RESEARCH PAPER

Accepted 20 September 2000
Revised 4 September 2000
Received 6 March 2000
Indian J. Pharm. Sci. 2001, 63(1) 15-19

Preparation and Evaluation of Sustained Release Nimesulide Microspheres Prepared from Sodium Alginate

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Nimesulide-loaded calcium alginate beads were prepared. In this investigation, a 2^3 full factorial design was used to study the joint influence of three variables, the polymer concentration (X_1), the drug concentration (X_2) and the cross-linker (calcium chloride) concentration (X_3), on various dependent variables like per cent entrapment, sphericity, particle size and amount of drug released during the initial (t=60 min). and final stages (t=360 min) of release. A statistical model with a significant interaction term is obtained to predict the results. The drug release mechanisms from microspheres were studied using the Peppas equation.

Nimesulide or N-(4-Nitro-2-phenoxyphenyl) methanesulphonamide, is a non-steroidal antiinflammatory drug administered orally or rectally for a variety of inflammatory and pain states associated with osteoarthritis, cancer, thrombophlebitis, oral surgery and dismenorrhoea in adults, reducing pain associated with general surgery, pain, fever and inflammation accompanying respiratory tract infections, otorhinolaryngological diseases and traumatic injury. Its biological half-life has been reported to be 3-4 h[1]. Although well tolerated by adults, elderly and pediatric patients, it exhibits the usual adverse effects associated with other NSAIDs such as gastrointestinal side effects (epigastralgia, heart burn, nausea, loose motion), dermatological effects (rash, pruritis) and central effects (somnolence, dizziness)[2]. Because of the above mentioned drawbacks, it was considered a good candidate for controlled drug delivery.

The purpose of the present study was to prepare nimesulide microspheres using the optimization methodology. A 2^3 factorial was employed to study the various effective factors such as polymer concentration, drug concentration and cross-linker ($CaCl_2$) concentration. The release mechanisms of nimesulide from the microspheres were also elucidated.

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MATERIALS AND METHODS

Nimesulide was received as a gift sample from Panacea Biotec Ltd., New Delhi, India. Calcium chloride dihydrate obtained from Sarabhai M. Chemicals, Baroda and sodium alginate procurred from Loba Chemie Pvt. Ltd., Mumbai, were used as received. The viscosity average molecular weight (M_v) of the alginate was found to be 60,000 calculated from viscosities of diluted solutions (<1% w/v) using a Ubbelhode viscometer employing the Mark Hauowink equation:

$$[\eta] = K (M_v)^a \qquad (1)$$

where $[\eta]$ is the intrinsic viscosity, $K = 2x10^{-5}$ and $a = 1$ (0.1 M NaCl solution)[3,4].

Preparation of Microspheres:

Calcium-induced ionotropic gelation of alginate was used for the preparation of nimesulide microspheres[5]. Small uniform sized droplets of nimesulide dispersed in alginate solution were produced using a purpose designed

coaxial needle assembly[6]. These droplets were dropped in a well-stirred solution of calcium chloride. The produced beads were kept in the same solution overnight for hardening. The microspheres were then filtered and washed with distilled water to remove excess calcium chloride and then air-dried, till constant weight.

Determination of Per cent Yield and Drug Content:

Per cent yield was calculated based on the total solid content of drug and polymer. For determination of drug content, 10 mg of microspheres were accurately weighed and dissolved in 100 ml of 0.1 M NaOH containing 1% w/v sodium citrate and kept on rotary shaker at 200 rpm overnight for complete extraction. It was then filtered and analyzed spectrophotometrically at 393 nm after suitable dilution.

