



Paclitaxel loaded transfersomal vesicular drug delivery for the treatment of melanoma skin cancers

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ABSTRACT

Over the last few years, there is tremendous growth of melanoma skin cancer in all around the world. Paclitaxel is an antineoplastic drug which is widely used for breast cancer, lung cancer, and Kaposi sarcoma. It is available only in the form of intravenous (IV), which produces tissue damage and hypersensitivity in some patients if the dose is over. In the present article, the new initiative formulation is done for melanoma skin cancer with transfersomes drug delivery systems. A different formulation of transfersomes is prepared using Span. The various characterization is done for the transfersomes like morphology, entrapment efficacy, in vitro drug release dialysis bag method. By using the Carbopol 940, the transfersomes gel was prepared. Paclitaxel-loaded transfersomes (FS-2) shows the positive entrapment efficacy 68.2% and the vesicle size 200 nm. From the in vitro dialysis bag method, FS-2 showed the high rate of drug release by nearly 72% in 12 hrs. We conclude that this will be the initial step for the further studies of melanoma skin cancer and provide the better treatment for skin cancer.



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INTRODUCTION

Skin cancer is the most dangerous leading cause of death worldwide. The term melanoma indicates aggregation lumps of melanin-forming cells in the surface of the skin majorly develops in the melanocyte. According to WHO, nearly 132,000 people get affected by melanoma skin cancer every year ([American Cancer Society, 2017](#)). In USA, 9320 people get affected by melanoma skin cancer

every year of that 5990 male and female 3330. The major cause of this cancer is due to overexposure of the UV radiation (ultraviolet radiation) from the depleted ozone layer. Unlikely other cancer, the melanoma skin cancer is readily visible. It can be easily identified and treated at an early stage. The notion of prevention of this cancer is very important because it has the capacity to spread all over the body once it reaches the dermis layer. The major factors which cause melanoma skin cancer are chemical carcinogens, a radioactive substance, x rays and phenotype susceptibility, and immunosuppression ([Kricker et al., 1994](#)). Paclitaxel is from the Taxol family, where it is widely used to cure various cancers like breast cancer, ovarian cancer, lung cancer, and Kaposi sarcoma. Paclitaxel is available only in the form of IV route according to BCS classification it falls under the category IV low (solubility and low permeability) ([Chavda et al., 2010](#)). Paclitaxel is mostly irritant to a patient who takes through a vein, and it is having a huge chance of the tissue damage. Paclitaxel has other major side effects if the dose is exceeding inpatients like hypersensitivity

reaction, low blood count, and peripheral neuropathy (Myers Squibb Bristol, 2010). The paclitaxel has no other dossier form except Intravenous route (IV). For melanoma skin cancer, a new approach aimed to improve through the transdermal drug delivery system. In transdermal drug delivery system, various techniques are used; one of the techniques is vesicular drug delivery. In vesicular drug delivery system, the lipid plays the major role in taking the drug to the site of action. The new concept has been introduced by Cevc and Blume in 1992, ultra-deformable vesicle or transfersomes. The transfersomes are composed of phospholipids, Edge Activator (EA), and alcohol. Transfersomes majorly works on the principle osmotic gradient (Elsayed et al., 2006). In transfersomes edge activator, Span, or Tween, plays a vital role in taking the drug across the stratum corneum to the cancer cells (Badran et al., 2012). In this present study, the concept is to formulate a transfersomal gel of paclitaxel for better penetration across the stratum corneum to melanocyte.

MATERIALS AND METHODS

Paclitaxel (PTX) from NACTO-Pharma. Ltd, Phosphatidyl-choline (PC) from SISCO Research Lab.Pvt.Ltd. Ltd, Ethanol from Changshu Hong sheng Fine, Chloroform from SISCO Research Lab.Pvt.Ltd. Ltd, Sorbitan monooleate (Span 80).

