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FORMULATION AND EVALUATION OF TOPICAL NIOSOMAL GEL OF ROXITHROMYCIN

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ABSTRACT

Roxithromycin is a macrolide antibiotic,which is used commonly for the treatment of soft tissue infection either single or in combination. Niosomes, a vesicular formulation, has been explored extensively for topicalapplication to enhance skin penetration as well as to improve skin retention of drugs. In the present investigation, Roxithromycin was entrapped intoniosomes by a thin film hydration technique and various process parameters were optimized by partial factorial design. The optimized niosomalformulation was incorporated into carbopol gel and extensively characterized to Percentage Drug Entrapment (PDE) and in-vitro releaseperformance. The stability of above formulation was studied at different temperatures. The present study demonstrates prolongation of drug release after encapsulation of Erythromycin intoniosomal topical gel.

Key word- Soft tissue infection, Roxithromycin, Macrolide antibiotic,Niosomal gel.

1. INTRODUCTION

Drug delivery systems using vesicular carriers such as niosome, which consists of distinct advantages over conventional dosage forms since the vesicle can act as drug containing reservoirs. Niosomes are unilamellar or multilamellar vesicles including an aqueous phase that is encapsulated in highly ordered bilayer made up of nonionic surfactant with or without other components like, cholesterol and dicetyl phosphate. Niosomes showa desired interaction with human skin when applied through topical preparation by improving especially the horny layer characteristics, which in turn, due to reduction in transdermal water loss and increase in smoothness via replenishing skin lipids. Niosomes are preferred because of their low cost and higher stability of lipids which have been replaced by non-ionic

surfactants. Niosomes loaded with drugs for dermal application shows interactions with the epidermal tissue without exerting immediate or strong systemic action.[1] Roxithromycin is macrolide antibiotic which may be either bacteriostatic or bactericidal depending on the sensitivity of the microorganism and the concentration of the drug. Present study is based on the hypothesis that incorporateRoxithromycin into niosomesthat can improve the amount and time of drug retention within the skin; in turn it will increase the therapeutic efficacy of the drug and reduce the toxicity.[2] In topical drug delivery, niosomes are preferred because they enhance skin penetration of drugs andimprove the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biologicalenvironment and restricting effects to target the cells.[3]

2. MATERIALS AND METHODS

2.1. Materials

Roxithromycin was obtained as gift sample from Calyx chemical and Pharmaceutical Pvt Ltd (Mumbai, India). Span20, Span60, Span80 and Cholesterol were purchased from Oxford Laboratory (Mumbai, India). Chloroform and methanol were purchased from Rankem (Mumbai, India).Carbopol 934 K was purchased from Titan biotech Limited. All the other reagents were used without furtherpurifications. Phosphate Buffer Saline pH 7.4 (PBS pH 7.4) was prepared asdescribed in the Indian Pharmacopoeia (2007) and necessarychemicals were obtained from LobaChem (Mumbai, India).

2.2. Method

Preparation of Niosomes-

Niosomes were prepared through a thin film hydration technique.Different niosomal formulations were prepared by the lipid film hydration technique reported by accurately weighted quantities of surfactant (either span 20,40 or 60), roxithromycin (drug) and cholesterol in different molar ratios, were dissolved in 10ml of a chloroform / methanol mixture (1:1, v/v) in a round bottom flask. The solvent mixture was evaporated in a rotary flash evaporator under a vacuum of 20 inches of Hg at a temperature of 60 ± 2 ^{o}C and the flask rotated at 100 rpm until a smooth, dry lipid film was obtained. The film was hydrated with 5 ml of PBS 7.4 for 60 minute at 60 ^{o}C with gentle shaking on a water bath.[4]

3. Characterization of Niosomes

3.1. Drug entrapment efficiency

Roxithromycinniosomal formulations were centrifuged at 15,700×*g* for 90 min at 4°C using a

cooling centrifuge to separate niosomes from non-entrapped drug.[5] Concentration of the free drug in the supernatant was determined by measuring absorbance at 203 nm with a UV spectrophotometer. The percentage of drug entrapment in niosomes was calculated.