Determination of Particle Size and Sphericity:

The particle size was determined microscopically. Particle shape was measured by computing shape/circularity factor[7]. The tracings obtained from photomicrograph were used to calculate area (A) and Perimeter (P). The circularity factor(s) was calculated as :

$$(S) = P^2/(12.56 \times A) \qquad (2)$$

***In vitro* Release Studies:**

Dose calculation for *in vitro* release studies was done using Dobrinska and Welling equation (Table 1)[8,9]. Microspheres equivalent to 100 mg of nimesulide was taken for *in vitro* dissolution studies. The study was carried out in an USP XXII basket apparatus at a rotational speed of 50 rpm at 37° in 1000 ml phosphate buffer (pH 8.0). Samples (5 ml) were withdrawn at regular time intervals. Precaution was taken to avoid particles in the aliquot by using a pipette tied with muslin cloth. The amount of drug released was determined spectrophotometrically at 393 nm after appropriate dilution with dissolution medium. Release studies were carried out in duplicate.

Factorial Design:

The levels of factors were independently varied, each at two levels (Table 2). The responses Y_i were measured for each trial. A polynomial equation was constructed where the coefficients in the equation were related to the effects and interactions of the factor. Interactive statistical first order complete model (equation 3) were first generated to evaluate the selected response.

$$Y=b_o+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+b_{123}X_1X_2X_3 \quad (3)$$

Where b_o is the arithmetic mean response of 8 runs and b_i is the coefficient of factor X_i. The main effects (X_1, X_2 and X_3) represent the average result of changing one factor at a time from its low to high value. The interaction (X_1X_2, X_1X_3, X_2X_3 and $X_1X_2X_3$) show how the dependent variable changes when two or more factors are simultaneously changed. UNISTAT® version 3 for Windows™ (copyright © 1994 MEGALON SA and UNISTAT Ltd.) was used to obtain the equation. Multiple regression analysis

TABLE 1: PHARMACOKINETIC DATA AND CALCULATED PARAMETERS OF NIMESULIDE FOR *IN VITRO* RELEASE STUDIES

Pharmacokinetic Data for Nimesulide	Reported Value	Value Taken For Calculation
Elimination Half Life ($t_{1/2}$)	3.74±0.5 h	3.46 h
Elimination Rate constant (K_{el})	0.21±0.03 h^{-1}	0.2 h^{-1}
Peak Plasma Level (C_{max})	2.62±0.27 mg/L	2.5 mg/L
Apparent Volume of Distribution (V_d)	0.19-0.39 L/Kg	0.3 L/Kg
Calculated Parameters	**Calculated Values**	**Formula[8,9]**
Amount of drug in body at steady state (A_{ss})	52.5 mg	$A_{ss}=C_{ss}\ V_d$
First order elimination rate constant (K_{el})	0.2 h^{-1}	$K_{el}=0.69/t_{1/2(el)}$
Instantaneous dose D_{fi}	52.5 mg	$D_{fi}=A_{ss}=K_0/K_{el}$
Zero order release rate constant K_0	10.5 mg/h	$K_0=K_{el}.\ A_{ss}$
Maintenance dose D_{fs}	105 mg	$D_{fs}=K_0T$

and f statistics were used to identify statistically significant terms[10]. Only those terms, which were statistically significant were retained in the equation.

RESULTS AND DISCUSSION

Calcium-induced ionotropic gelation of sodium alginate was successfully used as a method for preparation of nimesulide microspheres. The data obtained for various batches prepared using a 2^3 factorial design is depicted in Table 2. The results obtained for various parameters after stepwise multiple regression analysis are shown in Table 3. The interpretations are based on the value and sign of coefficient for the different parameters individually or in combination.

of microspheres decreases due to shrinking. Thus, with the increase in drug concentration (X_2), particle size increases because of the higher solid content. Also, a higher drug release was observed from microspheres with larger particle size. This was not it accordance with the general theory of microspheres. Since, in this case the increase in particle size is essentially due to the higher drug content, it leads to higher release. The negative value of coefficient b_{13} indicates a decrease in particle size when the two factors are simultaneously increased. This may be attributed to the greater extent of cross-linking leading to significant shrinkage to occur.

