Development and Evaluation of Implanted In Situ Gel Formulation for Treatment of Osteoarthritis

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Abstract: The primary aim of the present study was to develop and assess a topical gel containing Diacerein-loaded transferosomes in order to address the limitations of oral administration of Diacerein in the treatment of Osteoarthritis. Osteoarthritis, the prevailing kind of arthritis, is the primary cause of disability and lacks effective symptomatic treatment options. Diacerein hinders the interaction with the function of interleukin 1-β and is beneficial in the palliative management of osteoarthritis, while it also induces diarrhoea when taken orally. Diacerein is rapidly eliminated from the body since it has a short half-life. Therefore, a sustained release formulation is necessary for long-term administration in order to enhance patient adherence. Osteoarthritis, unlike other joint disorders, specifically affects one or a few joints. This characteristic allows for targeted Intra-articular treatment, minimising the potential for systemic adverse effects. An enhanced delivery mechanism is urgently required to enhance bioavailability and mitigate drug-related toxicity and the inefficacy of anti-inflammatory medicines. An investigation was conducted to assess the efficacy of Diacerein in situ gel as a potential treatment for Osteoarthritis. The formulation consists of poloxamer 407 as the primary polymer, hydroxypropyl methylcellulose K4, and carbopol 934 as the copolymer. It is manufactured using the conventional cold technique and is distinguished by multiple properties. All characterizations yielded satisfactory outcomes. The drug release is conducted in vitro using a phosphate buffer solution with a pH of 7.4, which indicates a regulated release of the medication.

Keywords: Osteoarthritis, Gel, Diacerein, Topical, Anti-inflammatory, Drug release


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**Introduction**

Osteoarthritis (OA) is the predominant rheumatologic condition and form of arthritis in India, affecting from 22% to 39% of the population. Osteoarthritis is associated with the degeneration of cartilage in the joints and can develop in almost any joint in the body (Christian *et al.*, 2019). Typically, it manifests in weight-bearing joints such as the hips, knees, and spine. Additionally, it impacts the digits, thumb, cervical region, and hallux. Osteoarthritis often does not impact more joints unless there has been a prior accident, high stress, or underlying damage to the cartilage (Felson, 1988).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a type of medication used to alleviate inflammation and pain. However, their effectiveness is overshadowed by the increased risk of adverse effects on the upper gastrointestinal tract. Additionally, NSAIDs do not address the root causes of joint diseases, so they have limited impact on modifying the progression of the disease or improving quality of life. Diacerein, an anthraquinone compound, is employed for the treatment of osteoarthritis (Salve *et al.*, 2010). Its mechanism of action involves the inhibition of interleukin-1 beta, a protein that plays a role in the inflammatory process and the degradation of cartilage. Diacerein is characterised by its quick elimination from the body, which is attributed to its relatively short half-life. Therefore, the utilisation of a sustained-release formulation is justified in order to prolong its effects and improve patient adherence. Hence, it is appropriate to devise a method for administering the medication in a consistent and prolonged manner (Kempe and Mäder 2012, Janakiraman *et al.*, 2018).

Typically, Diacerein is given orally to treat osteoarthritis (OA). However, the oral administration of Diacerein has a low bioavailability, resulting in a limited absorption of the drug. This leads to unwanted side effects such as diarrhoea or loose stools due to the unabsorbed substance. These issues can be resolved by applying medication directly to the affected area of osteoarthritis. The topical formulation exhibits superior permeability, resulting in enhanced therapeutic efficacy and absence of gastrointestinal disturbances in comparison to oral administration. Moreover, the amount of medication needed to achieve therapeutic effects by topical administration is anticipated to be lower compared to the oral route (Pal *et al.*, 2016; Salgado *et al.*, 2021).

Considerable enhancements can be achieved in order to extend the length of action of intra-articular treatment. This is desirable in order to minimise the frequency of intra-articular injections per year, as they can cause discomfort and agony during administration, and also pose a danger of infection (Mathers *et al.*, 2000). This underscores the necessity for the development of intra-articular drug delivery systems that enable sustained drug release from the depot for an extended duration of several weeks. *In situ* injectable thermo-reversible gels can enhance the efficacy of both newly developed Osteoarthritis medications and existing treatments by prolonging the release of drugs within the joint space (Akinpelu *et al.*, 2009; Moghddam *et al.*, 2016). The primary aim of the present research was to develop and assess a topical gel containing Diacerein loaded transferosomes, in order to address the limitations associated with the oral administration of Diacerein for the treatment of Osteoarthritis.

