PUBLICATIONS AND POSTER PRESENTATION
Development and Evaluation of Lipospheres of Diclofenac Sodium

M.C. GOHEL AND AVANI AMIN
Dept. of Pharmaceutics and Pharmaceutical Technology, L.M. College of Pharmacy, Ahmedabad - 380 009

Received 17 June 1996
Accepted 2 March 1997

Lipospheres of diclofenac sodium were prepared by melt dispersion technique using triple pressed stearic acid. Free flowing lipospheres were obtained by congealing the microemulsion. The amount of water, Tween 20 (surfactant) and butyl alcohol (co-surfactant) were identified as the key variables affecting the formation of discrete spherical lipospheres. More than 70% of the isolated lipospheres were of the size range 180-250 μ. The amount of drug entrapped in the lipospheres was found to be dependent on the lipid to drug ratio and the drug loading was further increased by using caranuba wax coated particles of diclofenac sodium. The in vitro drug release study was conducted in phosphate buffer (pH 7.2). Dissolution of the entrapped drug was greatly retarded. The results of the F-statistics revealed that the drug was released by anomalous diffusion.

DICLOFENAC sodium is frequently prescribed for the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Its biological half-life has been reported as 1-2 h. Gastrointestinal side effects such as bleeding, ulceration or perforation of intestinal wall are commonly seen. Thus, a controlled release dosage form of diclofenac sodium is required to be formulated to minimize the damage to the gastro-intestinal mucosa and to reduce the frequency of dosing.

A survey of the lipid materials contained in drug products marketed in the United States showed that stearic acid or its salts are widely used in dosage forms. The low cost, low toxicity and ease of fabrication have been stated as the major advantages of lipids by Kabwvichii. Studies have been reported on the formulation development and dissolution testing of wax microspheres of different drugs. The aim of the present investigation was to develop controlled release lipospheres of diclofenac sodium using melt dispersion technique, which does not require organic solvents.

Diclofenac sodium (J.P.) was received as a gift sample from Sharda Drugs. Triple pressed stearic acid, Tween 20 and butyl alcohol were purchased from local market.

Lipospheres were prepared from microemulsions as reported by Dino and co-workers. The formulation of different batches is depicted in Table 1. Briefly, triple pressed stearic acid was melted on a water bath maintained at 70-72°. Finely powdered drug particles (90#) were dispersed in the molten wax. Aqueous phase was prepared by heating a blend of water and Tween 20 (surfactant, HLB 16.7) to 70-72°. Butyl alcohol (co-surfactant) was
Table 1: Formulation Variables and Drug Content of Lipospheres

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Butyl Alcohol (ml)</th>
<th>Drug (mg)</th>
<th>Water (ml)</th>
<th>Drug Content (%)</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>0.6</td>
<td>--</td>
<td>60</td>
<td>--</td>
<td>AP</td>
</tr>
<tr>
<td>B2</td>
<td>0.8</td>
<td>--</td>
<td>60</td>
<td>--</td>
<td>AP</td>
</tr>
<tr>
<td>B3</td>
<td>1.0</td>
<td>--</td>
<td>60</td>
<td>--</td>
<td>AP</td>
</tr>
<tr>
<td>B4</td>
<td>1.2</td>
<td>--</td>
<td>60</td>
<td>--</td>
<td>AP</td>
</tr>
<tr>
<td>B5</td>
<td>1.5</td>
<td>--</td>
<td>60</td>
<td>--</td>
<td>DP</td>
</tr>
<tr>
<td>B6</td>
<td>5.0</td>
<td>--</td>
<td>60</td>
<td>--</td>
<td>DP</td>
</tr>
<tr>
<td>B7</td>
<td>10.0</td>
<td>--</td>
<td>60</td>
<td>--</td>
<td>DP</td>
</tr>
<tr>
<td>1</td>
<td>1.5</td>
<td>500</td>
<td>60</td>
<td>5.8</td>
<td>DP</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>750</td>
<td>60</td>
<td>11.7</td>
<td>DP</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>1000</td>
<td>60</td>
<td>17.5</td>
<td>DP</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>500</td>
<td>60</td>
<td>5.5</td>
<td>DP</td>
</tr>
<tr>
<td>5</td>
<td>1.5</td>
<td>500</td>
<td>60</td>
<td>12.7</td>
<td>DP</td>
</tr>
<tr>
<td>6</td>
<td>1.5</td>
<td>1000</td>
<td>60</td>
<td>27.6</td>
<td>DP</td>
</tr>
<tr>
<td>7</td>
<td>1.5</td>
<td>1000</td>
<td>60</td>
<td>27.4</td>
<td>DP</td>
</tr>
<tr>
<td>8</td>
<td>1.5</td>
<td>500</td>
<td>40</td>
<td>9.9</td>
<td>DP</td>
</tr>
<tr>
<td>9</td>
<td>1.5</td>
<td>500</td>
<td>20</td>
<td>11.8</td>
<td>AP</td>
</tr>
<tr>
<td>10</td>
<td>1.5</td>
<td>500</td>
<td>10</td>
<td>13.4</td>
<td>AP</td>
</tr>
</tbody>
</table>

Note: Two % Tween 20 was used as a surfactant except in Batch 7, where 5% Polaxomer was used. All batches were prepared using 2 gm of triple pressed stearic acid. AP = Aggregated Product, DP = Discrete Product.

