Fully Supramolecular Vesicles as Anticancer Drug Delivery Systems
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Experimental

Materials and methods
Glycidol was purchased from Aldrich. Sodium methoxide, ethanol, acetone, tetrahydrofuran, methanol and cation-exchange resin were purchased from Merck. β-cyclodextrin was purchased from Fluka. Linear polystyrene (Mn=16000) was prepared by ATRP method.

Characterization
Infrared (IR) experiments were performed using a Nicolet 320 FT-IR. Ultraviolet (UV) spectra were recorded on a Shimadzu (1650 PC) scanning spectrophotometer. Ultrasonic bath (Model: 5RS, 22 KHZ, Made in Italy) was used to disperse materials in solvents. The particle size, polydispersity and zeta potential of materials were determined using Dynamic Light Scattering (DLS) (zetasizer ZS, Malvern Instruments). Molecular weights were determined using Knauer Gel permeation chromatography (GPC) equipped with Smartine Pump 1000. Surface imaging studies were performed using atomic force microscopy (AFM) to estimate surface morphology and particle size distribution. The samples were imaged with the aid of Dualscope/Rasterscope C26, DME, Denmark, using DS 95-50-E scanner with vertical z-axis resolution of 0.1 nm.

Thermogravimetric analysis (TGA) were carried out in a thermal analyzer (model: DSC 60, shimadzu, Japan) under dynamic atmosphere of an inert gas (i.e. N\textsubscript{2}) at 30 ml/min (room temperature). The Transmission electron microscopy (TEM) LEO 912AB electron microscope with accelerating voltage of 200 kV. The HPLC system (KNAUER) was
equipped with a C18 column and a UV/visible light detector with a mobile phase of acetonitrile/water (50:50 vol.%). The column SN was XL81 (Packing:100-5-C18).

Preparation of (β-CD-HPG)
The reaction was carried out in a glass reactor equipped with a mechanical stirrer. In a typical synthesis, β-CD (0.5 g, 0.5 mmol) was first added to a methanol suspension of sodium methoxide (3 mL, 0.78g) and mixture was stirred for 1 h at room temperature for partial deprotonation of β-CD. Then methanol was vaporized using vacuum oven and glycidol (6.17 mL, 92 mmol) was added to the deprotonated β-CD gradually at 100 °C over 2 h. Mixture was stirred at this temperature for 12 h. Then it was cooled and dissolved in methanol and neutralized by filtration over cation-exchange resin. The product was twice precipitated from methanol into acetone as a viscose light yellow compound and dried using vacuum oven at 80 °C for 6 h.

Spectral characterization: IR ν\text{max}/cm^{-1}: 1047(C=OH); 1108(C-O-C); 2931(C-H); 3400(O-H).

^{1}H NMR (400MHz, δ, ppm): 3.57C_{4}(H), 3.63C_{2}(H), 3.83C_{5}(H), 3.87C_{6}(H), 3.93C_{3}(H), 5.06C_{1}(H), 3.2-3.9 (polyglycerol).

Preparation of supramolecular linear-dendritic copolymers
Polystyrene (0.1g) was added to water (50 ml) and it was sonicated for five minutes. Then β-CD-HPG (0.1 g) was added to this mixture and it was sonicated for 15 minutes and stirred for one hour. A milky homogenous solution was obtained. Solution was filtered and it was stored for further studies.

Preparation of β-CD-HPG-PS-PTX
Paclitaxel (0.01 g) was added to 2 ml of above solution and it was sonicated for 15 min and stirred for 1 hour. Mixture was filtered and a homogenous solution was obtained.

In vitro release of paclitaxel from the micelles
The pattern of drug release from the β-CD-HPG-PS-PTX was studied in phosphate buffers at pH 5 and 7.4 and 37 °C. β-CD-HPG-PS-PTX (containing 1 mg of paclitaxel)
dispersed in 10 mL of buffer was placed in a dialysis bag immersed in 100 mL of the release media, and was then placed in an incubator shaker set at 100 rpm and 37°C. At predetermined time intervals, certain volumes of the buffer in the outside of dialysis bag was remover and injected to the high-pressure liquid chromatography (HPLC).