Preparation of Transfersomes

Transfersomes were processed by a conventional rotary vacuum evaporator (Cevc et al., 1997). The major part of the preparation is choosing the accurate ratio between surfactant and Phosphatidyl-choline (95:05%w/w, 90:10 w/w, 85:15% w/w, 80:20% w/w PC: surfactant). In a clean round bottom flask, the pure drug was dissolved completely at first with a small quantity of chloroform from the ratio. Then the lipid mixture was dissolved in a solvent mixture of chloroform and ethanol (2:1 v/v). By rotating the rotary flask at 80 rpm and maintaining the temperature about 50 for 1 hr shown in Figure 1. After getting the thin film, it is allowed to dry overnight to remove the remaining traces of organic solvent. The phosphate buffer solution pH-6.8 is prepared to hydrate the lipid film by rotating the flask at 30 rpm for 40 min at room temperature. Then it is kept in room temperature for 1 hr to form multilamellar vesicles. The thick milky suspension is then kept in a high-speed homogenizer (Ingenieurburu cat) for three cycles each 10 min to get the transfersomes vesicles completely.

Evaluation of Transfersomes

Vesicle Size and Morphology

The Transfersomes solution is diluted with a phosphate buffer solution at first then subjected to vesicle size and zeta potential by using Malvern zeta sizer Nano.

Entrapment Efficacy

The transfersome preparation was kept overnight at room temperature, and 10ml of the solution were ultra-centrifuged at 30,000 rpm for 35 min. Then the supernatant solution was collected separately (if necessary, dilution is done). The sample was analyzed for drug content at 229 nm by ultraviolet, visible spectroscopy (UV) (UV-1700 series, Shimadzu). The entrapment efficacy was calculated by using the formula

In-vitro Drug Release Using Dialysis Bag Method

The *in vitro* drug release study was carried in a phosphate buffer solution (pH-7.4) using dialysis bag technique (Nounou et al., 2006). Prior to the experiment, the bag is thoroughly equilibrated in the dissolution method for 8 h, then 10ml of the transfersome solution is transferred into the dialysis bag, and both ends were properly tied. The dialysis bag was suspended in 40ml of phosphate buffer solution and continuously maintained at room temperature at 37 ± 1 shown in Figure 2. The phosphate buffer solution was rotated at 200 rpm by using magnetic stirrer. The aliquots solution 1ml was analyzed for drug release in the frequency interval of time (2,4,6,8,10 and 12 h) by using UV-spectrophotometry at 229 nm and replaced with 1ml of fresh pH-7.4 solution which is maintained at 37 ± 1 . The beaker/receptor compartment was closed with aluminum foil to prevent the evaporation loss from the medium.

Preparation of the Transfersomal Gel

The transfersomal gel is prepared by using the ingredient Carbopol 940. The 1g of Carbopol 940 is taken separately, and 100 ml (1%) distilled water is taken in a clean beaker. By using the Remi laboratory stirrer by setting 700 rpm, the Carbopol 940 was added slowly into the distilled water to mix uniformly shown in Figure 3. Then the prepared transfersome solution was added into the gel slowly and allowed to mix well completely for 10 min.

Evaluation of the Transfersomal Gel

Viscosity and pH

The transfersomal gel formulation is subjected to determine the viscosity of the gel by Brook field viscometer. The pH of the transfersomal gel was measured by a pH meter at room temperature.

