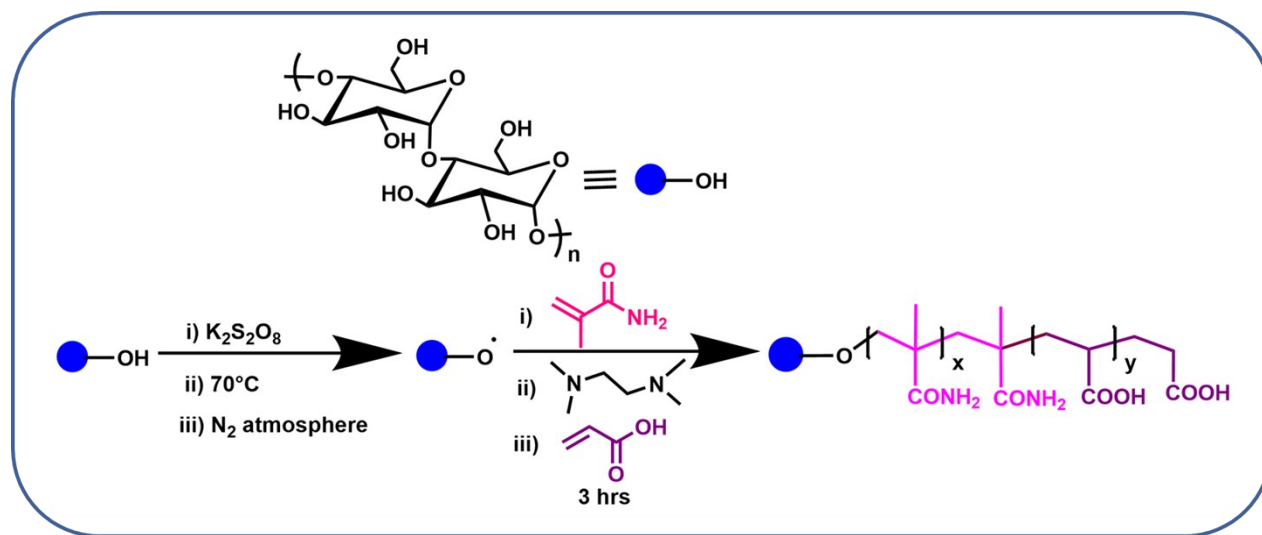
Here, $OD_{405} [\text{Sample}]$, $OD_{405} [\text{Control}]$, and $OD_{405} [\text{Blank}]$ are the optical density of the cells with sample, cells without sample and blank respectively.

Determination of the T_{CP}. The T_{CP} was determined from the plot of % transmittance vs. temperature. Firstly, after plotting, two tangents were drawn and from the intersection point of

these two tangents one perpendicular line was drawn to the temperature axis. The temperature in which the perpendicular lines touches is called as T_{CP} ^{1,2}.

RESULTS AND DISCUSSION

Synthesis. The UCST copolymer Dxt-g-(PMAM-co-PAA) was synthesized by FRP through the grafting of MAM and AA simultaneously on Dxt. At first, in presence of N_2 atmosphere KPS undergoes decomposition and generates free radical. Then these free radicals again produce free radical active sites on the Dxt backbone by abstracting the protons from the said polysaccharide moiety³. After that the active free radical of Dxt further reacts with both the vinylic monomers (MAM and AA) concurrently to develop Dxt-g-(PMAM-co-PAA). In this reaction, TEMED was used as the reaction enhancer (Scheme S1, ESI)⁴.



Scheme S1. Synthesis route of UCST based graft copolymers

Table S1. Table for theoretical content of monomers.

Material	Amount of Dextran (mole)	Theoretical MAM content (mole)	Theoretical AA content (mole)
Dxt-g-(PMAM-co-PAA)-1	1.25×10^{-5}	5.87×10^{-3}	4.37×10^{-2}
Dxt-g-(PMAM-co-PAA)-2	1.25×10^{-5}	1.17×10^{-2}	4.37×10^{-2}
Dxt-g-(PMAM-co-PAA)-3	1.25×10^{-5}	1.76×10^{-2}	4.37×10^{-2}
Dxt-g-(PMAM-co-PAA)-4	1.25×10^{-5}	2.35×10^{-2}	4.37×10^{-2}

Polymer characterization.

CHN analysis. Table S2 shows the elemental analysis data of the Dxt and four UCST polymers. From the data it is noticeable that, Dxt does not comprise of N whereas Dxt-g-(PMAM-co-PAA)-1 contain 2.28 % of N which concludes that PMAM has been successfully grafted to Dxt moiety to form the copolymer. Also, the elemental analysis reveals that the N-content of the copolymers increases from 2.28 to 6.02 % with increase in the MAM content from Dxt-g-(PMAM-co-PAA)-1 to Dxt-g-(PMAM-co-PAA)-4.

Table S2. CHN analysis data.

Materials Name	N content (%)	C content (%)	H content (%)
Dxt	0.00	41.50	6.502
Dxt-g-(PMAM-co-PAA)-1	2.28	45.17	6.491
Dxt-g-(PMAM-co-PAA)-2	3.34	44.29	6.605
Dxt-g-(PMAM-co-PAA)-3	5.16	47.85	7.126
Dxt-g-(PMAM-co-PAA)-4	6.02	47.76	7.194

FTIR analysis. FTIR spectrum of Dxt (Fig. S1(a), ESI) shows the characteristic peaks at 3452, 2929 and 1656 cm^{-1} , which corresponds to -O-H, -C-H and $>\text{C}=\text{O}$ stretching vibrations respectively. The other peaks appeared at 1158, 1108, 1008 and 917 cm^{-1} are due to the -C-O-C stretching, glycosidic -C-O linkage, α -(1,6) glycosidic linkage and α -glycosidic linkage respectively. The FTIR spectra of Dxt-g-(PMAM-co-PAA)-3 (Fig. S1(b), ESI) contains all the characteristic peaks of Dxt moiety with two additional peaks at 1717 and 1602 cm^{-1} , which corresponds to $>\text{C}=\text{O}$ of -COOH group and amide-II vibrations, respectively. The amide-I vibration merge with the $>\text{C}=\text{O}$ stretching vibrations, which appears at 1659 cm^{-1} .

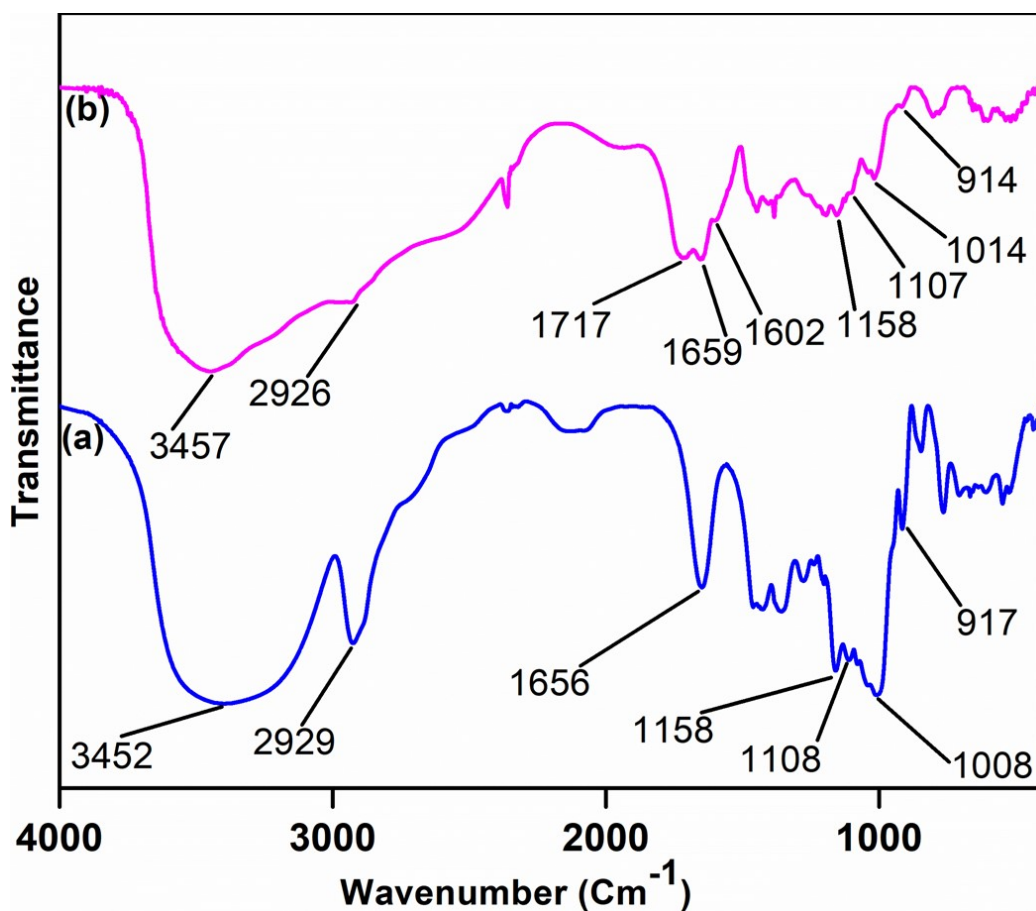


Fig. S1. FTIR spectra of (a) dextran (b) Dxt-g-(PMAM-co-PAA)-3.

¹H NMR analysis. Fig. S2, ESI demonstrates the ¹H NMR spectrum of Dxt. The peak corresponds to $\delta = 4.89$ ppm is related to anomeric protons (a_h). The NMR peak at $\delta = 3.43$ ppm is responsible for the -OH protons on the ring of the said polysaccharide moiety. The peaks at $\delta = 3.60$ and 3.72 ppm are allotted to f_h and c_h, b_h protons respectively. The peaks present at $\delta = 3.18$ and 4.65 ppm are due to d_h and e_h protons respectively. In the ¹H NMR spectrum of Dxt-g-(PMAM-co-PAA)-1 (Fig. S3, ESI) the signal at $\delta = 4.88$ ppm is assigned to the anomeric proton (a_h) of Dxt. The existence of the peak at $\delta = 3.44$ ppm is attributed to the -OH protons of Dxt and c_h, b_h, f_h, d_h protons of Dxt. The signal corresponds to $\delta = 4.64, 4.54$ ppm is due to e_h protons. The newly appeared peaks at $\delta = 2.18, 2.09, 1.47, 1.69$ and 0.92 ppm are related to k_h, j_h, h_h protons respectively. Additionally, the presence of the peaks at $\delta = 7.05$ and 12.22 ppm are due to the -NH (i_h) and -COOH (l_h) protons respectively which confirms the successful grafting of MAM and AA on the back of the Dxt moiety.

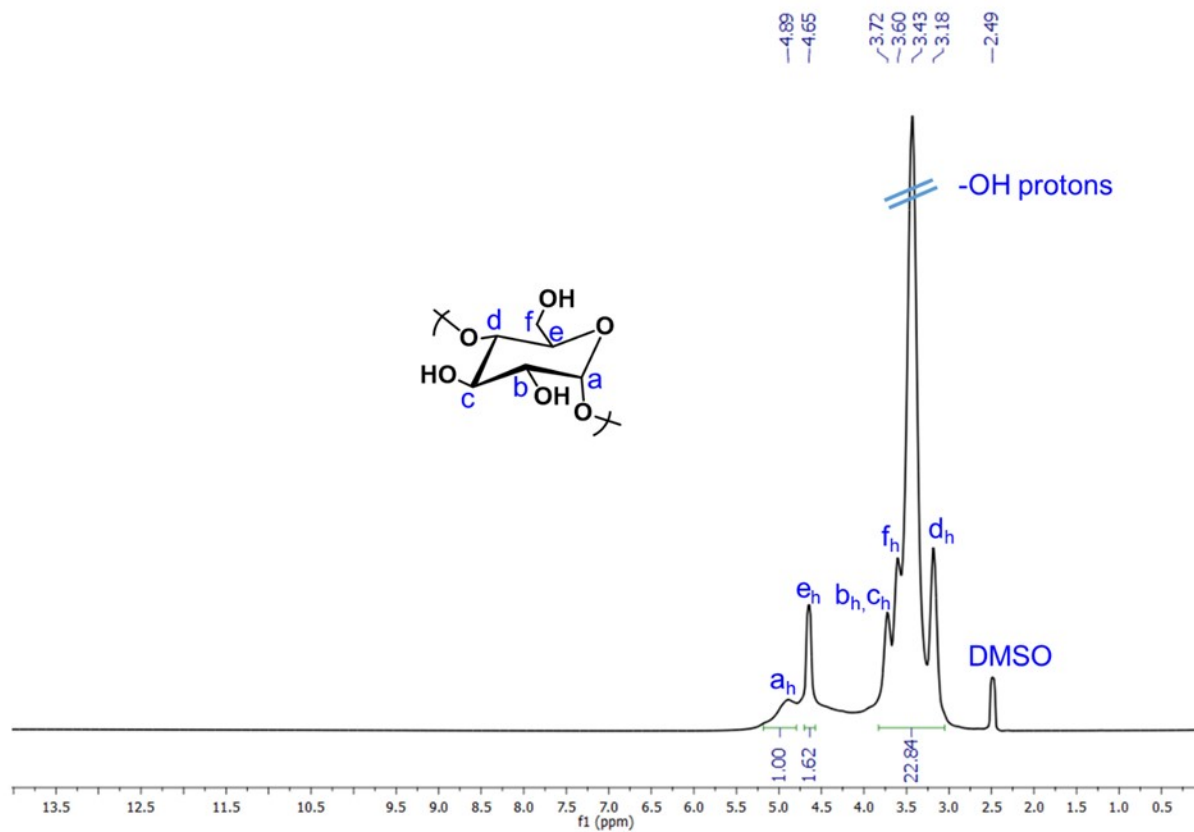


Fig. S2. ^1H NMR spectrum of Dxt.