The release profile of nimesulide from various batches is illustrated in fig. 1. The amount of drug release during

TABLE 2: DATA ALONGWITH CODED VALUES FOR 2^3 FACTORIAL DESIGN

Batch	Variable level in coded form*			Per cent yield	Per cent Efficiency	Sphericity	Size (μm)	Amount released at time t	
	X_1	X_2	X_3					$t_{60\ min}$	$t_{360\ min}$
1	-1	-1	-1	95.54	88.60	1.140	503.5	47.43	89.36
2	-1	-1	+1	112.0	90.28	1.267	527.1	21.76	94.34
3	-1	+1	-1	99.38	96.41	1.422	625.9	72.64	94.45
4	-1	+1	+1	97.97	93.15	1.289	674.1	60.69	84.06
5	+1	-1	-1	108.2	95.26	1.133	547.6	9.023	65.62
6	+1	-1	+1	96.38	91.06	1.078	511.2	20.13	96.41
7	+1	+1	-1	90.65	84.56	1.167	666.5	60.41	79.09
8	+1	+1	+1	95.12	89.40	1.092	653.5	20.29	88.92

*Coded values	Actual values		
	X_1	X_2	X_3
-1	1.2% w/v	0.5% w/v	1.0% w/v
+1	2.0% w/v	2.0% w/v	3.0% w/v

Where X_1 is the polymer concentration, X_2 is the drug concentration and X_3 is the calcium chloride concentration.

Table 2 indicates that both the yield (> 95%) and the entrapment efficiency (>85%) were satisfactory. The following equation was obtained for the particle size.

$$Y = 588.6 + 66.2\ X_2 - 15.2\ X_1X_3 \quad (4)$$

The arithmetic mean particle size was 588.6 μm. The droplet generator produced droplets of uniform size, which undergo cross-linking as soon as they enter the calcium chloride solution. During the cross-linking and subsequent drying significant synerisis occurs and the particle size

initial and final stages of release profile is affected by all the three variables (Table 2). The amount released at time t=60 min is given by the equation 5.

$$Y = 39.2-11.7X_1+14.6X_2-8.5X_3-1.7X_1X_2+1.2X_1X_3-4.8X_2X_3-7.9X_1X_2X_3 \quad (5)$$

The value of b_0=39.2 is indicative of a burst effect. The negative value of coefficients of X_1 and X_3 indicate that these variables retard the release considerably in the initial stages. This is in accordance with the earlier

TABLE 3: STEP-WISE MULTIPLE REGRESSION ANALYSIS OF MEASURED RESPONSES

Coefficient	% efficiency	Particle size (μm)	Sphericity	Amount released at time t	
				$t_{60\,min}$	$t_{360\,min}$
b_0	-	588.56	1.21	39.16	86.53
b_1	-	-	-0.09	-11.71	-4.021
b_2	-	66.22	0.05	14.58	-
b_3	-	-	-	-8.46	-4.40
b_{12}	-	-	-0.04	-1.70	-
b_{13}	-	-15.24	-0.03	1.19	5.75
b_{23}	-	-	-	-4.82	-4.54
b_{123}	-	-	-	-7.99	-
r_2	-	0.98	0.97	0.99	0.87
Significance	-	0.00	0.02	0.00	0.00

Equation $Y = b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+b_{123}X_1X_2X_3$

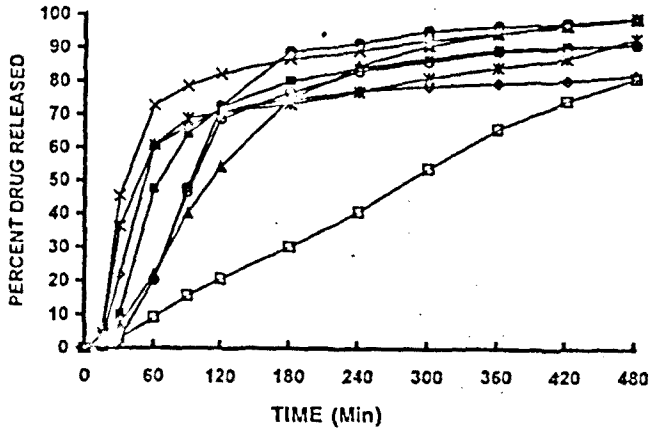

Fig. 1 : *In vitro* drug release profile of nimesulide from different batches of microspheres. (-□-) batch 1, (-▲-) batch 2, (-✳-) batch 3, (-✳-) batch 4, (⊟) batch 5, (-O-) batch 6, (◈) batch 7, (O) batch 8.

studies, where increase in polymer concentration as well as cross-linking agent concentration causes a decrease in drug release[5,11,12]. An increase in drug concentration causes an increase in release as indicated by the positive value of coefficient b_2. This is because as the drug concentration increases, the drug to polymer ratio increases leading to a greater release[11].

