Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

of

Dextrin and Poly (lactide) based Biocompatible and Biodegradable Nanogel for Cancer Targeted Delivery of Doxorubicin

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In Vitro Cytocompatibility Study and Morphological Assessment

In brief, the flat pellets of the n-Dxt-p(lactide) nanogel was prepared by using hydraulic press. For pellet preparation, the equivalent weight of powder samples was employed. The pellets were sterilized by 70% ethanol and UV followed by frequent washing with sterilized phosphate buffer solution (pH 7.4). 2.5×10^4 no of human mesenchymal stem cells (hMSCs; Advanced Neuro-Science Allies, Bangalore, India) were seeded in sample pellets using a 24-well tissue culture plates and cultivated for 1, 3 and 7 days, respectively. Tissue culture plates (TCP) were treated as control. On completing the respective cultivation period, samples were washed thrice in PBS and the number of viable cells was calculated using Vybrant® MTT Cell Proliferation Assay Kit (Invitrogen) according to the manufactures instruction. Graphs show mean ± standard deviation (n = 3).[1]

For morphological assessment, hMSCs were cultured in the n-Dxt-p(lactide) nanogeletor pellets using a 12 well plates. Cells were also seeded on lysine coated slides for control. The coverslips were removed at desired time of interest fixed and permeabilized using Triton-X-100 followed by blocking the non-specific sites using bovine serum albumin (Sigma) and stained with rhodamine–phalloidin (catalog no. R415, Invitrogen) and DAPI (4, 6-diamidino-2-phenylindole, catalog no. D1306, Invitrogen). The cells were then imaged using fluorescence microscope (Zeiss Axio Observer Z1, Carl Zeiss, Germany) on required time of interest.[1]

Biodegradation study

The enzymatic degradation study of dextrin (10 mg/mL) was performed in 5 mL of lysozyme-buffer (pH 7.4) solution (1.5 μg/mL) at 37 ± 0.1 ºC. Owing to the soluble nature of dextrin in aqueous media, the degradation phenomena was analysed using GPC study at various time intervals (0, 3, 14 and 21 days). The n-Dxt-p(lactide) nanogeletor film was investigated using lysozyme chloride as illustrated in the previous literature.[1] Briefly, the known amount of n-Dxt-

p(lactide) nanogeletor films (10 × 10 × 0.1 mm^3) were immersed in lysozyme-buffer (pH 7.4) solution (1.5 μg/mL) at 37 ± 0.1 ºC. The solution was changed regularly after 24 h to keep the enzyme activity constant. After 3, 7, 14 and 21 days, the swollen nanogels were taken out from the solution, cleaned with double distilled water and dried in vacuum oven for 72 h. After that, it was reweighted. The *in vitro* degradation has been represented as % weight loss vs. time. Results expressed as mean ± standard deviation.[1] Also the degradable nature of PLA (100 mg/mL) was executed in lysozyme-buffer (pH 7.4) solution (1.5 μg/mL) at 37 ± 0.1 ºC in a similar way as of nanogel. The weight loss was measured after 3, 7, 14 and 21 days. The *in vitro* degradation has been represented as % weight loss vs. time. Results expressed as mean ± standard deviation.[1]

Cells viability study using MG cancer cells

MG 63 cancer cells lines were acquired from the national centre for cell sciences (NCCS), Pune, India and grown in Dulbecco's Modified Eagle's medium containing 10% fetal calf serum, penicillin (100 units/mL), streptomycin (100 mg/mL), and 4 mM L-glutamine (Himedia, India) at 37 °C in a 5% CO_2 and 95% air humidified atmosphere. Approximately, 1 x 10^4 number of cells were trypsinised from cell culture flasks and were plated on lysine coated coverslips for 12 h.

After 12 h, the MG 63 cancer cells were incubated with different concentrations (20, 50 and 100 μg/mL) of free nanogel and DOX-loaded nanogel at 37 °C in a humidified incubator for 6,12 and 24 h. MG 63 cells grown on lysine coated coverslips without addition of nanogels in the same experimental conditions were treated as positive controls. The cell viability was estimated by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, Sigma Aldrich) assay. The % cell viability was calculated using eq. (1):

$$\%\ Cell\ viability = \frac{No.of\ cells\ on\ free\ \ nanogel\ or\ loaded\ nanogel}{No.of\ cells\ on\ positive\ control} \times 100 \qquad (1)$$

Further, images of the cells were captured in Zeiss Axiovision (Zeiss Axio Observer Z1, Carl Zeiss, Germany) microscope.

Swelling study and its kinetics:

The equilibrium swelling ratio (ESR) of n-Dxt-p(lactide) nanogeletor was assessed at 37 ± 0.5 ºC in pH 5.5 and pH 7.4 buffer solutions. In brief, 0.25 g of n-Dxt-p(lactide) was immersed in buffers and then left to swell for 8 h to attain equilibrium swelling (equilibrium swelling was achieved at ~ 6 h). The nanogels were quiet after regular time intervals (after every 1 h) and the excess surface water was removed cautiously with tissue paper and reweighed. The % ESR has been calculated using eqn. (2):[2]

$$ESR(\%) = \frac{W_{eq} - W_d}{W_d} \times 100 \qquad (2)$$

Where W_d, W_{eq} are weights of dried gel, and swollen gel at equilibrium respectively.