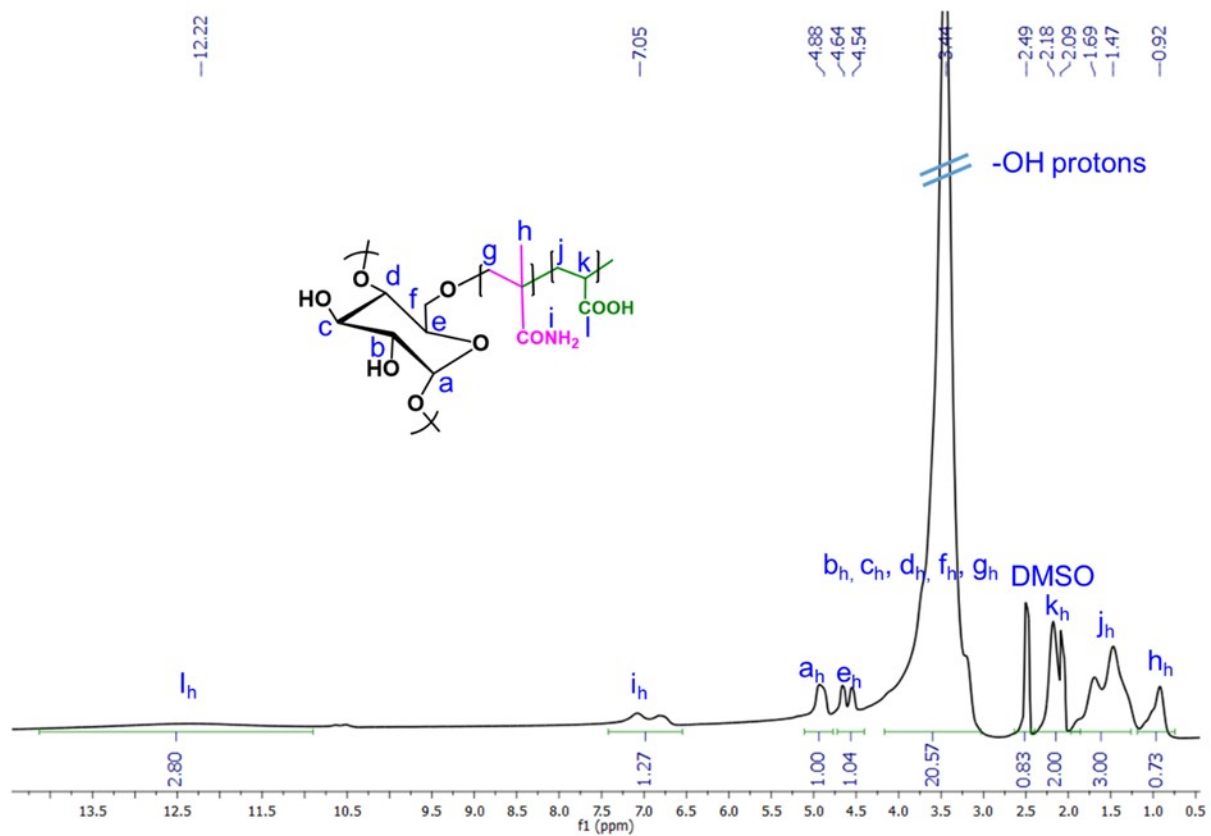


Fig. S3. ^1H NMR spectrum of Dxt-g-(PMAM-co-PAA)-1.

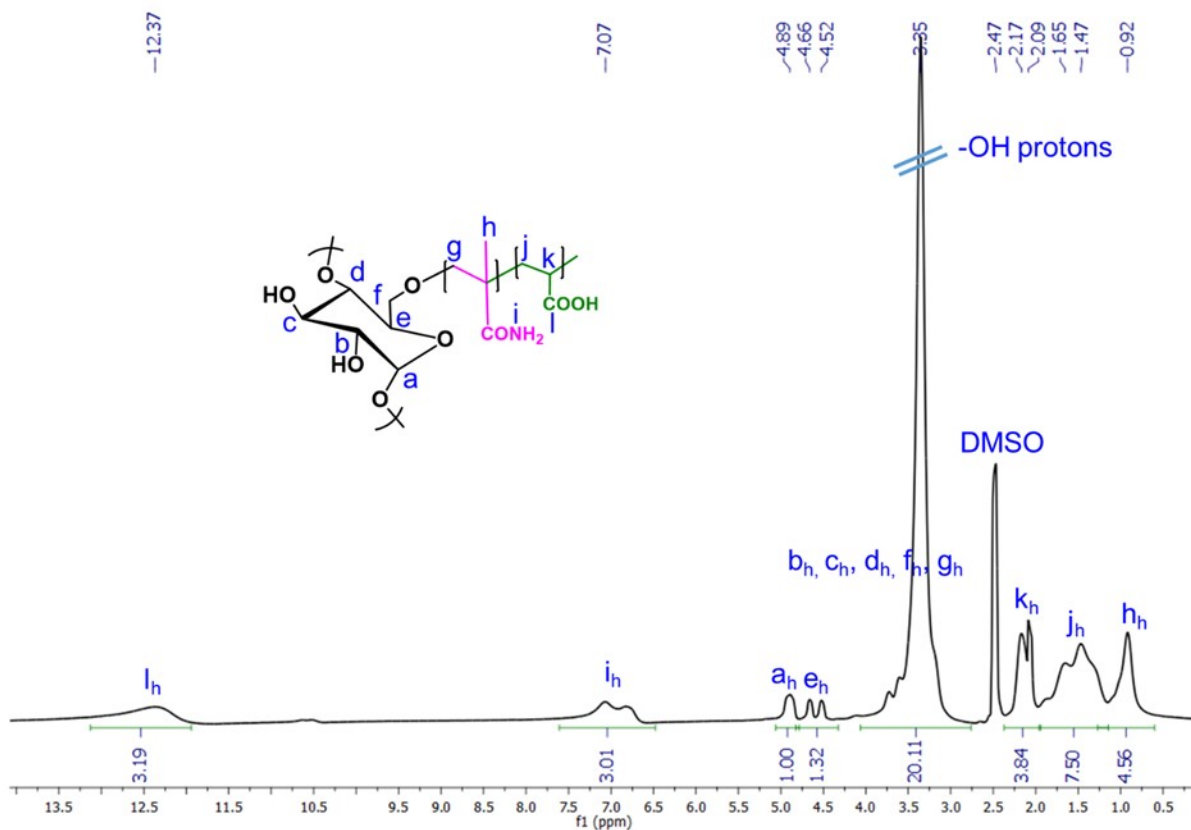


Fig. S4. ^1H NMR spectrum of Dxt-g-(PMAM-co-PAA)-2.

Dxt-g-(PMAM-co-PAA)-2: ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 4.89 (a_h), 3.35 (b_h, c_h, d_h, f_h, g_h), 4.66 and 4.52 (e_h), 2.17 and 2.09 (k_h), 1.65 and 1.47 (j_h), 0.92 (h_h), 7.07 (i_h) and 12.37 (l_h).

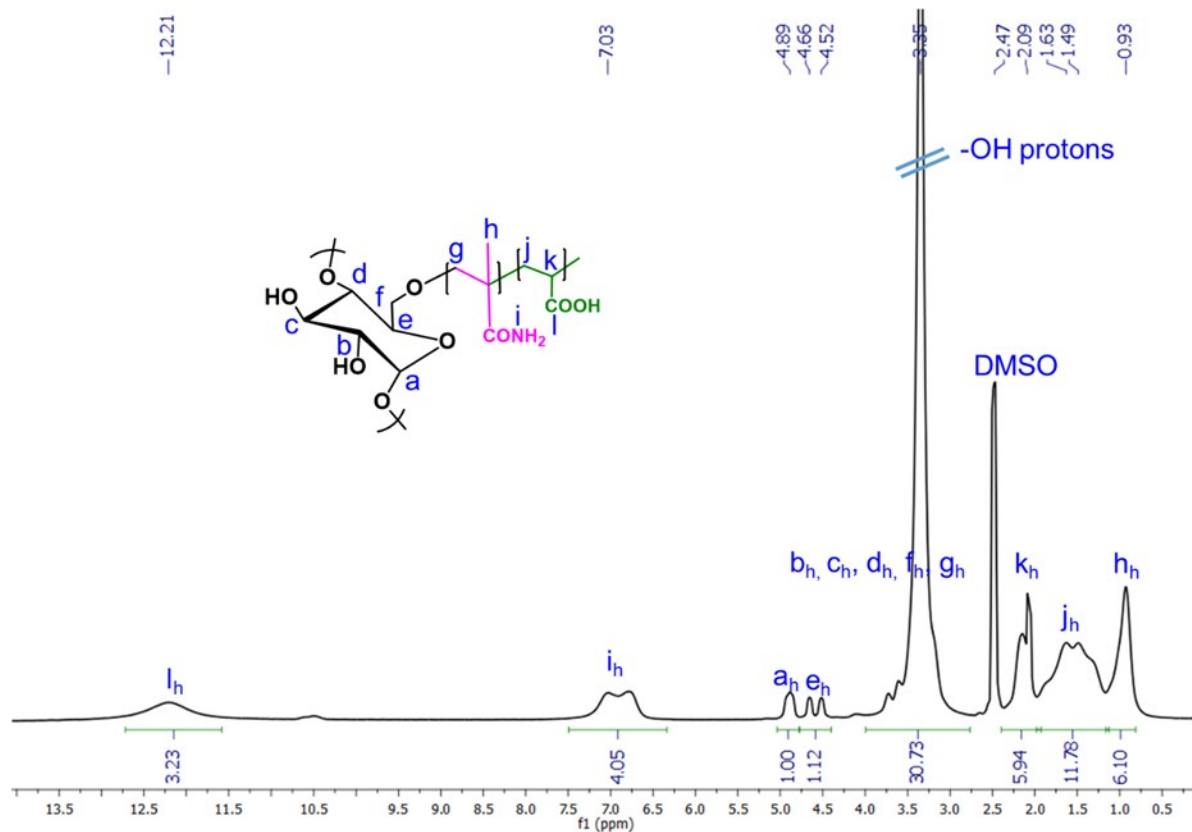


Fig. S5. ^1H NMR spectrum of Dxt-g-(PMAM-co-PAA)-3.

Dxt-g-(PMAM-co-PAA)-3: ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.89 (a_h), 3.35 (b_h , c_h , d_h , f_h , g_h), 4.66 and 4.52 (e_h), 2.09 (k_h), 1.63 and 1.49 (j_h), 0.93 (h_h), 7.03 (i_h) and 12.21 (l_h).

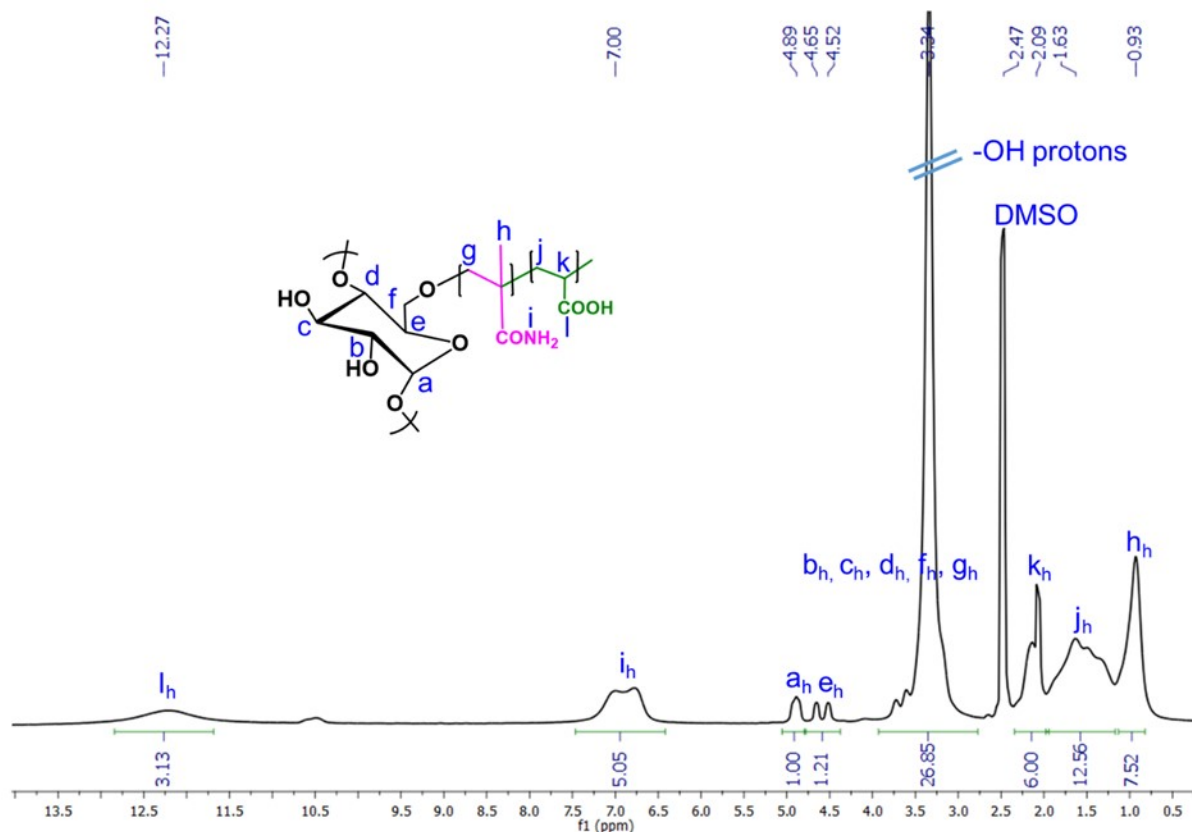


Fig. S6. ^1H NMR spectrum of Dxt-g-(PMAM-co-PAA)-4.

Dxt-g-(PMAM-co-PAA)-4: ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.89 (a_h), 3.34 (b_h, c_h, d_h, f_h, g_h), 4.65 and 4.52 (e_h), 2.09 (k_h), 1.63 (j_h), 0.93 (h_h), 7.00 (i_h) and 12.27 (l_h).

Determination of monomer ratio by ^1H NMR spectra.

The ratio of acrylic acid / methacrylamide with respect to dextran has been calculated by comparing the integration values of characteristic peaks of polymerized acrylic acid and methacrylamide moiety on the dextran backbone. From Fig. S6, it is assumed that half of the peak intensity (integration value) of amide proton (i_h) of methacrylamide would be equal to peak intensity (integration value) of one carboxylic acid proton (l_h) of acrylic acid with respect of peak intensity (integration value) of one anomeric proton (a_h) of dextran. Hence, ratio of acrylic acid / methacrylamide is calculated using the following equation (S2)⁵.

$$\text{acrylic acid / methacrylamide} = \frac{I_{lh}}{\frac{1}{2}(I_{ih})} \dots\dots\dots (S2)$$

It is obvious from Fig. S6 that

$(I_{ah}) = 1.00$ (considered as base value of anomeric proton of Dextran), $(I_{lh}) = 3.13$ (one proton of carboxylic acid of acrylic acid) and $(I_{ih}) = 5.05$ (two protons of amide for methacrylamide). Hence the ratio of acrylic acid / methacrylamide, present in Dxt-g-(PMAM-co-PAA)-4 is 3:2.5.