Hot water was added to the finely powdered lipospheres (100 mg) and the resultant dispersions were exposed to ultrasonic treatment (Vibrcnis, Bombay) for 20 min. The ultrasonic treatment was repeated thrice with a resting period of 30 min. between treatments. The absorbance measurements were done on a Hitachi double beam UV/VIS spectrophotometer at 276 nm. Corresponding concentrations in the samples were calculated from the standard plot generated by fitting weighted linear regression model. The results of the analysis are depicted in Table 1.
Lipospheres containing 100 mg of diclofenac sodium were filled in hard gelatin capsules and evaluated for in vitro dissolution studies. The dissolution studies (n=3) were performed using USP XXII basket apparatus at a rotational speed of 50 RPM, at 37° in 900 ml phosphate buffer (pH 7.2). Samples (10 ml) were withdrawn at regular time intervals and filtered through 0.45 μ membrane filter. The drug content was determined in the filtrate.

The amount of water used for the preparation of lipospheres was found to influence the characteristics of the product. Large and aggregated lipospheres were obtained when the level of water was used in the range of 10 to 30 ml. Superior quality of lipospheres i.e. small in size and discrete in nature were obtained when 60 ml of water was used. Sphericity of the product was lost when 100 ml of water was used. The drug loading was found to be inversely related with the amount of water used in the preparation. It is therefore concluded that morphological characters and the drug content in the lipospheres may be optimized by using appropriate quantity of aqueous phase.

The presence of Tween 80 (2%) was found to be essential to obtain a discrete product. Butyl alcohol, a co-surfactant, was also found to influence the characteristics of the product. Aggregated lipospheres with non-spherical shape were obtained in batches B1 to B4 probably due to faster evaporation of butyl alcohol. Hence, it may be concluded that over and above the amount of butyl alcohol, other processing conditions such as stirring speed and temperature also play a critical role in the preparation of lipospheres. It was difficult to remove the excess amount of butyl alcohol from batches B6 and B7. Hence, 1.5 ml of butyl alcohol is suggested as the correct amount. Washing with water (3 x 50 ml) was found to be essential to avoid lumping of lipospheres.

The results depicted in Table 1 demonstrates that the drug content in the lipospheres was found to be dependent on the lipid:drug ratio. The most probable reason for lower percentage of drug loading is the higher aqueous solubility of diclofenac sodium (10 mg/ml). A higher drug to lipid ratio yielded a faster drug release. The surface characteristic of diclofenac sodium particles was modified by treating the drug particles with wax solution in an effort to increase the percentage drug loading in the lipospheres. It may be expected that if wax treated drug particles are used for the preparation of lipospheres, less leakage of the water soluble drug would occur in the outer aqueous phase.

The method used by Kawashima and co-workers was modified and used for coating the drug particles. The drug particles were suspended in a 3% w/v solution of caranuba wax (M.P. 31-86°). Chloroform (batch 4) and ethyl alcohol (batches 5, 6, 7) were tried as a solvent for coating the drug particles. The drug particles coated using alcoholic caranuba wax solution yielded lipospheres containing higher percentage of drug loading (12.7%, 4:1 lipid to drug ratio) whereas only 5.5% of the drug was loaded when chloroformic solution of caranuba wax was used at the same lipid to drug ratio.
The results of sieve analysis of batch I (uncoated diclofenac sodium particles, lipid to drug ratio 4:1) showed that about 70% of the lipospheres were of particle size range 180 to 250 µ. The results of batch 6 (wax coated diclofenac sodium particles, lipid to drug ratio 4:2) showed that about 70% of the lipospheres were found to be in the range of 250 to 500 µ. The main factors influencing the size distribution were the surface characteristics of the drug particles, stirring rate, cooling rate and congealing process. The in vitro drug release profile of pure diclofenac sodium powder and that of lipospheres of batch 3 (lipid to drug ratio of 4:2) and that of batch 6 (lipid to drug ratio of 4:2 containing wax coated drug particles) is depicted in Fig. 1. From the figure, one can conclude that sustained release of diclofenac sodium was obtained from the lipospheres. Batch 7 was prepared using 5% poloxamer as the surfactant instead of Tween 20. Satisfactory lipospheres were obtained, confirming that the wax coated drug particles helps in obtaining higher percentage of drug loading (Table 1). The drug release was greatly retarded when wax coated drug particles were used (Fig. 1).

The goodness of fit test proposed by Bamba and co-workers13 was used to determine the mechanisms of drug release. The data of Batch 6, a potential candidate for 12 h in vitro release, was fitted to the different models. The release profile fitted best to Korsmeyer and Peppas equation (log time vs. log fraction of drug released, F = 2.43) showing the least residual sum of square as compared with Higuchi equation (sq. rt. of time vs percentage drug released, F = 4.96) or Weibull equation (log log plot of time vs -ln (1-m), where m=fraction dissolved at time t, F= 10.23). This superiority is, however statistically insignificant as shown by F-ratio test. The values of correlation coefficient were found to be 0.9979, 0.9965, and 0.9868 for Korsmeyer and Peppas, Higuchi and Weibull models respectively. The values of slope and intercept were found to be 0.5547 and - 1.6020 for Korsmeyer model respectively. From the value of the slope, it may be concluded that the drug is released by diffusion of anomalous type (non-Fickian).

