DESIGN AND EVALUATION OF SUSTAINED RELEASE POTENTIAL OF DICLOFENAC POTASSIUM CONTAINED IN BEESWAX MATRIX

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ABSTRACT

Conventional dosage forms such as normal release tablets and capsules have many drawbacks such as repeated administration, unavoidable fluctuations of plasma concentration levels, high incidence of adverse effects and poor patient compliance. The present study is aimed at examining the controlled release potential of granules of diclofenac potassium contained in beeswax matrix, formulated in polysorbate 80 and poloxamer 188. The granules were formed by melt granulation technique and characterized for size and swell ability. The drug release characteristics from different matrices were evaluated using different kinetic models. Analgesic effect was investigated by formalin-induced paw licking and Eddy hot plate latency models. Results show that combination of poloxamer 188 and polysorbate 80 in beeswax matrix formed granules with good flow properties and moderate swelling (< 3 %) within 1 h and excellent release retardation. However, formulations C and D containing poloxamer 188 or polysorbate 80 alone in combination with beeswax did not perform well in this study. Formulations A and B were found to have significant (p<0.05) analgesic effect in the tested models compared with controls. The significant (p<0.05) analgesic activity of formulations A and B at the late phase of formalin-induced paw licking model and high MDT are indications of the sustained release effect of the diclofenac in beeswax matrix. The study showed that the order of sustained analgesic effect of the matrices is B>A>C>D.  

(Keywords: delayed release, diclofenac potassium, analgesic, paw licking)

1. INTRODUCTION

Oral drug delivery is the most convenient route of drug administration regardless of the inherent limitation common with it. Some of the challenges encountered are gastrointestinal erosion, drug-food interaction, poor absorption and poor patient compliance. To ensure optimum delivery without compromising the efficacy, potency and stability, drugs are modified by coating, matrix formation or encapsulation by considering the physicochemical properties of drug, physiology of mucosal membrane and delivery devices or matrices [1-6]. The choice of matrices depends on sustainability of drug release, stability of the matrix, safety profile, release of molecules from the matrix, biocompatibility and biodegradability of the matrix components. Modified-release dosage forms are preparations that regulate the rate and/or time and/or site of release of the active ingredient, in order to achieve specific therapeutic objectives, which cannot be achieved by conventional immediate release dosage forms when similarly administered [7]. Matrix formulations are type of controlled drug delivery systems which release the drug (dispersed in swellable hydrophilic substances, an insoluble matrix of rigid non-swellable hydrophobic materials or plastic materials) in continuous manner by dissolution controlled as well as diffusion controlled mechanisms. Dissolution-limited drugs like diclofenac have attracted researchers’ interest in a bid to improve their solubility and hence bioavailability.
Che
c
mically, diclofenac is [o-(2,6-
dichloranilino)phenyl]acetate with molecular
weight of 318.10. It is a potent non selective
cyclooxygenase (COX) enzyme inhibitor and least
soluble among the phenylacetic acid derivative
even though present as sodium and potassium salts
[8-12]. The poor solubility in lower pH medium
and simulated gastric fluid (SGF), and high
solubility in high pH medium and simulated
intestinal fluid (SIF) [13] necessitate its formulation
as delayed release using release retardants to
enhance solubility of hydrophobic drugs upon oral
administration [14].

Previous studies have shown that
hydrophobic wax matrix systems are being widely
used in oral controlled drug delivery [15] because
of their flexibility to obtain a desirable drug release
profile, cost-effectiveness, and wide regulatory
acceptance [16]. The current research, therefore, is
tailored to examine the effects of beeswax matrix
on analgesic effect of diclofenac potassium
containing non-ionic surfactant, polysorbate 80 and
non-ionic triblock polymer, poloxamer 181 (P188).

2. MATERIALS AND METHODS

2.1 Materials

Poloxamer 188 (Sigma, USA) and polysorbate 80
(Merck, Germany) were used as received, beeswax
was prepared from fresh honey combs harvested in
Nsukka, Nigeria as described below. Diclofenac
potassium was obtained from Norvatis Pharma
Services, Lagos. All other reagents and chemicals
used were of analytical grade.

