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PRE-FORMULATION STUDY OF NANDROLONE DECANOATE FOR FORMULATION AS TRANSFEROSOMES FOR TDDS

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ABSTRACT

The first and foremost aim of the study was to prepare transferosomes which are recent approaches in modulating vesicle compositions. The second approach was to prepare a formulation of nandrolone decanoate in the form of transferesome which can be potentially useful for delivery of this drug and also overcome the binding affinity with the receptors. Transferosomal formulation of Nandrolone decanoate was prepared by using the lipid film hydration technique. For optimization of transfersome, different formulations (N1 to N6) were prepared using the various quantities of lipid & surfactant. The formulation (N3) was found with maximum entrapment efficiency. The shape and size of the optimized N3 formulation was confirmed through microscope and particle size and found that most of the particles were well identified. For Topical drug delivery of transfersomal gel of optimized N3 formulation was prepared by dispersing the formulation successfully in 1%, 1.5%, 2% carbopol 934. Formulation N9 (2% Carbopol 934) is optimized for further study. The % EE of optimized formulation (89.825 ± 0.100) with fruitful viscosity as well as spreadability. Apart from it, the particle size with polydispersity index of optimized formulation is 242.3nm with PDI 0.246. Optimized formulation *in vitro* drug release was studied in phosphate buffer (PB) pH 7.4 using Franz-type diffusion cells. To know precisely, the rate and mechanism of drug release, the *in vitro* data was fitted to zero order, first order, Higuchi and Korsmeyer-Peppas model. The results showed that the drug release of N9 formulation followed Korsmeyer-Peppas order which describes that the Nandrolone decanoate follows a controlled mechanism for release from transfersome. Thus, it can be concluded from the result obtained that the transferosomal gel formulation developed for

transdermal delivery of Nandrolone decanoate possessed better skin permeation potential, and higher entrapment efficiency, as well as had ability as a self penetration enhancer.

KEYWORDS: transferosomes, TDDS, nadrolone decanoate, skin permeation.

INTRODUCTION

Transfersomes

Transferosome means “carrying body” which is derived from the Latin word 'transferre', it means 'to carry across', and the Greek word 'soma', means 'a body'. A Transferosomes carrier is an artificial vesicle which is especially designed to exhibit the characteristics of a cell vesicle or a cell engaged in exocytosis. These are suitable for controlled and, potentially, targeted drug delivery. This novel concept was introduced in 1991 by Gregor Cevc. Till then numerous groups have since been working with similar carriers, with different names such as elastic vesicle, flexible vesicle, Ethosomes, etc.) to describe them.

Transferosomes are recent approaches in modulating vesicle compositions. These have been investigated to develop systems that are capable of carrying drugs and macromolecules to deeper tissues which have been resulted in the design of two novel vesicular carriers;

1. Ethosomes and
2. Ultra flexible lipid-based elastic vesicles transferosomes.[1]

Out of which transferosomes do ultra deformable vesicles possess an aqueous core surrounded by the complex lipid bilayer. Interdependency of local composition and shape of the bilayer makes the vesicle both self-regulating and self-optimizing.[2] These have been recently introduced techniques, which are capable of transdermal delivery of low as well as high molecular weight drugs.[3] Walve et al suggest that the transferosomes are specially optimized, ultra flexible lipid supra molecular aggregates, which are able to penetrate the mammalian skin intact and then act as a drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents.[4]

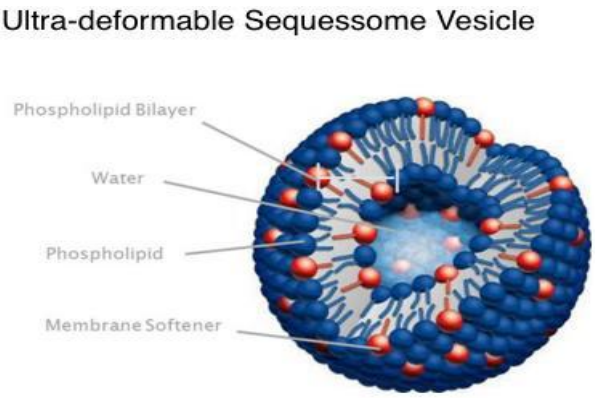


Figure 1: Structure of Transferosome.

Transferosomes are generally consists of two distinct region as hydrophobic and hydrophilic moieties together and as a result it can accommodate drug molecules with wide range of solubility. The uniqueness of transferosomes is it can deform and pass through narrow constriction (5-10 times less than their own diameter) without measurable loss. This high deformability gives better penetration of intact vesicles. The second peculiar things is that it can act as a carrier for low as well as high molecular weight drugs such as analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin. They are also biocompatible and biodegradable and they are made from natural phospholipids similar to liposomes. They have very high entrapment efficiency, in case of lipophilic drug approximately 90%. They have capacity to protect the encapsulated drug from metabolic degradation and it act as depot and releasing their contents slowly and gradually. They can be easily employed for both systemic as well as topical delivery systems of drug. They are also easy to scale up, because their procedure is simple and it does not involve and unnecessary use or pharmaceutically unacceptable additives.[5]

Mechanism of Penetration of Transfersome

Current investigations indicate that the transport of transfersomes across the skin involves the amalgamation of two methods, which depends on the affinity of the therapeutic agent towards water and the structure of transfersomes. The hydration or osmotic force has been reported to be involved in the transport of transfersomes across the stratum corneum.[6] Because of the lack of any internal source of energy, a transfersome follows naturally occurring energy gradients, such as transepidermal water activity gradient, for its transportation.[7] The ability of the skin to control water loss as described above aids in establishing a difference in the water movement between the accessible part of the epidermis (75% water content) and the almost completely dry stratum corneum (15% water content), leading to formation of the

water activity gradient. Air in the atmosphere surrounding the skin contributes towards the stability of this gradient by behaving as an ideal sink for the water molecules even in conditions of high transdermal water loss. Transfersomes are believed to be drawn into the body by hydrotaxis. Elastomechanics is considered to have a significant role in the movement of transfersomes through the normally confining pores. It also offers a rationale for the development of ultradeformable vesicles as drug carriers. Drying and partial dehydration of vesicles are thought to be the initial events in skin permeation by the transfersome upon topical administration. As a consequence, the transfersome becomes compressed or curved. The phospholipid component of the transfersome has a tendency to evade a dry environment. Thus, to stay fully swollen, the transfersome follows the local hydration gradient and penetrates more strongly the hydrated layers of skin, reaching the epidermis and dermis.[8] The decrease in flux observed upon wetting of the skin establishes the hydration theory.[9] Hydrotaxis of these ultradeformable systems enables them to carry in excess of 50% of the administered therapeutic agent through the skin. Transfersomes can penetrate the intact stratum corneum spontaneously along two routes in the intracellular lipid so as to differ in their bilayers properties. When a transfersome reaches a pore, it is able to change its membrane composition reversibly as a result of its self-optimizing deformability (Figure 5). To pass through the pore, components of the transfersome responsible for its deformability start accumulating at the site of stress, whereas the less flexible components undergo dilution, this significantly reduces the active rate of embrace deformation and allows the highly flexible particles to pass through the pores. The passage of transfersomes through the skin and the epithelial barrier is greatly influenced by the flexibility of their membrane, which can be achieved using an appropriate ratio of surfactants. The resulting flexibility of the transfersomal membrane also reduces the possibility of its rupture in the skin[10, 11]

