ORIGINAL RESEARCH

DEVELOPMENT OF MUCOADHESIVE MICROSPHERES OF METHYL PREDNISOLONE BY USING HYDROPHILIC POLYMERS AND ITS EVALUATION

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ABSTRACT: In the present work, bioadhesive microspheres of Methylprednisolone were formulated using Sodium alginate along with Methocel, Carbopol 971, and pectin as copolymers. Methylprednisolone is a synthetic glucocorticoid or corticosteroid drug. The results of this investigation indicate that ionic cross linking technique Ionotropic gelation method can be successfully employed to fabricate Methylprednisolone microspheres. Micromeritic studies revealed that the mean particle size of the prepared microspheres was in the size range of 512-903m and are suitable for bioadhesive microspheres for oral administration. The in-vitro mucoadhesive study demonstrated that microspheres of Methylprednisolone using sodium alginate along with Carbopol934 as copolymer adhered to the mucus to a greater extent than the microspheres of Methylprednisolone using sodium alginate along with Carbopol 971 and HPMC K4M as copolymers. Analysis of drug release mechanism showed that the drug release from the formulations followed non-Fickian diffusion and the best fit model was found to be Korsmeyer-Peppas. Based on the results of evaluation tests formulation coded T4 was concluded as best formulation.

KEY WORDS: Methylprednisolone, Corticosteroid, Microspheres, Ionic cross linking technique, Non-Fickian diffusion.

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INTRODUCTION:

Mucoadhesive drug delivery system utilizes the property of bio adhesion in which certain polymers become adhesive on hydration and are used for targeting a drug to a particular region of the body for extended periods of time[1,2].

These hydrated polymer molecules have the ability to bind to various biological membranes by interacting with the mucin component and thus can remain attached to the membranes allowing the drug to release in a slow fashion into the underlying tissues, thus enhancing the activity of the drug molecules[3]. Polymers have played an important role in designing such systems so as to increase the residence time of the active agent at the desired location. Mucoadhesive polymers are water-soluble and water insoluble polymers, which are swellable networks, joined by cross-linking agents. These polymers possess optimal polarity to make sure that they permit sufficient wetting by the mucus and optimal fluidity that permits the mutual adsorption and interpenetration of polymer and mucus to take place[4].

The advantages associated with these mucoadhesive polymers makes the mucoadhesive drug delivery system a promising delivery means for various drugs by using various novel approaches[5].

Methylprednisolone is a synthetic glucocorticoid or corticosteroid drug which is widely used in the treatment of various inflammatory conditions. It is well absorbed after oral administration but it is subjected to first pass hepatic metabolism making it less available at the site of action[6-8]. Thus in the present study methyl prednisolone is formulated into mucoadhesive microsphere which prevents the drug from undergoing hepatic metabolism and helps in achieving the desired blood concentration and prolong their efficacy.

MATERIALS AND METHODS:

Methylprednisolone was obtained from Amoli Organics Pvt.Ltd India. Methocel was purchased from Spectrum reagents and chemicals Pvt. Ltd. Pectin was purchased from Sd fine chem limited, Mumbai, India. Carbopol 971p was purchased from Qualigens fine chemicals, Mumbai, India and sodium alginate was purchased from Loba chemie Pvt, Ltd, Mumbai, India.

Methods:

Various batches of microspheres were prepared by ionotropic gelation method which involved reaction between sodium alginate and polycationic ions like calcium to produce a hydrogel network of calcium alginate. Sodium alginate and the mucoadhesive polymer as mentioned in table-1 were dispersed in purified water (10 ml) to form a homogeneous polymer mixture. Methylprednisolone (100 mg) was added to the polymer premix and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added through a 22G needle into calcium chloride (4% w/v) solution. The addition was done with continuous stirring at 200rpm. The added droplets were retained in the calcium chloride solution for 30 minutes to complete the curing reaction and to produce rigid spherical microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with purified water to remove excess calcium impurity deposited on the surface of microspheres and then air-dried[9].