For swelling kinetics, water absorption of nanogels have been measured at consecutive time intervals until and unless equilibrium was achieved. The pH-sensitivity of the nanogel was determined using the equilibrium swelling ratio in various buffer media. The Voigt model (eq. 3) is used to determine the rate swelling of the nanogels.[2]

$$S_t = S_e\left(1 - e^{-t/\tau}\right) \qquad (3)$$

Where, S_t (g g^{-1}) is the swelling at time t, S_e (g g^{-1}) is the equilibrium swelling, t is the time (min) for swelling and s (min) stands for the rate parameter. The rate parameter (s) has been determined using eq. (3), which is a measure of the swelling rate i.e. the lower the rate parameter value (s) the higher be the swelling rate.[2]

Drug release kinetics and mechanism models:

The zero order kinetic model (eq. 4) explains the drug dissolution from transdermal systems, as well as matrix tablets with low soluble drugs, coated forms, osmotic systems, etc.[3]

$$Q_t = Q_0 + K_0 t \qquad (4)$$

Where Q_t is the amount of drug release in time *t*, Q_0 the initial amount of drug in solution (most times, Q_0=0) and K_0 is the zero order release constant expressed in units of concentration/time and *t* is the time.

The first order kinetic model (eq. 5) describes the release from systems those containing water soluble drugs in porous matrices, where the drug release is proportional to the amount of drug remaining in its interior of the matrix.[4]

$$\log Q_t = logQ_0 - \frac{K_1 t}{2.303} \quad (5)$$

Where, Q_t, Q_0 is the amount of drug released in time t and the amount of initial drug in tablet respectively, K_1 is first order rate constant.

The Korsemeyer-Peppas model (eq. 6),[5] Higuchi model (eq.7)[6] are given below:

$$\frac{M_t}{M_\infty} = Kt^n \quad (6)$$

Where M_t/M_∞ 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 '*n*' value is used to characterize different release mechanisms. $n \leq 0.45$ 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.

$$Q_t = Q_0 + K_H t^{1/2} \quad (7)$$

Where, Q_t is the amount of drug release in time *t*, Q_0 the initial amount of drug in solution, K_H is the Higuchi dissolution constant.

The Hixson-Crowell cube root law (eq. 8) illustrates the release from systems where there is a change in surface area and diameter of particles or tablets i.e. it supports erosion of matrix is the main principle of drug release .[7]

$$W_0^{1/3} - W_t^{1/3} = K_{HC} t \quad (8)$$

Where, W_t is the amount of drug remaining after time t, W_0 is the initial amount of the drug in tablet and K_{HC} is the rate constant for Hixson-Crowell rate equation.[8]

