Formulation, Characterization and Evaluation of Liposomal Hydrogel for the Treatment of Antibiotic Resistant Propionibacterium Acne

Pankaj Kumar Pandey and Ashish Kumar Parashar

ABSTRACT

This work aimed to prepare and evaluate the topical liposomal hydrogel to treat antibiotic-resistant propionic bacterium acne. Nadifloxacin-loaded liposomes were prepared by thin-film hydration technique. Nadifloxacin, soya lecithin, cholesterol were dissolved in a mixture of chloroform and taken in different levels, and liposomes were prepared. The prepared liposomes were evaluated for in-vitro drug release. Formulation F2 was the highest percentage entrapment of 71±1.50% and released 58.12±1.2% of the drug in 6hrs. Minocycline hydrochloride-based hydrogel was prepared using the methylcellulose gelling agent, and the drug concentration was kept constant at 0.25%. The concentration of propylene glycol and methylparaben was kept constant at 15% and 0.3%. The hydrogel formulation was evaluated for various physicochemical parameters like percentage drug content, spreadability, and drug release. Formulation H9 was the highest drug content, 98.23±0.031%, and the drug released 90.96±2.6% of the drug in 6hr. Out of these formulations, F2 from liposomes and H9 from hydrogel formula was selected to prepare the final liposomal hydrogel formulation. Developed liposomal hydrogel (F2H9) were evaluated like size, drug content and drug entrapment, spreadability, homogeneity, washability, pH, etc. In-vitro drug release from liposomal hydrogel in Nadifloxacin release 54.86±3.1% and Minocycline hydrochloride 91.24±1.82% in 6hr. Developed liposomal hydrogel formulation can better treat acne due to high drug retention and permeation in skin layers.

Key words: Acne, antibiotics, liposomes, hydrogel, Nadifloxacin, Minocycline hydrochloride.

1. INTRODUCTION

Acne is an inflammatory follicular disorder, approximately 85% affecting teenagers, and 95% of the population suffers from acne. Endogenous and exogenous hormones respond to acne. It is pilosebaceous units on the skin consisting of the open follicle, the group of sebaceous gland surrounds that. Acne is a common skin disease. Acne is a cutaneous pleomorphic situation of the pilosebaceous component involving the unbalance in sebum production. Inflammatory and non-inflammatory lesions categorize acne. Acne is mainly caused by the propionibacterium and staphylococcus bacteria present in the epidermis, and pus forms on the skin. Propionibacterium and staphylococcus bacteria are responsible for several lesions of acne. It is also called pimples, zits, or blemishes.

Several clinical management included seborrhea, comedones, erythematous papules, and pustules, less frequent nodules, deep pustules or pseudocysts, and final scarring in a few of them. Acne has four main pathogenic mechanisms- abnormalities in sebum production, follicular hyperkeratinization, Propionibacterium acne (P. acne) colonization, and the products of inflammation.
Liposomal hydrogels have an advantage over other conventional formulations such as creams, ointments, and gels. They enhance the skin retention of drugs, higher drug concentrations in the skin, and at the same time, slow down the systemic absorption of drugs.[7-8] They also act as a drug depot and provide sustained localized drug delivery, and liposomal hydrogels deliver an adequate quantity of drugs for their therapeutic activity. The composition and concentration of lipid in liposomes incorporated into hydrogels are the two significant factors that play an important role in the rheological properties of hydrogels.[9-12]

Nadifloxacin is a synthetic bactericidal fluoroquinolone and a broad spectrum of antibacterial activity against aerobic Gram-positive and Gram-negative and anaerobic bacteria, including P. acnes and Staphylococcus epidermidis. It is antibacterial but also anti-inflammatory actions, which may have effects on some aspects of inflammatory acne.[13-14]

Minocycline is a broad-spectrum tetracycline. Tetracycline antibiotic that fights bacteria into the body. Minocycline hydrochloride is helpful in bacterial infections, such as urinary tract infections, respiratory infections, skin infections, severe acne, gonorrhea, tick fever, chlamydia.[15] It is a bacteriostatic antibiotic, categorized as a long-acting type due to its long half-life. It normally has serum levels 2-4 times that of the simple water-soluble tetracyclines.[16-17]

2. MATERIAL AND METHODS

2.1 Material

Nadifloxacin was gift sample from Wockhardt Limited, Aurangabad. Minocycline-hydrochloride was gift sample from Sun Pharma Ltd Dewas, potassium dihydrogen phosphate, sodium hydroxide, methanol, chloroform, cholesterol, soya lecithin, agar, methylcellulose, methyl paraben, triethanolamine, propylene glycol, distilled water.

