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FORMULATION AND EVALUATION OF GENTAMICIN SULFATE NIOSOMALGEL FOR TOPICAL APPLICATION

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ABSTRACT

The purpose of the present study was to formulate and evaluate Gentamicin Sulfate niosomal gel. Gentamicin Sulfate is an aminoglycoside antibiotic used to prevent or treat wide variety of bacterial infections. Also used to treat minor skin infections (such as impetigo, folliculitis) eczema, psoriasis, minor burns/cuts/wounds. Topically applied niosomes can increase the residence time of drugs in the stratum corneum and epidermis while reducing the systemic absorption of the drug. The niosomal dispersion was evaluated for surface morphology, drug content, entrapment efficiency and *in-vitro* drug release. Among the six formulations of niosomes, F6 formulation showed 75.86% entrapmentefficiency and the F4 formulation the drug content was found to be 79.39% and *in-vitro* drug release was found to be 87.01% at the end of 8 hrs. F4 was selected as the best formulation and this formulation was incorporated niosomal gel and evaluation for niosomal gel was determined by physical appearance, pH, viscosity, spreadability, drug content and *in-vitro* diffusion studies. The percentage of drug release from the niosomal gel was found to be 89.51%. The present study demonstrates prolongation of drug release, an increase the amount of drug retention into skin and improved permeation across the skin after encapsulation of Gentamicin Sulfate into topical gel.

KEYWORDS

Niosomes, Gentamicin Sulfate, Span 40/60 and Thin film hydration.

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INTRODUCTION

In the past few decades, considerable attention has been on advancing novel drug delivery systems (NDDS) recently. These aim to regulate drug delivery to match the needs of the body and target active ingredients effectively. However, traditional methods like prolonged-release formulas fall short. Currently, no delivery systems meet these standards perfectly, but innovative approaches are being pursued to achieve them[1].

Targeted drug delivery concentrates medication in specific tissues, minimizing its impact on surrounding areas. This localization maximizes efficacy by preventing drug loss. Various carriers like immunoglobulin, liposomes and microspheres achieve this targeting[2].

Topical drug delivery applies medication directly to the skin for treating cutaneous disorders like psoriasis and acne. Semisolid formulations such as gels, creams and lotions are commonly used, along with other formulations like foams and sprays. Topical agents may include astringents, rubefacients and keratolytic agents, delivering drugs locally or systemically.

This route is increasingly significant for systemic drug administration, although primarily used for local effects. While some systemic absorption may occur, it is typically at sub-therapeutic levels, with minimal concern for systemic effects in most cases[3,4].

Niosomes are vesicles composed of non-ionic surfactants that form when synthetic non-ionic surfactants are hydrated, sometimes with the addition of cholesterol or other lipids[5]. They arise from the self-organization of non-ionic surfactants in aqueous environments, forming closed bilayer structures, which can be either unilamellar or multi-lamellar[6].

Niosomes can encapsulate hydrophilic drugs within their core cavity and hydrophobic drugs in the nonpolar region of the bilayer. This allows for the incorporation of both hydrophilic and hydrophobic drugs into niosomes[7].

The present study is Investigation on niosomal gel containing Gentamicin sulphate for topical application

MATERIAL AND METHODS

Materials

Gentamicin sulfate, span 40, span 60, cholesterol, carbopol, diethyl ether, chloroform, methanol.

Methods

Preformulation studies

Preformulation testing is the first step in rational development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of drug substance alone and when combined with excipients. Preformulation investigations are designed to deliver all necessary data especially physiochemical, physico-mechanical and biopharmaceutical properties of drug substances, excipients and packaging materials.

Organoleptic properties

The colour, odour and taste of Gentamicin Sulfate was recorded using descriptive terminologies.

Determination of melting point

Determination of melting point of Gentamicin Sulfate was carried out by using capillary method. Fine powder of Gentamicin Sulfate was filled in glass capillary tube (previously sealed at one end). The capillary tube was tied to thermometer and it was placed in the Theil's tube and this tube was placed on fire. The powder at what temperature is melted was noticed[8].

