

Supporting Information

Creation of Dextrin Vesicles and Their Loading-Delivering Capabilities

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Experimental Procedures

Materials: Dextrin (Type 1 from corn, $M_w=7700$ g/mol), 3-pentadecylphenol, dicyclohexyl carbodiimide, 4-dimethylaminopyridine, pyrene, rhodamine-B, uranyl acetate and horse liver esterase were purchased from Aldrich Chemicals. Dry DMF was purchased from Finar reagents and distilled using calcium chloride and calcium hydride. Ethyl chloroacetate, K_2CO_3 , KOH, and other solvents and reagents were purchased locally and purified following the standard procedure. Dialysis membrane made of regenerated cellulose materials were purchased from Spectrum Laboratories, USA and used for *in vitro* release studies. PDP ester and PDP acid were synthesized as described earlier.¹⁸

Methods: NMR was recorded in a 400 MHz Jeol NMR spectrometer in $CDCl_3$ (for PDP-ester) and $dms\text{-}d_6$ (for PDP-acid and DEX derivatives) containing a small amount of TMS as internal standard. FT-IR spectra of all compounds were recorded on a Thermo Scientific Nicolet 6700 FTIR spectrometer using potassium bromide (KBr) disks prepared from powdered samples (3 mg) mixed with dry KBr. The spectra were recorded in absorbance mode from 4000 to 400 cm^{-1} . Thermal gravimetric analysis (TGA) was performed on a Perkin Elmer STA 6000 instrument. Mass of PDP-derivatives was confirmed using the Applied Biosystems 4800 PLUS MALDI TOF/TOF analyzer. Absorption and Emission studies were performed on a Perkin-Elmer Lambda 45 UV-Visible spectrophotometer and SPEX Fluorolog HORIBA JOBIN VYON fluorescence spectrophotometer with a double grating 0.22 m spex 1680 monochromator and a 450 W Xe lamp as the excitation source at RT. The excitation spectrum was collected at 375 and 420 nm and emission was collected by exciting the sample at the excitation maxima. The size of the DEX-PDP amphiphiles was determined by DLS using a Nano ZS-90 apparatus using a 633 nm red laser at 90 ° angle from Malvern instruments. The sample was dispersed in water (or PBS) to obtain a concentration of 0.5 mg/ml and then sonicated, heated and filtered using a 0.45 μm filter to afford a clear solution. For the DEX-PDP derivatives encapsulated with Rhodamine-B, the solution from the dialysis bag was filtered and diluted before analysis. The fluorescent micrographs of Rhodamine B and CPT loaded DEX-PDP-7 was recorded using Carl Zeiss Axiovert 200 microscope. It consists of HXP 120 C metal halide light source. For rhodamine B DEX-PDP-7, the image were collected using RFP filter (excitation BP572/25, Beam splitter FT 590 and Emission BP 629/ 62) and for CPT loaded DEX-PDP-7, the images were collected using DAPI filter (Excitation G365, beam splitter FT 395 and emission BP 445/50). AFM images were recorded by drop casting the samples on a freshly cleaved mica surface using Carl Zeiss AFM setup and the experiment was performed

in tapping mode. FE-SEM images were recorded on a Zeiss Ultra Plus scanning electron microscope with samples prepared by drop casting on a silicon wafer and air dried. TEM images were recorded using a Technai-300 instrument by drop casting and air drying the sample on formvar coated copper grid.

Synthesis of DEX-PDP-x: Dextrin (type 1 from corn, $M_w=7700$, 0.5 g, 0.00310 mol of anhydroglucose unit) was dissolved in 20 ml dry DMF and refluxed for 1 hour at 90°C under nitrogen atmosphere. It was then cooled to RT and purged with nitrogen for 10 min. Following this, PDP-acid **2** (0.57 g, 0.00155 mol for DEX-PDP-13) dissolved in dry DMF (3 ml) was added to the reaction mixture and it was cooled to 0°C. DCC (0.39 g, 0.00186 mol) and 4-DMAP (0.047 g, 0.000310 mol) were added to the reaction mixture and the reaction mixture was stirred at 90°C for 24 hours under nitrogen atmosphere. The reaction mixture was cooled, filtered to remove dicyclohexylurea and the filtrate was poured into ice-cold methanol. The precipitate was then filtered and washed with methanol. It was dissolved again in DMF and precipitated using methanol and dried in the vacuum oven to afford a crystalline brown solid. Yield=50 %. $^1\text{H NMR}$ (400 MHz, $\text{dms}\text{-d}_6$): 7.13 ppm (t, 1H, Ar-**H**), 6.67 ppm (m, 3H, Ar-**H**), 5.42,5.50 ppm(s,2,3-hydroxyl of dextrin), 4.72 ppm (s, 2H, O-**CH**₂ of ester linkage), 2.49 ppm (t, 2H, Ar-**CH**₂), 1.52 ppm (m, 2H, Ar-**CH**₂-**CH**₂), 1.28 ppm (m, 27H, Aliphatic protons), 0.87 ppm (s, 3H, -**CH**₃). FT-IR ((KBr), cm^{-1}): 3438 (O-H stretch), 2922, 2852 (Aliphatic C-H stretch), 1765 (Ester C=O stretch), 1693 (ring C=C stretch), 1450 (O-H bending), 1207 (C (=O)-O stretch).

DEX-PDP with different degree of substitutions i.e DEX-PDP-7, 13, 25, 33 and 50 were thus, synthesized by changing the mole ratio of dextrin to PDP-acid as 0.25, 0.5, 1, 1.5 and 2 in the feed.

Determination of critical vesicular concentration (CVC): The critical vesicular concentration was determined using Pyrene as a probe. In a typical experiment, 1 ml of Pyrene in acetone (0.6 μM) was added to 3 ml glass vials and the acetone was allowed to dry completely. Concentrations of DEX-PDP derivatives varying from 0.5 mg/ml to 0.00033 mg/ml were then added to these vials. The vials were sonicated for 1 hour and the samples were left to equilibrate overnight. The excitation wavelength was set to be 334 nm and the excitation and emission slit width were fixed as 3 nm. The ratio of fluorescence intensity at I_1 (375 nm) and I_3 (386 nm) was calculated and plotted against the logarithm of

concentration to obtain a graph where the onset of the slope gave the critical vesicular concentration (CVC).