% Drug Entrapment= (Total Drug-drug in supernatant/Total drug)$_{X}$100[6]

3.2.Drug Entrapment

Drug entrapments of different niosomes formulation were as follow

Table no.1 Drug entrapment efficiency of different niosomes formulation

S.No	Formulation code	Surfactant used	Drug:Surfactant:Cholestrol ratio(weight ratio)	%Entrapment effciency±SD
1	N1	Span 20	1:1.0:1	28.93%±0.48
2	N2	Span20	1:2.0:1	36.91%±0.23
3	N3	Span20	1:1.0:2	38.78%±0.92
4	N4	Span20	1:2.0:2	42.75%±0.45
5	N5	Span60	1:1.0:1	56.62%±0.43
6	N6	Span60	1:2.0:1	63.66%±0.45
7	N7	Span60	1:1.0:2	69.75%±0.53
8	N8	Span60	1:2.0:2	66.72%±0.30
9	N9	Span40	1:1.0:1	45.34%±0.33
10	N10	Span40	1:2.0:1	48.24%±1.0
11	N11	Span40	1:1.0:2	50.90%±0.82
12	N12	Span40	1:2.0:2	50.32%±0.49

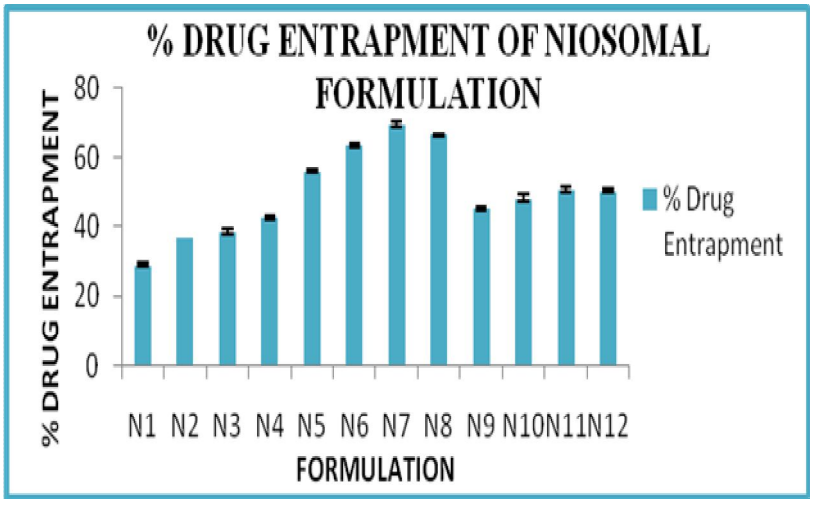

Fig no. 1% Drug entrapment efficiency of different niosomes formulation

Order of % drug entrapment of different surfactant was as follow

Span20> span40>span60

3.3.Optimization of ratio of Drug and cholesterol

Batch N7 was taken and experiments were conducted by varying the proportion of cholesterol and surfactant. Optimize the ratio of cholesterol and span 60 to get maximum % drug Entrapment.

Table no. 2 Data of optimization of surfactant: cholesterol ratio

S.No	Formulation code	Drug	Cholesterol	Span 60	%Drug Entrapment ±%SD
1	N7.1	1	1	1	71.03%±0.65
2	N7.2	1	1	2	73.28%±0.81
3	N7.3	1	2	1	64%±77
4	N7.4	1	2	2	67.56%±0.44

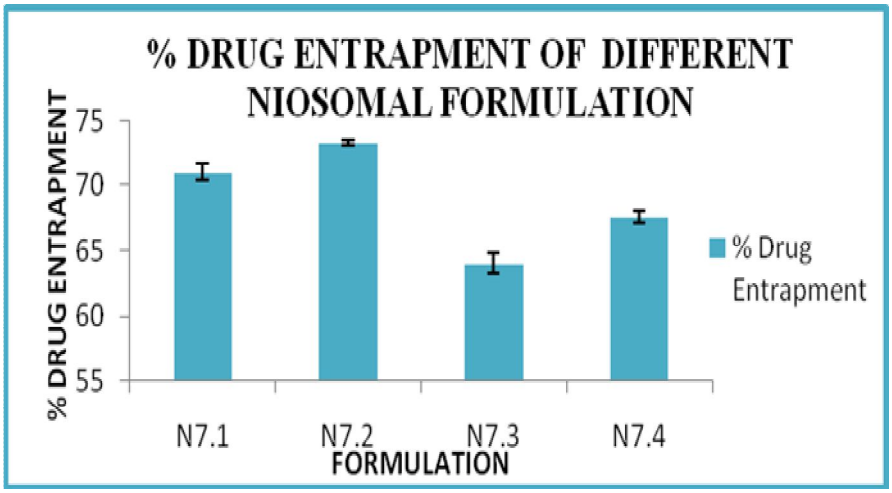

Fig no.2 % Drug entrapment efficiency of different niosomes formulationwhich are optimize of surfactant: cholesterol ratio

3.4.Optimization of solvent system ratio

Batch N7.2 Formulation was takenand experiments were conducted by varying the proportion of solvent (methanol and chloroform). Optimize the ratio of solvent system to get maximum % drug entrapment.