Table S3. Table of the intensity and monomer ratio of different grades of copolymers

Materials	Intensity Ratio	Monomer Ratio
Dxt-g-(PMAM-co-PAA)-1	$\frac{I_{lh}}{\frac{1}{2}(I_{ih})}$	3:0.5
Dxt-g-(PMAM-co-PAA)-2	$\frac{I_{lh}}{\frac{1}{2}(I_{ih})}$	3:1.5
Dxt-g-(PMAM-co-PAA)-3	$\frac{I_{lh}}{\frac{1}{2}(I_{ih})}$	3:2
Dxt-g-(PMAM-co-PAA)-4	$\frac{I_{lh}}{\frac{1}{2}(I_{ih})}$	3:2.5

¹³C NMR analysis. From the solid state ¹³C NMR spectrum of Dxt (Fig. S7, ESI) it can be shown that the peak at $\delta = 72.5$ ppm is attributed to the ring carbons (C2 – C6) present in the Dxt moiety. On the other hand, the peak at $\delta = 99.7$ ppm corresponds to the anomeric carbon (C1) of the Dextran⁶. Fig S8, ESI demonstrates the solid state ¹³C NMR spectrum of Dxt-g-(PMAM-co-PAA)-

1 in which all the peaks of Dxt were present along with some additional peaks were observed. The peaks appear at $\delta = 72.5$ and 98.9 ppm are due to the presence of C2 – C6 and C1 carbons of dextran, respectively. Other peaks at $\delta = 39.5$ and 17.5 ppm are attributed to C8 – C10 and C13 carbons respectively. The remaining peak at $\delta = 184.6$ ppm is assigned to C11 and C12 carbons which is coming from the attached monomer to the dextran moiety. From these peaks it can be confirmed that the monomers have been grafted successfully on the Dxt backbone.

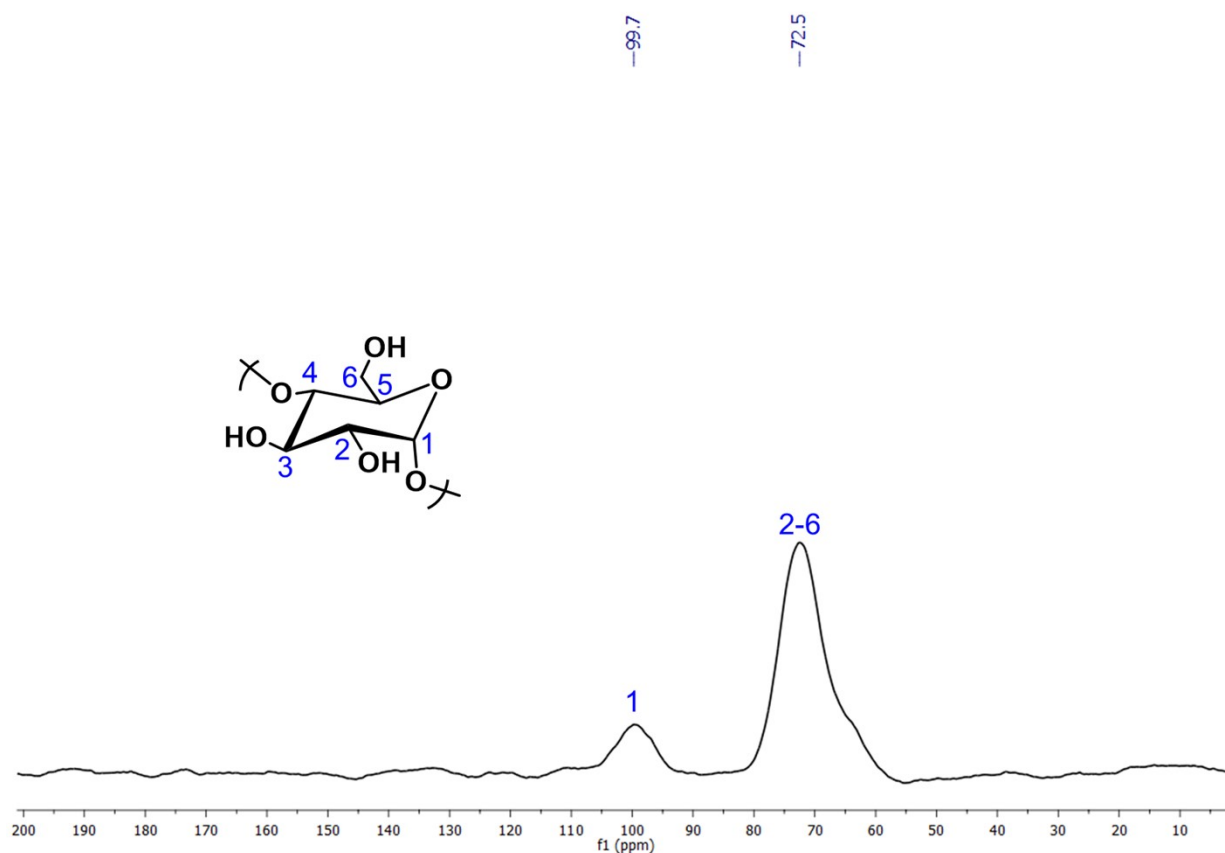
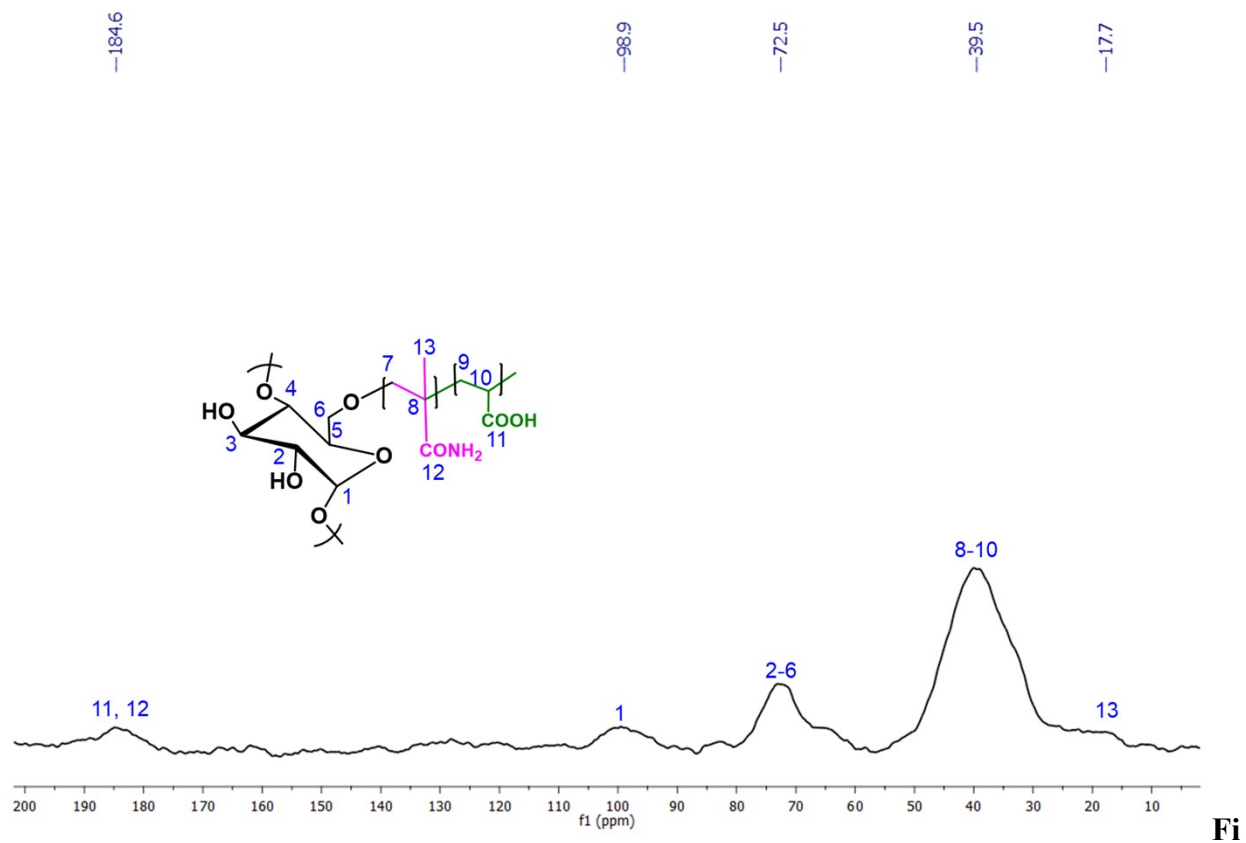
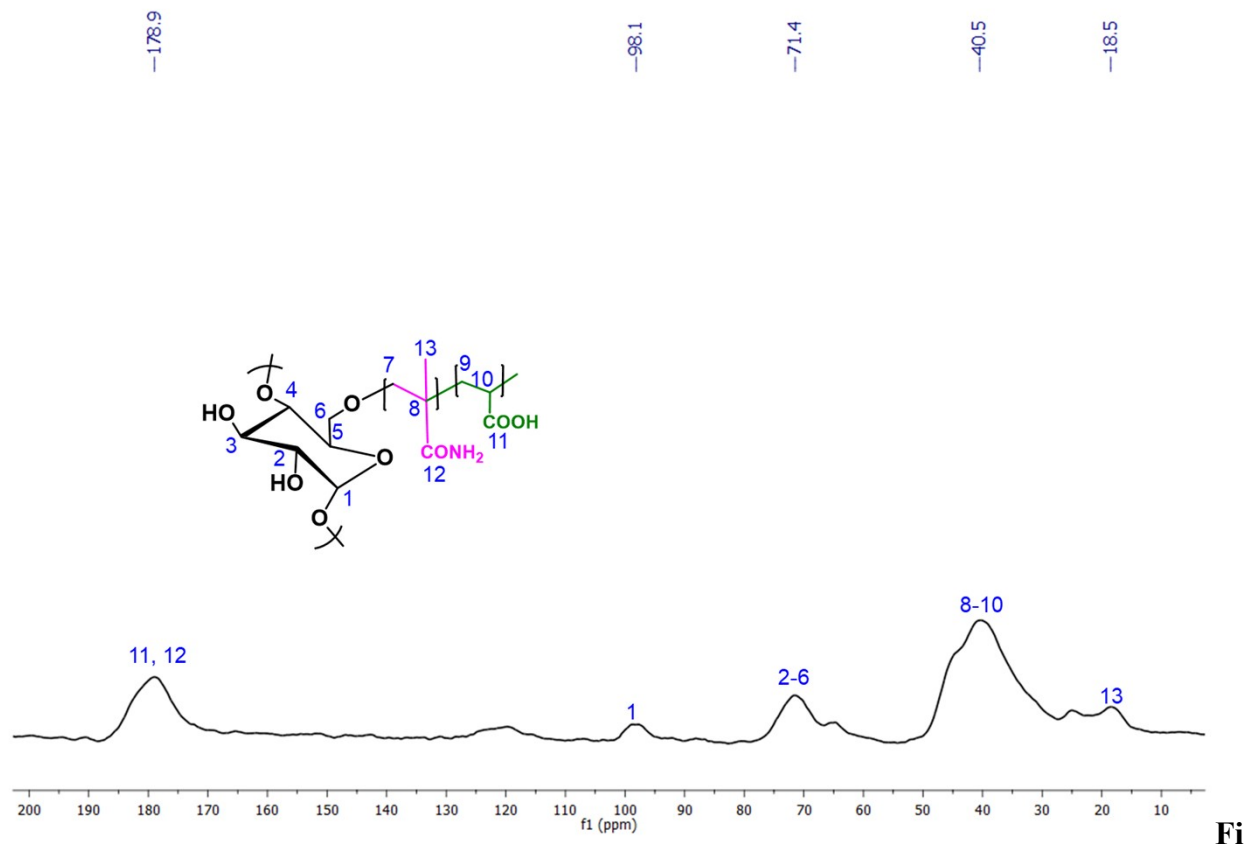


Fig. S7. ^{13}C NMR spectrum of Dxt.

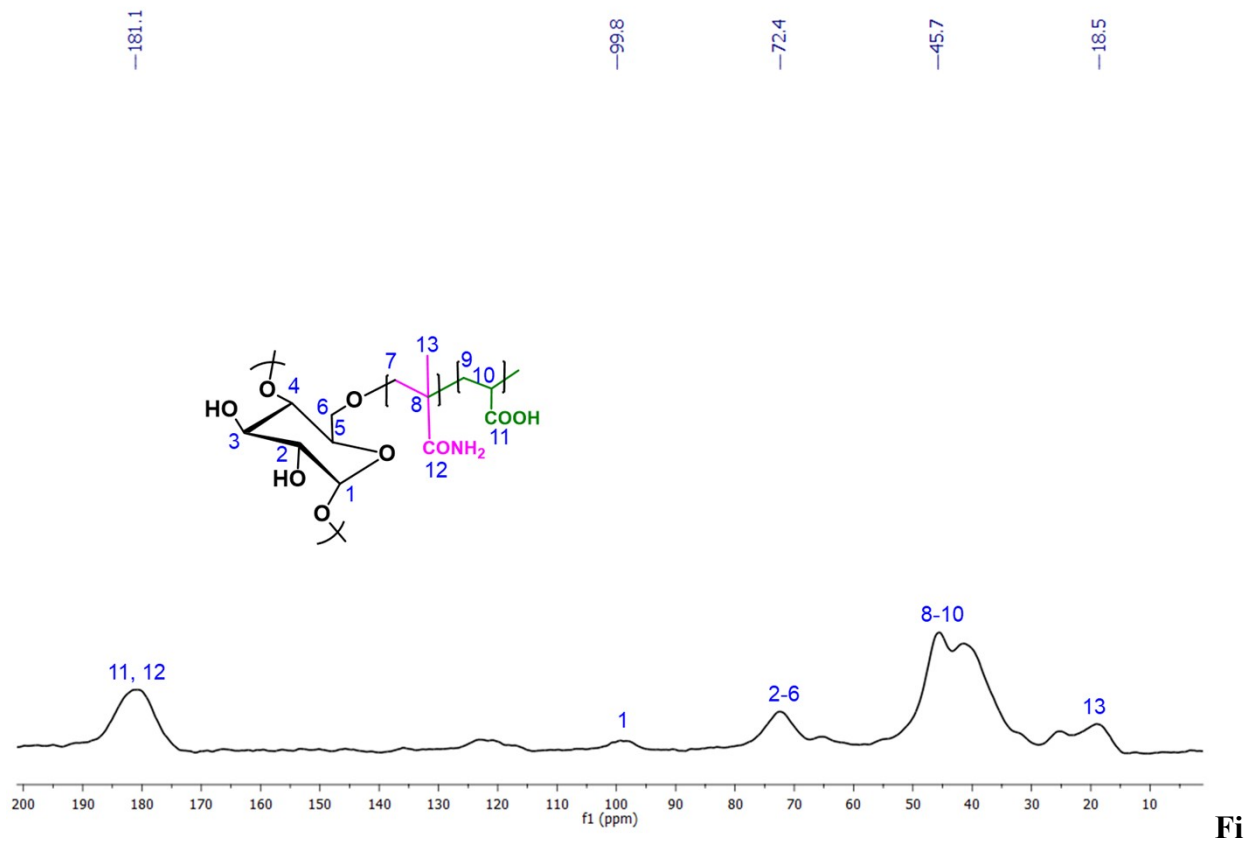


g. S8. ^{13}C NMR spectrum of Dxt-g-(PMAM-co-PAA)-1.



g. S9. ^{13}C NMR spectrum of Dxt-g-(PMAM-co-PAA)-2.