REFERENCES
1 Todd, P A and Sorkin, E M, Drugs, 1988, 35, 244
6 Christianah, M A, and James, C P, Pharm. Res., 1994, 11, 575
7 Vilvalam, V D, and Adeyeye, C M J. Micrcencapsulation, 1994,11, 455
May 16, 1997

Dr. Mukesh Gohel  
Department of Pharmaceutical Technology  
L.M. College of Pharmacy  
Ahmedabad-380 009  
India

Ref: 96-208H(R)

Dear Dr. Gohel:

Your manuscript entitled "Formulation Optimization of Controlled Release Diclofenac Sodium Microspheres Using Factorial Design", has been accepted for publication in the Journal of Controlled Release.

Many thanks for an excellent contribution.

Best personal regards.

Sincerely yours,

J. Heller

JH:rmt
Formulation optimization of controlled release diclofenac sodium microspheres using factorial design

M.C. Gohel*, A.F. Amin

Department of Pharmaceutics and Pharmaceutical Technology, L.M. College of Pharmacy, Navrangpura, Ahmedabad 380 009, India

Received 23 December 1996; received in revised form 28 April 1997; accepted 16 May 1997

Abstract

Diclofenac sodium is an ideal candidate for incorporation in a controlled release device to diminish its adverse effects after oral administration. Microspheres were prepared by using sodium alginate as a polymer and CaCl₂ as a cross-linking agent. In this investigation, 3² full factorial design was used to investigate the joint influence of three variables: the stirring speed (X₁), concentration of CaCl₂ (X₂) and % of heavy liquid paraffin in a blend of heavy and light liquid paraffin in the dispersion medium (X₃) on the time for 80% drug dissolution (t₈₀). Potential variables such as concentration of sodium alginate and drug: sodium alginate ratio were kept constant in the experimental design. A statistical model with significant interaction terms is derived to predict t₈₀. The results of multiple linear regression analysis and F-statistics revealed that for obtaining controlled drug release, the microspheres should be prepared using relatively lower stirring speed, higher concentration of CaCl₂ and higher percentage of heavy liquid paraffin in the dispersion medium. The X₁X₂ and X₁X₃ interactions were found to be statistically significant in nature. A response surface plot is presented to show the effects of X₁, X₂ and X₃ on t₈₀. The drug was released by diffusion of anomalous type. A model was validated for accurate prediction of drug release profile. Acceptable batches were identified in the experimental design with constraints on percentage drug released in 1, 6 and 8 h. © 1997 Elsevier Science B.V.

Keywords: Microspheres; Diclofenac sodium; Sodium alginate; Factorial design; Response surface plot

1. Introduction

Diclofenac sodium, a potent non-steroidal anti-inflammatory drug with pronounced analgesic properties, is used in the long term treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Its biological half-life has been reported as 1–2 h [1]. Gastrointestinal side effects such as bleeding, ulceration or perforation of intestinal wall are commonly seen [2]. Due to short biological half-life and associated adverse effects, it is considered as an ideal candidate for controlled drug delivery.

Over the last few years medical and pharmaceutical industries have shown increased interest in the use of biopolymers in general, and for alginates in particular. The reasons for this increased interest is their usefulness in specific applications and long term safety aspects. Rajanarivo and co-workers reported that alginates are haemocompatible and did not accumulate in any of the major organs [3]. The quality of the alginates has improved in the recent years due to advancement in technology. A ginate
are used in areas such as matrixing agent, encapsulating agent, etc.

The cross-linking of alginate is a function of both the alginate composition and the length of the molecule. The affinity of the cross-linking cation for the alginate is also of great importance in the cross-linking reaction. The preparation of alginate particles described here is based on the cross-linking properties of this polysaccharide with CaCl$_2$. The calcium ion is believed to interact with five different oxygen atoms of two adjacent guluronate units in intra-chain bindings. In addition, it makes an "egg-box model" through inter-chain binding of calcium to two or more alginate chains. The encapsulated drug is released from the alginate matrix at a controlled rate [4].

It is well known that traditional experimentation involves a good deal of efforts and time especially when complex formulations are to be developed. It is desirable to develop an acceptable pharmaceutical formulation in shortest possible time using minimum number of man hours and raw materials. In addition to the art of formulation, the technique of factorial design is an efficient method of indicating the relative significance of a number of variables and their interactions. The response surface method, first reported by Box and Wilson [5], has been applied to dosage form design for various kinds of drugs by many researchers [6-8].

In this study, factorial design based on response surface method was adopted to optimize microspheres of model drug diclofenac sodium. A 3$^2$ full factorial design was employed to evaluate the combined effect of the selected independent variables on the time for 80% of drug dissolution ($t_{80}$) and percentage drug released in 60, 360 and 480 min.