Solubility

It is determined by dissolving Gentamicin Sulfate in water, phosphate buffer pH 7.4 and methanol. The solubility study was conducted by taking excess quantity of the drug in 10ml of solution. Then the sample were kept in water bath shaker and agitated for 12 hrs at 37±0.5^{o}C. The sample were filtered and diluted suitably with buffer solution. The samples were analysed spectrophotometrically at λ max. The concentration of Gentamicin Sulfate was determined using respective standard graph[9].

Preparation of standard plot of Gentamicin Sulfate in PBS pH 7.4

UV Spectrophotometric estimation method was used for the preparation of standard plot of Gentamicin Sulfate. 100mg of Gentamicin Sulfate was dissolved in PBS solution of pH 7.4 and volume was made up to 100ml with further addition of buffer to give concentration of 1000µg/ml in 100ml volumetric flask and labelled as stock a solution. From the stock A solution, 10ml was pipetted out and diluted to 100ml with PBS pH 7.4 to get concentration of 100µg/ml and labelled as stock B solution. From the stock B solution aliquots of 2ml, 4ml, 6ml, 8ml and 10ml were pipetted and diluted up to 10ml with PBS pH 7.4 to get

concentration of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml respectively. The absorbance values were recorded against PBS pH 7.4 as blank[10].

METHOD OF PREPARATIONS OF NIOSOMES

Niosomes were prepared by thin film hydration method as follows: The niosomal formulations were prepared by thin film hydration technique. Accuratelyweighed quantities of drug (100mg), non-ionic surfactant (Span 40, 60) and Methanol (2:1)) to give a clear solution. The resulting solution is poured into a 1000 ml rotary flask and evaporated under vacuum (20-25mm Hg) at 60°±2°C with the rotation speed of 100 rpm to form a uniform thin dryfilm. The rotary flask was removed from the bath and allowed to return to room temperature. The thin film formed was hydrated with 20 ml of PBS pH 7.4 and while rotating the flaskat 50rpm (gentle agitation) at a temperature 60°±2°C. And sonicated in prob sonicator for 20 min. Then niosome suspension transferred into container and stored in refrigeratorat 4°C[11].

In Vitro characterization of niosomes

Drug content analysis

The amount of drug in the formulation was determined by lysing the niosomes using 50% npropanol. 1ml of the niosomal preparation was pipetted out, sufficient quantity of 50% npropanol was added and shaken well for the complete lysis of the vesicles. After suitable dilution with the phosphate buffered saline of pH 7.4. The absorbance of the solution was measured at 202 nm in the UV- Visible Spectrophotometer[12].

Estimation of entrapment efficiency

The entrapment efficiency of the formulations was determined by centrifuging 1ml of the suspension diluted to 10 ml with distilled water at 15,000rpm for 60 minutes at 4°C using a high-speed cooling centrifuge in order to separate niosomes from unentrapped drug. The free drug concentration in the supernatant was determined at 202nm using UV-Visible Spectrophotometer after suitable dilution. The percentage of drug entrapment in niosomes was calculated using the following formula. Drug entrapment = (Total drug- Drug in supernatant liquid)/ Total drug X 100

In-vitro diffusion study

In- vitro drug release pattern was studied using cellophane membrane. The niosomal preparation after separation of unentrapped drug was placed in an open-ended glass tube, one end of which was tied with the cellophane membrane. This acted as the donor compartment. Then the open-ended tube was placed in a beaker containing 100ml phosphate buffered saline pH 7.4, which acted as receptor compartment. The temperature of the receptor medium was maintained at 37°±2°C and the medium was agitated at a speed of 100rpm using a magnetic stirrer. 5ml of the samples were collected at a predetermined time and replenished immediately with the same volume of fresh buffer PBS pH 7.4. The sink condition was maintained throughout the experiment. The collected samples were analysed spectrophotometrically at 202nm using UV-Visible spectrophotometer[13].

Method of preparations of niosomes

A gel base was prepared by soaking the weighed amount of Carbopol 940 (1% w/v) inappropriate amount of distilled water to form an aqueous dispersion. The prepared niosomal suspension (equivalent to 0.1% w/w) was incorporated into Carbopol 940 base and properly mix by mechanical stirring until homogenous gel was formed. Then propylene glycol (5%) was added in stirring to obtain the desired consistency. Drops of triethanolamine (q.s) were added to adjust pH. And make up with distilled water up to 15g. The samples were stored in refrigerator (4°C) for at least 24 hours prior to performing rheological measurement[14].