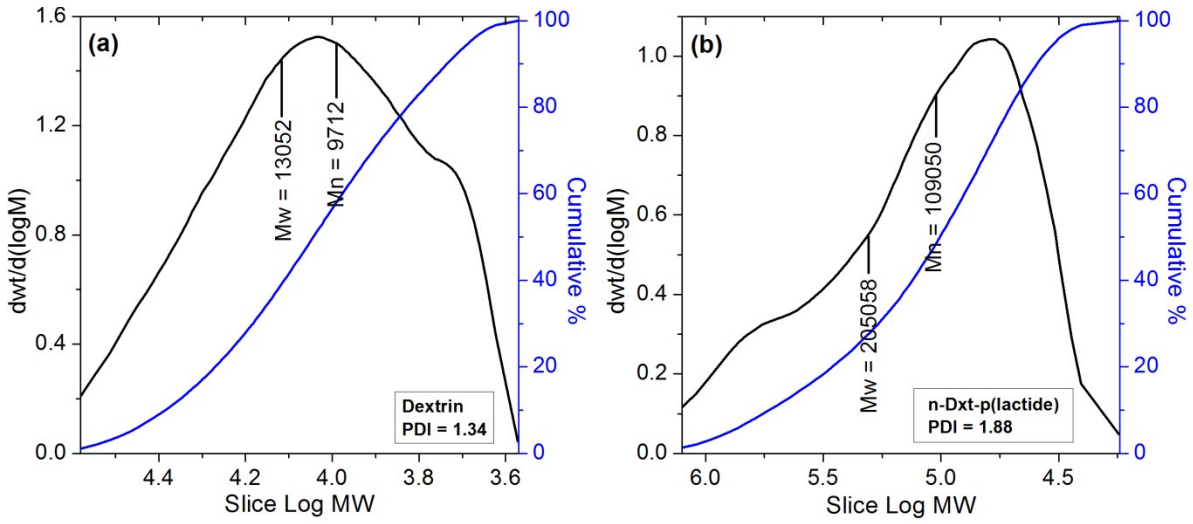

Fig. S1: GPC analyses results of (a) dextrin and (b) n-Dxt-p(lactide) nanogel

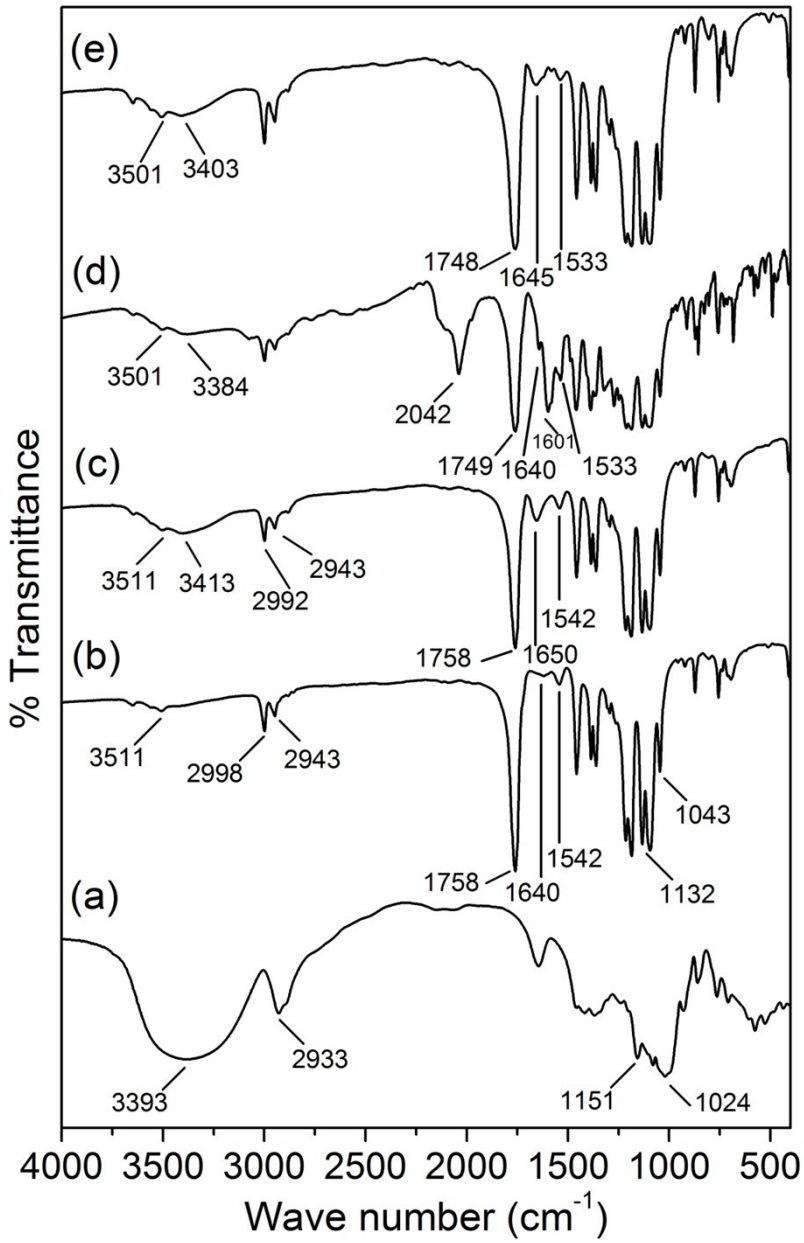

Fig. S2: FTIR spectra of (a) dextrin, (b) n-Dxt-p(lactide) nanogeletor, (c) n-Dxt-p(lactide)-FA, (d) FTIC-loaded n-Dxt-p(lactide)-FA, and (e) Dox-loaded n-Dxt-p(lactide)-FA