Table no.3 Data of optimization of solvent system ratio

S.No	Formulation Code	Chloroform (ml)	Methanol (ml)	Volume of Solvent system(ml)	%Drug entrapment±%SD
1	N7.2.1	1	1	10	71.03%±0.65
2	N7.2.2	2	1	15	73.28%±0.18
3	N7.2.3	1	2	15	56.67%±0.41
4	N7.2.4	2	2	20	67.56%±0.44

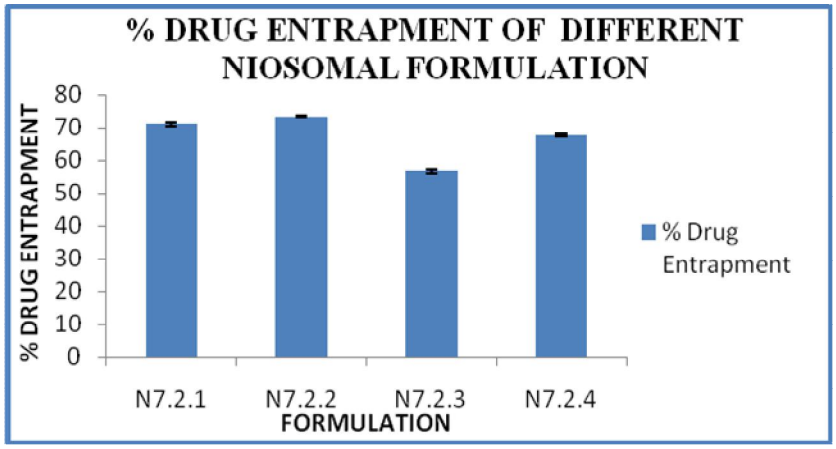

Fig no. 3 % Drug entrapment efficiency of different niosomes formulation which are optimize of solvent system ratio

3.5.Optimization of volume of hydration and time of hydration

Batch N7.2.2Formulation was takenand experiments were conducted by varying the proportion of volume of hydration and time of hydration. Optimize the ratio volume and time of hydration to get maximum % drug entrapment.

Table no. 4 Optimization of volume of hydration and time of hydration ratio

S.No	Formulation code	Volume of hydration(ml)	Time of hydration(hour)	%drug entrapment±%SD
1	N7.2.2.1	5	1.0	56.67%±0.41
2	N7.2.2.2	7	2.0	69.75%±0.75
3	N7.2.2.3	7	1.0	73.28%±0.18
4	N7.2.2.4	5	2.0	63.66%±0.26

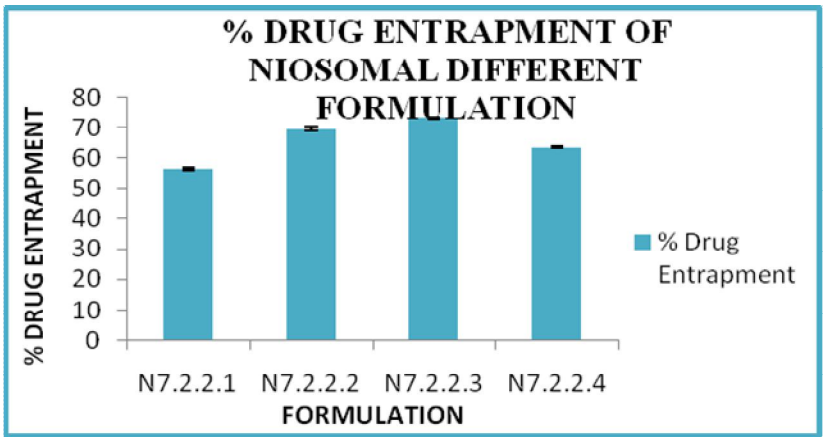

Fig no. 4% Drug entrapment efficiency of different niosomes formulationwhich are optimize of volume of hydration and time of hydrationratio

3.6.Optimization of speed of rotation, intensity of vacuum, temperature and annealing time

Batch N7.2.2Formulation was takenand experiments were conducted by varying the proportion of Speed of rotation, Intensity of vaccum, temperature and annealing time. Optimize the ratio of all parameters to get maximum % drug entrapment.

