Novel Albumin Self-Assembled Liposomes for Drug Delivery Applications

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Statement of Purpose:

The fate of intravenously administered colloidal drug carriers like liposomes, nanoparticles depends upon the surface adsorption of proteins. Albumin adsorbing surfaces are found to be highly blood compatible, and it reduces macrophage activation[1]. Here we are devising a novel strategy to self-assemble albumin onto this colloidal drug carrier surface, based on receptor-ligand interactions. For that the liposomes are coated using polymeric chains (Polyvinyl alcohol) (PVA) with tethered model ligand, Diclofenac Sodium (DIC) (It is a non steroidal anti inflammatory drug, and is highly albumin specific (>99% is bound to albumin)). The PVA- DIC complex coated liposome is further coated with albumin. It is further characterized by Fourier Transform Infrared (FTIR) spectroscopy, Circular Dichroism (CD) spectroscopy and Transmission Electron Microscope (TEM).

Materials & Methods:

The PVA- DIC complex was prepared by esterification using PVA (CDH, India) (Mol. Wt. 1, 25, 000D) (8%w/v) and DIC (Ranbaxy Lab Ltd., India) (200 mg) in presence of 3ml of concentrated hydrochloric acid SD Fine, India) and acetone (SD Fine, India) (1:1v/v) under stirring at 80°C for three hours. The modified polymer was reprecipitated using acetone. Thus obtained PVA-DIC complex was studied by FTIR analysis (FTIR Impact 410 spectrometer).

The liposomes were prepared by using a lipid solution of egg- Phosphatidylcholine: Cholesterol (Sigma Chemicals Co. St Louis Mo, USA) (1:0.35) mg% w/ v in 3ml of a mixture of Cyclohexane/ n- Butanol (SD Fine, India) (5: 2) by film rehydration method using a round bottom flask.

The polymer-coated liposomes were prepared by adding (1:1) mixture of PVA- DIC 1%w/v solution to homogenized liposomal suspensions, incubated at 10 ºC for 2 h. The uncoated polymer from the liposomes was separated by ultracentrifugation at 10,000 rpm for 10 min and then it is diluted with 2ml of albumin (100 mg%w/v) solution. This solution was stored at 4-8 °C for further analysis.

The structure of the albumin bound to the liposomes was studied by CD spectroscopy (Jasco- 810 spectropolarimeter, using a 0.1cm path length quartz cuvette). The final spectrum represents in mean θ residue ellipticity. The spectral analysis was done using the algorithm K2d kohonen neural algorithm[2]. Further the surface morphology of the liposomes was studied by using TEM.

Results / Discussion:

The Figure-1 shows the immobilization of the drug onto the PVA chain. Earlier our studies from mixture of proteins revealed that the drug have high albumin specificity in the bound form [3].

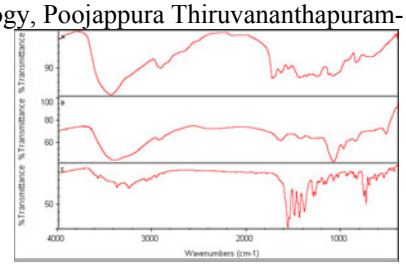

Figure-1 FTIR Spectro scopy of PVA- DIC complex. PVA (A), (PVA- DIC (B), DIC (C).

The Figure-2 demonstrates that the bound albumin shows least conformational change.

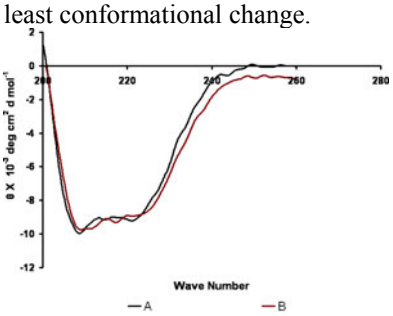

Figure-2 CD Spectroscopy of the ligand bound albumin. Albumin (A), Bound albumin (B).

The spectral analysis, table-1, also suggested the same.

Table-1 Secondary structure of the albumin molecule (%)

	Native	Bound to PVA-DIC
α	31.5	31
β	11	11
Random	57.5	58
Squaredistance (Å)	309.54	289.21

The figure-3 shows the characteristic brush border around the liposome membrane.

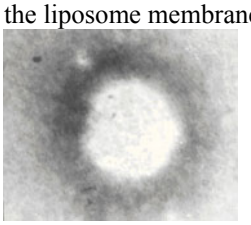

Figure-3 TEM of the albumin coated liposomes. (Mag = X 50, 000), (75nm).

Conclusions:

Our studies reveal that the DIC is covalently immobilized onto the PVA polymeric chains. This allows the ligand to expose its albumin binding domain to the exterior. The bound albumin shows least conformational change. Albumin forms a continuous self assembled coat over the liposome surface. We hypothesize here that such a system can effectively prolong the circulation time of these colloidal particles, due to the non- opsonizing nature of the self- assembled albumin in its native conformation.

References:

(1) Jenney CR, Anderson JM. J Biomed Mater Res. 2000 Mar 15; 49(4):435-47.
(2) Kaladhar, K; Sharma, CP, International Symposium on Advanced Materials and Processing; Dec 6-8, (2004)., IIT Kharagpur., India.
(3) Andrade MA, Chacon P, Merelo JJ, Moran F. Protein Eng. 1993; 6: 383- 390.