Encapsulation of a hydrophilic dye Rhodamine (Rh-B) in DEX-PDP vesicles: The ability of the DEX-PDP-derivatives to stabilize Rh-B was investigated using the solvent exchange/dialysis method. In a typical experiment, 20 mg of the polymer and 2 mg of Rhodamine-B was dissolved in 2 ml DMSO. Following this, 2 ml of distilled water was added drop wise into the polymer solution stirring at 25° C and further stirred for 12 hours. The solution was transferred to a dialysis bag (MWCO=2000), stirred for 24 hours and dialyzed against distilled water upto 7 days to check the stabilization of the hydrophilic dye.

Encapsulation of a hydrophobic Camptothecin into DEX-PDP vesicles

Water insoluble Camptothecin (CPT) anticancer drug was loaded into vesicles by dialysis method similar to RhB encapsulation. Briefly, 50 mg DEX-PDP-7 and 5 mg CPT was dissolved in 3 ml DMSO and stirred for 15 min. To this solution 3 ml of water was added drop wise and the resulting suspension was stirred at room temperature for 12h. This solution was taken in dialysis tube (MWCO 3,500) and dialysed for 24 h with 4 times water exchange. After dialysis, the solution was filtered and lyophilized to receive drug loaded polymer powder. The drug loading content (DLC) was determined by absorption spectroscopy, where absorbance obtained from 1 mg of lyophilized powder dissolved in 3 ml DMSO was substituted in Beers equation. The molar absorption coefficient of CPT was kept as 11,250. The DLE and DLC was calculated by using following equation.

$$\text{DLE (\%)} = \{ \text{Weight of drug in vesicles} / \text{Weight of drug in Feed} \} \times 100 \%$$

$$\text{DLC (\%)} = \{ \text{Weight of drug in vesicles} / \text{Weight of Drug loaded vesicles} \} \times 100 \%$$

***In vitro* studies:** To study the release profile of Rh-B and CPT , 3 ml of the solution in the dialysis bag above was immersed in 100 ml PBS buffer (pH 7.4) in a beaker. At specific time intervals, 3 ml of the dialysate was withdrawn and replaced with an equal volume of fresh buffer. The amount of Rh-B and CPT present in each aliquot was measured using UV-Visible spectroscopy and quantified using Beer-Lambert's law in terms of weight percentage. To ascertain the effect of esterase enzyme on the release of Rh-B and CPT, 10 U of horse liver esterase was added to the dialysis bag and the above procedure was repeated.

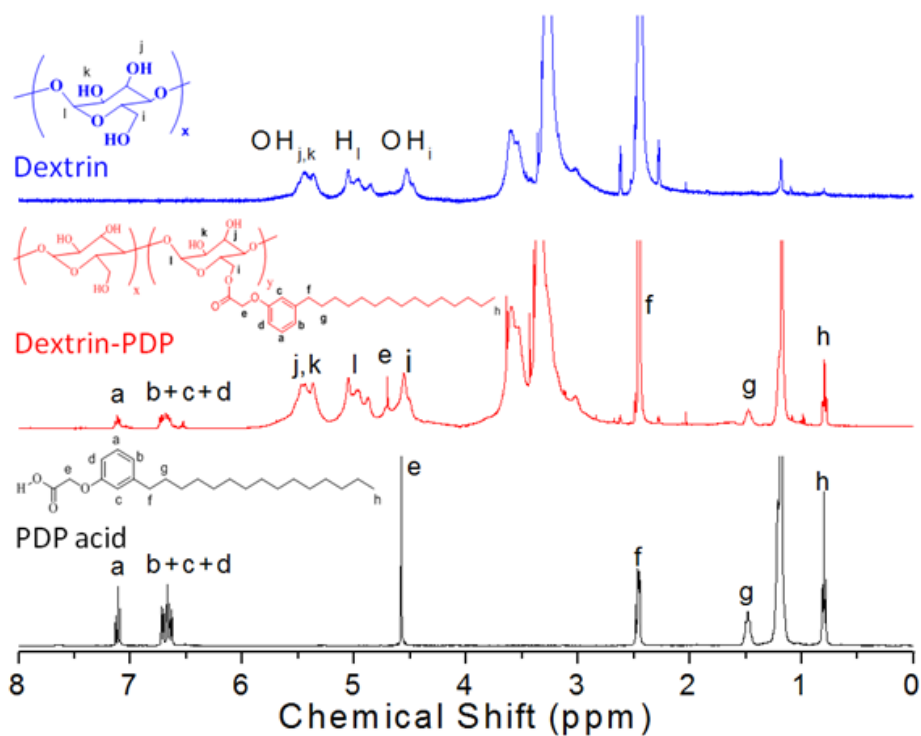


Figure SF1. ^1H spectra of *Dextrin*, *DEX-PDP* and *PDP acid* (in d_6 -DMSO)

Note: The dextrin of the substituted dextrin showed peaks at 6.67 and 7.13 ppm and 0.5 to 3.00 ppm for the PDP-aryl and aliphatic protons respectively. The protons from dextrin units appeared from 3.30-5.50 ppm. Upon the formation of ester linkage, the protons Ar-OCH₂-COO-DEX appeared at 4.67 ppm. The degree of substitution (DS) was calculated by comparing the peak intensities of anomeric proton in dextrin at 5.11 ppm with the PDP aryl protons at 7.14 ppm.

