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Original Article

FORMULATION DEVELOPMENT, EVALUATION AND OPTIMIZATION OF MEDICATED LIP ROUGE CONTAINING NIOSOMAL ACYCLOVIR FOR THE MANAGEMENT OF RECURRENT HERPES LABIALIS

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ABSTRACT

Objective: Aim of the study was to formulate, evaluate and optimize medicated Lip rouge containing acyclovir encapsulated inside a novel vesicular carrier, niosome so that the formulation can improve its membrane penetration. Formulating as a cosmetic Lip rouge formulation will also improve patient compliance in the treatment of herpes labialis.

Methods: Acyclovir niosomes were prepared by thin film hydration method. Niosomes were evaluated and were optimized by considering the entrapment efficiency and *in vitro* release profile. The optimized niosomes were incorporated into lipstick, lip balm and lip rouge for selecting the best lip formulation. Based on the *in vitro* release profile, ease of application and properties of prepared formulations lip rouge was selected and further evaluations were carried out.

Results: Among the six formulations of niosomes NF2 has showed 88.49 % entrapment efficiency and 86.97% cumulative drug release in 8 h. The formulation was optimized considering both entrapment efficiency and *in vitro* release. The optimized formulation of niosomes was incorporated into Lipstick, lip balm and lip rouge. The evaluation results of lipstick, lip balm and lip rouge for *in vitro* release suggested lip rouge as the best formulation. The percentage cumulative release of drug from optimized lip rouge at the end of 8 h was 84.77%. The percentage cumulative drug release in *ex vivo* studies for 8 h was 60.88 %.

Conclusion: The results suggested that prepared lip rouge containing acyclovir niosomes can effectively deliver the drug than the marketed acyclovir cream and successful therapy of Recurrent Herpes labialis can be achieved.

Keywords: Acyclovir, Niosomes, Herpes Labialis, Lip, Cosmetics

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INTRODUCTION

Herpes labialis (cold sores) is a condition which affects the skin and mucous membranes (particularly, the lips) that are preceded and accompanied by burning pain. The recurrent infections occurring due to different stimuli like sunlight, fever, menstruation, etc are difficult to manage. Acyclovir is the common antiviral agent that is used for the treatment of Herpes labialis. Acyclovir 5% cream is used for the treatment of herpes labialis along with oral dosage forms like tablets. These formulations show poor therapeutic outcome due to the poor bioavailability (15-20%) of oral dosage forms and poor penetration of the drug from topical formulations across the lip membrane. Acyclovir is a BCS class III Drug having limited permeability. Enhancement of penetration of Acyclovir through the lip membrane will increase the therapeutic outcome [1].

Enhancement of penetration of drugs can be achieved by encapsulating such drugs inside a lipophillic vesicular carrier. Niosomes have been claimed to improve the topical delivery of compounds, by increasing penetration of the active ingredient through the site of application.

Niosomes are nonionic surfactant based unilamellar or multilamellar vesicles in which an aqueous solution of solute is entirely enclosed by a membrane resulting from the organization of surfactant macromolecules as bilayers. It is possible to encapsulate hydrophilic as well as lipophophillic drugs in niosomes. The lipophilic nature of constituent lipids in niosomes helps in the penetration of the drugs encapsulated inside into the deeper tissues of the lip [2, 3]. Niosomes are widely used in the formulation of cosmetic products like lip balms, creams, liprouge, lipsticks, etc.

The cosmetic formulations which are applied to the lips include lipsticks, lip balms, lip jellies, lip salves, lip gloss, lip rouge etc. These formulations impart attractive colour along with gloss to the lips. Lip rouge [4] is used as an alternative to lipstick. They are almost semisolid or liquid in nature. They can be prepared by incorporating pigments in a base that can incorporate the suitable amount of aqueous phase. Such a formulation is suitable for incorporating niosome encapsulated drugs. The consistency of this type of formulation allows easy application with the help of a brush attached to the lid of the container. It also helps to achieve better permeation of the active medicament into the lip membrane. The formulation is a cosmetic with good aesthetic appeal and ease of application. The more accurate application of the active ingredient to the area involved leads to a more effective treatment of the disease. The cosmetic formulation will be more acceptable to the patient compared to the existing formulation and this may improve patient compliance.