2.1.1 Preparation of liposomes

Nadifloxacin loaded liposomes were prepared by thin film hydration technique. The lipid mixtures and drug were placed in the 250 ml round bottomed flask and dissolved by using in 5ml chloroform. The organic solvent was evaporate by the rotatory evaporator (100 RPM) and maintain the temperature 50°C for 2 hours then thin film obtained and hydrated with 10-15 ml of phosphate buffer 7.4pH for a period of 1h. 18

| Batch No. | Nadifloxacin (mg) | Soya lecithin (mg) | Cholesterol (mg) | Chloroform (ml) | RPM | Temp °C |
|-----------|------------------|--------------------|------------------|-----------------|-----|---------|
| F1        | 10               | 100                | 5                | 5               | 100 | 50      |
| F2        | 10               | 100                | 10               | 5               | 100 | 50      |
| F3        | 10               | 100                | 50               | 5               | 100 | 50      |

2.1.2 Preparation of hydrogel

Distilled water in added methyl cellulose placed over a night then 0.25gm of minocycline hydrochloride and added propylene glycol, methyl paraben for the preservative, hydrogel neutralized with triethanolamine[19] (Table 2). All the trial batches of hydrogel were evaluated by various parameters like appearance, pH, washability, viscosity, spreadability, homogeneity, drug content.

2.1.3 Preparation of liposomal hydrogel

Nadifloxacin loaded liposomal suspension in added methyl cellulose over a night for swelling and add the 0.25gm of minocycline hydrochloride then added propylene glycol. Neutralized the hydrogel with triethanolamine and last in add methyl paraben (Table 3). 20-21

2.2 Characterization of liposomes

2.2.1 Vesicle shape determination

The determination of shape and surface morphology was done by scanning electron microscope Jeol JSM-5600, Japan. SEM analysis of the samples revealed that all liposome prepared at UGC-DAE Consortium for scientific Research, Indore.

2.2.2 Entrapment efficiency

Liposomal formulations were subjected to centrifugation using cooling centrifuge at 2,500 rpm for about 2 h. The clear supernatant was separated then carefully to separate the un-entrapped nadifloxacin and sediment was then treated with 1 ml of methanol to lyse the vesicles and diluted to 10 ml with methanol and absorbance of both solutions was observed at 294 nm. The amount of nadifloxacin in supernatant and sediment gave a total
amount of nadifloxacin in 1 ml of dispersion. The entrapment efficiency was calculated using this formula.

\[ \text{% Entrapment efficiency} = \frac{\text{Entrapped drug}}{\text{Total drug added}} \times 100 \]

2.2.3 In vitro drug diffusion studies

The release of drug was determined by using the dialysis membrane mounted on the one end of open tube, containing 5ml of liposomal suspension (10 mg of Nadifloxacin). The dialysis tube was suspended in 200ml beaker containing 100 ml of PBS (pH 7.4).

2.3 Evaluation of liposomal hydrogel

2.3.1 Percentage Drug content

The liposomal hydrogel sample (1 gm) was withdrawn and dissolved in 100ml of phosphate buffer (pH 7.4). The volumetric flask containing liposomal hydrogel solution shaken for 2hr. This solution was filtered and estimated using UV spectrophotometer at 294 and 384nm.

2.3.2 Visual inspection

All developed liposomal hydrogel formula was checked for their homogeneity, color and presence of lumps by visual inspection after the gels have been set in the container.

2.3.3 Measurement of pH

The pH of various gel formulations was determined by using digital pH meter. One gram of liposomal hydrogel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values are calculated.

2.3.4 Spreadability

A sample of 0.5 g of each formula was pressed between two slides (divided into squares of 5 mm sides) and left for about 5 minutes where no more spreading was expected. Diameters of spreaded circles were measured in cm and were taken as comparative values for spreadability. The results obtained are average of three determinations.

2.3.5 Viscosity determination

The viscosity of gel was determined by using a Brookfield DV-E viscometer model with a T-Bar spindle in combination with a helipath stand. The spindle#6 was used for determining the viscosity of the gels the factors like temperature, pressure and sample size etc. Which affect the viscosity was maintained during the process.

2.3.6 Homogeneity

After the liposomal hydrogel have been set in the container, all developed liposomal hydrogel were tested for homogeneity by visual inspection. They were tested for their appearance and presence of any aggregates.

2.3.7 Extrudability study

The liposomal hydrogel were set in the container, the formulations were filled in the collapsible tubes. The extrudability of the formulation was determined in terms of weight in grams required to extrude a 0.5 cm. ribbon of gel in 10 second.

2.4. In vitro Drug release studies

The liposomal hydrogel in release of drug was determined by using the dialysis membrane mounted on the one end of open tube, containing 1gm of liposomal hydrogel. The dialysis tube was suspended in 200ml beaker containing 100 ml of PBS (pH 7.4).

