Preparation and Evaluation of Spray-on Bandage Incorporated With NSAID

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Abstract

The preparations in pressurized packaging are referred to as Spray-on bandages. They are usually used for the purpose to protect and cover small cuts and minor scrap but, in this article, a preparation was developed by the incorporation of an NSAID, i.e., Diclofenac Sodium for its inflammatory actions and then, its evaluations were carried out. Further, this topical formulation also minimizes the adverse effects associated with its oral administration. Polymers like Ethyl cellulose and Acrylic acid were used along with PEG 200 as plasticizer. The solvent-system used was acetone and ethanol with the ratio of 1:1 to obtain fast dried films when sprayed on the skin. The film was formed immediately once sprayed on skin and then dried to transparent, tack-free film in less than 7 min.

Abbreviations: NSAID; Non-Steroidal Anti-Inflammatory Drug; FTIR: Fourier Transform Infrared Spectroscopy

Keywords: Diclofenac sodium; ethyl cellulose; acrylic acid; PEG 200; ethanol; acetone

Introduction

The Topical drug delivery system which follows, Spray-on bandages, is a recent approach to drug delivery. Drugs which are being administered through conventional routes as conventional dosage forms like tablets and capsules posses several disadvantages like hepatic first pass metabolism and undesirable toxicity due to fluctuations in drug concentrations [1,2]. Diclofenac sodium, which is a Non-Steroidal Anti-Inflammatory Drug (NSAID), shows its therapeutic effects in the system by inhibiting a biochemical pathway, the biosynthesis of pain inducer such as prostaglandins. Diclofenac sodium is used for treating pain caused by minor sprains, strains or bruising, inflammation and is widely applicable in rheumatoid arthritis, osteoarthritis, spastic spondylitis, acute gout etc [3,4]. However, if diclofenac is taken orally for a longer periods, there are chances of ulcer formation along with complications like anemia by hemorrhage. Thus topical application of diclofenac may avoid such undesirable effects [5,6]. By mimicking the first pass metabolic effect it avoids drug wastage and also it is a non-invasive process and needs no hospitalization. Spray on bandages, which readily forms films containing drug on the intact skin surface when sprayed, deploys its therapeutic effect at a pre-determined rate by delivering a predetermined amount of the drug so as for a controlled management of pain and inflammation [7-10]. Hence in the present work spray on bandages of diclofenac has been prepared to avoid dose related adverse of diclofenac with oral route.

Materials and Methods

Materials

Diclofenac sodium was obtained from Baja drugs, Chennai, India. Ethyl cellulose, acrylic acid and PEG 200 were procured from SD Fine chemicals, Mumbai, India. All other regents used were of analytical grade.

Pre-formulation studies

Development of calibration curve

A standard stock solution of diclofenac sodium (1000μg/ml)
was prepared by taking 100 mg of drug in 100 ml of 7.4 pH phosphate buffer. From this, 10 ml solution was diluted to 100 ml using 7.4 pH phosphate buffer solutions (100 μg/ml). From this, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml solutions were taken into different volumetric flasks and made up to 10 ml with phosphate buffer solution (pH 7.4) so as to get the concentrations of 2 μg/ml, 4 μg/ml, 6 μg/ml and 8 μg/ml, respectively. The absorbance of these solutions was measured at a λ max of 276 nm against a blank. Standard curve between concentration and absorbance was plotted.

**Solubility Studies**

The solubility of Diclofenac sodium was determined by adding excess amount of drug to measured volume of distilled water in a glass vial to get a saturated solution. The solution was solicited and kept at room temperature to attain equilibrium. The concentration of Diclofenac sodium was determined spectrophotometrically after 24 h by measuring at 276 nm.

**Partition coefficient studies**

A known quantity of the drug i.e, Diclofenac sodium was taken in a separating funnel and to it 50 ml of 1-octanol and 50 ml of water/ phosphate buffer pH 7.4 was added in a separating funnel. Then, the two phases were allowed to equilibrate at 37°C for 24 h with intermittent shaking. The concentration of the drug in the aqueous phase and organic phase was determined by UV spectroscopic method after necessary dilution. The apparent partition coefficient (Kp) was calculated as the ratio of drug concentration in each phase by the following equation:

\[
\text{Partition coefficient of the drug (Kp)} = \frac{\text{concentration of the drug in organic phase}}{\text{concentration of the drug in aqueous phase}}
\]