The mobile phase consisted of a mixture of acetonitrile and water (50:50, v/v), was delivered at a flow rate of 1.0 mL/min. The column effluent at 6.2 min was detected at 227 nm with a variable wavelength detector. The calibration curve for the paclitaxel was linear over the concentration range of 0.01–0.1 mg/mL. The solvent for calibration was the mixture of acetonitrile and water (50:50, v/v).

**Result and discussion**

Comparison the IR spectra of polystyrene, paclitaxel and \( \beta \)-CD-HPG with those for \( \beta \)-CD-HPG-PS and \( \beta \)-CD-HPG-PS-PTX show that supramolecules and drug delivery systems are prepared successfully.

Appearance the characteristic absorbance bands of polystyrene (C=C), paclitaxel (C=O) and \( \beta \)-CD-HPG (OH) in the IR spectra of \( \beta \)-CD-HPG-PS-PTX confirm preparation of the micelles containing encapsulated PTX.
Fig. ESI1. IR spectra of a) polystyrene, b) paclitaxel, c) β-CD-HPG and d) β-CD-HPG-PS-PTX.

GPC diagram show 7800 molecular weight for synthesized β-CD-HPG. The monomodality of GPC diagram show that there is not homopolymer or any other impurities. Since the molecular weight of beta cyclodextrin is 1135 the molecular weight for all conjugated polyglycerols to each beta cyclodextrin is around 6700.

Fig. ESI2. GPC diagram for β-CD-HPG.

TEM images for β-CD-HPG-PS show two phases including inner and a thick shell. Inner phase is the cavity of micelles where paclitaxel molecules will encapsulate and the shell is consisting supramolecular linear-dendritic copolymers in which polyglycerol is
directed toward outside (water) and hydrophobic polystyrene is directed toward inside (far from water).

![TEM image of micelle created by self-assembly of supramolecular linear-dendritic copolymer.](image)

**Fig. ESI3.** TEM image of a micelle created by self-assembly of supramolecular linear-dendritic copolymer.

Nanoparticles of $\beta$-CD-HPG-PS-PTX are smaller than $\beta$-CD-HPG-PS nanoparticles, due to the stronger hydrophobic interactions between paclitaxel molecules and hydrophobic polystyrene inside probably.

![TEM image and photograph of milky water solution.](image)

**Fig. ESI4.** a) TEM image of micelles created by self-assembly of supramolecular linear-dendritic copolymer which are containing encapsulated paclitaxel. b) Photograph of milky water solution of $\beta$-CD-HPG-PS-PTX.

Comparison the topographic AFM images for $\beta$-CD-HPG-PS and $\beta$-CD-HPG-PS-PTX show that there is a difference between their shell and topology so that in $\beta$-CD-HPG-PS-
PTX a shell has surrounded an inner particle. Phase contrast AFM images show that \( \beta \)-CD-HPG-PS are particles with inner cavity.

**Fig ESI5.** a) and c) topographic AFM images for \( \beta \)-CD-HPG-PS and \( \beta \)-CD-HPG-PS-PTX respectively. d) and e) phase contrast images for \( \beta \)-CD-HPG-PS and \( \beta \)-CD-HPG-PS-PTX respectively.

As it can be seen there is a big difference between thermal behavior of a mixture of \( \beta \)-CD-HPG, polystyrene and paclitaxel and supramolecular \( \beta \)-CD-HPG-PS-PTX confirming strong non-covalent or supramolecular interactions between all segments of supramolecules.
Fig. ESI6. DTA diagrams for paclitaxel, $\beta$-CD-HPG-PS supramolecular linear-dendritic copolymers and physical mixture of $\beta$-CD-HPG, polystyrene and paclitaxel.