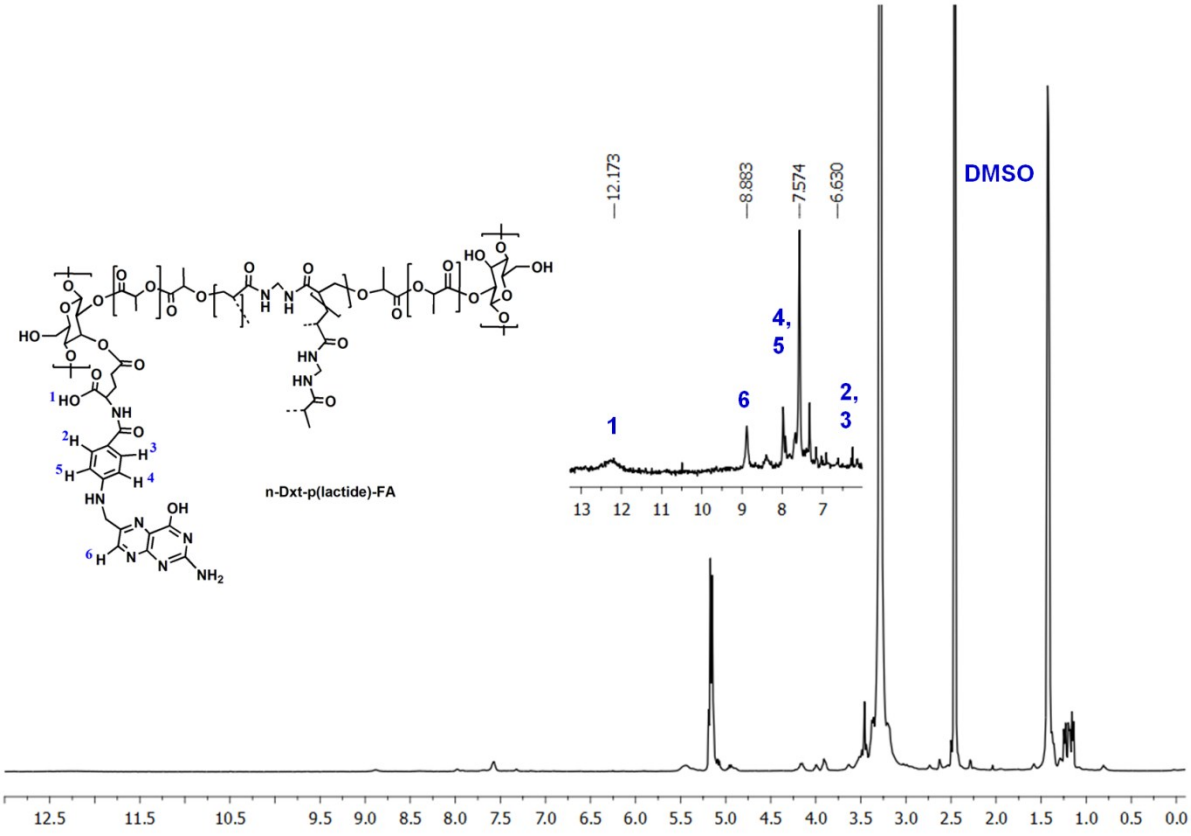

Fig. S3: ^{1}H NMR spectrum of n-Dxt-p(lactide)-FA adduct in DMSO-d_6

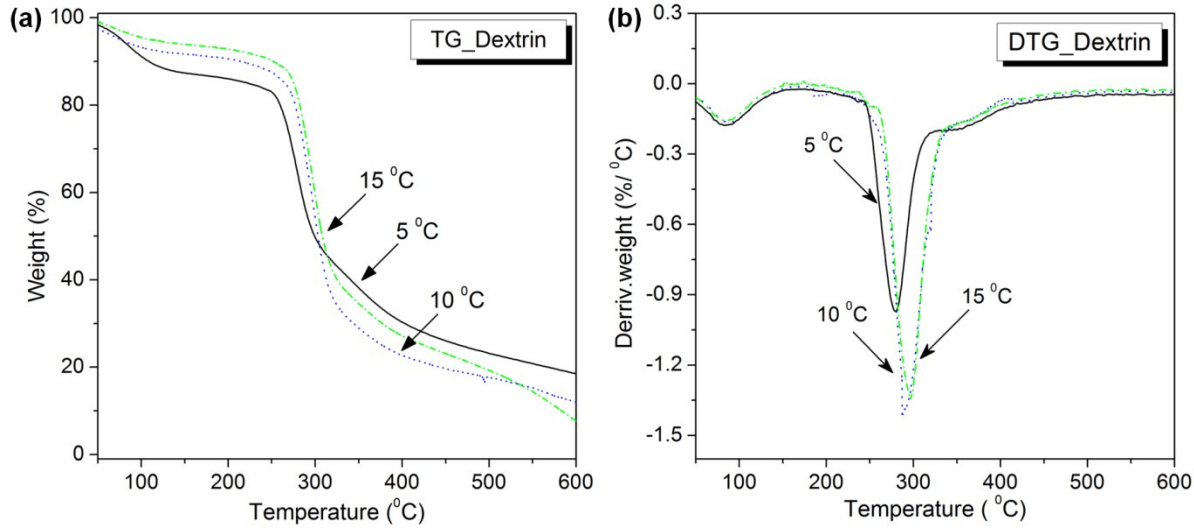

Fig. S4: (a) TG and (b) DTG curve of dextrin at 5 °C, 10 °C and 15 °C

