INTERNATIONAL RESEARCH JOURNAL ON ADVANCED SCIENCE HUB
e-ISSN : 2582 - 4376
Open Access
RSP SCIENCE HUB
(The Hub of Research Ideas)
Available online at www.rspsciencehub.com

Development and Evaluation of Niosomal Gel for Transdermal Application of steroidal API

Sharma Rupali [1], Diwan Anupama[2], Sardana Satish[3], Sharma Shekhar[4], Vyas Amisha[5]

[1]Associate Professor, Amity Institute of Pharmacy, Amity University, Amity Education Valley, Manesar Panchgaon, Gurugram-122413-Haryana.

[2]Director, School of Pharmaceutical Sciences, ApeejayStya University Sohna Palwal Road, Gurugram - 122018, Haryana.

[3]Director, Amity Institute of Pharmacy, Amity University, Amity Education Valley, Manesar Panchgaon, Gurugram-122413-Haryana.

[4,5]Assistant Professor, School of Pharmaceutical Sciences, ApeejayStya University Sohna Palwal Road, Gurugram -122018, Haryana.

rsharma9@ggn.amity.edu[1]

Abstract

Niosomal drug delivery system serves as drug depots within the body that release the drug in a controlled manner through its bilayer, providing the enclosed drug to be released with sustainedaction.Transdermal drug delivery system for steroidal drug molecules through the development of a niosomal gel is needed for an optimized drug delivery system due to various problems associated with conventional treatment of steroidal molecule. The objective of this study was to develop a niosomalgel-based formulation system for testosterone steroidal molecule dissolved in a mixture of non-ionic surfactant and cholesterol in nanoparticulate form and evaluation of niosomal gel by different optimization parameters. The niosomal gel dispersion was prepared by heating system technique.The drug loaded niosomes showed much less vesicle size [10 - 500 nm] and good PDI, which means that the drug loaded can easily permeate the skin. Niosomal based gel was formulated by using the xanthan gum as gelling agent by optimizing different concentration of different gelling agents to get the best consistency of final niosomal gel. Various measurement parameters such as product quality, pH, purity, homogeneity, spread ability, viscosity, In vitro drug release, particle size determination, zeta potential, FTIR studies and TEM analysis were done to optimize the best batch.Entrapment efficiency was very good with value of 92.17 ± 0.02 percent. Niosomal gel has been found to exhibit strong consistency, good homogeneity, spread ability, and viscosity parameters.Studies of FTIR showed no excipient interaction with the API molecule. TEM images showed that all the particles were in uniform range in niosomal dispersion.Data on the release of in vitro drugs also showed that the release pattern was comparable to the industry formula. The niosomalbased gel formulation developed can be a promising alternative to delivering steroidalbased molecule to minimize the side effects due to skin problems as well as to increase the permeation rate of steroids by using transdermal drug delivery.

Keywords: Niosomal Gel, Steroid, Dispersion, Particle size

1. INTRODUCTION

Niosomes are non-ionic surfactants with multi-lamellar vesicles obtained on hydration of synthetic non-ionic surfactants, with or without incorporation of sterol such as cholesterol or other lipids. Recently niosomes have been extensively studied for their potential to serve as a carrier for the delivery of drugs, hormones, antigens and other biologically active drugs. It has also been used to solve the problem of insolubility, instability and rapid degradation of

drugs. The presence of the steroidal system in the niosomes improves the rigidity of the bilayer, affect the bilayer fluidity and permeability andprotect the drug molecules from the degradation due to some unwanted biological effects. Mainly, Niosomes are preferred over liposomes because the former exhibit high chemical stability and economy. In general, niosomal drug delivery has been studied using various routes of administration including intramuscular, intravenous, peroral and transdermal [1].

Niosomes serve as drug depots in the body which release the drug in a controlled manner through its bilayer providing sustained release of the enclosed drug. Targeted drug delivery can also be achieved using niosomes the drug is delivered directly to the body part where the therapeutic effect is required. Thereby reducing the dose required to be administered to achieve the desired effect [2]. The therapeutic efficacy of the drugs isimproved by reducing the clearance rate, targeting to the specific site and by protecting the encapsulated drug. Drug targeting reduces the dose which leads to subsequent decrease in the side effects. The vesicular system in the niosomes make them capable off encapsulating hydrophilic and lipophilic substances. Hydrophilic drugs are usually encapsulated in the inner aqueous core or adsorbed on the bilayer surfaces, while lipophilic substances are entrapped by their partitioning into the lipophilic domain of the bilayers [Figure 1][3].

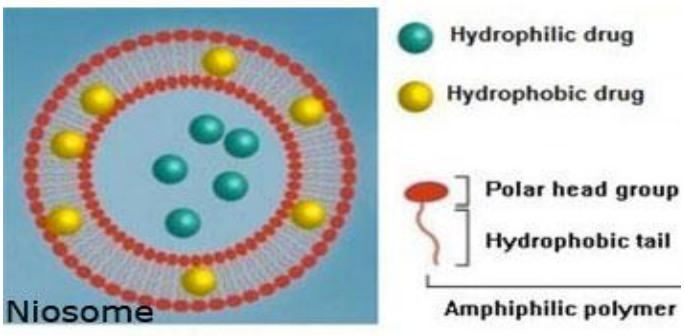


Fig.1. Schematic representation of a niosomal vesicle

The formation of vesicular assemblies requires the input of some form of energy, and all the experimental methods investigated involve hydration of a mixture of surfactants above the gel to liquid phase transition temperature of the system, followed by optional size reduction to obtain a colloidal dispersion. Because of their potential ability to carry a variety of therapeutics, these vesicles have been widely used as drug delivery systems to achieve drug targeting, controlled release, and permeation enhancement. In fact, niosomes can act as therapeutic reservoirs for delivery of a drug in a controlled manner to enhance bioavailability, obtaining a therapeutic effect over a longer period of time, and can be modified by altering the composition, concentration of various additives, and surface charge of vesicle components and membrane additives. Moreover, drug ionization has been found to modulate the physicochemical properties of the vesicles and their percutaneous permeation profiles.In recent decades, niosomes have been investigated in-depth as potential carriers for sustained and targeted drug delivery, since they are easily derivatized to enhance vesicles versatility to improve the affinity for the target site. Surfactant forming niosomes are biodegradable, non-immunogenic and biocompatible. Incorporating them into niosomes enhances the efficacy of drug, such as nimesulide, flurbiprofen, piroxicam, ketoconazole and bleomycin exhibit more bioavailability than the free drug. [4,5]

1.1 Advantages of Niosomes:

The niosomal drug delivery is a potential drug delivery method for controlled and targeted drug delivery, the major advantages of these vesicular drug carriers are[6].

- Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external nonaqueous phase.

- As comparison with oily dosage forms, niosomes offers high patient compliance because, niosomal vesicle suspension is water –based vehicle.
- They are osmotically active and stable, as well as they increase the stability of entrapped drug.
- Handling and storage of surfactants requires no special conditions.
- They improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs.
- They can be made to reach the site of action by oral, parenteral as well as topical routes.
- The surfactants are biodegradable, biocompatible and non-immunogenic.
- They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.
- Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non-aqueous phase.
- They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.[4]

At the same time, disadvantages associated with niosomes areless shelf life, and small physical and chemical instability, aggregation, fusion of vesicles, and leaking or hydrolysis of the encapsulated drug. Moreover, the methods required for preparation of multilamellar vesicles, such as extrusion or sonication, are time-consuming and may require specialized equipment for processing.