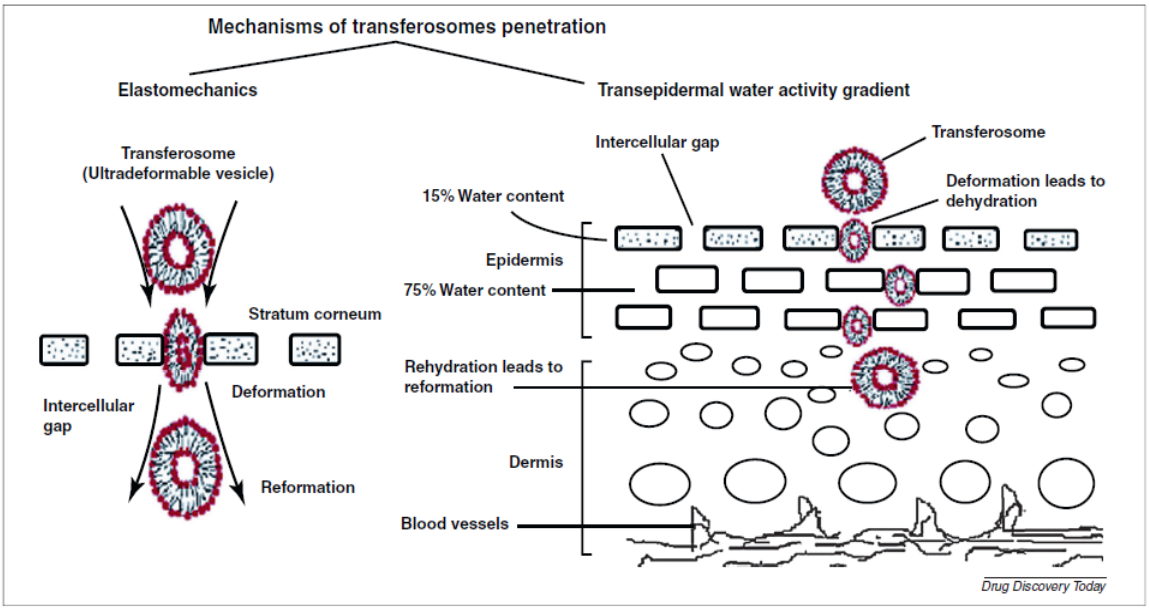


Figure 2: Mechanisms of penetration of transfersomes (ultradeformable vesicle) through the skin.

Methods of Preparation of Transferosomes

A. Thin Film Hydration Techniques: These are comprised of following three steps:

1. First of all a thin film is prepared from the mixture of vesicles forming ingredients such as phospholipids and surfactant by dissolving in volatile organic solvent (e.g. chloroform methanol). The solvent used are evaporated at transition temperature (room temp. for pure PC vesicles, or 50°C for dipalmitoyl phosphatidyl choline) using rota evaporator followed by evaporation of final traces of solvent under vacuum for overnight.
2. Then the prepared thin film is hydrated with buffer (pH 6.5) by rotation at 60 rpm for 1 hr at the corresponding temperature. The resulting vesicles were swollen for 2 hr at room temperature.
3. To prepare small vesicles, resulting vesicles were sonicated at room temperature or (50°C) for 30 min. by using a bath sonicator or if probe sonicated then at 4°C for 30 min. followed by homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membranes.[12,13,14]

B. Modified Hand shaking Lipid film hydration Technique: These are comprised into following steps:

- Take Drug, lecithin (PC) and edge activator and dissolved in ethanol: chloroform (1:1) mixture. Then removed the organic solvent by evaporation while hand shaking above

lipid transition temperature (43°C). A thin lipid film was formed inside the flask wall with rotation. kept the film overnight for complete evaporation of solvent.

- The film was then hydrated with phosphate buffer (pH 7.4) with gentle shaking for 15 minute at corresponding temperature. The transfersome suspension further hydrated up to 1 hour at 2-8°C.[12,13,14]

C. Over all, transferosomes were prepared by mixing an ethanolic SPC solution with appropriate amount of an edge-active molecule, mainly sodium cholate. The resulting solution formed in ratio of 8.7 wt % SPC and 1.3 wt% cholate as well as approx. 8.5 vol% ethanol. Then the solution is buffered with triethanolamine–HCl buffer to yield total lipid concentration of 10 wt%. The resulting suspension was sonicated, frozen, and thawed 2–3 times. The desired was confirmed by using photon correlation spectroscopy, by ultrasonication or intermediate-pressure homogenization. Vesicle suspension was ultimately sterilized by filtration through a 0.2 mm micro-porous filter Poretics, CA, USA. The final vesicle size was confirmed by the dynamic light scattering in different suspensions. Preparation of drug-Loaded Liposomes, Transferosomes, and Suspensions. Liposomes containing a controlled amount of PC and various amounts of drug were formulated.

- The drug concentration was varied from 2.5 to 70.0 wt. % of the PC.
- The sonication method was used to prepare different formulations; they were composed of bilayer-forming PC and either Chol, NaO, NaChol, or DCP in a molar ratio of 10: 2.
- The PC, Chol, NaO, NaChol, DCP, and drug were each briefly dissolved in chloroform: methanol (2: 1 v/v).
- In preparing drug-loaded liposomes and transferosomes, the materials were deposited in a test tube, and the solvent was evaporated with nitrogen gas.
- The lipid film was placed in a desiccator connected to a vacuum pump for a minimum of 6 hrs to remove the remaining organic solvent.
- The dried lipid film was hydrated with tris buffer. Following hydration, the dispersion was sonicated in a bath for 30 min and then probe-sonicated for 2 cycles of 30 min.