Table no. 5 Data of optimization of speed of rotation, intensity of vacuum, temperature and Annealing time

S.No	Formulation code	Speed of rotation (rpm)	Intensity of vaccum (mmHg)	Temperature (^{o}C)	Annealing time(Hour)	%Drug Entrapment± %SD
1	N7.2.2.3.1	100	20	60	1	73.28%±0.18
2	N7.2.2.3.2	125	25	70	2	71.03%±0.65
3	N7.2.2.3.3	100	20	60	1	69.75%±0.75
4	N7.2.2.3.4	125	25	70	2	63.74%±0.45

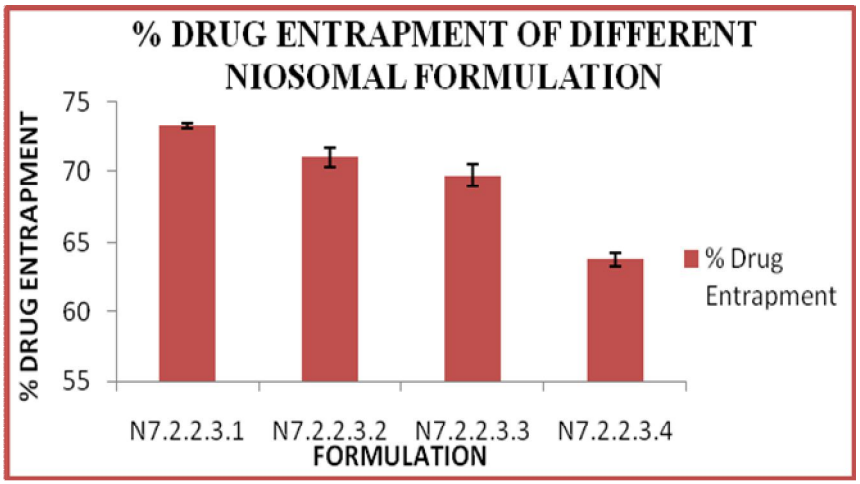

Fig no. 5 % Drug entrapment efficiency of different niosomes formulation optimization of speed of rotation, intensity of vacuum, temperature and annealing time

3.7.Final optimized batch of formulations

Optimized formulations batch shows maximum % drug entrapment and all parametres compare to other formulations[7]

Table no. 6 Final optimized batch of % drug Entrapment efficiency of different niosomes formulation

S.No	Formulation code	Parameters	Optimized value
1	N7	Nonionic surfactant	Span 60
2	N7.2	Drug:cholestrol:surfactant ratio	1:1:2
3	N7.2.2	Solvent system	Chloroform:methanol (2:1)ratio
4	N7.2.2.3	Hydration temperature	60^0C
5	N7.2.2.3.1	Vaccum	20mmHg
6	N7.2.2.3.1	Speed of Rotation	125rpm
7	N7.2.2.3	Hydration volume	7ml
8	N7.2.2.3	Hydration Time	1 Hour
9	N7.2.2.3.1	Annealing Time	2Hour

3.8.Particle size analysis

Particle size of niosomes plays an important role in determining the release characteristics. The average hydrodynamic diameter of niosomes was determined by HORIBA scientific nano particle size analyzer. Samples were preparedin phosphate buffered saline solution at pH 7.4.[8]