**Materials and Methods**

**Material:**
The compounds Diacerein, HPMC K4, and
Carbopol 934 were provided as a gift by Ami Life Sciences Ltd, India. The purchase of Poloxamer 407 was made from Loba Chemicals Pvt. Ltd. in Mumbai, India. The chemicals, including DMSO, benzoyl alcohol, benzalkonium chloride, and triethanolamine, were acquired from Thomas Baker in Mumbai, India. All the remaining chemicals and solvents utilised were of analytical grade.

**Pre-formulation study:**

10 mg of diacerein was weighed and then placed into a standard flask with a volume of 100ml. Subsequently, the substance was mixed with 10ml of phosphate buffer pH 7.4 and 10ml of DMSO, and the total volume was adjusted to 100 ml using phosphate buffer. 7. A 10 ml aliquot of the aforementioned stock solution I was transferred into a 100 ml standard flask and the volume was adjusted to 100 ml using phosphate buffer (pH 7.4). 2, 4, 6, 8, and 10 ml of solution were transferred from stock solution II to a set of 10 ml volumetric flasks. The volume was composed of a phosphate buffer solution at pH 7.4. The absorbance of these solutions was measured at a wavelength of 258 nm relative to a blank sample. FT-IR spectroscopy was utilized to evaluate the compatibility between the medicine and excipients. The spectral range and spectral resolution are 400-4000 cm\(^{-1}\) and 4 cm\(^{-1}\), respectively (Dhaneshwar et al., 2009a; Elsayed et al., 2014).

**Evaluation of Prepared Formulation:**

**Viscosity:**

The viscosity of Diacerein in situ gels was measured at a temperature of 5 ± 1 °C and at 37 ± 1 °C using a Brookfield viscometer. The samples were immersed in a water tank with controlled temperature for a duration of 10 min. The viscosity was measured at a rotational speed of 50 rpm using spindle number 5 (Falgarone and Dougados, 2001Wu et al., 2014).

**Syringeability:**

The syringeability test equipment consists of a Diacerein in situ gelling solution, which is placed into a 5 ml glass syringe. The syringe is equipped with an 18G needle that is securely held in place. The duration needed for a gelling solution to be able to be injected using a syringe at a specific pressure was documented by Jain et al. (2013) and Ahirrao et al. (2022).

**Gelation temperature and time:**

The gelation temperature was assessed by placing a thin-walled tube in a water bath that was controlled at a specific temperature. The temperature of the water bath was gradually increased at a constant rate of 2°C every 5 min, while gently shaking the tube at regular intervals, until it solidified into a gel. The temperature at which the material transitioned from a "flow liquid sol" to a "no flow solid gel" following the movement of the tube was regarded as the gelation temperature. Gelation time refers to the
duration needed for a sol to undergo a transition into a gel state when exposed to the temperature at which gelation occurs. The gelation time was determined using the test tube transposition method, which involves placing a thin-walled glass tube in a thermostatically regulated water bath at the gelation temperature. The tube is gently shaken at regular intervals during the process. The gelation time, defined as the duration (in seconds) required for the transition to gel, was determined by observing whether the test tube exhibited flow or no-flow characteristics when inverted (Keservani et al., 2010; Rehman et al., 2015).

**Drug content:**

We mixed 1 ml of in situ gel with a phosphate buffer solution at pH 7.4 and adjusted the total volume to 50 ml. Then, we took 1 ml of this solution and adjusted the volume to 10 ml using phosphate buffer at pH 7.4. The drug content was determined by employing a UV-visible spectrophotometer at a wavelength of 258 nm, following appropriate dilution (Dougados et al., 2001; Brahmachari et al., 2009).

**Sterility test:**

An analysis was conducted to assess the efficacy of the sterilisation procedure for gamma irradiation Diacerein in situ gels. The sterile gels were incubated in fluid thioglycolate media containing aerobic and anaerobic bacteria at a temperature of 37 ± 1 °C. Additionally, the gels were exposed to soybean casein-digested media for fungus at a temperature of 25 °C for a duration of 14 days. Following the incubation time, the proliferation of bacteria or fungi is seen (El-Say et al., 2016; Rohmani et al., 2021).

**Bacterial endotoxin test:**

The process of bacterial endotoxin testing entails examining the diacerein in situ gel sample using the Limulus Amebocyte Lysate (LAL) reagent. This reagent is derived from the horseshoe crab. When exposed to pyrogenic substances, the lysate undergoes clotting or colour alterations (Pelletier et al., 2000; Patel et al., 2014).

