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OPTIMIZED DEVELOPMENT AND EVALUATION OF MICROBIALLY TRIGGERED BASED ORNIDAZOLE MICROSPHERES FOR COLON TARGETING

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ABSTRACT

The objective of the present study is to formulate and evaluate microbially triggered based colon targeted microspheres using two carriers tragacanth gum and inulin individually which would protect the release of the drug in the physiological environment of the stomach and small intestine and release the drug in the colon providing local action for the treatment of the amoebiasis. Ornidazole is a 5-nitroimidazole derivative and is used in the treatment of susceptible protozoal infections and also in anaerobic bacterial infections mainly for amoebiasis, giardiasis, vaginitis and duodenal ulcers. It is more effective against amoebiasis than metronidazole. The research involves the formulation, optimization and evaluation of colon targeting microspheres. The colon targeting microspheres were successfully developed by Ionotropic gelation technique, using tragacanth gum and inulin polymers in various proportions. Further, the prepared microspheres were characterized for particle size, morphology, entrapment efficiency, in vitro drug release, release kinetics, compatibility studies (DSC) and stability studies, Best formulations were selected from both type of microspheres as ORT_4 and ORI_4 based on amount of tragacanth gum or inulin, sodium alginate and glutaraldehyde affecting % CDR and % EE. Drug release kinetics of ORT_4 as well as ORI_4 system best corresponds to koresmeyer peppas kinetics in absence of rat caecal content while in presence of rat caecal contents, ORT_4 followed zero order kinetics and ORI_4 still followed koresmeyer peppas kinetics and the mechanism of drug release of all batches obtained from koresmeyer peppas kinetics corresponding to non Fickian diffusion showing Super Case II Transport mechanism.

KEYWORDS: Amoebiasis, Colon targeted microspheres, Ornidazole, Koresmeyer peppas kinetics.

INTRODUCTION

Drug targeting into the colon is highly desirable for local treatment of a variety of bowel disease such as ulcerative colitis, crohn's disease, amoebiasis, colonic cancer, local treatment of colonic pathologies, systemic delivery of proteins & peptide drug.[1] Rectal administration offers the shortest route for targeting drugs to the colon. However, reaching the proximal part of colon via rectal administration is difficult. Rectal administration can also be uncomfortable for patients and compliance may be less than optimal.[2] The colon is rich in lymphoid tissue uptake of antigens into mast cells of the colonic mucosa produces rapid local production of antibodies and this helps in efficient vaccine delivery. The colon in attraction interest as a site where poorly absorbed drug molecule may have an improved bioavailability .This reason of colon is pioneer of having a somewhat less hostile environment with less diversity and intensity of activity then the stomach and small intestine. Additionally, the colon has a longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drug apart from retarding of targeting dosage forms, a reliable colonic drug delivery could also be important starting position for the colonic absorption of per orally applied, undigested, unchanged and fully active peptide drugs.[3] The human colon has over 400 distinct species of bacteria as resident flora, a possible population of up to 1010 bacteria per gram of colonic contents. Among the reactions carried out by these gut flora are azoreduction and enzymatic cleavage i.e. glycosides. These metabolic processes may be responsible for the metabolism of many drugs and may also be applied to colon- targeted drug delivery of peptide based macromolecules such as insulin by oral administration.[2]

MATERIALS AND METHOD

Material

Ornidazole was obtained as a gift sample from K. Pharma Chem, Ambala, Haryana and Sodium alginate, Inulin, Calcium Carbonate AR, Glutaraldehyde, Acetone, Isopropyl alcohol was purchased from Loba Chemie Mumbai and Tragacanth Gum were purchased from S.D. Fine Chem Limited Mumbai and Eudragit S 100 was purchased from Degussa Pvt. Ltd. Mumbai.

Method

Ionotropic gelation method was selected for preparation of microspheres. Tragacanth gum and inulin were selected individually as microbial degradable polymer along with Sodium alginate.

