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# Supporting Information

# POLY(N-VINYLCAPROLACTAM) CONTAINING SOLID LIPID POLYMER HYBRID NANOPARTICLES FOR THE CONTROLLED DELIVERY OF GEMCITABINE HYDROCHLORIDE

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**Cloud point determination**

Aqueous polymer solution (10 mg/mL) was prepared and the change in absorbance was recorded with respect to temperature. The temperature was increased at the rate of 1$^{o}$C/min from 25 $^{o}$C to 45 $^{o}$C. The temperature at which 10% decrease in the transmittance occurred was taken as the cloud point.

**Differential scanning calorimetry**

Drug and excipients were mixed using mortar and pestle. Typically, 4-5 mg of the sample was placed in aluminium pan with a lid having pinhole at the middle. The sample was heated from -50 $^{\circ}$C to 300 °C with heating rate of 10 °C/min under nitrogen atmosphere with a flow rate of 50 mL/min. An empty pan was used as reference.

**Determination of encapsulation efficiency**

SLNs were dissolved in ethyl acetate and methanol (1:1) mixture. The solution was vortexed for 2 min and centrifuged at 15000 rpm for 30 min at 10 $^{o}$C. Supernatant was diluted with DI water and filtered through 0.45 µm membrane filter. The filtrate was analyzed on UV-visible spectrophotometer at 268 nm to determine the amount of drug present.

Encapsulation efficiency was determined using the formula given below.

$$\%EE = \text{Amount of drug entrapped in particles/ Initial amount of drug added} \times 100$$

***In-vitro*** **hemolysis study**

Hemolysis study was conducted by reported procedure with minor modifications.[38,39] Human blood sample was collected in an ethylenediamine tetraacetic acid (EDTA) coated tube. RBCs were separated from plasma by centrifugation at 3000 rpm for 10 minutes. The obtained RBCs were washed for three times with PBS (Phosphate Buffer Saline). A 2% RBC suspension was incubated with samples at different concentrations (1, 2, 3, 4, 5 mg/mL) for 2 h. RBCs treated with PBS were taken as negative control and RBC treated with triton X-100 were taken as positive control. After 2 h incubation, samples were taken and centrifuged for 10 min at 3000 rpm. Supernatant from the samples was collected and absorbance was

measured at 576 nm using UV-vis spectrophotometer (Spectramax, microplate reader, Molecular Devices, California, USA).

The percentage hemolysis was calculated using the following formula

$$\text{haemolysis (\%)} = (A_s - A_o)/(A_{100} - A_o) \times 100$$

where As is absorbance of the sample, Ao is absorbance of sample treated with saline, and $A_{100}$ is absorbance of sample treated Triton X-100.

***In-vitro* cytotoxicity studies**

*In-vitro* cytotoxicity assay (MTT assay) was performed as per reported procedure with slight modifications. [40] MDA-MB-231 triple negative breast cancer cell line was used and maintained in DMEM medium with 10% FBS in an incubator at 37 $^oC$, 5% $CO_2$. The cells ($10^4$ cells/ well) were seeded in 96 well tissue culture treated plate and incubated for 24 h. UV sterilized samples (equalent to 4, 8, 12, 16, 20 and 24 µg/ mL of gemcitabine) prepared in media were added and incubated for another 24 h. At the end of the 24 h medium was replaced by working 10% MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution prepared in complete media and incubated for 4h with 5% $CO_2$ at 37 $^oC$. At the end of incubation MTT solution was discarded and formazan crystals were dissolved in DMSO and absorbance was determined at 550 nm in UV-visible spectrophotometer. Relative %cell viability was calculated using following formula

$$\text{Relative percent cell viability} = (A_{test}/A_{control}) \times 100$$

where $A_{test}$ is the absorbance of cells treated with sample, and $A_{control}$ is the absorbance of the untreated cells (control). Experiments were performed in triplicate.