Transdermal drug delivery being more popular in last few years and niosomal use has been studied in several disease models, and more efforts are being done on optimization of procedures, new compositions, and final formulations. As, new highly flexible niosomes, known as elastic vesicles, have been proposed and are reported to be effective at delivering molecules through the skin, since edge activators [ie, ethanol] provide vesicles with elastic characteristics, which allow them to penetrate more easily into the deeper layers of the skin. Moreover, the drawback only associated with liquid nature of niosomes because when applied they may leak from the application site. So due to keeping this drawback in mind every researcher having an aim to formulate a niosomal based gel system for transdermal drug delivery. Niosomal gels system enhance retention of therapeutic agent on the skin by providing high and sustained drug concentrations in the skin. Now a day'sinnovation in niosomes is represented by proniosomes or "dry niosomes", which have been proposed as niosomal formulations; these need to be hydrated before use, and hydration results in formation of an aqueous niosomal dispersion. Proniosomes decrease the aggregation, leakage, and fusion problems associated with traditional niosomes and offer a versatile transdermal drug delivery system because, upon application to the skin, they becomehydrated with water from the skin under occlusion.[5]

The objective of this study was to develop a niosomal based gel drug delivery system for testosterone for transdermal application, which enhances drug absorption through skin and minimize the side effects of drug also. Due to low oral bioavailability of model drug molecule i.e testosterone, niosomal gel system was developed because of good penetration power of niosomes through skin and more stable than any other transdermal drug delivery system. Because of amphiphilic nature of niosomes, Testosterone water insoluble drug molecules was easily entrapped in niosomes.

2. Materials and Methods

Testosterone was obtained as a gift sample from Martin & Harris Pvt Ltd. India. All the chemicals and cholesterol and surfactant were purchased from Sigma Aldrich and of analytical grade.

2.1 Pre- formulation Study:

Pre-formulation studies was done to optimize the different physicochemical properties of API molecule as well as to design and formulate the niosomal dispersion and niosomal gel. The data collected with preformulation study gave a possible no chemical and physical interaction of all excipients used for formulation the dosage form. [8]

The reformulation studies were performed by U.V.spectroscopy,HPLCandExcipient compatibility with the drug by using FTIR.

3. Formulation Development

Preparation of Niosomal dispersion

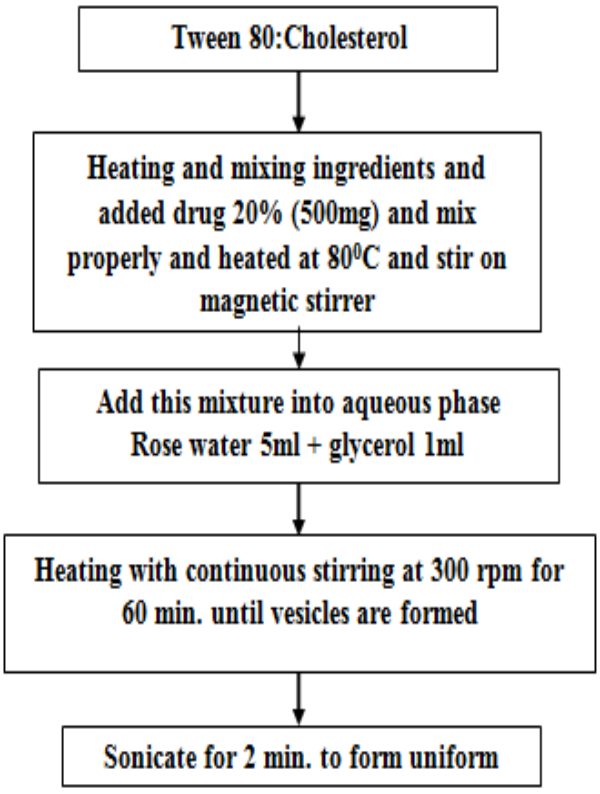


Fig.2.Formation of Niosomes by heating method

Niosomes were prepared by the heating method as shown in Fig 1 Accurately weighed quantity of drug, surfactant & cholesterol were mixed by heating at temp 800 C and then this mixture was added in the aqueous phase. The mixture was heated while stirring [at low shear forces] until vesicles are formed. Formed vesicle are sonicate for 2 min to form uniform sized vesicle. Flow Diagram of formation of niosomes shown in Fig 2

3.1 Formulation of Niosomal gel

Prepared niosomes were dispersed in distilled water and then preservatives were added in formulation. Desired xanthan gum [3%] was used for making the dispersion in gel like consistency. [9]

Niosomal gel was prepared by adding niosomal dispersion into suitable gel base like carbopol U21, carbopol 934, carbopol 974 NF and xanthan gum to optimize the best gelling agent and stable nature of final dosage form. [10]

3.2 Niosomes in Carbopol U21 Gel

Niosomal gel was prepared by adding niosomal dispersion into carbopol U21 gel base. Various batches of the gels were prepared using carbopol at varied concentrations [0.1%, 0.5%, 1% and 1.5%]. Definite amount of polymer was sprinkled into the vortex created by stirring double distilled water and stirred for 25-30 min. Gelling was induced by neutralization using triethanolamine. Niosomal dispersion was then added to the hydrated gel with stirring. [11,12]

3.3. Niosomes in Carbopol 934 Gel

Niosomes were prepared by using drug, span and cholesterol. The obtained niosomes were incorporated in carbopol-934 gel [1% w/w] base containing propylene glycol [10% w/w] and glycerol [30 % w/w].[13]

3.4. Preparation of Carbopol 974 NF Based Topical Gel:

The optimized niosomes were incorporated into a topical gel using Carbopol 974 NF. Weighed quantity of Carbopol 974 NF was dispersed in water [2% w/w]. The dispersion was stirred for 2–3 h. niosomes [equivalent to 1% w/w of drug] were incorporated into the gel base. It was stirredfor 1 h. pH was adjusted to 6.0 ± 0.05, using sodium hydroxide solution [1 N]. [14]

3.5 Preparation of Niosomal Gel by Xanthan Gum:

Optimized niosomal formulation was gelled by dispersing xanthan gum [2%w/w]. Briefly, niosomes pellet quantity equivalent to 1% w/w drug was weighed and transferred to a 50mL beaker containing 5mL of distilled water. Mixture was stirred to obtain homogenous dispersion. Gelling agent, xanthan gum, was weighed accurately and added with stirring to niosomal dispersion. Weight of mixture was adjusted up to 10 gm by adding drop by drop distilled water. After completion of addition the mixture could hydrate to its maximum capacity by keeping it at room temperature for 2 hrs. [15,16]

3.6 Evaluation of Drug loaded Niosomal gel:

The niosomal gel were evaluated for the following properties like pH, entrapment efficiency, drug content, viscosity study spreadability of gel, extrudability of gel, consistency of gel, particle size and polydispersity index of niosomal dispersion as well as gel. Most important parameters like optical microscopy, zeta potential, TEM study, in-vitro drug release study and stability study. [17]

3.7 Measurement of pH

The pH of niosomal based gel formulations were determined by digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values were calculated by using pH Meter. [18]

3.8. Entrapment Efficiency

Entrapment efficiencies of niosomal formulations were determined by centrifugation method. For this, 10 mL niosomal suspension was poured into a centrifugation tube and centrifuged by using cooling centrifuged [REMI cooling centrifuge] at 10000 rpm at 4°C for 10 min.

The clear fraction was further used for the determination of free drug by using UV/visible spectrophotometer [19]. The entrapment efficiency was calculated using the following formula: where is the concentration of total drug and is the concentration of unentrapped drug.

3.9. Drug content

Drug content of niosomal based gel formulations was optimized by mixing 1 g of the niosomal gel with 100ml of suitable solvent. Aliquots of different concentration were prepared by suitable dilutions after filtering the stock solution and absorbance was measured. Drug content was calculated using the equation, which was obtained by linear regression analysis of calibration curve.