2.2 Preparation of Beeswax

The honeycomb was harvested in November, 2011
and squeezed to remove the honey and other
extraneous materials. The comb was boiled to melt
in distilled water at 100 ºC, filtered through a sieve
and allowed to cool overnight. The refined wax was
collected floating on water, dried and stored in a
desiccator for further use.

2.3 Preparation of Matrix Granule

Matrix granules were prepared by melt granulation
method [12]. Fixed amount of beeswax (15 g) and
diclofenac potassium (0.5 g) were weighed to
prepare four different formulations of the granules
(Table 1).

<table>
<thead>
<tr>
<th>Formulation Codes</th>
<th>Beeswax (g)</th>
<th>Diclofenac potassium (g)</th>
<th>Polysorbate 80 (g)</th>
<th>Poloxamer 188 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15.00</td>
<td>0.50</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>B</td>
<td>15.00</td>
<td>0.50</td>
<td>2.50</td>
<td>7.50</td>
</tr>
<tr>
<td>C</td>
<td>15.00</td>
<td>0.50</td>
<td>10.00</td>
<td>0.00</td>
</tr>
<tr>
<td>D</td>
<td>15.00</td>
<td>0.50</td>
<td>0.00</td>
<td>10.00</td>
</tr>
</tbody>
</table>

For each formulation, the beeswax was melted at 70
°C and all the ingredients (drug, polymer and/or
surfactant) incorporated slowly to the molten wax.
After cooling, each batch was granulated by
passing through the sieve No 16 and the screened
fractions collected and kept in a hot air oven at 50
°C for 12 hours to dry completely.

2.4 Swelling Studies on the Granules

10 mg quantity of granules from each formulation
was added into a calibrated micropipette blocked at
one end. A measured volume of distilled water was
added to each granule and allowed to stand for 60
minutes. The swelling level of the granule in each
micropipette was recorded. Percentage swelling
was calculated from equation below:

Percentage swelling = (Final granule level-Initial
granule level)/Initial granule level x100

2.5 Physical Characteristics of the Granules

Various physical characteristics of the granules:
bulk and tapped density, Hausner’s ratio, angle of
repose and compressibility index [17] were
determined.

2.6 In-Vitro Release Studies of Granules
The guide contained in British Pharmacopoeia [18] was modified and adopted in this study with distilled water at 37.5 ºC as dissolution medium. At predetermined time intervals, 2 ml samples was withdrawn from the medium and assayed spectrophotometrically at 266 nm for diclofenac. The amount of diclofenac released at each time interval was calculated from pre-calibrated Beer’s plot and release data analyzed according to zero order, first order, Higuchi, Nixson-Crowell and Korsemeyer-Peppas equation models to ascertain the equation with the best fit [19].

2.7 Animal Studies

30 male albino rats weighing 150 – 180 g purchased from Department of Veterinary Medicine, University of Nigeria, Nsukka and kept at well ventilated Animal house in a photo period controlled environment (12 hours light/dark cycle) were used for each model of the study. The rats were divided into 6 groups (A-F) of 5 rats each. Rats in groups A-D received 10 mg/kg of formulations A-D respectively while rats in group E and F received equivalent doses of blank formulation (10 mg/kg) and standard diclofenac potassium (0.2 mg/kg) respectively.

2.7.1 formalin-induced paw licking test

The method adopted was described by Hunskaar and Hole [20]. Freshly prepared formalin solution (0.2mL of 3% formalin) was injected subcutaneously into the sub-planter surface of the left hind paw of rats in each group 1 hour after the oral administration of blank formulation, diclofenac potassium, or formulations A-D. The time spent by each rat in licking the injected paws within the first 5 minutes and 20–30 minutes after formalin injection was recorded. The mean of the time spent in licking the paw in each phase was determined for each group of animals.