D. In this method Soya-phosphatidylcholine was taken in a round bottom flask then added Span 80 or Tween 80 in the same round bottom flask in ethanol. The drug was also loaded in the same RBM and mixed the solution by vigorous shaking. The resulting solution was formed as thin evaporated the organic solution by using rotatory vaccum evaporator at 60C. Then hydrated the thin film by using phosphate buffer saline to get the transferosomes.[6]

E. They could be formulated by the conventional rotary evaporation sonication method.[4,6] Transferosomes containing could be prepared by taking phospholipids (soya lecithin), surfactant (span 80), and the drug (sertraline) and were formulated. The usuall concentration was used in the range of drug concentration varied from 0.5 to 1.8 wt. % and the surfactant concentration varied from 5 to 25 wt % of the phospholipids. Ingredients were taken in a clean, dry, round-bottom flask and this lipid mixture was dissolved in a small quantity of ethanol. The organic solvent was removed by rotary evaporation under reduced pressure at 40°C followed by final removal of solvent under vacuum at overnight. The deposited lipid film was hydrated with 7% v/v ethanol *i.e.* solution of the drug by rotation at 60 rpm for 1 hr. The resulting vesicles were kept for 2 hrs at ambient temperature to swell and form into large multi lamellar vesicles. These were sonicated in a bath for 10 min to achieve smaller vesicles. The varied amounts of the drug were added (0.5 to 1.8%) to determine the drug-loading capacity of transferosomes. All of the drug-loaded vesicular formulations were examined for maximum entrapment efficiency and for the appearance of drug crystals over a period of 14 days using an optical microscope. Table 1 show the Different additives used in formulation of Transferosomes.

EXPERIMENTAL

MATERIALS AND METHODS

All the materials are purchased and used as such. The equipments used for pre-formulationstudy of nandrolone decanoate for formulation of transferosomes for TDDS are calibrated and followed standard operating procedure. During the experiments following parameters are studied

Pre-formulation studies[15-16]

Organoleptic Characteristics

The drug sample was characterized for the physical characterization like appearance, color and odor.

1. Melting point.[17]
2. UV spectrum of Nandrolone decanoate.[18]
3. Estimation of Nandrolone decanoate
4. Estimation of Nandrolone decanoate by UV-visible spectrophotometer
5. Solubility Studies.[19]
6. Partition Coefficient of Drug.[20]

7. FTIR of Nandrolone decanoate and Excipients.[21]

8. Drug-excipients Compatibility Study by FTIR

9. Preparation, optimization and characterization of transfersome.[22]

Table 1: Composition of Different Formulation Transfersomes with different surfactant.

Formulation Code	Drug (mg)	Phospholipid (mg)	Sodium deoxycholate (mg)	Tween 80(mg)	Span 80 (mg)
A1	38	85	15	-	-
A2	38	85	-	15	-
A3	38	85	-	-	15

Table 2: Composition of Different Formulation Transfersomes with Optimized surfactant.

Formulation Code	Drug (mg)	Phospholipid (mg): Tween 80 (mg)
N1	38	100:00
N2	38	90:10
N3	38	80:20
N4	38	70:30
N5	38	60:40
N6	38	50:50

Evaluation of Transfersome[22-23]

Photomicroscope study

A selected Transfersomal formulation was chosen for microscopic investigation due to its optimum relative deformability. The vesicles were examined under a binocular optical microscope and photographed at a magnification of 100× by means of a fitted camera.

Particle size

Measurement of Particle size of the Transfersome was performed using a Zetasizer.

Transmission Electron Microscopy (TEM) Analysis

Drug Entrapment Efficiency

The %EE was calculated as:

$$\%EE = \frac{\text{Amount of drug added} - \text{Amount of drug unentraped}}{\text{Amount of drug added}} X100$$

Incorporation of Transfersome into gel[24][25]

The developed transfersomal gel was evaluated for various parameters.

Table 3: Composition of different transfersomal gel.

S.no.	Formulation code	Carbapol 934(%w/w)
1	N7	1
2	N8	1.5
3	N9	2

RESULT AND DISCUSSION

Result of Pre-formulation study of drug

The aim of pre-formulation studies is to investigate the physical and chemical properties of a drug substance. The selected drug nandrolone decanoate was subjected for investigation of physical characterization parameters such as:

- Organoleptic properties
- Melting point
- UV-visible spectra
- Solubility Study
- Partition coefficient
- FT-IR spectra

Organoleptic properties

Organoleptic properties of drug Nandrolonedecanoate found to be as per USP monograph. The Organoleptic properties of Nandrolonedecanoate were found to the given **Table 8**.

Table 4: Organoleptic Properties of Nandrolonedecanoate.

Sr. No.	Properties	Inferences
1.	Colour	White
2.	Odour	Odourless
3.	Form	Powder

7.1.2 Melting Point

The melting point of a substance is the temperature at which the solid phase gets converted to liquid phase under the one atmosphere of pressure. The melting point determination implies the purity of drug. Melting point of Nandrolonedecanoate was determined by capillary tube method and was found to be quite similar to the reported melting point as shown in **Table 9.**[25]

Table 5: Melting Point of Nandrolonedecanoate.

Drug	Observed melting point	Reference melting point
Nandrolonedecanoate	35.300±0.755°C	33-37 °C

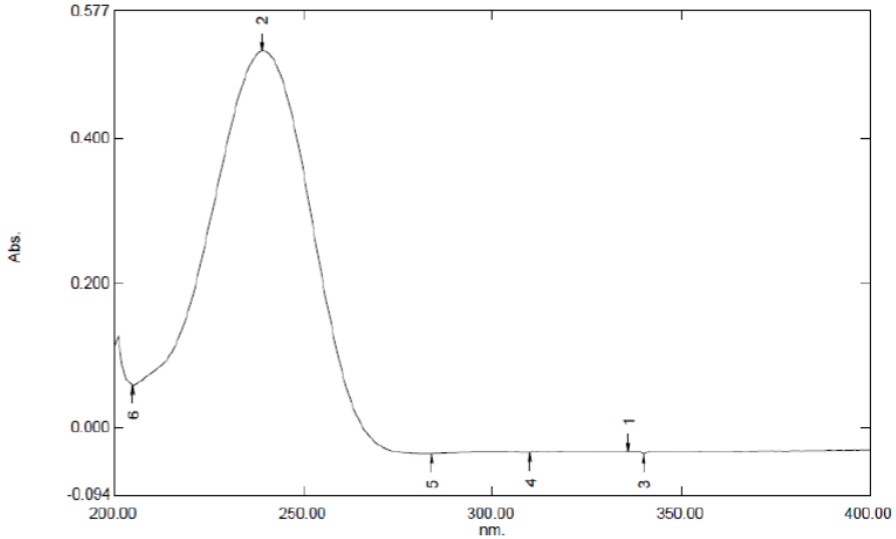
DISCUSSION

The melting point of Nandrolonedecanoate was found to be in range 35.300±0.755°C which is of the pure drug. Hence drug sample was free from any type of impurities.