3.10 Viscosity study

Viscosity of the niosomal gel formulations were measured by using Brookfield Viscometer. Rotations of gel are done at 0.3, 0.6 and 1.5 rotations per minute and at each speed, the corresponding dial reading was noted. The viscosity of the gel was obtained by multiplication of the dial reading with factor given in the Brooke field Viscometer catalogue. [20,21]

3.11 Spreadability

The spreadability of the niosomal gel formulations were determined by taking 0.5 g of gel. It was placed within a circle of 1 cm

diameter premarked on a glass plate over which a second glass plate was placed. A weight of 500 g was put on the upper glass plate. The increase in the diameter due to spreading of the gels was noted.[22]

3.12. Extrudability study

The niosomal gel formulations were filled in the collapsible tubes after the gels set in the container. The extrudability of the formulation was determined in terms of weight in grams required to extrude a 0.5 cm. ribbon of gel in 10 second. [23]

3.13.Consistency

The measurement of consistency of the prepared gels is done by dropping a cone attached to a holding rod from a fix distance of 10cm in such way that it should fall on the centre of the glass cup filled with the gel. The penetration by the cone is measured from the surface of the gel to the tip of the cone inside the gel. The distance travelled by the cone is noted down after 10 sec.[24]

3.14.Optical Microscopy

A drop of niosomal suspension was placed on the microscopic glass slide. Photographs of niosomes formulations were taken at 10x magnification using the digital camera [digicam 5 mega pixel] attached to the eye piece of the Nikon projection microscope. Shape and lamellar nature were confirmed with this photograph.[25]

3.15.Vesicle Size Determination and Polydispersity Index

Vesicle size and polydispersity index of testosterone niosomal dispersion and testosterone niosomal gel was determined by zeta sizer. Zeta sizer is based on principle of dynamic light scattering. Take a little amount of sample and dilute it with distilled water, then this sample was filled in cuvet and place into zeta sizer. Run the equipment and size of vesicles are plotted in a graph, peak of graph shows the value of vesicle size. The polydispersity is the ratio of standard deviation to mean droplet size, so it indicates the uniformity of vesicle size within the formulation. The higher the polydispersity, the lower the uniformity of vesicle size in formulation.[26,27]

3.16.Tem [Transmission Electron Microscopy]

A drop of the sonicated niosomal sample was placed onto a copper grid to leave a thin film. Before drying of this film on the grid, it was negatively stained with 1% phosphotungstic acid [PTA]. For this, a drop of staining solution was added onto the film and the excess of the solution was drained-off with a Whatman filter paper. The grid could air dry thoroughly and was then visualized using a Transmission Electron Microscope.[28]

3.17. In Vitro Release Studies

In vitro release pattern of niosomes dispersion and niosomal gel was carried out by dialysis bag method. A dialysis sac was washed and soaked in distilled water. The niosomal dispersion was pipette into a bag made up of tubing and sealed followed by placing the dialysis bag into a beaker containing 200 mL of PBS 7.4 The vessel was placed over magnetic stirrer [50 rpm] and the temperature was maintained at 37°C ± 0.5°C. Samples were withdrawn at predetermined time intervals and immediately replaced with the fresh medium to maintain the sink condition throughout experiment. Samples were diluted and analyzed for drug release by using UV/visible spectrophotometer [29]

3.18.Stability

The stability studies are carried out for all the gel formulation by freeze - thaw cycling. In this syneresis is observed by subjecting the product to a temperature of 4° C for 1 month, then at 25°C for 1 month, then at 40°C for 1 month. After this

gel is exposed to ambient room temperature and liquid exudates separating is noted. [30]

4. RESULTS AND DISCUSSIONS

4.1. Preparation of Standard calibration curve

The analytical wavelength and calibration data for testosterone in methanol was determined. The λ max of testosterone was found to be 241 nm, which was selected as the analytical wavelength for further analysis. The calibration curve for testosterone were given in figure 4 and figure 5.Linearity range was obtained in the concentration between 0, 2, 4, 6, 8, & 10 µg/ml.[31]

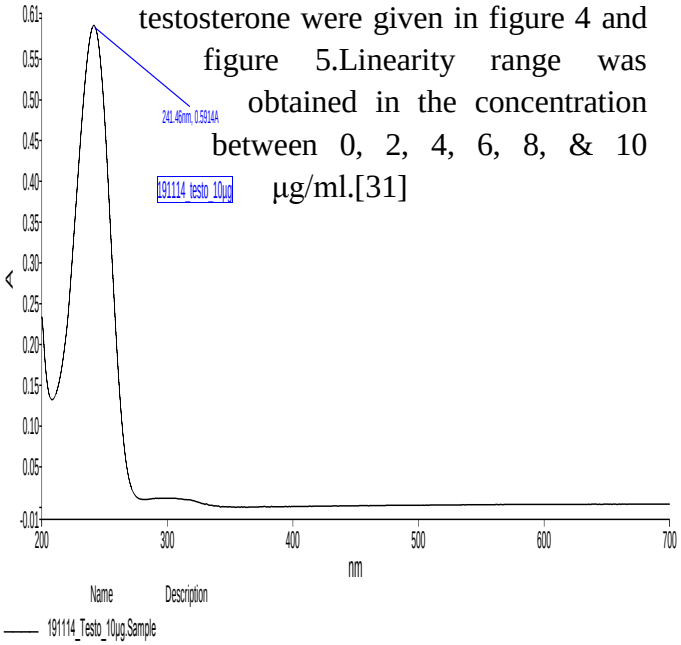


Fig. 4.UVCalibration spectra

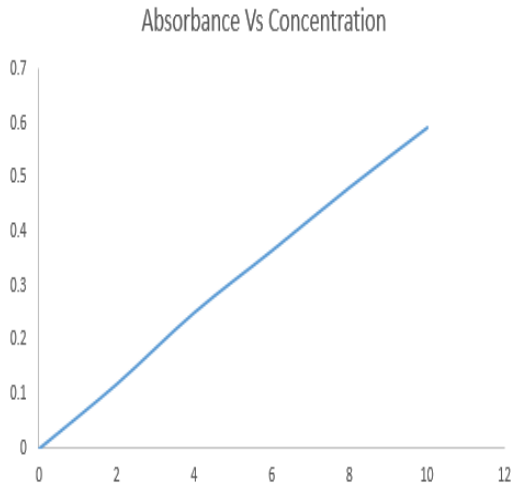


Fig. 5. Calibration curve

4.2. FTIR Spectroscopy:

The FTIR spectrum as shown in Fig 6 and 7, along with interpretation of FTIR spectrum of testosterone showed all the peaks corresponding to the functional groups present in the structure of testosterone. Spectra of drug and niosomal gel show that no interactions between the drug molecule andexcipients used for niosomal gel formulation.[32,33]

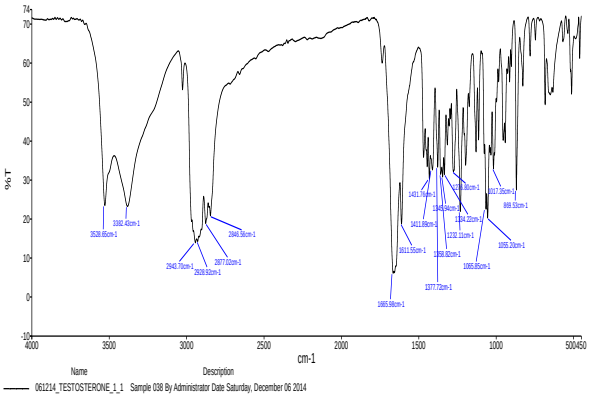


Fig. 6. FTIR Spectra of API

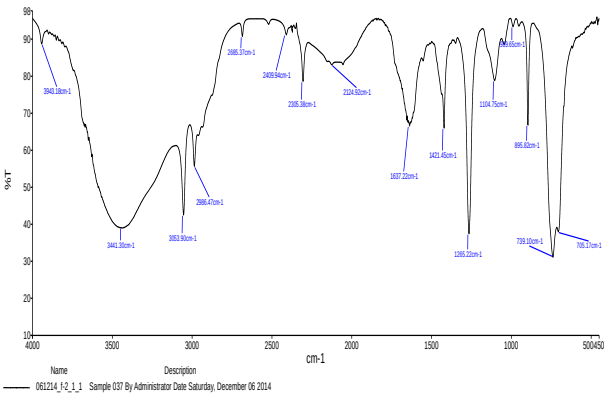


Fig. 7. FTIR Spectra of Niosomal gel

The possible interaction between drug and excipients used was studied by FTIR data. The results revealed no considerable changes in the IR peaks of drug molecule when mixed with excipients. So, there is no chemical interaction between drug and excipients. In FT-IR studies, the characteristic tautomeric testosterone C=O stretching at around 1660 cm–1 was clearly distinguishable in the testosterone loaded

formulation Additionally, O=C—H stretching of testosterone at 2871 cm–1 was also observed unchanged in formulation NTGX, suggesting no drug-excipients chemical interactions in loaded niosomal gel.