The amount released during later stages (at t=360 min) is given by equation 6.

$$Y = 86.5-4.0X_1+4.4X_3+5.7X_1X_3-4.5X_2X_3 \quad (6)$$

The average drug release after 6 h time interval is 86.5% The negative value of coefficient b_1 indicates that the polymer does retard the drug release even at later stages. The release in the later stages depends essentially on the amount released in the initial stages. This is reflected from the sign of coefficient b_3 during initial and final stages. As described earlier, the cross-linking agent concentration (X_3) retards the drug release during initial stages, leaving a considerable amount of drug to be released in the later stages. After subsequent interaction with the dissolution medium, the cross-links break significantly leading to higher drug release in the later stages. The negative value of coefficient b_{23} may be because of predominant effect of variable X_2 in the interaction term. Since, in the initial stages significant amount of drug is released, the drug content in the later stages is low leading to a lower release. The diminished effect of X_3 is due to significant breakdown of cross-links occurring in later stages.

In order to ascertain the mechanism of drug release, the release data was fitted into the general equation (equation 7)[13].

$$M_t/M_\infty = K\,t^n \quad (7)$$

Where M_t is amount of drug released at time t and M_∞ is amount of drug released at infinite time, K is the constant incorporating characteristics of the polymer

TABLE 4: MODEL FITTING FOR *IN VITRO* RELEASE STUDIES

Runs	1	2	3	4	5	6	7	8
n	0.927	1.287	0.6343	0.6873	1.2015	1.273	0.8485	1.539
K	0.4945	15.7	2.798	1.842	0.056	0.072	0.7252	0.0156
r	0.8898	0.9268	0.7929	0.8076	0.9935	0.932	0.8092	0.9072

Model fitting was done using equation $M_t/M_\infty=Kt^n$. Where 'M'_t and 'M'_∞ is the amount of drug released at time 't' and at '∞'. 'n' is the diffusional exponent indicative of the release mechanism. 'K' is the constant incorporating characteristics of the polymer network system and the drug and 'r' is the coefficient of correlation.

network system and the drug and n is the diffusional exponent indicative of the transport mechanism. The value of the exponent n varies from 0.6 to 1.5 indicating a non-Fickian to super case II transport mechanism (Table 4)[14]. From the graph of percent released versus time, it can be seen that almost all the batches show an initial lag followed by a sudden release of drug (Fig. 1) This behavior can be explained taking into account the chemistry of the alginate gel matrix. The calcium ions are bound to carboxylate residues of both mannuronic acid and guluronic acid, but only those calcium ions that are attached to guluronic acid take part in cross-linking mechanism (egg box model)[15,16]. During the initial stages of dissolution, those calcium ions that are not taking part in the egg box formation (Ca^{++} interacting with polymannuronate sequences) are preferentially released by diffusion through ion exchange with sodium ions present in the medium. Almost negligible alginate disintegration occurs at this stage due to relatively stable association of calcium ions with the polyguluronate sequences. Further alginate disintegration occurs when the calcium ions in the egg box structure are released along with the electrostatic repulsion between carboxylate anion, which enhanced swelling of alginate gel and eventually facilitates their erosion[17]. At this stage a burst is seen. This burst is less when drug to polymer ratio is low (batch 5). Thus release from alginate gel matrix can be considered as a combination of swelling, erosion, disintegration/disentanglement of polymer along with dissolution and diffusion of the drug.

ACKNOWLEDGEMENTS

The authors thank Panacea Biotec. Ltd. (New Delhi, India) for providing a gift sample of nimesulide. VP wishes to thank the AICTE, New Delhi for providing a Junior Research Fellowship.

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