UV Spectroscopy

Determination of absorption maxima in ethanol

Absorption maxima of Nandrolonedecanoatewere found to be at 239 nm similar to literature as shown in **Figure 7.**[15]



No.	P/V	Wavelength	Abs.	Description
1	↑	336.00	-0.034	
2	↑	239.00	0.521	
3	↓	340.00	-0.038	
4	↓	310.00	-0.036	
5	↓	284.00	-0.038	
6	↓	205.00	0.057	

Figure 3: UV spectrum of Nandrolonedecanoatein ethanol.

Preparation of standard curve of Nandrolonedecanoatein ethanol

Table 6: Calibration curve of Nandrolonedecanoate in ethanol (λmax= 239nm).

Sr. no.	Concentration µg/ml	Absorbance
1	2.5	0.111±0.001
2	5.0	0.187±0.003
3	7.5	0.285±0.001
4	10.0	0.400±0.001
5	12.5	0.521±0.001
6	15.0	0.613±0.003

7	17.5	0.699±0.001
8	20.0	0.816±0.001
9	22.5	0.920±0.001
10	25.0	0.995±0.001

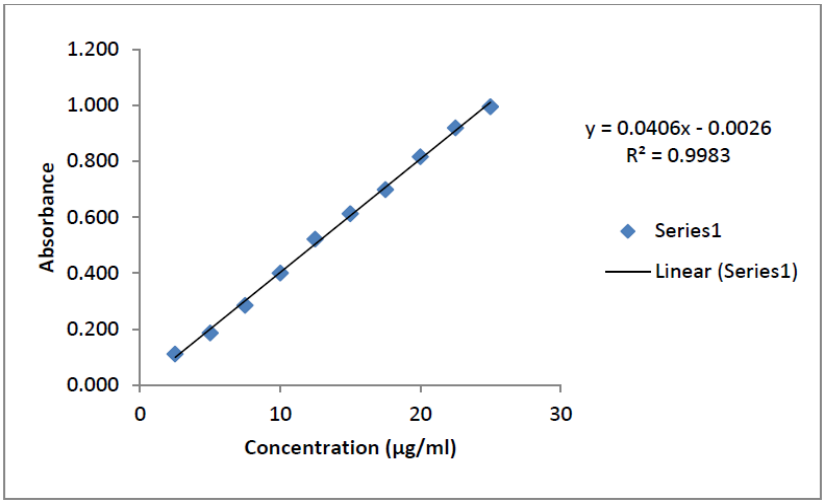


Figure 4: Graph of standard calibration curve of Nandrolonedecanoate in ethanol.

Table 7: Result of regression analysis of UV method for estimation of Nandrolonedecanoate.

Statistical Parameters	Results
λ max	239 nm
Regression equation ** Y=mx+C	Y= 0.040x - 0.002
Slope (b)	0.040
Intercept (C)	0.002
Correlation coefficient (r2)	0.998

Discussion: The calibration curve for Nandrolonedecanoate was obtained by using the 2.5 to 25 µg/ml concentration of Nandrolonedecanoate in ethanol. The absorbance was measured at 239 nm. The calibration curve of Nandrolonedecanoate as shows in graph indicated the regression equation Y=0.040x-0.002 and R2 value 0.998, which shows good linearityas shown in

Solubility studies

Solubility of drug in solvents was carried out in order to screen for the components to be used for formulation development. Analysis of the drug was carried out on UV Spectrophotometer at 239nm.[108]

Solubility Studies of Nandrolonedecanoate in various Solvents

Table 8: Solubility studies of Nandrolonedecanoate for different solvents.

Sr.no	Solvent	Solubility in (mg/ml) (mean±SD) *
1	0.1N HCl	0.014±0.001
2	DMSO	0.128±0.0004
3	DMF	0.140±0.0007
4	PBS 7.4	0.284± 0.002
5	Water	5.550±0.087
6	PBS 6.8	7.250±0.150
7	Chloroform	19.667±1.041
8	Acetone	27.500±0.866
9	Methanol	85.000±0.500
10	ethanol	151.333±0.764

* Each value is mean of three independent determinations

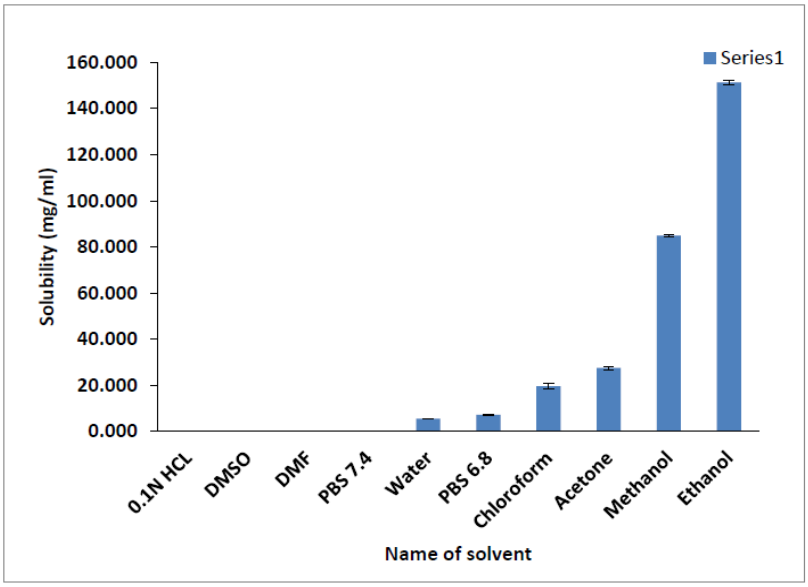


Figure 5: Solubility study of drug in different solvents.

Discussion: From the above data, it was clearly seen that Nandrolonedecanoateis highly soluble in ethanol, methanol, and acetone followed by chloroform.

7.1.5 Partition coefficient determination

Partition coefficient of the Nandrolonedecanoate was determined using n-octanol and water. Log P greater than one indicates that the drug is lipophilic in nature, whereas those with partition coefficients less than one are indicative of a hydrophilic drug. This indicated the lipophilicity and purity of drug.

Table 9: Partition coefficient determination of Nandrolonedecanoate.

