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Research Article

FORMULATION AND IN *VITRO* AND SKIN PERMEABILITY EVALUATION OF DEXAMETHASONE LOADED NIOSOMAL GEL

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ABSTRACT

The aim of this work was to prepare a reservoir type transdermal delivery system with the drug core being formed of a Niosomal Gel and to evaluate the permeation. The present study was focused on the screening of dexamethasone loaded Niosomal Suspension and formulation of a Niosomal Gel. Niosomal Suspension of Dexamethasone prepared by Hand Shaking Method using Span 60, Tween 20 and Tween 80 with addition of Charge Inducer. Prepared Niosomal Suspension selected batch show following results Drug Content (98.10±0.885 %), % Entrapment Efficiency (85.27±0.476), Zeta Potential (-23.0 mV) and Invitro Drug Release (74.168± 0.551). Niosomal Gel Prepared by Carbopol 940 and characterized by Drug Content (98.730±0.259), Invitro Cumulative Drug Release (52.962 ±0.226 %). Dexamethasone released from the Plain Gel and permeated 13.499 in 8 hrs; niosomal formulations were able to delay the process up to 29.910 in 8 hrs. The skin permeation increase of dexamethasone was recorded from vesicle Gel in comparison to plain gel. The best fit correlation was found in the Zero order with the R^2 value of 0.997. Kinetic model described the release of drug in zero order release model which states the release rate from insoluble matrix is independent of drug concentration. The results indicate that noisome formulation decreased the permeability across gout skin compared with Plain Gel. Niosomal Gel storage under refrigeration showed greater stability. The results suggested that Niosomes could better promote the transdermal delivery of dexamethasone, by their ability to control drug release.

KEYWORD: *Niosomal Gel, Span, Dexamethasone, Dicetyl Phosphate, Stearylamine.*

INTRODUCTION

Niosomes are a novel delivery system, in which drug is incorporate into a vesicle. Niosomes are self-assembled vesicles mainly composed of synthetic surfactants and cholesterol in aqueous media. They are bilayer non-ionic surfactant vesicles [1]. Niosomal Vesicles act as a rate controlling membrane which regulate systemic absorption drug. Delivery of Drug though the Niosomal vesicles have many advantages as compared to conventional dosage forms because the vesicles can act as drug reservoirs [2]. Niosomal Vesicles and its Components showed greater solubility of drugs and increase transdermal absorption of lipophilic and hydrophilic drugs [3].

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Niosomal Gel is designed to develop a continuous delivery of drugs at predictable kinetics over an extended period of time in the systemic circulation [4]. In Stratum corneum Niosomes vesicles bind with aggregation, fusion and adhesion to the cell surface and provides an efficient transdermal (systemic or local) drug delivery system [5]. Niosomes deliver incorporated drug molecules into or across the skin. Stratum Corneum is an important site for the application of drugs [6]. Drugs Permeation through the skin is the base of transdermal delivery. Topical application of Niosomes was increased by formulation niosomal gel using carbomers [7]. Transdermal drug delivery having some advantages such as continuous controlled drug delivery, avoid intestinal and hepatic first-pass, avoid GI irritation, which is common associated with oral Drug delivery, and facilitate of drug target [8]. Number of drug categories is being added to the list of therapeutic agents that can be delivered

into systemic circulation, in clinically effective concentrations, via the transdermal route [9]. Drug targeting with minimum toxicity and bioavaibility has been core of development of therapeutic pathway [10]. Dexamethasone is a Corticosteroids commonly used in inflammatory condition, cerebral edema, in alcohol withdrawal syndrome, and congenital adrenal hyperplasia, cerebral malaria, especially associated with a high dose of anticancer agents, Respiratory disorders, opportunistic mycobacterial infections, skin disorders and rheumatism [11]. Dexamethasone has following properties, such as $t_{1/2}$ of 2 to 5 h, plasma protein binding about 67%, and daily dose ranging from 0.5 - 9 mg, with the associated drawbacks such as hepatic first pass effect of the drug and gastric irritation upon oral administration which can be overcome by transdermal delivery [12]. Various methodologies have been reported for increasing the transdermal absorption of drugs from formulation by employing deformable carriers, patches, ointment, Transfersomes, ocular microemulsion, Phonophoresis and Iontophoresis [13], [14].