2.5. Washability

Liposomal hydrogel formulation was applied on the skin and then ease and extent of washing with water were check manually.

3. RESULTS AND DISCUSSION

3.1 Evaluation of Nadifloxacin liposomes

3.1.1 Vesicle shapes determination

The shape of liposomal (F1-F3) formulations was uniform and spherical showing multilamellar, onion peel structure to large and small unilamellar vesicles. The shape and size of the vesicles were dependent on the ratio of phospholipid and cholesterol used in the formulation (Table 4).

3.1.2 Entrapment efficiency

The entrapment efficiency of all the formulations was determined using UV-Visible spectrophotometer at a wavelength of 294 nm. Formulation (F2) showed highest percent entrapment of drug i. e 71±1. 50% as compared to other formulation F1, F3 that...
showed 61±1.12%, 60±2.35%, respectively (Table 5). The concentration of cholesterol and phospholipid has a great influence on entrapment efficiency. Formulation F2 consisting of an equimolar concentration of cholesterol and phospholipid showed high entrapment efficiency. Phospholipid is important for bilayer formation while cholesterol increases the bilayer hydrophobicity as well as improves the stability of liposomes.

3.1.3 In vitro drug diffusion study

The cumulative amount of drug permeated across dialysis membrane was plotted as a function of time and % drug release was calculated from the slope of linear portion. The release profile was shown in the figure. In all the formulations, formulation F2 with drug: lecithin: cholesterol ratio 10:100:10 was found to give a better result i.e.; drug 58.12±1.2% release in 6 hours. The amount of nadifloxacin permeated in 6 hours was found to be 58.12±1.2% from liposomes.

3.2 Evaluation of Liposomal hydrogel

3.2.1 Drug content

After various formulation of liposomal hydrogel the drug content of the formulated gel (F2H9) was estimated and the results were 70.25±0.121% of nadifloxacin and 98.16±0.221% of minocycline hydrochloride. The drug content determination also showed that the drug was uniformly distributed throughout the gel (Table 7).

3.2.2 Measurement of pH

The pH of the liposomal hydrogel was found in between 6.8. This pH is found to be close with the pH of human skin and hence it can be assumed that no skin irritation will occur after application of gel (Table 8).

3.2.3 Spreadability

The spreadability is very much important as show the behavior of gel comes out from the tube. The values of spreadability shown in table indicate that polymer used gave gels spread by small amount of shear. The diameter of the spreaded circle was 4.1gm.cm/sec (Table 8).

3.2.4 Viscosity

Brookfield digital viscometer was used to measure the viscosity (in cps) of the prepared gel formulation. The spindle no. 6 was rotated at 10 rpm. The torque % of the formulation was near to 30%. Reading was detected 30 sec after measurement was made, when the level was stabilized (Table 8).

3.2.5 Homogeneity

The prepared liposomal hydrogel of F2H9 formula was inspected visually for their color and syneresis. The developed preparation of F2H9 is much clear and transparent. The developed gel F2H9 showed good homogeneity with absence of lumps and syneresis.

3.2.6 In vitro Drug release studies

In vitro drug release of Nadifloxacin from liposomal hydrogel was found to be 54.86±3.1% in 6h, while 91.24±1.82% of minocycline hydrochloride was released from the final formulation in 6h (Table 9).

3.3 Stability study of liposomal hydrogel formulation

The prepared liposomal hydrogel formulation was stored at room temperature and refrigeration for a period of 30 days and then visually observed for clearance of every week. No significant visual change was observed during the study period.

| Table 2: Formulation batches for Hydrogel |
|-----------------------------------------|
| **Formulation batches** | **Minocycline hydrochloride(mg)** | **MC (gm)** | **Propylene glycol(ml)** | **Methyl paraben(gm)** | **TEA (ml)** | **Distilled water** |
|-----------------------------------------|-----------------------------------|-------------|--------------------------|------------------------|-------------|---------------------|
| H1                                      | 0.25                              | 1           | 5                        | 0.3                    | Qs          | 100                 |
| H2                                      | 0.25                              | 2           | 5                        | 0.3                    | Qs          | 100                 |
| H3                                      | 0.25                              | 3           | 5                        | 0.3                    | Qs          | 100                 |
| H4                                      | 0.25                              | 1           | 10                       | 0.3                    | Qs          | 100                 |
| H5                                      | 0.25                              | 2           | 10                       | 0.3                    | Qs          | 100                 |
| H6                                      | 0.25                              | 3           | 10                       | 0.3                    | Qs          | 100                 |
| H7                                      | 0.25                              | 1           | 15                       | 0.3                    | Qs          | 100                 |
| H8                                      | 0.25                              | 2           | 15                       | 0.3                    | Qs          | 100                 |
| H9                                      | 0.25                              | 3           | 15                       | 0.3                    | Qs          | 100                 |
Table 3: Formulation batch for liposomal hydrogel