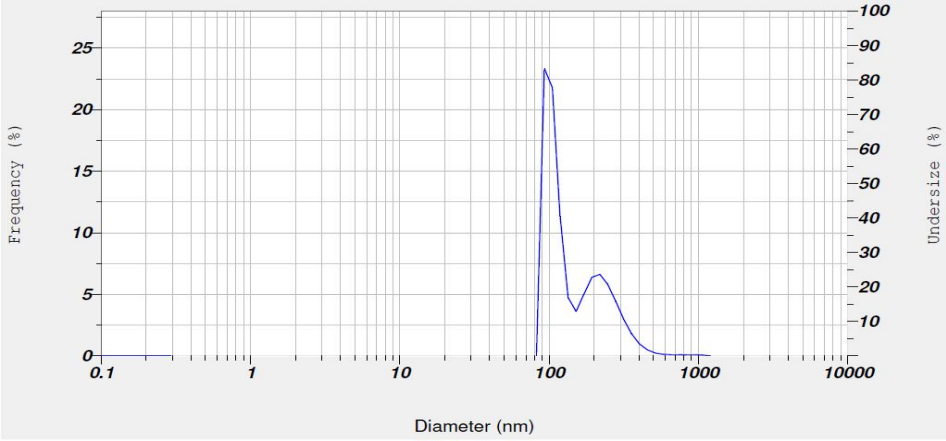

Figure no. 6 Particle size distribution of N-1 niosome formulation

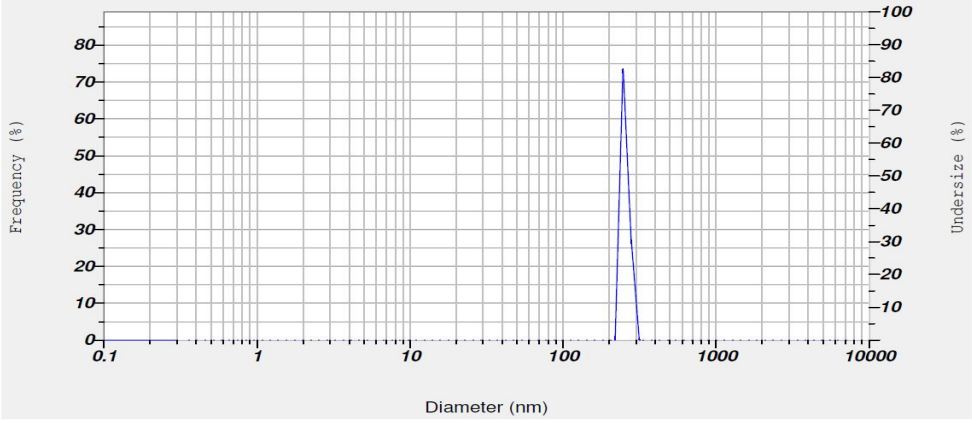

Figure no. 7Particle size distribution of N-2niosome formulation

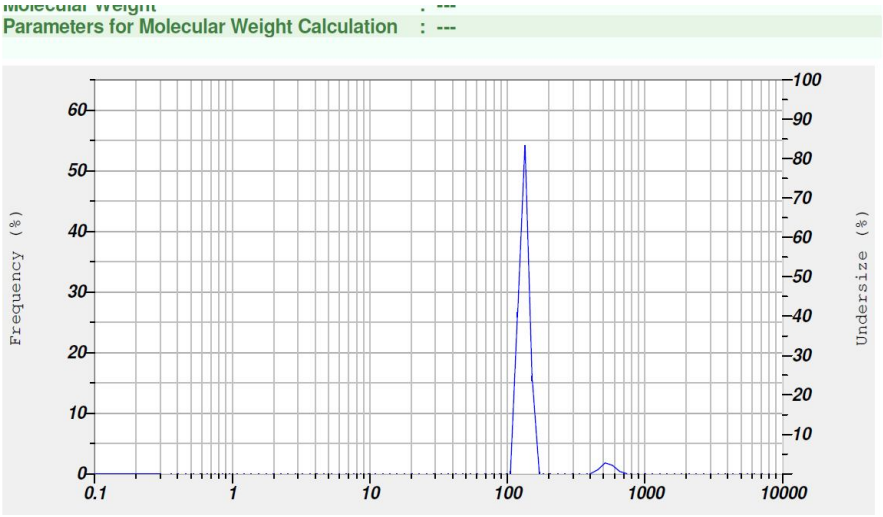

Figure no. 8Particle size distribution of N-3niosome formulation

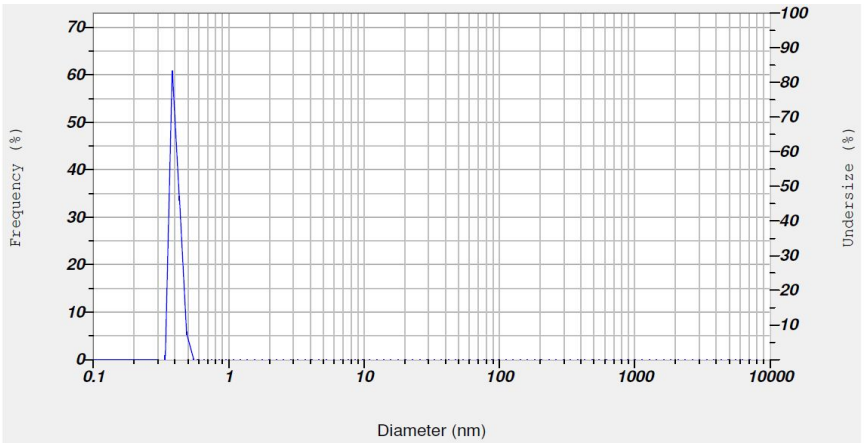

Figure no. 9Particle size distribution of N-4niosome formulation

Table no.7 Particle size analysis of niosomal formulation

S.NO	FORMULATION	PARTICLE SIZE(nm)
1	N1	100.6nm
2	N2	240.3nm
3	N3	124.9
4	N4	0.4nm