2.7.2 hot plate latency test

The method of Eddy [21] was used for this study. Animals were divided into six groups. The formulations A - D, blank formulation or standard diclofenac was administered to 12 h fasted animals after which the animals were placed on the hot plate maintained at 55±1 ºC. The reaction time characterized by jumping off of the animals from the hot plate to the thermal stimuli was noted at 30, 60 and 120 minutes post formulation administration. The mean of the latencies + SEM of the animals on the hot plate were determined for each group.

2.8 Statistical Analysis

Values were expressed as mean ± standard error of mean (S.E.M). Statistical significance was determined by using student t-test; values with $p < 0.05$ compared with control were considered as significant.

3 RESULTS AND DISCUSSION

Physical characterization of the granule matrix is a pre-formulation study that determines the flow properties, uniformity and particle distribution within the matrix. All parameters show that the matrix conforms to standard values (Table 2).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% swelling at 30 min</th>
<th>% swelling at 60 min</th>
<th>Bulk Density (gc/m$^3$)</th>
<th>Tauped Density (gc/m$^3$)</th>
<th>Hausner Ratio</th>
<th>Carr’s Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.68 ±0.3 4</td>
<td>2.69 ±0.0 8</td>
<td>0.3 11</td>
<td>0.3 46</td>
<td>1.2</td>
<td>16 .4</td>
</tr>
<tr>
<td>B</td>
<td>1.20 ±0.9 1</td>
<td>1.20 ±0.1 7</td>
<td>0.3 22</td>
<td>0.3 65</td>
<td>1.3</td>
<td>15 .8</td>
</tr>
<tr>
<td>C</td>
<td>6.80 ±0.3 2</td>
<td>7.15 ±0.0 5</td>
<td>0.3 56</td>
<td>0.3 84</td>
<td>1.0</td>
<td>16 .2</td>
</tr>
<tr>
<td>D</td>
<td>6.10 ±0.1 2</td>
<td>6.20 ±0.3 9</td>
<td>0.3 80</td>
<td>0.4 10</td>
<td>1.1</td>
<td>17 .0</td>
</tr>
</tbody>
</table>

(n=3; mean±SEM)
Formulation B (containing the highest amount of P188) has the finest granules while Formulation C with no P188 has the largest granule size. The fineness of the granule is an indication of the uniformity in distribution of the drug within the matrix. The swelling rate of formulation also maintained a constant value over 1 h as compared to increasing swelling of formulation C and D. The granule size and swelling rate depend on the surfactant-polymer ratio in the formulation. Matrices with higher concentration of P188 have lower size and swelling rate due to its amphiphilic nature.

The release characteristics of diclofenac from all the matrices within 24 h are excellent (Figure 1). To understand the release mechanism of diclofenac from the matrices, release data was filled into various kinetic models.

Figure 1: Release Profiles of Diclofenac from Matrix Granules

The mean diffusion time, MDT, which explains the diclofenac release rate and the release retarding efficiency of polymer/waxes, was above 100 h for matrices A and B and below 20 h for matrices C and D. From the kinetic models, the best fit with higher $R^2$ value ($R^2 \geq 0.98$) was found with Higuchi’s equation but its applicability in describing release mechanism was limited by poor swelling of matrices (<3 % for matrices A and B) upon hydration and gradual erosion of matrices. Thus, a plot of log percentage release against log time for all formulations was carried to obtain n values. Previous studies [22] show that n value of 0.45 indicate Fickian release, 0.45<n<0.89 indicate non-Fickian release, n value of 0.89 show case II type (zero order) release while a value higher than 0.89 represent a super case II type of release. This study show that diclofenac release from matrices A and B follows Korsmeyer-Peppas model and occurs via classical Fickian diffusion controlled release, matrix C Higuchi, via non-Fickian or anomalous release while matrix D follows first order and super case II relaxation transport model (Table 3).