Table 1: Formulations of Methylprednisolone Mucoadhesive Microspheres

Formulation code	Drug: Polymer ratio	Polymer ratio
T_1	1:2.5	Na alginate:Methocel (1.5:0.5)
T_2	1:3	Na alginate:Methocel (2:1)
T_3	1:3.5	Na alginate:Methocel (2.5:1)
T_4	1:4	Na alginate:Methocel (3:1)
T_5	1:2.5	Na alginate:Carbopol 971 (1.5:0.5)
T_6	1:3	Na alginate:Carbopol 971 (2:1)
T_7	1:3.5	Na alginate:Carbopol 971 (2.5:1)
T_8	1:4	Na alginate:Carbopol 971 (3:1)
T_9	1:2.5	Na alginate:Pectin (1.5:0.5)
T_{10}	1:3	Na alginate:Pectin (2:1)
T_{11}	1:3.5	Na alginate:Pectin (2.5:1)
T_{12}	1:4	Na alginate:Pectin (3:1)

CHARACTERIZATION OF MICROSPHERES[10]

Percentage Yield:

The percentage yield was calculated from the weight of dried microspheres recovered from each batch and the sum of the initial weight of starting materials. The percentage yield was calculated using the formula

$$\% \text{ Yield} = \frac{\text{Practical mass(Microspheres)}}{\text{Theoretical mass(polymer + drug)}} \times 100$$

Drug entrapment efficiency:

Microspheres equivalent to 100 mg of Methylprednisolone were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres. The powder was transferred to a 100 ml volumetric flask and dissolved in 10ml of methanol and the volume was made up using 0.1N HCL. After 24 hours the solution was filtered through Whatmann filter paper and the absorbance was measured after suitable dilution spectrophotometrically at 250 nm. The amount of drug entrapped in the microspheres was calculated by the following formula,

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100$$

Particle size analysis:

Samples of the microspheres were analyzed for particle size by optical microscope. The instrument was calibrated and found that 1unit of eyepiece micrometer was equal to 12.5µm. Nearly about 100 microspheres sizes were calculated under 45 x magnifications. The average particle size was determined by using the Edmondon's equation:

$$D_{Mean} = \frac{nd}{n}$$

Where,

n – Number of microspheres observed and d – Mean size range

Swelling study:

Swelling ratio of dried microspheres were determined gravimetrically in 0.1N HCL .The microspheres were removed periodically from the solution, blotted to remove excess surface liquid and weighed on balance. Swelling ratio (% w/v) was determined from the following relationship:

$$\text{Swelling ratio} = \frac{W_t - W_o}{W_t} \times 100$$

Where, W_0 & W_t are initial weight and Final weight of microspheres respectively.

Scanning Electron Microscopy (SEM):

Scanning electron microscopy (Hitachi S-3600N, Japan) was done to characterize surface topography of the microspheres. Photomicrograph of the microspheres before and after the release of drugs was taken. The quality of the microspheres (with respect to surface properties) and the nature and size of pores developed on the surface can be studied. The changes that occur during in-vitro dissolution studies may have implications to the performance of the microspheres.

Evaluation of mucoadhesive property[11,12]:

The mucoadhesive property of microspheres was evaluated by an *in-vitro* adhesion testing method known as wash-off method. Freshly excised pieces of goat stomach mucous were mounted on to glass slides with cotton thread. About 20 microspheres were spread on to each prepared glass slide and immediately thereafter the slides were hung to USP II tablet disintegration test apparatus, when the test apparatus was operated, the sample is subjected to slow up and down movement in 0.1N HCL at 37ºC contained in a 1-litre vessel of the apparatus. At an interval of 1 hour upto 8 hours, the apparatus is stopped and number of microspheres still adhering to mucosal surface was counted.

$$\% \text{ Mucoadhesion} = \frac{\text{Number of microspheres adhered}}{\text{Number of microspheres applied}} \times 100$$

***In-vitro* drug release study:**

The dissolution studies were performed in a fully calibrated eight station dissolution test apparatus (37 ± 0.5^{0}C, 50 rpm) using the USP type – I rotating basket method in 0.1N HCL (900ml). A quantity of accurately weighed microspheres equivalent to 100mg Methylprednisolone each formulation was employed in all dissolution studies. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 250nm. At the same time the volume withdrawn at each time intervals were replenished immediately with the same volume of fresh pre-warmed 0.1N HCL maintaining sink conditions throughout the experiment.