Calcium chloride was used for hardening of microspheres. Glutaraldehyde was used as cross linking agent. Tragacanth gum and Inulin, individually, of different concentration were dissolved in distilled water and allow to swell for few hours. (I) Sodium alginate dissolved in distilled water and drug dispersed into this solution (II). I and II solution were mixed kept on magnetic stirrer at room temperature with constant speed. Added few ml of glutaraldehyde into mixture and allowed to crosslink for 1 hour. The bubble free dispersion was dropped into calcium chloride solution with a needle of 21 gauze. The microspheres allowed to harden in solution for 15 minutes. Then microspheres were filtered and dried primarily at room temperature and after it, in hot air oven at 35°C for about 30 min. Microspheres were then stored in glass bottles, capped tightly.

EVALUATION PARAMETERS OF MICROSPHERES[4, 5, 6]

Flow properties of microspheres

Bulk Density

The bulk density was calculated using the formula.

$$\delta_b = M/V_0$$

Where, δ_b = Bulk density

M = Mass of sample in gm

V_0 = Bulk volume of microspheres in cc

Tapped Density

The tapped density was calculated using the formula

$$\delta_1 = M/V_a$$

Where, δ_1 = Tapped density

M = Mass of sample

V_a = Tapped density of microspheres in cc

Angle of Repose

The angle of repose, θ, was calculated using the formula.

$$\theta = \tan^{-1} h/r$$

Where, θ = angle of repose

h= height of cone

r = radius of the cone base

Table 1: Angle of repose as an indication of powder flow properties

S.No.	Angle of repose (θ)	Type of flow
1.	<20	Excellent
2.	20-30	Good
3.	30-40	Passable
4.	>40	Very poor

Compressibility Index or Carr's index

Compressibility index is calculated using the formula.

% Compressibility = $(\delta_t - \delta_0/\delta_t)$ x 100

Where, δ_t = Tapped density

δ_0 = Bulk density

Table 2: Relationship between % compressibility and flowability

S.No.	Compressibility Index	Flow character
1.	<10	Excellent
2.	11-15	Good
3.	16-20	Fair
4.	21-25	Passable
5.	26-31	Poor
6.	>31	Very poor

Hausner's ratio

Hausner's ratio is calculated using the formula.

Hausner's ratio = δ_t / δ_0

Where, δ_t = Tapped density

δ_0 = Bulk density

Lower Hausner's ratio (<1.25) indicates better flow properties than higher ones (>1.25).

CHARACTERIZATION OF MICROSPHERES[7, 8, 9]

Characterization of microspheres was done by visual observation, optical microscopy, entrapment efficiency, drug loading, % yield of microspheres and *In-vitro* release studies.

Visual observation

Surface and shape characteristics of microspheres were described by visual observations.

Optical Microscopy

Particle size analysis of drug loaded microspheres was performed by optical microscopy using compound microscope calibrated with eyepiece and stage micrometre.

Encapsulation efficiency

Practical or actual drug content was determined by accurately weighed amount (10 mg) of the formulation of microspheres in 20 ml phosphate buffer pH 6.8. It was left to equilibrate for 24 hrs at room temperature. The suspension was then centrifuged at 3000 rpm for 15 minutes. The supernatant was diluted appropriately with phosphate buffer and analyzed for concentration of drug using UV spectrophotometrically (318.6nm) at suitable wavelength (USP, NF 2005).

Drug release study[10]

In Vitro Drug release study in pH 1.2 (Acidic buffer) and pH 7.4 (Phosphate buffer)

The ability of the formulated microspheres to prevent the drug release in the physiological environment of the stomach and retard drug release in small intestine was assessed by conducting drug release studies in simulated stomach and simulated intestinal pH respectively. In vitro dissolution studies or drug release studies were performed for microspheres of ornidazole using USP dissolution apparatus II (Paddle type, Electrolab tablet dissolution apparatus) at 50 rpm and temperature was maintained upto 37 ± 0.5^{o}C, with dissolution medium of 250 ml. The microspheres were enclosed in empty teabags to prevent slippage of microspheres from basket. Using 250 ml of 0.1 N HCl for first 2 hr and phosphate buffer of pH 7.4 for next 3 hrs. An aliquot (5 ml) of the sample solution was withdrawn at predetermined time intervals, filtered through whatman filter paper and analyzed spectrophotometrically at 277.4 for 0.1N HCl buffer and at 307.6 for phosphate buffer of pH 7.4. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample.