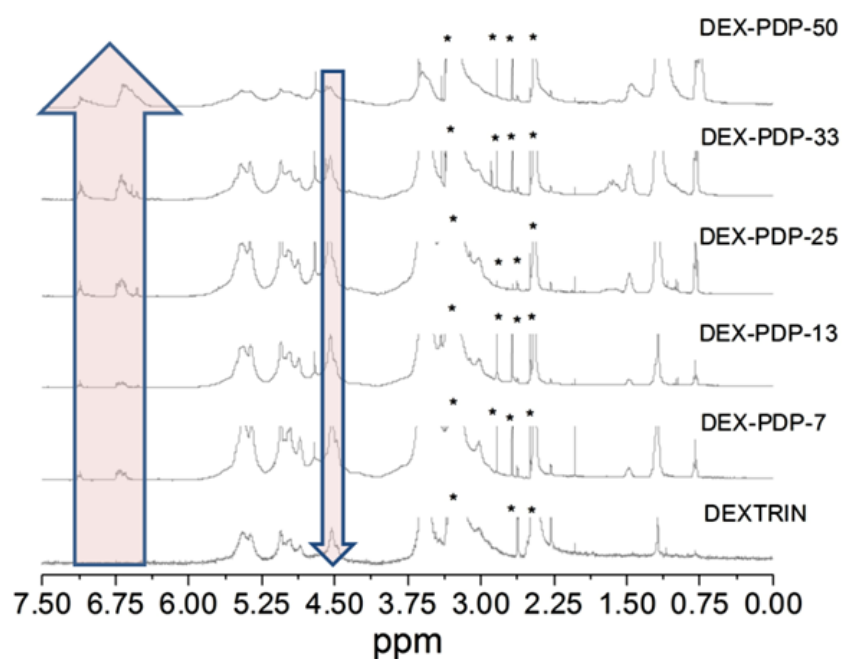


Figure SF2. *Stack plot of NMR of Dextrin and various Dextrin substituted amphiphiles in DMSO (d6).*

Note: The intensity of the aromatic peaks was found to increase with an increase in the substitution. But intensity of primary hydroxyl group, which involved in the esterification reaction, of dextrin was reduced with substitution. This is a clear evidence for the formation of the dextrin-PDP amphiphiles.

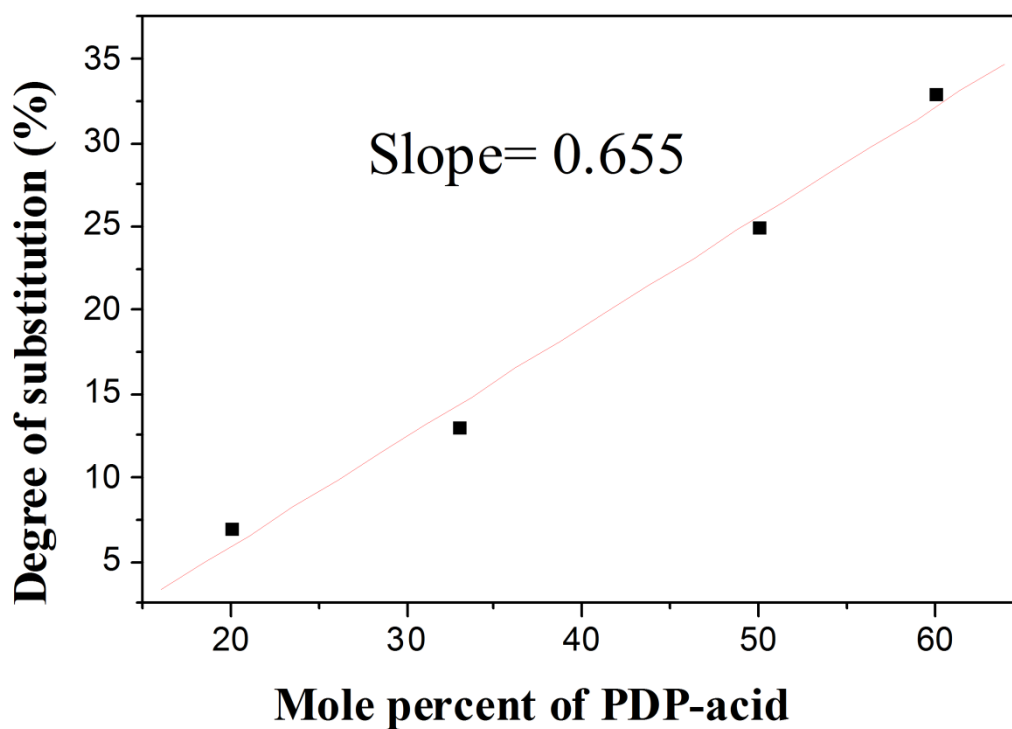


Figure SF3. Plot of mole percent of PDP-acid used vs degree of substitution

Note: A plot of the mole percent of PDP-acid added in the reaction versus the actual degree of substitution obtained on the dextrin backbone showed predominantly a linear trend with a slope value of 0.655. This confirms that dextrin amphiphiles with required amount of hydrophobicity can be synthesized by changing the feed ratio of PDP acid.

