Research Article

Formulation and Evaluation of Colon Specific Microsphere Containing Bicalutamide

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ABSTRACT

The aim of the present work is to develop the Bicalutamide microsphere using Guar gum as a polymer by O/W emulsion solvent diffusion method for Sustained effect. The drug–polymer ratio is use in various concentrations, to obtain the desire sustained formulation. The microsphere was evaluated for physical characterizations angle of repose, particle size, drug entrapment efficiency, in-vitro dissolution. Results of all the physical and in-vitro dissolution data concluded in that formulation AB-4 was the most promising formulation. AB-4 which contains 2.5% of guar gum, 0.25% span 80 as dispersing agent showed the optimum cumulative % drug release of 94.22 % in 24 hrs. Compared to other batch. SEM photographs revealed the absence of crystals of drug on the surface of Microsphere and uniform distribution of the drug within the Microsphere. FTIR Studies shows there was no interaction found between any excipient and drug.

Key words: Bicalutamide, Microsphere, Guar gum, % Drug release.

INTRODUCTION

Microencapsulation is a process whereby relatively thin coating of polymers are applied to small particles of solid or droplets of liquid and dispersions. Microencapsulation leads to microcapsules or micro spheres, which are reservoir type and matrix type respectively. In either case, one or more active ingredient (core) is entrapped within matrix, shell or coat which is usually composed of one or more polymers.[1-2] Drugs from many different pharmacological classes have been microencapsulated, particularly analgesics, antibiotics, antihistamines, cardiovascular agents, iron salts, tranquilizers and vitamins.

There are many reasons why drugs and related chemicals have been microencapsulated. Toxic chemicals such as insecticides may be microencapsulated to reduce hazards to operators. Also the hydroscopic properties of many core materials such as sodium chloride may be reduced by microencapsulation. Many drugs have been microencapsulated to reduce gastric irritation and other gastrointestinal (GI) tract irritation, including ferrous sulfate and potassium chloride. Sustained – release aspirin preparations have been reported to causes significantly less gastric bleeding than conventional aspirin preparation. [3, 4]

Bicalutamide is Antineoplastic agent that is primarily used in the treatment of metastatic colorectal cancer. Mechanism of It is a semisynthetic, water-soluble derivative. Action camptothecin, a cytotoxic alkaloid extracted from plants such as Camptotheca acuminata. Irinotecan and its active metabolite, SN-38, inhibit the action of topoisomerase I, an enzyme that produces reversible single-strand breaks in DNA during DNA replication. These single-strand breaks relieve torsional strain and allow DNA replication to proceed. Bicalutamide is a nonsteroidal pure antiandrogen given at a dosage of 150 mg once daily as monotherapy for the treatment of early (localised or locally advanced) nonmetastatic prostate cancer. Bicalutamide is well-absorbed following oral administration, Protein Binding 96%. [5]

METHOD AND MATERIALS

MATERIALS

Bicalutamide was obtained as a kind gift sample from Cipla pvt ltd, Mumbai. Guar gum has been purchased from Centre drug Lab, Delhi .All other chemicals, reagents and solvents used are of analytical grade.

FORMULATION OF MICROSPHERE

Microsphere containing Bicalutamide as a core material was prepared by O/W emulsion solvent diffusion method. The polymer guar gum dissolves in the mixture of Calcium chloride and acetone (1:1). The core material bicalutamide was dispersed in polymer solution with constant stirring to get uniform mixture. This mixture poured drop wise into stirring 50 ml, 0.25 % w/v aqueous solution of span 80. The emulsion was stirred at 1500 rpm for 1 Hr. then the prepared microsphere was collected by filtration and wash with N-hexane, microsphere dried at room temperature and store in desiccators.

Table 1: Schematic representation of formulation of microsphere

Sr. No.	Ingredients	AB-1	AB-2	AB-3	AB-4	AB-5
1.	Bicalutamide	1	1	1	1	1
2.	Guar gum	1	1.5	2	2.5	3
3.	Calcium chloride	10	10	10	10	10
4.	Acetone	10	10	10	10	10
5.	Span 80	0.4	0.4	0.4	0.4	0.4
6.	Distilled Water	250	250	250	250	250

Evaluation of Microsphere

Angle of repose

Angle of repose is defined as the maximum angle possible between the surface of pile of powder and horizontal plane. The angle of repose for the granules of each formulation was determined by the funnel method. The microspheres were allowed to flow out of the funnel orifice on a plane paper kept on the horizontal surface. It forms a pile of microsphere on the paper. The angle of repose was calculated by substituting the values of the base radius 'R' and pile height 'H' in the following equation [6]

$$\mathbf{Tan\theta = H/R}$$

Where,

H = Pile Height.

