Development of diclofenac sodium-loaded alginate-PVP K 30 microbeads using central composite design

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ABSTRACT

Background and the purpose of the study: Diclofenac sodium is a non-steroidal anti-inflammatory agent with a short biological half-life (1-2 hr) and requires multiple dosing. This research was carried out to develop and optimize diclofenac sodium loaded alginate-PVP K 30 microbeads to eliminate the need for multiple dosing and adverse effects.

Methods: Diclofenac sodium loaded alginate-PVP K 30 microbeads were prepared by ionotropic gelation. Particle size, drug release, swelling, FTIR and SEM analyses were performed.

Results: Optimized microbeads showed particle size of 0.589 ± 0.054 to 0.620 ± 0.067 mm, and drug entrapment efficiency of 97.88 ± 2.86 to 98.60 ± 3.55 %. The in vitro drug release from microbeads was sustained over 10 hrs and followed controlled-release pattern. FTIR analysis indicated the possibility of intermolecular hydrogen bonding interactions, i.e., –OH…O=C in microbeads.

Conclusion: Microbeads for oral controlled delivery of diclofenac sodium were successfully developed by ionotropic gelation.

Keywords: Controlled release, Optimization, Ionotropic gelation, Polymer blend, FTIR.

INTRODUCTION

Alginate, the monovalent form of alginic acid, belong to the family of linear copolymers composed of β-D-mannuronic acid monomers (M), regions of ∞-L-guluronic acid residues (G), and regions of interspersed M and G units (1). It is used as matrix material in various formulations due to its hydrogel-forming properties (2). Alginates undergo gelation due to ionic interaction between carboxylic acid groups located on polymer backbone and these cations like Ca\(^{2+}\), Al\(^{3+}\), etc (3-4). Various drugs have been successfully incorporated in alginate hydrogels and have exhibited different drug release profiles (1, 4-6).

Diclofenac sodium (DS) is a non-steroidal anti-inflammatory agent widely used as analgesic (7). Its biological half-life is 1-2 hr (2) and requires multiple dosing to maintain therapeutic concentration in blood. Hence, the controlled release systems of DS can eliminate the need for multiple dosing and adverse effects. Several investigations on formulation of DS-loaded alginate-based microparticles/beads using different polymer-blend have been reported (7-10). However, no attempt has been taken to formulate DS-loaded bead system using alginate-polyvinyl pyrrolidone (PVP) blend. Therefore in the present investigation, an attempt was made to develop and optimize DS-loaded alginate-PVP K 30 microbeads. The effects of ratios of sodium alginate to PVP K 30 and CaCl\(_2\) (cross-linker) concentrations on drug entrapment and release were analyzed using central composite design (CCD).

MATERIAL AND METHODS

Materials

DS (Techno Remedies, India), calcium chloride, sodium alginate and PVP K 30 (Loba Chemie, India) were used in this study. Other chemicals used were of analytical grades.

Microbead preparation

DS-loaded alginate-PVP K 30 microbeads were prepared by ionotropic gelation (2). Sodium alginate and PVP K 30 aqueous solutions were mixed together. Then, DS was added to the mixture and homogenized (for 10 min, 1000 rpm). The resulting mixture (drug : polymer = 1:2) was dropped into CaCl\(_2\) solution via 26-gauge needle. After 15 min, beads were collected by decantation, washed repeatedly with deionized water and dried at 45°C.
Microbeads (100 mg) were soaked in phosphate buffer of pH 7.4 and 0.1 N HCl of pH 1.2. Swelled microbeads were removed and weighed. Swelling indexes were determined using the formula:

\[
\text{Swelling index} = \frac{\text{Weight of swelled microbeads}}{\text{Dry weight}} \times 100
\]  