Table 1: Various formulations prepared for optimizing process variables

Formulation	Composition PC: Sc (%w/w)	Vesicle size (nm)	Zeta potential	Entrapment efficiency (%)
FS-1	95:05	220	-29	65.2 %
FS-2	90:10	200	-26	68.2 %
FS-3	85:15	263	-24	58.8 %
FS-4	80:20	224	-25	46.8 %

Table 2: *In vitro* drug release data performed using dialysis bag

Time (h)	Percentage of drug release (%)			
	FS-1	FS-2	FS-2	FS-4
0	0	0	0	0
2	15	19	18	16
4	29	33	31	24
6	46	50	42	40
8	58	60	55	48
10	60	68	61	55
12	67	72	64	64

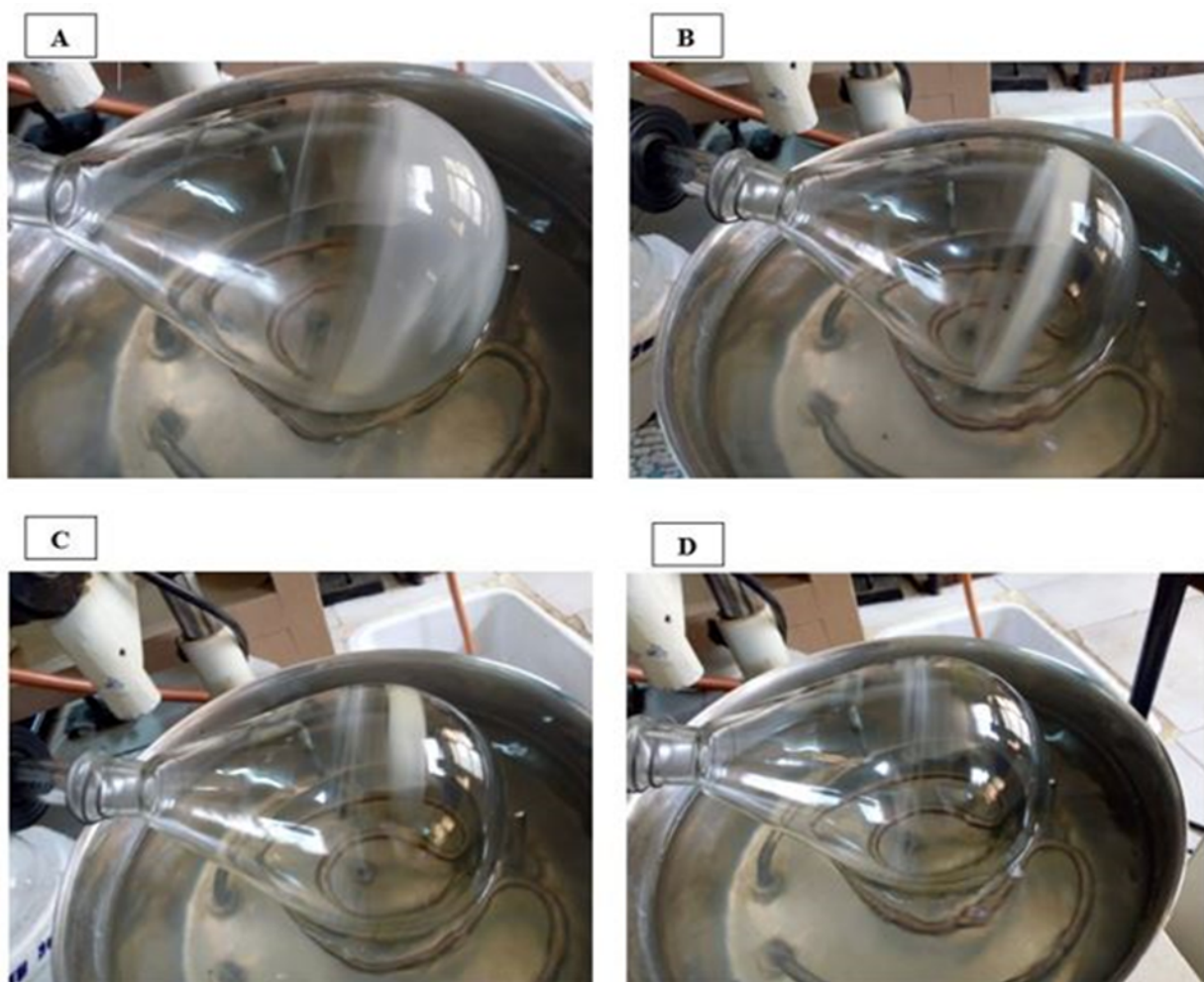


Figure 1: Preparation of Transfersomal Formulation by rotary vacuum evaporator

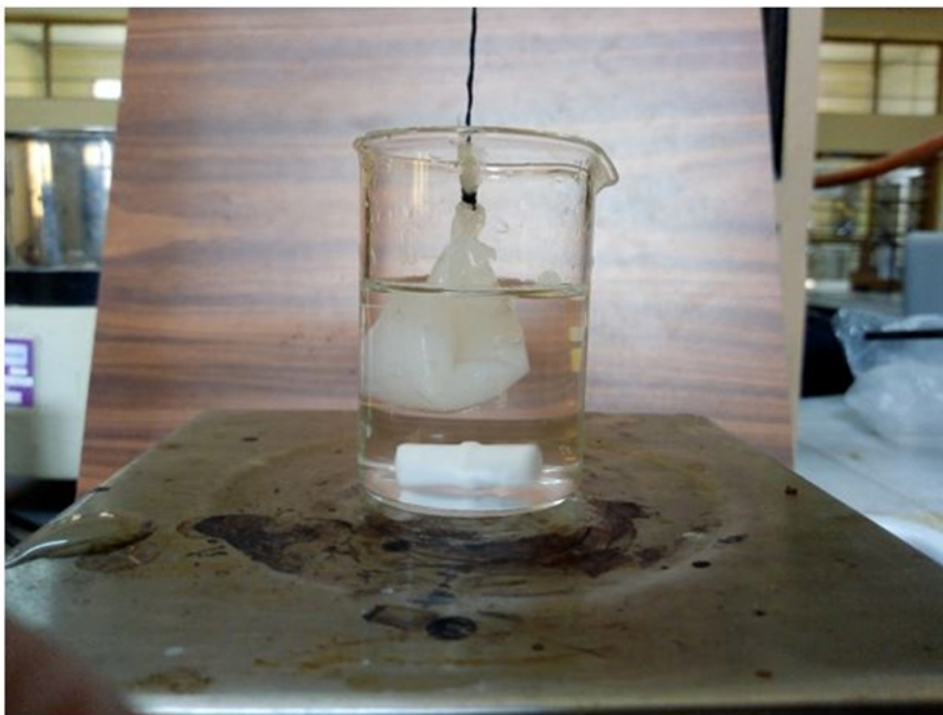


Figure 2: Drug release from Transfersomal formulation through dialysis bag



Figure 3: Preparation of Transfersomal gel

RESULTS AND DISCUSSION

Preparation of Transfersomes

Transfersomes were prepared by the rotary evaporation sonication method to yield the multilamellar vesicles. This technique has the ability of high entrapment efficacy. The formulated transfersomes FS-1-FS-4 are white in color. The formulation is optimized by using various ratios of the ingredients, but FS-2 was found to be most appropriate. The amount of the Paclitaxel added in the formulation

was 1% w/v. Then the FS-2 formulation subjected to the various evaluation process.

Evaluation of Transfersomes

Vesicle Size and Morphology

The drug-loaded transfersome FS-2 showed a balanced ratio of increased phosphatidylcholine and surfactant. The morphology of the transfersomes is shown in Figure 4. The Span-80, which has the HLB value of 4.3 in a combination of the Phosphatidylcholine, which helps to form the multilamellar vesicles. The vesicles size was found in the range of 220-

Results

	Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 92.07	Peak 1: 200	100.0	196.3
Pdl: 0.432	Peak 2: 0.000	0.0	0.000
Intercept: 0.669	Peak 3: 0.000	0.0	0.000
Result quality : Good			