From graphs as well as observation it was comprehensible that particle size of different niosmesformulatin increase in order are as follow

N2 <N3<N1< N4

3.9.ZetaPotential Measurement

The niosomes surface charge was assessed by zetapotential measurements, using a ZetasizerNanoseriesinstrument.Samples were preparedin phosphate buffered saline solution

at pH 7.4.Thezeta potential measurements were performed at 25ºC with the help of HORIBA scientific nano particle Analyzer.[8]

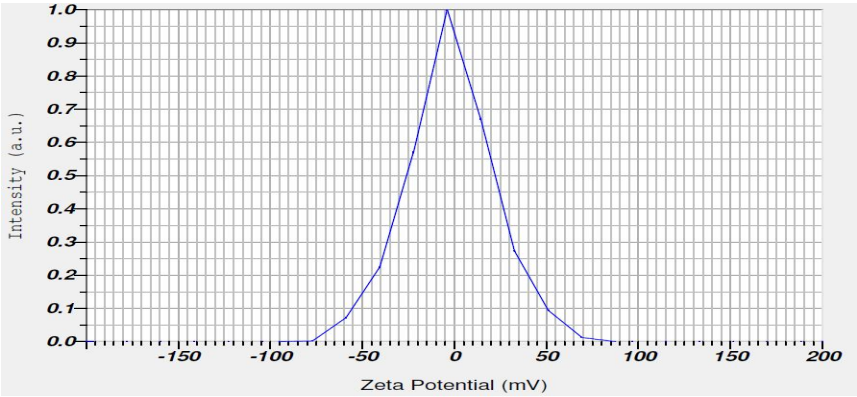

Fig no. 10Zeta potential of N-1 Formulation

Table no. 8 zeta potential of niosomal formulation

S.No	Formulation	Zeta potential
1	N1	-20.1v

3.10.*In vitro* drug release ofniosomes

The release of roxithromycin from niosomes was determined using the membrane diffusion technique, 1 ml of niosomal suspension was placed in a diffusion cell (glass tube) of diameter 2.5 cm, the lower open end of the glass tube was covered with soaked cellulose membrane. This cell then suspended in the beaker containing PBS pH 7.4 (100 ml). This was constantly stirred at speed 50 rpm at 37 ±1 0C on a magnetic stirrer with a thermostat. Aliquots were withdrawn at hourly intervals and replaced simultaneously with equal volume of fresh PBS. The roxithromycin concentration in the samples was analyzed spectrophotometrically[9]

Table no. 9% cumulative drug release of Different niosomes formulation

S.No	Time (min)	% cumulative Drug Release of formulation±S.D			
		$N1_{span60}$	$N2_{span60}$	$N3_{span60}$	$N4_{span60}$
1	0	0±0	0±0	0±0	0±0
2	15	2.3±0.47	2.25±0.20	3.28±0.49	5.32±0.40
3	30	4.5±0.32	5.56±0.45	6.33±0.31	8.21±0.21
4	60	7.43±0.37	8.26±0.25	10.26±0.1	11.61±0.36
5	90	9.35±0.11	10.92±0.61	13.37±0.30	14.58±0.33
6	120	11.42±0.11	13.27±0.35	15.25±0.45	16.30±0.40
7	180	13.52±0.70	15.18±0.41	17.21±0.36	18.24±0.33
8	240	15.65±0.3	17.32±0.27	19.10±0.58	20.23±0.41
9	300	17.34±0.25	19.25±0.11	20.35±0.20	21.45±0.45