**RESULTS AND DISCUSSION**

DS-loaded alginate-PVP K 30 microbeads were prepared by ionotropic gelation according to CCD (Table 2). Quadratic models were selected as better-fit models due to smaller PRESS, and insignificant lack of fit (Table 3). Results of ANOVA indicated all models were significant (Table 4). After eliminating non-significant \((p > 0.05)\) terms, the models became:

\[
\text{DEE} = 58.67 - 0.66 X_1 + 8.12 X_2 - 1.28 X_1^2 - 0.41 X_2^2
\]  

\[
R_{10h} = 99.43 - 1.13 X_1 - 3.54 X_2 - 0.40 X_1 X_2 - 0.06 X_1^2 X_2
\]  

Three-dimensional response surface (Fig. 1), and contour plots (Fig. 2) demonstrate changes in DEE, and \(R_{10h}\) due to variation of factors. For optimization, numerical analysis was performed by restricting desirable ranges to 95 ≤ DEE ≤ 100 %, and 60 ≤ \(R_{10h}\) ≤ 65 %. The optimized microbeads were formulated and evaluated (Table 5). Models to produce optimized responses were well fitted \((R^2 = 0.9912\) for DEE, and 0.9967 for \(R_{10h}\)). Optimized microbeads showed DEE of 97.88 ± 2.86 to 98.60 ± 3.55 %. Among DS-loaded alginate microbeads, highest DEE of 85.77 ± 2.92 % was observed in the case of S-3 (Table 6). The particle sizes of alginate-PVP K 30 microbeads decreased by increase in the amount of incorporated PVP K 30 (due to decrease in viscosity and droplet sizes of polymer solution), and the CaCl\(_2\) concentration (due to high degree cross-linking) (Table 7). The surface morphology of optimized beads was visualized by SEM, which showed rough surface (Fig. 3).

In FTIR spectra, incorporation of DS in alginate showed characteristic peaks of DS, and alginate (Fig. 4). However, in DS-loaded alginate-PVP K 30 microbeads, two characteristic shifts for C=O stretching of PVP, and –OH groups of alginate compared to pure component (Fig. 4) appeared which strongly supports the idea of an intermolecular hydrogen-bonding between C=O groups of PVP, and –OH groups of alginate in alginate-PVP K 30 microbeads.

Microbeads were found to release negligible amounts of DS in acidic medium which probably could be the surface adhered drug. Alginate-PVP K 30 microbeads showed prolonged drug release over 10 hrs (Figs. 5 and 6). In contrast,
Table 1. Factors and levels of the circumscribed central composite design.

| Experimental Settings | Normalized levels of factors | Responses | SP-1 | SP-2 | SP-3 | SP-4 | SP-5 | SP-6 | SP-7 | SP-8 | SP-9 | SP-10 | SP-11 | SP-12 | SP-13 |
|-----------------------|-----------------------------|-----------|------|------|------|------|------|------|------|------|------|------|-------|-------|-------|
| SA:PVP K 30<sup>a</sup> (X<sub>1</sub>) | 1.00 | 1.50 | 2.80 | 4.00 | 1.414 | 0.00 | 2.80 | 4.00 | 1.414 | 0.00 | 1.00 | 1.50 | 2.80 | 4.00 |
| CaCl<sub>2</sub> (% w/v) (X<sub>2</sub>) | 1.00 | 2.50 | 6.30 | 10.00 | 11.60 | 1.00 | 6.30 | 10.00 | 11.60 | 1.00 | 2.50 | 6.30 | 10.00 | 11.60 |

<sup>a</sup>Observed response values: Mean ± SD (n = 3); <sup>b</sup>SA:PVP K30 = Sodium alginate to polyvinyl pyrrolidone K 30 ratio; <sup>c</sup>DEE = Drug entrapment efficiency (%); <sup>d</sup>R<sub>10hr</sub> (%) = % Drug released in 10 hrs

Table 2. Experimental plan and observed response values from randomized run in central composite design.

