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EFFECT OF TWEEN 20 AND TWEEN 40 SURFACTANTS ON 5-FLUOROURACIL NIOSOMES

Pharmaceutics

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ABSTRACT

Niosomes (Non-ionic surfactant vesicles) have become promising alternatives to liposomes because they are more economic and chemically stable. Hence, Niosomes of 5-Fluorouracil were prepared by using Tween 20 and Tween 40, by ether injection method (EIM). Niosomes of Tween 40, which proved to be the best formulation, showed a mean vesicle size of 5µm and entrapment efficiency of Tween 20 and Tween 40 were found to be 46.40% and 39.20%. The cumulative % drug release of Tween 20 and Tween 40 were 86.41% and 92.84% respectively in 12 h. The tissue distribution studies of Tween 40 formulation reveal that the drug loaded niosomes showed preferential drug targeting to liver followed by spleen and lungs.

KEYWORDS

Introduction

To pursue optimal drug action, the functional molecule would be transported by a carrier to the site of action and release to perform their task.[1] non-ionic surfactant based vesicle (niosomes) are formed from the self-assembly of non-ionic amphiphiles in the aqueous media, resulting in closed bilayer structures.. *In vivo* niosomes behave like liposomes[3]. Niosomes are biodegradable, biocompatible, non-toxic and capable of encapsulating large quantities of material in relatively small volume of vesicles. Niosomes may reduce the systemic toxicity of anti-cancer drugs and improve the therapeutic index of drugs by restricting the effect to targeted cells[4,5,6].

5-Fluorouracil is an anti-neoplastic agent used in the treatment of malignant neoplasm of breast, colon, urinary bladder, liver, pancreas and ovary. Its oral absorption is incomplete and unpredictable. Therefore, it is administered parenterally but has side effects on bone marrow and gastrointestinal tract, due to its fast metabolism, non-specific distribution of drugs in tumour tissue as well as in normal tissue[7,10]. The present work deals with the formulation of 5-Fluorouracil niosomes by ether injection method, its characterization, entrapment efficiency, *in vitro* release and bio-distribution Studies.

MATERIALS AND METHODS

Dicetyl Phosphate was purchased from Sigma Chemicals, Mumbai. Cholesterol, Tween 20 and Tween 40 were procured from S. D. Fine Chemicals, Mumbai. All other Chemicals and solvents were of analytical or pharmacopoeial grade. 5-Fluorouracil was supplied as a gift sample by Dabur Research Foundation Ghaziabad (U.P.).

Preparation of niosomes:[4,7]

The reported method of ether injection by Baillie et al was followed for the preparation of niosomes. [4] Surfactants, Cholesterol and Dicetyl Phosphate (47.5:47.5:5) were dissolved in 20 ml of Diethyl ether and injected slowly (0.25 ml/min) into 5 ml aqueous phase (PBS pH 7.4) containing drug (5 mg/ml)[7]

Particle size analysis:

Scanning electron microscopy, using JEOL JSM-330A scanning microscope was used for particle size analysis. Pictures of niosomes were taken by random scanning of the stub.[8] The diameter of about 30 niosomes was measured from the photomicrograph of each batch. And finally, average mean diameters were obtained.

Entrapment efficiency:

The entrapment efficiency of niosomes was determined by the dialysis method. A known amount of niosomal suspension was exhaustively dialyzed by placing it inside the tube and suspending it in phosphate buffer saline (PBS) solution pH 7.4 with constant stirring of the external phase.[7] The receiver solution was completely withdrawn and replaced with fresh phosphate buffered saline pH 7.4. At each schedule time interval, the vesicles were disrupted with 50% n-propan-1-ol in PBS. The entrapment efficiency was measured spectrophotometrically at 266 nm using 50%n-propan-1-ol as blank.[7,11]

In vitro release studies:

The *in vitro* release of the drug from niosomal formulation was determined by using the membrane diffusion technique. Briefly 4 ml of niosomal suspension containing known amount of drug was placed in a sac of semi permeable membrane. The sac was placed in 400 ml of PBS (pH 7.4) maintained at 37C and stirred with the help of magnetic stirrer. Aliquots (4 ml) of the release medium were withdrawn every hour and the sample was replaced with fresh PBS (pH 7.4) to maintain constant volume. The absorbance of the sample was measured at max 266 nm, after suitable dilution if necessary, using appropriate blank[7,11].