The aim of the study was to develop medicated lip rouge containing niosomal acyclovir for the treatment of recurrent herpes labialis. This study was an approach for the development of a formulation capable of providing an increased concentration of the drug at the dermal tissue for the effective management of cold sores. As the formulation is developed by encapsulating the drug inside a niosomal carrier which is lipophillic, it may be able to penetrate deep into the lip membrane and release the drug at the site of action.

MATERIALS AND METHODS

Materials

Acyclovir was gifted from U square life sciences, Ahmadabad span 60 and span 80(Nice chemicals kochi, kerala), cholesterol, (central drug house pvt. Ltd, New Delhi), dichloromethane (Nice chemicals Pvt. Ltd, Kochi), white beeswax (Central drug house Pvt. Ltd, New Delhi), carnauba wax (oxford laboratory, mumbai), cetyl Alcohol (Qualigens Fine Chemicals, Mumbai), hard paraffin (southern union pharmaceuticals pvt. Ltd, Kochi), anhydrous lanolin (Loba chemie Pvt. Ltd, Mumbai), white soft paraffin (Southern union pharmaceuticals. pvt. Ltd, Kerala), castor oil (Central drug house pvt. Ltd, New Delhi),

light liquid paraffin (Nice chemicals pvt. Ltd. kochi), propylparaben was gifted from chethana pharmaceuticals Pvt. Ltd. Kerala.

Methods

Formulation development

Preparation of niosomes containing acyclovir by thin film hydration method

Weighed accurately the required quantity of cholesterol, surfactant, in a clean and dry round-bottomed flask, dissolved it by adding required quantity of dichloromethane. The flask was attached to a rotary vaccum evaporator and allowed to rotate at 150 rpm until a thin layer of dry film was formed on the inner wall of the flask [3]. Dissolved the required quantity of acyclovir in phosphate buffer pH 7.4 containing a required quantity of 0.1N sodium hydroxide [5].

The thin film formed on the inner wall of the flask was hydrated with this aqueous phase containing the drug. The flask containing niosomes was shaken well and placed in a bath sonicator to reduce the size of vesicles. Unentrapped drug was removed by centrifugation method using a cooling centrifuge at 4 °C and 11000rpm

Table 1: Compositions of the niosomes of acyclovir

Formulation code	Drug: surfactant: cholesterol ratio	Drug (g)	Span 60 (g)	Span 80 (g)	Cholesterol (g)
NF1	1:1:1	1		1	1
NF2	1:2:1	1		2	1
NF3	1:1:2	1		1	2
NF4	1:1:1	1	1		1
NF5	1:2:1	1	2		1
NF6	1:1:2	1	1		2

Evaluation of niosomes

Physical appearance

All the batches of prepared niosomes were observed visually for physical appearance, and homogeneity [6].

Drug content and entrapment efficiency

The unentrapped drug was separated by centrifugation in a cooling centrifuge at 11000 rpm and at 4 °C for 30 min [7]. The supernatant was separated. The resultant niosomal pellets were taken and a known amount of pellets was disrupted by adding 2-propanol and diluted with phosphate buffer pH 7.4. The absorbance was measured using UV spectrophotometer at λ max 251 nm. The drug content and percentage entrapment efficiency was calculated using the following equations

$$\text{Percentage entrapment efficiency} = \frac{\text{weight of entrapped drug}}{\text{weight of total drug}} \text{ X } 100$$

***In vitro* drug release study**

The *in vitro* release studies on niosomal suspension was performed using open-ended diffusion tube having an internal diameter of 2.5 cm and length of about 10 cm which acts as donor compartment. 50 ml pH 7.4 phosphate buffer was used as the receptor medium. The Hi-media dialysis membrane 60 was mounted between the donor and receptor compartment [8]. A measured amount of niosomal suspension was placed on one side of the membrane. The receptor medium was stirred by a magnetic bead fitted to a magnetic stirrer at a speed of 600 rpm. At each sampling interval, 2 ml were withdrawn and were replaced by equal volumes of fresh receptor fluid. The sink condition was maintained throughout the experiment. Samples withdrawn were suitably diluted and analyzed spectrophotometrically at λmax 251 nm. The percentage drug release was calculated using calibration curve of the drug in phosphate buffer pH 7.4 containing 0.1N Sodium hydroxide.