R = Radius of Pile

Therefore; $\theta = \tan^{-1}\frac{H}{R}$

Bulk Density

Bulk density of all batches of microsphere was determined by pouring gently 0.5 g of sample through a glass funnel into a 10 ml graduated cylinder. The volume occupied by the sample was recorded. Bulk density was calculated as per given formula. [7]

$$\text{Bulk density} = \frac{\text{Weight of sample}}{\text{Volume occupied by the sample}}$$

Tapped Density

The tapped density was determined by pouring 0.5 g of microsphere through a glass funnel into a 10 ml graduated cylinder. The cylinder was tapped from height of 2 inches until a constant volume was obtained. Volume occupied by the sample after tapping was recorded .The values for tapped density was calculated as per given formula**.**

$$\text{Tapped density (g/ml)} = \frac{\text{Weight of sample}}{\text{Volume occupied by the sample}}$$

Compressibility index

The compressibility indices of the formulation blends were determined using Carr's compressibility index formula.

$$\text{Carr's Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Determination of particle size

The particle size was determined using stage micrometre. The diameters of about 300 microspheres were measured and the average particle size determined.[8,9]

Percentage yield

The percentage yield of different formulations was determined by weighing the floating microspheres after drying. The percentage yield was calculated as follows. [10, 11.]

$$\% \text{ Yield} = \frac{\text{Total weight of floating microspheres}}{\text{Total weight of drug and polymer}} \times 100$$

Drug entrapment

The various batches of the floating microspheres were subjected to estimation of drug content. The floating microspheres equivalent to 50 mg of bicalutamide from all batches were accurately weighed and crushed. The powdered of microspheres were dissolved in ethanol (10 ml) in volumetric flask (100ml) and made the volume with 0.1 N HCl. This solution is then filtered through Whatmann filter paper No. 44. After filtration, from this solution accurate quantity (10 ml) was taken and diluted up to 100 ml with 0.1 N HCl. From this solution, accurate volume (2 ml) was pipette out and diluted up to 10 m1 with 0.1 N HCl and the absorbance was measured at 272 nm against 0.1 N HCl as a blank. The percentage drug entrapment was calculated as follows.

$$\% \text{ Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

***In-Vitro* Dissolution Study**

The study was carried out using dissolution apparatus USP Type-I (Rotating Basket type) accurately weighed microsphere equivalent to 50 mg of Bicalutamide were taken in muslin cloth and it was kept in baskets. Dissolution study was carried out in 1.2 & 6.8 pH phosphate buffer at 50 rpm at temp 37 ^{o}C ± 0.5^{0}C. During dissolution study 10 ml aliquot was withdrawn at a time intervals of 1 to 24 hrs and same was replaced with equal volume of fresh medium. The withdrawn samples were filtered through Whatmann filter paper and absorbances were measured at 272 nm. Drug concentration in the samples was determined from the standard calibration curve. Cumulative percent of drug dissolved was found out at each time point.

Surface Morphology

This study was performed at Diya Labs, Mumbai by Scanning Electron Microscopy (SEM) using JSM 6380 A(JOEL, Japan).The microspheres were coated with Platinum by ion sputtering using Autofine coater JFC-1600 (JOEL, Japan) The microspheres were kept on the sample holder and the scanning electron micrographs were taken [12].

Stability Study

Stability testing of formulations was carried out to determine the stability of drug and carrier and also to determine the physical stability of formulation under accelerated storage condition at various temperatures. The prepared microspheres were placed in borosilicate screw capped glass containers. The samples were kept at condition of 45°C/75% RH and were analyzed at 42 days for their physical changes and in drug content [13].

IR Analysis

The spectral analysis was done using FT-IR (Schimadzu 8400 SCCE).The dry sample of Bicalutamide, Guar gum, Ethyl cellulose, physical mixture, optimized formulation (AB-4) was mixed by triturating with dry potassium bromide (A.R. Grade) and placed in sample cell.[14-16]

RESULTS AND DISCUSSION

All the formulations show angle of repose value in the range of 25.74± 0.732 -22.78±0.534. These values for angle of repose (< 30) indicated good flow properties. The values for bulk density were found to range from 0.354±0.035 to 0.475±0.073. The values for tapped density were found to range from 0.395±0.064 to 0.548±0.064. Compressibility Index values were found in the range of 08.00±0.18 to 13.32±0.024 respectively.