***In-Vitro* Drug Release Kinetics**

The release data obtained was fitted into various mathematical models as mentioned in the table-2 to examine the release mechanism of Methylprednisolone from the microspheres.

Table 2: Release mechanism based on release exponent

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
$0.5<n^{*}<1.0$	Anomalous transport or non-Fickian	t^{n-1}
1.0	Case-II transport	Zero-order release
Higher than 1.0	Super Case-II transport	t^{n-1}

If n < 0.5, the polymer relaxation does not affect the molecular transport, hence diffusion is Fickian.
If n > 0.5, the solid transport will be non-fickian and will be relaxation controlled.

RESULTS AND DISCUSSION

Percentage Yield

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to blocking of needle and wastage of the drug- polymer solution, adhesion of polymer solution to the magnetic bead and microspheres lost during the washing process. The percentage yield was found to be in the range of 80 to 88% for microspheres containing sodium alginate along with Methocel as copolymer, 62.22 to 87% for microspheres containing sodium alginate along with carbopol 971 as copolymer and 80 to 87.5% for microspheres containing sodium alginate along with pectin as copolymer, these are represented in the table-3.

Drug Entrapment Efficiency

The results of drug entrapment efficiency are given in the table-3. Percentage Drug entrapment efficiency of Methylprednisolone ranged from 82.66 to 88.66% for microspheres containing sodium alginate along with Methocel as copolymer, 53.2 to 76.66% for microspheres containing sodium alginate along with carbopol 971 as copolymer and 66.73 to 79.2% for microspheres containing sodium alginate along with pectin as copolymer. The drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers. Increase in the polymer concentration increases the viscosity of the dispersed phase. The particle size increases exponentially with viscosity. The higher viscosity of the polymer solution at the highest polymer concentration would be expected to decrease the diffusion of the drug into the external phase which would result in higher entrapment efficiency.

Particle Size Analysis

The mean size increased with increasing polymer concentration which is due to a significant increase in the viscosity, thus leading to an increased droplet size and finally a higher microspheres size. Microspheres containing sodium alginate along with Methocel as copolymer had a size range of 512µm to 826µm, microspheres containing sodium alginate along with carbopol 971 as copolymer exhibited a size range between 517µm to 834µm and microspheres containing sodium alginate along with pectin as copolymer had a size range of 664µm to 903µm. The particle size as well as % drug entrapment efficiency of the microspheres increased with increase in the polymer concentratio n, the results are given in the table-3.

Swelling study

Swellability data as represented in the table-4 revealed that amount of polymer plays an important role in solvent transfer. It can be concluded from the data that with an increase in polymer concentration, the percentage of swelling also increases. Thus we can say that amount of polymer directly affects the swelling ratio. As the polymer to drug ratio increased, the percentage of swelling increased from 28 to 85% for microspheres containing sodium alginate along with methocel as copolymer, 24 to 64% for microspheres containing sodium alginate along with carbopol 971 as copolymer and 31 to 85% for microspheres containing sodium alginate along with pectin as copolymer.

Shape and surface morphology: SEM analysis was performed on the prepared sustained release microspheres of Methylprednisolone to accesses their surface morphological characteristics. SEM was performed for best formulations T4 and shown

in the figure-1&2. The polymer surface of the microspheres appeared spherical with smooth texture surface and found to be within optimum size range.

In-Vitro Mucoadhesion Test

As the polymer to drug ratio increased, microspheres containing sodium alginate along with Methocel as copolymer exhibited % mucoadhesion ranging from 65 to 85%, microspheres containing sodium alginate along with carbopol 971 as copolymer exhibited % mucoadhesion ranging from 60 to 75% and microspheres containing sodium alginate along with Pectin as copolymer exhibited % mucoadhesion ranging from 60 to 80%. The rank of order of mucoadhesion is methocel > pectin > carbopol 971. The results are presented in the table-5.