Optimization of niosomes

Optimization of formula for niosomes was carried out based on the results of percentage entrapment efficiency and *in vitro* drug release [9].

Microscopic evaluation

Niosomal suspension was also viewed by a binocular microscope at 40x magnification, to observe characteristics of suspension by spreading a thin layer of niosomal suspension on a slide [9].

Vesicle size, size distribution analysis

The vesicle size of the sample of niosomes was analysed by using particle size analyser (Malvern instrument) [9].

Zeta potential

The optimized niosomal dispersions were characterised for zeta potential by dynamic light scattering technology using Malvern Instruments.

Formulation of lip cosmetic formulations containing acyclovir niosomes [10, 11]

Table 2: Composition of lip formulations containing acyclovir niosomes

S. No.	Ingredients	Quantity		
		Lipstick	Lip balm	Lip rouge
1	White beeswax	15%	-	-
2	Hard paraffin	-	5%	-
3	Carnauba wax	5%	-	-
4	White soft paraffin	-	10%	30%
4	Anhydrous lanolin	30%	30%	35%
5	Cetyl alcohol	5%	-	-
6	Castor oil	35%	30%	15%
7	Light liquid paraffin	-	15%	10%
8	Strawberry flavour	q. s	q. s	q. s
9	Carmine red	q. s	q. s	q. s
10	Propyl paraben	0.05%	0.05%	0.05%

The required quantity of optimized niosomes was incorporated in all the formulations which will give 5% Acyclovir in the final formulation.

Evaluation of lip cosmetic formulations containing acyclovir niosomes

***In vitro* release study**

The *in vitro* release of Acyclovir niosome loaded lipstick, lip balm and lip rouge were compared using open-ended diffusion tube. The capacity of receptor compartment was 50 ml pH 7.4 phosphate buffer placed in 100 ml beaker. The temperature was maintained at 37±0.5 °C.

The hi media dialysis membrane 60 was mounted between the donor and receptor compartment. Accurately weighed quantities of formulations were separately applied on one side of the membrane. The receptor medium was stirred by a magnetic bead fitted to a magnetic stirrer. At each sampling interval, (2 ml) were withdrawn and replaced by equal volumes of fresh receptor fluid on each occasion. The Sink condition was maintained throughout the experiment. Samples withdrawn were suitably diluted and analyzed spectro-photometrically at λ_{max} 251 nm.

Optimization of formulations

Among the three formulations a suitable type of formulation that is easy to apply on lips of affected patients, easy to prepare and handle, and which on the application will release sufficient quantities of the drug and hence provide a better therapeutic effect was selected. *In vitro* drug release profile was also considered.

Evaluation of optimized medicated lip rouge containing Acyclovir niosomes

Physical appearance

The prepared lip rouge was evaluated for colour and physical appearance

Spreadability [12]

The formulation was applied to a glass slide. The time taken by the other slide attached to a pan containing fixed weights to slide over a particular distance was noted. Spreadability was calculated using the formula,

$$S = M X \frac{L}{T}$$

Where S is the Spreadability, M is the weight in the pan (tied to the upper slide), L is the length moved by the glass slide and T is the time taken to separate the slide completely from each other.

Determination of pH

pH of the selected formulation was determined using digital pH meter at room temperature.

Determination of viscosity

The viscosity of the formulation was analysed using Brookfield DV-E viscometer using spindle S-64 at various rpm ranging from 2 to 10. The value of viscosity was measured in terms of cps.