4.3.Optimization of concentration of surfactant and cholesterol for niosomal dispersion

Various concentrations of non-ionic surfactant, tween 80 was taken for niosomal dispersions while the concentration of cholesterol was kept constant. Niosomes were prepared by heating method by keeping different parameters mentioned in table 2 constant and vesicles formed were observed under microscope.[34]

Table 1: Optimization of concentration of Tween 80 and cholesterol

Formulation batch	Tween 80 [gm]	Cholesterol [gm]
TNGV 11 [1:1]	2.5	2.5
TNGW 21 [2:1]	5.0	2.5
TNGX 31 [3:1]	7.5	2.5
TNGY 41 [4:1]	10	2.5

Table 2: Different Parameters for Formulation of Niosomal dispersion

Heating Temperature	80^0C
Hydration Medium	Rose Water [5ml] + Glycerol [1ml]
Hydration Time	6 [ml]
Hydration Volume	30 [min.]
Rotation Speed of Magnetic Stirrer	100 [rpm]
Bath Sonication Time	2 [min.]

Fig.8 (a)

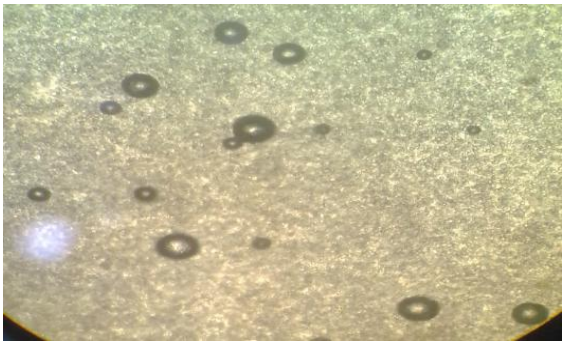


Fig.8 (b)

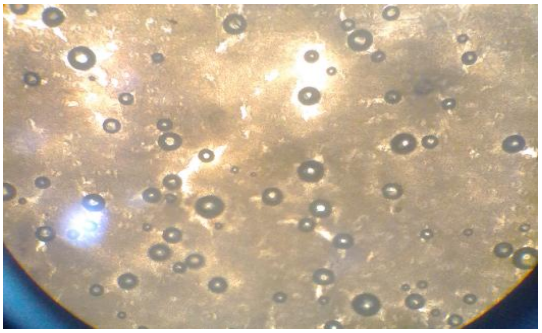


Fig.8 (c)

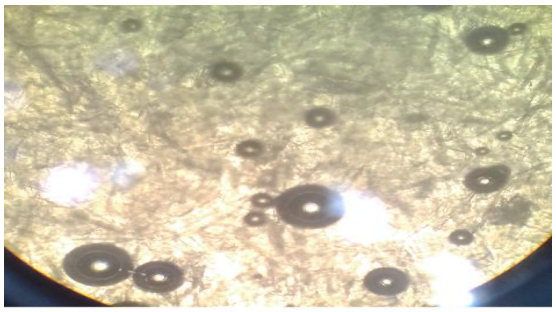


Fig.8 (d)

Fig.8(a,b,c,d). Microscopic images of Niosomes using various concentrations of surfactant and cholesterol by Nikon projection Microscope scale 10X

Four ratios of tween 80 and cholesterol was taken i.e. 1:1, 2:1, 3:1, 4:1. Niosomes were prepared keeping parameters of process like Heating temperature, Hydration medium, Hydration volume, Hydration time, Rotation speed of magnetic stirrer, Bath sonication, constant. The prepared vesicle size and lamellarity were studied by optical microscopy method. Ratio 1:1 the niosomes vesicles formed per unit area were very less, Ratio 2:1 and 4:1 spherical vesicles were seen along with aggregates and were unstable during storage, but in ratio 3:1 spherical vesicles without aggregates were obtained and number of vesicles seen per unit area were also

comparatively more and were stable during storage. TNGX 3:1 was most optimized batch, as the shape & size of vesicles were better even the yield of vesicles was also better.The concentration of tween 80 and cholesterol for TNGX 31 batch was 3:1, so 3:1 was the most optimized concentration from all the concentrations to be taken.[35]

4.4.Optimization of Process Variables[36]

A. Optimization of hydration time
B. Optimization of rotation speed of magnetic stirrer

A. Optimization of hydration time

Hydration time for formulation was selected based on microscopic observation. Hydration time was increased for Batch TNGX31. More the hydration time lead to vesicles aggregation. More the vesicles structure formed, without aggregation showed most optimized hydration time for the formulation

Table 3: Optimization of Hydration time

Formulation batch	Hydration time [min]
TNGX 31 [A][3:1]	30
TNGX 31 [B][3:1]	60
TNGX 31 [C][3:1]	120

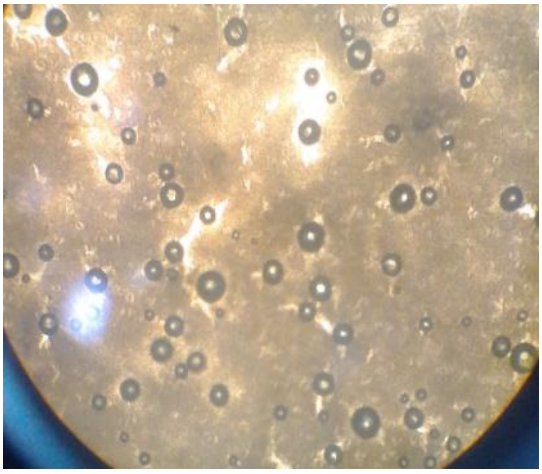


Fig.9 (a)

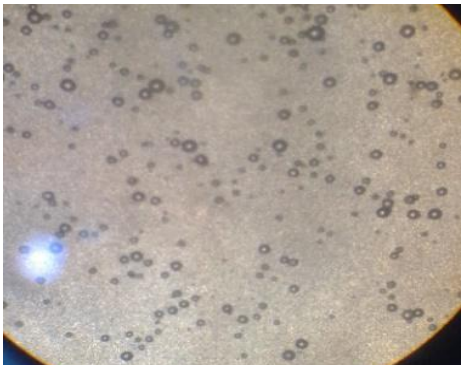


Fig.9 (b)

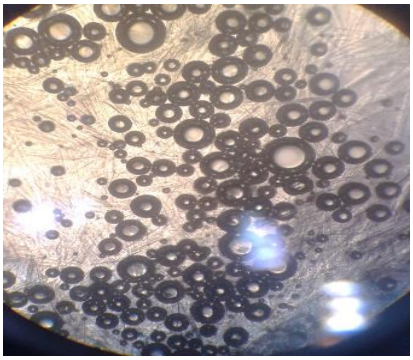


Fig.9 (c)

Fig.9 (a,b,c). Microscope Images of niosomes at different hydration time.

