Formulation, Development and Evaluation of Ketoprofen Loaded Transethosomes Gel

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Abstract

Ketoprofen (RS2)-[3-benzoylphenyl]-propionic acid) is widely employed as a non-steroidal anti-inflammatory drug (NSAID). Ketoprofen is offered in a variety of forms in the pharmaceutical industry, including coated tablets, capsules, topical gels, transdermal patches, liquid spray, and injection solutions. The purpose of present work was to look at the possibility of transethosomes gel formulations for transdermal distribution of ketoprofen and to see effect of lipid concentration, ethanol concentration, drug concentration, and stirrer time affected the results. Viscosity, size surface charge, entrapment efficiency, and stability studies were employed to characterise transethosomes. The viscosity, pH, drug content, extrudability, spreadability, and in vitro drug diffusion studies were employed to characterise the transethosomes containing gel. The optimised formulation of transethosomes had an average vesicle size (nm), percent EE, and zeta potential (mV) of 135.65, 76.65, and -39.90, respectively. The prepared gel TG-12 had a viscosity of 3540±15cps, a percent assay of 99.05±.045, an extrudability of 175±0.25g, and a spreadability of 13.65±0.35 (g.cm/sec), respectively. Employed the Franz diffusion cell method, in vitro drug release from transethosomes gel was measured and determined to be 99.12 percent in 10 hours. The liberate of drugs from transethosomes gel formulations was establish to be exceedingly consistent and regulated. The ketoprofen-loaded transethosomes formulation in the gel was refined, and it may now be employed as a topical medication for its non-steroidal anti-inflammatory effects. The findings revealed that transethosomes gel was a viable choice for transdermal medication administration with tailored and long-term release. It also improves the penetration of many medications through the skin.

Keywords: Transethosomes gel, Ketoprofen, Franz diffusion cell, NSAID

INTRODUCTION

Ketoprofen is a powerful nonsteroidal anti-inflammatory drug (NSAID) that is employed to treat rheumatoid arthritis, osteoarthritis and other joint diseases. It is a selective inhibitor of cyclooxygenase-2 and has analgesic and anti-inflammatory assets. Ketoprofen’s low water solubility and wettability make it challenging to formulate oral and topical formulations. According to the Biopharmaceutics Classification Scheme ketoprofen is a class II medication. Because dissolution is the rate-limiting stage in medication absorption, ketoprofen’s poor water solubility causes inadequate absorption, resulting in low bioavailability. In accumulation to issues with absorption, oral ketoprofen formulations can harm the gastrointestinal mucosa, resulting in ulcers and bleeding. As a consequence, improving the H2O solubility of medications can lead to improved oral or topical formulations. Topical NSAID management on the inflamed area has the benefit of delivering a medicine directly to the disease site and causing its local action. This is accomplished by preventing gastrointestinal discomfort and reducing negative systemic effects. As a result, the current research aims to provide an alternate medication delivery technique that can successfully distribute this drug while also improving patient compliance. The topical route for drug delivery is seen to be one of the most promising for such medications since it avoids hepatic first-pass metabolism and allows the agent to function locally in the targeted location. It not only improves the drug’s therapeutic efficacy, but it also reduces the drug’s side effects through systemic circulation. Despite the potential benefits of topical delivery systems, traditional topical delivery methods have difficulties in penetrating the profounder layers of the skin. As a result, vesicular DDS such as niosomes and liposomes are ideal for achieving localised drug activity for effective treatment. Niosomes and liposomes, on the additional hand, are unable to penetrate deep into the skin due to their less flexible structure. Formulation of elastic vesicles has been attempted in recent years to circumvent these restrictions. These elastic vesicles are divided into two types: transfersomes, which are made up of an edge activator and lipid, and ethosomes, which are made up of lipid and ethanol. These aid in reducing drug washout through the bloodstream, which is a prevalent issue when medications are administered topically by penetration enhancers, ionophoresis, or electrophoresis processes. Apart from these benefits, elastic vesicles have the possible to bypass the stratum corneum and the capillary bed of the skin, depositing medication in the profounder layers of the skin. Transfersomes increase vesicle flexibility by redistributing the edge activator and lipid in their environment, whereas ethosomes work by fluidizing both...
the skin and the vesicles’ lipids. Transethosomes were created as unique vesicles holding both lipids and ethanol, which mimicked the features of both ethosomes and transfersomes. As a consequence, the present study centers on the formulation of transethosomes, in which the established carrier system was successfully used to create a gel for topical distribution of ketoprofen.