| S.No. | Ingredients                  | Quantities |
|-------|-----------------------------|------------|
| 1.    | Nadifloxacin(mg)            | 10         |
| 2.    | Soya lecithin(mg)           | 100        |
| 3.    | Cholesterol(mg)             | 10         |
| 4.    | Chloroform(ml)              | 5          |
| 5.    | Minocycline hydrochloride(mg)| 250        |
| 6.    | Methylcellulose(gm)         | 3          |
| 7.    | Propylene glycol(ml)        | 15         |
| 8.    | Methyl paraben(mg)          | 300        |
| 9.    | Triethanolamine(ml)         | q.s        |
| 10.   | Distilled water             | 100ml      |

Table 4: Vesicle sizes of liposomes

| S. No. | Formulation batch | Vesicle size(µm) |
|--------|-------------------|------------------|
| 1.     | F1                | 6.23             |
| 2.     | F2                | 5.1              |
| 3.     | F3                | 7.51             |

Table 5: Entrapment efficiency of Nadifloxacin

| S. No. | Formulation batch | % Entrapment efficiency |
|--------|-------------------|-------------------------|
| 1.     | F1                | 61±1.12                 |
| 2.     | F2                | 71±1.50                 |
| 3.     | F3                | 60±2.35                 |

Table 6: Release of Nadifloxacin from liposomes

| Time[hr] | % Cumulative Drug release |
|----------|---------------------------|
| 15min    | 6.42±1.7                  |
| 30min    | 7.86±2                    |
| 1hr      | 11.46±1.34                |
| 2hr      | 25.97±1.65                |
| 4hr      | 42.80±2.6                 |
| 6hr      | 58.12±1.2                 |

Table 7: % Drug content (Mean±S.D.: n=3)

| S. No. | Drug                          | Drug content % |
|--------|-------------------------------|----------------|
| 1.     | Nadifloxacin                  | 70.25±0.121    |
| 2.     | Minocycline hydrochloride     | 98.16±0.221    |

Table 8: Evaluation parameter for liposomal hydrogel

| S.No. | Evaluation parameters | Result |
|-------|-----------------------|--------|
| 1.    | pH                    | 6.8    |
| 2.    | Spreadability (gm.cm/sec) | 4.1±0.12 |
| 3.    | Viscosity(cps)        | 24200±0.120 |
| 4.    | Extrudability         | ++     |
| 5.    | Washability           | ++     |

Excellent: +++, Good: ++, Average: +, Poor: -

Table 9: Release of Nadifloxacin and minocycline hydrochloride from liposomal hydrogel (Mean±S.D. n=3)

| Time (h) | % Cumulative Drug release |
|----------|---------------------------|
|          | Nadifloxacin              | Minocycline hydrochloride |
| 0.25     | 4.64±2.31                 | 15.6±2.1                  |
| 0.5      | 6.22±1.26                 | 20.68±2.32                |
| 1        | 8.3±1.24                  | 37.36±1.32                |
| 2        | 23.53±1.85                | 54.44±1.96                |
| 4        | 37.42±1.94                | 67.72±2.36                |
| 6        | 54.86±3.1                 | 91.24±1.82                |

Figure 9: Release of Nadifloxacin and minocycline hydrochloride from liposomal hydrogel

Stability study of liposomal hydrogel formulation

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Stability study of liposomal hydrogel formulation
4. CONCLUSION

The prepared liposomes were tested for drug release in vitro. Formulation F2 had the highest percentage of entrapment (701.50%) and released the most drug (58.121.2%) in 6 hours. The methylcellulose gelling agent was used to create a hydrogel containing minocycline hydrochloride, and the drug concentration was kept constant at 0.25 percent. Propylene glycol and methylparaben concentrations were kept constant at 15% and 0.3 percent, respectively. The hydrogel formulation was tested for physicochemical parameters such as percentage drug content, spreadability, and drug release. Formulation H9 had the highest drug content, 98.230.031 percent, and the drug released 90.962.6 percent in 6 hours. F2 from the liposome formulation and H9 from the hydrogel formulation were chosen to prepare the final liposomal hydrogel formulation. Size, drug content and drug entrapment spreadability, homogeneity, washability, pH, and other properties of the developed liposomal hydrogel (F2H9) were evaluated. In-vitro drug release from liposomal hydrogel was 54.863.1 percent for Nadifloxacin and 91.241.82 percent for Minocycline hydrochloride in 6 hours. Because of the high drug retention and permeation in skin layers, the developed liposomal hydrogel formulation may have a better effect in treating acne.

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