Partition coefficient of Drug	Solvent System	Log P Value
Nandrolonedecanoate	Water:n-octanol	3.254± 0.0233

Value is expressed as mean ± SD; n = 3

Discussion: The partition coefficient of Nandrolonedecanoate in n- Octanol: Water was found to be 3.254± 0.0233 this indicates that the drug is lipophilic in nature.

7.1.6 FTIR of Nandrolonedecanoate and Excipients[26]

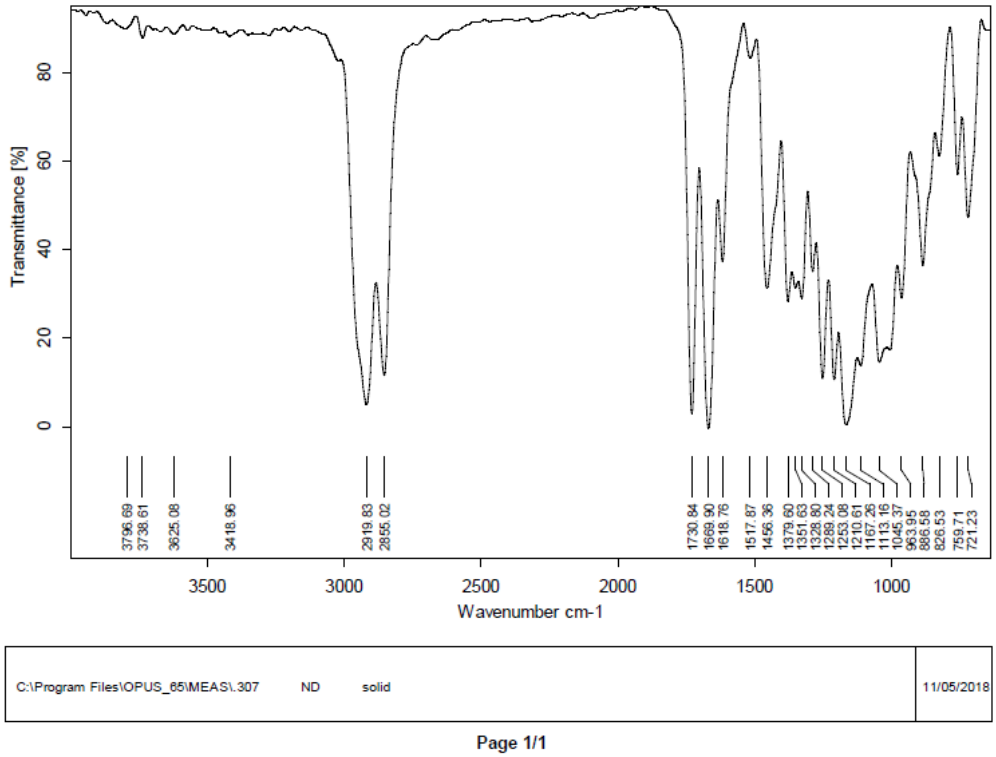


Figure 6: FTIR spectrum of Nandrolonedecanoate.

Table 10: FTIR interpretation of Nandrolonedecanoate.

Characteristics Peaks	Reported (cm-1)	Observed(cm-1)
C=O Stretching esters, saturated aliphatic	1750–1735	1730.84
C=O stretch α,β–unsaturated aldehydes, ketones	1710–1665	1669.90
C–C stretch (in–ring) aromatics	1500–1400	1456.36
C–H bend alkanes	1470–1450	1456.36
C–H rock alkanes	1370–1350	1351.63

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The FTIR spectra of Nandrolonedecanoate were shown in the **Figure 10**; **Table17**. The principal IR absorption peaks of Nandrolonedecanoate at 1730.84cm-1 (C=O stretching), 1669.90cm-1 (C=O stretch α,β–unsaturated aldehydes, ketones), 1456.36cm-1 (C–C stretch (in–ring) aromatics), 1456.36cm-1 (C–H bend alkanes) and 1351.63 (C–H rock alkanes) were all observed in the spectra of Nandrolonedecanoate. These observed principal peaks. This observation confirmed the purity and authenticity of the Nandrolonedecanoate.

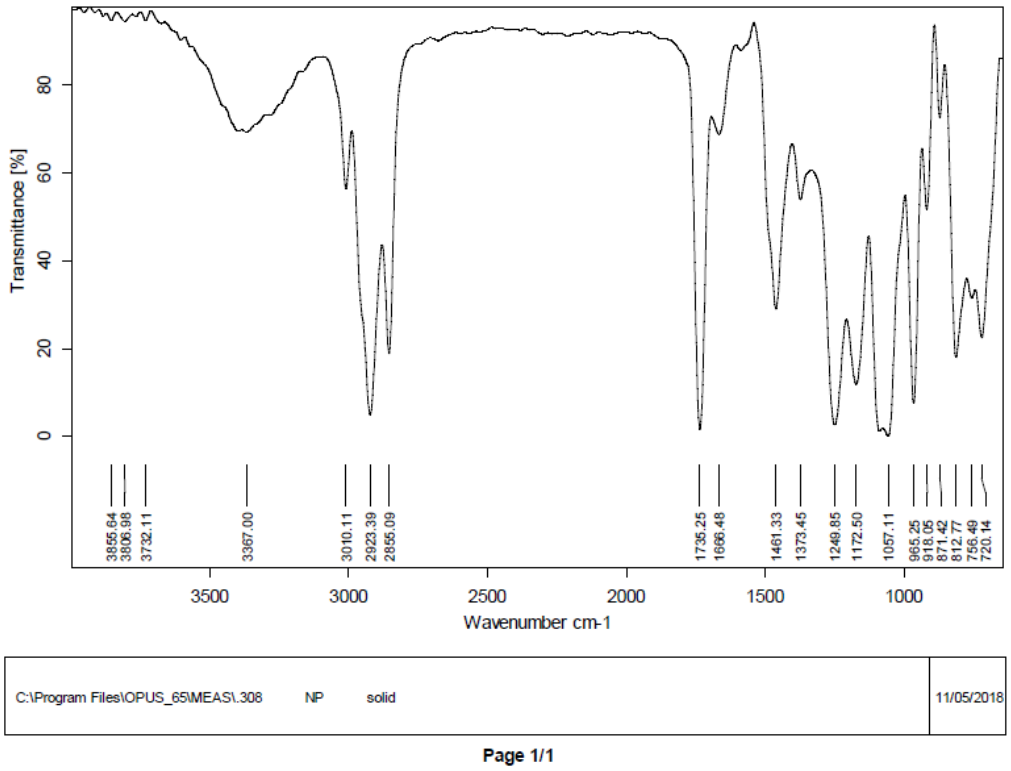

Figure 7: FTIR spectrum of Phosphatidylcholine.