**EXPERIMENTAL**

**Materials**

Ketoprofen as a gift obtained from Shreyas Life Sciences (Aurangabad, Maharashtra, India). Soya PC and Span 20 from Himedia. CDH Chemical Pvt. Ltd., New Delhi, provided ethanol, chloroform, and carbopol-934. Himedia Laboratory in Mumbai provided a Mol Wt cutoff 1200 dialysis membrane. Freshly prepared demineralized and deionized water was utilized whenever necessary. All of the other chemicals and reagents used were of analytical quality.

**Formulation, development ketoprofen loaded transethosomes**

In a beaker, carbopol 934 (1 percent w/v) was precisely weighed and disseminated in deionized water (80ml). Before adding 10 ml of propylene glycol, the solution was agitated continuously at 800 rpm for 1 hour. To remove air bubbles, the gel was diluted to 100 ml and sonicated on a bath sonicator for 10 minutes. The pH of the gel base was finally set to 6.8. To attain the necessary drug concentration in the gel basis, a transethosomal preparation equivalent to 0.1 percent w/w of Ketoprofen was introduced into the gel base.

**Optimization of transethosomes formulation**

The formulation of transethosomes was optimized based on the consequences of the above-mentioned strategy technique, which was supported on the source of average vesicle size and percent EE. The ratio of soya pc: span 20 in the transferosomal formulation was optimised by taking multiple ratios such as 9:5:0.5, 9:1, 8:2, and 7:3 percent w/v ratio and keeping all other parameters constant. The ethanol content was optimised by varying the amount of ethanol used, such as 5, 10, 15, and 20, while keeping all other factors constant. Drug concentration was tuned by preparing formulations with varied concentrations of drug, such as 1, 1.5, and 2.0 percent w/v, while all other parameters, such as soya pc: span 20, stirrer time, were constant. Stirrer time was optimized by stirring the formulation for different time, i.e., 5, 10, and 15 min.

**Characterization of ketoprofen vesicles loaded transethosomes**

**Surface charge and vesicle size**

The size and size distribution of the vesicles in addition to their surface charge were assessed using the Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK).

**Zeta potential**

From their electrophoretic mobility, the zeta potential was determined according to Helmholtz-Smoluchowsky. A zetasizer was employed on a large bore measuring cell to measure zeta potential with field strength of 20 V/cm. The samples were adjusted to a conductivity of 50 IS/cm by diluting them with 0.9 percent NaCl.

**Entrapment efficiency**

To separate the entrapped drug from the unentrapped drug, one millilitre of transethosomes suspension was spun at 15000 rpm for one hour. The silt was lysed with methanol after the supernatant was removed, and then spectrophotometrically examined at 256nm employed a UV spectrophotometer (Labindia 3000+). The percentage of drug entrapment efficiency in the prepared transethosomes was estimated using the following equation:

\[
\% \text{ Entrapment Efficiency} = \frac{\text{Theoretical drug content} - \text{Practical drug content}}{\text{Theoretical drug content}} \times 100
\]

**Characterization of transethosomes containing gel**

**Measurement of viscosity**

The viscosity of the produced topical transethosomes-based gel was determined employing a Brookfield viscometer with spindle no. 63 and a 10rpm optimal speed; viscosity 15-16.

**pH measurements**

A digital pH metre was used to determine the pH of selected optimal formulations. pH metres should be calibrated with buffer solutions of pH 4, pH 7, and pH 9.2 before each measurement of pH. The electrode was dipped into the vesicles after calibration for as long as the vesicles covered it. The pH of a particular formulation was then determined, and the findings were displayed on the screen.

**Drug content**

100 mg of topical transethosomes gel was precisely weighed in a beaker, and 20 ml of methanol was added. This solution was carefully mixed before being filtered via Whatman filter paper no. 1. Then, 1.0 ml of the filtered solution was placed in a volumetric flask with a capacity of 10 ml, and the volume was raised to 10 ml using methanol. A UV-Spectroscope with a maximum wavelength of 256nm was used to examine this solution.

**Extrudability study**

The amount of gel extruded from a collapsible tube when a given weight was applied was used to measure extrudability. As the amount of gel extruded increases, extrudability improves. It was indomitable by weighing a gel-filled collapsible tube and noting the weight at which the gel was extruded.

**Spreadability**

The formulation must be spreadable to ensure that an appropriate dosage is available for absorption through the skin, resulting in a beneficial therapeutic response. The top slide is moveable, with one end of the movable slide coupled to a weight pan, and it is put on a wooden block. To test spreadability, 2-5 g of gel was placed between two slides, and the weight was gradually increased by adding it to the weight pan, with the top plate’s time to cover a distance of 6cm after adding 20g of weight being recorded. The term “spreadability” refers to the ability to spread in a short amount of time.

\[
\text{Spreadability} = \frac{\text{Weight to Upper Slide} \times \text{Length moved on the glass slide}}{\text{Time taken to slide}}
\]

**In vitro drug diffusion study**

The In-vitro diffusion study is carried by using Franz Diffusion Cell. The removed samples are spectrophotometrically examined at the drug’s wavelength of 256nm.
Stability Studies
For three weeks, drug-loaded transethosomes were studied at two dissimilar temperatures: refrigerated temperature (4.0±0.2°C) and room temperature (25-28±2°C). The stability study formulation was stored in a borosilicate container to avoid any contact between the formulation and the container glass. The formulations were tested for physical changes and pharmacological content.