Drug content

Weighed accurately about 1 gm of lip rouge in a 100 ml standard flask and 1 ml of 2-propanol was added to break the niosomal vesicles and made up to volume with phosphate buffer pH 7.4 and absorbance of the resulting solution was measured at 251 nm [13].

Comparison of medicated lip rouge containing acyclovir niosomes with marketed formulation

***In vitro* permeation study**

The *in vitro* release of acyclovir niosome loaded lip rouge was compared with marketed formulation using open-ended diffusion tube which acts as donor compartment. The hi media dialysis membrane 60 [14] was mounted between the donor and receptor compartment. Accurately weighed lip rouge formulation and marketed formulation was suitably applied on one side of the membrane. The *in vitro* release of the acyclovir niosome loaded lip rouge was compared with the marketed formulation as described in the previous section.

***Ex vivo* permeation study**

Accurately weighed quantities of marketed formulation and optimized formulation of lip rouge were separately applied on a porcine snout membrane [14]. A blank experiment was also performed simultaneously. The *ex vivo* permeation of Acyclovir niosome loaded lip rouge was compared with marketed formulation using open-ended diffusion tube [15, 16].

The receptor medium was stirred by a magnetic bead fitted to a magnetic stirrer. 2 ml was withdrawn and replaced by equal volumes of fresh receptor fluid on each occasion. The Sink condition was maintained throughout the experiment. Samples withdrawn were suitably diluted and analyzed spectrophotometrically at λmax 251 nm.

Skin retention study

After *ex vivo* permeation studies of prepared Lip rouge dosage form and a marketed formulation containing an optimized formulation of niosomes [17], the amount of drug in the donor and receptor compartments were analysed spectrophotometrically and the difference between the above two parameters was considered as the drug retained on the porcine skin.

RESULTS AND DISCUSSION

Formulation and evaluation of niosomes

Physical appearance

All the batches of prepared niosomes appeared as white coloured uniform dispersion, without any agglomeration.

Drug content and entrapment efficiency

The percentage entrapment efficiency of niosomal formulation was found to be greatest for formulations NF2 and NF5. Niosomes containing span 60 showed greater percentage entrapment efficiency compared to the niosomes made of span 80 in similar ratios.

The nature of the hydrophobic alkyl chain affects the encapsulation efficiency of the drug. Vesicles obtained from the long alkyl chain surfactants give slightly higher entrapment efficiency than those obtained from the shorter alkyl chain surfactants [18].

This results were found to be in conformance with a previous study done by M. A Linghan *et al*.

Table 3: Drug content and percentage entrapment efficiency

Formulation code	Drug content* (mg)	% entrapment efficiency*
NF1	371.8±1.84	37.47±0.420
NF2	885.6±2.08	88.49±0.600
NF3	604.3±1.52	60.40±0.418
NF4	379.3±2.08	37.98±0.179
NF5	886.6±1.58	88.63±0.209
NF6	675.8±2.00	67.63±0.276

*(n =3) (mean±SD)

Table 4: *In vitro* drug release data of niosome formulations

Time (H)	Percentage cumulative drug release (% CDR)					
	NF1	NF2	NF3	NF4	NF5	NF6
0	0	0	0	0	0	0
0.5	23.05±1.14	28.86±1.00	25.94±1.64	28.35±1.07	31.66±1.37	17.69±1.26
1	38.69±1.11	38.56±1.58	31.11±1.39	37.29±1.12	40.58±2.09	28.66±1.32
2	47.09±1.73	47.91±2.22	36.08±1.50	45.26±2.08	47.48±1.53	36.34±1.73
3	52.94±1.94	55.53±2.54	41.43±1.67	51.39±0.91	54.76±1.88	42.03±1.70
4	57.83±1.73	62.04±1.25	45.06±1.55	56.85±1.04	60.50±1.54	45.23±2.67
5	62.04±1.21	68.57±1.14	49.78±2.40	60.93±1.09	65.07±1.55	47.84±1.28
6	67.47±1.51	74.6±1.58	53.43±1.99	64.27±1.49	70.54±1.63	49.74±1.57
7	70.89±1.45	80.57±1.32	57.17±1.28	67.75±2.08	75.48±1.11	51.32±1.73
8	72.73±2.61	86.97±1.28	58.62±1.03	69.81±1.94	77.73±1.80	52.74±1.75

*(n =3) (mean±SD)

In vitro drug release study

Formulation NF2 showed the highest percentage cumulative drug release at the end of 8 h All niosome formulations containing span 80 showed the better release of the drug compared to the corresponding niosome formulations made in the similar ratios of span 60.