***In Vitro* Drug Release Study in Phosphate Buffer Saline pH 6.8**

After performing the *in vitro* drug release studies in the stimulated dissolution medium of pH 1.2 and 7.4, same formulation were tested in the dissolution medium having phosphate saline buffer of pH 6.8 for stimulation of the colonic medium. All the conditions were same used for *in vitro* drug release. Drug content was also determined by the same method at 318.6nm.

CHARACTERIZATION OF BEST BATCH OF MICROSPHERES

Swelling behaviour of microspheres[11]

The swelling properties of optimized batches of microspheres were investigated in phosphate buffer 7.4 and phosphate buffer 6.8. Sample of microspheres equivalent to 10 mg were placed in petri plates containing 20 ml of swelling solution (6.8 or 7.4 buffer). After 2 hours, swollen microspheres were removed and reweighed. The wet weight of swollen microspheres was determined by blotting them filter paper to remove moisture, followed by weighing them on electronic balance. The percentage of swelling of microspheres was calculated from equation.

Percentage of swelling = $[(W_s - W_i)/W_i)] \times 100$

Where, Ws = weight of microsphere in swollen state

Wi = initial weight of the microspheres.

Ex-vivo drug release studies in presence of Rat Caecal Content[10, 12]

As the tragacanth & inulin, which was used as a polymer to make formulations were susceptible to microbes present in the colon. Therefore, dissolution rate studies were also performed using rat caecal content because of similarity with human colonic microflora to simulate microbial environment of colon.

The experimental protocol was under strict compliance of the CPCSEA guidelines as IPS/AH/278. Albino rats which were maintained on normal diet were used and to simulate enzymes which specifically hydrolyze tragacanth gum, enzyme induction was done. For enzyme induction, tragacanth gum aqueous dispersion (1 ml of 2% w/v dispersion) was administered to the rats daily for 6-7 days. Thirty minutes before the commencement of study, four rats were killed, their abdomen were opened, caecai were isolated, ligated from both ends, cut loose and transferred immediately into phosphate saline buffer pH 6.8 bubbled with CO_2 gas. Afterwards, caecal bags were opened and their contents were weighed and transferred to phosphate saline buffer to obtain 4% w/v rat caecal content. Due to the anaerobic nature of the bacterial content, all the operations were performed under the environment of CO_2 gas.

The drug release studies were carried out by using the same USP dissolution rate test apparatus. The capsules were placed in basket and basket was enveloped in empty tea bags to prevent clogging of basket by rat caecal content. The experiments were carried out in a 250 ml beaker immersed in water maintained in the jars of the dissolution test apparatus. Initial

studies were carried out in 0.1 N HCl (pH 1.2) for 2 hrs followed by phosphate saline buffer at pH 7.4 for 3 hrs. Afterwards, drug release studies were performed using 250 ml of pH 6.8 phosphate saline buffer having 4% w/v of rat caecal content prepared by adding 10 gm of caecal content to the dissolution medium of pH 6.8. The experiment was performed for 7 hrs in pH 6.8 completing an overall time period of 12 hrs with continuous supply of CO_2 to provide environment.

At different time intervals, samples were withdrawn without a prefilter and was replaced with the same dissolution medium freshly bubbled with CO_2 gas to maintain the sink condition. Afterwards, each withdrawn samples were diluted with phosphate saline buffer pH 6.8. Then samples were centrifuged and supernatant was removed using bacteria proof filters (G5) and the filtrates were analyzed for drug concentration by UV spectroscopy.