MATERIAL AND METHOD

Materials

Dexamethasone gift sample was obtained from Alpa Lab. Indore (M.P.). Span 60 and Stearylamine Purchased from Sisco Research Lab. Mumbai, Tween 20 and Tween 80 purchased from LOBA Chemie, Mumbai, Dicetyl Phosphate (DCP) purchased from Sigma Aldrich, Mumbai, Chloroform and Methanol of Analytical grade was used.

Preparation of Niosomal Suspension

Dexamethasone containing Niosomes were prepared by hand shaking Method. The Surfactants, Cholesterol, Stearylamine and Dicetyl Phosphate in different ratio were accurately weight and dissolved in Chloroform: Methanol (1:1) in Round Bottom Flask (Table 1). Organic solvent was removed by Hand shaking on a water bath at 40°c to form a thin film on the wall of flask. Thin film dry overnight then hydrated with PBS (7.4) containing drug and sonicated in Bath sonicator for 5 min. Then niosome suspension transferred into Container and stored in refrigerator at 4°C [15].

Table 1: Composition of Niosomal Suspension

Formulations	Drug (mg)	Cholesterol (mg)	Surfactant (mg)	Dicetyl Phosphate (mg)	Stearylamine (mg)	Chloroform: Methanol (ml)
T80(F1)	50	50	25	5	-	1:1
T80(F2)	50	50	50	-	5	1:1
T80(F3)	50	50	75	-	-	1:1
S60(F4)	50	50	25	5	-	1:1
S60(F5)	50	50	50	-	5	1:1
S60(F6)	50	50	75	-	-	1:1
T20(F7)	50	50	25	5	-	1:1
T20(F8)	50	50	50	-	5	1:1
T20(F9)	50	50	75	-	-	1:1

Preparation of Niosomal gel and Plain Gel

1% W/V Carbopol 940 swells in distilled Water with the help of Vortex Shaking and allowed to hydrate 3-4 h. 2ml of niosomal suspension was added into weighed quantity of Carbopol 940 base and properly mix by Mechanical Stirring. Glycerine, Methylparaben was added. Then Triethanolamine was added to adjust pH. Niosomal gel was sonicated for 15mins and kept overnight to remove air bubbles. Equivalent to 20 mg of drug was added into Carbopol 940 base and properly mixes by Mechanical Stirring then same process follows as Niosomal Gel [14], [16].

Characterization of Niosomes and Niosomal Gel

Vesicle Shape

The vesicles shape was measured by scanning electron microscopy. Take the small amount of Niosomes suspension in covered with cover slip on the specimen stub. Coat it with carbon and then with gold vapor using vacuum evaporator. The samples are examining under scanning electron microscope, and then photograph it [17], [18].

Entrapment efficiency

Dexamethasone (5 mg) containing niosome Dispersion are centrifuged at 5,000 rpm for 90 minutes at 4ºC using a refrigerated centrifuge then separate unentrapped drug from niosomes. After further dilution concentration of free drug in supernatant layer was determined at 240 nm using UV-Visible Spectrophotometer (Shimadzu UV-1800) against blank solution. The percentage of drug entrapment in niosomes was calculated by using the following formula. [19]

$$\%\ Entrapment\ efficiency = \frac{Total\ Drug - drug\ in\ supernatant\ liquid}{Total\ drug} X\ 100$$

Determination of Zeta Potential

The zeta potential of the selected niosomal formulation was determined at 25ºC by using Zeta Sizer (Malvern Instruments). Niosomes were diluted 100 times with double-distilled water and voltage was set at 50 or 100 V and electrodes place in dispersion for the measurement of zeta potential [20], [21], [22].