Table 11: FTIR interpretation of Phosphatidylcholine.

Characteristics Peaks	Reported (cm-1)	Observed(cm-1)
C–H stretching band of long fatty acid chain	2918.3 and 2854.96	2923.39 and 2856.09
Carbonyl stretching band in the fatty acid ester	1728.22	1735.25
C=O stretch α,β–unsaturated aldehydes, ketones	1710–1665	1666.48
P=O stretching band	1236.37	1249.85
P–O–C stretching band	1093.65	1057.11
N+(CH3)3 stretching	966.34	985.25

The FTIR spectra of phosphatidylcholinewere shown in the **Figure 11**; **Table 18**. The principal IR absorption peaks of phosphatidylcholine at 2923.39 and 2856.09cm-1 (C–H stretching band of long fatty acid chain), 1735.25cm-1 (Carbonyl stretching band in the fatty acid ester), 1249.85cm-1 (P=O stretching band), 1057.11cm-1 (P–O–C stretching band) and

985.25cm-1 (N+(CH3)3 stretching) were all observed in the spectra of phosphatidylcholine. These observed principal peaks. This observation confirmed the purity and authenticity of the phosphatidylcholine.[38]

- FTIR of Pure drug and physical mixtures:

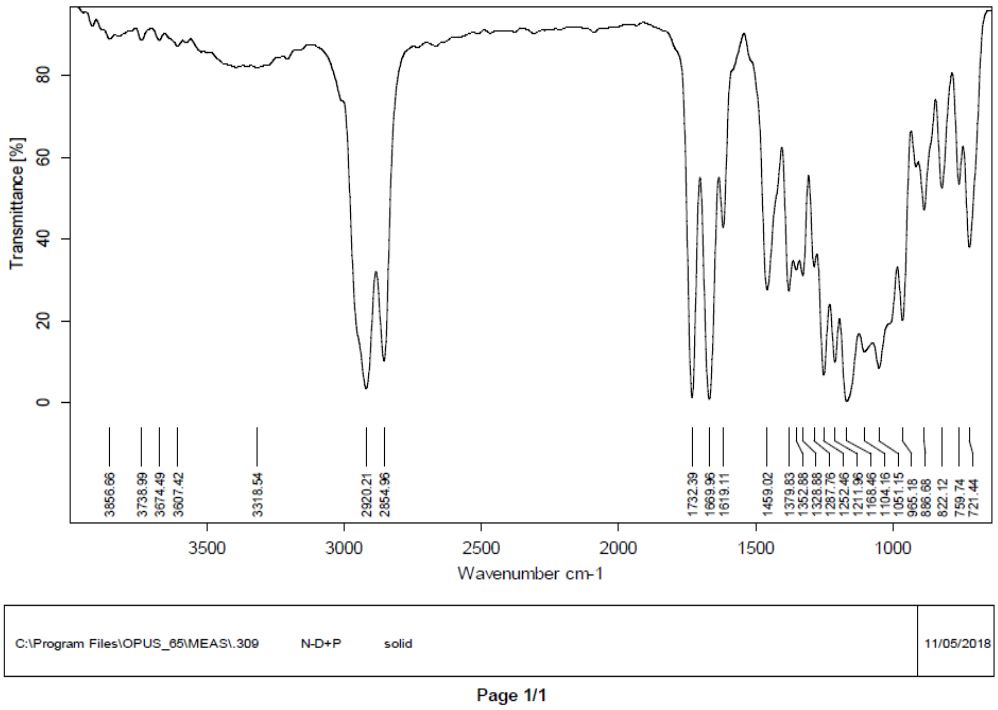

Figure 8: FTIR spectra of Nandrolonedecanoate and phosphatidylcholine.

Table 12: FTIR interpretation of Nandrolonedecanoate and phosphatidylcholine.

Characteristics Peaks	Reported (cm-1)	Observed(cm-1)
C=O Stretching esters, saturated aliphatic	1750–1735	1732.39
C=O stretch α,β–unsaturated aldehydes, ketones	1710–1665	1669.96
C–C stretch (in–ring) aromatics	1500–1400	1459.02
C–H bend alkanes	1470–1450	1459.02
C–H rock alkanes	1370–1350	1352.88
C–H stretching band of long fatty acid chain	2918.3 and 2854.96	2920.21 and 2854.96
P=O stretching band	1236.37	1252.46
P–O–C stretching band	1093.65	1051.15
N+(CH3)3 stretching	966.34	965.18

FTIR of Pure drug and physical mixture studies **(Figure 12**; **Table 19)** were carried out to eliminate the possibility of interaction between drug and excipients. All the spectrum peaks revealed that corresponding peaks of drugs are present in the above spectra along with excipients peaks. Hence no interaction was observed in this mixture.

Preparation, optimization and characterization of transfersome

A number of novel drug delivery systems have emerged to achieve controlled and targeted drug delivery. Transfersome is a novel provesicular carrier for drug delivery. Transfersomes are liquid crystalline flexible lipid vesicles that will be converted into the ultraflexible vesicles transfersomes*in-situ* by absorbing water from the skin. This system is more stable, having higher entrapment efficiency, can be used as self penetration enhancer, easy to scale up, better for dermal and transcutaneous delivery.[39]

Transfersomes loaded with Nandrolonedecanoatewere successfully prepared using lipid film hydration technique with slight modifications. Various formulations were prepared using different types of edge activators (sodium deoxycholate, Span 80 and tween 80) and lipids (soyalecthin). These transferosome were characterised w.r.t. %EE and Appearance.

Table 13: Composition of Different Formulation Transfersomes with different surfactant.