Three batches TNGX 31 [A], TNGX 31 [B], TNGX 31 [C], were prepared with different hydration time of 30, 60, 120 respectively. At 30 min Both Batches TNGX 31 [A] and TNGX 31 [C], the vesicle size and shape were disagreeable. However, in 60 min batch no. TNGX 31 [B], the vesicle size shape and lamellarity was most optimum. Therefore, hydration time of 60 min was optimized for final formulation.[36]

B. Optimization of rotation of speed of magnetic stirrer

By using optimized concentration of 3:1 and hydration time 60 min. rotation speed of magnetic stirrer was optimized by keeping all remaining parameters like, hydration volume, heating temperature, hydration medium, bath sonication, constant. Observe each formulation batch under microscope.[37]

Table 4: Optimization of rotation speed of magnetic stirrer

Formulation batch	Speed of magnetic stirrer[rpm]
TNGX 31 [1]	100
TNGX 31 [2]	200
TNGX 31 [3]	300
TNGX 31 [4]	400
TNGX 31 [5]	500

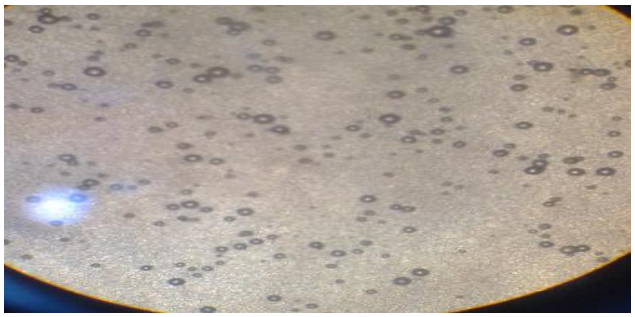


Fig.10 (a)

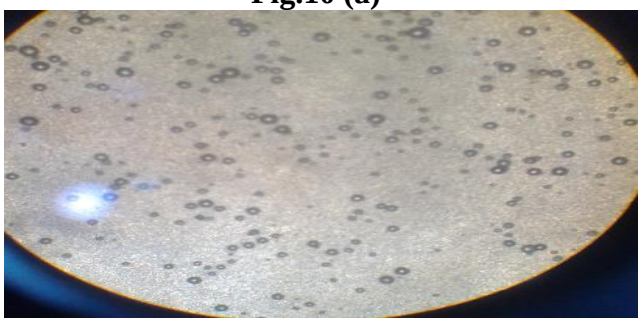


Fig.10 (b)

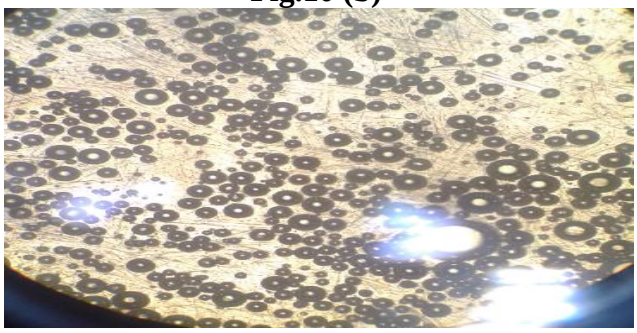


Fig.10 (c)

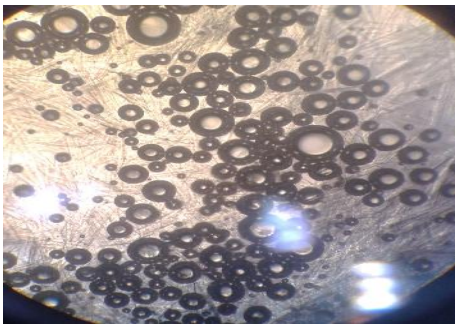


Fig.10 (d)

Fig.10 (a,b,c,d).Microscopic images of niosomes at various rpm

The objective was to optimize rotation speed of magnetic stirrer with optimized concentration of tween 80 and cholesterol, 3:1 keeping parameters, hydration volume, hydration medium, heating temperature, bath sonication, constant and by using optimized hydration time of 60 min. the various batches TNGX 31 [1], TNGX 31 [2], TNGX 31 [3], TNGX 31 [4], TNGX 31 [5], were prepared at various rpm 100,200,300,400,500. Out of various batches prepared withincrease of speed from 100 to 500 rpm, batch prepared with 300 rpm was most optimized as shown in microscopic images. It showed uniform lamellarity and vesicle size and shape under microscopic observation.[38]

TNGX 31 - Testosterone niosomal dispersion, TWN – Tween 80 , CHOL – Cholesterol , TEMP – Temperature , HM – Hydration medium , RW – Rose Water , GLY – Glycerine , HV – Hydration volume HT – Hydration time , RSMS – Rotation speed of magnetic stirrer , BST- Bath sonication time.

Table No 5: Table showing optimized Process related variable in the Formulation

S.NO.	Batch No.	TWN 80: CHOL [gm][gm]	Temp [^{0}C]	HM	HV in ml	HT in min.	RSMS Rpm	BST min
1.	TNGX 31	7.5 : 2.5 [3:1]	80^{0}C	R W + GLY [5ml][1ml]	6	60	300	2

4.5Heating Temperature

Temperature plays a major role in vesicle formation in heating method of mozafari et al., heat acts as a energy source for vesiculation, the cholesterol and tween 80 was mixed properly with drug testosterone and make a clear solution. Heating of surfactant tween 80 and cholesterol on 80^0C was increase the stability of the formulation.

4.6 Hydration medium and Time

Tween 80 formulations hydrated with mixture of 5 ml of rose water and 1 ml of glycerine formed stable vesicles. Increasing the volume of hydration medium increased drug leakage. Increasing the hydration time from 60 min to 120 min causes aggregation of vesicles.

4.7.Rotational speed of magnetic stirrer

A speed of 300 rpm yielded a uniformly spherical vesicles on hydration. Lower and higher rpm produced thick films that formed aggregates of vesicles on hydration.

4.8.Effect of Sonication Time

Spherical niosome vesicles were not observed after 5 min or 15 min sonication suggesting that ≥5 min exposure to ultrasound may damage the vesicles. 2 min of sonication produced vesicles with a uniform unilamellar structure.

4.9.Vesicle Size and lamellarity

Entrapment Efficiency

Drug entrapment of the optimized batch TNGX loaded with drug was determined at wavelength 241 nm with the help of UV spectroscopy. Entrapment efficiency was found to be 69 %, which describes an appreciable drug entrapment in niosomes vesicles.[40]

Determination of pH

Testosterone niosomes shape and lamellar structure were determined by optical microscopy Photomicrographs revealed that niosomes formulated with tween 80 and cholesterol were spherical and multilamellar. Evaluation of Niosomal dispersion and Niosomal gel

4.10.Determination of drug content in testosterone niosomal gel

Drug content was determined immediately after formation of testosterone niosomal gel. 1gm of testosterone niosomal gel contains 20mg of drug and this amount was dissolved in 100ml of methanol, the formed solution was 200µg/ml. make serial dilutions of this solution up to 10µg/ml. and check the absorbance in triplicates by UV Spectrophotometer at λmax 241 nm.[39] From table no. 6, Drug content of final formulation batch TNGX was found to be 99.21%

Table No. 6: Drug Content

Absorbance of drug molecule [10 µg/ml]	Absorbance final formulation batch TNGX [10 µg/ml]	Average
0.5914	0.5866	0.5867
	0.5868	
	0.5868	

Testosterone niosomal gel pH was observed and adjusted up to skin pH by using triethanolamine. The pH of final formulation was calculated 7.2.

4.11.Spreadability

[dish plate which shows that the spreadability of gel was good.[41]

4.12.Viscosity

Viscosity of testosterone niosomal gel formulations was determined using Brookfield viscometer.

Table 7: Viscosity of Niosomal gel

Sr.no.	Speed of rotation	Viscosity in [cp]	Torque
1.	0.5	55908	23.4
2.	1.0	38752	32.3
3.	2.0	27114	45.2
4.	2.5	24091	50.2
5.	4.0	19316	64.4
6.	5.0	17324	72.3

Viscosity calculated for Niosomal gel was 12,500 cps after calculating by factor given in Brookfield viscometer catalogue.[42]

4.13.In vitro drug release studies

Drug release of the niosomes loaded with API was carried out of PBS 7.4 and was found to be 71.9 % after 24 h. An appreciable release was observed with approximate first-order kinetics for up to 8 h and then gradually tills toward a pseudo-first-order phase.