The release of drug from niosomes depends upon the chain length of surfactant and concentration of cholesterol. This can be explained by the fact that niosomes exhibit an alkyl chain length-dependent release. The higher the chain length, the lower the release rate. span 60 thus retards the drug release. This is in accordance with a previous study done by Ruckmani *et al.* (2000) [19]. This can be explained by the fact that niosomes exhibit an alkyl chain length-dependent release. The higher the chain length, the lower the release rate (Devaraj *et al.*, 2002) [20] But in the case of niosomes containing span 80, the highest release of 86.97% was obtained. This higher release rate may be due to the unsaturation present in alkyl chain of span 80 which causes loosening in the structure leading to increased drug release. Span 60 have the same head group with different alkyl chain but span 80 has an unsaturated alkyl chain so the double bond made the chain bend. This means that the adjacent molecules cannot be tight when they form the membrane of niosome and might be the reason for the higher drug release from span 80 systems.

Optimization of niosomal formulations

From the evaluation results of 6 formulations of Niosomes (NF1, NF2, NF3, NF4, NF5, NF6) optimization was done based on the results of Entrapment efficiency and *In vitro* drug release studies. The results of entrapment efficiency of formulation NF2 and NF5 showed the highest values of 88.49% and 88.63% respectively. But *in vitro* release of 86.97% was exhibited by formulation NF2. Therefore NF2 was selected for further studies.

Microscopic evaluation

The optimized niosomal suspension (NF2) was observed under a binocular microscope. The fully formed niosome vesicles were visible and the image is as shown in fig. 4.

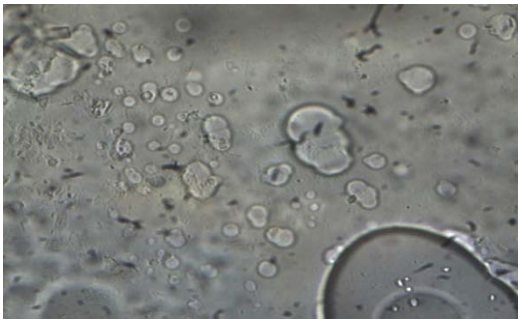

Fig. 1: Microscopic image of Niosomes under binocular microscope

Vesicle size, size distribution analysis

The particle size of the sample of niosomes was analysed by using particle size analyser (malvern instrument). The results of particle size analysis of blank niosomes and optimized formulation NF2 are shown in fig.