Formulation Code	Drug (mg)	Phospholipid (mg)	Sodium deoxycholate (mg)	Tween 80 (mg)	Span 80 (mg)	%EE	Appearance
A1	38	85	15	-	-	89.211±0.114	Stable
A2	38	85	-	15	-	91.118±0.174	Stable
A3	38	85	-	-	15	93.026±0.263	Phase separation

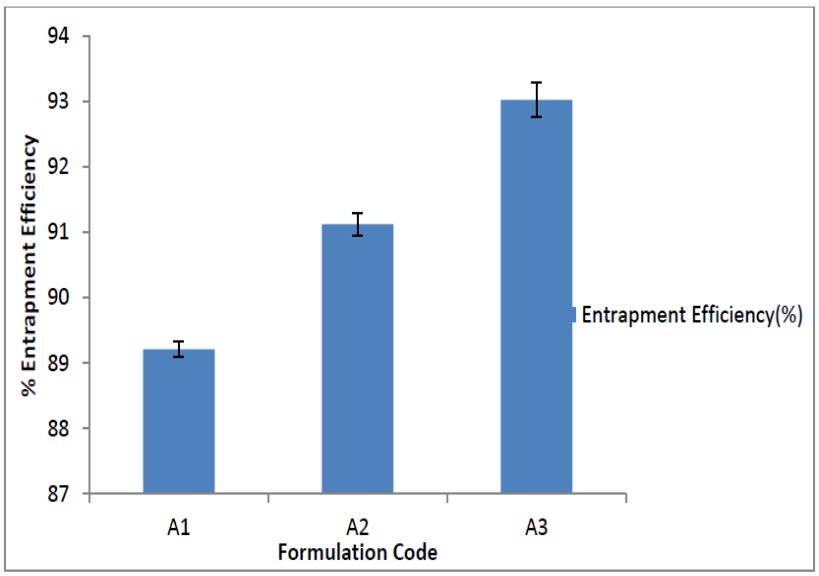

Fig. 9: Percentage entrapment efficiency of transfersome formulations.

One way to explain these findings is to consider the HLB of these edge activators are 4.3, 15, 16.7 for span 80, tween 80 and sodium deoxycholate, respectively. Based on these HLB

values, the affinity for lipids was expected to be in the order of span 80>tween 80 > and sodium deoxycholate. This explanation verifies the higher EE% encountered withspan 80, tween 80 and sodium deoxycholate. But span 80 showed phase separation not stable.[128]

These results the deformability of Transfersomes is also affected by the type of edge activator used, which could be interpreted by the differences in their chemical structures. By comparing the optimum deformability ratio (85:15%, w/w), it was found that, showed the highest deformability. This could be attributed to the highly flexible and nonbulky hydrocarbon chains of tween 80.[117]

On the basis of %EE & Appearance Tween 80 has been selected for further optimization of formulation and proceed for further studies (Table 21).

Table 14: Composition of Different Formulation Transfersomes with Optimized surfactant.

Formulation Code	Lipid: Tween 80 (w/w)	Drug	%EE	Appearance
N1	100:00	38	82.917±0.090	Phase separation
N2	90:10	38	87.803±0.203	Stable
N3	80:20	38	89.825±0.100	Stable
N4	70:30	38	79.627±0.137	Stable
N5	60:40	38	77.303±0.174	Stable
N6	50:50	38	74.539±0.132	Stable

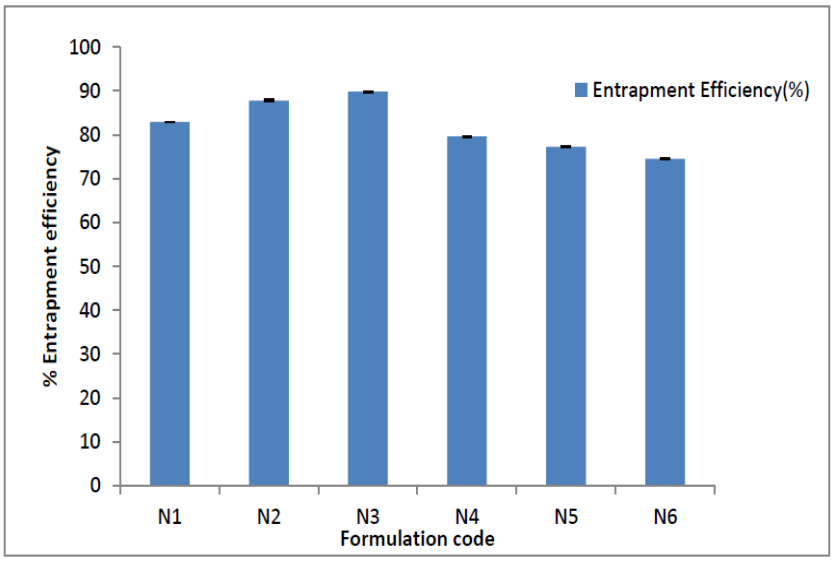

Fig.10: Percentage entrapment efficiency of transfersome formulations.

Initially, the EE% increased with increasing edge activator concentration from 10-20mg (w/w) (i.e. from 90:10to 80:20). After that EE% started decrease while increasing the

concentration of edge activator 30 mg to 50 mg (w/w) Table 22. The ratio 80:20 (w/w) showed optimum EE%. Upon incorporation of edge activator in low concentration, growth in vesicle size occurred whereas; further increase in the content of edge activator may have led to pore formation in the bilayers.[129-130]

EVALUATION OF TRANSFERSOME

Photomicroscopicstudy

The photomicrograph of N3 formulationrevealed that particles present in uniform shape without any aggregation.

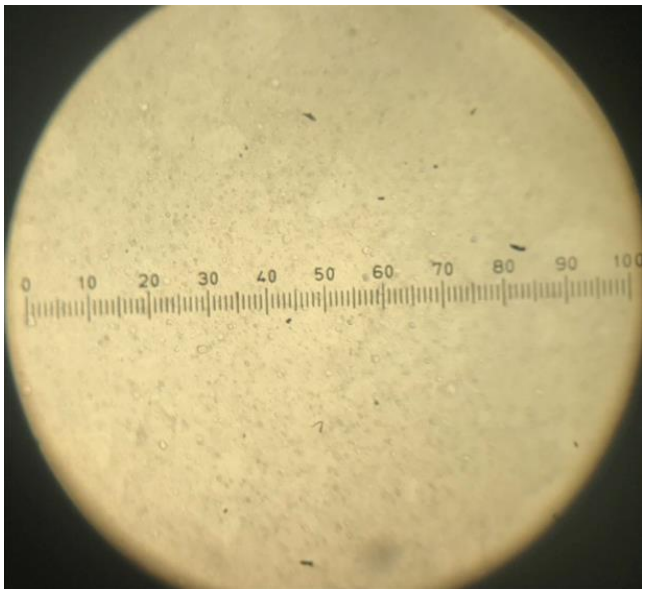

Figure 11: The photomicrograph of N3 formulation transferosome.

7.3.2. Determination of particle size

The prepared N3 Formualtionwas tested for particle size and polydispersity index, as shown in Figure 16.