**Compatibility studies**

Fourier Transform Infrared Spectroscopy (FTIR) can be used to investigate and predict any physicochemical interaction between different components in a formulation and therefore it can be applied to the selection of suitable chemical compatible excipients while selecting the ingredients, we would chose, those which are stable, compatible, cosmetically and therapeutically acceptable. Infrared spectra matching approach was used for detection of any possible chemical interaction between the drug, polymers and the plasticizer used. As there were liquid samples too, so, for liquid samples a suitable solvent was taken and dissolved the desired drug, polymers and plasticizer in that solvent, while keeping that solvent as blank. For solid samples, physical mixture of drug, polymers and plasticizer was prepared and mixed with suitable quantity of potassium bromide. This mixture was compressed to form a transparent pellet using a hydraulic press at 15 tons pressure. It potassium bromide. This mixture was compressed to form a plasticizer was prepared and mixed with suitable quantity of for solid samples, physical mixture of drug, polymers and plasticizer in that solvent, while keeping that solvent as blank. As there was used for detection of any possible chemical interaction between the drug, polymers and the plasticizer used. As there were liquid samples too, so, for liquid samples a suitable solvent was taken and dissolved the desired drug, polymers and plasticizer in that solvent, while keeping that solvent as blank. For solid samples, physical mixture of drug, polymers and plasticizer was prepared and mixed with suitable quantity of potassium bromide. This mixture was compressed to form a transparent pellet using a hydraulic press at 15 tons pressure. It was scanned from 4000 to 400 cm⁻¹ in a FTIR spectrophotometer (FTIR 8400 S, Shimadzu). The IR spectrum of the physical mixture and the liquid samples was compared with those of pure drug, polymers and plasticizer peak matching was done to detect any appearance or disappearance of peaks.

**Preparation of topical films of Diclofenac sodium**

Weighed amount of the drug, ethyl cellulose and measured volume of PEG 200, acrylic acid were taken as mentioned in table 1 for the different formulations. To these mixtures required quantity of solvents ethanol and acetone were added. Then, they were stirred continuously until all the ingredients dissolved in the solvent and were sprayed using a nozzle directly on the hands or backside of the Petri dishes to form films. These films were then evaluated for film forming properties and also for further studies.

**Table 1:** Composition of various topical films of Diclofenac sodium.

<table>
<thead>
<tr>
<th>Formulations (mg/ml)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac sodium</td>
<td>40mg</td>
<td>40mg</td>
<td>40mg</td>
<td>40mg</td>
</tr>
<tr>
<td>Ethyl cellulose</td>
<td>0.8mg</td>
<td>1.2mg</td>
<td>1.2mg</td>
<td>0.8mg</td>
</tr>
<tr>
<td>Acrylic acid</td>
<td>1ml</td>
<td>2ml</td>
<td>1ml</td>
<td>1ml</td>
</tr>
<tr>
<td>PEG 200</td>
<td>2ml</td>
<td>2ml</td>
<td>1ml</td>
<td>1ml</td>
</tr>
<tr>
<td>Ethanol</td>
<td>8ml</td>
<td>8ml</td>
<td>8ml</td>
<td>8ml</td>
</tr>
<tr>
<td>Acetone</td>
<td>8ml</td>
<td>8ml</td>
<td>8ml</td>
<td>8ml</td>
</tr>
</tbody>
</table>

**Evaluation of topical films of Diclofenac sodium**

**Film strength:** The film formed was cut into 2X2 cm² size after they were dried and was repeatedly folded at the same place till it breaks.

**Tackiness:** when sprayed on the back of the petri dishes, the film formed was examined for its adhesive nature by gently touching the formed film.

**Adherence time:** the film formed on the petri dishes was measured for its adherence time till the film came off of the petri dishes.

**Flexibility:** the films were examined by viewing the ease at which the films could be moved from there sprayed position on the petri dishes.

**Water resistance:** Initially, weight of the formed films was taken and then they were subjected to spray on by a water spray upon them and again their weight was taken (final weight). The films were then examined by, % Moisture content= (initial weight-final weight)/(final weight) ×100

**Drug content**

Drug content estimation was carried out in triplicate on each formulation. Each film from different formulations was taken, cut into small pieces and was added to a beaker containing 40 ml of Phosphate buffer pH 7.4. It was then stirred on a magnetic stirrer and filtered. From the filtrate, 1 ml was withdrawn and diluted to 10 ml with phosphate buffer pH 7.4 and the absorbance was measured at λmax 276 nm using UV – spectrophotometer against phosphate buffer pH 7.4 as blank and the concentration was calculated. By correcting the dilution factor, the drug content was determined.