Table 2: Data for evaluation of Microsphere

Formulation code	Angle of Repose (θ)	Bulk Density(g/m+l)	Tapped Density(g/ml)	Carr's Index (%)	Hausner's Ratio
AB-1	23.32±0.645	0.475±0.073	0.548±0.064	13.32±0.024	1.15±0.0675
AB-2	22.78±0.534	0.452±0.024	0.521±0.036	13.24±0.032	1.15±0.064
AB-3	23.95±0.633	0.358±0.024	0.403±0.014	11.16±0.075	1.12±0.014
AB-4	25.74±0.732	0.354±0.035	0.395±0.064	10.37±0.075	1.15±0.046
AB-5	24.68±0.590	0.471±0.025	0.512±0.012	08.00±0.180	1.52±0.019

n=3

Determination of Particle Size

By keeping drug ratio constant and varied polymer ratio as the polymer concentration increases viscosity increases which influences the interaction between disperse phase and dispersion medium that affects the size distribution of particle. If there was increase in the amount of polymer concentration, there was increase in relative viscosity so as a result increases in mean particle size. The average particle size of Microsphere is found to be within 112.546± 1.42 to 171.342± 1.36 µm.

Analysis of Drug Content

The drug % encapsulation efficiency of guar gum, ethyl cellulose Microsphere is shown in Table No. 11. The drug: polymer ratio showed significant effect on the encapsulation efficiency of Microsphere. The increase in concentration of drug showed the increase in drug encapsulation efficiency. The Microsphere formulated using acetonitrile and dichloromethane as internal organic phase or solvent showed better encapsulation efficiency than other Formulations. The % encapsulation efficiency is found to be in the range of 65.36 to 75.58 %.

Table 3: Data for Percentage yield, percentage loading and encapsulation efficiency of Bicalutamide Microsphere

Formulation Code	Drug : Polymer	Theoretical loading (%)	Actual Drug Loading (%)	Encapsulation Efficiency (%)	Yield (%)
AB-1	1:1	46.53	30.81 ± 0.355	69.43 ± 1.06	83.96
AB-2	1:1.5	50	35.29 ± 0.35	75.58 ± 0.7	88.2
AB-3	1:2	45	28.30±0.33	72±0.77	84.3
AB-4	1:2.5	44	32.30±0.45	73±1.3	85.6
AB-5	1:3	48	34.73±0.72	74±1.07	87.1

IN *VITRO* DRUG RELEASE

The dissolution profiles of all formulations were demonstrates in Table No.4. From the results it can be concluded that formulation AB-4 which contain 2.5% of guar gum showed the optimum cumulative % drug release of 94.22 % in 24 hrs. as compared to other formulation So formulation AB-4 is selected for the further study.

Table 4: In-*vitro* Drug Release from All Formulations of Microsphere

S.N	Time in hrs	AB-1	AB-2	AB-3	AB-4	AB-5
1	0	0	0	0	0	0
2	1	13.01 ±0.234	12.94 ±0.35	9.21 ±0.20	6.89 ±0.22	4.32±0.01
3	2	26.71 ±0.981	24.32 ±0.24	12.36 ±0.63	10.66 ±0.21	6.89±0.02
4	3	36.22 ±0.742	33.36 ±0.23	16.32 ±0.78	13.85 ±0.55	9.21 ±0.23
5	4	42.66 ±1.367	40.87 ±0.26	21.74 ±0.02	19.31 ±0.04	12.36 ±0.33
6	5	48.83 ±0.843	46.63 ±0.85	25.63 ±0.96	23.53 ±0.22	16.32 ±0.23
7	6	56.85 ±0.51	50.63 ±0.33	29.25 ±0.99	27.96 ±0.21	21.74 ±0.65
8	7	59.45 ±1.09	55.89 ±0.78	33.56 ±0.22	31.65 ±0.21	25.63 ±0.33
9	8	63.81 ±0.98	69.0±0.96	37.33 ±0.52	35.96 ±0.04	29.25 ±0.21
10	9	65.50 ±0.87	63.63±0.25	41.93 ±0.99	38.96 ±0.23	33.56 ±0.21
11	10	68.38 ±1.08	66.32±0.21	45.88 ±0.09	42.22 ±0.89	37.33 ±0.33
12	11	71.01 ±0.02	69.20±0.56	52.89 ±0.08	4574 ±0.63	41.93 ±0.33
13	12	74.75 ±0.57	73.32±0.89	56.01 ±0.05	49.32 ±0.54	45.88 ±0.32
14	13	76.50 ±0.59	77.20±0.32	60.99 ±0.74	56.32 ±0.41	52.89 ±0.22
15	14	78.36 ±0.78	81.23 ±0.36	64.59 ±0.65	61.25 ±0.52	56.01 ±0.12
16	15	82.36 ±0.89	86.30 ±0.89	67.63 ±0.21	64.96 ±0.88	60.99 ±0.25
17	16	86.21 ±0.23	85.32 ±0.25	71.21 ±0.25	68.36 ±0.88	64.59 ±0.89
18	17	90.28 ±0.98	89.45 ±0.85	75.89 ±0.99	71.36 ±0.32	67.63 ±0.87
19	18	94.21 ±0.96	91.25 ±0.63	79.36 ±0.88	74..2 ±0.41	71.21 ±0.14
20	19	96.25 ±0.30	94.22 ±0.24	83.92 ±0.33	78.20 ±0.41	75.89 ±0.89
21	20	98.21 ±0.32	97.32±0.48	86.26 ±0.25	82.50 ±0.88	79.36 ±0.05
22	21	------	---------	89.23 ±0.35	86.22 ±0.74	83.92 ±0.21
23	22	------	--------	92.32 ±0.12	89.23 ±0.42	86.26 ±0.54
24	23	------	-------	95.56 ±0.21	91.02 ±0.82	89.23 ±0.24
25	24	-------	------	97.12 ±0.85	94.21 ±0.22	92.32 ±0.21