Evaluation of niosomal gel

Physical appearance

The prepared gel formulations were inspected visually for clarity, colour, homogeneity, texture and presence of foreign particles.

Determination of pH

2.5gm of gel was dispersed in 25ml distilled water and the pH was examined using digital pH meter.

Determination of Viscosity

Viscosity of niosomal gel was determined by Brookfield Viscometer. 10g of gel was taken into a beaker and the spindle was dipped into the gel formulation. Viscosity of the gel formulation was measured by rotating the spindle (S-64) at 100rpm.

Spreadability

The formulation was applied to the glass slide. The time taken by the other slide attached to the pan containing fixed weight to slide over a particular distance was noted. Spreadability was calculated using the formula, S=M×L/T Where, S= Spreadability M= Weight in the pan (tied to upper slide) L= Length moved by the glass slide T= Time taken to separate the slide completely from each other.

Drug content

The drug content of the prepared gel was carried out by dissolving accurately weighed quantityof gel equivalent to 100mg of the drug in 100ml volumetric flask and suitable volume of 50% n-propanol for the lysis of the vesicles. The volume was made up to 100ml with phosphate buffer pH 7.4. The solution was filtered through Whatman filter paper (No.41). 5ml of above solution was taken into a 50ml volumetric flask and volume was made up to mark with phosphate buffer pH 7.4 and was analyzed spectrometrically by using UV Visible Spectrophotometer at 202nm.

Kinetic studies

Zero order= % Cumulative drug release v/s time First order= log % drug remaining to release v/s time The mathematical models such as zero order and first order are used to understand the kinetic and mechanism of drug release. The various kinetic equations are as follows:

Zero order kinetics

Drug dissolution from pharmaceutical dosage forms that release the drug slowly, assuming thatarea does not change and no equilibrium conditions and are represented by the equation: $Qt = Q0 + K0t$ Where, Qt =amount of drug dissolved in time t, Q0 =initial amount of drug in the solution, K = zero order release constant

First order kinetics

The application of this model to drug dissolution studies used to describe absorption and/ or elimination of drugs. To study the first order release rate kinetics the release rate data were fitted to the following equation: $LogQt = logQ0 + K1t/2.303$ Where, Qt is the amount of drug released in time t, Q0 is the initial amount of drug in the solution, K1 is the first order release constant.

Stability studies

Stability of drug can be defined as the time from date of manufacture and the packaging of the formulation, until its chemical or biological activity is not less than predetermined level of labelled potency and its physical characteristics have not changed appreciably. The purpose of the stability studies is to provide evidence on how quality of the drug substance or drug product varies with time under the influence of variety of environmental factors such as humidity, temperature and light. The formulated niosomal gel was kept in the air tight containers and stored in the ICH certified stability chamber maintained at room temperature 30℃±2℃/ 65%±5%RH and refrigeration 4.0±2.0°C for three months. The sample was withdrawn at different time interval over a period of 1, 2 and 3 months and the sample was analysed for physical appearance, pH and drug content.

RESULTS AND DISCUSSION

In the present study total six niosomal formulations loaded with Gentamicin sulfate were prepared by thin film hydration technique with varying surfactants ratio. Priorly the preformulation studies for the drug was carried followed by preparation of formulation and their evaluation. The results for the following experiment conducted are as follows.

Preformulation studies of pure drug Gentamicin sulfate

Organoleptic properties

Organoleptic properties like general description, colour and odour of Gentamicin sulfate were characterized. It was found that Gentamicin Sulfate

is a white amorphous powder, odourless and was found to be within the reported literature limits.

Determination of melting point

The study was carried out and found that the drug melted at 235ºC which is in the reported range of 218-237ºC and thus was within the reported literature limits indicating that the drug is pure.