***In vivo* studies**[13, 14]

The polymers used in the formulation are based on microbially triggered release mechanism Tragacanth and inulin individually used in the formulations is capable of microbial growth in the colon. Therefore *in-vivo* studies were also performed using mice stools to test the efficacy of polymers to increase the quantity of enzymes present in the colonic microflora.

The experimental protocol was under strict compliance of the CPCSEA guidelines as IPS/AH/278. Enterococci stool colonization method is followed for *in vivo* studies.

Male mice weighing 25±5gm were housed in individual cages and fed rodent chow and water. 1ml of coated microspheres dispersion was orally fed to mice (in a group of six) using an oral feeding canula. The stools were collected periodically at 8 hrs intervals for 50 hrs and subjected for Enterococci colonization density study.

Quantification of stool organisms

For quantification of microorganisms, stool samples in normal saline were heated to 80°C for 20 min in order to limit growth of other organisms. These samples were serially diluted in saline, poured on agar plates and incubated at 37°C for 48 h. Bacillus colonies were identified by their unique morphology.

FTIR studies

The FTIR spectra of the pure drug ornidazole, microspheres of optimized batch were recorded with FTIR spectrometer. FT-IR Nicolet-87000 instrument was used for this purpose.

The samples were prepared by using potassium bromide and scanned for the absorbance at 4000-400/cm^{-1}.

SEM analysis[15]

The optimized batches were analyzed by SEM for surface morphology before and after dissolution. SEM studies were carried out with scanning electron microscope. Samples were sticked on double sided carbon tape on electron microscope brass tab and coated with gold in ion sputter. Picture of microspheres were taken by random scanning of stub.

RESULT AND DISCUSSION

In the present study an attempt was made to formulate ornidazole as multiparticulate drug delivery system for colon targeting using Tragacanth and inulin as biodegradable natural polymer.

Morphological Study

All the prototype batches of tragacanth gum microspheres were evaluated on basis of morphological characteristics like shape, colour, stickiness odour and mentioned in Table: 3.

Table 3: Morphological characters of ornidazole microspheres with tragacanth gum

S. No.	Formulation code	Shape	Colour	Stickiness	Odour
1	ORT_1	Slightly Spherical	Brown	Absent	Odourless
2	ORT_2	Slightly irregular	Brown	Absent	Odourless
3	ORT_3	Spherical	Brown	Absent	Odourless
4	ORT_4	Spherical	Brown	Absent	Odourless
5	ORT_5	Spherical	Brown	Absent	Odourless
6	ORT_6	Slightly irregular	Brown	Absent	Odourless
7	ORT_7	Spherical	Brown	Absent	Odourless
8	ORT_8	Spherical	Brown	Absent	Odourless

All the prototype batches of inulin microspheres were evaluated on basis of morphological characteristics like shape, colour, stickiness odour and mentioned in Table: 4.

Table 4: Morphological characters of ornidazole microspheres with inulin

S. No.	Formulation code	Shape	Colour	Stickiness	Odour
1	ORI_1	Spherical	Brown	Absent	Odourless
2	ORI_2	Spherical	Brown	Absent	Odourless
3	ORI_3	Spherical	Brown	Absent	Odourless
4	ORI_4	Slightly irregular	Brown	Absent	Odourless

5	ORI_5	Spherical	Brown	Absent	Odourless
6	ORI_6	Spherical	Brown	Absent	Odourless
7	ORI_7	Slightly irregular	Brown	Absent	Odourless
8	ORI_8	Spherical	Brown	Absent	Odourless

Determination of particle size.

Determination of particle size is determined by optical microscopy. Photographic images are shown in "Fig": 1.