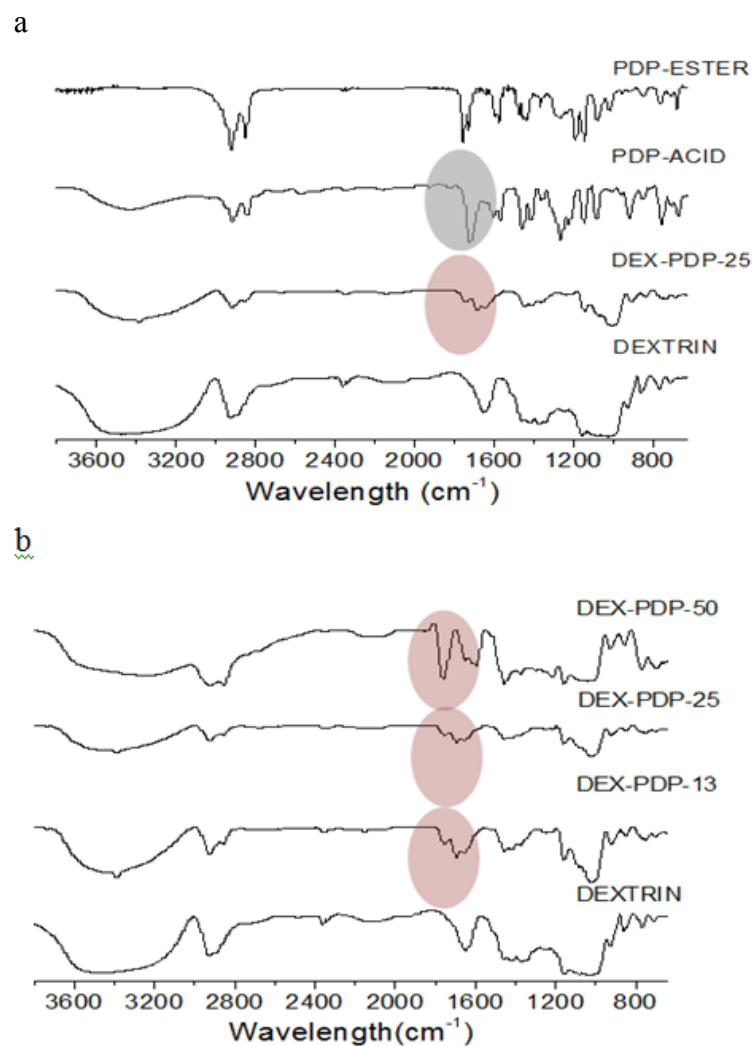


Figure SF4. a. FT-IR plot of Dextrin, Dextrin PDP, PDP ester and PDP acid. **b.** FTIR comparison plot of Dextrin and various substituted dextrans.

Note: The carbonyl (C=O) ester linkage in PDP ester showed a distinct band at FT- 1760 cm⁻¹. This peak got shifted to 1730 cm⁻¹ in PDP acid. But in case of dextrin-PDP amphiphile, -C=O stretching frequency again appeared at 1765 cm⁻¹ which confirm the formation of ester linkage between dextrin and PDP unit. It was also noticed that the intensity of -C=O stretching frequency at 1765 cm⁻¹ increased with an increase in the substitution.

Sample Name	PDP in Feed (mol %)	Actual PDP in DEX-PDP (mol %)	DLS Size (nm)	FE-SEM Size (nm)	CVC or CAC (mg/ml)	Type of Self-assembly
DEX-PDP-7	20	7	160	100	8.33×10^{-3}	Vesicle
DEX-PDP-13	33	13	186	160	5.0×10^{-2}	Nanoparticle
DEX-PDP-25	50	25	320	135	5.0×10^{-2}	Nanoparticle
DEX-PDP-33	60	33	500	120	--	Nanoparticle

Table ST 1. Table showing mole percent of PDP acid used for different dextrin derivatives and its corresponding self assembly characteristics.

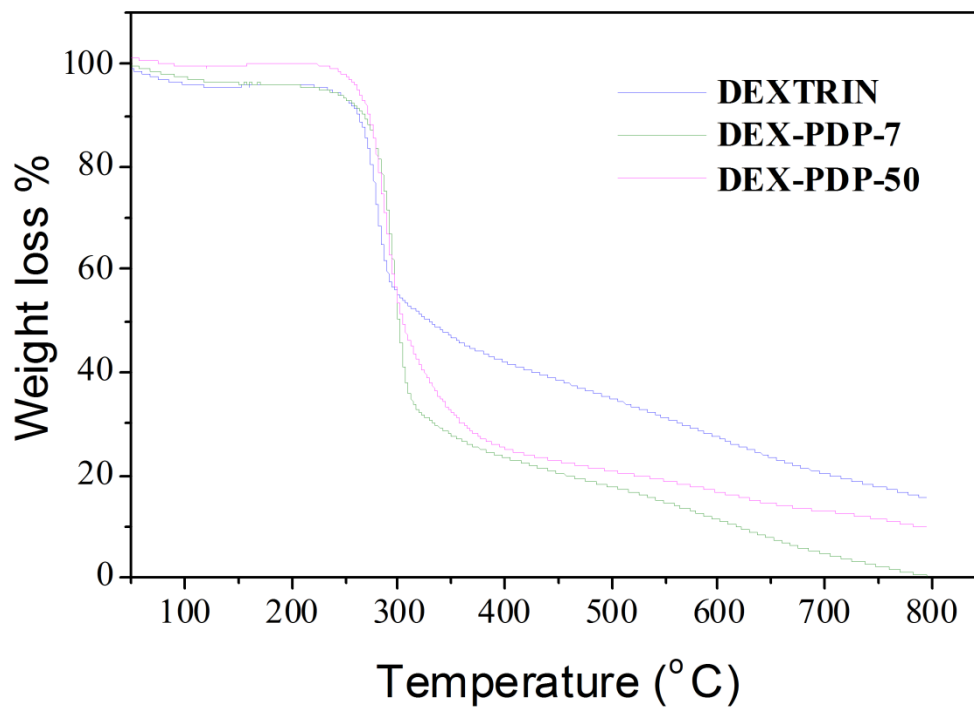


Figure SF5. Thermo gravimetric plot of dextrin, DEX-PDP-7 and DEX-PDP-50

Note: The Thermal gravimetric analysis of the DEX-PDP derivatives showed thermal stability up to 262 °C with a small increment upon varying the degree of substitution.