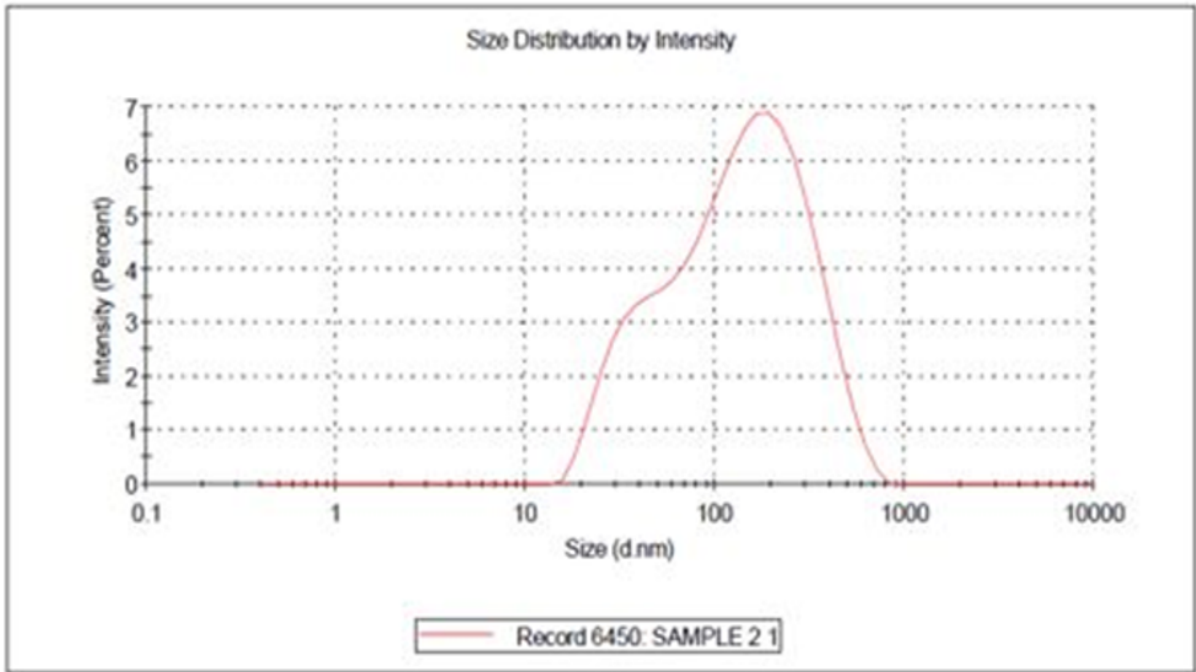


Figure 4: Particle size analysis

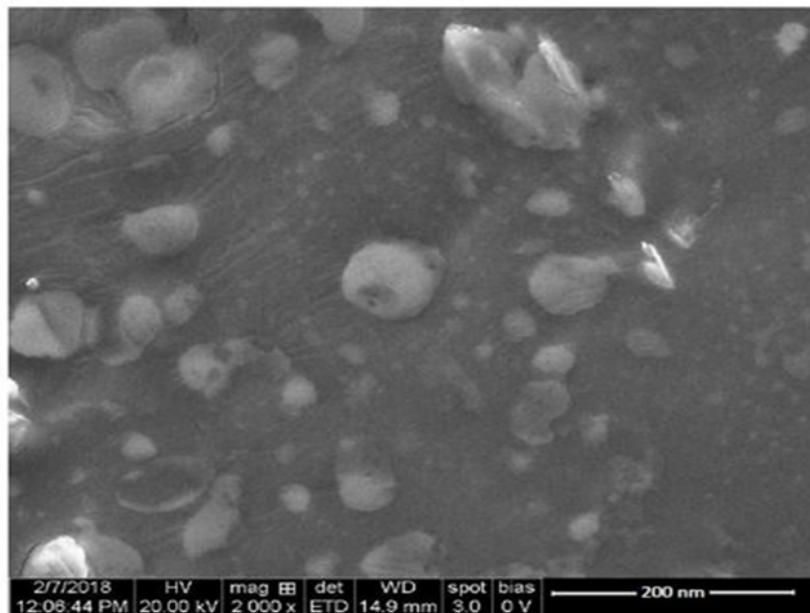


Figure 5: SEM Image

Results

	Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -26.0	Peak 1: -26.0	100.0	6.19
Zeta Deviation (mV): 6.19	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.0422	Peak 3: 0.00	0.0	0.00
Result quality : Good			