SURFACE MORPHOLOGY

SEM photographs showed that the Microsphere were spherical in nature and had a smooth surface. SEM photographs revealed the absence of crystals of drug on the surface of Microsphere and uniform distribution of the drug within the Microsphere.

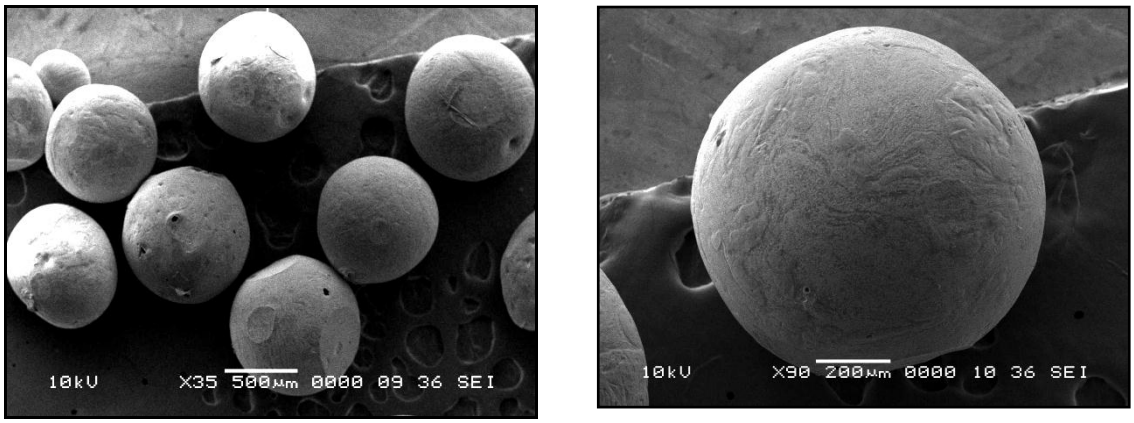

Fig.1: SEM Photographs of Microsphere of Bicalutamide formulation AB-4

STABILITY STUDY

The stability study was conducted and changes in poperties like drug content and maximum drug release was measured. There was no significant change in % drug content of the formulation and hence sit can be concluded that formulation was stable for 60 days.

Table 5: Properties of batch AB-4 of microsphere after stability

Sr. No	Parameter	Days				
		0	15	30	45	60
1	Drug Content (%)	99.56	99.48	98.97	98.86	98.70
2	Drug Release (%)	94.21	94.00	93.56	93.56	93.45
3.	Color	No change	No change	No change	No change	No change

FT-IR Analysis

The FT-IR spectra of pure drug - Bicalutamide, guar gum, ethyl cellulose and physical mixture and formulation were taken by preparing KBr pellets. (Disk method) Scanning Range: 4000 – 500 cm^{-1}

