Formulation and Evaluation of Parenteral Sustained Release Microspheres of Diclofenac Sodium

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Summary

The aim of this study was to formulate and evaluate microsphere based depot type parenteral sustained release formulation of diclofenac sodium (DFS). Drug was formulated in the form of microspheres, using varying proportion of ethylcellulose (EC) as the retardant material to extend the release, by phase separation-coacervation technique. The in vitro release pattern of the designed formulations was studied using modified Franz diffusion cell. In vivo pharmacodynamic study was carried out by determining the index of analgesia (increase in response time to thermal stress as percentage of basal response time). Tail flick method was employed to measure both the degree of analgesia and its duration of action. The prepared microspheres were white, free flowing, and spherical in shape with a mean particle size of 50 μm. In vitro release study of the micro-spheres in aqueous media was found to extend the release of DFS beyond 24 hours with DFS and EC ratio 1:3. The plot of log percentage remaining to be released vs. time gave a linear relationship indicating first-order release kinetics. The in vitro release rate constant ($K_r$) for different microspheres varied between 0.1448 hr$^{-1}$ and 0.0256 hr$^{-1}$. A good correlation was obtained between $K_r$ and proportion of EC in the microspheres. In vivo pharmacodynamic studies indicated that the duration of analgesic action is prolonged beyond 24 hrs in case of microsphere products of 1:3 ratio of DFS to EC, whereas administration of marketed parenteral preparation showed activity only up to 11hrs. Also, a good correlation was obtained between analgesic activity in vivo and cumulative percentage of drug release from the formulations.

Keywords: Diclofenac sodium, Ethyl cellulose, Parenteral sustained release, Microspheres.

1. Introduction

DFS, an effective non-steroidal anti-inflammatory and analgesic drug with its low oral bioavailability (60%), short plasma half-life (1.1 –1.8 hrs), and low dose (25-75 mg thrice daily) is an ideal candidate for the formulation of parenteral sustained release drug delivery system in the
management of acute and chronic pain and traumatic conditions\textsuperscript{1,2}. A good amount of work have been reported in the literature on the development of oral sustained release drug delivery system for Diclofenac Sodium (DFS), including the work by our group\textsuperscript{3-5}. However, very little work has been reported on the development of parenteral sustained release formulation. The efficacy of dosage regimen in such conditions depend on the ability of the parenterally administered sustained release formulation to provide an initial burst release of drug to facilitate rapid onset of action and then maintain a constant plasma drug level for prolonged period of time. Such a formulation will thereby decrease the dosing frequency, alleviate pain and suffering for longer duration and at the same time avoid systemic accumulation of the drug and related side effects\textsuperscript{6}. Increased need for patient compliance, especially in chronic pain or postoperative conditions, and improving the therapeutic efficacy of the drug suggest the need for sustained release parenteral drug delivery system for DFS.

Sustained release parenteral formulations of DFS have been attempted at by only few workers and are not simple. Various techniques which have been utilized for this purpose include multiple emulsion system\textsuperscript{7}, poly-lactic-co-glycolic acid copolymer microcapsules\textsuperscript{8}, and in situ gel forming systems\textsuperscript{9}. Ethyl cellulose (EC) has been widely used for the microencapsulation of a number of water-soluble drugs to retard the release rate or to improve the stability\textsuperscript{5,10-13}.

In the present study microsphere based depot type formulations were designed for DFS, using varying proportion of EC as the retardant material, by phase separation coacervation technique. Physical characteristics, micromeritics, and in vitro release studies were carried out to evaluate the release characteristics of DFS from these microsphere-based formulations. In vivo pharmacodynamic studies were carried out on animal (albino rat) model to measure the degree and sustained nature of the analgesia produced by tail-flick method (analgesiometer), wherein, the
index of analgesia was calculated as increase in response time as percentage of basal response time and compared with conventional market preparation. A regression analysis was performed between index of analgesia and cumulative percentage drug released in vitro, up to time for 60% of drug release.