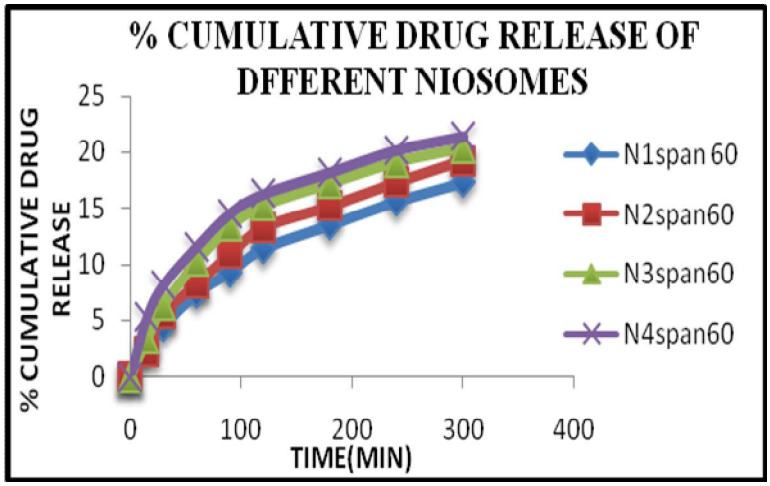

Fig no.11Rate of roxithromycin release from niosomes prepared by different ratio

4.Niosomal Topical gel preparation

a)Preparation of carbopol gel

Carbopol 934 (1.5%w/w) was dispersed in distilled water by stirring at 800 rpm for 20minutes. Then, propylene glycol (1%w/w) was added and the mixture was neutralized by dropwise addition of triethanolamine.[10] Mixing was continued until a transparent gel appeared, while the amount of the base was adjusted to achieve a gel with pH 5.5.[11]

b) Incorporation of Roxithromycin niosomes of optimized batch into carbopol gel

Niosomes containing Roxithromycin (separated from the unentrapped drug) were mixed into the 1.5% (w/w) Carbopol gel with a magnetic stirrer (1250 rpm, 20 min), the amount of Niosomes of optimized batch added into the gel.[12]Roxithromycin equivalent to 2%w/w was incorporated into the gel base composed of Carbopol 934(150mg),Triethanolamine (quantity sufficient) and distill water up to 15 gm.[13]

4.1.) Characterization of Gel

4.1.1.) organoleptic properties

The prepared gel formulations were inspected visually for their color, homogeneity, consistency, and spreadability.

Color-off white

Homogeneity-Good

Odor-odorless

4.1.2.)Ph

The pH of the dispersion was measured by using a digital pH metre. 2.5gm of gel were accurately weighed and dispersed in 25 ml of distilled water. The pH of the dispersion was measured by using a digital pH meter.[14]

4.1.3) Drug Content-

The drug content of the prepared gel was carried out by dissolving accurately weighed quantity of gel equivalent to 10mg of the drug in 100ml volumetric flask and volume was made up to 100ml with methanol.[15] The content was filtered through Whattman filter paper of above solution was taken into a 25ml volumetric flask and volume was made up to mark with methanol. The content of Roxithromycin was determined at 203 nm against blank using the Shimadzu UV/visible spectrophotometer.[16]

Table no. 12% Drug content of niosomal gel

S.No	Formulation	%Drug content±SD
1	G1	59.07 ± 0.22
2	G2	53.16±1.02
3	G3	64.43±0.40
4	G4	66.28±0.33
5	G5	68±0.1

4.1.4.) *In-vitro* drug release studies: *In-vitro* skin permeation studies of Roxithromycinniosomes were done using modified vertical Franz-diffusion cell.[17]Gel equivalent to 10mg of Roxithromycin is taken inside the cell (donor compartment) and the cell is immersed in a beaker containing 100 ml of PBS pH7.4 [18]

4.1.4.1.) Drug Release from different Niosomal gel Formulation

Roxithromycin release from different niosomal gel formulations were given in observation table as well as graph form.[19]

Table no. 14% cumulative drug release of Different niosomal gel formulation

S.No	Time (min)	% Cumulative Drug release of Formulations Code ±%S.D			
		NG1	NG2	NG3	NG4
1	0	0%±0	0±0	0%±0	0%±0
2	15	2.41±0.88	5.49±0.78	9.7±0.79	11.58±0.58
3	30	6.25±0.70	10.56±0.89	15.26 ±0.57	19.32 ±0.69
4	60	10.30±0.82	15.35±0.72	21.84 ±0.88	26.54 ±0.88

5	90	14.49±0.63	20.61±0.80	27.21 ±0.79	31.20 ±0.80
6	120	17.9±0.61	25.84 ±0.74	32.65 ±0.38	35.84 ±0.68
7	180	21.4±0.83	30.82 ±0.74	38.2 ±0.73	43.87 ±0.80
8	240	25.0±0.59	34.89 ±0.85	42.61 ±0.94	49.78 ±0.93
9	300	28.4±0.94	39.41±0.87	47.32 ±0.96	56.22 ±0.93