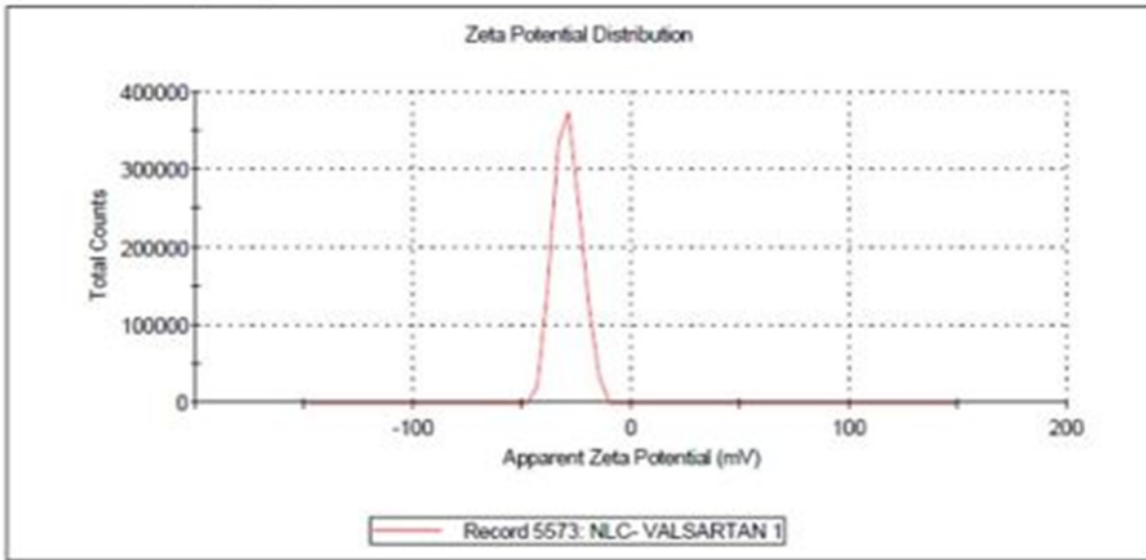


Figure 6: Zeta potential

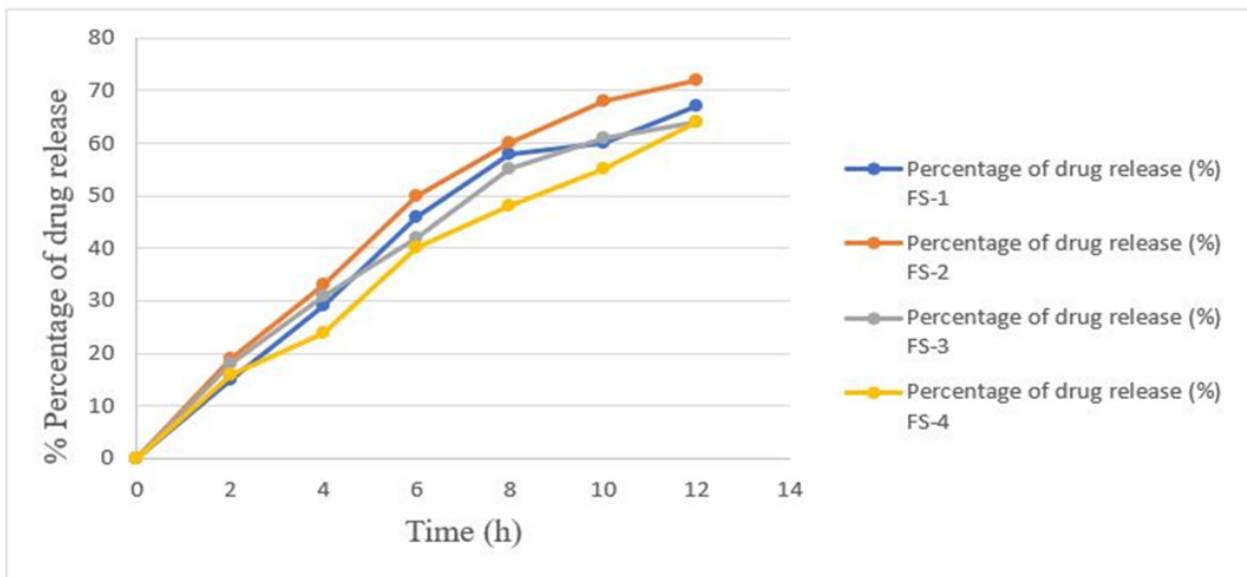


Figure 7: In vitro drug release dialysis bag

263 nm shown in Table 1. The FS-2 shows an average globule size of 200 nm with PI 0.432 shows the unimodal distribution Figure 5. The zeta potential of the transfersomes showed the negatively charged vesicular surface. The zeta potential of the FS-2 formulation showed -26 mV shown in Figure 6.

Entrapment Efficacy

The term entrapment denotes the amount of the paclitaxel bounded into the transfersome formulation. The span 80 plays a major role in the entrapment efficacy shown in the Table 1. The various concentration ratio was used in the four formulations the entrapment of the paclitaxel in the transfersome formulation ranges from 46.8% - 68.2%, were FS-2 shows 68.2% has higher entrapment efficacy due to the ratio (90:10% w/w). Majorly HLB plays a vital role in the effect on entrapment efficacy of the drug.

In-vitro Drug Release Using Dialysis Bag Method

The drug release from the dialysis bag method was carried out by suspending the transfersomal solution in a pH-7.4 buffer solution under the constant magnetic stirring and maintaining constant temperature 37 ± 1 . The paclitaxel-loaded transfersomal solution was measured in the frequency interval of time (2,4,6,8,10 and 12 h) by UV spectrophotometer at 229 nm. The four formulations FS-1, FS-2, FS-3, and FS-4, were kept in the dialysis bag to find which transfersomal formulation shows a better release of the drug Table 2. That FS-2 shows 72% of drug release, which comparatively higher than the other three formulations shown in Figure 7.