The optimized formulation of alginate-PVP K 30 microbeads showed high percent of DS release (Fig. 6). The drug release from optimized microbeads was evaluated using various kinetic models, and it was found that the drug release followed zero-order model (Table 8), indicating controlled-release pattern. The Korsmeyer-Peppas model was employed to distinguish Fickian-release when n ≤ 0.43 and case-II transport when n ≥ 0.85 (in Korsmeyer-Peppas Model, n is diffusional exponent) (12, 13). The values of n ranged between 0.9632 to 0.9950 (Table 8), indicating the drug release followed case-II transport.

The swelling of optimized DS-loaded alginate-PVP K 30 microbeads was lower in acidic medium (pH 1.2) in comparison with that of alkaline medium (pH 7.4) (Fig. 7). Maximum swelling was noticed after 2-3 hrs in alkaline pH after which, erosion and dissolution took place. The swelling of optimized microbeads in alkaline pH could be explained by the exchange of ions between the calcium ion of microbeads and the sodium ions present in phosphate buffer, under influence of calcium-sequestrant phosphate ions, which could result in disaggregation of alginate-PVP K 30-matrix structure leading to matrix erosion and dissolution of swollen microbeads (14, 15).

**CONCLUSION**

The optimized formulation of alginate-PVP K 30
Table 3. Summary of the model analysis (A), lack of fit (B), and $R^2$ analysis (C) for the measured responses.

| Source | DEE (%) | $R_{10hr}$ (%) |
|--------|---------|----------------|
|        | Sum of squares | $p$-value | Sum of squares | $p$-value |
| (a) Model analysis | | | | |
| Mean vs Total | 92855.36 | | 93597.19 | |
| Linear vs Mean | 1771.28 | $< 0.0001$ | 1296.88 | $< 0.0001$ |
| 2FI vs Linear | 4.18 | 0.7105 | 13.99 | 0.0028 |
| Quadratic vs 2FI | 241.45 | $< 0.0001$ | 4.57 | 0.0395 |
| Cubic vs Quadratic | 4.82 | 0.3780 | 2.01 | 0.0635 |
| Residual | 10.14 | | 1.00 | |
| Total | 94887.24 | | 94915.63 | |

(b) Lack of fit |

| Source | DEE (%) | $R_{10hr}$ (%) |
|--------|---------|----------------|
|        | Sum of squares | $p$-value | Sum of squares | $p$-value |
| Linear | 257.83 | 0.0007 | 21.04 | 0.0034 |
| 2FI | 253.65 | 0.0005 | 7.05 | 0.0195 |
| Quadratic | 12.20 | 0.0600 | 2.49 | 0.0533 |
| Cubic | 7.37 | 0.0310 | 0.48 | 0.1293 |
| Pure error | 2.77 | | 0.52 | |

(c) $R^2$ analysis

| Source | DEE (%) | $R_{10hr}$ (%) |
|--------|---------|----------------|
|        | Adjusted | Predicted | Adjusted | Predicted |
|        | $R^2$ | $R^2$ | $R^2$ | PRESS$^d$ | $R^2$ | $R^2$ | $R^2$ | PRESS$^d$ |
| Linear | 0.8717 | 0.8461 | 0.7762 | 454.69 | 0.9836 | 0.9804 | 0.9651 | 45.98 |
| 2FI | 0.8738 | 0.8317 | 0.6734 | 663.68 | 0.9943 | 0.9923 | 0.9860 | 18.52 |
| Quadratic | 0.9926 | 0.9874 | 0.9552 | 91.05 | 0.9977 | 0.9961 | 0.9860 | 18.49 |
| Cubic | 0.9950 | 0.9880 | 0.7656 | 476.18 | 0.9992 | 0.9982 | 0.9763 | 31.24 |

$^d$DEE = Drug entrapment efficiency (%); $^b$R$_{10hr}$ = % Drug released in 10 hrs; $^c$2FI = Two factor interaction; $^d$PRESS = predicted residual sum of squares.