Preparation of standard plot of Gentamicin Sulfate in PBS pH 7.4

The values of the absorbance at different concentration (µg/ml) in phosphate buffer pH 7.4 are given in Table No.2 and the standard plot is shown in Figure No.1. The absorbance value remained linear and obeyed Beer's Lamberts Law in the range of 20-100µg/ml with the R2 value of 0.9995.

Preparation of niosomes

Total six niosomal formulations loaded with Gentamicin Sulfate were prepared by thin film hydration technique with varying surfactant ratio. As per the procedure given in the methodology. On physical evaluation all the six-formulation appeared as milky white dispersion.

***In-vitro* characterization of niosomes**

Drug content

Niosomes prepared by using drug: span 60: Cholesterol (1:1:1) give the highest drug content was found to be 79.39%

Entrapment efficiency

Niosomes prepared using Drug: Span 60: Cholesterol (1:3:1) gave the best entrapment efficiency 75.86%. As the concentration of span 60 increased, there was significant increase in the entrapment efficiency of the drug. Hence the formulations with higher concentration of Span 60 showed greater percentage of entrapment efficiency.

***In-vitro* diffusion study**

Niosomal formulation F4 showed highest percentage cumulative drug release at the end of 8hrs. Niosomal formulation containing Drug: Span 60: cholesterol in the ratio (1:1:1) showed higher drug release of 87.01% compared to the other ratios.

Evaluation of niosomal gel

Physical appearance

The prepared niosomal gel was evaluated for physical appearance. The formulation was clear, homogenous without any presence of foreign particles.

Determination of pH

The pH of prepared niosomal gel found to be 5.3±0.21 which is related to the pH of the skin. Thus, the niosomal gel was compatible with skin.

Determination of viscosity

Viscosity for the prepared niosomal gel was found to be 304 cps.

Determination of spreadability

The spreadability of the prepared niosomal gel was determined and it was found to be 21.43±0.25 (g.cm/sec). This showed that niosomal gel has a good spreadability so that it is apply to skin.

Determination of drug content

The niosomal formulation F4 that showed highest drug content was incorporated to niosomal gel and drug content of the niosomal gel was determined. The drug content from the niosomal gel was found to be 78.63%.

***In-vitro* drug permeation study**

The niosomal formulation F4 that showed highest drug release was incorporated to niosomal gel and the *in vitro* drug permeation of the niosomal gel was determined. The *in vitro* drug permeation from the niosomal gel was found to be 89.51% at the end of 8 hrs.

Kinetic study

The various kinetic models were applied to in-vitro release data for prediction of the drug release kinetic mechanism. In order to determine the release mechanism that provides the best description to the pattern of drug release, the in vitro release data were fitted to Zero order and First order. The data were processed for regression analysis using MS-EXCEL statistical function. The release constants was calculated from the slope of appropriate plots, and the regression coefficient (R2) was determined. It was found that in vitro drug release of niosomal gel was best explained by zero order kinetic model as the plots shows highest linearity. Correlation

coefficient (R2) was found to be 0.9915 for niosomal gel indicating that the drug release was nearly independent of concentration.

Stability studies

It was conformed from the stability studies for formulated niosomal gel was stable at at room temperature 30°C±2°C/65% RH±5% RH and refrigeration 4.0±2.0°C. The formulation was evaluated for physical appearance, pH and drug content.

Table No.1: Pre-formulation studies of Gentamicin Sulfate

S.No	Properties	Reported		Observed	
1	Description	White to almost white colour		White to buff colour	
2	Colour	White		White	
4	Odour	Odourless		Odour	
5	Melting Point	218-237^{o}C		237^{o}C	
6	Solubility	Water	50mg/ml	Water	48mg/ml
		Methanol	23mg/ml	Methanol	20mg/ml
		Phosphate buffer saline pH 7.4	46mg/ml	Phosphate buffer saline pH 7.4	43mg/ml

Table No.2: Calibration data of Gentamicin sulfate in phosphate buffer of pH 7.4

S.No	Concentration	Absorbance
1	0	0.000
2	20	0.1413±0.125
3	40	0.3034±0.163
4	60	0.4347±0.118
5	80	0.5907±0.102
6	100	0.7285±0.137

Table No.3: Drug content of niosomal formulation

Formulation Code	Drug Content (%)
F1	74.52
F2	71.34
F3	76.33
F4	79.39
F5	73.74
F6	77.96