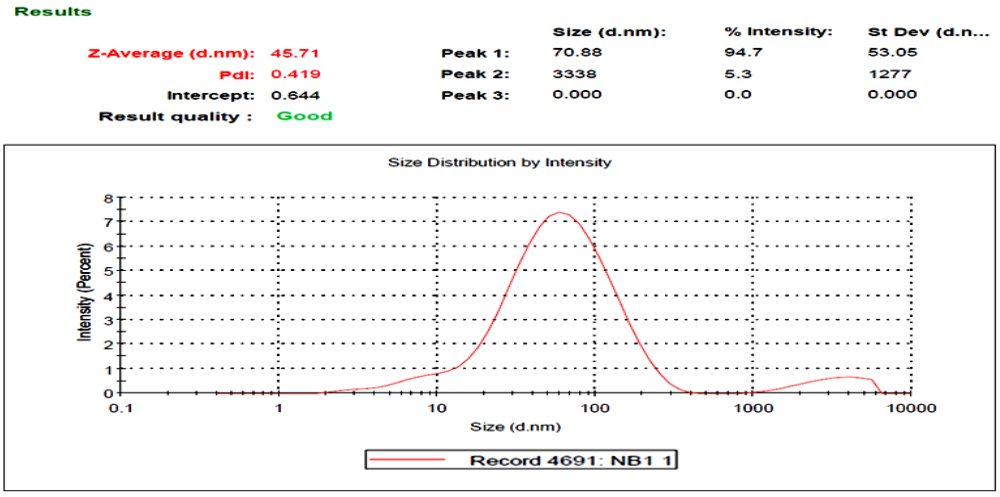

Fig. 2: Vesicle size and size distribution of blank niosomes

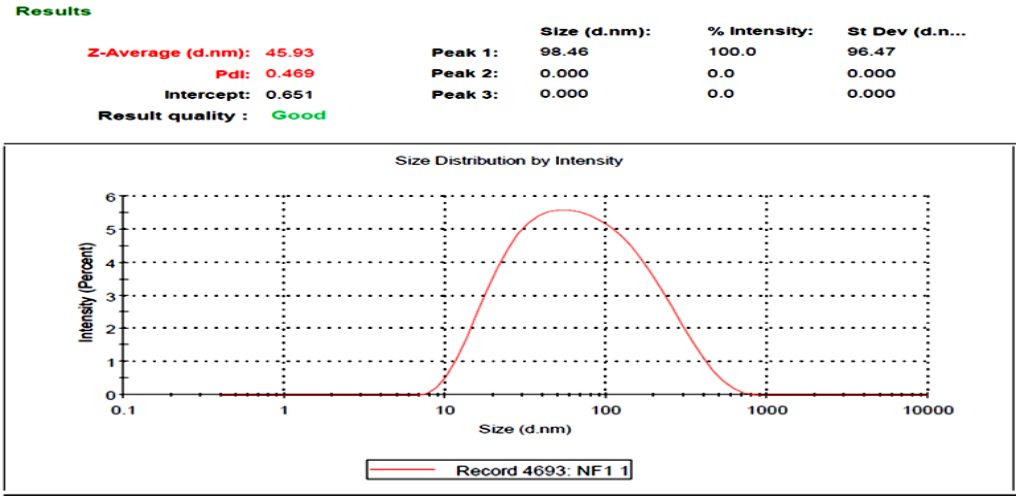

Fig. 3: Vesicle size and size distribution of optimized niosomal formulation NF2

Polydispersity Index (PdI) is a dimensionless measure of the broadness of the size distribution calculated from the cumulants analysis. In the zetasizer software, it ranges from 0 to 1 values greater than 1 indicate that the distribution is so polydisperse the sample may not be suitable for measurement by dynamic light scattering. PdI of blank niosomes and optimized niosomes NF2 were found to be 0.419 and 0.469 respectively.

Table 5: Standards for polydispersity index

Polydispersity index value	Comments
<0.05	Normally encountered with particls made to be monodisperse
<0.08	Nearly monodisperse sample
0.08 to 0.7	A mid-range value of PdI.
>0.7	Indicates a very broad distribution of particle sizes

The results suggested a moderately broad size distribution of particles

Zeta potential

The optimized niosomal dispersion was characterised for zeta potential by dynamic light scattering technology using malvern Instruments. Zeta potential was found to be-53.5mV.

It indicates good stability of niosomal dispersion.

Formulation and evaluation of lipstick, lip balm and lip rouge containing optimized acyclovir niosomes

Optimized niosomal formulation of acyclovir was incorporated into lipstick base. Similarly, lip balm and lip rouge were prepared by incorporating optimized niosomal formulation of acyclovir. The prepared formulations were evaluated for *in vitro* release profile.