***In-Vitro* Drug Release Studies**

Dissolution studies of all the formulations were carr ied out using dissolution apparatus USP type I. The dissolution studies were conducted by using dissolution media, pH 1.2 and the results are given in the tables-6, 7 and 8.

The formulations T_1, T_2, T_3 and T_4 containing Sodium alginate along with Methocel as copolymer showed a maximum release of 92.66% after 9 hours, 90.66% after 10 hours, 90.6% after 11 hours and 97.66% after 12 hours respectively.

The formulations T_5, T_6, T_7 and T_8 containing Sodium alginate along with Carbopol 971 as copolymer showed a maximum release of 92.22% after 9 hours, 91.33% after 10 hours, 89.55% after 11 hours and 90.66% after 12 hours respectively.

The formulations T_9, T_{10}, T_{11} and T_{12} containing Sodium alginate along with PECTIN as copolymer showed a maximum release of 92.6% after 9 hours, 91.3% after 10 hours, 90% after 11 hours and 92.44% after 12 hours respectively.

This shows that more sustained release was observed with the increase in percentage of polymers. As the polymer to drug ratio was increased the extent of drug release decreased. A significant decrease in the rate and extent of drug release is attributed to the increase in density of polymer matrix that results in increased diffusion path length which the drug molecules have to traverse. The release of the drug has been controlled by swelling mechanism. Additionally, the larger particle size at higher polymer concentration also restricted the total surface area resulting in slower release.

***In-vitro* drug release kinetics**

For understanding the mechanism of drug release a nd release rate kinetics of the drug from dosage form, the in-vitro drug dissolution data obtained was fitted to various mathematical models such as zero order, first order, higuchi and Krosmeyer-Peppas model. The values are compiled in Table 9. The coefficient of determination (R^2) was used as an indicator of the best fitting for each of the models considered. The kinetic data analysis of all the formulations reached higher coefficient of determination with the Korsmeyer-Peppas model (R^2 = 0.914 to 0.996) whereas release exponent value (n) ranged from 0.498 to 0.743. From the coefficient of determination and release exponent values, it can be suggested that the mechanism of drug release follows Korsmeyer-Peppas model along with non-Fickian diffusion mechanism which leading to the conclusion that a release mechanism of drug followed combination of diffusion and spheres erosion.

Table 3: Percentage yield and percentage drug entrapment efficiency of the prepared microspheres

Formulation code	% yield	Drug Content (mg)	Drug entrapment efficiency (%)	Average Particle Size (µm)
T_1	80	12.40	82.66	512
T_2	83.33	12.66	84.4	617
T_3	85	12.70	84.66	711
T_4	88	13.29	88.66	826
T_5	62.22	8.07	53.2	517
T_6	80	8.25	55	642
T_7	80	10.33	68.86	792
T_8	87	11.5	76.66	834
T_9	80	10.01	66.73	664

T_{10}	86	10.5	70	774
T_{11}	86.66	11.25	75	814
T_{12}	87.5	11.88	79.2	903

Table 4: Percentage swelling of the prepared microspheres

Formulation Code	Initial (Wt)	Final (Wt)	% Swelling
T_1	10	12.8	28
T_2	10	14.2	42
T_3	10	16.2	62
T_4	10	18.5	85
T_5	10	12.4	24
T_6	10	13.9	39
T_7	10	15.5	55
T_8	10	16.4	64
T_9	10	13.1	31
T_{10}	10	15.3	53
T_{11}	10	16.7	67
T_{12}	10	18.5	85

Table 5: Percentage mucoadhesion of the prepared microspheres

Formulation Code	No. of Microspheres		% Mucoadhesion
	Initial	Final	
T_1	20	13	65
T_2	20	14	70
T_3	20	15	75
T_4	20	17	85
T_5	20	12	60
T_6	20	13	65
T_7	20	14	70
T_8	20	15	75
T_9	20	12	60
T_{10}	20	14	70
T_{11}	20	15	75
T_{12}	20	16	80