Evaluation of niosomal, Plain gel

Physical appearance

The physical appearance of the prepared gels was determined by visual observations after the gels have been set in the container and presence of any aggregates was tested [23].

Homogeneity

The Homogeneity of the prepared gels was determined by visual observations after the gels have been set in the container [23].

Clarity

The clarity was determined by visual inspection under white and black background [24].

Viscosity

Viscosity of Niosomal Gel was determined by Brookfield Viscometer (DV-E viscometer INC, USA). 10g of gel was taken into a beaker and the spindle was dipped into the gel formulation, viscosity of the gel formulation was measured by rotating the spindle (s96) at 10 rpm [24].

pH

Accurately weighed 10 mg gel dispersed in 10 ml of distilled water. Electrode of pH meter placed in beaker. The pH of the dispersion was measured by using a digital pH meter (DPH-115, Kota) [25].

Spreadability

1g gel was placed in 1 cm of premarked circle on a glass slide over which a second glass slide was placed. A weight of 10 g is allowed to rest on the upper glass slide for 5 min. The increase in the diameter due to spreading of the gels was determined using formula [26].

$$S = \frac{mXL}{t}$$

Where, **S**: Spreadability, **m**: mass of the gel formulation, **L**: length travel by upper slide, **t**: time.

Extrudability

The formulation was filled into collapsible aluminum tubes. The tubes were pressed to extrude the 0.5 cm ribbon of the gel in 10 second and the Extrudability of formulations is checked [26].

Drug content

1 gm gel was taken and dissolved in 100 ml of phosphate buffer of pH 7.4. The volumetric flask containing gel solution was shaken for 1hr on mechanical shaker in order to get complete solubility of drug. This solution was filtered and estimated spectrophotometrically (Shimadzu-1800, Japan) at 240 nm using phosphate buffer (pH 7.4) as blank [26]. The percentage of drug content was calculated by using the following formula:

$$\%\ Drug\ Content = \frac{Sample\ Absorbance}{Standard\ Absorbance} X \frac{Standard\ Dilution}{Sample\ Dilution} X\ 100$$

In vitro release studies

Membrane diffusion technique was used for the study of Dexamethasone release from Niosomal Gel. The Niosomal Gel equivalent to 1mg of Dexamethasone was placed in a Dialysis bag was tied with glass tube which acts as a donor compartment. The glass tube was placed in a beaker containing 50 ml of phosphate buffer (pH 7.4), acting as a receptor compartment. The whole assembly was fixed in such a way that the lower end of tube containing gel was just touching (1-2 mm depth) the surface of diffusion medium. The temperature of receptor medium was maintained at 37 ± 5^0C and was agitated at the speed of 50 rpm using magnetic stirrer. Aliquots of 3 ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analyzed at 240 nm in UV spectrophotometer (Shimadzu UV-1800) using phosphate buffer (pH 7.4) as blank [23].

Drug release kinetic studies

It was used to investigate the possible mechanisms of Dexamethasone released from the prepared Niosomal Gel, the release data were analyzed mathematically according to different kinetic models such as, zero order kinetics, first order kinetics, Higuchi's model, Korsmeyer-peppas model and Hixson Crowell model [24].

In vitro permeability study

The permeability study was carried out on dorsal skin (freshly killed goat) obtained from the local slaughterhouse. First Skin was flushed with physiological solution for 2 h at room temperature. For removal of subcutaneous tissue skin was shaved using a hand razor and adhering fat was removing from the dermis layer by wiped with isopropyl alcohol. Then cleaned skin was washed with distilled water. A circular piece of skin about 3 cm diameters was placed between donor and receptor Chamber of the vertical diffusion chamber and Niosomal Gel (1 gm) was placed on the mucosal side. The whole assembly placed on magnetic stirrer and receptor chamber was filled with 55 ml PBS pH (7.4) at 37°C, speed maintained at 50 rpm. 3 ml sample were withdrawn from the receptor chamber after each withdrawal to maintain sink conditions with fresh (7.4) PBS. Samples drug content was analyzed by UV–Vis spectrophotometry (Simadzu – 1800, Japan) at 240 nm. Permeability of Plain Gel was also investigated in the same way [27].