Table 1. Physical characteristics of the microspheres

<table>
<thead>
<tr>
<th>Formulations</th>
<th>DMS1</th>
<th>DMS2</th>
<th>DMS3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Components</td>
<td>DFS:EC (1:1)</td>
<td>DFS:EC (1:2)</td>
<td>DFS:EC (1:3)</td>
</tr>
<tr>
<td>Physical Properties of Microspheres</td>
<td>White, Spherical, Free flowing</td>
<td>White, Spherical, Free flowing</td>
<td>White, Spherical, Free flowing</td>
</tr>
<tr>
<td>Particle Size&lt;sup&gt;a&lt;/sup&gt; (mean diameter in μm)</td>
<td>49.94 ± 12.83</td>
<td>51.03 ± 11.19</td>
<td>52.72 ± 14.15</td>
</tr>
<tr>
<td>Drug Content&lt;sup&gt;b&lt;/sup&gt; (in mg/ g of the microspheres)</td>
<td>585.23 ± 9.8</td>
<td>389.11 ± 3.2</td>
<td>324.78 ± 7.4</td>
</tr>
<tr>
<td>In-vitro release rate constant (K&lt;sub&gt;H&lt;/sub&gt;)</td>
<td>0.1418</td>
<td>0.0998</td>
<td>0.0256</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average of hundred microspheres ± S.D.
<sup>b</sup> Average of three batches ± S.D.

2. Results and discussions

Supplied DFS characterized by various official tests of identification<sup>14</sup> and analyzed by UV spectrophotometric method passed the tests of identification and analysis. Studies on effect of various formulation excipients like ethyl cellulose and cyclohexane on the stability of DFS suggest that the formulation additives, in the concentration used, did not affect the stability and UV absorbency profile of DFS. Physical characteristics and drug content per gram of formulated microspheres are presented in Table 1. The microspheres were white, free flowing and spherical in shape. The mean particle size varied between 49.94 μm to 52.72 μm for different formulations.
of DFS-EC microspheres (Table 1). The drug content per gram of the microspheres, as expected, decreased as proportion of the EC was increased.

Table 2. Regression equation between index of analgesia and cumulative percentage of drug released from designed microspheres

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Equation*</th>
<th>n</th>
<th>r</th>
<th>s</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMS1</td>
<td>IA = 5.2094(± 0.7995)× % DR − 57.7214 (± 33.3964)</td>
<td>6</td>
<td>0.9559</td>
<td>20.4131</td>
<td>42.4560</td>
</tr>
<tr>
<td>DMS2</td>
<td>IA = 3.5463 (± 0.1268) × % DR − 33.1170 (± 6.3886)</td>
<td>9</td>
<td>0.9956</td>
<td>8.3438</td>
<td>781.7274</td>
</tr>
<tr>
<td>DMS3</td>
<td>IA = 3.0568 (± 0.1547) × % DR − 12.7540 (± 6.3781)</td>
<td>12</td>
<td>0.9874</td>
<td>9.3725</td>
<td>390.2293</td>
</tr>
</tbody>
</table>

IA—Index of analgesia
%DR—Cumulative percentage of drug release at different time up to time for 60% DFS release
n—Number of data points used in the correlation
r—Correlation coefficient
s—Residual standard deviation
F—5-ratio between the variance of calculated and observed values of the dependent variable
* Data in the parentheses of the equation indicate the 95% confidence intervals

In vitro release study of the microspheres, performed in phosphate buffer of pH 6.8 showed that the duration of DFS release was extended beyond 24 hrs in case of DMS3 whereas in DMS1 and DMS2 the release were much faster. The plot of log percentage remaining to be released vs. time gave a linear relationship (Figure 1) suggesting first-order release kinetics of DFS from the EC microspheres. The in vitro release rate constant (Ks), as obtained from the slope of the plot and enlisted in Table 1, varied between 0.1448 hr⁻¹ (DMS1) and 0.0256 hr⁻¹ (DMS3). Since the EC barrier layer is insoluble in water, the release of the drug probably occurred through partitioning and/or diffusion. Increasing the proportion of EC slowed the rate of release. About 98% and 93% DFS was released in 24 hours in case of DMS1 [DFS: EC (1:1)] and DMS2 [DFS: EC (1:2)] respectively. However, in case of DMS3 [DFS: EC (1:3)] the duration of release was extended beyond 24 hrs, with only 56% release at the end of 24 hrs.
Correlation equation obtained between $K_r$ and proportion of EC (%EC) in the microspheres (calculated from the drug content data of table 1) is presented below:

$$K_r = -0.0045 \pm 0.0018 \times \%EC + 0.3184 \pm 0.1013$$

$n=9$, $r=0.9169$, $s=0.0339$, $F=5.2810$

In the above equation, $n$ is the number of data points used in the correlation, $s$ is the residual standard deviation, $r$ is the correlation coefficient, and $F$ is the $\delta$-ratio between the variance of calculated and observed values of the dependent variable. Data in the parentheses indicate the 95% confidence intervals. A good correlation was obtained between $K_r$ and proportion of EC (%EC) in the microspheres, obtained from the drug content data, as is evident from high correlation coefficient value of 0.9169 and very low residual standard deviation value. In addition, no significant difference was observed in the release profile of different batches of microspheres, indicating that the manufacturing process employed is reliable and reproducible. In addition, the study of stability and release profile at different interval of time, up to 12 months of storage, indicated the stability of the drug, unaltered physical characteristics of microspheres and release profiles.

The in vivo pharmacodynamic studies further confirmed the observed sustained release character of DFS from the designed formulations. Group 0 animals (0.6 mL of sterile water for injection) showed a response time of 4.0 (± 0.1) sec throughout the duration of the studies which was considered the basal response time. The plot between index of analgesia and time in rat upon i. m. administration of aqueous dispersion of the designed microspheres of DFS are presented in Figure 2. An immediate release parenteral preparation of DFS selected from the Indian market was used as positive control in the study. The studies revealed that in case of group 1 animals (market preparation) the time for onset of action is 0.5 hrs with a $t_{max}$ (time for peak analgesic
effect) of 2 hrs and the duration of action was around 11 hrs as is usually seen. However, in case of group 2 animals [DFS: EC (1:1)] the time for onset of action was 0.5 hr with a $t_{\text{max}}$ of about 6 hrs, but the duration of action was not extended beyond 12 hrs. In case of group 3 animals [DFS: EC (1:2)] the time for onset of action was 0.5 hr but the duration of action was extended beyond 20 hrs. Whereas, in case of group 4 animals [DFS: EC (1:3)] the time for onset of action was around 1 hr but the duration of action was extended beyond 24 hrs with only 56% release and near constant analgesic activity. The results indicate slow and extended duration of action for all the microsphere products. However, in case of DMS3 the release pattern became nearly zero order around 24 hours time as observed in both in vitro and in vivo studies. Further, on continuous macroscopic and microscopic observation of the site of administration of the aqueous dispersion of designed DFS microspheres, no tissue necrosis was observed.

Further, regression equations between index of analgesia (IA) and cumulative percentage of drug release (%DR) at different time up to time for 60% DFS release in vitro for various formulations were calculated and are presented in Table 2. A good correlation was obtained between analgesic activity in vivo and cumulative percentage of drug release from the formulations, up to a period for 60% of the drug release. The correlation coefficient ($r$), as given in table 2, varied from 0.9874 to 0.9956 indicating a very high in vivo and in vitro correlation. A significant difference (at $p < 0.05$) was observed in the in vitro release data as well as in vivo pharmacodynamic profile between various designed formulations using one-way analysis of variance and Tukey's multiple range tests.

It can be concluded that microsphere based depot type formulation is a feasible technique for preparing parenteral sustained release preparation of DFS. The EC based microspheres of DFS were stable, of good physical properties and exhibited reproducible release kinetics across the batches, indicating the reliability and reproducibility of the manufacturing process. The duration
of DFS release from the microspheres was extended upon increasing the proportion of EC. The result of this study should advance the rational basis to simulate the benefits of intravenous drug infusions without its potential hazards, discomforts to the patients through the development of depot type parenteral sustained release formulations.

![Graph](image1.png)

**Figure 1.** Release profile of DFS microspheres with varying proportion of EC. Data represents the average of triplicate release studies with standard deviation.

![Graph](image2.png)

**Figure 2.** Profile of index of analgesia in rat upon i. m. administration of the aqueous dispersion of the designed formulations of DFS and an ordinary i. m. market preparation. Data represents the average of six animals with standard deviation. Average basal response time of the animals used in the study was 4.0 ± 0.1 sec.
4. Experimental

4.1 Materials

Diclofenac sodium I.P. was obtained as gift sample from Torrent Pharmaceuticals Ltd., Ahmedabad, India. Ethyl cellulose and all other chemicals and reagents used in the study were of pharmaceutical or analytical grade.