**In vitro drug release:**

The investigation employed a diffusion medium of pH 7.4 phosphate buffer. The Franz diffusion cell was positioned within a magnetic field. The recipient compartment was filled with phosphate buffer solution at a pH of 7.4 and maintained at a temperature of 37 ± 0.5°C. Subsequently, a meticulously mounted goat skin was affixed to the cell, ensuring the prevention of any air bubble entrapment. The goat skin membrane was securely affixed to the receptor compartment fluid by tightly fastening it with a clamp or rubber band, ensuring intimate contact. The stirring velocity was maintained at a consistent rate throughout the duration of the experiment. A volume of 1 ml of the diacerein in situ gel was applied onto the surface of the goat skin membrane located within the donor compartment. The sample was extracted at specific time intervals from the sampling port of the receptor compartment using a micropipette. An equal volume of fresh buffer solution was then added to ensure that the sink condition was maintained. The samples were
Table 2: Analysis of Diacerein's FT-IR spectra and physical mixes

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Observed Peak</th>
<th>Interpretation</th>
<th>Formulation B1</th>
<th>Formulation B5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2850.41</td>
<td>N-H stretching</td>
<td>2860.21</td>
<td>2881.33</td>
</tr>
<tr>
<td>2.</td>
<td>2561.39</td>
<td>S-H stretching</td>
<td>2588.82</td>
<td>2591.58</td>
</tr>
<tr>
<td>3.</td>
<td>2589.58</td>
<td>S-H stretching</td>
<td>2538.66</td>
<td>2552.89</td>
</tr>
<tr>
<td>4.</td>
<td>1589.88</td>
<td>C-H bending</td>
<td>1589.78</td>
<td>1560.77</td>
</tr>
<tr>
<td>5.</td>
<td>1680.72</td>
<td>C-H bending</td>
<td>1680.62</td>
<td>1670.34</td>
</tr>
<tr>
<td>6.</td>
<td>1770.41</td>
<td>C-H bending</td>
<td>1769.51</td>
<td>1570.74</td>
</tr>
</tbody>
</table>

diluted and subjected to analysis using a UV spectrophotometer to determine the concentration of diacerein at a wavelength of 258 nm. The cumulative percentage medication release was determined by utilising the calibration curve of diacerein. The drug release profile from the gels was determined by plotting the in vitro drug release data and fitting it into different mathematical models to determine the mechanism of drug release (Kumar et al., 2011; Aziz et al., 2018; Kumar and Kumar, 2021).

Results and Discussion

Pre-formulation study:

The FT-IR spectra of the pure drug and formulation F4 and F5 demonstrated that the distinctive peaks of Diacerein remained unchanged in terms of their position, suggesting the absence of any chemical interactions between the medicine and the polymers utilised (Table 2). The FT-IR investigation revealed the compatibility between Diacerein and Poloxamer-407, HPMC K4, and Carbopol 934.

Diacerein solutions in phosphate buffer pH 7.4 were appropriately diluted to produce a range of concentrations, from 2 to 10 μg/ml. At 258 nm, the absorbance was measured (Soliman et al., 2021).

Formulation Development of Diacerein:

Using the thermosensitive polymer Poloxamer-407 and other copolymers like HPMC K4 and Carbopol 394, five formulations of diacerein in situ gelling systems were created using the conventional "cold method" in a controlled setting. Each formulation's medication concentration was maintained at the same level. The compositions had a clear, yellow colour free of any contaminants or suspended particles (Dhaneshwar et al., 2009).

Evaluation of Prepared Formulation:

Viscosity:

A study on the viscosity of produced diacerein in situ gels was conducted at 37±0.5°C and 5±3°C, the storage temperature. At body temperature, all of the formulations changed from their solid state in the refrigerator to a transparent stiff gel.

Gelation temperature and time:

The diacerein in situ gels that were made had their gelation temperature and gelation time measured. The diacerein in situ gels that were synthesised demonstrated sol-to-gel transition. The gelation temperature ranged from 34.8°C to 37.9°C, which is relatively near to physiological temperature. The gelation time values were found to be between 58 and 61 sec, according to the data. The syringeability of produced diacerein in situ gels was assessed. The syringeability time is the amount of time needed to inject the syringe's contents with a steady force. The results of the syringeability test showed that an 18G needle could be used to easily syringe the manufactured diacerein in situ gels. The syringeability times reported for gel formulations were found to fall between 6 and 7 seconds (Table 3).