Stability Study

Stability of Niosomal Gel was performed 1 month. In 5 gm collapsible aluminium tube sufficient quantity of the Niosomal gel was sealed and stored at room temperature (25±2◦C) and refrigerated temperature (2–8◦C). Specimen (0.1 gm) from each sample was withdrawn after one month for analysis of drug content, Physical appearance, Homogeneity, Viscosity [14], [16].

RESULT & DISCUSSION

Vesicle Shape

Scanning electron microscopy for the selected formulation F4 was carried out. The results were shown in the following SEM photographs (Fig 1).

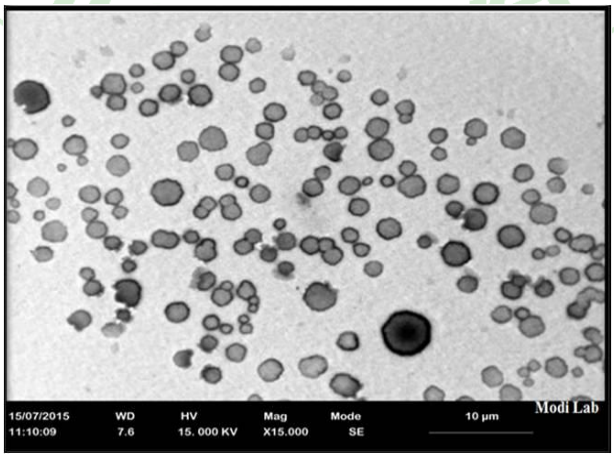

Fig. 1: Niosome (SEM)

Entrapment Efficiency

Formulation (F4 containing Span 60) was showed highest Entrapment Efficiency at 85.27±0.476.

Zeta Potential Analysis

Formulation (F4) was subjected to zeta potential analysis had a zeta value of -23.0 mV, which is a measure of net charge of niosomal suspension (Figure 2). Zeta potential magnitude gives an indication of stability. The higher charge on the surface of vesicle produces a repulsive force between the vesicles which made them stable.