Dxt-g-(PMAM-co-PAA)-2: ^{13}C NMR (400 MHz, Solid State) δ (ppm): 98.1 (C1), 71.4 (C2 – C6), 40.5 (C8 – C10), 18.5 (C13) and 178.9 (C11, C12).



g. S10. ^{13}C NMR spectrum of Dxt-g-(PMAM-co-PAA)-3.

Dxt-g-(PMAM-co-PAA)-3: ^{13}C NMR (400 MHz, Solid State) δ (ppm): 99.8 (C1), 71.4 (C2 – C6), 45.7 (C8 – C10), 18.5 (C13) and 181.1 (C11, C12).

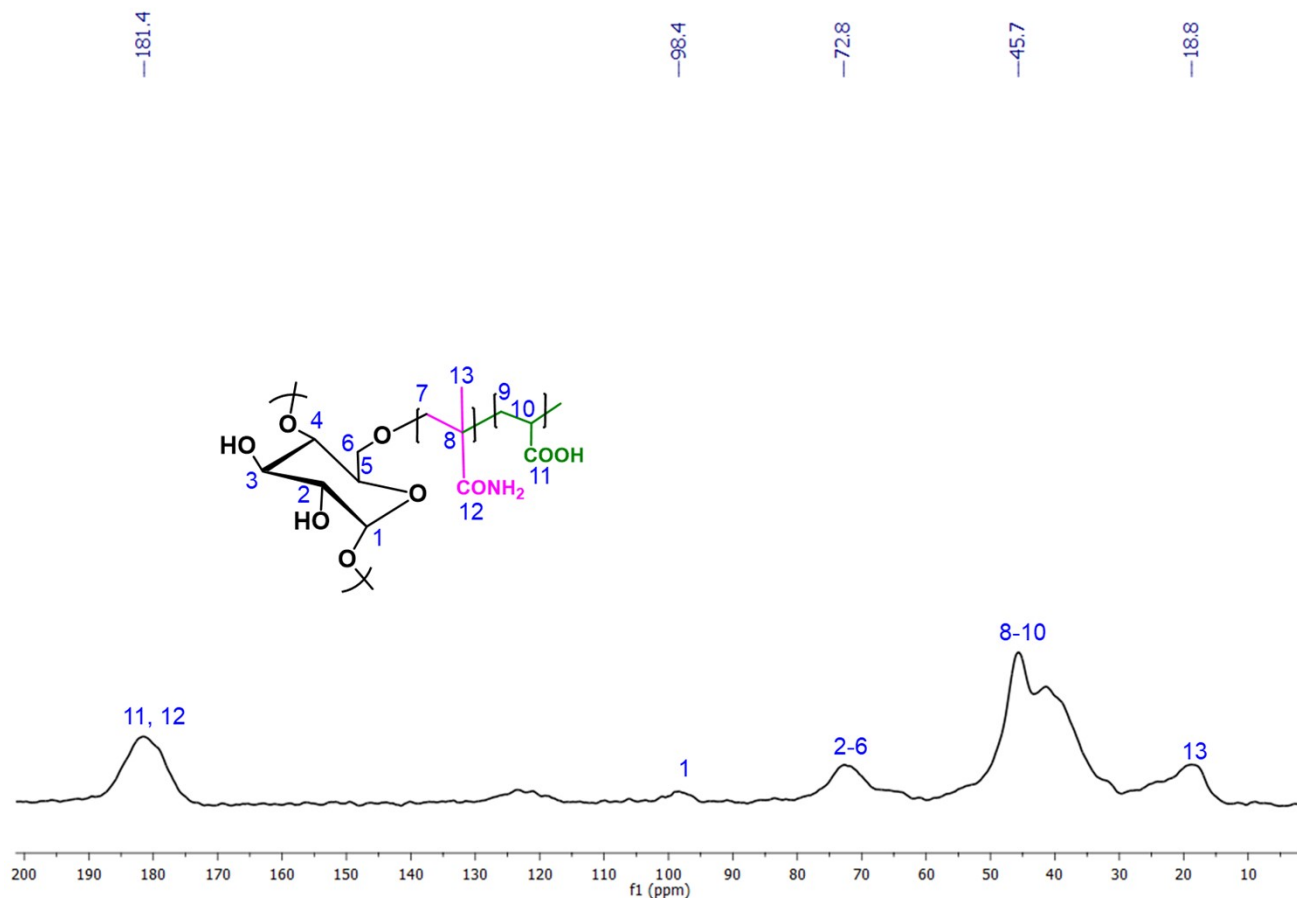


Fig. S11. ¹³C NMR spectrum of Dxt-g-(PMAM-co-PAA)-4.

Dxt-g-(PMAM-co-PAA)-4: ¹³C NMR (400 MHz, Solid State) δ (ppm): 98.4 (C1), 72.8 (C2 – C6), 45.7 (C8 – C10), 18.8 (C13) and 181.4 (C11, C12).

COSY Spectra analysis: From the COSY spectra (Fig. S12-S15, ESI) analysis of all the copolymers it can be shown that a_h , e_h , b_h , c_h , d_h , f_h , g_h protons are coupled with each other. From these spectra it is confirmed that all the copolymers are successfully synthesized.

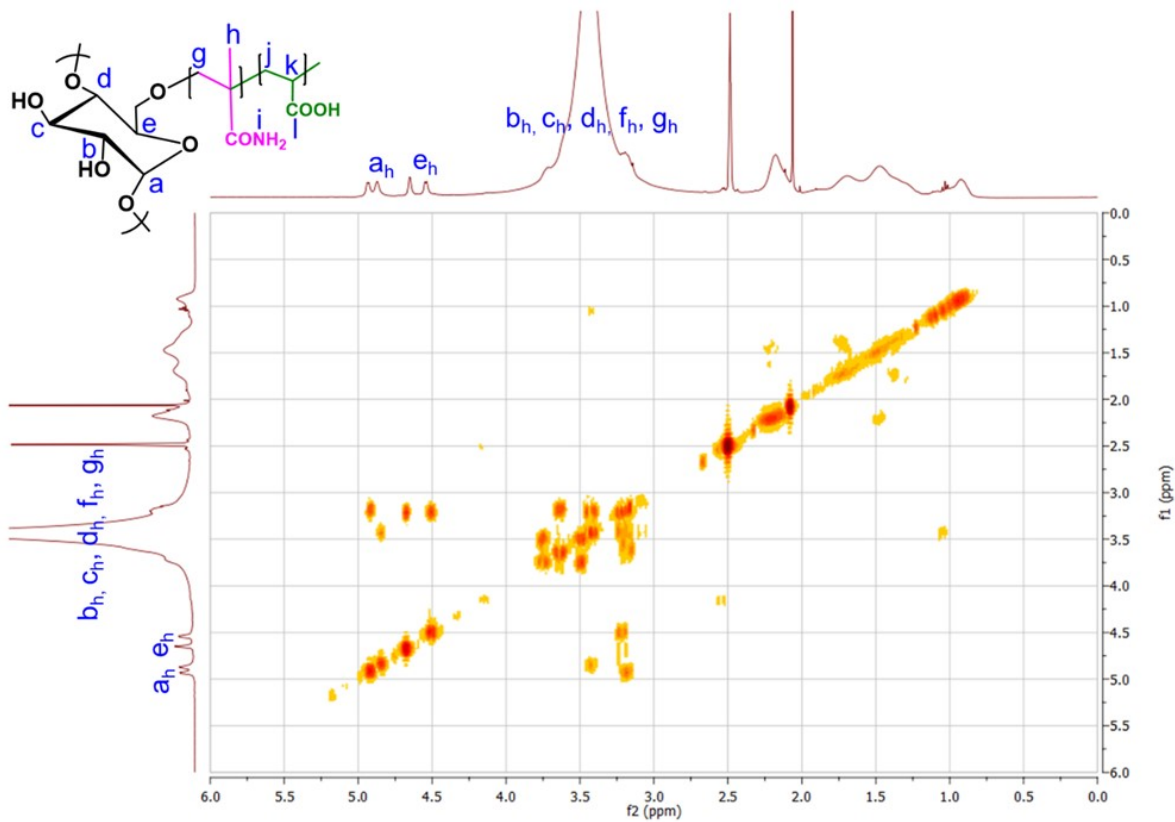


Fig. S12. COSY spectrum of Dxt-g-(PMAM-co-PAA)-1.

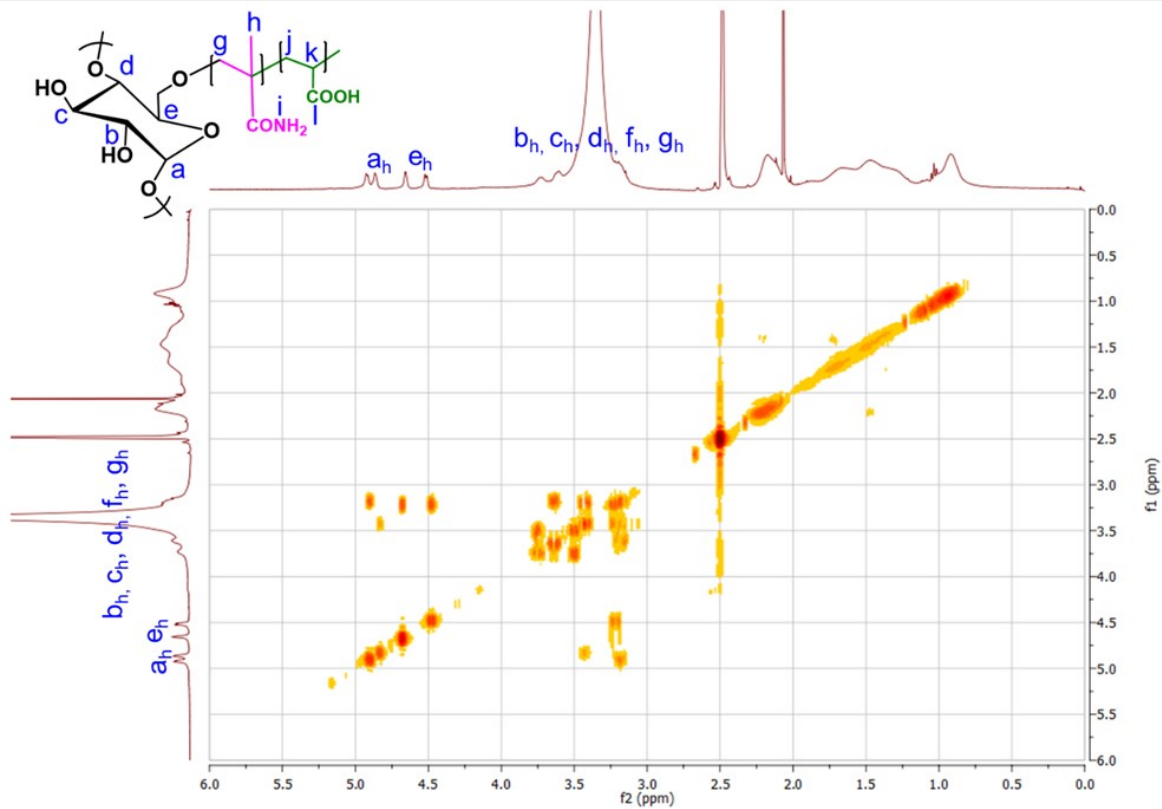


Fig. S13. COSY spectrum of Dxt-g-(PMAM-Co-PAA)-2.