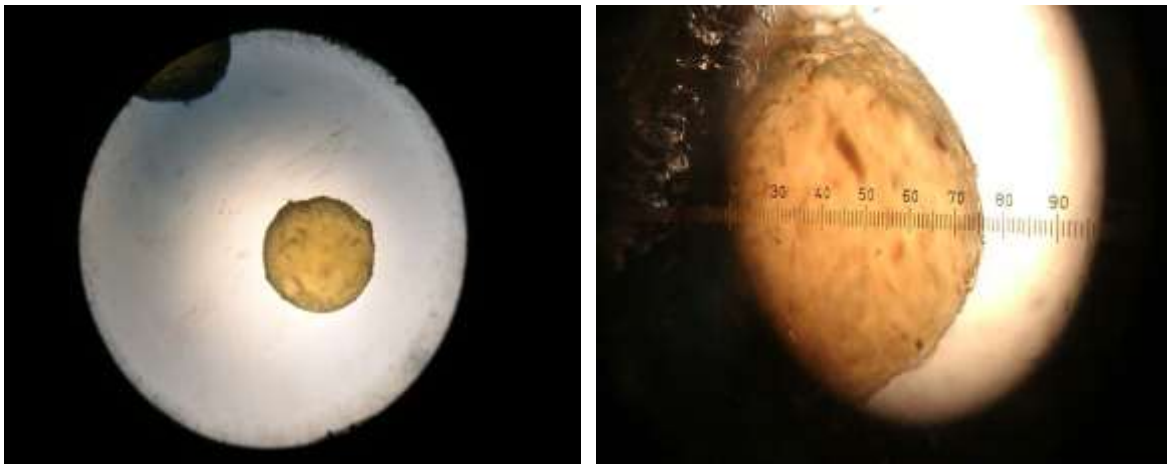

Figure 1: Optical photograph of microsphere with & without eyepiece micrometer

The mean particle size of microspheres (in µm) of both the batches are depicted in Table: 5.

Table 5: Mean particle size of ornidazole microspheres with tragacanth gum and Inulin

Formulation code	Microspheres with tragacanth gum (µm)	Formulation code	Microspheres with inulin (µm)
ORT_1	512.0	**ORI_1**	473.5
ORT_2	562.1	**ORI_2**	458.1
ORT_3	481.2	**ORI_3**	442.7
ORT_4	519.7	**ORI_4**	454.3
ORT_5	506.3	**ORI_5**	423.5
ORT_6	483.2	**ORI_6**	450.4
ORT_7	477.4	**ORI_7**	435.0
ORT_8	523.6	**ORI_8**	462.0

The graph plotted in "Fig": 2 shows the comparison of microspheres size prepared with tragacanth gum.

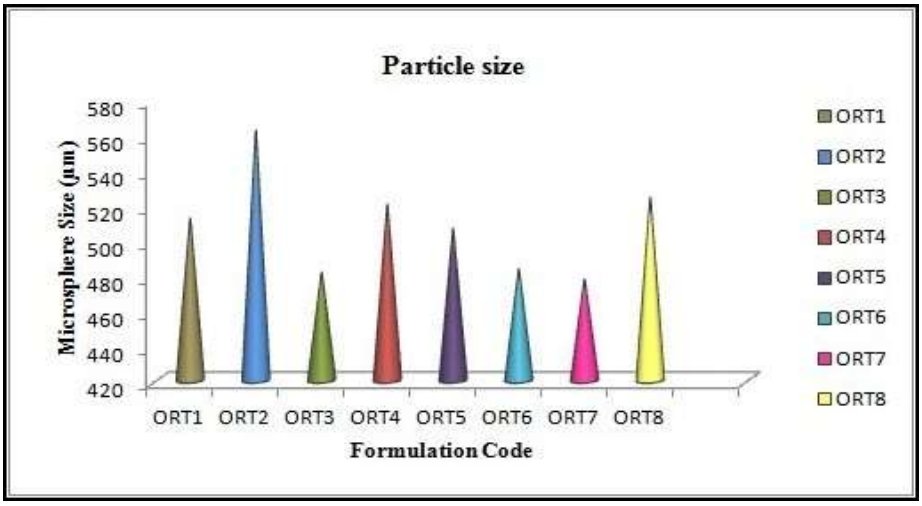

Figure 2: Particle size of microspheres prepared with tragacanth gum

The graph plotted in "Fig": 3 shows the comparison of microspheres size prepared with inulin.