The ability of gel formulation to deliver testosterone was examined by determining its drug release rate. Phosphate buffer [pH 7.4] was used in receiver compartment and sink conditions were maintained. Figure 11 shows the cumulative percentage release of drug from NG at different sampling intervals. After 24 h, in vitro drug release from niosomal gel was found to be 60.4%, respectively.[43,49]

4.14. Determination of vesicle size and polydispersity index.

The vesicle size and polydispersity index were measured by zeta sizer as mentioned in methods.[44]

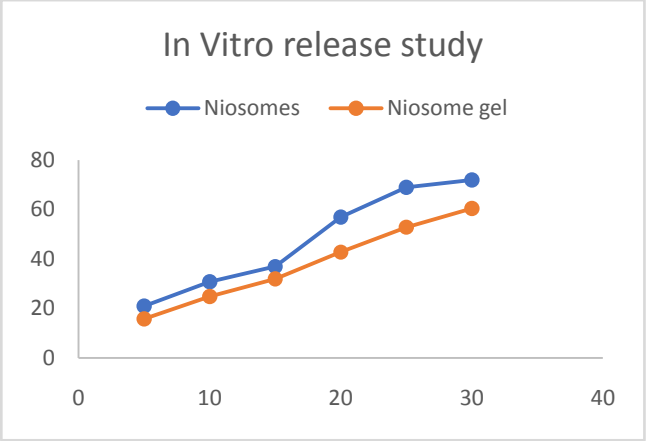


Figure 11: Cumulative percentage release Vs Time [hrs] graph

Table 8: Vesicle size and Polydispersity index of niosomal dispersion and niosomal gel

Sr.no.	Formulations	Vesicle size [nm]	Polydispersity index
1.	TNGX 31	4251	0.243
2.	TNGX	4414	0.992

TNGX 31- Testosterone niosomal dispersion, TNGX- Testosterone niosomal gel.

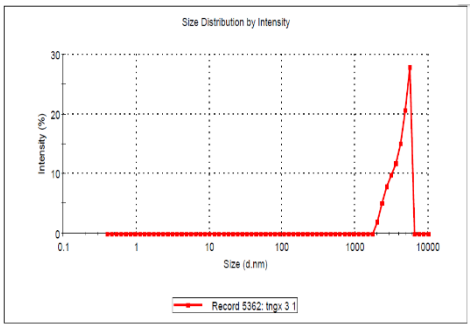


Fig.12 Size distribution pattern of TNGX 31

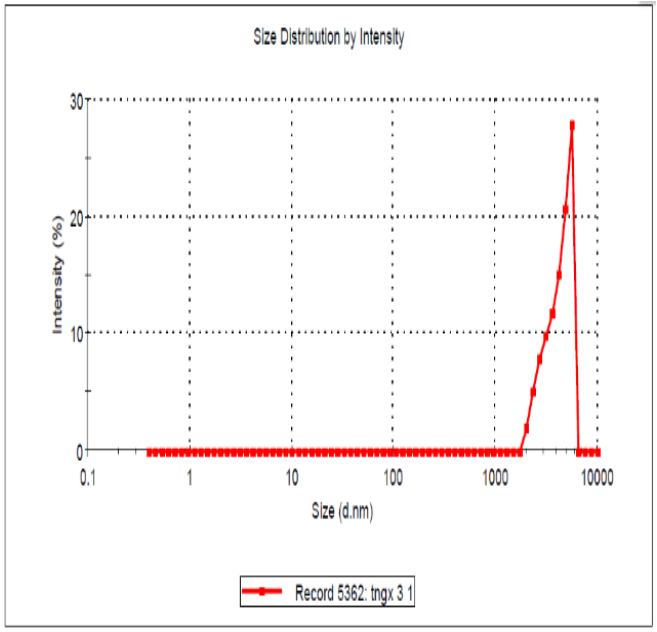


Fig.13: Size distribution pattern of TNGX

The mean vesicle size for niosomal dispersion was 4251 nm and niosomal gel was 4414 nm. The polydispersity is the ratio of standard deviation to mean droplet size, so it indicates the uniformity of vesicle size within the formulation. The higher the polydispersity, the lower the uniformity of vesicle size in formulation. The PDI for niosomal dispersion was very low so indicating higher uniformity in comparison to gel formulation. On gelling of niosomal dispersion the vesicle size and PDI of formulation increases.[50,51]

4.15.Transmission Electron Microscopy

TEM is an ultrasensitive technique for the evaluation and analysis of the morphology of nanogel. Precise particle size of bright field images as well as dark field images are provided by the TEM, and it provides details regarding nanoparticles as it utilizes energetic electrons to provide information regarding morphologic, compositional, and crystallographic information. TEM provides qualitative digital images and supplies the view of morphological particles of nanogel. [45]The images of TEM analysis shown in Figure 14 and Figure 15 at different scales.

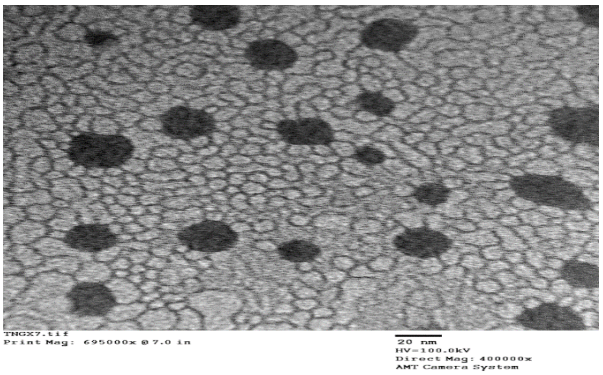


Fig.14. (a)

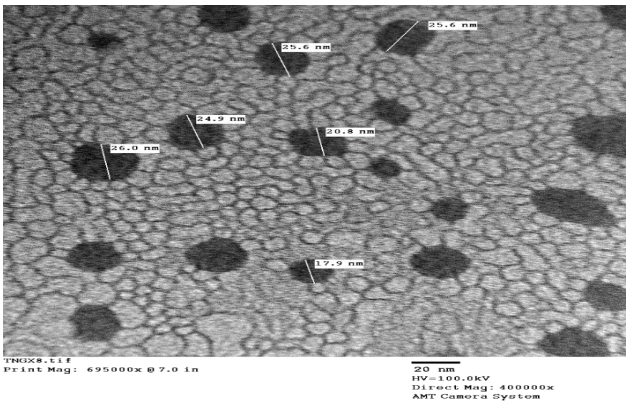
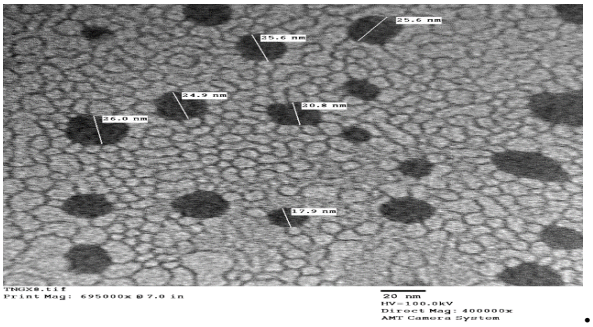


Fig.14. (b)

Fig 14 (a,b). TEM image of drug loaded niosomes at 20 nm scale

Fig.15



(a)

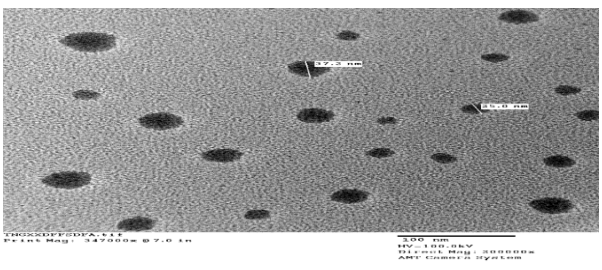


Fig.15. (b)

Fig 15: TEM image of drug loaded niosomes at 100 nm scale

TEM images showed that sonicated niosomes vesicles loaded with the drug were in nanosized range with mean diameter of 10 nm to 150 nm.