2. Materials and method

Diclofenac sodium (J.P.) was received as a gift sample from Sharda Drugs. Sodium alginate (Loba-Chemie Pvt. Ltd., Bombay), dioctylsulphosuccinate sodium (Aldrich Chemical Co., USA) and ethyl cellulose (Laser Laboratories, Ahmedabad) were used as received. All the other solvents and chemicals were of analytical grade and were used without further purification. The viscosity of aqueous sodium alginate solution (1% w/v) was found to be 30 cP, and that of ethylcellulose solution (5% w/v in toluene:ethanol 80:20) was found to be 14 cP at 25°C.

2.1. Preparation of microspheres

In the present investigation, stirring speed, concentration of calcium chloride and percentage of heavy liquid paraffin in the dispersion medium were selected as independent variables, whereas $t_{80}$ and percentage drug released in 60, 360 and 480 min were chosen as dependent variables. The formulations and processing conditions in the factorial design are shown in Table 1. Potential variables such as concentration of sodium alginate solution, drug to sodium alginate ratio and amount of dispersion medium were kept constant. The formulations were prepared according to the experimental design.

Diclofenac sodium (10g) was suspended in hot aqueous solution of sodium alginate (50°C, 20% w/v, 100 ml). The mixture was carefully added to the dispersion medium (500 ml) consisting of a mixture of heavy and light liquid paraffin in a specific ratio and 400 mg dioctylsulphosuccinate sodium. The dispersion was stirred for 5 min. using a propeller stirrer to obtain a water in oil emulsion. The droplets were crosslinked by the addition of CaCl$_2$ solution (100 ml) of specific strength. The stirring was continued for 10 min. and then the microspheres were coated by adding solution of ethyl cellulose (10% w/v in acetone, 200 ml). Petroleum ether (100 ml) was added after 5 min. to rigidize the coat. The microspheres were filtered under vacuum and washed with cold petroleum ether (3×100 ml) to remove the adhered liquid paraffin. The microspheres were allowed to air dry.

2.2. Size distribution of microspheres

The separation of the microspheres into various size fractions, was carried out using a mechanical sieve shaker (Industrial Combustion Ltd., U.K.). A series of six standard stainless steel sieves (10C-720 μm) were arranged in the order of decreasing aperture size. Ten grams of drug loaded microspheres were placed on the uppermost sieve. The sieves were shaken for a period of about 15 min.
<table>
<thead>
<tr>
<th>Batch No</th>
<th>Variable level in coded form</th>
<th>( t_{ad} ) (min)</th>
<th>( Y_{400} ) (min)</th>
<th>( Y_{s} ) (min)</th>
<th>( Y_{1050} ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(-1) (-1) (-1) (-1)</td>
<td>397</td>
<td>40.29</td>
<td>76.38</td>
<td>83.71</td>
</tr>
<tr>
<td>2</td>
<td>(-1) (-1) (-1) (0)</td>
<td>434</td>
<td>42.35</td>
<td>73.86</td>
<td>80.28</td>
</tr>
<tr>
<td>3</td>
<td>(-1) (-1) (-1) (1)</td>
<td>453</td>
<td>37.26</td>
<td>70.84</td>
<td>81.77</td>
</tr>
<tr>
<td>4</td>
<td>(-1) (-1) (0) (-1)</td>
<td>477</td>
<td>40.00</td>
<td>69.29</td>
<td>80.31</td>
</tr>
<tr>
<td>5</td>
<td>(-1) (-1) (0) (0)</td>
<td>483</td>
<td>37.32</td>
<td>69.17</td>
<td>77.79</td>
</tr>
<tr>
<td>6</td>
<td>(-1) (-1) (0) (1)</td>
<td>499</td>
<td>36.82</td>
<td>68.36</td>
<td>76.26</td>
</tr>
<tr>
<td>7</td>
<td>(-1) (-1) (1) (-1)</td>
<td>454</td>
<td>40.65</td>
<td>70.57</td>
<td>81.62</td>
</tr>
<tr>
<td>8</td>
<td>(-1) (-1) (1) (0)</td>
<td>532</td>
<td>41.84</td>
<td>66.84</td>
<td>73.54</td>
</tr>
<tr>
<td>9</td>
<td>(-1) (-1) (1) (1)</td>
<td>579</td>
<td>29.71</td>
<td>61.82</td>
<td>73.07</td>
</tr>
<tr>
<td>10</td>
<td>(0) (-1) (-1) (-1)</td>
<td>347</td>
<td>45.88</td>
<td>81.14</td>
<td>88.02</td>
</tr>
<tr>
<td>11</td>
<td>(0) (-1) (-1) (0)</td>
<td>375</td>
<td>40.72</td>
<td>76.80</td>
<td>85.00</td>
</tr>
<tr>
<td>12</td>
<td>(0) (-1) (-1) (1)</td>
<td>385</td>
<td>40.84</td>
<td>77.43</td>
<td>84.86</td>
</tr>
<tr>
<td>13</td>
<td>(0) (-1) (-1) (1)</td>
<td>395</td>
<td>42.14</td>
<td>76.58</td>
<td>89.62</td>
</tr>
<tr>
<td>14</td>
<td>(0) (-1) (-1) (1)</td>
<td>429</td>
<td>38.83</td>
<td>72.98</td>
<td>83.42</td>
</tr>
<tr>
<td>15</td>
<td>(0) (-1) (-1) (1)</td>
<td>448</td>
<td>37.48</td>
<td>71.27</td>
<td>82.26</td>
</tr>
<tr>
<td>16</td>
<td>(0) (-1) (-1) (0)</td>
<td>462</td>
<td>40.34</td>
<td>70.02</td>
<td>80.98</td>
</tr>
<tr>
<td>17</td>
<td>(0) (-1) (-1) (1)</td>
<td>475</td>
<td>39.98</td>
<td>69.41</td>
<td>80.49</td>
</tr>
<tr>
<td>18</td>
<td>(0) (-1) (-1) (1)</td>
<td>503</td>
<td>39.50</td>
<td>67.38</td>
<td>75.80</td>
</tr>
<tr>
<td>19</td>
<td>(1) (-1) (-1) (-1)</td>
<td>296</td>
<td>51.79</td>
<td>88.00</td>
<td>93.25</td>
</tr>
<tr>
<td>20</td>
<td>(1) (-1) (-1) (0)</td>
<td>307</td>
<td>48.13</td>
<td>86.15</td>
<td>93.52</td>
</tr>
<tr>
<td>21</td>
<td>(1) (-1) (-1) (1)</td>
<td>321</td>
<td>45.42</td>
<td>81.84</td>
<td>95.63</td>
</tr>
<tr>
<td>22</td>
<td>(1) (-1) (-1) (0)</td>
<td>348</td>
<td>40.32</td>
<td>79.25</td>
<td>91.75</td>
</tr>
<tr>
<td>23</td>
<td>(1) (-1) (-1) (0)</td>
<td>370</td>
<td>40.97</td>
<td>77.27</td>
<td>85.52</td>
</tr>
<tr>
<td>24</td>
<td>(1) (-1) (-1) (0)</td>
<td>382</td>
<td>41.02</td>
<td>77.77</td>
<td>85.23</td>
</tr>
<tr>
<td>25</td>
<td>(1) (-1) (-1) (0)</td>
<td>415</td>
<td>40.21</td>
<td>75.23</td>
<td>83.46</td>
</tr>
<tr>
<td>26</td>
<td>(1) (-1) (-1) (0)</td>
<td>436</td>
<td>42.25</td>
<td>73.69</td>
<td>80.09</td>
</tr>
<tr>
<td>27</td>
<td>(1) (-1) (-1) (0)</td>
<td>511</td>
<td>38.50</td>
<td>66.91</td>
<td>73.87</td>
</tr>
</tbody>
</table>