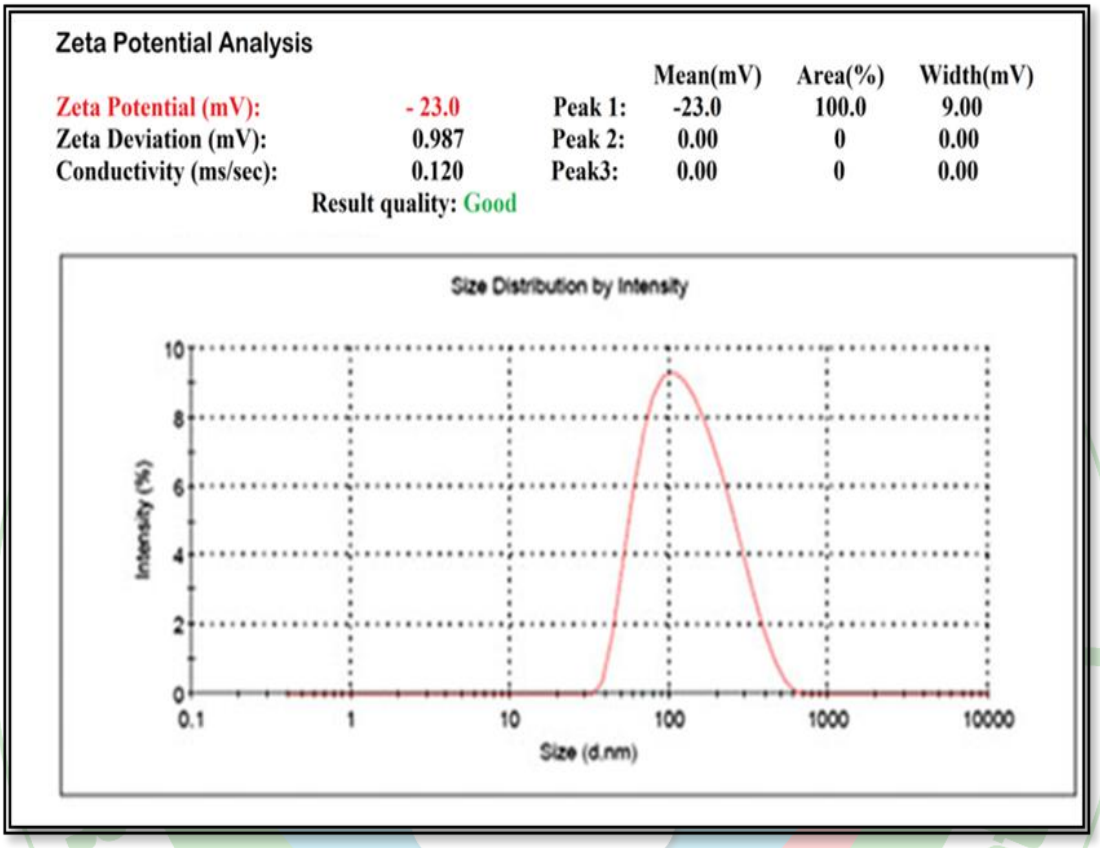

Fig 2: Zeta Potential Analysis

Evaluation of niosomal & Plain gel

Physical appearance & Homogeneity

All formulation of Niosomal gel was colourless and showed absence of lumps and having good homogeneity (Table 2).

Clarity

All gels were found to be transparent and were no presence of particles (Table 2).

Measurement pH

The pH of the Niosomal gel formulations which lies between the normal skin pH ranges (Table 2).

Viscosity

Viscosity of various Niosomal gels was found in the range of 9000 to 9880 cps (Table 3).

Spreadability & Extrudability

Spreadability diameter for different Niosomal Gel formulations showed good spreadability i.e. gel is easily spreadable. Extrudability of various formulations was satisfactory, good and excellent (Table 3).

Drug Content

Drug content of all the formulations was within the acceptable range which shows the proper mixing of the drug with the excipients (Table 3).

Table 2: Physical Appearance, Homogeneity, Clarity and pH

Formulations	Physical Appearance	Homogeneity	Clarity	pH
F1	Colourless	Good	++	7.3±0.244
F2	Colourless	Good	++	7.36±0.169
F3	Colourless	Good	++	7.33±0.094
F4	Colourless	Good	++	7.23±0.094
F5	Colourless	Good	++	7.2±0.081
F6	Colourless	Good	++	7.26±0.188
F7	Colourless	Good	++	7.3±0.216
F8	Colourless	Good	++	7.33±0.047
F9	Colourless	Good	++	7.16±0.169
Plain Gel	Colourless	Good	+++	7.23±0.876

Turbid +, Clear ++, Very Clear +++

Table 3: Viscosity (cps), % Drug Content, Spreadability (g.cm/sec) and Extrudability

Formulations	Viscosity (cps)	% Drug Content	Spreadability (g.cm/sec)	Extrudability
F1	9280±5.677	96.931±0.791	3.3±0.081	++
F2	9880±8.76	97.248±1.224	3.26±0.124	+++
F3	9000±8.564	98.730±0.259	3.33±0.169	++
F4	9440±7.658	99.15±0.395	3.23±0.205	+++
F5	9940±9.656	96.613±1.330	3.3±0.081	++
F6	9690±9.465	97.460±0.897	3.3±0.081	+
F7	9280±7.576	96.402±0.395	3.36±0.262	++
F8	9280±8.656	97.142±1.187	3.26±0.169	++
F9	9000±12.87	97.566±0.910	3.1±0.081	+
Plain Gel	9280±4.867	98.73± 0.259	3.23±0.205	++

\+ Satisfactory, ++ Good, +++ Excellent

In vitro release studies

Dexamethasone After incorporation of Niosomal vesicles into gel base formulations with Span 60, Tween 20 and Tween 80 show significant reduction in *Invitro* drug release in 8 hours compared with Niosomal suspension Formulations. This confirms that a sink condition for Dexamethasone release was accomplished and the dialysis bag used in the dissolution procedure does not limit Dexamethasone release (Fig 3).