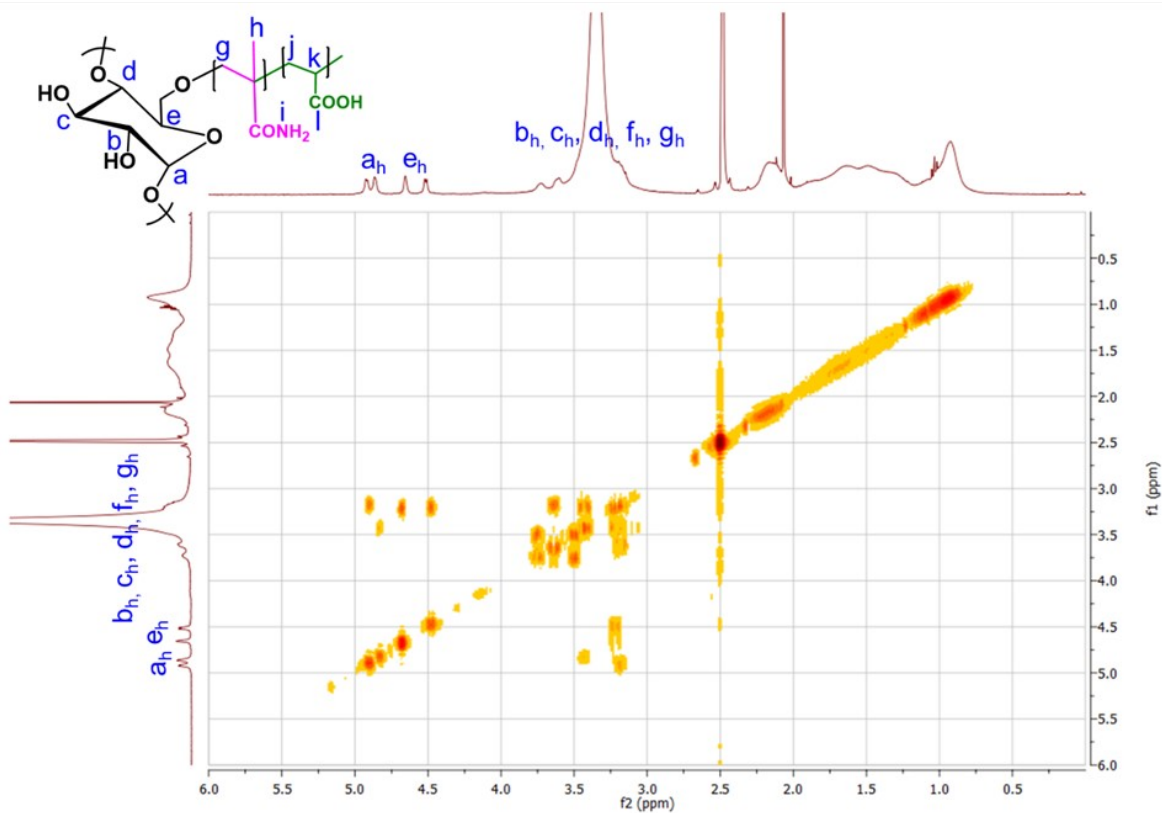


Fig. S14. COSY spectrum of Dxt-g-(PMAM-Co-PAA)-3.

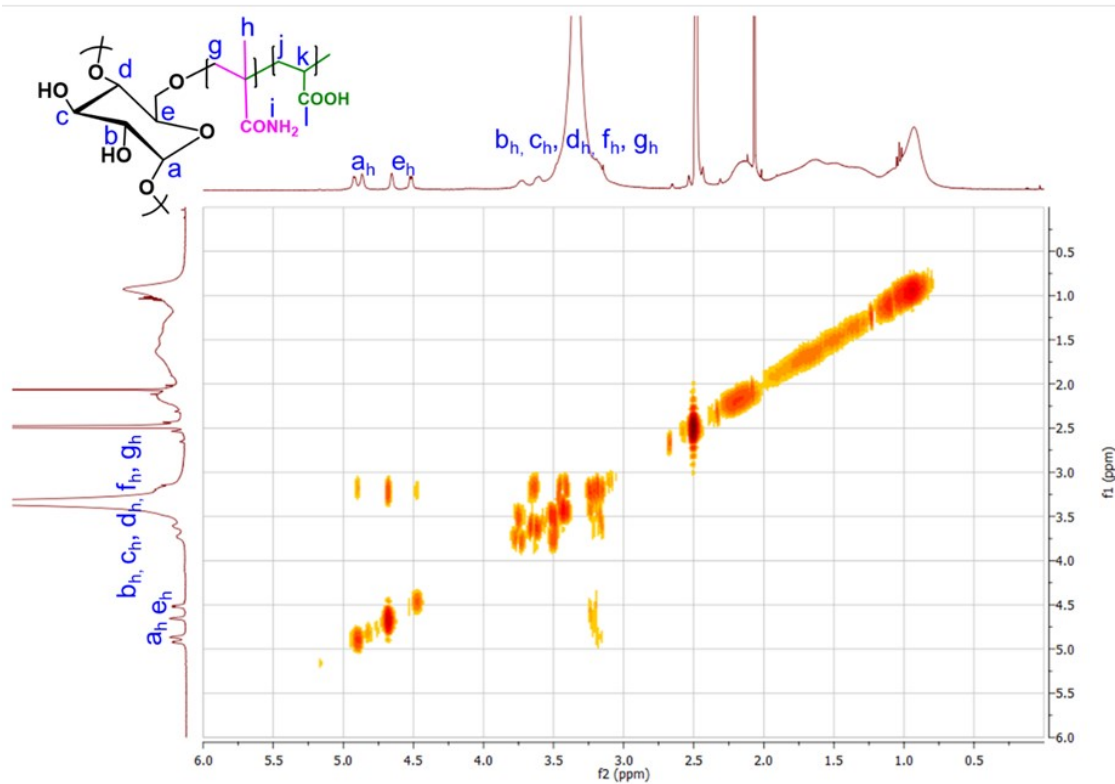


Fig. S15. COSY spectrum of Dxt-g-(PMAM-Co-PAA)-4.

HSQC spectra analysis: From the below HSQC spectra (Fig. S16-S19, ESI) of all the copolymers it can be shown that specific H-atoms are interrelated to specific C-atoms which also confirms the successful synthesis of the copolymers.

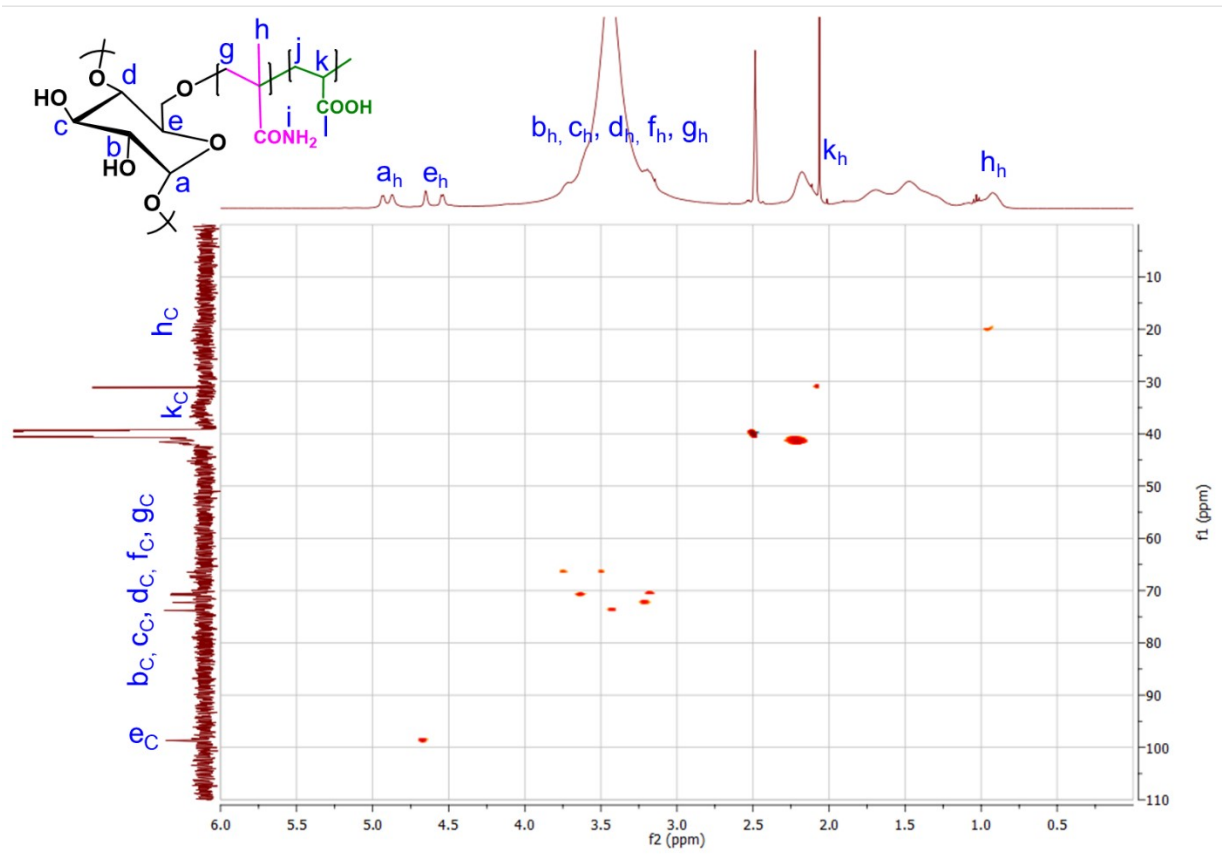


Fig. S16. HSQC spectrum of Dxt-g-(PMAM-Co-PAA)-1.

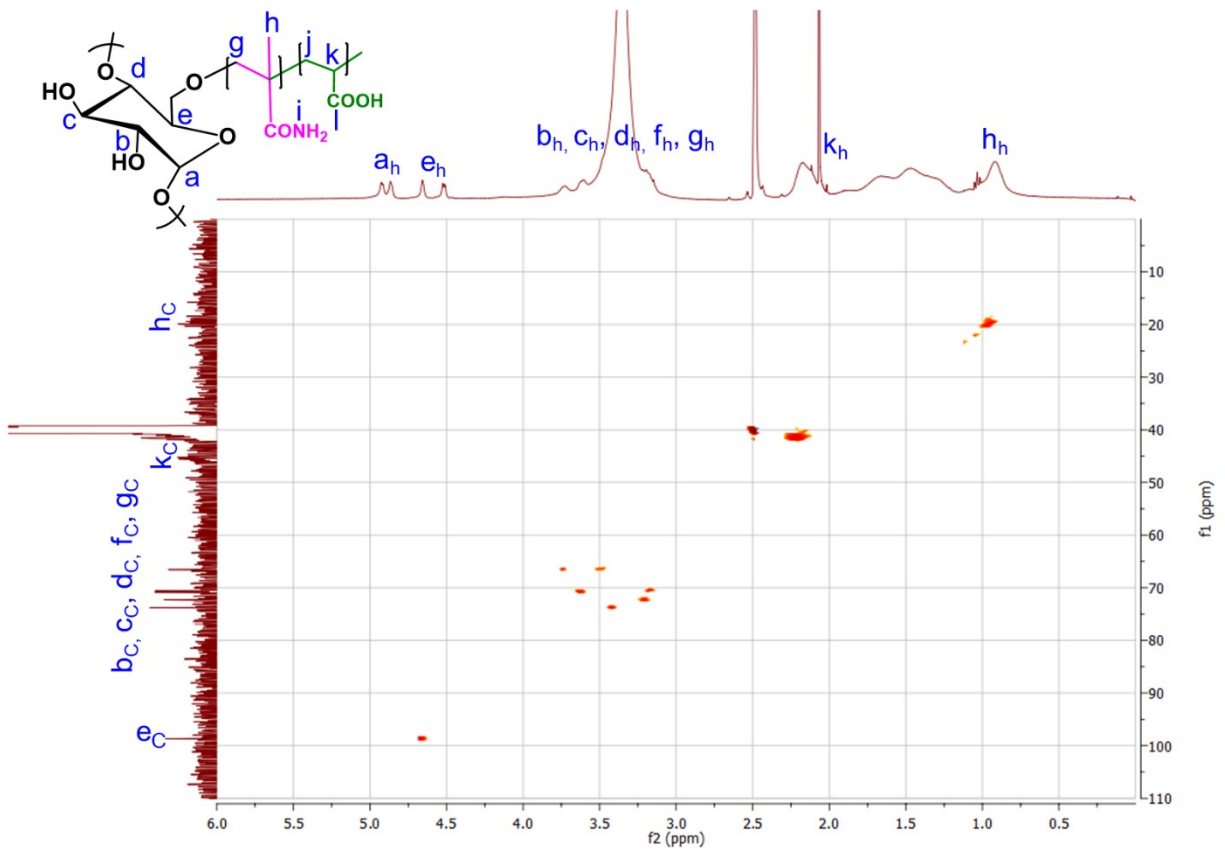


Fig. S17. HSQC spectrum of Dxt-g-(PMAM-Co-PAA)-2.

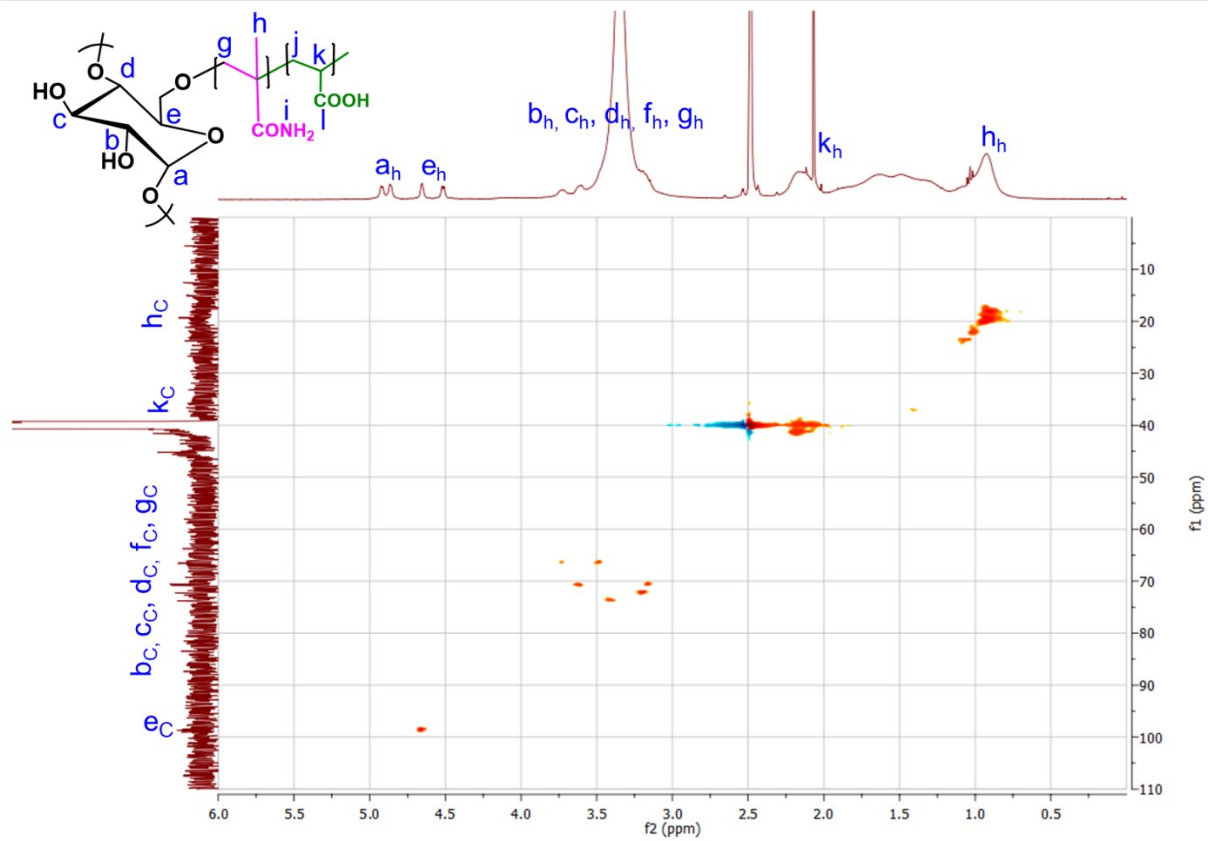


Fig. S18. HSQC spectrum of Dxt-g-(PMAM-Co-PAA)-3.