Table 4. Summary of ANOVA for the response parameters.

| Source | DEE (%) | $R_{10hr}$ (%) |
|--------|---------|----------------|
|        | Sum of squares | Degree of freedom | Mean square | $F$ value | $p$-value | Prob > $F$ |
| (A) For DEE (%) | | | | | |
| Model | 2016.91 | 5 | 403.38 | 188.70 | $< 0.0001$ | |
| $X_1$ | 314.12 | 1 | 314.12 | 146.94 | $< 0.0001$ | |
| $X_2$ | 1457.15 | 1 | 1457.15 | 681.64 | $< 0.0001$ | |
| $X_1X_2$ | 4.18 | 1 | 4.18 | 1.96 | 0.2046 | |
| $X_1^2$ | 27.77 | 1 | 27.77 | 12.99 | 0.0047 | |
| $X_2^2$ | 230.44 | 1 | 230.44 | 107.80 | $< 0.0001$ | |

(B) For R$_{10hr}$ (%) | | | | | |
| Model | 1315.43 | 5 | 263.09 | 612.17 | $< 0.0001$ | |
| $X_1$ | 181.04 | 1 | 181.04 | 421.25 | $< 0.0001$ | |
| $X_2$ | 1115.84 | 1 | 1115.84 | 2596.44 | $< 0.0001$ | |
| $X_1X_2$ | 13.99 | 1 | 13.99 | 32.55 | 0.0007 | |
| $X_1^2$ | 0.02 | 1 | 0.02 | 0.05 | 0.8376 | |
| $X_2^2$ | 4.3 | 1 | 4.39 | 10.22 | 0.0151 | |

DEE = Drug entrapment efficiency (%); R$_{10hr}$ = % Drug released in 10 hrs. $X_1$ and $X_2$ represent the factors; $X_1^2$ and $X_2^2$ are the quadratic effect; $X_1X_2$ is the interaction effect.
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Table 5. Results of experiments for confirmation of the optimization capability.

| Trial Code | Factors | Responses |
|------------|---------|-----------|
|            | SA:PVP K 30 | CaCl₂ (% w/v) | DEE (%) | R₁₀hr (%) |
|            | Predicted | Observed | Predicted | Observed |
| O-1        | 1.00     | 8.60    | 99.45 | 98.27 ± 3.42 | 69.55 | 71.28 ± 2.22 |
| O-2        | 1.50     | 9.20    | 99.79 | 98.60 ± 3.55 | 69.41 | 71.02 ± 2.67 |
| O-3        | 2.20     | 10.30   | 99.16 | 97.88 ± 2.86 | 68.46 | 69.88 ± 2.82 |

aSA:PVP K 30 = Sodium alginate to polyvinyl pyrrolidone K 30 ratio; bDEE = Drug entrapment efficiency (%); cR₁₀hr = % Drug released within 10 hrs; dObserved response values: Mean ± SD (n = 3).

Table 6. Processing parameters and responses of plain alginate microbeads containing diclofenac sodium.

| Trial Code | Processing parameters | Responses |
|------------|-----------------------|-----------|
|            | SA b | CaCl₂ (% w/v) | DEE (%) | R₁₀hr (%) |
| S-1        | 2.00 | 8.60    | 78.23 ± 2.06 | 95.38 ± 3.62 |
| S-2        | 2.00 | 9.20    | 81.48 ± 3.15 | 91.22 ± 3.23 |
| S-3        | 2.00 | 10.30   | 85.77 ± 2.92 | 81.03 ± 3.05 |

bObserved response values: Mean ± SD (n = 3); cSA = Sodium alginate; bDEE = Drug entrapment efficiency (%); cR₁₀hr = % Drug released in 10 hrs

Table 7. Average diameter of alginate-PVP K 30 and plain alginate microbeads containing diclofenac sodium, measured by optical microscopic method.