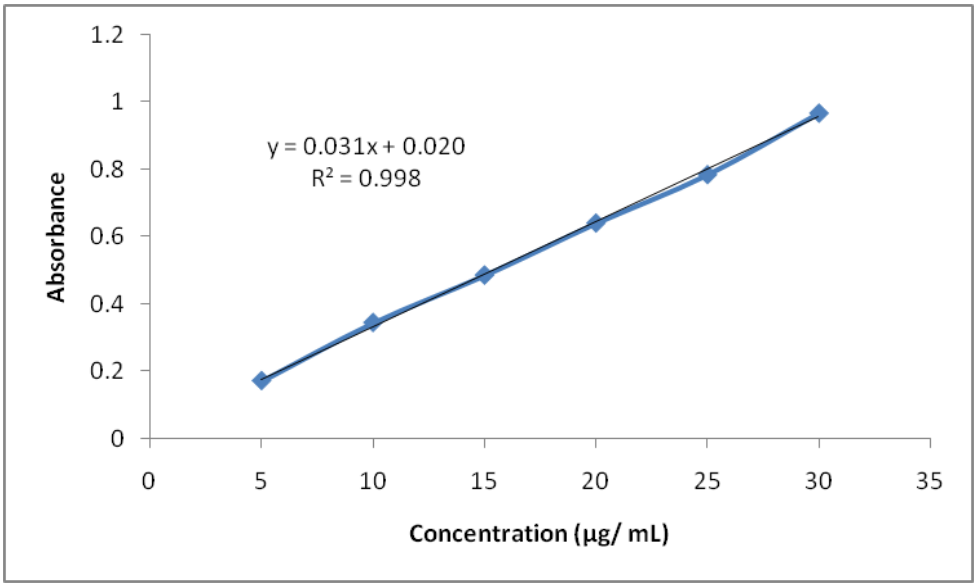


**Figure S1.** UV-vis calibration curve for gemcitabine in pH 7.4 buffer

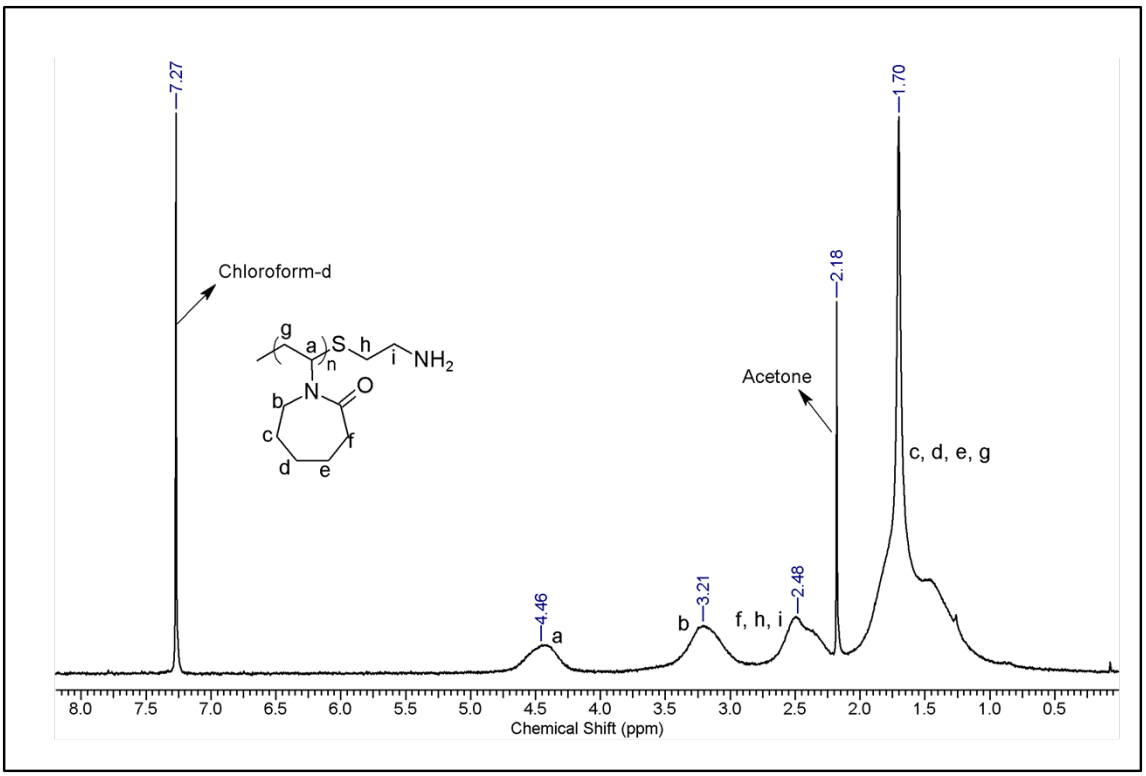


**Figure S2.** 200 MHz $^{1}$H NMR spectrum of PVCL in $CDCl_3$

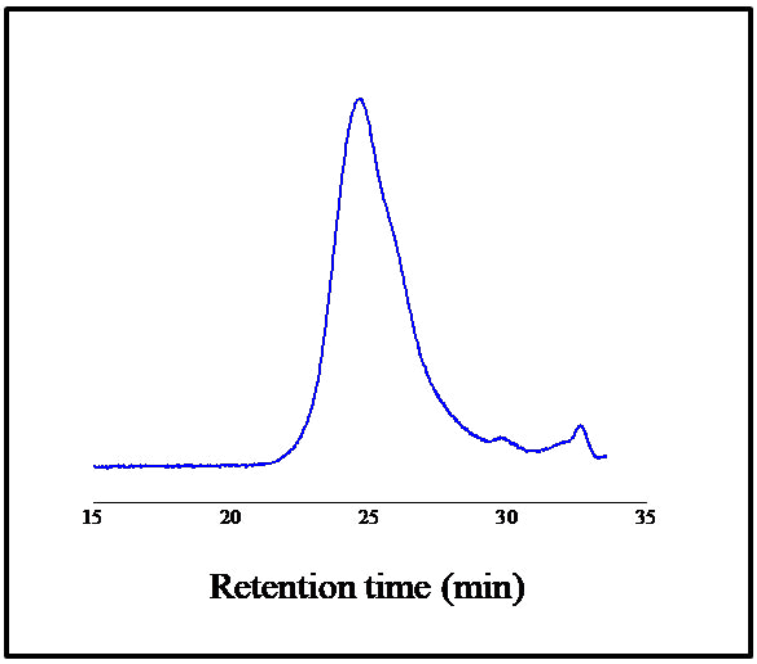


**Figure S3**. GPC chromatogram of PVCL (eluant Chloroform, PS Std.)

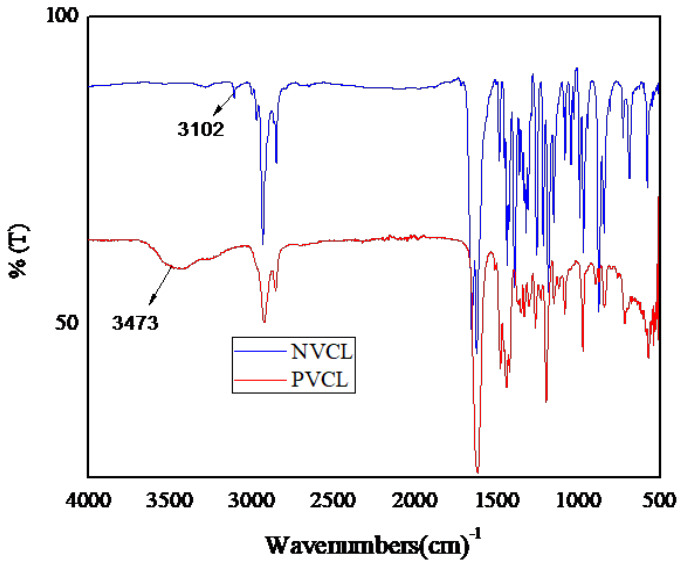