### Table I

<table>
<thead>
<tr>
<th>X1</th>
<th>X2</th>
<th>X3</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>1000</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>1500</td>
<td>15</td>
<td>50</td>
</tr>
</tbody>
</table>

2.3. Assay

Aqueous solutions of diclofenac sodium (3 to 30 \mu g/ml) in phosphate buffer (pH 7.2) were prepared and the absorbance was measured on a Hitachi double beam U-2000 spectrophotometer at 276 nm [9]. An equation was generated by fitting weighted linear regression model to the data obtained in triplicate (Absorbance=0.029 * Concentration – 0.0062) [10].

2.4. Drug content

To determine the efficiency of entrapment, the microspheres were assayed for the drug content. Drug loaded microspheres (100 mg) of each batch were assayed for the drug content.
were finely powdered in a glass mortar. Hot water (60°C) was added to the powder and the resultant dispersions were exposed to ultrasonic treatment (Vibronics, Bombay) for 20 min. This treatment was repeated thrice with a resting period of 30 min. The drug content was determined in the solutions after filtering the dispersion through 0.45 μm filter.

2.5. Dissolution study

Microspheres equivalent to 100 mg of diclofenac sodium were filled in hard gelatin capsules and were evaluated for in-vitro dissolution studies. The study was carried out in USP XXII basket apparatus at a rotational speed of 50 rpm at 37°C in 900 ml phosphate buffer (pH 7.2). Samples (10 ml) were withdrawn at regular time intervals and filtered through 0.45 μm membrane filter. The drug content was determined in the filtrate either directly or after appropriate dilution with the dissolution medium. The \( \bar{r}_n \) was calculated for each formulation. The average values of \( \bar{r}_n \) and percentage drug released in 60, 360 and 480 min (n=2) are depicted in Table 1.

2.6. Factorial design

Traditionally pharmaceutical formulations are developed by changing one variable at a time approach. The method is time consuming in nature and requires a lot of imaginative efforts. Moreover, it may be difficult to evolve an ideal formulation using this classical technique since the joint effects of independent variables are not considered. It is therefore very essential to understand the complexity of pharmaceutical formulations by using established statistical tools such as factorial designs.

The number of experiments required for these studies is dependent on the number of independent variables selected by the pharmacist. The response/f (Y) is/are measured for each trial and then either simple linear (\( Y=b_0+b_1X_1+b_2X_2+b_3X_3 \)) or interactive (\( Y=b_0+b_1X_1+b_2X_2+b_3X_3+b_12X_1X_2+b_13X_1X_3+... \)) or quadratic (\( Y=b_0+b_1X_1+b_2X_2+b_3X_3+b_12X_1X_2+b_13X_1X_3+...+b_{123}X_1X_2X_3 \)) model is fitted by carrying out multiple regression analysis and F-statistics to identify statistically significant terms.