| Formulation codes | Average diameter (mm) |
|-------------------|-----------------------|
| SP-1              | 0.93 ± 0.08           |
| SP-2              | 0.59 ± 0.07           |
| SP-3              | 0.95 ± 0.10           |
| SP-4              | 0.84 ± 0.07           |
| SP-5              | 0.79 ± 0.05           |
| SP-6              | 0.87 ± 0.09           |
| SP-7              | 0.97 ± 0.07           |
| SP-8              | 0.60 ± 0.06           |
| SP-9              | 0.86 ± 0.08           |
| SP-10             | 0.86 ± 0.10           |
| SP-11             | 0.86 ± 0.08           |
| SP-12             | 0.84 ± 0.06           |
| SP-13             | 0.85 ± 0.09           |
| O-1               | 0.59 ± 0.05           |
| O-2               | 0.62 ± 0.08           |
| O-3               | 0.62 ± 0.07           |
| S-1               | 0.87 ± 0.07           |
| S-2               | 0.83 ± 0.08           |
| S-3               | 0.82 ± 0.08           |

SP-1 to O-3 were alginate-PVP K 30 microbeads; whereas S-1-S-3 were plain alginate microbeads containing diclofenac sodium. Mean ± SD (n = 3).

microbeads containing DS was developed based on central composite design. The DEE of optimized alginate-PVP K 30 microbeads containing DS were found to be 97.88 ± 2.86 to 98.60 ± 3.55 % with a controlled-release pattern (zero-order) and case-II transport drug release. The swelling behaviour of the developed microbeads was influenced by the pH of the test medium. The FTIR spectroscopy showed an intermolecular hydrogen-bonding which could be formed between C=O groups of PVP K 30 and –OH groups of alginate in alginate-PVP K 30 microbeads which might sustain drug release from alginate-PVP K 30 microbeads and minimize drug leaching during preparation to facilitate increase in DEE. The methods of preparation of DS-loaded alginate-PVP K 30 for controlled release characteristics were found to be simple and reproducible. In conclusion, an oral alginate-PVP K 30 microbeads for controlled delivery system of DS was successfully developed by alginate-PVP K 30 blending using ionotropic gelation.

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Table 8. Results of curve fitting of the in-vitro diclofenac sodium release data from different optimized alginate-PVP K30 microbeads.

| Formulation codes  | Zero-order Model | First-order Model | Higuchi Model | Korsmeyer-Peppas Model |
|--------------------|------------------|------------------|--------------|------------------------|
|                    | $K_o$            | $K_{1st}$        | $K_i$        | $K_p$                  |
| O-1                | 0.0711           | 0.1426           | 0.3486       | 0.0779                 |
| O-2                | 0.0705           | 0.1394           | 0.3456       | 0.0724                 |
| O-3                | 0.0730           | 0.1427           | 0.3579       | 0.0728                 |
| $R^2$              | 0.9962           | 0.9795           | 0.9870       | 0.9632                 |
|                    |                  | 0.9736           | 0.9825       | 0.9884                 |
|                    |                  |                  | 0.9845       | 0.9950                 |

Figure 1. Effects of experimental factors presented by response surface plots (a, b).
Figure 2. Effect of main effects on responses presented by contour plots (a, and b).
Figure 3. Photomicrograph of the surface morphology of DS-loaded alginate-PVP K 30 microbead (O-2).

Figure 4. FTIR spectra of DS, sodium alginate, PVP K 30, DS-loaded alginate (S-3) and DS-loaded alginate-PVP K 30 microbeads (O-2).
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Figure 5. (a). In vitro drug release from alginate-PVP K 30 microbeads containing DS (SP-1 to SP-6) (Mean ± SD, n = 3). (b). In vitro drug release from alginate-PVP K30 microbeads containing DS (SP-7 to SP-13) (Mean ± SD, n = 3).

Figure 6. In vitro drug release from optimized alginate-PVP K 30 microbeads (O-1 to O-3) and alginate microbeads (S-1 to S-3) containing DS (Mean ± SD, n = 3).
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