4.16.STABILITY STUDIES

Stability studies were carried out as per the procedure specified in section materials and methods. Samples were analyzed for the parameters such as colour, pH and vesicle size. The colour of testosterone niosomal gel was unchanged during and after 1 month accelerated stability studies.

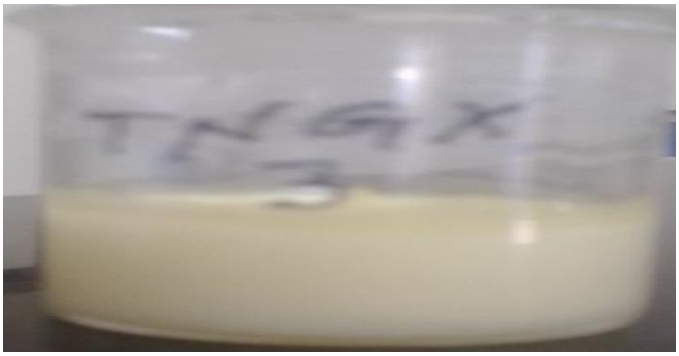


Fig 16: Testosterone niosomal gel, colour observed after 4weeks accelerated stability studies

Table 9: Table showing Effect on pH on accelerated storage condition

Sr.no.	Code	First day	After 7 days	After 14 days	After 21 days	After 28 days
1.	TNGX	7.2	7.1	6.9	7.0	6.8

Table 10: Table showing Vesicle size on Storage

Sr.no.	Formulation	First day	After 7 days	After 14 days	After 21 days	After 28 days
1.	TNGX	4414	4474	4521	4530	4528

Table 11: Drug content

Absorbance of drug molecule [10 µg/ml]	AverageAbsorbance 0f final formulation batch TNGX [10 µg/ml]	Drug content
0.5914	[after 1st week] 0.5857	99.05%
	[after 2nd week] 0.5848	98.90%
	[after 3rd week] 0.5804	98.15%
	[after 4th week] 0.5786	97.85%

Accelerated stability studies resultin Table 9,10,11 showed that no change in colour and slight changes in pH and vesicle size, which showed the formulation was stable. The drug content in formulation stored at accelerated temperature conditions was found to be 97.85% after 4 weeks. These results revealed that drug present in formulation was stable, and not degraded during accelerated temperature conditions.[46,47]

Conclusion:

The testosterone loaded Niosomes were prepared using optimized concentrations of non-ionic surfactant [Tween 80] and cholesterol by the Heating method technique. Niosomes are more stable than liposomes because cholesterol formed stable vesicles in niosomes. Niosomes have better penetration through skin epidermis because of their nanosized vesicles and lipophilicity. Formed drug loaded niosomes shows very less vesicle size [10 – 150 nm] and good PDI , it means the loaded drug can easily permeate through the skin and give desired pharmacological action which was not studied yet in this work, it can be further attempted in future . These results indicated that niosomes is a suitable carrier oftestosterone with improved permeation through the skin due to nanosized vesicles and uniform distribution of

drug due to good PDI value and make stable formulation for long time as compare to liposomes and any other nanocarrier.[48,49]

Therefore, niosomes was selected and niosomal based gel containing testosterone was formulated by using the gelling agent xanthan gum. It has been observed that niosomal gel showed good consistency, homogeneity, spreadability, and viscosity. The pH was modified by using triethanolamine and adjusted towards neutral nearby skin pH, adjusted to 7.2.

The formulation TNGX selected from all formulations based on various processing parameters studied. Because this formulation having high concentration of dug content [99.21%] immediately after preparation and 98.86% after stored in room temperature for 4 weeks. The formulation having 20 mg/gm drug. formulation was noted good spreadability hence easily applied on skin. Formulation was stable at room temperature and on accelerated temperature conditions. PDI value of formulation was less than 1 it was 0.992 which shows vesicles are uniformly distributed in the formulation and so the drug was uniformly distributed in the formulation. PDI of the niosomal dispersion of this formulation was noted 0.243 it was increases on gelling. And vesicle size was also increasing on gelling from 4251 nm to 4414 nm. TEM results shows niosomes size lies in 10nm to 150nm. So, the formulation may be shows enhanced permeation of testosterone through transdermal route due to very less size of niosomes

The present study concluded that niosomal based gel containing testosterone dissolved in a mixture of non - ionic surfactant and cholesterol in the nanoparticulate form helped us to attain the objective of transdermal delivery of testosterone and reduce the side effects by decreasing dose of drug and helpful to reduce the cost of treatment.

References

1. Mujoriya Rajesh, Bodla Ramesh Babu, Dhamande Kishor, Singh Devendra, Patle Lokesh. Niosomal drug delivery system: The magic bullet. Japsonline 01 [09]; 2011 : 20-23
2. Madhav NVS, Saini A. Niosomes: a novel drug delivery system. IJPRC;2011, 1[3]
3. S Gopalakrishnan, A Chenthilnathan. Niosomes – A novel drug delivery device. RJPBS 2012; 3 [3] : 1090
4. TangriPranshu, Khurana Shaffi. Niosomes formulation and evaluation.IIB, 2011; 2 [1]: 47-53
5. SankhyanAnchal, PawarPravin. Recent trends in niosome as vesicular drug delivery system.JAPS02 [06]; 2012 : 20-32
6. Chauhan S and Luorence MJ. The Preparation of Polyoxyethylene Containing Non-Ionic Surfactant Vesicles. J Pharm. Pharmacol. 1989;41:6.
7. Yoshioka T, Stermberg B and Florence AT. Preparation and Properties of Vesicles [Niosomes] of Sobitan Monoesters [Span 20, 40, 60, and 80] and a SorbitanTriester[Span 85]. Int J Pharm. 1994;105:1-6.
8. Gayatri DS, Venkatesh P and Udupa N. Niosomal Sumatriptan Succinate for Nasal Administration. Int J Pharm Sci. 2000;62[6]:479-481
9. Hu C and Rhodes D.G. Proniosomes: a novel drug carrier preparation. Int J Pharm. 1999;185:23-35.
10. B.L. Silver Ed., The Physical Chemistry of Membranes, Alan & Unwin and Soloman Press. New York, USA. 1985;209-230.
11. N.0 Sahin , Niosomes as nanocarrier system, M.R. Mozafari[ed.], Nanomaterials and Nanosystems for Biomedical Applications, 67–81. © 2007 Springer
12. D.Krishangopal ,Alpana Ram. Niosomes as a novel drug delivery system a review. Int J App Pharm 2013 ; 6[1]: 1-7
13. Desai Sanjeevani ,DokeAjit, Disouza John , Athawale Rajani. Development and evaluation of antifungal topical niosomal gel formulation . Int J Pharm Pharm Sci. 2011 ; 3[5] : 224-231
14. Chawla V, Saraf SA. Rheological studies on solid lipid nanoparticle based carbopol gels