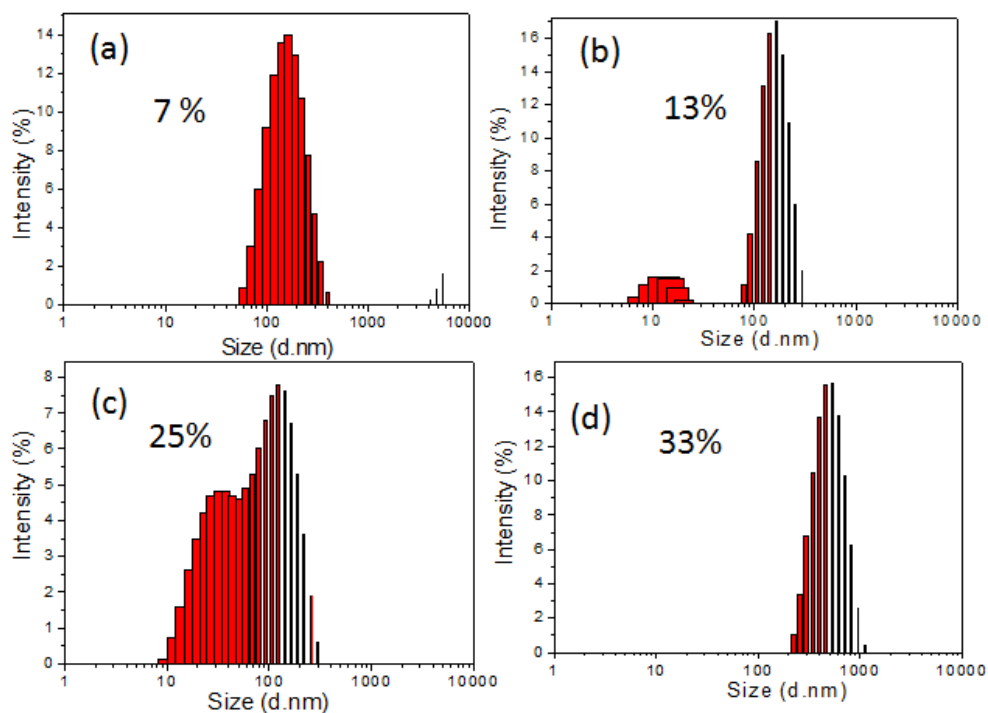


Figure SF6. DLS histogram of DEX-PDP-7 (a)DEX-PDP-13 (b), DEX-PDP-25 (c), DEX-PDP-33 (d)

Note: it can be observed that self assembly of the DEX-PDP derivatives changes drastically upon changing the degree of substitution i.e. it changes from 160 nm to 500 nm upon changing the substitution from 7% to 50 %.

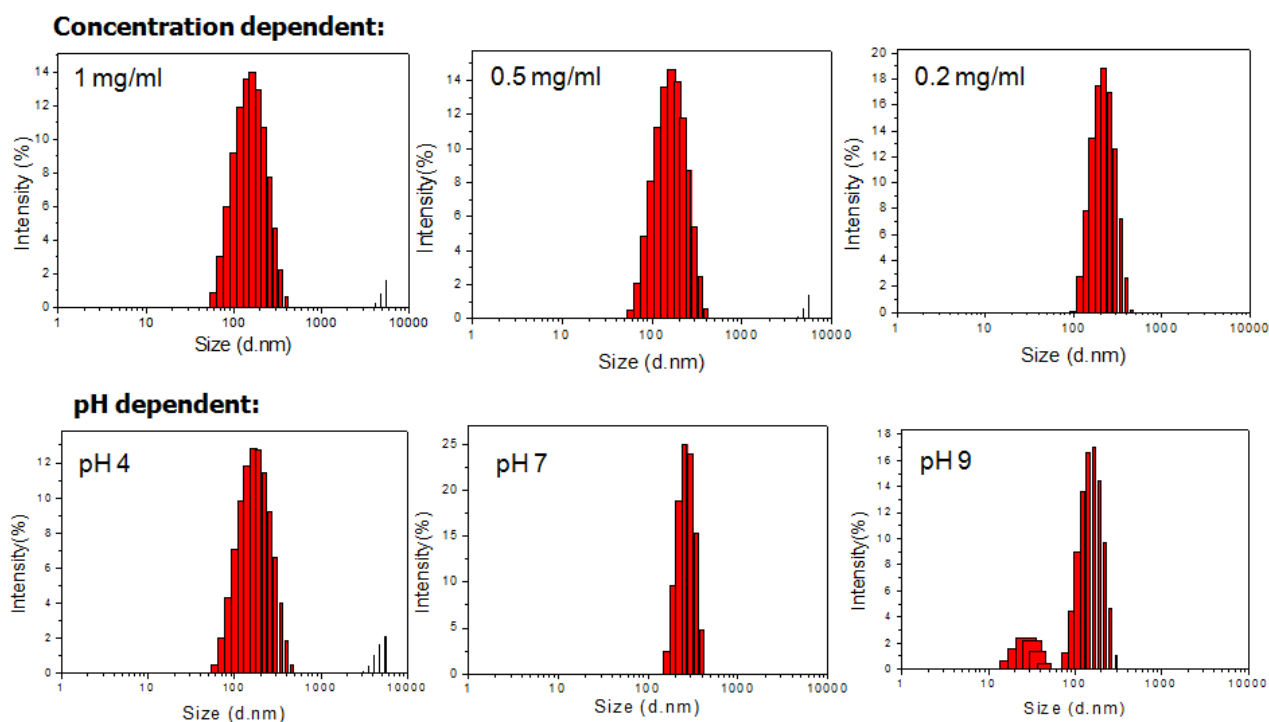


Figure SF7. DLS histogram of DEX-PDP-7 at different concentrations and pH.

Note: The DLS measurement of DEX-PDP-7 was performed at different concentrations and at different pH values. The DLS profile of DEX-PDP-7 did not change appreciably with concentration. But in pH dependent studies, it was observed that the mean hydrodynamic diameter of DEX-PDP-7 was larger in case of basic solutions than in case of neutral or acidic solutions. The size obtained for pH 4 and pH 7 were in agreement with that obtained in water.