**In vitro release studies**

The films were cut into 1 cm² and placed on the commercial semi- permeable membrane (cellophane membrane) and attached to the diffusion cell such that the cell’s drug releasing surface towards the receptor compartment which was filled with phosphate buffer solution of pH 7.4 at 37±0.5°C. The elution medium was stirred magnetically. The aliquots (2 ml) was withdrawn at predetermined time intervals and replaced with same volume of phosphate buffer pH 7.4. The samples were analyzed for drug content using UV spectrometer.
Kinetics of In vitro drug release

In vitro drug release kinetic of diclofenac sodium loaded spray on bandage was calculated by fitting into various drug release models.

In vitro Cytotoxicity studies/ skin irritation studies

Cell Line Used: Vero (African Green Monkey, Kidney)

Assay: MTT Assay

Concentration used: 500 – 62.5 μg/ml

In vitro cytotoxicity assay

Determination of mitochondrial synthesis by MTT assay

The percentage growth inhibition was calculated using the following formula and concentration of drug or test samples needed to inhibit cell growth by 50% values were generated from the dose-response curves for each cell line.

% Growth Viability=(Mean OD of individual test group)/(Mean OD of control group)×100

Results and Discussion

Preformulation studies

Calibration curve of Diclofenac Sodium

From the Diclofenac sodium standard stock solution (1000μg/ml), 10 ml solution was diluted to 100 ml using 7.4 pH phosphate buffer solutions (100μg/ml). From this 0.2ml, 0.4ml, 0.6ml, 0.8ml of solutions were taken into different volumetric flasks and made up to 10ml with phosphate buffer solution (pH 7.4) so as to get the concentrations of 2μg/ml , 4μg/ml , 6μg/ml and 8μg/ml, respectively. The absorbance of these solutions were measured at λmax 276 nm.

It was found that the calibration curve (Fig.1) between absorbance taken and concentration gave a straight line with R² = 0.999 which is approximately = 1, i.e., the limit value.

**Figure 1: Calibration Curve of Diclofenac Sodium using pH 7.4.**

Solubility Studies

The saturation solubility of Diclofenac Sodium was determined in different solvents and in different pH. The results are given in (Table 4,5) The solubility in phosphate buffer pH 7.4 and acetone was found to be 56.12μg/mL, and 74.38μg/mL respectively. From the study, Diclofenac was found to be highly soluble in acetone from table 2 and from table 3 in Phosphate buffer pH 7.4, as per I.P and B.P limits.

**Table 2: Solubility profile of Diclofenac Sodium with different solvents.**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solvents</th>
<th>Solubility (μg/ml)</th>
<th>Solubility (AS PER I.P &amp; B.P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water</td>
<td>53.78</td>
<td>Sparingly soluble</td>
</tr>
<tr>
<td>2</td>
<td>Acetone</td>
<td>74.38</td>
<td>Soluble</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>70.04</td>
<td>Soluble</td>
</tr>
</tbody>
</table>

**Table 3: Solubility profile of Diclofenac Sodium in different pH.**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>pH</th>
<th>Solubility (μg/ml)</th>
<th>Solubility (AS PER I.P &amp; B.P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>4.024</td>
<td>Very slightly soluble</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>4.609</td>
<td>Very slightly soluble</td>
</tr>
<tr>
<td>3</td>
<td>6.2</td>
<td>14.829</td>
<td>Slightly Soluble</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>55.365</td>
<td>Sparingly soluble</td>
</tr>
<tr>
<td>5</td>
<td>7.4</td>
<td>56.12</td>
<td>Sparingly soluble</td>
</tr>
</tbody>
</table>

**Table 4: Partition coefficient values (n-Octanol: distilled water).**

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Solvent system</th>
<th>Log P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n- octanol : water</td>
<td>1.15</td>
</tr>
<tr>
<td>2</td>
<td>n- octanol : phosphate buffer 7.4</td>
<td>1.14</td>
</tr>
</tbody>
</table>

Partition coefficient studies

Diclofenac sodium is a hydrophilic drug with a log P value of 1.12. The partition coefficient was found in order of water (1.15) > phosphate buffer pH 7.4 (1.14). Phosphate buffer pH 7.4 is more lipophilic of the two and had higher affinity for Diclofenac sodium.

Compatibility Studies

The spectra obtained from IR studies at wavelength from 4000 cm⁻¹ to 400 cm⁻¹ are shown in Fig 2-5. After interpretation of the above spectra it was confirmed that there was no major shifting, loss or appearance of functional peaks between the spectra of drug, polymer, physical mixture of drug and polymer (1249.91cm⁻¹, 1724.42cm⁻¹, 2236.54cm⁻¹, 2885.60cm⁻¹, 3358.60cm⁻¹, 3509.60cm⁻¹). From the spectra it was concluded that the drug and polymer mixture lack of any chemical interaction. From the IR study it was concluded that, the selected polymers, acrylic acid and ethyl cellulose and plasticizer peg 200 were found to be compatible with the selected drug Diclofenac sodium. (Figure 2-4)
**Evaluations of topical films of Diclofenac sodium**