RESULTS AND DISCUSSIONS
The absorption maxima of ketoprofen were establish to be 256 when the spectra of the drug solution were put through a double beam ultraviolet spectrophotometer (Labindia UV 3000 * ) with a concentration range of 5-25g/ml ketoprofen in 7.4phosphate buffers. All of the data from the preformulation study matched those in the standard monograph, indicating that the medication was authenticated and pure in form, and that it could be utilized in the formulation of ketoprofen-loaded transethosomes. Optimization of the transethosomes to produce the formulation code was done using the strategy as reflected in Table 1 optimization of lipid concentration, Table 2 optimization of ethanol concentration, Table 3 optimization of drug concentration and Table 4 optimization of stirrer time. It was scrutinized that the vesicles dimension of transethosomes was increased with raising the concentration of soya pc: span 20 and ethanol. The average vesicle size did not change significantly as the drug concentration was improved, but the size of the vesicle fell from 135.65 to 112.20 after 15 minutes of stirring as the stirrer time was improved. When using the EE, it was discovered that as the ethanol concentration and stirring time were increased, the % drug entrapment dropped. It’s because raising the mechanical force with a stirrer causes the medicine to leak out of the vesicles, and increasing the concentration of ethanol causes the size of transethosomes to shrink. It was clearly shown when formulation was stirred for 5, 10, and 15 min then the % EE was 76.65, 68.85, and 61.12. 5 mins selected as optimized time for stirrer because it provided the required size of vesicle 135.65 nm and good % EE, i.e., 76.65. The resulted formulation code F-12 was considered as the optimized formulation. The average vesicle size of optimized formulation (F-12) observed as 135.65nm, zeta potential observed as -39.98 mV and %EE was found as 76.65%. Stability study was executed on optimized formulation (F-12) and its characterization depicted in Table 5. Stability study data exposed that the optimized formulation (F-12) was stable after 3 months of storage at 4.0°C ± 0.2°C while at 25-28±2°C, the formulation was found unstable. Stability of formulation was experiential on the basis of % drug remain, average vesicles size and physical appearance. The percent drug content, extrudability, spreadability, viscosity, pH and drug release study of transethosomes loaded with ketoprofen (TG-12) were all assessed. The viscosity of the prepared gel TG-12 was establish to be 354015cps, the percent assay to be 99.050.45, the extrudability to be 1750.25g, and the spreadability to be 13.650.35 (g.cm/sec), respectively. The Franz diffusion cell method was employed to test in vitro drug release (Table 6 & Figure 2) from transethosomes gel and discovered 99.12 percent in 10 hours. It was 26.65 percent drug release in the first hour, which was slightly high. It was caused by the leaching of free drug from transethosomes, which led to the release of free drug in the bag. The release of drugs from transethosomes gel formulations was establish to be exceedingly consistent and regulated. When the regression coefficient values of were compared, it was scrutinized that ‘r’ values of Higuchi’s model was maximum i.e. 0.985 hence indicating drug release from formulations was establish to follow Higuchi’s model release kinetics Table 7.

Table 1 Optimization of ratio of lipid concentration

| Formulation code | Soya PC: Span 20 (% w/v) | Ethanol (% w/v) | Drug (% w/v) | Average vesicle size (nm) | % entrapment efficiency |
|------------------|--------------------------|-----------------|--------------|--------------------------|------------------------|
| F1               | 9.5:0.5                  | 10              | 1.0          | 325.65                   | 63.36                  |
| F2               | 9:1                      | 10              | 1.0          | 285.65                   | 68.85                  |
| F3               | 8:2                      | 10              | 1.0          | 245.65                   | 75.65                  |
| F4               | 7:3                      | 10              | 1.0          | 275.65                   | 70.25                  |

Table 2 Optimization of ethanol concentration

| Formulation code | Soya PC: Span 20 (% w/v) | Ethanol (% w/v) | Drug (% w/v) | Average vesicle size (nm) | % entrapment efficiency |
|------------------|--------------------------|-----------------|--------------|--------------------------|------------------------|
| F5               | 8:2                      | 5               | 1.0          | 285.65                   | 68.65                  |
| F6               | 8:2                      | 10              | 1.0          | 224.45                   | 76.65                  |
| F7               | 8:2                      | 15              | 1.0          | 265.58                   | 69.98                  |
| F8               | 8:2                      | 20              | 1.0          | 271.65                   | 63.32                  |
Table 3 Optimization of drug concentration

| Formulation code | Soya PC: Span 20 (% w/v) | Drug (% w/v) | Ethanol (ml) | Average vesicle size (nm) | % Entrapment efficiency |
|------------------|--------------------------|--------------|--------------|--------------------------|------------------------|
| F9               | 8:2                      | 1.0          | 10           | 165.58                   | 76.65                  |
| F10              | 8:2                      | 1.5          | 10           | 198.85                   | 70.23                  |
| F11              | 8:2                      | 2.0          | 10           | 185.65                   | 69.95                  |