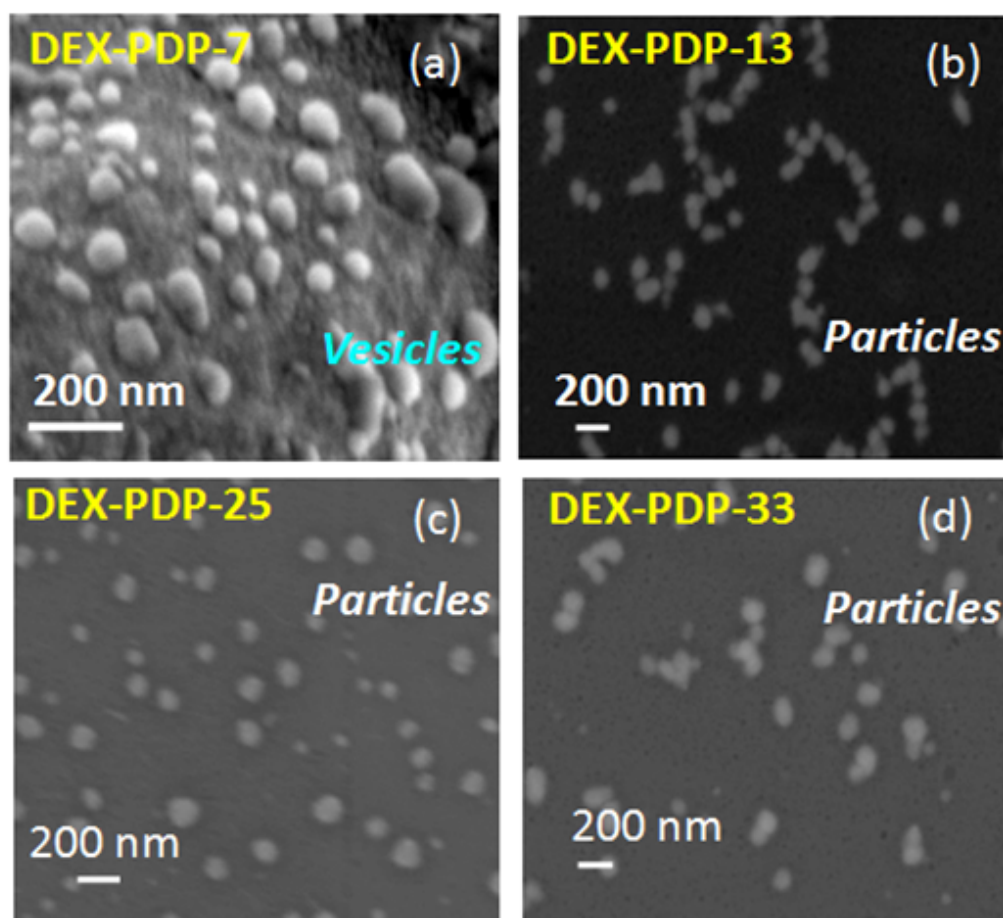


Figure SF 8a. FE- SEM image of DEX-PDP-7 (a) DEX-PDP-13 (b), DEX-PDP-25 (c) DEX-PDP-33(d)

Note: Scanning electron microscopy (SEM) analysis indicates that the morphology of these vesicles is indeed spherical. The results obtained from DLS are also in agreement with the size obtained from Scanning electron microscopy (SEM). SEM analysis also revealed that the morphology of the scaffolds changed from vesicular to nanoparticles upon increasing the degree of substitution.

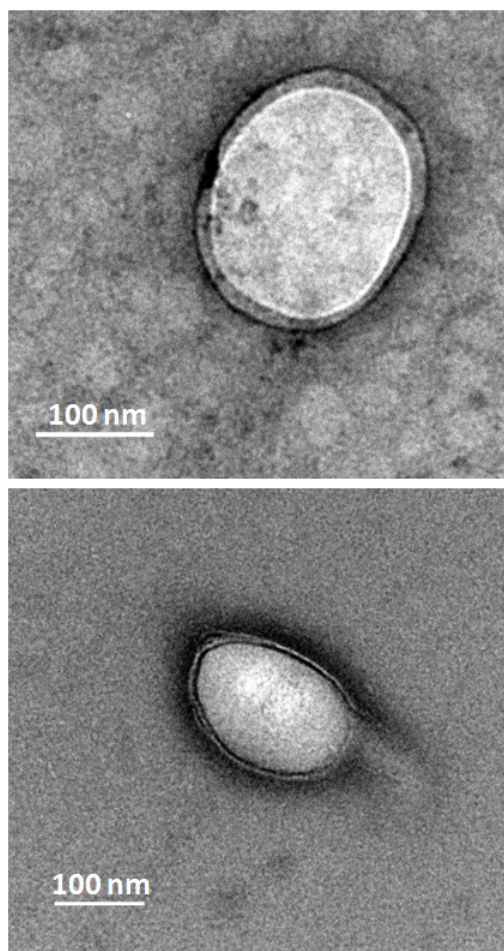


Figure SF 8b: *Transmission Electron Microscopic images of DEX-PDP-7 vesicles negatively stained using 0.2 % uranyl acetate.*

Note: 10 μ L of DEX-PDP-7 vesicular solutions in water were drop casted on a formvar coated copper grid. Samples were kept for 1 hour and the remnants were wicked off by using filter paper and then 10 μ L freshly prepared 2.0 % uranyl acetate solution was placed on the grid. Uranyl acetate solution was wicked off from the grid after 30 seconds and the grid was washed twice using 10 μ L dd water each time. The sample was then air dried over a dust free surface under funnel. The vesicles were then imaged by using Tecnai T300 HR-TEM instrument.