The reduced equation, an equation containing only statistically significant terms, is then used for drawing response surface plots to visualize the impact of changing variables at a glance. The optimum point may be identified from the plot and replicate trials may be run to verify the prediction of optimum response. For simplicity, it was decided to perform a three variable study at three experimental levels to achieve the set objectives efficiently.

3. Results and discussion

Polymer concentration of 10, 15 and 20% w/v were selected for carrying out preliminary trials. Formation of flakes was noticed when sodium alginate was used at a level of 10%, whereas maximum sphericity was obtained when the alginate concentration was kept at 20%. The solution of sodium alginate was found to be too viscous to handle when tried at a level greater than 20% and hence sodium alginate concentration was fixed at 20% for the experimental design. The solution of sodium alginate was heated to 50°C before dispersing it in the liquid paraffin blend to improve pourability and to facilitate mixing of phases. The addition of 400 mg of dioctyl sulphosuccinate sodium to the dispersion medium was found to be essential to minimize aggregation of microspheres. The drug was released at a comparatively faster rate when the microspheres were not coated with ethyl cellulose. For preliminary screening, three batches of microspheres were prepared using polymer to drug ratio of 1:1, 2:1 and 1:2. The formulation prepared using 2:1 ratio showed highest \( \bar{r}_n \) and hence this ratio was selected for the experimental design. All the formulations prepared within the experimental design layout yielded free flowing microspheres.

Interactive statistical first-order complete model (Eq. (1)) was first generated to evaluate the selected response.

\[
Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3
\]
and $X_2X_3$ show how the $t_{80}$ value changes when two or more factors are simultaneously changed. The fitted equation relating the response ($t_{80}$) to the transformed factors is shown in Eq. (2).

$$
t_{80} = 426.296 - 51.22X_1 + 58.28X_2 + 27.22X_3 + 13.08X_1X_2 + 11.92X_2X_3 + 0.25X_1X_3 + 2X_3$$  (2)

The $t_{80}$ values for the twenty seven batches show a wide variation i.e. the response ranges from a minimum of 296 to a maximum of 579 min. The data clearly indicates that the $t_{80}$ value is strongly dependent on the factors.

The significance test for regression coefficients was carried out by applying Student’s $t$-test. A coefficient is significant if the calculated $t$ value is greater than the critical value of $t$. The significance level of coefficients $b_{12}$ and $b_{23}$ were found to be 0.3841 and 0.9642 respectively and hence they were removed from the regression Eq. (2) to generate the reduced model. The coefficients $b_1$, $b_2$, $b_3$, $b_{12}$, $b_{23}$ showed significant values of less than 0.05 and hence they were retained in the reduced model. (Eq. (3))

$$
t_{80} = 426.296 - 51.22X_1 + 58.28X_2 + 27.22X_3 + 13.08X_1X_2 + 11.92X_2X_3$$  (3)

The reduced model was tested in portions to determine whether the coefficients $b_{12}$ and $b_{23}$ contribute significant information for the prediction of $t_{80}$ or not [11]. This was done by testing the hypothesis that the two interaction terms were equal to zero (simple model: $Y=b_0+b_1X_1+b_2X_2+b_3X_3$). The results of regression analysis for the simple model are depicted in Table 2. The critical value of $F$ for $\alpha=0.05$ is equal to 3.47 ($DF=2$, 21). Since the calculated value ($F=8.24$, refer Table 2) is greater than the critical value (3.47), it may be concluded that the interaction terms i.e. $b_{12}$ and $b_{23}$ contribute significantly to the prediction of $t_{80}$ and hence they should be retained in the reduced model.

It may be concluded that the low level of $X_1$ (stirring speed) and high levels of $X_2$ (concentration of CaCl$_2$) and $X_3$ (percentage of heavy liquid paraffin in the dispersion medium) appear to favour the preparation of controlled release microspheres of diclofenac sodium. The Eq. (3) is presented in the form of a response surface plot in Fig. 1 to visualize the impact of changing independent variables on $t_{80}$. The factor $X_1$ is fixed at a high level (+1) for drawing the response surface plot since it exhibits the most significant effect on $t_{80}$. 

It may be concluded that the general theory of microspheres. The drug is released at a slower rate from larger microspheres because of aggregate effect of decreased surface area and increased diffusion path length. Similarly, when higher level of CaCl$_2$ is used cross-linking reaction is favoured and hence slower drug release is observed. When heavy liquid paraffin is used at a higher level, greater resistance is offered in the process of dispersion leading to the formation of

\[ F = (3755/2)/228 = 8.24 \]

<table>
<thead>
<tr>
<th>Response</th>
<th>$b_0$</th>
<th>$b_1$</th>
<th>$b_2$</th>
<th>$b_3$</th>
<th>$b_{12}$</th>
<th>$b_{13}$</th>
<th>$b_{23}$</th>
<th>$b_{123}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{80}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sample calculation for testing the model in portions:

<table>
<thead>
<tr>
<th></th>
<th>$DF$</th>
<th>$SS$</th>
<th>$MS$</th>
<th>$F$</th>
<th>$R$</th>
<th>$SSE1 - SSE2 = 8546 - 4788 = 3758$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>RM</td>
<td>5</td>
<td>125457</td>
<td>25091</td>
<td>110.4</td>
<td>0.9632</td>
</tr>
<tr>
<td></td>
<td>SM</td>
<td>3</td>
<td>121699</td>
<td>40566</td>
<td>109.17</td>
<td>0.9343</td>
</tr>
<tr>
<td>Error</td>
<td>RM</td>
<td>21</td>
<td>4788</td>
<td>228</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SM</td>
<td>23</td>
<td>8546</td>
<td>371</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results are in agreement with the general theory of microspheres. The drug is released at a slower rate from larger microspheres because of aggregate effect of decreased surface area and increased diffusion path length. Similarly, when higher level of CaCl$_2$ is used cross-linking reaction is favoured and hence slower drug release is observed. When heavy liquid paraffin is used at a higher level, greater resistance is offered in the process of dispersion leading to the formation of
RESPONSE SURFACE PLOT

Fig. 1. Response surface plot.

The microspheres of batch 9 were found to be spherical in shape and exhibited highest $t_{sw}$ amongst all the batches. Moreover, about 30% of the drug was released in the first hour (loading dose) and thereafter the drug was released at a fairly controlled rate. About 85% of the microspheres were found in the size range of 250 to 500 $\mu$m. The dissolution data of microspheres of batch 9, a potential candidate for 12 hour in-vitro release, was further analysed to ascertain the mechanism of drug release [12]. The release profile fitted best to Korsmeyer and Peppas equation [13] ($F = 1.0334$) giving the least residual sum of square as compared with Higuchi equation [14] (square root of time versus % drug released, $F = 3.1867$) or Weibull equation [15] ($F = 3.3925$). This superiority is, however statistically insignificant as shown by $F$-ratio test. The values of correlation coefficient were found to be 0.9973, 0.9925, and 0.9666 for Korsmeyer and Peppas, Higuchi and Weibull models respectively. For the Korsmeyer model, the values of slope and intercept were found to be 0.4240 and $-1.2837$ respectively. From the value of slope, it can be concluded that the drug is released by diffusion of anomalous type (non-Fickian).

Peck and co-workers [16] derived mathematical relationship for the expression of entire dissolution profile from matrix tablets. An effort was made in the present investigation to derive similar type of relationship. A linear interactive model was generated using data of percentage drug released at 60, 180, 300, 360, and 480 min from all the 27 batches. The Higuchi model fitted well to the data set and hence square root of time was chosen as an additional independent variable. The multiple linear regression analysis was performed using the actual values. Microsoft EXCEL® was used to derive the equation. The derived equation describing the dissolution pattern is shown in Table 3 where, $Y$ is the percentage drug dissolved at time $\tau$. The $R^2$ was found

<table>
<thead>
<tr>
<th>Response</th>
<th>Coefficients for mathematical models</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y$</td>
<td>$b_0 = 1$  $b_1 = 0.0144$ $b_2 = -0.0287$ $b_3 = -0.0297$ $b_4 = -0.0007$ $b_5 = -2.6E-05$ $b_6 = -0.0073$ $b_7 = 4.99E-06$ $b_8 = 3.009$ $b_9 = 0.9979$</td>
</tr>
<tr>
<td>$Y_{sw}$</td>
<td>$b_0 = 32.347$ $b_1 = 0.0149$ $b_2 = 0.07282$ $b_3 = 0.1022$ $b_4 = -0.0012$ $b_5 = -0.0001$ $b_6 = -0.0245$ $b_7 = 2.52E-05$ $b_8 = -0.7357$</td>
</tr>
<tr>
<td>$Y_{sw}$</td>
<td>$b_0 = 70.132$ $b_1 = 0.0155$ $b_2 = -0.2718$ $b_3 = -0.0405$ $b_4 = -0.0007$ $b_5 = -2.6E-05$ $b_6 = 0.00497$ $b_7 = 2.11E-06$ $b_8 = -0.9528$</td>
</tr>
<tr>
<td>$Y_{sw}$</td>
<td>$b_0 = 76.704$ $b_1 = 0.0145$ $b_2 = 0.0658$ $b_3 = -0.0733$ $b_4 = -0.0006$ $b_5 = 0.0001$ $b_6 = -0.00299$ $b_7 = -1.08E-05$ $b_8 = -0.9212$</td>
</tr>
</tbody>
</table>
to be 0.9779, indicating a good fit. The F-test was found to be significant at $P<0.05$. The derived equation may be used for calculating percentage drug release from different batches within the factor space. The residuals were chosen as one of the options while carrying out multiple linear regression analysis in EXCEL® and hence calculated percentage drug release from all the 27 batches is obtained in the output. The following constraints were chosen for the selection of acceptance batches: $20\% < Y_{60} < 40\%$; $50\% < Y_{150} < 70\%$; $65\% < Y_{480} < 80\%$. The batches 5, 6, 8, 9, 17 and 18 met the selection criteria. But, the batches 5 and 17 showed greater than 69% drug release after 360 min. Therefore, they are considered as borderline cases. Hence, the final selection was done from batches 6, 8, 9 and 18. The drug release rate from batches 6, 8, and 18 was found to be relatively slow in the terminal phase of the dissolution test (i.e. 4.5% per h in between 6 and 8 h), whereas it was found to be 6.5% per hour from batch 9. Therefore, a check-point ($X_1=550$ RPM, $X_2=14.5\%$ CaCl$_2$ solution and $X_3=47.5\%$ heavy liquid paraffin in the dispersion medium) was selected close to the settings of batch 9 to validate the derived equation. The predicted and observed dissolution profile for the check-point is depicted in Fig. 2. The experimental release data compare quite well to the release profile predicted from the mathematical model. To widen the scope of selection, co-efficients were calculated for the percentage drug released at 60, 260 and 480 min. The data are shown in Table 3. Two-dimensional contour plots for each response were drawn separately and then overlapped as shown in Fig. 3 for the selection of acceptable region (ABCD). In conclusion, this study demonstrates the use of factorial design for the preparation of controlled release diclofenac sodium microspheres. This statistical technique allows scientists to examine more than one independent variable at a time. The desirable goals can be obtained by systematic formulation approach in shortest possible time.