Table 4 Optimization of stirrer duration

| Formulation code | Soya PC: Span 20 (% w/v) | Drug (% w/v) | Stirrer duration (min) | Average vesicle size (nm) | % Entrapment efficiency |
|------------------|--------------------------|--------------|------------------------|--------------------------|------------------------|
| F12              | 8:2                      | 1.0          | 5                      | 135.65                   | 76.65                  |
| F13              | 8:2                      | 1.0          | 10                     | 125.45                   | 68.85                  |
| F14              | 8:2                      | 1.0          | 15                     | 112.20                   | 61.12                  |

Table 5 Characterization of optimized formulation of transethosomes formulation

| Characteristic                  | Time (Month)                      |
|--------------------------------|-----------------------------------|
|                                | 1 Month                           |
|                                | 2 Month                           |
|                                | 3 Month                           |
| Temperature                    | 4.0±0.2°C                         |
|                                | 25-28±2°C                         |
|                                | 4.0±0.2°C                         |
|                                | 25-28±2°C                         |
| Average vesicles size (nm)     | 135.65                            |
|                                | 225.65                            |
|                                | 146.21                            |
|                                | 245.65                            |
|                                | 298.85                            |
| % EE                           | 76.25                             |
|                                | 68.85                             |
|                                | 75.45                             |
|                                | 60.45                             |
|                                | 73.14                             |
|                                | 54.12                             |
| Physical Appearance            | Normal                             |
|                                | High turbid                       |
|                                | Normal                             |
|                                | High turbid                       |
|                                | Normal                             |
|                                | High turbid                       |

Table 6 In-vitro drug release data for gel formulation

| Time (h) | Square Root of Time(h) | Log Time | Cumulative* % Drug Release | Log Cumulative % Drug Release | Cumulative % Drug Remaining | Log Cumulative % Drug Remaining |
|----------|------------------------|----------|-----------------------------|-------------------------------|-----------------------------|--------------------------------|
| 0.5      | 0.707                  | -0.301   | 11.12                       | 1.195                         | 84.35                       | 1.926                          |
| 1        | 1.000                  | 0.000    | 26.65                       | 1.426                         | 73.35                       | 1.865                          |
| 1.5      | 1.225                  | 0.176    | 39.98                       | 1.589                         | 61.15                       | 1.786                          |
| 2        | 1.414                  | 0.301    | 46.65                       | 1.669                         | 53.35                       | 1.727                          |
| 4        | 2.000                  | 0.602    | 59.98                       | 1.778                         | 40.02                       | 1.602                          |
| 6        | 2.449                  | 0.778    | 76.65                       | 1.859                         | 27.77                       | 1.444                          |
| 8        | 2.828                  | 0.903    | 89.98                       | 1.949                         | 11.02                       | 1.042                          |
| 10       | 3.162                  | 1.000    | 99.12                       | 1.992                         | 1.88                        | 0.274                          |

*Average of three reading (mean ±SD)

Table 7 Regression analysis data of transethosomes gel formulation

| Batch            | Zero Order | First Order | Higuchi’s Model | Korsmeyers Peppas Equation |
|------------------|------------|-------------|-----------------|----------------------------|
| Optimized gel formulation | 0.937      | 0.869       | 0.985           | 0.939                      |
CONCLUSION

The % drug entrapment and average vesicle size were employed to get ready and optimise transethosomes. The improved formulation was then mixed with a gel base (Carbopol gel) and tested for percent drug content, extrudability, spreadability, viscosity, pH and drug release. Optimized formulation (F-12) of transethosomes resulted in average vesicle size as 135.65nm, zeta potential as -39.98mV and % EE as 76.65% and stability study data exposed that the optimized formulation was stable after 3 months of storage at 4.0° ±0.2°C. Prepared gel of optimized formulation viscosity was 35±1.5cp, % drug content was 99.05±0.45, extrudability was 175±0.25g and spreadability (g/cm/sec) was 13.65±0.35 (g/cm/sec) and in vitro drug release establish as 99.12 % in 10h, respectively. It can be accomplished that prepared gel containing ketoprofen-loaded transethosomes formulation was optimized and can be of use for topical preparation.

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