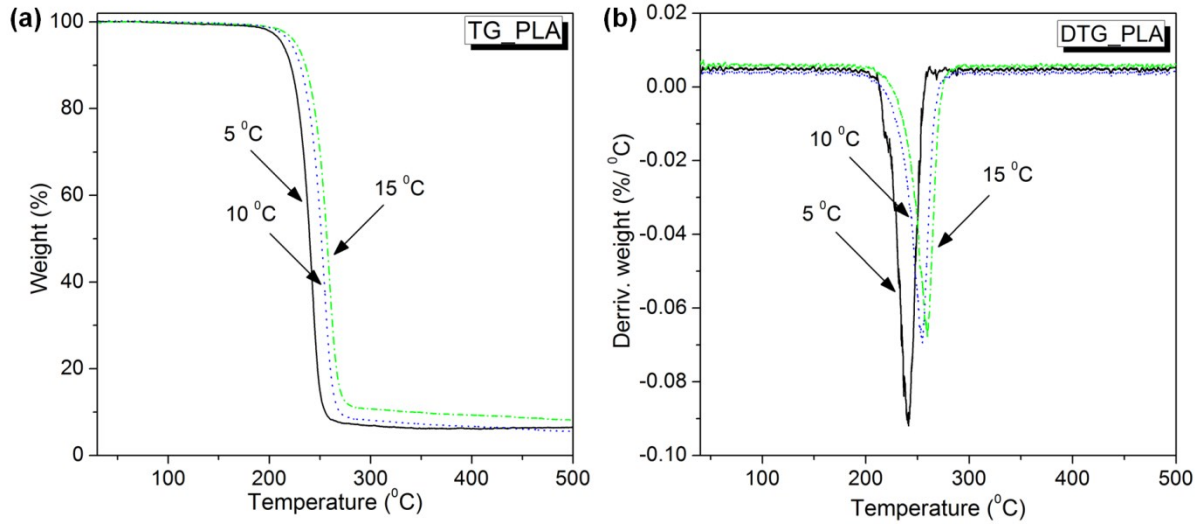

Fig. S5: (a) TG and (b) DTG curve of PLA at 5 °C, 10 °C and 15 °C

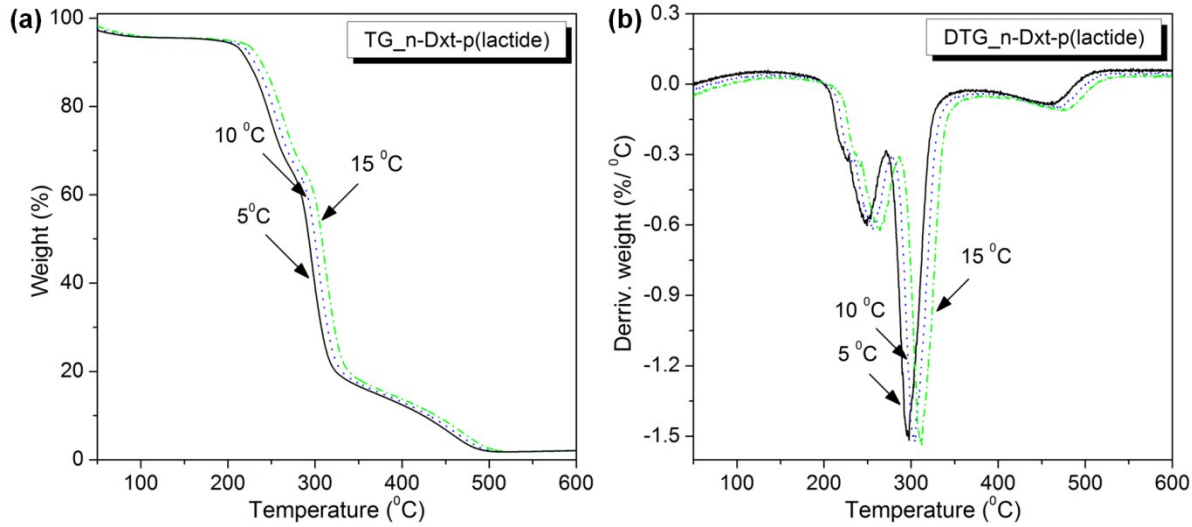

Fig. S6: (a) TG and (b) DTG curve of n-Dxt/p(lactide) nanogeletor at 5 °C, 10 °C and 15 °C