Drug content:

Using a UV-visible spectrophotometer set to 258 nm, the drug content of each formulation was
Table 3: Syringeability time, gelling time, and gelation temperature of formulas

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulations</th>
<th>Temperature (Gelation °C)</th>
<th>GellinG Time (Sec.)</th>
<th>Syringeability Time (Sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>B1</td>
<td>36.8</td>
<td>59</td>
<td>7</td>
</tr>
<tr>
<td>2.</td>
<td>B2</td>
<td>37.6</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>3.</td>
<td>B3</td>
<td>35.8</td>
<td>59</td>
<td>7</td>
</tr>
<tr>
<td>4.</td>
<td>B4</td>
<td>36.5</td>
<td>60</td>
<td>7</td>
</tr>
<tr>
<td>5.</td>
<td>B5</td>
<td>36.7</td>
<td>59</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 4: Drug content of the formulations

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulations</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>B1</td>
<td>93.34%</td>
</tr>
<tr>
<td>2.</td>
<td>B2</td>
<td>96.12%</td>
</tr>
<tr>
<td>3.</td>
<td>B3</td>
<td>92.23%</td>
</tr>
<tr>
<td>4.</td>
<td>B4</td>
<td>93.49%</td>
</tr>
<tr>
<td>5.</td>
<td>B5</td>
<td>94.67%</td>
</tr>
</tbody>
</table>

calculated and found to be within the standard range (Table 4).

**Sterility test:**

Before the dosage form is administered to the body, gamma irradiation sterilisation aids in the dosage form's final step of sterilisation. Gamma irradiation was used to sterilise the best formulation, B1. Gamma irradiation was chosen above other sterilisation techniques due to its great penetration rate, which allows sterilisation of even sensitive materials without raising the temperature. Gamma irradiation, however, might have had an impact on how the in situ gels were characterised. Furthermore, the impact of gamma irradiation on injection time, gelation temperature and duration, in vitro drug release, and drug content has been assessed. The outcomes demonstrated that the established parameters remained mostly unchanged across the irradiated and non-irradiated formulations. One crucial requirement for the parenteral formulation is sterility. Diacerein in situ gel is intended to be administered parenterally to the intended spot. After exposure, the optimised formulation B2 was checked for sterility using gamma irradiation.

The microbiological assessment guarantees the sterility of the product and the efficacy of the sterilisation process. There was no turbidity during the post-incubation phase.

**Bacterial endotoxin test:**

The LAL reagent did not coalesce in the bacterial endotoxin test result. The test verified that there was no bacterial endotoxin material in the diacerein in situ gel and that the gel was appropriate for injection.

**In vitro drug release:**

The zero-order, first-order Higuchi model and the Korsmeyer-Peppas model were fitted to the in vitro release data in order to identify the release mechanism that best describes the drug release pattern. The zero-order kinetics are followed by the Diacerein in situ gels. The case II transport was supported by the values of every formulation. Table 5 depicts the in vitro drug release data and the mathematical model fitting.

**Conclusion**

In order to offer a unique injectable and biodegradable method for treating osteoarthritis, Diacerein in situ gels were developed. The
Table 5: Data on in vitro drug release and fitting mathematical models

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulation Code</th>
<th>Drug release (Order)</th>
<th>Drug release Mechanism</th>
<th>Value (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>B1</td>
<td>Zero order</td>
<td>Case II transport</td>
<td>1.006</td>
</tr>
<tr>
<td>2.</td>
<td>B2</td>
<td>Zero order</td>
<td>Case II transport</td>
<td>1.003</td>
</tr>
<tr>
<td>3.</td>
<td>B3</td>
<td>Zero order</td>
<td>Case II transport</td>
<td>1.005</td>
</tr>
<tr>
<td>4.</td>
<td>B4</td>
<td>Zero order</td>
<td>Case II transport</td>
<td>1.007</td>
</tr>
<tr>
<td>5.</td>
<td>B5</td>
<td>Zero order</td>
<td>Case II transport</td>
<td>1.050</td>
</tr>
</tbody>
</table>

formulation with the greatest duration of drug release, B2, was determined to be the optimal one. The ideal physicochemical parameters, including viscosity, syringeability time, gelation temperature, and gelation time, have also been demonstrated. When the gel reached body temperature, the Diacerein in situ gel changed into a gel while remaining stable and thermo-responsive. The treatment modality was enhanced by the controlled release of Diacerein from the optimised in situ gel. The process used for the in situ gelling system is simple, practical, and economical. The development of a unique drug delivery method for delayed release of Diacerein has the potential to increase patient compliance by lowering dosage, frequency of administration, related side effects, and therapy costs. In order to overcome the shortcomings of the currently available formulation, this research illustrated the benefits of Diacerein in situ gel and suggests that it could be used as an injectable drug delivery system for the treatment of osteoarthritis.

References