Evaluation of Transfersomal Gel

The viscosity of the paclitaxel-loaded transfersomal gel was determined using Brookfield viscometer. The viscosity ranges from 4890-4952 cps. The pH of the gel by using the FS-2 formulation was pH-6.8 neutral, which is acceptable to avoid the risk of irritation upon the application on the surface of the skin were skin pH is 5.5.

CONCLUSION

In this study, a brief investigation was carried out on the paclitaxel-loaded transfersomes vesicular drug delivery system for melanoma skin cancer. The transfersomes was formulated and subjected to various evaluation studies. The result shows that surfactant Span 80 plays a vital role in entrapping the drug into the small multilamellar vesical. The transfersomal invitro dialysis bag method shows a positive result, which will be the path for further studies. In *in vivo* study, there is a huge chance of drug deposition on the melanoma site due to small vesicle size. Only Ketoprofen in transfersome formulation

gained approval under the Swiss regulatory agency (Swiss medic).

Conflict of interest statement

The authors declare that there are no conflicts of interest involved in this study. The authors alone are responsible for the content and writing of the paper.

Author's contribution

Raahulan S was the lead author and synthesized the literature. Bharat Kumar Reddy. S involved in drafting the paper. Karri V V S R provided conceptual input, design, and critical revision of the manuscript. All authors read and approved the final paper.

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