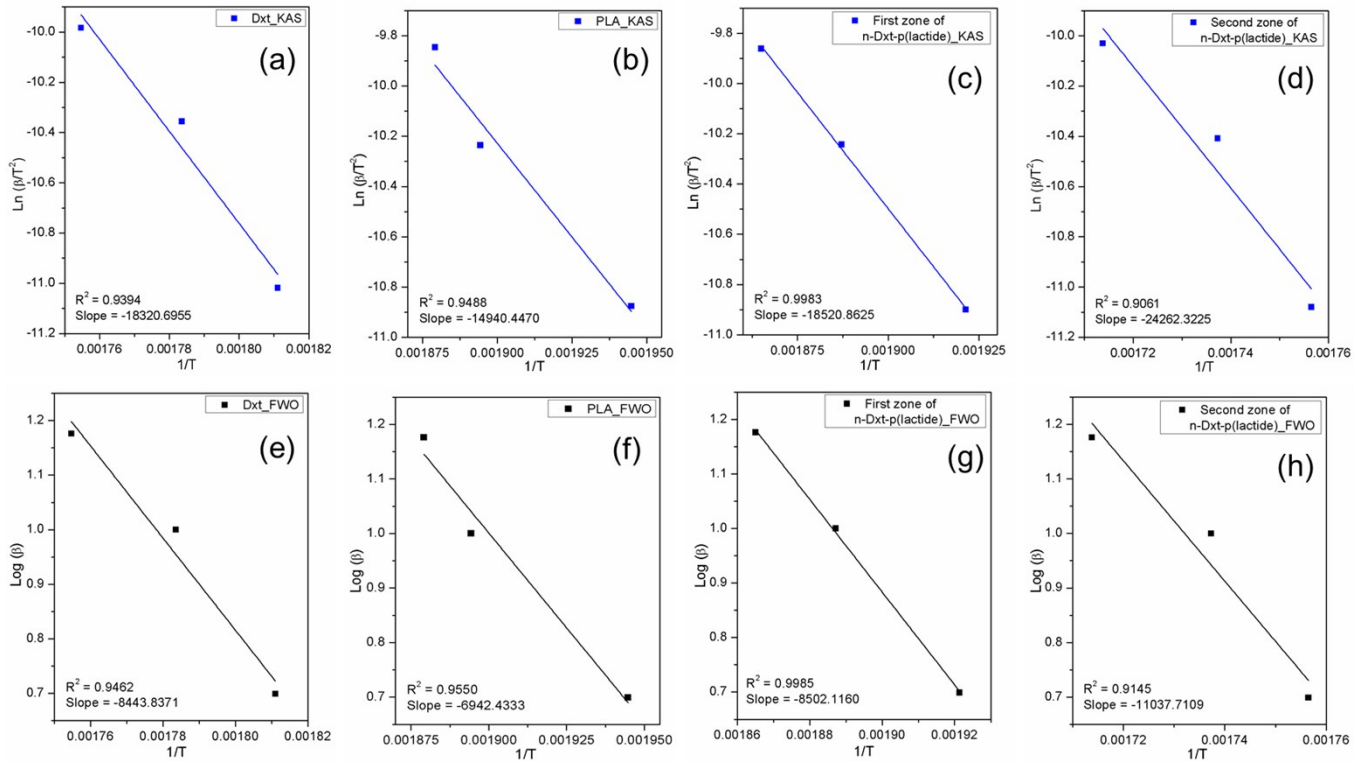

Fig. S7: Fitted graphs for dextrin, PLA and nanogel obtained from Kissinger-Akahira-Sunose (KAS) method (a-d) and Flynn-Wall-Ozawa (FWO) method (e-h).

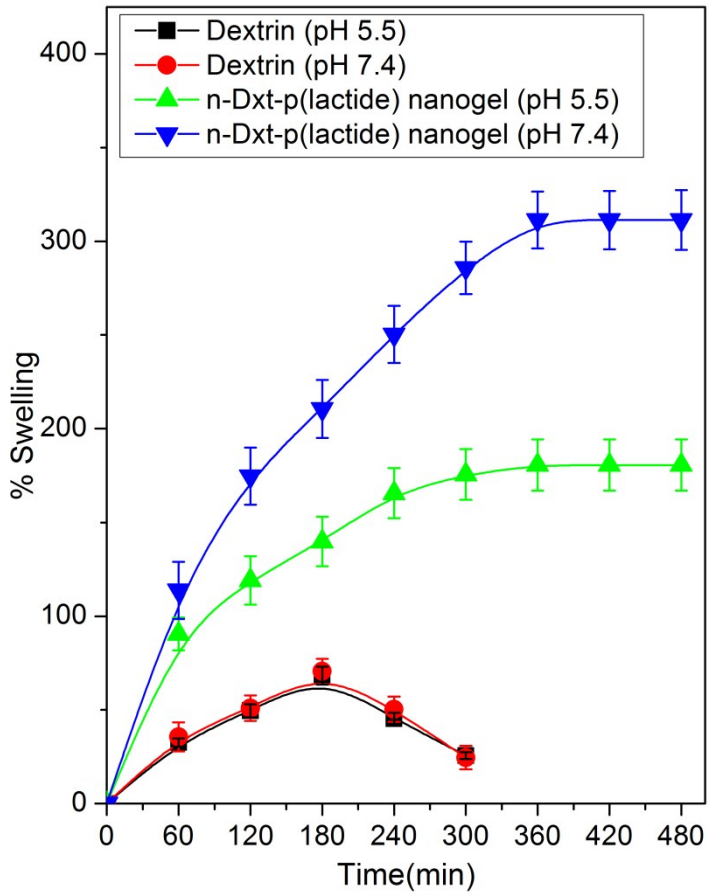

Fig. S8: Swelling characteristics of dextrin and n-Dxt-p(lactide) nanogel at pH 5.5 and 7.4 in 37 ± 0.5 °C

Table S1: % Equilibrium swelling ratio (ESR) and rate parameter value (***τ***) at different pH and 37 °C.

Polymer	% Equilibrium swelling ratio (ESR)		Rate parameter value (***τ***)	
	pH 5.5	pH 7.4	pH 5.5	pH 7.4
n-Dxt-p(lactide) nanogel	180 ± 13.69	311 ± 16.08	500.00	444.40

Table S2: Values of yield stress and gel strength of n-Dxt-p(lactide) nanogel at various frequencies.

Polymer	Applied frequency (Hz)	Yield stress (Pa)	Gel strength (G'/G")
n-Dxt-p(lactide) nanogel	1	625	3.36
	5	750	3.62
	10	833	3.73