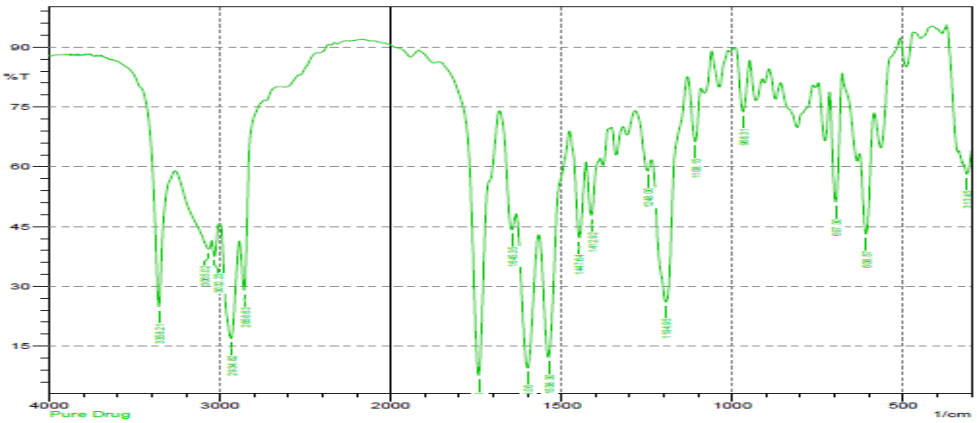

Fig. 2: IR spectrum of Bicalutamide

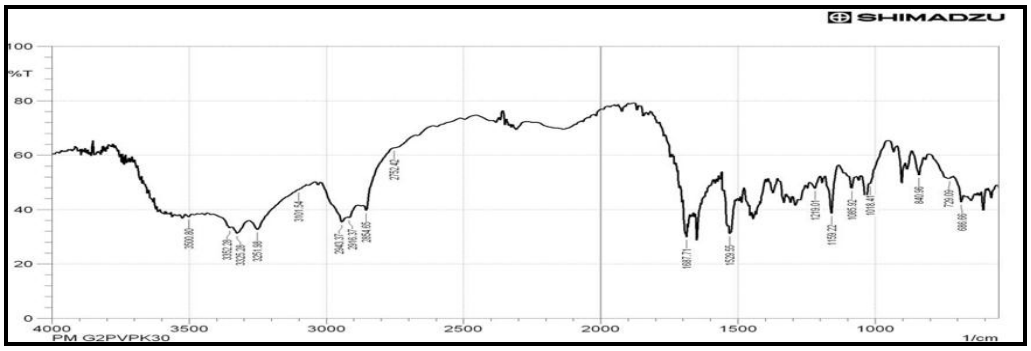

Fig. 3: IR spectrum of Physical mixture

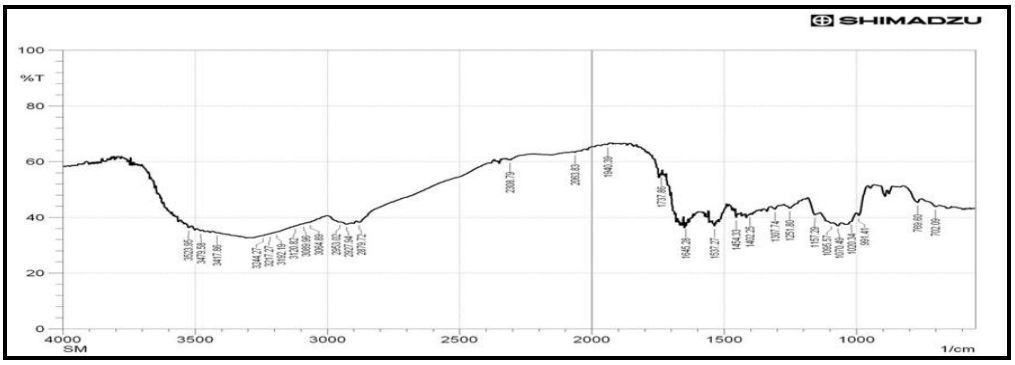

Fig. 4: FT-IR of Bicalutamide, Ethyl cellulose, Formulation AB-4

IR spectra for Bicalutamide, guar gum, ethyl cellulose and formulation AB-4. Major functional groups of Bicalutamide (di-substituted aromatic ring, ketone bond.) can be seen in Fig. No.2-4. spectra of individual drugs as well as in spectra of formulation. So there is no interaction between Bicalutamide and ethyl cellulose.denser peaks compared to plain Bicalutamide. This indicates that Bicalutamide is dispersed at the molecular level in the blend polymeric matrix.

CONCLUSION

The results obtained from this investigation are interesting and promising. The objective of the present investigation was to improve oral bioavailability of the poorly water soluble drug. For better absorption and enhanced bioavailability of some drug, prolongation of retention time of the dosage form in the gastro-intestinal tract is essential. This problem can be solved by preparation of colon drug delivery systems. An attempt was made to prepare microspheres of Bicalutamide using Guar gum. Ideal properties of microspheres sufficient release of drug in basic condition. The prepared formulation (AB-4) showed best drug release rate.

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