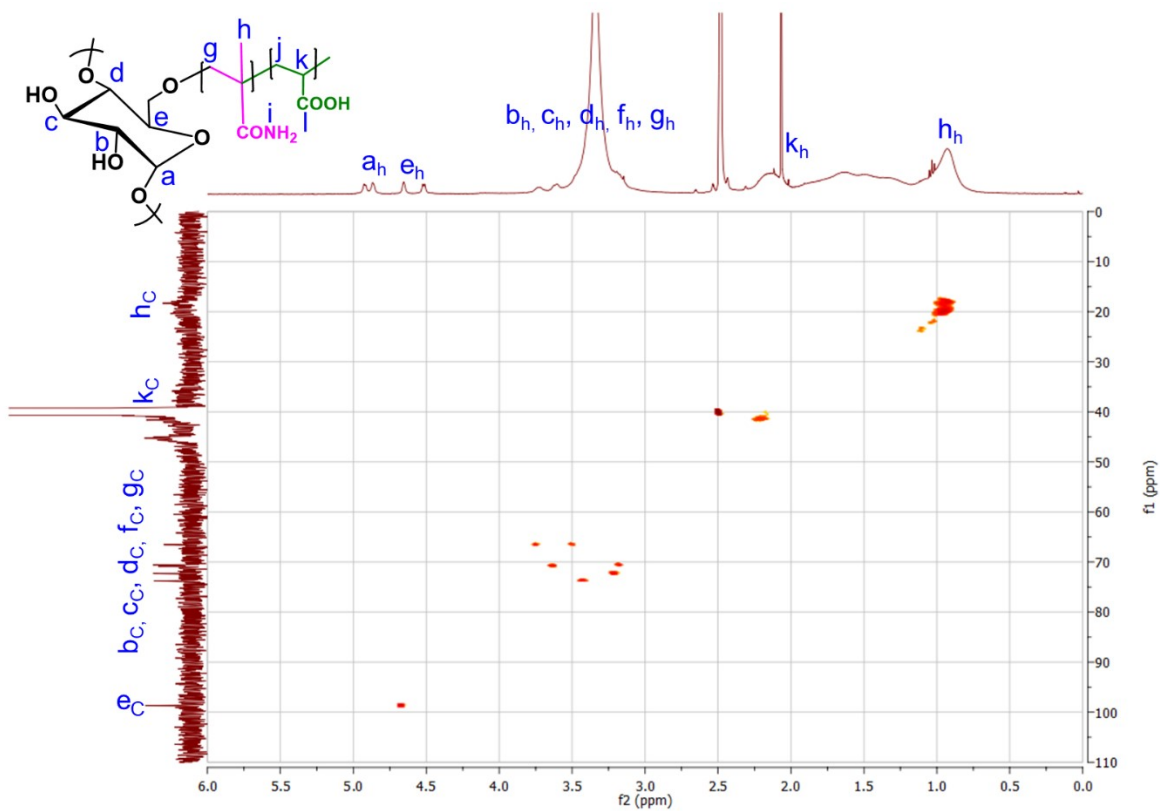


Fig. S19. HSQC spectrum of Dxt-g-(PMAM-Co-PAA)-4.

Cytocompatibility Study. From the cytocompatibility study (Fig. S20, ESI), it is obvious that the copolymers are cytocompatible in nature. From the study, it is also clear that the copolymers are noncytotoxic in nature even after 72 h of culture. The % of cell viability mostly increases or remains same w.r.t. TCP, even when time passes from 24 h to 72 h. This confirms that the copolymers derived from dextran (which is also biocompatible) is biocompatible in nature^{5,7}.

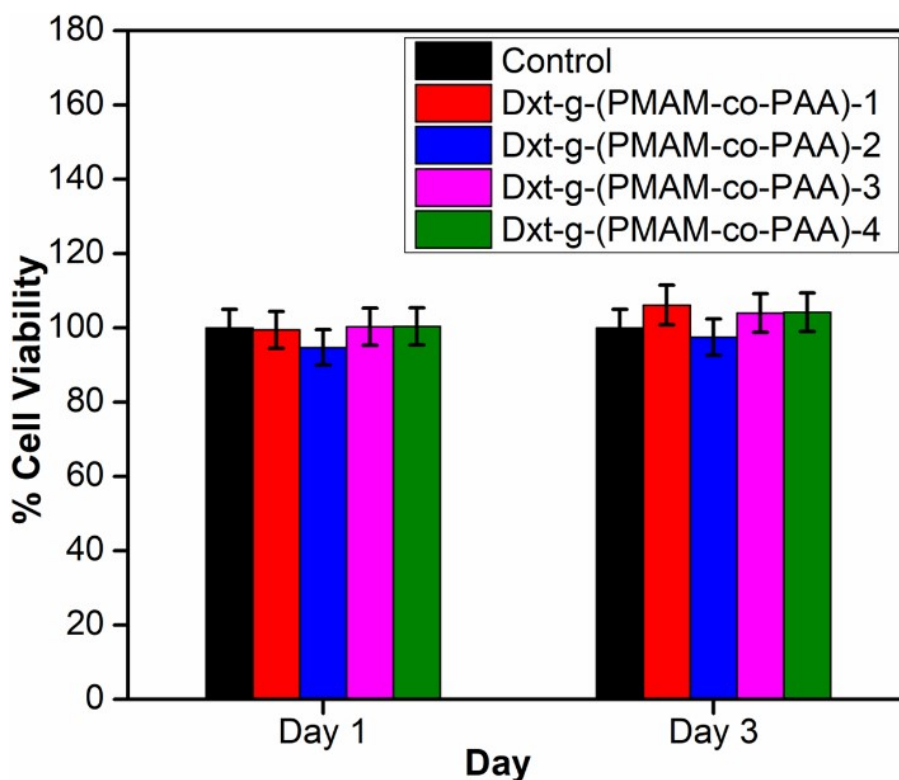


Fig. S20. Plot of % Cell viability vs. Day for various copolymers.

MALS/GPC analysis. From the MALS/GPC results (Table S4, Fig. S21 – S25, ESI) it can be resolved that the molecular weight of the copolymers is higher than that of Dxt, which indicates that polymerization has been happened successfully. The number average (\bar{M}_n) and weight average (\bar{M}_w) molecular weight of the polymers varies in the range of 1.212×10^6 to 4.428×10^6 g/mol and

1.475×10⁶ to 5.330×10⁶ g/mol, respectively. The dispesity index (PDI) of the copolymers was around ~1.20.

Table S4.

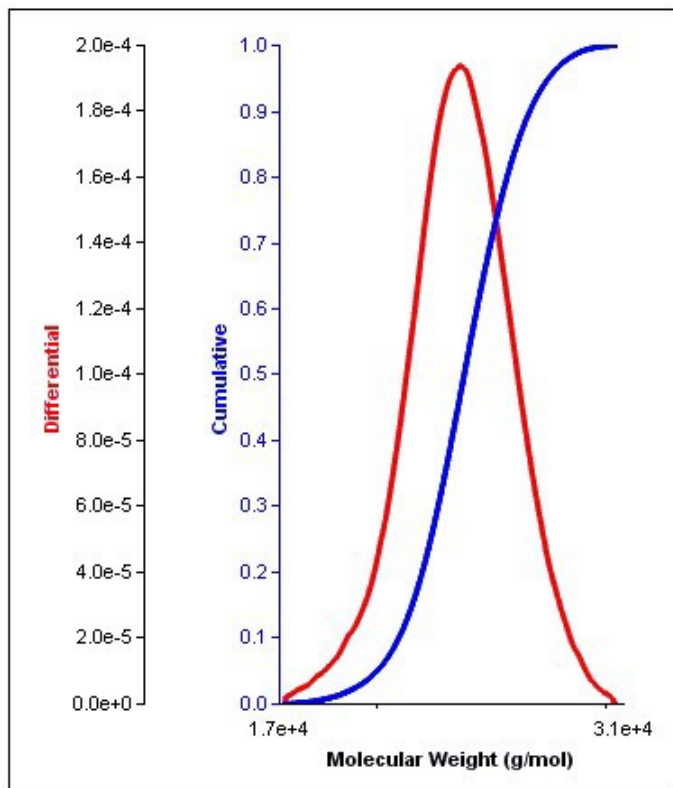
Material	\bar{M}_w	\bar{M}_n
Dxt	2.326×10 ⁴	2.306×10 ⁴
Dxt-g-(PMAM-co-PAA)-1	1.475×10 ⁶	1.212×10 ⁶
Dxt-g-(PMAM-co-PAA)-2	2.248×10 ⁶	1.885×10 ⁶
Dxt-g-(PMAM-co-PAA)-3	5.330×10 ⁶	4.428×10 ⁶
Dxt-g-(PMAM-co-PAA)-4	3.248×10 ⁶	2.617×10 ⁶

Molecular weight data of the various UCST polymers

Sample ID: Dextran. 11/10

Data Collection Date: Oct 11, 2021 14:01:47

Operator: sajjad



Results

M_n	2.306e+04	g/mol
M_w	2.326e+04	g/mol
M_z	2.346e+04	g/mol
M_p	2.317e+04	g/mol
M_w/M_n	1.009	
M_z/M_w	1.008	
$<M_{10}>$	2.077e+04	g/mol
$>M_{50}$	2.622e+04	g/mol
V_p	1.82	mL
$Area_{RI}$	1.904e-02	V*mL
Concentration	undetermined	mg/mL
dn/dc	0.2000	mL/g
Sample Recovery	undetermined	%
$[\eta]$	undetermined	mL/g
$<Rg>_n$	1.692e+02	nm
$<Rg>_w$	1.740e+02	nm
$<Rg>_z$	1.789e+02	nm

Sample Parameters

Concentration	1.930	mg/ml (given)
dn/dc	unknown	mL/g
Duration	40.0	min
A_2	5.00e-04	mL mol/g ²
Injection Volume	20.000	μ L

Calculation Options

Conc. Signal From	RI
Method	SLS
Concentration	given
dn/dc	measured

System Information

System ID	ISM Dhanbad First System Setup
Group ID	SHAON
Eluent	Water
Eluent Additive	
Flow Rate	0.100 mL/min

Calculation Limits

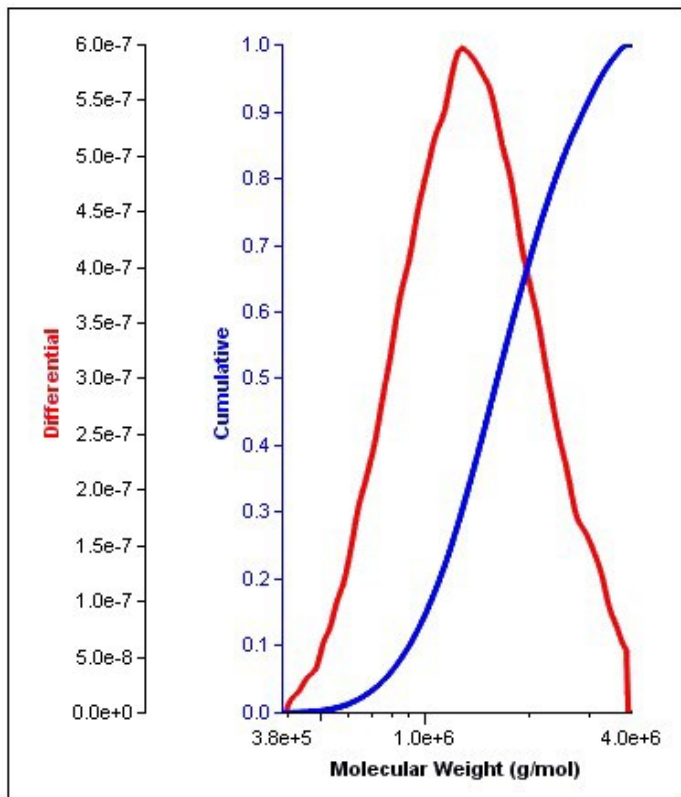
Baseline	0.75 to 2.19 mL
Integration	1.59 to 2.07 mL
Fit	1.59 to 2.07 mL

Sample ID:Dextran. 11/10

Fig. S21. GPC analysis of Dextran.