Table 6: *In-Vitro* drug release data of Methylprednisolone microspheres containing sodium alginate along with Methocel as copolymer

Time (h)	Cumulative percent of drug released			
	T_1	T_2	T_3	T_4
0	0	0	0	0
1	24.88	21.11	18.66	15.88
2	31.55	31.55	25.11	24.22
3	42.44	39.77	35.44	32.66
4	53.55	47.77	40.66	39.33
5	62	56.66	52	47.55
6	74.66	62.44	57.33	55.77
7	83.55	69.55	63.11	61.77
8	89.33	75.33	69.11	69.55
9	92.66	84.66	75.33	77.55
10	85.55	90.66	82.66	85.55
11	80.22	84.22	90.66	90.66
12	78.88	80.88	89.55	97.66

Table7: *In-Vitro* drug release data of Methylprednisolone microspheres containing sodium alginate along with carbopol 971 as copolymer

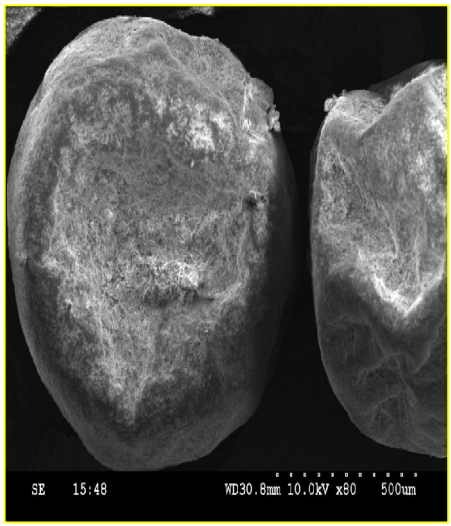
Time (h)	Cumulative percent of drug released			
	T_5	T_6	T_7	T_8
0	0	0	0	0
1	27.77	22.44	18.44	17.11
2	36.44	32.22	29.33	26.44
3	43.77	40.88	39.55	37.55
4	54.66	48.66	45.55	46.88
5	64.01	57.55	57.33	55.77
6	75.77	63.55	65.33	63.55
7	84.65	70.44	71.55	71.33
8	90	76.55	77.56	75.77
9	92.22	85.55	81.55	79.77
10	84.88	91.33	83.33	82.44
11	79.55	85.77	89.55	86.88
12	77.55	81.11	87.55	90.66

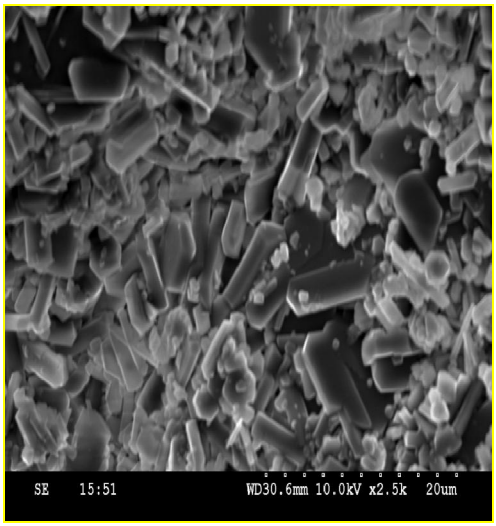
Table 8: *In-Vitro* drug release data of Methylprednisolone microspheres containing sodium alginate along with Pectin as copolymer

Time (h)	Cumulative percent of drug released			
	T_9	T_{10}	T_{11}	T_{12}
0	0	0	0	0
1	25.77	21.55	18.66	16.44
2	35.33	31.77	26.55	27.11
3	43.55	40.44	36.55	36.44
4	54	48.44	43.66	45.55
5	63.55	57.11	54.55	55.33
6	75.33	63.11	62.33	63.11
7	84	70.22	67.68	71.55
8	89.77	76	73.55	76.44
9	92.66	85.11	78.55	80.66
10	85.11	91.33	83	85.55
11	80.66	85.33	90	89.55
12	78	81.11	87.55	92.44