Study of drug release kinetics

The all kinetics models were represented in its respective graphs. The best fit with higher correlation was found with the Zero order with the R^2 value of 0.997. Niosomal Gel Formulation follows Zero Oder Release Kinetics (Fig 4, 5).

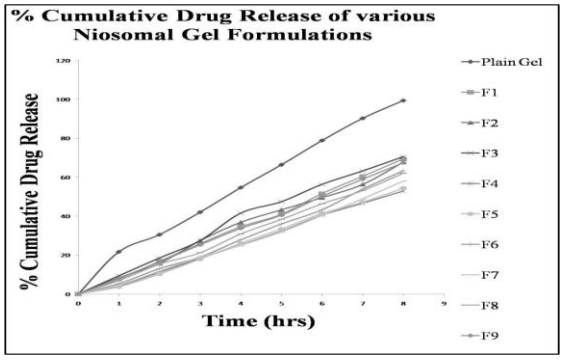

Fig. 3: % Cumulative Drug Release of Niosomal & Plain Gel

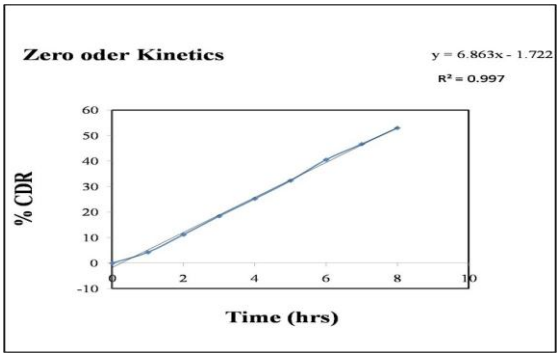

Fig. 4: Zero Order

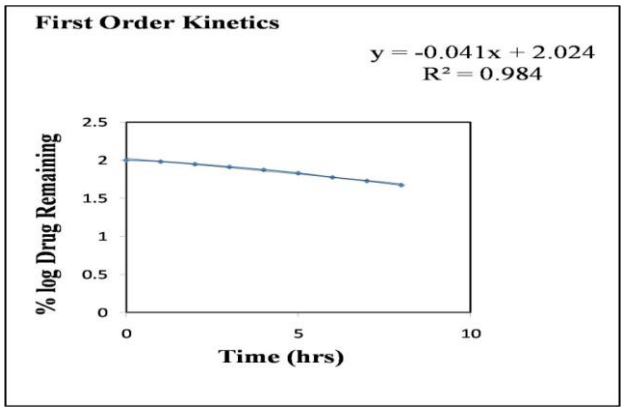

Fig. 5: First Order

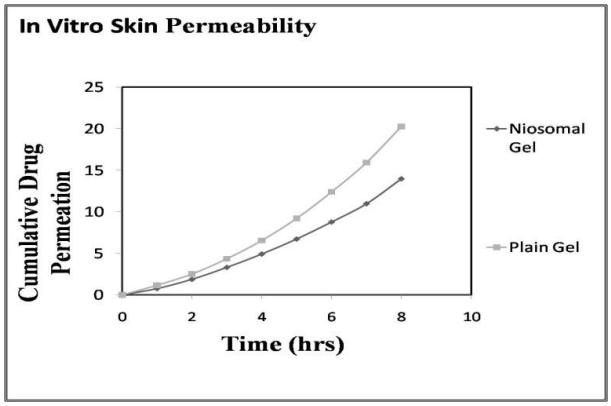

Fig. 6: % Cumulative Drug Permeation

In Skin permeability study

Skin permeation of the Dexamethasone in the form of Niosomal gel and plain Carbopol gel was evaluated using Franz diffusion cell.