In vivo drug targeting studies:

Niosomal F2 (Tween 40) formulation was selected for the tissue-distribution study on the basis of their *in vitro* release performance. The experimental protocol was approved by the Institutional animal ethical committee. Sprague-Dawley rats of either sex weight 200-225 g were used in the study. A constant day and night cycle was maintained and they were fasted for 12 h. The animals were divided in two groups, each containing five rats. Group I received niosomal suspension equivalent to 2.5 mg of 5-Fluorouracil in caudal vein after redispersing them in sterile phosphate buffer saline solution. Group II received 2.5mg of pure 5-Fluorouracil intravenously. After 6 h the rats are sacrificed and their liver, lungs and spleen were excised, rinsed, blotted, dry and weighed. The individual organs of each mouse were homogenized in 8 ml PBS (pH 7.4) and centrifuged to obtain a supernatant solution. The supernatant of tissue homogenate was mixed with 0.1 ml of 0.25M NaH_2PO_4 Buffer and extracted with 6.0 ml of ethyl acetate. After centrifugation at 5000 rpm for 5 min, 3 ml of the organic layer was evaporated at 55. The residue was redissolved in triple distilled water and analyzed with by HPLC with UV detector at 280 nm.[7,9]

RESULTS AND DISCUSSION

Niosomes were prepared by ether injection method using surfactant, cholesterol and dicetyl phosphate in the molar ratio of 47.5:47.5:5. Scanning electron photomicrograph of selected formulation is shown in slide no 1 and 2.

Different magnifications were used while taking the photomicrographs. Average particle size of non-ionic surfactant vesicles of 5-Fluorouracil were found to be 5 m, 10 m, 15 m and 20 m. Particles of F2 formulation were smooth, oval and discrete.

The entrapment efficiency was studied for both formulations of niosomes prepared by using Tween 20 and Tween 40. The amount of drug bound per 4 ml of niosomal suspension was determined in both formulations. Drug entrapment efficiency was calculated from drug content. The Drug entrapment was found to be 46.4% in F1 (Tween 20) followed by 39.2% in F2 (Tween 40).

Both formulations were subjected to *In vitro* release studies. These studies were carried out by subjecting the formulations to dialysis exhaustively against phosphate buffer saline pH 7.4. The cumulative

percent drug release of pure drug was found to be 91.3% at 3 h. while that for F1 and F2 after 12 h was 86.41% and 92.84% respectively. When compared with the pure drug formulation, the release of niosomal 5-Fluorouracil was prolonged over a period of 12 h or more. The results of cumulative drug release study were shown in table no. 2, 3, 4 and graph No.1.

Formulation F2, with high drug content, was selected for tissue distribution study. The average targeting efficiency of drug-loaded niosomes was found to be 26.16% of the injected dose in liver, 11.40% in lungs and 15.08% in spleen, whereas the concentration of pure drug was 15.52% in liver, 9.0% in lungs and 9.5% in spleen. These results reveal that the drug loaded niosomes showed preferential drug targeting to liver followed by spleen and lungs. It was also revealed that as compared to pure drug, higher concentration of the drug was targeted to the organs after administering the dose in form of niosomes. Higher drug targeting in liver and spleen as compared to lungs may be attributed to higher macrophage load in these organs and large size of liver as compared to spleen and lungs. Accumulation of drugs in lungs could be also due to physical entrapment of the niosomes. The results of tissue distribution study were shown in table no. 5, 6,7 and graph no.2.

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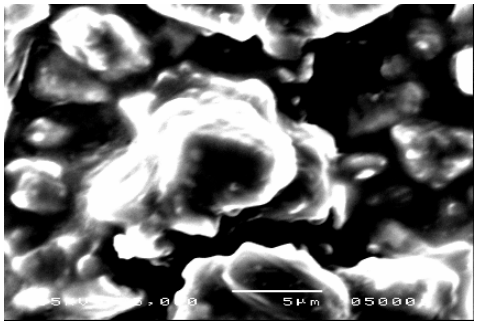
TABLE NO.1 *IN VITRO* RELEASE PROFILE FOR PURE 5-FLUOROURACIL, TWEEN 20 AND TEEEN 40.