**Figure S4**. FT-IR spectra of NVCL and PVCL.

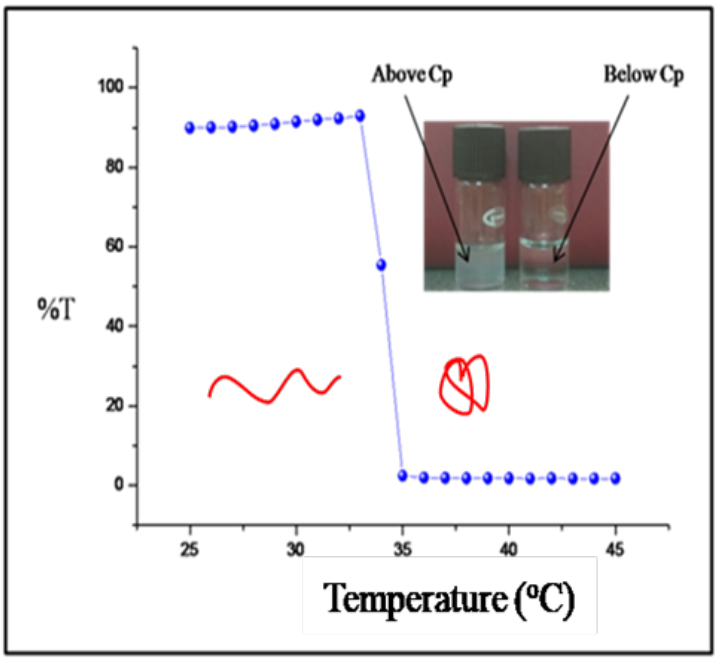


**Figure S5**. Temperature dependent transmittance of PVCL in DI water at 10 mg/ mL

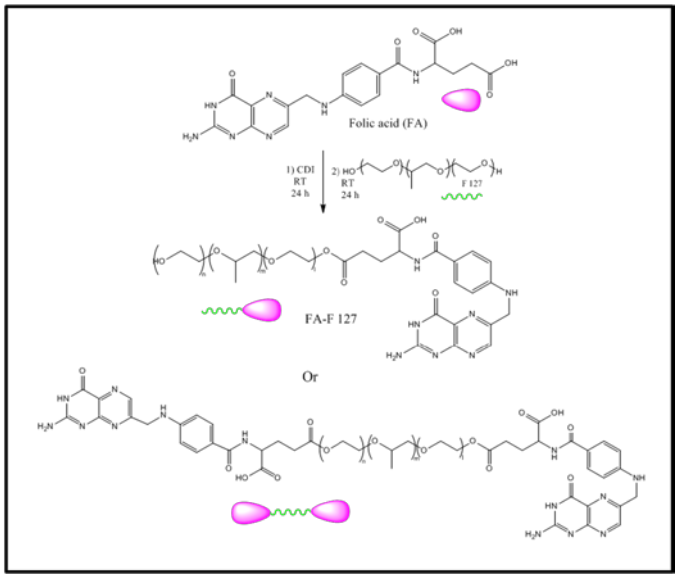


**Figure S6.** Scheme for the synthesis of folic acid-F127 conjugate

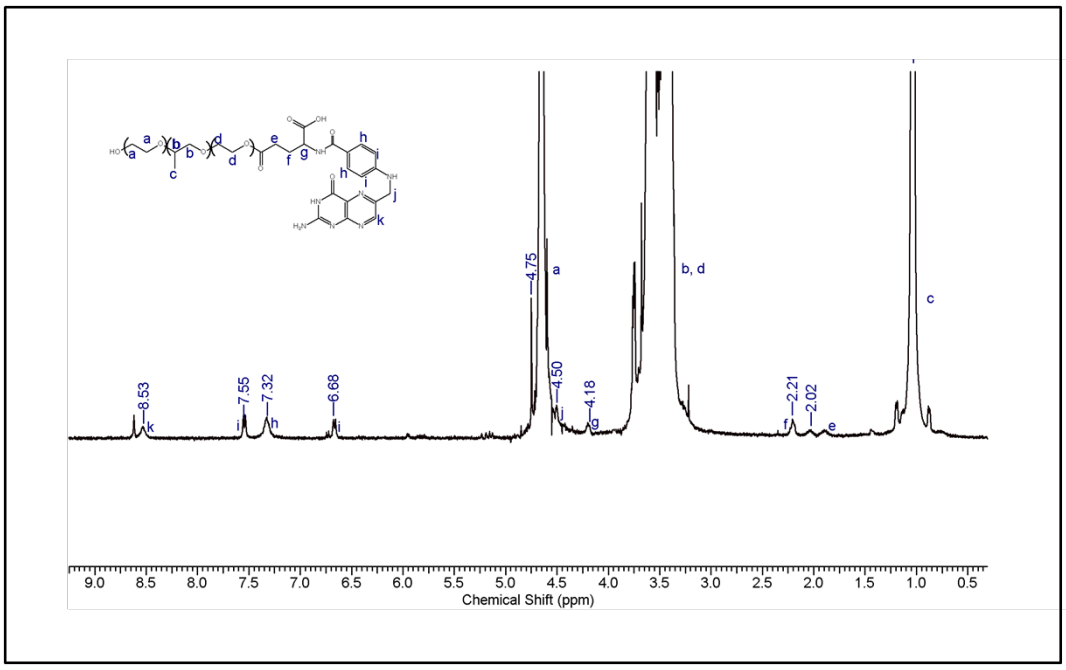