References

surface methodology to design optimization in formulation
of a typical controlled release system, Pharm. Ind. 57(12)


FORMULATION DESIGN AND OPTIMIZATION
OF MODIFIED RELEASE MICROSPHERES
OF DICLOFENAC SODIUM

M.C. Gohel and A.F. Amin
L.M. College of Pharmacy, Ahmedabad.

Computer optimization technique was employed for developing microspheres of diclofenac sodium. A central composite design consisting of a two-level full factorial design superimposed on a stand design was employed. Two additional centre point replicates were also used to determine reproducibility of the system. The drug to polymer ratio and amount of cross-linking agent were chosen as independent variables, and time for 80% drug dissolution ($t_{80}$) was selected as the dependent variable. Eleven batches of microspheres of diclofenac sodium were prepared using polyvinyl alcohol as a matrixing agent and glutaraldehyde as a cross-linking agent. The microspheres were evaluated for drug content, drug release studies, particle size distribution and swelling studies. An optimum polynomial equation was generated for the prediction of the response variable. The results revealed that sustained drug release can be obtained by appropriate selection of the variables. The in vitro data were subjected to F-test for determining mechanism of drug release. It may be concluded that a broader understanding of the system can be investigated using statistical tools.

Note: *Certificate for above Poster Presentation attached herewith
  * The same under publication in Drug Development and Pharmacy - Acknowledgement letter attached
  Paper accepted for publication in DDIP - Acceptance letter attached
B.V. Patel Pharmaceutical Education & Research Development (PERD) Centre
Ahmedabad, India

THIS IS TO CERTIFY THAT

DR./MR./MS. A.F. Amin

HAS PARTICIPATED
AND PRESENTED A POSTER
AT THE

3rd International Symposium
on
INNOVATIONS IN PHARMACEUTICAL SCIENCES & TECHNOLOGY
7th - 9th February, 1997

Mr. Ajit Singh
CHAIRMAN
Organising Committee

Dr. Anisha Pargal
CONVENER
Scientific Programme
Dear Dr. Gohel,

This letter acknowledges receipt of a manuscript titled:

Formulation Design and Optimization of Modified Release

which you have submitted for consideration as regards its suitability for publication in Drug Development and Industrial Pharmacy / Biotechnology Drug Development. It will be sent for review. We hope to be in contact with you again within about twelve weeks. All correspondence concerning your manuscript must refer to the manuscript number which appears at the head of this letter.

You are reminded that our current instructions to authors require that if our reviewers recommend publication a floppy disk of the paper, incorporating all changes required by the reviews, must be sent to the Editor at the above address before publication of your paper can be effected.

If your paper is accepted and you wish us to consider your paper for early publication, you should write to the editor giving specific detailed reasons as to why it would be advantageous to our readership for your proposal to be adopted.

A reprint order form and price list will be sent to you with proofs and should be returned directly to the publisher.

All correspondence concerning advertising, subscriptions, and reprints should be sent directly to our publishers, Marcel Dekker, Inc., New York City, N.Y 10016.

Sincerely,

C.T. Rhodes, Ph.D.
Editor, Drug Development and Industrial Pharmacy
President, PharmaCon Inc. of R.I.

Jan 19, 1998

PharmaCon, Inc. is registered in the State of Rhode Island
Phone (401) 782-3705 • Fax (401) 782-9837
Dear [Name],

1) Your paper has been provisionally accepted for publication subject to compliance with the following changes:

Once these changes have been made, please send a disk and two hard copies within six weeks to:

Dr. C. T. Rhodes, Editor
400 Dugway Bridge Rd
P.O.Box 360
West Kingston, Rhode Island 02892, USA

(If no changes are required, you only need to send the disk)

2) Once the disk has been received in this office, it will be sent to our Publishers within about two months. You will receive a reprint order form from the Publisher in about five months.

3) Once you have sent the disk to this office in Rhode Island all further correspondence should be addressed to:

Ms Iris Accordino
The Journal Editor
Marcel Dekker Inc.
270 Madison Avenue
New York, NY 10016, USA

Sincerely,

C.T. Rhodes, Ph.D.
Editor