Table 9: Release kinetics studies of the prepared formulations

Formulation code	Release model									Best fit model
	Zero order		First order		Higuchi matrix		Koresmeyer-peppas			
	K	R^2	K	R^2	K	R^2	N	K	R^2	
T_1	21.6	0.797	1.923	0.720	-0.313	0.912	0.556	1.388	0.925	Peppas
T_2	16.39	0.908	1.991	0.890	-3.945	0.970	0.595	1.326	0.983	Peppas
T_3	10.45	0.976	2.062	0.945	-8.966	0.975	0.673	1.233	0.991	Peppas
T_4	7.434	0.990	2.118	0.914	-12.25	0.962	0.743	1.171	0.996	Peppas
T_5	24.34	0.768	1.897	0.689	2.624	0.903	0.498	1.442	0.914	Peppas
T_6	17.19	0.904	1.990	0.885	-3.333	0.971	0.579	1.346	0.981	Peppas
T_7	14.53	0.936	2.018	0.985	-6.239	0.983	0.655	1.278	0.990	Peppas
T_8	13.06	0.948	2.032	0.990	-7.587	0.984	0.690	1.241	0.991	Peppas
T_9	23.20	0.783	1.909	0.704	1.336	0.909	0.526	1.418	0.925	Peppas
T_{10}	16.73	0.906	1.992	0.885	-3.771	0.970	0.591	1.334	0.982	Peppas
T_{11}	12.50	0.957	2.036	0.974	-7.640	0.982	0.667	1.253	0.993	Peppas
T_{12}	11.94	0.959	2.061	0.982	-8.986	0.981	0.712	1.226	0.995	Peppas





External morphology of the microsphere **Drug-polymer distribution in the microspheres**

Fig 1: SEM Photo graphs for Methylprednisolone Microspheres

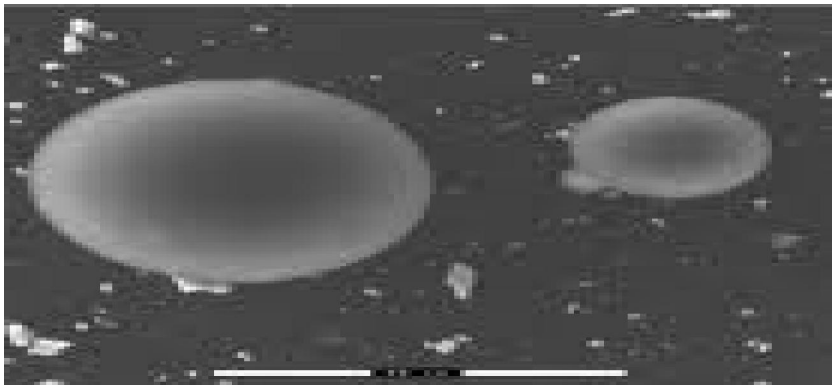


Fig 2: SEM studies for the optimized formulation T4

CONCLUSIONS:

In the present work, mucoadhesive microspheres of Methylprednisolone using Sodium alginate along with Methocel, Carbopol 971, Pectin as copolymers were formulated to deliver Methylprednisolone via oral route. The Ionotropic gelation technique provides characteristic advantage over conventional microsphere method, which involves an "all-aqueous" system, avoids residual solvents in microspheres. Other methods utilize larger volume of organic solvents, which are costly and hazardous because of the possible explosion, air pollution, toxicity and difficult to remove traces of organic solvent completely. In the present study it was found that increase in the polymer concentration led to increase in % Yield, % Drug entrapment efficiency, Particle size, % swelling and % Mucoadhesion. The *invitro* drug release decreased with increase in the polymer and copolymer concentration. Based on the results obtained the formulation T4 containing sodium alginate and methocel in 1:3 ratio have shown better results as a mucoadhesive delivery system when compared with other developed formulations thus the formulation T4 was optimized.

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