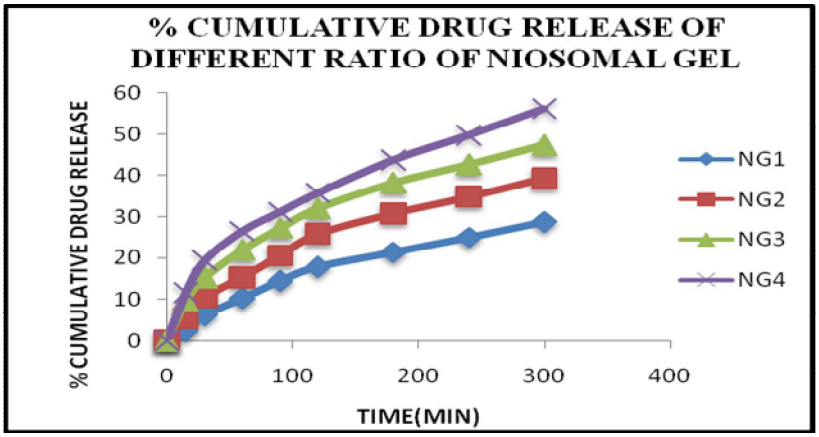

Fig no.14Rate of roxithromycin release from niosomal gel prepared by different ratio

NG4 Formulations was taken and experiment was conducted to get maximum % drug release.

4.1.4.2.Drug release of different Niosomal gel Formulation

Plain gel of roxithromycin was compared with optimized NG4 niosomal gel formulation.[20]

Table no. 15% cumulative drug release of Different niosomal gel formulation

S.No	Time(min)	%Cumulative Drug release of Formulations±S.D	
		Plain gel	NG4
1	0	0±0	0±0
2	15	2.24±0.25	11.58±0.58
3	30	5.38±0.33	19.32 ±0.69
4	60	9.45±0.43	26.54 ±0.88
5	90	13.61±0.30	31.2 ±0.80
6	120	15.37±0.20	35.84 ±0.68
7	180	17.34±0.31	43.80 ±0.80
8	240	19.6±0.43	49.78 ±0.93
9	300	22.32±0.35	56.22 ±0.93

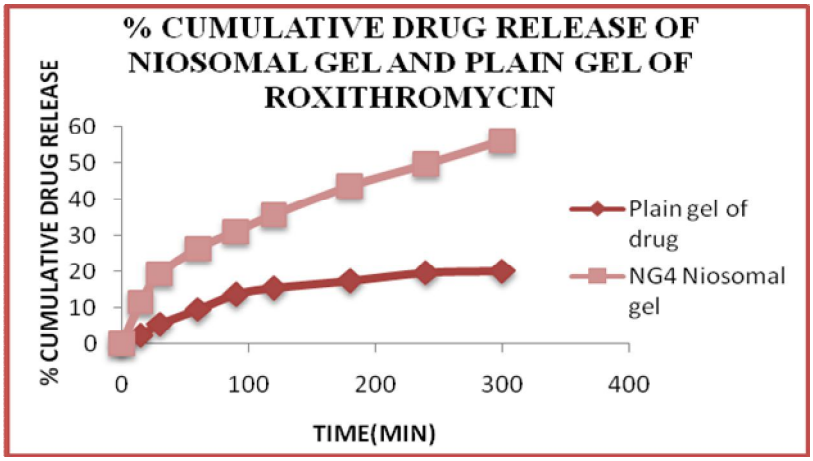

Fig. no. 15Rate of roxithromycin release from optimized NG4 niosomal gel andPlaingel of roxithromycin.

4.1.5.Stability studies

The niosomal gel was packed in aluminum tubes andsealed properly. The required numbers of tubes werecharged at different accelerated and non-acceleratedconditions 2-8°C and 40±2°C for 1 month.[21]The samples were withdrawn after each week andanalyzed for percentage drug retained in niosome vesicles.[22]

Table no. 18Stability Data for Optimized niosomal gel of roxithromycin

S. No.	Time	Storage conditions	Absorbance At 203nm	% Drug Entrapment
1.	Initial	Room Temperature	0.623	74%
2.	15 days	2-8˚C	0.526	74%
		Room Temperature	0.465	72%
		40˚C	0.362	71%
3.	1 month	2-8˚C	0.532	74%
		Room Temperature	0.413	69%
		40˚C	0.311	67.7%

CONCLUSION

Niosomes are vesicles composed of non-ionic surfactant that have been evaluated as carriers for a number of drugs and cosmetics application. The finding of this investigation have conclusively demonstrated that the encapsulation of Roxithromycin into niosomal gel formulationimproves In vitro drug release which may be reflected, based on priorhypothesis, as significantly improved therapeutic response andconsiderably reduced adverse symptoms.[23] However, the role ofniosomal Erythromycin gel of this study can only be settled afterclinical

evaluation of the product by large number of patient withspecial focus on the adverse symptoms of the therapy.[24]

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