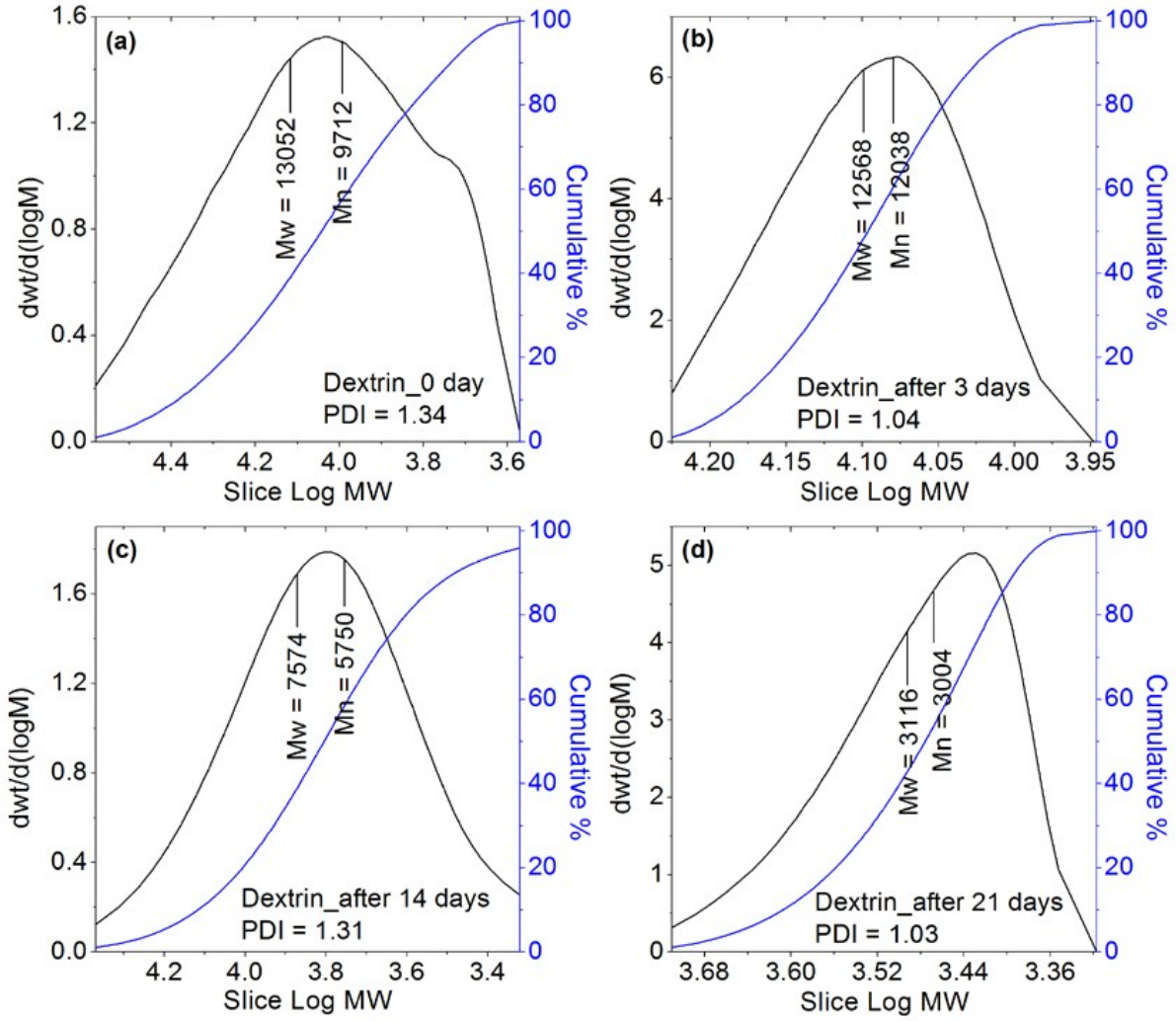

Fig. S9: GPC analyses of (a) dextrin, (b) dextrin after 3 days of degradation study (c) dextrin after 7 days of degradation study, and (d) dextrin after 21 days of degradation study

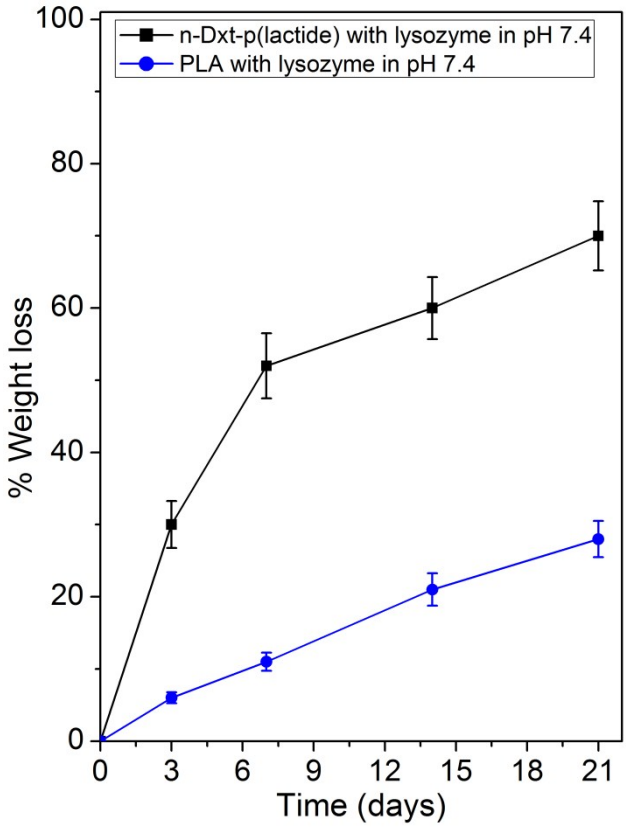

Fig. S10: Results of biodegradation study for PLA and n-Dxt-p(lactide) nanogel (SD, n = ±3)