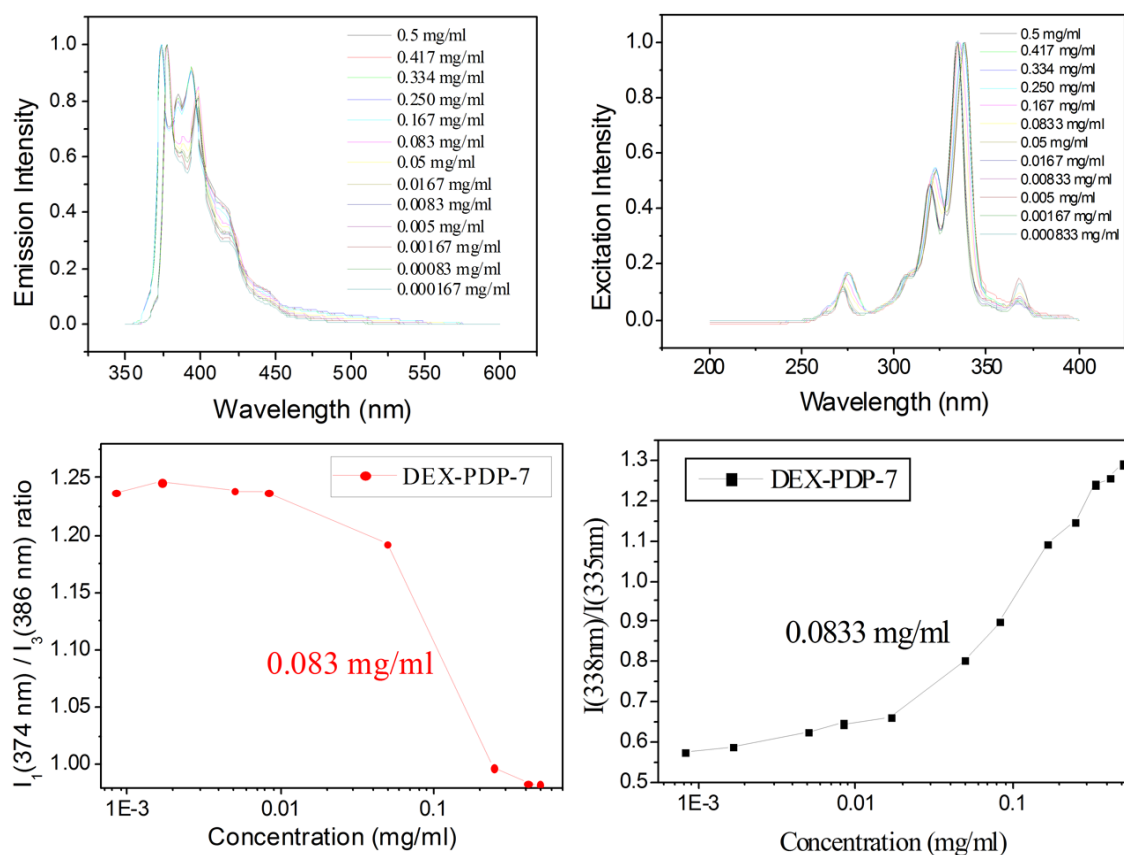


Figure SF9. Emission, and Excitation spectra of pyrene with DEX-PDP-7 at different polymer concentration and CVC determination.

Note . The concentration of Pyrene was fixed to be 0.6 μM so as to prevent excimer formation and polymer concentration varied. At higher polymer concentration, i.e. above CVC, pyrene will prefer to stay in the hydrophobic layer of the vesicle..A plot of I_1/I_3 ratio was plotted against the log of concentration resulted in a sigmoidal curve. Here, we have chosen the onset of the slope since that indicates the onset of the association event. Excitation spectra collected at both monomer and excimer emission of pyrene shows two peaks at 334 and 338 nm that are characteristic of pyrene monomer absorption in the hydrophilic and hydrophobic environment. The ratio of I_{338}/I_{335} also plotted against concentration for calculating CVC as shown in the figure

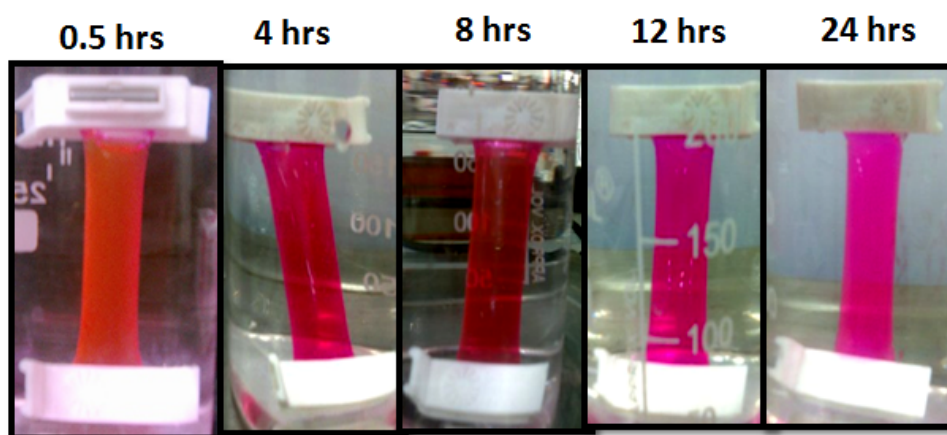


Figure SF10. *Photographs of dialysis bag carrying dextrin-PDP-7 with Rhodamine B at different time intervals.*

Note: The photographs prove the capability of vesicles formed from DEX-PDP-7 in stabilization of water soluble rhodamine B. Here water soluble dye is stabilized against water, which is considered as a difficult task in the area of self assembly. The dialysate was replaced with fresh water before taking images for better visibility.