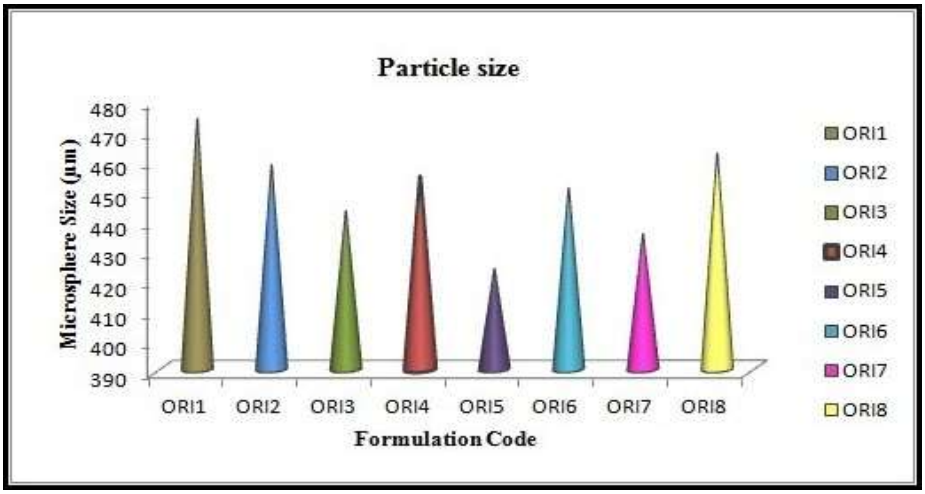

Figure 3: Particle size of microspheres prepared with inulin

It was found that average particle diameter from 423.5 µm to 562.1 µm in both type of microspheres and particle diameter distribution of each formulation was within narrow range. The diameter of particle size depends upon the needle size pore. Small decrease in particle was noted with increasing amount of glutaraldehyde. However composition of polymers affect the particle size as increase in particle size is noted with increase in sodium alginate with respect to tragacanth gum and inulin.

Evaluation of flow properties of all formulation batches

The microspheres of all the formulation batches with tragacanth gum and inulin were evaluated for the flow properties**.** In Table 6 and 7 all the flow properties determinant parameters of microspheres are given with value.

Table 6: Evaluation of flow properties of all formulation batches with tragacanth gum

Batch No.	Bulk Density (g/cm^3)	Tapped Density (g/cm^3)	Carr's Index (%)	Hausner's Ratio	Angle of Repose
ORT_1	0.896	1.008	11.11	1.12	14.84
ORT_2	0.956	1.092	12.45	1.14	13.49
ORT_3	0.865	0.910	4.94	1.05	15.26
ORT_4	0.840	0.925	9.19	1.10	16.27
ORT_5	0.804	0.877	4.22	1.09	14.43
ORT_6	0.855	0.915	6.55	1.07	13.60
ORT_7	0.847	0.957	11.50	1.12	17.22
ORT_8	0.935	1.039	10.01	1.11	15.00

Table 7: Evaluation of flow properties of all formulation batches with inulin

Batch No.	Bulk Density (g/cm^3)	Tapped Density (g/cm^3)	Carr's Index (%)	Hausner's Ratio	Angle of Repose
ORI_1	0.910	1.058	13.98	1.16	10.75
ORI_2	0.933	0.997	6.41	1.06	13.22
ORI_3	0.875	0.942	7.11	1.07	16.06
ORI_4	0.910	0.976	6.76	1.07	13.00
ORI_5	0.933	0.989	5.66	1.06	12.57
ORI_6	0.886	0.972	8.84	1.09	11.87
ORI_7	0.898	1.000	10.20	1.11	12.18
ORI_8	0.910	0.992	8.26	1.09	12.57

Bulk density values of both batches of microspheres were found to be in the range of 0.804-0.956 g/cm^3 while the corresponding tapped density values were in the range of 0.877-1.092 g/cm^3. The values of Carr's index for all the batches were found out to be less than 15, values of Hausner's ratio was also found to be less than 1.25 and values of angle of repose is less than 20 indicating that formulations of all the batches were excellent flow properties. Hence, it concluded that microspheres are easily filled in hard gelation capsules.