Sample ID: 0.1 N NaNO₃ poly 1. 11/10
Data Collection Date: Oct 11, 2021 16:17:26

Operator: sajjad



Results

M_n	1.212e+06	g/mol
M_w	1.475e+06	g/mol
M_z	1.780e+06	g/mol
M_p	1.307e+06	g/mol
M_w/M_n	1.217	
M_z/M_w	1.207	
$<M_{10}>$	8.980e+05	g/mol
$>M_{50}$	2.884e+06	g/mol
V_p	1.82	mL
$Area_{RI}$	2.953e-03	V*mL
Concentration	undetermined	mg/mL
dn/dc	0.0293	mL/g
Sample Recovery	undetermined	%
$[\eta]$	undetermined	mL/g
$<Rg>_n$	1.493e+02	nm
$<Rg>_w$	1.391e+02	nm
$<Rg>_z$	1.293e+02	nm

Sample Parameters

Concentration	2.040	mg/ml (given)
dn/dc	unknown	mL/g
Duration	40.0	min
A_2	5.00e-04	mL mol/g ²
Injection Volume	20.000	μ L

Calculation Options

Conc. Signal From	RI
Method	SLS
Concentration	given
dn/dc	measured

System Information

System ID	ISM Dhanbad First System Setup
Group ID	SHAON
Eluent	Water
Eluent Additive	
Flow Rate	0.100 mL/min

Calculation Limits

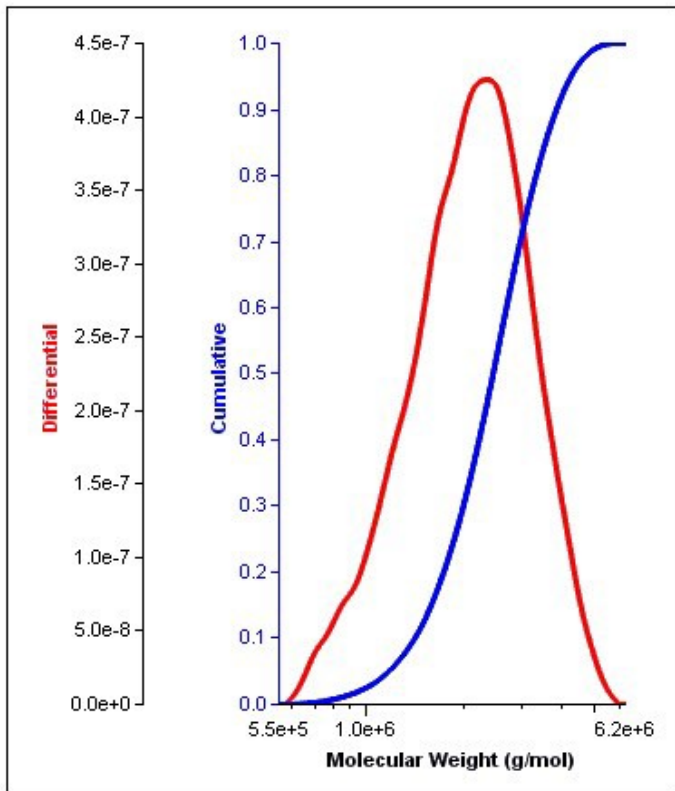
Baseline	1.56 to 2.20 mL
Integration	1.67 to 2.00 mL
Fit	1.67 to 2.00 mL

Sample ID:0.1 N NaNO₃ poly 1. 11/10

Fig. S22. GPC analysis of Dxt-g-(PMAM-co-PAA)-1.

Sample ID: 0.1 N NaNO₃ poly 2. 11/10
Data Collection Date: Oct 11, 2021 15:07:53

Operator: sajjad



Results

M_n	1.885e+06	g/mol
M_w	2.248e+06	g/mol
M_z	2.611e+06	g/mol
M_p	2.347e+06	g/mol
M_w/M_n	1.193	
M_z/M_w	1.161	
$<M_{10}$	1.418e+06	g/mol
$>M_{50}$	3.842e+06	g/mol
V_p	2.74	mL
$Area_{RI}$	8.271e-04	V*mL
Concentration	undetermined	mg/mL
dn/dc	0.0079	mL/g
Sample Recovery	undetermined	%
$[\eta]$	undetermined	mL/g
$<Rg>_n$	1.215e+02	nm
$<Rg>_w$	1.260e+02	nm
$<Rg>_z$	1.301e+02	nm

Sample Parameters

Concentration	2.130	mg/ml (given)
dn/dc	unknown	mL/g
Duration	40.0	min
A_2	5.00e-04	mL mol/g ²
Injection Volume	20.000	μ L

Calculation Options

Conc. Signal From	RI
Method	SLS
Concentration	given
dn/dc	measured

System Information

System ID	ISM Dhanbad First System Setup
Group ID	SHAON
Eluent	Water
Eluent Additive	
Flow Rate	0.100 mL/min

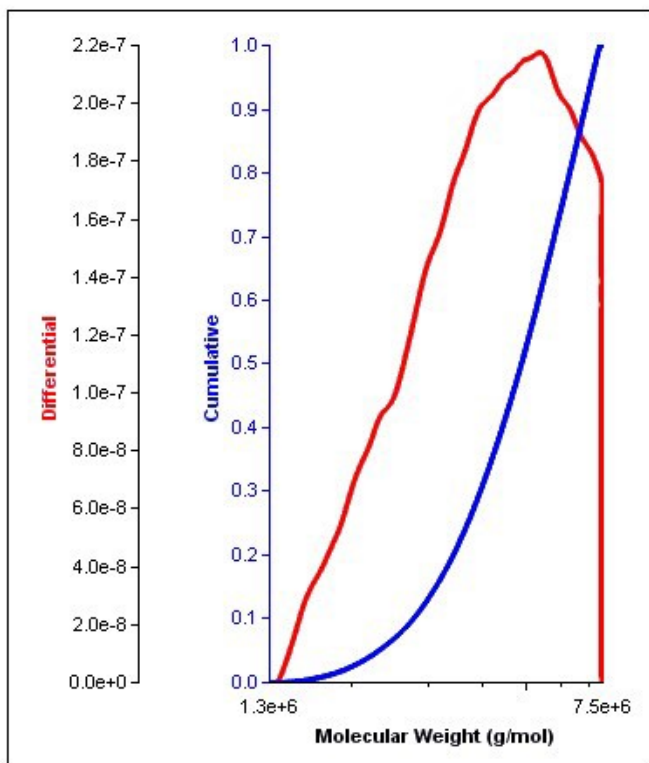
Calculation Limits

Baseline	2.60 to 3.45 mL
Integration	2.66 to 2.84 mL
Fit	2.66 to 2.84 mL

Fig. S23. GPC analysis of Dxt-g-(PMAM-co-PAA)-2.

Sample ID: 0.1N NaNO3 POLY 3
Data Collection Date: Oct 6, 2021 16:38:58

Operator: Sajjad



Results

M_n	4.428e+06	g/mol
M_w	5.330e+06	g/mol
M_z	6.039e+06	g/mol
M_p	5.224e+06	g/mol
M_w/M_n	1.204	
M_z/M_w	1.133	
$\langle M_{10} \rangle$	2.768e+06	g/mol
$\langle M_{50} \rangle$	6.816e+06	g/mol
V_p	1.85	mL
$Area_{RI}$	2.751e-03	V*mL
Concentration	undetermined	mg/mL
dn/dc	0.0273	mL/g
Sample Recovery	undetermined	%
$[\eta]$	undetermined	mL/g
$\langle Rg \rangle_n$	1.824e+03	nm
$\langle Rg \rangle_w$	1.064e+03	nm
$\langle Rg \rangle_z$	5.665e+02	nm

Sample Parameters

Concentration	2.040	mg/ml (given)
dn/dc	unknown	mL/g
Duration	60.0	min
A_2	5.00e-04	mL mol/g ²
Injection Volume	20.000	μ L

Calculation Options

Conc. Signal From	RI
Method	SLS
Concentration	given
dn/dc	measured

System Information

System ID	ISM Dhanbad First System Setup
Group ID	SAYAN
Eluent	Water
Eluent Additive	
Flow Rate	0.100 mL/min

Calculation Limits

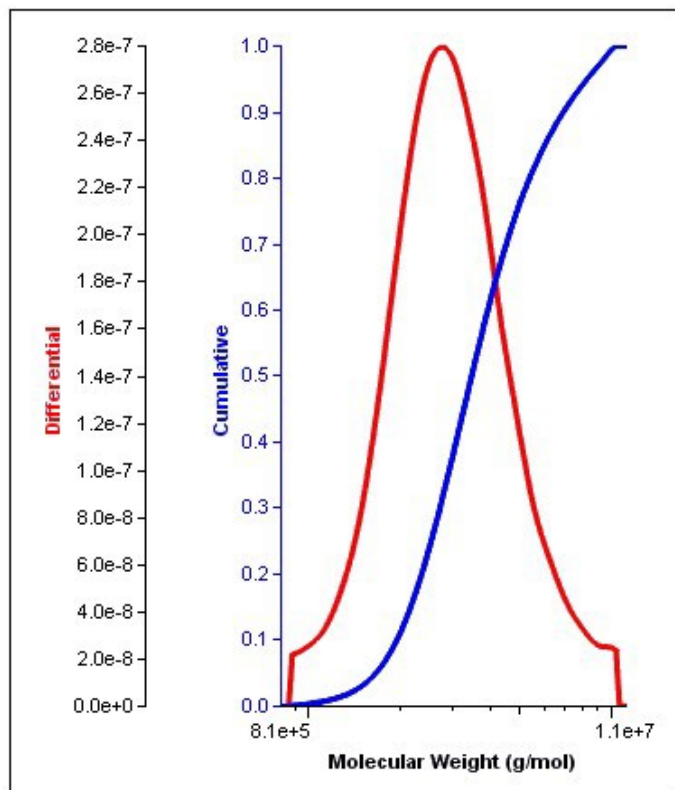
Baseline	1.24 to 3.06 mL
Integration	1.66 to 2.07 mL
Fit	1.66 to 2.07 mL

Sample ID:0.1N NaNO3 POLY 3

Fig. S24. GPC analysis of Dxt-g-(PMAM-co-PAA)-3.

Sample ID: 0.1 N NaNo3 poly4
Data Collection Date: Oct 7, 2021 14:38:38

Operator: Sajjad



Results

M_n	2.617e+06	g/mol
M_w	3.248e+06	g/mol
M_z	4.079e+06	g/mol
M_p	2.749e+06	g/mol
M_w/M_n	1.241	
M_z/M_w	1.256	
$<M_{10}$	1.952e+06	g/mol
$>M_{50}$	6.944e+06	g/mol
V_p	2.34	mL
$Area_{RI}$	2.901e-03	V*mL
Concentration	undetermined	mg/mL
dn/dc	0.0303	mL/g
Sample Recovery	undetermined	%
$[\eta]$	undetermined	mL/g
$<Rg>_n$	2.007e+02	nm
$<Rg>_w$	1.858e+02	nm
$<Rg>_z$	1.710e+02	nm

Sample Parameters

Concentration	1.940	mg/ml (given)
dn/dc	unknown	mL/g
Duration	50.0	min
A_2	5.00e-04	mL mol/g ²
Injection Volume	20.000	μ L

Calculation Options

Conc. Signal From	RI
Method	SLS
Concentration	given
dn/dc	measured

System Information

System ID	ISM Dhanbad First System Setup
Group ID	SHAON
Eluent	Water
Eluent Additive	
Flow Rate	0.100 mL/min

Calculation Limits

Baseline	0.00 to 3.19 mL
Integration	2.28 to 2.43 mL
Fit	2.28 to 2.43 mL

Sample ID:0.1 N NaNo3 poly4

Fig. S25. GPC analysis of Dxt-g-(PMAM-co-PAA)-4.

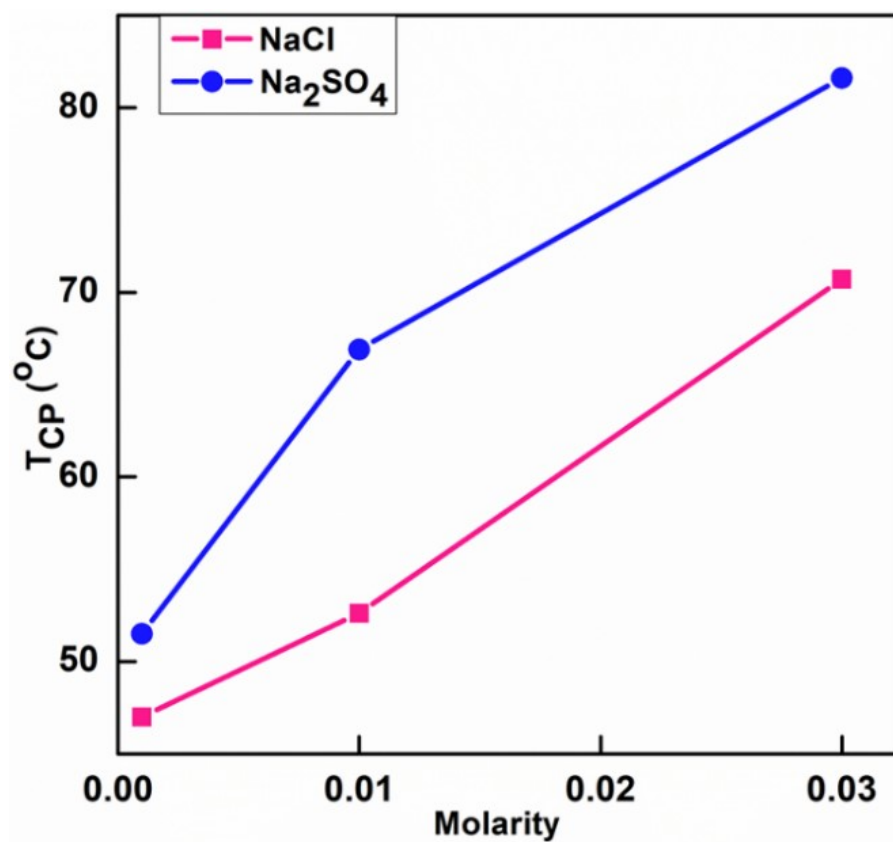
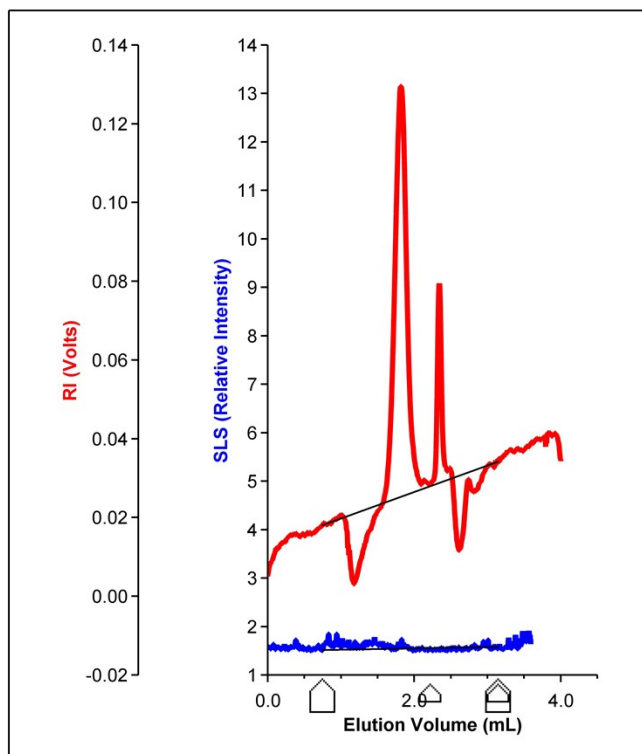


Fig. S26. Variation of cloud point T_{CP} with change in molarity of two different salts.