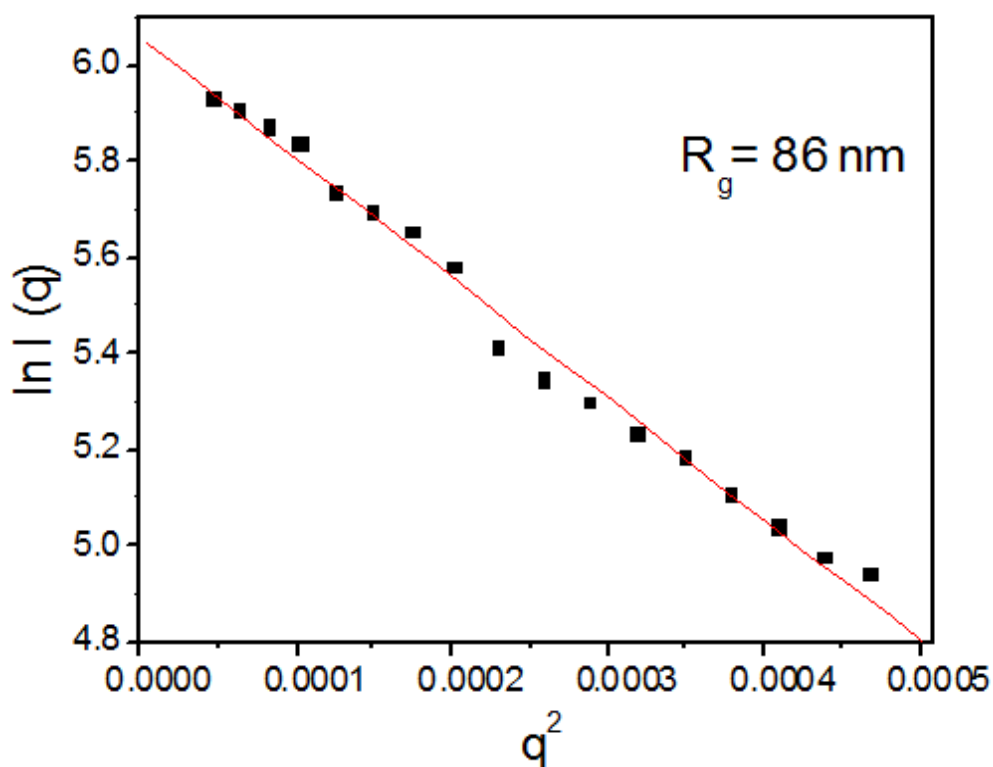


Figure SF 11. SLS plot of DEX-PDP-7 loaded with Rhodamine B

Note: Here the difference of scattering light intensity with scattering angle ranging from 15° to 130° was exploited to determine the size of the particle. The radius of gyration (R_g) was calculated as 86 nm from the slope of Guinier plot, where the natural logarithm of scattering intensity at different angles was plotted against square of scattering vector magnitude (q^2), where

$$q = \frac{4\pi n \sin(\theta/2)}{\lambda_o}$$

n is the refractive index of solution and λ_o is the incident light wavelength, ie: 632.8 nm. Guinier plot is given by:

$$\ln(I(q)) = \ln(I(0)) - \frac{q^2 R_g^2}{3}$$

Slope of the graph is $R_g^2/3$.

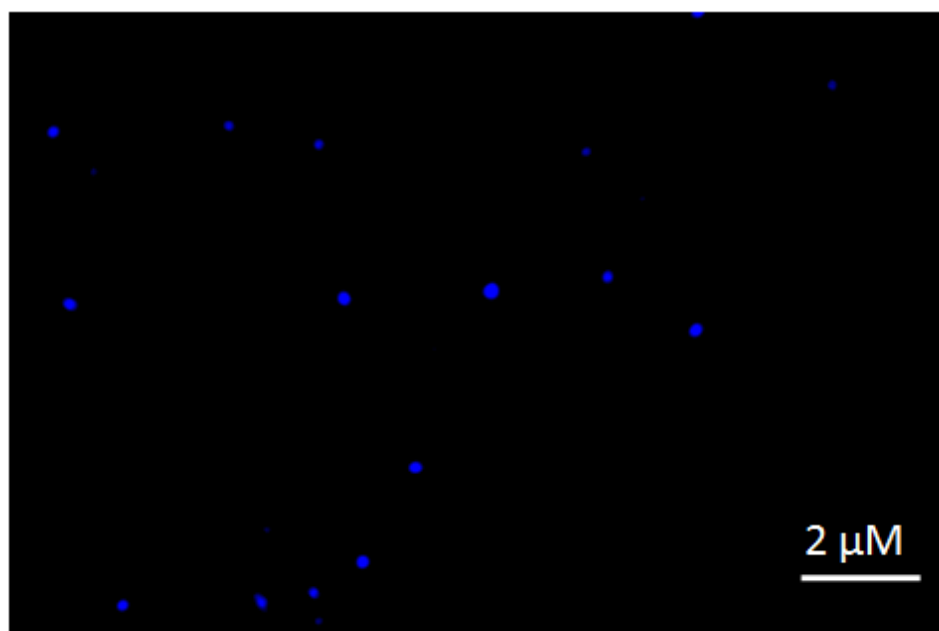
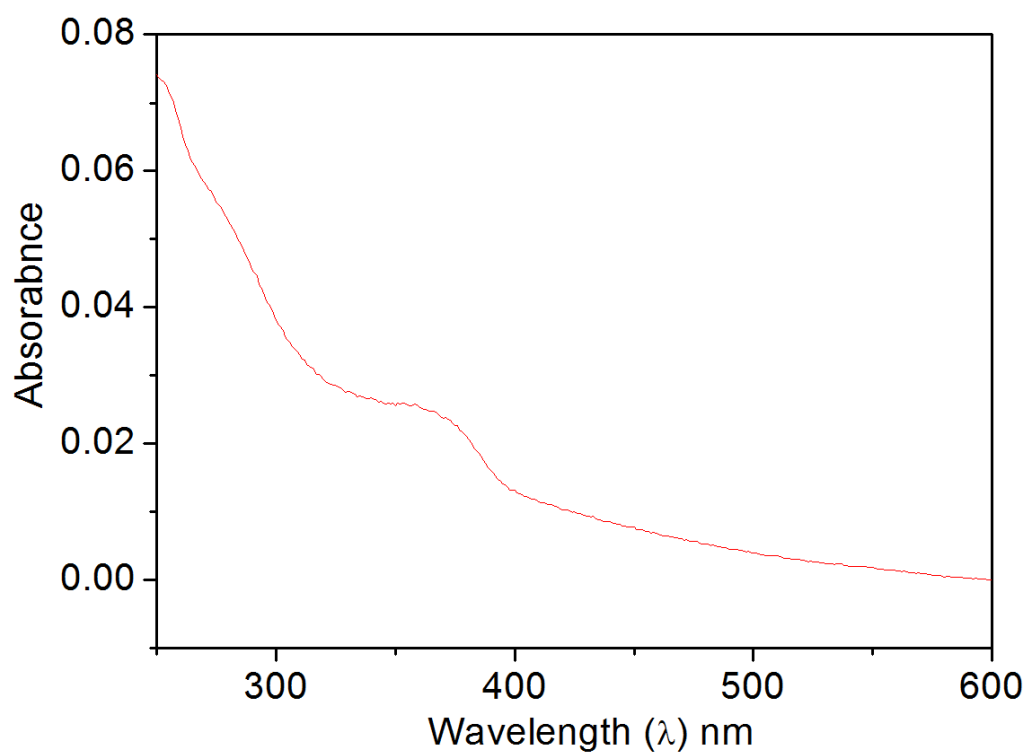


Figure SF 12. Absorbance plot of CPT in DEX-PDP-7 (top) and Fluorescence microscopic image of CPT loaded DEX-PDP (bottom).