The Cumulative mount of drug permeated per unit area was plotted as Function of time, and the steady state permeation rate (Jss) were calculated from the slope and Intercept of the linear portion, permeability coefficient. Dexamethasone released from the Plain Gel Jss (µg/h.cm^2) 2.656±0.578, permeability coefficient. (KP) (cm.h^{-1}) 0.00159±0.057 and Diffusion Coefficient (D) (cm^2.h^{-1}) 13.499*10^{-5}±0.487 in 8 hrs; niosomal formulations were able to delay the process up to Jss (µg/h.cm^2) 1.812±0.986, KP (cm.h^{-1}) 0.00181±0.894 and D (cm2.h^{-1}) 29.910*10^{-5}±0.568 in 8 hrs (Fig 6). The results indicate that noisome formulation decreased the permeability across gout skin compared with Plain Gel.

Stability Study of Niosomal Gel

The stability of niosomal Gel was carried out for 1 month. There is no evident for aggregation, fusion or disruption of the vesicles during the study period of 1 months and it was found that the prepared formulations were able to retain their multilamellar nature. Thus it was found that storage under refrigerated condition showed greater stability [Table 4].

Table 4: Effect of different storage temperature conditions on drug Content (F4)

Parameters	Initial	4^{0}C	Room Temp.
Colour	Colourless	NC	NC
pH	7.23±0.09	7.3±0.216	7.16±0.04
Clarity	Clear	Clear	Clear
Homogeneity	Good	Good	Good
%Drug Content	99.153±0.395	99.153±0.395	98.095±0.685

But in both the storage conditions drug content was found to be within the specification of 95-105% throughout the study period of 1 month (Fig 10).

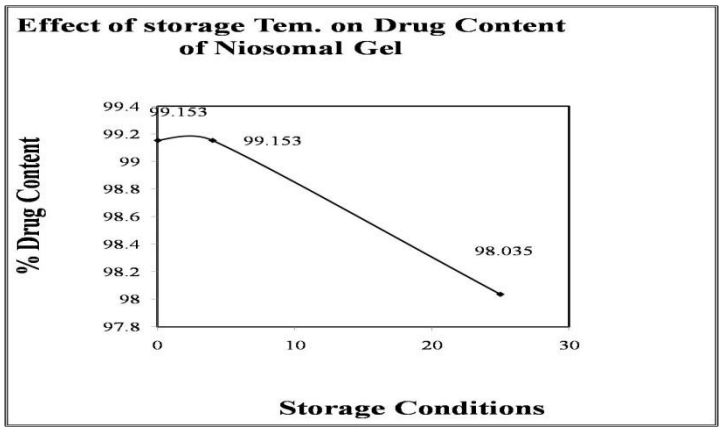

Fig. 7: Drug Content after 1 month

CONCLUSION

Results of the present investigation it may be concluded that formulation F4 was showing high Entrapment Efficiency and desired sustained release of Dexamethasone. Increase surfactant concentration leads to decrease in entrapment efficiency of the formulation while cholesterol content was constant. Formulation (F4 containing Span 60) was showed highest Entrapment Efficiency at 85.27±0.476. Dexamethasone After incorporation of Niosomal vesicles into gel base formulations with Span 60, Tween 20 and Tween 80 show significant reduction in *Invitro* drug release in 8 hours compared with Niosomal suspension Formulations. The best fit correlation was found in the Zero order with the R^2 value of 0.997. Kinetic model described the release of drug in zero order release model which states the release rate from insoluble matrix is independent of drug concentration. Dexamethasone released from the Plain Gel and permeated 13.499 $cm^2.h^{-1}$ in 8 hrs; niosomal formulations were able to delay the process up to 29.910 $cm^2.h^{-1}$ in 8 hrs. The results indicate that noisome formulation decreased the permeability across gout skin compared with Plain Gel. Niosomal Gel storage under refrigeration showed greater stability. But in both the storage conditions drug content was found to be within the specification of 95-105% throughout the study period of 1 month. The results suggested that Niosomes could better promote the transdermal delivery of dexamethasone, by their ability to prolong drug release.

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