**Figure S7.** 400 MHz $^{1}$H NMR spectrum of FA-F127 in DMSO-$d_6$

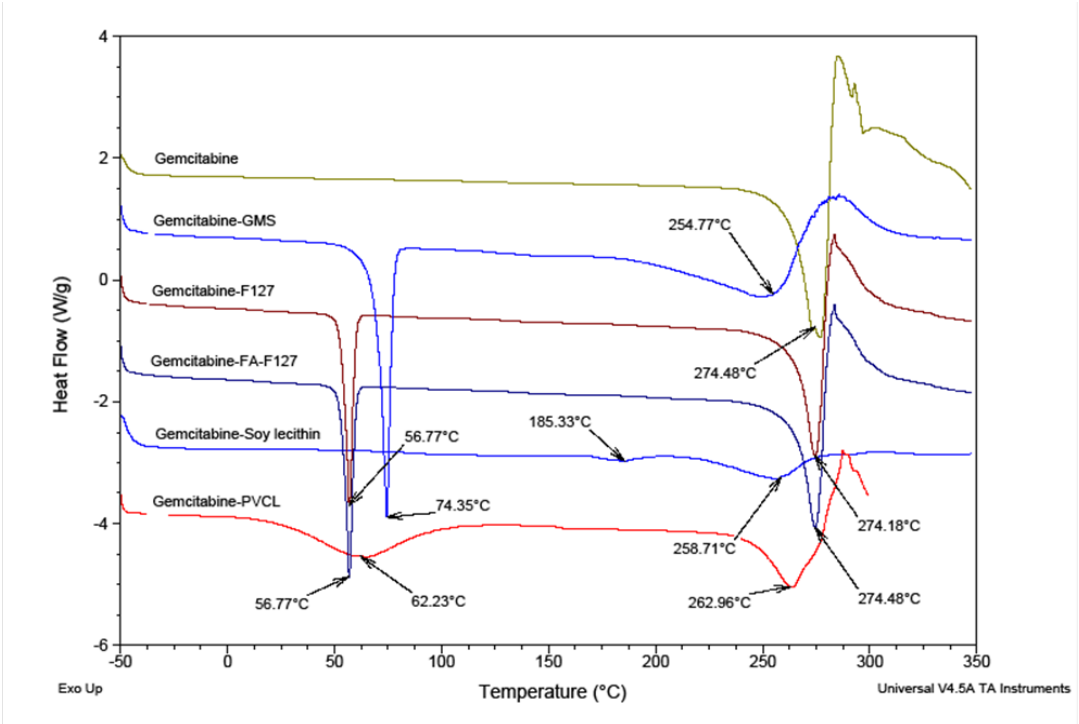


**Figure S8.** DSC curves of the drug-excipient physical mixture

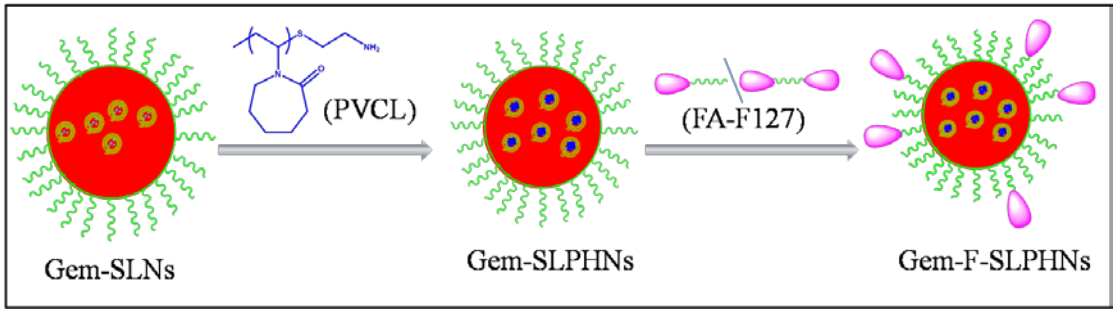


**Figure S9.** Schematic illustration of structure of nanoparticles formed.

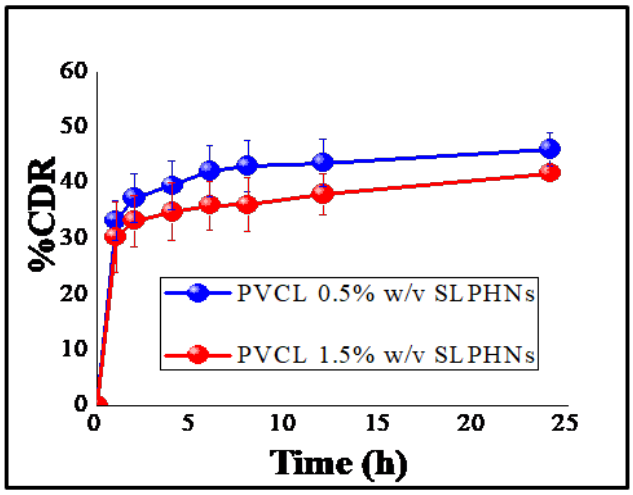