**Physical Evaluations**

From the table 5 of physical evaluations of all the four formulations, it was found that the formulation F3 was with most of the desirable characteristics than the other three formulations as it showed good film forming properties as compared to other formulations and were also within the limits. So, it was concluded from the study that the most optimized batch was found to be formulation F3 with all the film forming properties within the limits.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Film strength</th>
<th>Tackiness</th>
<th>Adherence</th>
<th>Flexibility</th>
<th>Water resistance (%)</th>
<th>Drug content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Poor</td>
<td>Good</td>
<td>10-Aug</td>
<td>Poor</td>
<td>2.69%</td>
<td>3.831</td>
</tr>
<tr>
<td>F2</td>
<td>Fair</td>
<td>Good</td>
<td>&gt;10</td>
<td>Good</td>
<td>2.87%</td>
<td>3.66</td>
</tr>
<tr>
<td>F3</td>
<td>Good</td>
<td>Fair</td>
<td>8-Jul</td>
<td>Fair</td>
<td>3.10%</td>
<td>4.148</td>
</tr>
<tr>
<td>F4</td>
<td>Poor</td>
<td>Fair</td>
<td>&gt;10</td>
<td>Fair</td>
<td>2.58%</td>
<td>3.486</td>
</tr>
</tbody>
</table>

Table 5: Evaluation parameters data of F1 to F4 formulations.

**Drug content uniformity**

Drug content of the films was carried out to ascertain that the drug is uniformly distributed into the formulation. The results obtained are represented in (Table 5). From the results obtained, it was clear that there was proper distribution of Diclofenac sodium in the film formulations. Hence it was concluded that drug was uniformly distributed in all the formulations. Delivery of drug through skin for systemic action is a promising route of administration. The purpose of this study was to investigate the in vitro release profile of Diclofenac sodium from various films containing different ratios of combination of polymers (Ethyl cellulose and Acrylic acid).

**In vitro release studies**

*In vitro* release Diclofenac films were carried out in diffusion cell using commercial available semi permeable membrane and phosphate buffer (pH 7.4) as diffusion medium. The release profile data of Diclofenac sodium were given in fig 6 for films F1 to F4. From the diffusion studies it was observed that, at the end of 8h, drug diffusion from the formulation F3 (79.94%) was observed to be maximum than F1 (65.48%), F2 (69.35%), F4 (58.28%). This shows that the most optimized batch was found to be F3. The kinetics of drug diffusion was found out by plotting different graphical models of the optimized batch i.e., F3. All the release profiles were calculated and the kinetic data is represented in respective (Figure 7-11) In order to understand the mechanism of action of drug release, in vitro release data were treated to kinetic models and linearity was observed with respect to equation. So, it was confirmed that the F3 was best fitted with zero order kinetics model and the mechanism was diffusion controlled (Figure 11)
The percentage growth inhibition was calculated using the following formula and concentration of drug or test samples needed to inhibit cell growth by 50% values were generated from the dose-response curves for each cell line.

The IC_{50} value of the formulation is about 825.4811 μg/ml and IC_{50} value of the drug is about 359.8086 μg/ml. So, it was confirmed from the data that there was no toxicity in the given optimized formulation F3, as the data falls under the limits. No irritation to the skin was observed from the study (Table 6-12).
Conclusion

The present studies have brought out that selected polymers were found to be compatible with Diclofenac sodium based on FT-IR peak matching method. Partitioning studies indicate that phosphate buffer pH 7.4 had a higher partition coefficient for Diclofenac compared to water. Higher partition coefficient indicates better penetrating efficiency. The prepared batch with 1:1 ratio of polymer concentration and 1% w/w plasticizer concentration showed desirable releasing rate and film forming properties and was identified as an ideal batch. Film formulations released the drug in controlled manner over a period of 8h. The formulation also showed effective release property with controlled release of the drug over a period of time and it does not cause any irritation to the skin. It has shown very good patient compliance and possessed a higher relative bioavailability than oral route due to avoidance of hepatic first-pass metabolism. It can be concluded that the present film formulation provided controlled release of drug and hence, these systems can be used for treating wounds, burns, inflammations and tissue repairing.

Table 6: In vitro Cytotoxicity Studies.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Sample Description</th>
<th>Vero ic50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Formulation</td>
<td>825.4811</td>
</tr>
<tr>
<td>2</td>
<td>Pure drug</td>
<td>359.8086</td>
</tr>
<tr>
<td>3</td>
<td>Blank</td>
<td>2782.304</td>
</tr>
</tbody>
</table>

References