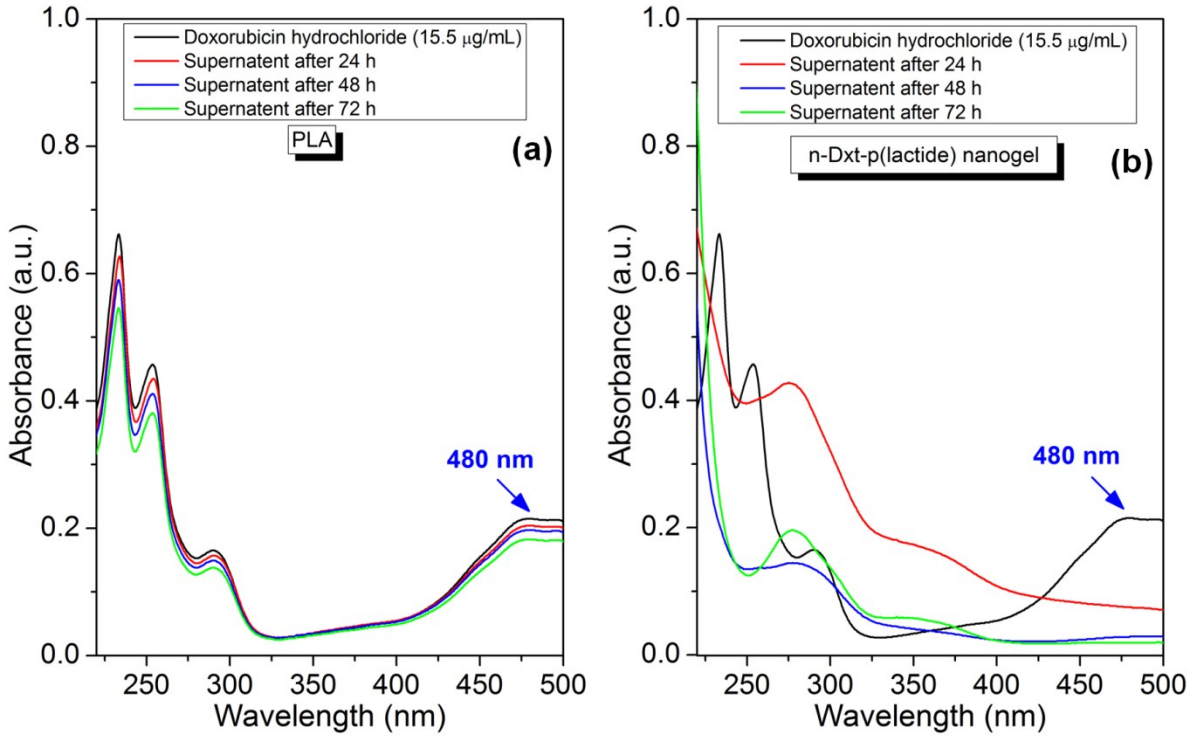

Fig. S11: Plots of doxorubicin hydrochloride loading in (a) PLA and (b) n-Dxt-p(lactide) nanogel after 24 h, 48 h and 72 h.

Table S3: Results of doxorubicin hydrochloride loading in PLA and nanogel after 24 h, 48 h and 72 h

Weight of PLA and n-Dxt-p(lactide) nanogeletor taken is 50 µg/mL Doxorubicin hydrochloride taken 15.50 µg/mL				
Time (h)	PLA		n-Dxt-p(lactide) nanogel	
	Loading (%)	Entrapment efficiency (%)	Loading (%)	Entrapment efficiency (%)
12	1.58 ± 0.008	5.12 ± 0.02	20.17 ± 0.07	65.12 ± 0.23
24	2.59 ± 0.01	8.37 ± 0.03	26.82 ± 0.12	86.52 ± 0.38
72	4.75 ± 0.05	15.35 ± 0.16	28.26 ± 0.20	91.16 ± 0.64

Table S4: Model fitted data for doxorubicin hydrochloride release from loaded PLA/nanogel

Polymers	Zero order		First Order		Higuchi Model		Korsemeyer-Peppas model			Hixson-Crowell Model	
	K_0	R^2	K_1	R^2	K_H	R^2	K_{KP}	R^2	n	K_{HC}	R^2
n-Dxt-p(lactide) (pH 5.5)	0.0067	0.9505	0.00008	0.9706	0.5850	0.9882	0.0321	0.9859	0.81	3.7722	0.8039
n-Dxt-p(lactide) (pH 7.4)	0.0036	0.9737	0.00004	0.9818	0.3127	0.9845	0.0150	0.9837	0.88	3.3373	0.8235
PLA (pH 5.5	0.5833	0.9864	0.0352	0.7849	12.1832	0.9912	-	-	-	0.0126	0.9277
PLA (pH 7.4)	0.4583	0.9660	0.0239	0.8339	10.7093	0.9830	-	-	-	0.0107	0.9056

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