Table No.4: Entrapment efficiency of niosomal formulation

Formulation Code	Drug Content (%)
F1	69.52
F2	71.69
F3	74.35
F4	70.64
F5	73.83
F6	75.86

Table No.5: *In vitro* drug diffusion profile of different niosomal formulations

Time (hrs)	%CDR					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	13.16±0.25	11.39±0.14	15.18±0.16	19.14±0.28	13.53±0.36	16.93±0.19
2	22.82±0.16	19.46±0.28	23.43±0.57	27.46±0.18	22.58±0.38	24.13±0.43
3	34.27±0.29	26.32±0.31	37.26±0.24	35.53±0.37	30.96±0.32	33.63±0.18
4	42.86±0.18	37.63±0.42	49.13±0.49	48.51±0.43	41.36±0.45	45.83±0.34
5	51.74±0.32	46.02±0.35	57.26±0.13	56.14±0.58	50.38±0.63	56.48±0.18
6	62.32±0.13	59.12±0.45	65.54±0.39	64.45±0.43	59.36±0.54	64.43±0.23
7	70.93±0.19	66.84±0.36	74.98±0.21	75.26±0.13	68.38±0.78	76.12±0.35
8	81.74±0.34	76.36±0.19	83.34±0.46	87.01±0.39	78.96±0.12	84.86±0.31

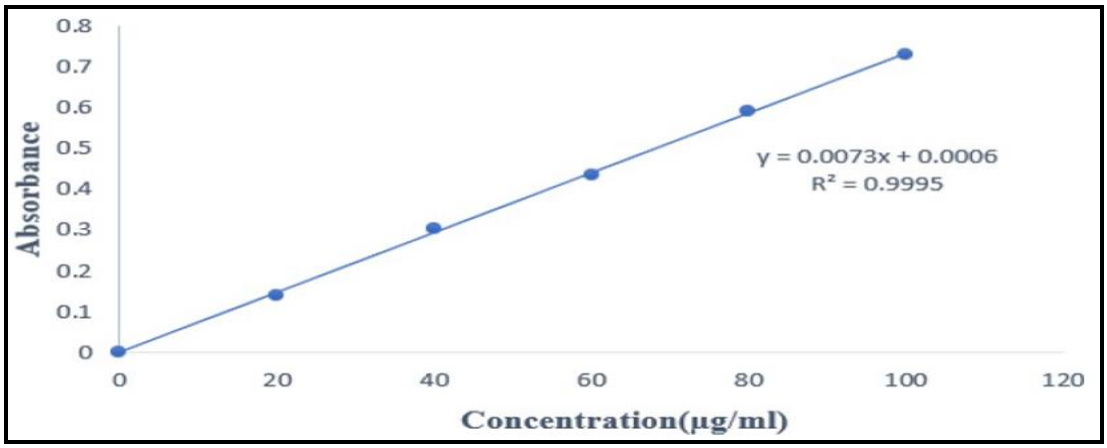

Figure No.1: Standard graph of Gentamicin Sulfate

CONCLUSION

The present study has been satisfactory attempt to formulation and evaluation of Gentamicin Sulfate niosomal gel for topical application. From the reproducible results of executed experiments: it can be concluded that; Preformulation studies of Gentamicin Sulfate comply with the reported literature limits. Niosomal formulation with Drug: surfactant: cholesterol ratio (1:3:1) showed significantly higher entrapment efficiency compared to that of the other niosomal formulations with different ratios of Drug: surfactant: cholesterol. Niosomal formulation with Drug: surfactant: cholesterol ratio (1:1:1) i.e., F4 showed higher cumulative drug release at 87.01% compared to the other formulations. The F4 formulation was selected as best formulation and conveniently incorporated intocarbopol 940 gel (1%). The gel formulation was evaluated for various parameters like physical appearance, pH, viscosity, spreadability, extrudability, drug content, *in-vitro* permeation study, kinetic studies, stability studies and results were found to be reproducible. *In-vitro* permeation studies of niosomal gel showed that the drug release maximum at8 hrs that is 89.51%.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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