of aceclofenac. Colloids Surf B Biointerfaces. Link: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3755149/
15. Park EK, Song KW. Rheological evaluation of petroleum jelly as a base material in ointment and cream formulations: steady shear flow behavior. Arch Pharm Res. Link: http://link.springer.com/article/10.1007%2Fs12272-010-2236-4
16. Chow KT, Chan LW, Heng PWS. Formulation of hydrophilic non-aqueous gel: drug stability in different solvents and rheological behavior of gel matrices. Pharm Res. Link: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3675732/
17. S.J. Shelke , D.M. Shinkar , R.B. Saudagar . Topical gel : A novel approach for development of topical drug delivery system . IJPT 2013 ; 5[3] : 2739-2763
18. Shindey A. Ujwala ,Kanojiya S. Shivkumar . Serratiopeptidase niosomal gel with potential in topical delivery . J Pharm 2014 ; 9 . Link: http://dx.doi.org/10.1155/2014/382959
19. Mozafari, M. R. [2005a]. A New Technique for the Preparation of non-toxic Liposomes and Nanoliposomes: the Heating Method. In: Mozafari MR and Mortazavi SM [eds.] Nanoliposomes: From Fundamentals to Recent Developments, Trafford Publishing Ltd, Oxford, UK, 91–98.
20. Mozafari, M. R., [2005b]. Method and Apparatus for Producing Carrier Complexes. UK Patent No. GB 0404993.8, Int. Appl. No. PCT / GB05 / 000825 [03 / 03 / 2005]
21. Baillie, A. J., Coombs, G. H., Dillan, G. D., Laurio, J. [1980]. Non-ionic Surfactant Vesicles, Niosomes as a Delivery System for the Anti-leishmanial Drug Sodium Stibogluconate. J. Pharm. Pharmacol., 38, 502.
22. Uchegbu, I. F., and Florence, A. T. [1995]. Nonionic Surfactant Vesicles [Niosomes]: Physical and Pharmaceutical Chemistry, Adv Coll Interf Sci., 58, 1-55.
23. Joshi M., Patravale V. Formulation and evaluation of nanostructured lipid carrier [NLC]-based Gel of Valdecoxib. Drug Development and Industrial Pharmacy. 2006;32:911–918. [PubMed]
24. SË˜kalko, N., CË˜ajkovac, M. and JalsË˜enjak, I. [1998]. Liposomes with Metronidazole for Topical Use: The Choice of Preparation Method and Vehicle. *Journal of Liposome Research*, 8[2], pp.283-293.
25. Agarwal, R., Katare, O. and Vyas, S. [2001]. Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. *International Journal of Pharmaceutics*, 228[1-2], pp.43-52.
26. Jain, S., Jain, N., Bhadra, D., Tiwary, A. and Jain, N. [2005]. Transdermal Delivery of An Analgesic Agent Using Elastic Liposomes: Preparation, Characterization and Performance Evaluation. *CDD*, 2[3], pp.223-233.
27. Nasseri, B. [2005]. Effect of cholesterol and temperature on the elastic properties of niosomal membranes. *International Journal of Pharmaceutics*, 300[1-2], pp.95-101.
28. Manconi, M., Sinico, C., Valenti, D., Lai, F. and Fadda, A. [2006]. Niosomes as carriers for tretinoin. *International Journal of Pharmaceutics*, 311[1-2], pp.11-19.
29. Khazaeli, P., Pardakhty, A. and Shoorabi, H. [2007]. Caffeine-Loaded Niosomes: Characterization and in Vitro Release Studies. *Drug Delivery*, 14[7], pp.447-452.
30. Mura, S., Pirot, F., Manconi, M., Falson, F. and Fadda, A. [2007]. Liposomes and niosomes as potential carriers for dermal delivery of minoxidil. *Journal of Drug Targeting*, 15[2], pp.101-108.
31. PAOLINO, D., COSCO, D., MUZZALUPO, R., TRAPASSO, E., PICCI, N. and FRESTA, M. [2008]. Innovative bola-surfactant niosomes as topical delivery systems of 5-fluorouracil for the treatment of skin cancer. *International Journal of Pharmaceutics*, 353[1-2], pp.233-242.
32. Manosroi, A., Jantrawut, P. and Manosroi, J.

[2008]. Anti-inflammatory activity of gel containing novel elastic niosomes entrapped with diclofenac diethylammonium. *International Journal of Pharmaceutics*, 360[1-2], pp.156-163.

33. Panyosak, A., Manosroi, J., Rojanasakul, Y. and Manosroi, A. [2009]. Safety assessment of azelaic acid and its derivatives entrapped in nanovesicles. *Human & Experimental Toxicology*, 28[6-7], pp.387-392.

34. Balakrishnan, P., Shanmugam, S., Lee, W., Lee, W., Kim, J., Oh, D., Kim, D., Kim, J., Yoo, B., Choi, H., Woo, J. and Yong, C. [2009]. Formulation and in vitro assessment of minoxidil niosomes for enhanced skin delivery. *International Journal of Pharmaceutics*, 377[1-2], pp.1-8.

35. Ataei S, Moazeni E, Gilani k, Ghaffari A, Asgharian R, Najafabi AR. In vitro evaluation of Itraconazole loaded vesicles prepared from non-ionic surfactants. JPHS. 2011; 1[1]:50-52.

36. Cosco, D., Paolino, D., Cilurzo, F., Casale, F. and Fresta, M. [2012]. Gemcitabine and tamoxifen-loaded liposomes as multidrug carriers for the treatment of breast cancer diseases. *International Journal of Pharmaceutics*, 422[1-2], pp.229-237.

37. Maheshwari, R., Tekade, R., Sharma, P., Darwhekar, G., Tyagi, A., Patel, R. and Jain, D. [2012]. Ethosomes and ultradeformable liposomes for transdermal delivery of clotrimazole: A comparative assessment. *Saudi Pharmaceutical Journal*, 20[2], pp.161-170.

38. Vimal K.R., Sankar V., Srinivas C.R. and kumaresan M. Enhancement of follicular delivery of Finasteride in Niosomes and Proniosomal Gel form for treating Androgenetic alopecia. Indian drugs. 2012; 49[05]:47-55.

39. Fang, J., Hong, C., Chiu, W. and Wang, Y. [2001]. Effect of liposomes and niosomes on skin permeation of enoxacin. *International Journal of Pharmaceutics*, 219[1-2], pp.61-72.

40. Bhavana Vora., Ajay J. Khopade., Jain, N.K. [1998]. Proniosome Based Transdermal Delivery of Levonorgestrel for Effective Contraception. J. Controlled Release, 54,149–165.

41. Jain, C. and Vyas, S. [1995]. Preparation and characterization of niosomes containing rifampicin for lung targeting. *Journal of Microencapsulation*, 12[4], pp.401-407.

42. Tejas R. Desai, Warren H. Finlay. [2002]. Nebulization of Niosomal All-trans-retinoic acid: an Inexpensive Alternative to Conventional Liposomes. Int. J. Pharm., 311–317.
43. Yongmei Hao., Fenglin Zhao., Na Li., Yanhong Yang., Kean Li. [2002]. Studies on a High Encapsulation of Colchicine by a Niosome System. Int. J. Pharm., 244 73–80.
44. Calum, J., Drummond, Celesta F. [2000]. Surfactant Self-assembly Objects as Novel Drug Delivery Vehicles. Curr. Opin Colloid In., 4, 449 - 456.
45. Himanshu, M., Varshney., Tanwar, Y. S., Rohit Lowalekar., Rathore, K. S. [2007]. Designing and Characterization of Verapamil Hydrochloride Niosomes, The Indian pharmacist, 6[57], 77-79
46. Ankur Gupta., Sunil Kumar Prajapati. [2007]. Design and Development of a Proniosomal Transdermal Drug Delivery System for Captopril. Trop. J. Pharm. Res., 6 [2], 687-693.
47. Alsarra, I., Bosela, A., Ahmed, S. and Mahrous, G. [2005]. Proniosomes as a drug carrier for transdermal delivery of ketorolac. *European Journal of Pharmaceutics and Biopharmaceutics*, 59[3], pp.485-490

48. Ainbinder, D. and Touitou, E. [2005]. Testosterone Ethosomes for Enhanced Transdermal Delivery. *Drug Delivery*, 12[5], pp.297-303.

49. Ainbinder D, Touitou E. Testosterone ethosomes for enhanced transdermal delivery. Drug Del. 2000;12:297–303.
50. Melton, L., Mindt, L. and Rees, D. [1976]. Covalent structure of the extracellular

polysaccharide from Xanthomonas campestris: evidence from partial hydrolysis studies. *Carbohydrate Research*, 46[2], pp.245-257.

51. 109. Jansson, P., Kenne, L. and Lindberg, B. [1975]. Structure of the extracellular polysaccharide from xanthomonas campestris. *Carbohydrate Research*, 45[1], pp.275-282.
52. 110. Clark, R.C. 1987. Viscoelastic response of xanthan gum/guar gum blends. In Gums and Stabilizers for the Food Industry, vol. 4. Edited by G.O. Phillips et al. Washington, D.C.: IRL Press, 1988 pp. 165-172.