Sample ID: Dextran. 11/10
Data Collection Date: Oct 11, 2021 14:01:47

Operator: sajjad



Results

M_n	undetermined
M_w	undetermined
M_z	undetermined
M_p	undetermined
M_w/M_n	undetermined
M_z/M_w	undetermined
$\langle M_{10} \rangle$	undetermined
$\langle M_{90} \rangle$	undetermined
V_p	undetermined
Area _{RI}	-4.271e-05 V*mL
Concentration	undetermined mg/mL
dn/dc	-0.0004 mL/g
Sample Recovery	undetermined %
$[\eta]$	undetermined mL/g
$\langle Rg \rangle_n$	undetermined nm
$\langle Rg \rangle_w$	undetermined nm
$\langle Rg \rangle_z$	undetermined nm

Sample Parameters

Concentration	1.930 mg/ml (given)
dn/dc	unknown mL/g
Duration	40.0 min
A_2	5.00e-04 mL mol/g ²
Injection Volume	20.000 μ L

Calculation Options

Conc. Signal From	RI
Method	SLS
Concentration	given
dn/dc	measured

System Information

System	ISM Dhanbad First System Setup
Group ID	SHAON
Eluent	Water
Eluent Additive	
Flow Rate	0.100 mL/min

Calculation Limits

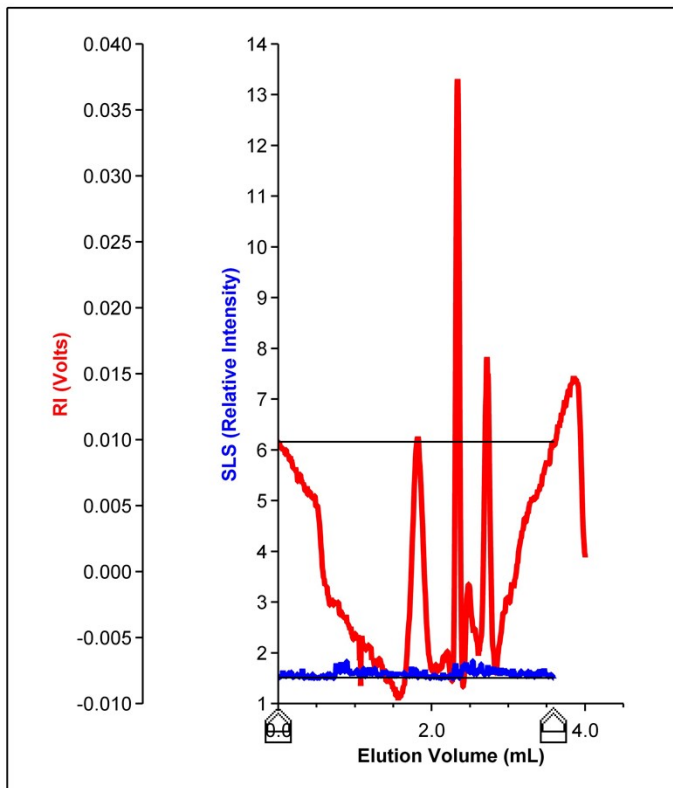
Baseline	0.75 to 3.15 mL
Integration	2.22 to 3.15 mL
Fit	2.22 to 3.15 mL

Sample ID:Dextran. 11/10

Fig. S27. Raw GPC-MALS data of Dxt.

Sample ID: 0.1 N NaNo3 poly 1. 11/10
Data Collection Date: Oct 11, 2021 16:17:26

Operator: sajjad



Results

M_n	undetermined
M_w	undetermined
M_z	undetermined
M_p	undetermined
M_w/M_n	undetermined
M_z/M_w	undetermined
$<M_{10}$	undetermined
$>M_{90}$	undetermined
V_p	undetermined
Area _{RI}	-3.660e-02 V*mL
Concentration	undetermined mg/mL
dn/dc	-0.3638 mL/g
Sample Recovery	undetermined %
$[\eta]$	undetermined mL/g
$<Rg>_n$	undetermined nm
$<Rg>_w$	undetermined nm
$<Rg>_z$	undetermined nm

Sample Parameters

Concentration	2.040 mg/ml (given)
dn/dc	unknown mL/g
Duration	40.0 min
A_2	5.00e-04 mL mol/g ²
Injection Volume	20.000 μ L

Calculation Options

Conc. Signal From	RI
Method	SLS
Concentration	given
dn/dc	measured

System Information

System	ISM Dhanbad First System Setup
Group ID	SHAON
Eluent	Water
Eluent Additive	
Flow Rate	0.100 mL/min

Calculation Limits

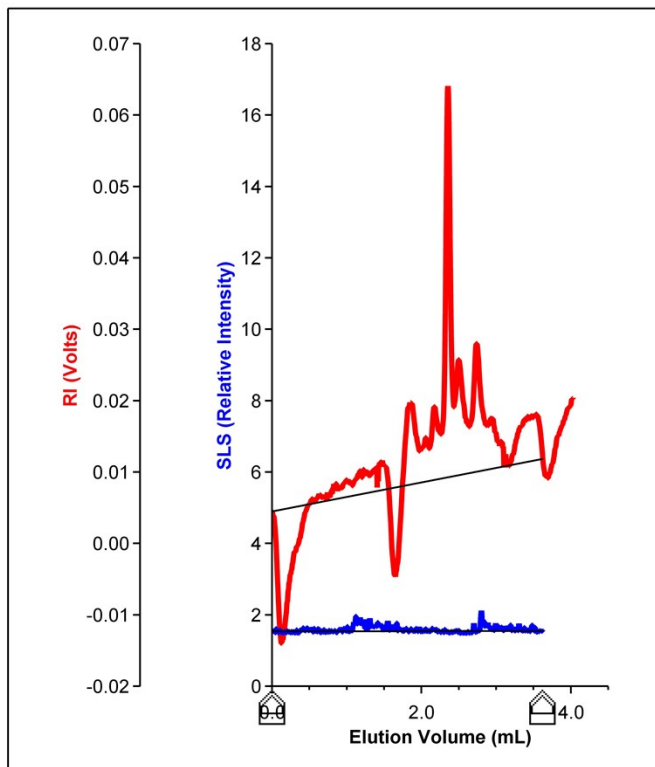
Baseline	0.00 to 3.59 mL
Integration	0.00 to 3.59 mL
Fit	0.00 to 3.59 mL

Sample ID:0.1 N NaNo3 poly 1. 11/10

Fig. S28. Raw GPC-MALS data of Dxt-g-(PMAM-co-PAA)-1.

Sample ID: 0.1 N NaNO3 poly 2. 11/10
Data Collection Date: Oct 11, 2021 15:07:53

Operator: sajjad



Results

M_n	1.649e+05	g/mol
M_w	2.400e+05	g/mol
M_z	3.244e+05	g/mol
M_p	1.950e+05	g/mol
M_w/M_n	1.456	
M_z/M_w	1.352	
$<M_{10}$	1.670e+05	g/mol
$>M_{90}$	5.995e+05	g/mol
V_p	2.35	mL
$Area_{RI}$	1.230e-02	V*mL
Concentration	undetermined	mg/mL
dn/dc	0.1171	mL/g
Sample Recovery	undetermined	%
$[\eta]$	undetermined	mL/g
$<R_g>_n$	1.212e+02	nm
$<R_g>_w$	1.270e+02	nm
$<R_g>_z$	1.319e+02	nm

Sample Parameters

Concentration	2.130	mg/ml (given)
dn/dc	unknown	mL/g
Duration	40.0	min
A_2	5.00e-04	mL mol/g ²
Injection Volume	20.000	μ L

Calculation Options

Conc. Signal From	RI
Method	SLS
Concentration	given
dn/dc	measured

System Information

System	ISM Dhanbad First System Setup
Group ID	SHAON
Eluent	Water
Eluent Additive	
Flow Rate	0.100 mL/min

Calculation Limits

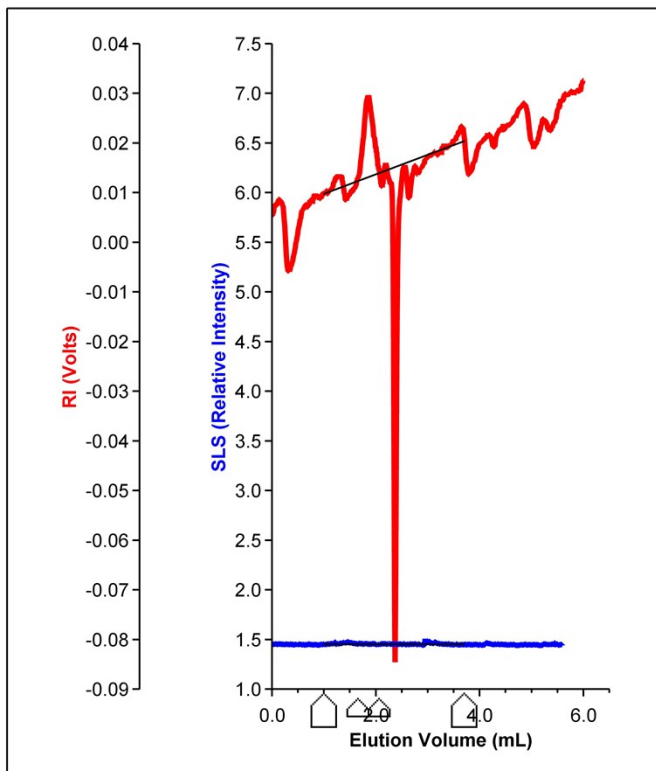
Baseline	0.00 to 3.62 mL
Integration	0.00 to 3.62 mL
Fit	0.00 to 3.62 mL

Sample ID:0.1 N NaNO3 poly 2. 11/10

Fig. S29. Raw GPC-MALS data of Dxt-g-(PMAM-co-PAA)-2.

Sample ID: 0.1N NaNO3 POLY 3
Data Collection Date: Oct 6, 2021 16:38:58

Operator: Sajjad



Results

M_n	9.270e+02	g/mol
M_w	7.399e+03	g/mol
M_z	3.056e+04	g/mol
M_p	2.248e+03	g/mol
M_w/M_n	7.981	
M_z/M_w	4.130	
$<M_{10}>$	3.287e+03	g/mol
$>M_{90}$	6.869e+04	g/mol
V_p	1.85	mL
$Area_{RI}$	3.386e-03	V*mL
Concentration	undetermined	mg/mL
dn/dc	0.0337	mL/g
Sample Recovery	undetermined	%
$[\eta]$	undetermined	mL/g
$<Rg>_n$	6.005e+01	nm
$<Rg>_w$	7.930e+01	nm
$<Rg>_z$	1.026e+02	nm

Sample Parameters

Concentration	2.040	mg/ml (given)
dn/dc	unknown	mL/g
Duration	60.0	min
A_2	5.00e-04	mL mol/g ²
Injection Volume	20.000	μ L

Calculation Options

Conc. Signal From	RI
Method	SLS
Concentration	given
dn/dc	measured

System Information

System ISM	Dhanbad First System Setup
Group ID	SAYAN
Eluent	Water
Eluent Additive	
Flow Rate	0.100 mL/min

Calculation Limits

Baseline	1.00 to 3.71 mL
Integration	1.66 to 2.07 mL
Fit	1.66 to 2.07 mL

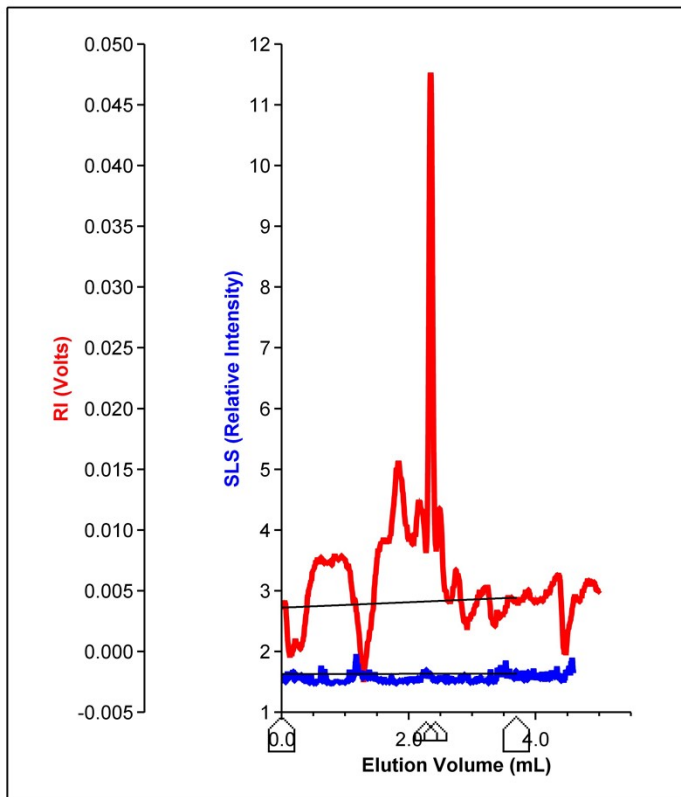
Sample ID:0.1N NaNO3 POLY 3

Fig. S30. Raw GPC-MALS data of Dxt-g-(PMAM-co-PAA)-3.

Sample ID: 0.1 N NaNo3 poly4

Data Collection Date: Oct 7, 2021 14:38:38

Operator: Sajjad



Results

M_n	undetermined
M_w	undetermined
M_z	undetermined
M_p	0.000e+00 g/mol
M_w/M_n	undetermined
M_z/M_w	undetermined
$<M_{10}$	undetermined
$>M_{90}$	undetermined
V_p	2.34 mL
$Area_{RI}$	3.005e-03 V*mL
Concentration	undetermined mg/mL
dn/dc	0.0314 mL/g
Sample Recovery	undetermined %
$[\eta]$	undetermined mL/g
$<R_g>_n$	undetermined nm
$<R_g>_w$	undetermined nm
$<R_g>_z$	undetermined nm

Sample Parameters

Concentration	1.940 mg/ml (given)
dn/dc	unknown mL/g
Duration	50.0 min
A_2	5.00e-04 mL mol/g ²
Injection Volume	20.000 μ L

Calculation Options

Conc. Signal From	RI
Method	SLS
Concentration	given
dn/dc	measured

System Information

System	ISM Dhanbad First System Setup
Group ID	SHAON
Eluent	Water
Eluent Additive	
Flow Rate	0.100 mL/min

Calculation Limits

Baseline	0.00 to 3.70 mL
Integration	2.28 to 2.43 mL
Fit	2.28 to 2.43 mL

Sample ID:0.1 N NaNo3 poly4

Fig. S31. Raw GPC-MALS data of Dxt-g-(PMAM-co-PAA)-4.

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