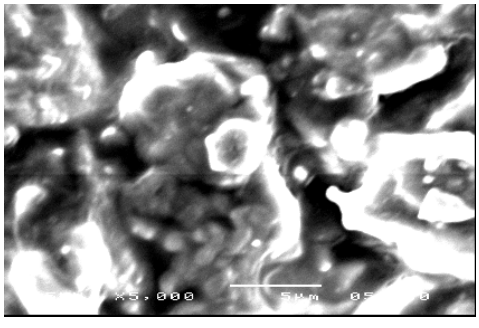
Time (min)	Drug Release (mg/ 400 ml)	CLA (mg)	Cumulative Drug Release (mg/400ml)	% Cumulative Drug Release (Free Drug)	% Cumulative Drug Release (Tween 20)	% Cumulative Drug Release (Tween 40)
30	8.16	_	8.16	40.80	18.40	20.00
60	11.04	0.081	11.12	55.60	24.15	27.4
90	12.8	0.110	12.91	64.55	42.60	51.74
120	14.4	0.129	14.52	72.64	55.62	64.51
150	16.16	0.145	16.30	81.32	68.55	77.51
180	18.08	0.180	18.26	91.30	81.48	84.77
					86.41	92.84

Where, CLA = Cumulative loss amount of drug added.
Amount of 5-Fluorouracil present in free drug solution – 20 mg

SCANNING ELECTRON MICROSCOPE (SEM) OF NIOSOMES FORMULATOINS

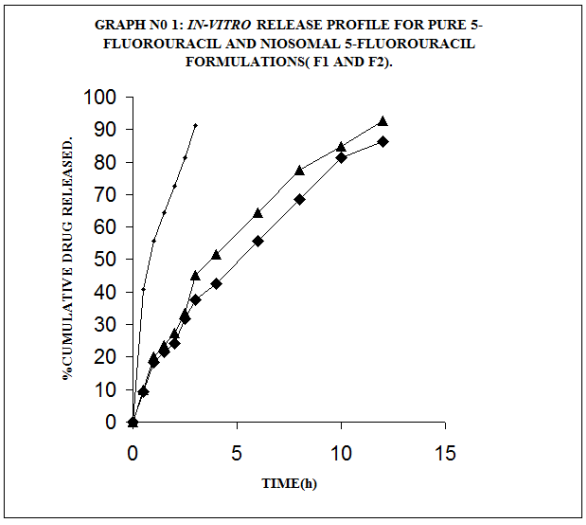


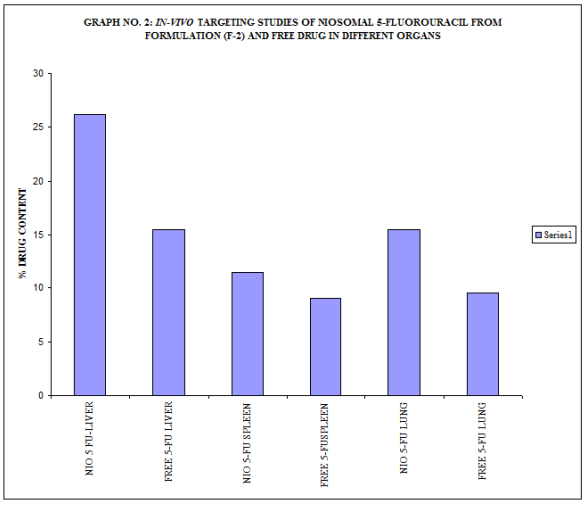
SLIDE NO: 1 F1 Formulation (Niosomes with Tween 20)



SLIDE NO: 2 F2 Formulations (Niosomes with Tween 40)

TABLE NO.1: FORMULATION PLAN OF 5-FLUOROURACIL NIOSOMES.





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