**Figure S10.** *In-vitro* drug release from SLPHNs at 37 $^{o}$C with increasing the concentration of PVCL

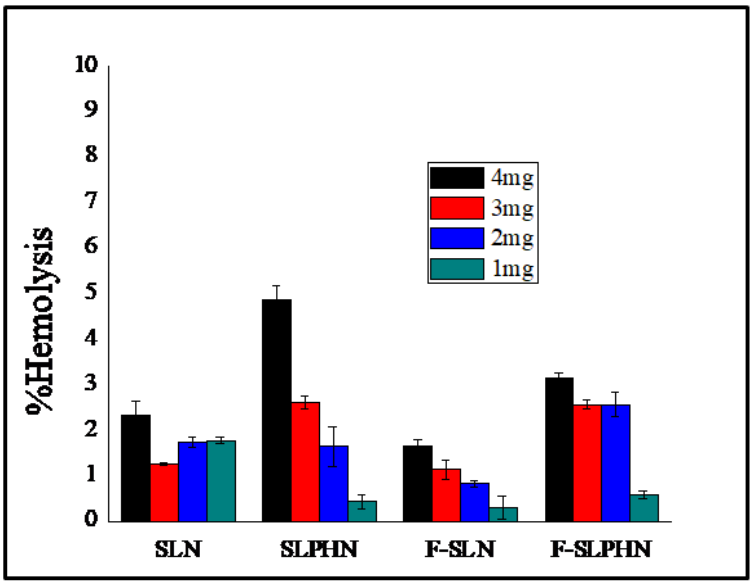


**Figure S11**. %Hemolysis of formulations at different concentrations

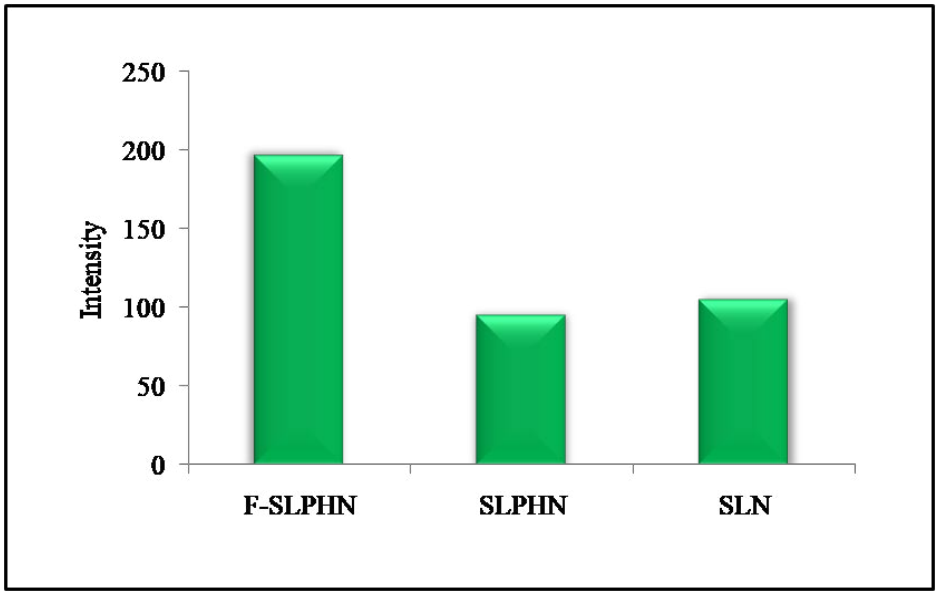


**Figure S12.** Fluorescence intensity of dye loaded SLNs, SLPHNs and F-SLPHNs inside the cells.

**Table S1.** Linearity coefficient and 'n' values of the formulations.

| Formulations | Zero order | First order | Higuchi | Peppas | n value |
|---|---|---|---|---|---|
| SLN | 0.705 | 0.628 | 0.726 | 0.835 | 0.108 |
| SLPHNs | 0.569 | 0.733 | 0.871 | 0.968 | 0.133 |
| F-SLPHNs | 0.908 | 0.919 | 0.974 | 0.941 | 0.078 |