4.2 Characterization of bulk drug

Bulk drug was characterized by various official tests of identification and analyzed by UV spectrophotometric method (Jasco, UV-Vis spectrophotometer; model -7800) at 276 nm in aqueous medium. Effect of various excipients like ethyl cellulose and cyclohexane on the stability and analysis of DFS was also studied.

4.3 Preparation of microspheres

Phase separation-coacervation method was employed to formulate microspheres of DFS using different ratio of DFS and EC [1:1 (DMS1), 1:2 (DMS2), and 1:3 (DMS3)]. EC was dissolved in cyclohexane by heating to a temperature of 80 °C. Finely pulverized DFS was dispersed in the solution of EC in cyclohexane with vigorous stirring. Phase separation was induced by reducing the temperature while maintaining vigorous stirring. The product obtained after drying was size reduced and passed through very fine sieve. The required dose of microspheres was dispersed in sterile triple distilled water at the time of administration. To study batch reproducibility three batches of each formulation were prepared and evaluated for release profile of DFS. These batches of the formulations were re-evaluated at 3, 6 and 12 months interval to study the effect of storage under ambient conditions on the stability and release profile of the drug from the EC microspheres.

4.4 Physical characterization of the microspheres

The microspheres prepared were studied for appearance and size distribution using optical
microscopy. The drug content in the microspheres was determined by dissolving accurately weighed amount of the formulation in little amount (<5 mL) of methanol to dissolve the EC coat. To this 20-25 mL of triple distilled water was added and then the solution heated to evaporate the methanol. The solution was filtered to remove insoluble EC, suitably diluted and analyzed by UV spectrophotometric method at 276 nm.

4.5 In vitro release study

The in vitro release from the designed formulations was studied using modified Franz diffusion cell. The formulation equivalent to 75 mg of DFS was placed in donor chamber in contact with the dissolution media (178 mL phosphate buffer of pH 6.8). The semipermeable membrane was pre-hydrated with dissolution media for 30 mins. A 3 mL sample were withdrawn and replaced with fresh media at different time interval and analyzed after suitable dilution. The in vitro studies were carried out in triplicate.

4.6 In vivo pharmacodynamic study

Thirty albino rats of either sex (200-250gms) were divided into five groups of six rats each and fasted for 4 hrs (water adlibitum) before the experiments. The practices of experimental animal handling were properly followed before, during and after experimentation according to the "Principles of Laboratory Animal Care" (NIH Publication # 85-23, revised 1985). The experimental protocol and usage of the animal was approved and monitored by the Institutional Animal Ethics Committee of Birla Institute of Technology and Science, Pilani. The index of analgesia was measured as the increase in response time to thermal stress as percentage of basal response time. Tail Flick Method (using analgesiometer) was employed to measure both the degree and sustained nature of the analgesia induced.

The volume of preparation injected was kept constant at 0.6mL for all the groups treatment. In case of treatment with the designed microspheres, the dose of the drug per Kg of the body
weight of the animal was doubled as compared to that of market preparation so as to get sufficient initial release for rapid onset as well as sustained nature of the analgesic action. The treatment given to the five groups were: Group 0 – placebo [0.6 mL sterile water for injection], Group 1 – market preparation [dose- 6.75 mg DFS/ Kg of the body wt. of 2.5mg DFS/ml of aqueous solution; i. m.], Group 2 – DMS1 [dose- 13.5 mg DFS/ Kg of the body wt. of 5.0 mg DFS/ml of aqueous dispersion; i. m.], Group 3 – DMS2 [dose- 13.5 mg DFS/ Kg of the body wt. of 5.0 mg DFS/ml of aqueous dispersion; i. m.], Group 4 – DMS3 [dose- 13.5 mg DFS/ Kg of the body wt. of 5.0 mg DFS/ml of aqueous dispersion; i. m.].

4.7 Data analysis

All values presented in this study are average of replicate experiments at the same time points. Least square regression equations and the correlation coefficients were calculated using Microsoft Office 2000, Excel package. Difference in in vitro release as well as in vivo pharmacodynamic profile of various designed formulations were tested statistically using one-way analysis of variance and Tukey’s multiple range test at p < 0.05.

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References


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