Table 3: In-Vitro Diclofenac Release Kinetics

<table>
<thead>
<tr>
<th>Formulation</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_0$ (h$^{-1}$)</td>
<td>0.82</td>
<td>0.23</td>
<td>1.93</td>
<td>0.28</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.935</td>
<td>0.978</td>
<td>0.963</td>
<td>0.983</td>
</tr>
<tr>
<td>First order</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_1$ (h$^{-1}$)</td>
<td>-2.38</td>
<td>-0.93</td>
<td>-1.02</td>
<td>-2.93</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.967</td>
<td>0.917</td>
<td>0.942</td>
<td>0.993</td>
</tr>
<tr>
<td>Higuchi</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_2$ (h$^{-1}$)</td>
<td>4.68</td>
<td>4.72</td>
<td>2.09</td>
<td>2.03</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.980</td>
<td>0.983</td>
<td>0.979</td>
<td>0.936</td>
</tr>
<tr>
<td>Peppas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>0.45</td>
<td>0.45</td>
<td>0.83</td>
<td>1.09</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.987</td>
<td>0.988</td>
<td>0.927</td>
<td>0.973</td>
</tr>
<tr>
<td>Best fit</td>
<td>Peppas</td>
<td>Peppas</td>
<td>Higuchi</td>
<td>First</td>
</tr>
<tr>
<td>MDT (h)</td>
<td>105</td>
<td>102</td>
<td>18.60</td>
<td>15.20</td>
</tr>
</tbody>
</table>

Formulations A and B significantly increased the reaction time to the thermal stimulus of the hot plate from 4.8 to 5.9 (30 minutes), 4.6 to 6.0 (60 minutes), 5.0 to 6.2 (90 minutes), 4.5 to 5.9 (120 minutes). Similar trend was observed for formulation B, though higher compared to blank formulation (Table 4).

Table 4: Results of Hot Plate Latency Test.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Reaction Time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Blank formulation</td>
<td>4.8±0.5</td>
</tr>
<tr>
<td>A</td>
<td>5.9±0.2</td>
</tr>
</tbody>
</table>
In the formalin-induced paw licking latency test, formulations A and B showed good responses compared with the controls (Table 5).

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Paw Licking Time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early Phase (1-5 minutes)</td>
</tr>
<tr>
<td>Blank formulation</td>
<td>123.9±5.2</td>
</tr>
<tr>
<td>A</td>
<td>35.2±1.9</td>
</tr>
<tr>
<td>B</td>
<td>16.3±0.4*&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>49.6±2.9</td>
</tr>
<tr>
<td>D</td>
<td>67.5±4.6</td>
</tr>
<tr>
<td>Standard Diclofenac</td>
<td>18.5±0.3*</td>
</tr>
</tbody>
</table>

Data expressed as mean ±SEM (n=5). *P<0.05 significantly different from control; <sup>a</sup>P<0.05 significantly different from standard drug group.

The results show that diclofenac can be delivered orally in a matrix comprising surfactants and/ or polymer with good hydrophilic-lipophilic balance (HLB) as release retardants. Polysorbate 80 used, in combinations with poloxamer188, provided a driving force for uniformity in the composition of the matrix and adequate solubilization of all the hydrophobic moieties in the matrix core of the delivery system. This arrangement ensures that the matrix core containing diclofenac depot is released slowly over a period to maintain a constant plasma concentration. P188 is a nonionic tri-block copolymer composed of a central hydrophobic chain of polyoxypropylene flanked by two hydrophilic chains of polyoxyethylene [23]. This amphiphilic nature and surfactant properties of the polymer, in addition to 70-75 percent of a mixture of various esters from C<sub>26</sub>-C<sub>32</sub> alcohols (particularly palmitic acid, hydroxyl palmitic, d-β-dehydropalmitic and cerotic acid) of beeswax, increases solubility of hydrophobic, oily substances thus increasing the miscibility of beeswax, diclofenac and polysorbate 80. This uniformity stabilizes the matrix (granules) with reservoir of the drug in the central core and ensures sustained delivery of the drug through the gradual and sustained erosion of the matrix. Another factor responsible for sustained release in granules A and B might be the weakening of matrix integrity due to decrease in tortuosity of diffusion path of drug, polymer and surfactant combination by their swelling ability upon hydration.

CONCLUSION

Beeswax matrix formulated in polymer, poloxamer188 using surfactant dispersion, polysorbate 80 provides reservoir for delayed release and sustained oral delivery of diclofenac potassium.

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