Table 6: *In vitro* drug release data of lipstick, lip balm and lip rouge

Time (H)	Percentage cumulative drug release* (%CDR)		
	Lipstick	Lip balm	Lip rouge
0	0	0	0
0.5	3.07±1.01	4.56±1.32	29.51±2.47
1	3.77±1.22	11.04±1.85	39.53±1.59
2	4.42±0.70	11.60±1.73	46.71±0.86
3	5.10±0.72	13.05±1.42	55.64±2.02
4	6.86±0.72	13.98±1.70	60.48±2.38
5	7.24±1.17	13.93±1.99	69.72±2.07
6	9.82±1.99	17.30±0.99	75.66±2.03
7	10.79±1.97	22.61±2.05	80.50±1.55
8	15.10±2.11	24.28±2.94	83.23±1.79

*(n =3) (mean±SD)

From the experimental data, it was found that lip rouge showed the highest percentage cumulative drug release compared to lip balm and lipstick. This suggested the possibility of formulating lip rouge for better release of drug and hence better therapeutic effect.

Optimization

Comparing the pharmaceutical elegance, ability to apply correct dose at the site of application and *in vitro* drug release profiles of the three types of formulations, it was found that the lip rouge formulation is superior to lip balm and lipstick. The lip rouge formulation had shown the better release of drug after 8 h of *in vitro* diffusion study. The results suggested the better release of drug from lip rouge among the three types of lip formulations.

Evaluation of optimized lip rouge containing acyclovir niosomes

Physical appearance

The prepared lip rouge was evaluated for physical appearance. The formulation was uniform in appearance without agglomerates.

Determination of spreadability

The spreadability data obtained for the optimized formulation of lip rouge was 8.043±1.02. This suggested that it has a good spreadability so that it is possible to apply the formulation easily on the lips with the brush or by roll-on applicator.

Determination of pH

pH of the optimized formulation of lip rouge was found to be 7.2±0.22. The results suggested that the lip rouge could be compatible with lips on application.

Table 7: Determination of viscosity

Sample	Viscosity(cps)
Lip rouge	3452±1.15

Drug content

Drug content of lip rouge containing the optimized formulation of niosomes (NF2) was found to be 46.28±1.24 mg/gm

Table 8: comparison of *in vitro* drug release data of optimized lip rouge with marketed formulation

Time (h)	Percentage cumulative drug release (% CDR) (mean±SD)	
	Lip rouge (NF2)	Marketed product
0	0	0
0.5	25.06±1.51	4.11±0.30
1	39.18±1.41	9.88±1.39
2	50.48±1.98	11.89±1.43
3	60.38±0.55	14.44±1.26
4	68.59±1.21	15.23±0.80
5	74.38±0.70	21.99±1.56
6	78.94±1.34	28.22±2.36
7	80.93±1.49	31.89±1.73
8	84.77±1.26	35.40±2.47

Comparison of medicated lip rouge containing acyclovir niosomes with marketed formulation

***In vitro* drug release study**

***Ex-vivo* permeability study**

Ex vivo permeability study using porcine snout membrane was carried out for optimized lip rouge formulation and for marketed acyclovir cream. In the case of *ex vivo* permeability study, lip rouge showed 60% drug release while it was 25% drug release for the marketed cream formulation.

Since drug release from formulated lip rouge is higher than that of the marketed formulation it will have increased therapeutic effect than the marketed formulation.

CONCLUSION

The goal of present study was to formulate a topical dosage form for the treatment of herpes labialis (cold sores) that will improve the penetration properties of the drug acyclovir which is a BCS class III drug, having a very low permeability to the dermal tissue. Acyclovir is a guanosine analog antiviral drug which is commonly used for herpes infections. Lipid vesicular carrier, niosome was used as a carrier for penetration enhancement of the drug, acyclovir. Since formulation of the dosage form as a cosmetic preparation can improve the patient acceptance, in this study acyclovir encapsulated niosomes were formulated as a lip rouge.

The formulated lip rouge containing acyclovir niosomes showed better penetration of drug than conventional formulation. Hence it can be concluded that the prepared lip rouge formulation showed better physicochemical properties and drug release compared to the marketed formulation. Since it is a cosmetic formulation with elegant appearance, colour and flavour, it will have a good cosmetic appeal on the application as well as improved therapeutic effect in the management of recurrent herpes labialis.

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CONFLICT OF INTERESTS

Declared none

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