Data output

Hydrodynamic diameter	242.3 nm	**Polydispersity index**	24.6 %
Intercept $g1^2$	0.8647	**Mean intensity**	315.8 kcounts/s
Filter optical density	1.4838	**Baseline**	1.004
Focus position	0.2 mm	**Angle used**	Side scatter
Processed runs	5	**Transmittance**	80.3 %
Diffusion Coefficient	2.0 $\mu m^2/s$		

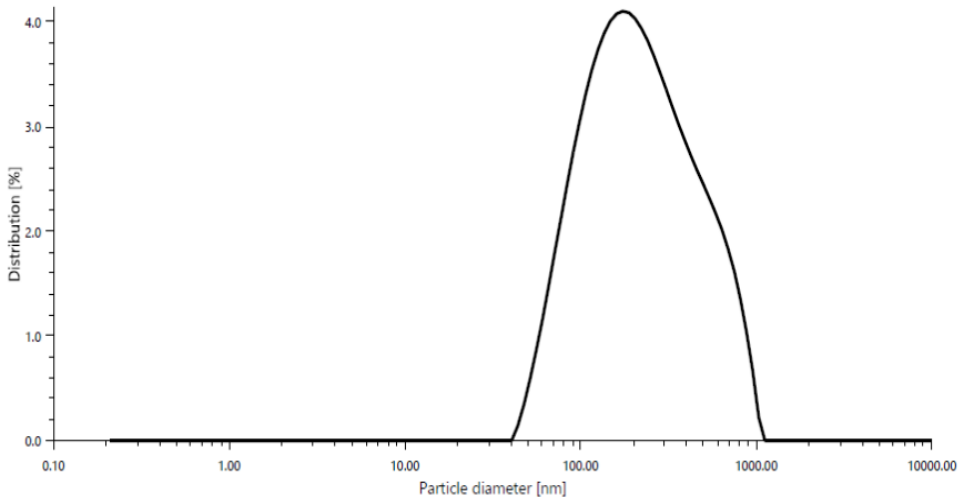

Figure 12: The Particle size with polydispersity index of N3 Formulation.

Discussion: TheParticle size with polydispersity index of N3 Formulation was 242.3nm with PDI 0.246.

- **7.3.3. Transmission electron microscopy (TEM) analysis**

The shapes of the optimized formulation were also confirmed through TEM study and are shown in Figure 17**.** Most of the vesicles is well identified, spherical and discreet having large internal aqueous space.

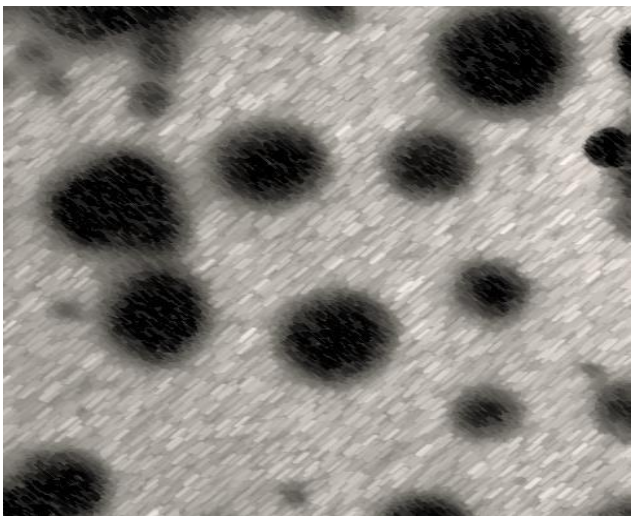

Figure 13: TEM analysis of Formulation of Transfersomal.

CONCLUSIONS

The results of the present investigation showed that the problems associated with the nandrolone decanoate conventioanal formulation could be overcome by incorporating it into

a new transdermal ultraflexible drug carrier, transfersomes. Nandrolone is an androgen receptor agonist. The drug bound to the receptor complexes which allow it to enter the nucleus and bind directly to specific nucleotide sequences of the chromosomal DNA. The areas of binding are called hormone response elements (HREs), and influence transcriptional activity of certain genes, producing the androgen effects. The half life of nandrolone decanoate and of distribution and elimination of nandrolone is 4.3 hours. Using lipid film hydration technique, nandrolone decanoate has been successfully incorporated in transfersomal formulations which can be potentially useful for delivery of this drug. Before transferosome development, preformulation studies were carried out to characterize the chemical and physical properties of drug substance. The FT-IR spectrum of drug samples was found to be in concordant with the reference chemical groups present in the structure of the Nandrolone decanoate. The UV spectrum of Nandrolone decanoate in Ethanol exhibited a broad band at 239 nm. The melting point was determined by capillary method which complies with the melting point given in reference. The solubility results showed that Nandrolone decanoate is soluble in Ethanol, Methanol and acetone, practically insoluble in 0.1NHCL. The solubility profile of drug in different solvents shows that drug is lipophilic in nature which is further confirmed by the partition coefficient study.

The standard curves of Nandrolone decanoate were prepared Ethanol and the absorbance data obtained subjected to linear regression. The correlation coefficients were found to be 0.998 for Nandrolone decanoate which is closed to one indicated for good linearity. The pre-formulation study (FT-IR spectrum, UV spectrum and melting point) results suggested that Nandrolone decanoate was pure and good in quality and the estimation procedure was found to be quite reliable, accurate and suitable for formulation development. Transferosomal formulation of Nandrolone decanoate was prepared by using the lipid film hydration technique. For optimization of transfersome, different formulations (N1 to N6) were prepared using the various quantities of lipid & surfactant. Formulation (N3) with maximum entrapment efficiency, No phase separation and optimum size considered as optimized formulation. The shape and size of the optimized N3 formulation was confirmed through microscope and particle size and found that most of the particles were well identified.

For Topical drug delivery of transfersomal gel of optimized N3 formulation is prepared by dispersing the formulation successfully in 1%, 1.5%, 2% carbopol 934. Formulation N9 (2% Carbopol 934) is optimized for further study. The % EE of optimized formulation

(89.825±0.100) with fruitful viscosity as well as spreadability. A part from it, the particle size with polydispersity index of optimized formulation is 242.3nm with PDI 0.246. Optimized formulation *in vitro* drug release was studied in phosphate buffer (PB) pH 7.4 using Franz-type diffusion cells. To know precisely, the rate and mechanism of drug release, the *in vitro* data was fitted to zero order, first order, Higuchi and Korsmeyer-Peppas model. The results showed that the drug release of N9 formulation followed Korsmeyer-Peppas order which describes that the Nandrolone decanoate follows a controlled mechanism for release from transfersome. Thus, it can be concluded from the result obtained that the transferosomal gel formulation developed for transdermal delivery of Nandrolone decanoate possessed better skin permeation potential, and higher entrapment efficiency, as well as had ability as a self penetration enhancer.

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