Determination of entrapment efficiency

The entrapment efficiency of microspheres is presented in Table 8 which indicates that the entrapment of ornidazole into microspheres was successful in all cases.

Table 8: Entrapment efficiency of alginate microspheres

Formulation code	Entrapment efficiency (%)	Formulation code	Entrapment efficiency (%)
ORT_1	91.62±0.28	**ORI_1**	84.60±0.26
ORT_2	88.87±0.30	**ORI_2**	89.44±0.41
ORT_3	92.80±0.08	**ORI_3**	83.02±0.15
ORT_4	94.99±0.21	**ORI_4**	86.47±0.25
ORT_5	85.82±0.18	**ORI_5**	81.60±0.18
ORT_6	75.07±0.31	**ORI_6**	78.10±0.13
ORT_7	77.13±0.21	**ORI_7**	74.18±0.09
ORT_8	87.83±0.47	**ORI_8**	82.58±0.33

Entrapment efficiency of ornidazole microspheres prepared with tragacanth gum and inulin was found to be 83.02% to 94.99% (ORT_1-ORT_4, ORI_1-ORI_4). Reduced entrapment efficiency was observed in microspheres prepared with increased amount of glutaraldehyde (ORT_5-ORT_8, ORI_5-ORI_8) ranging from 74.18% to 87.83%.

The entrapment efficiency of ornidazole microsphere for all the batches prepared with tragacanth gum are shown in “Fig”: 4.

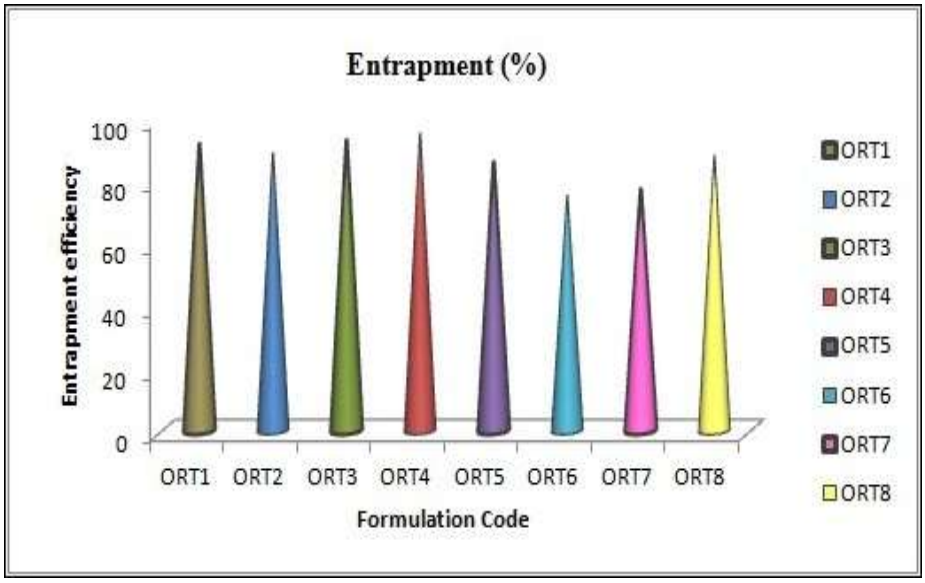

Figure 4: Entrapment efficiency of ornidazole microspheres prepared with tragacanth gum.

The entrapment efficiency of ornidazole microsphere for all the batches prepared with inulin are shown in “Fig”: 5.

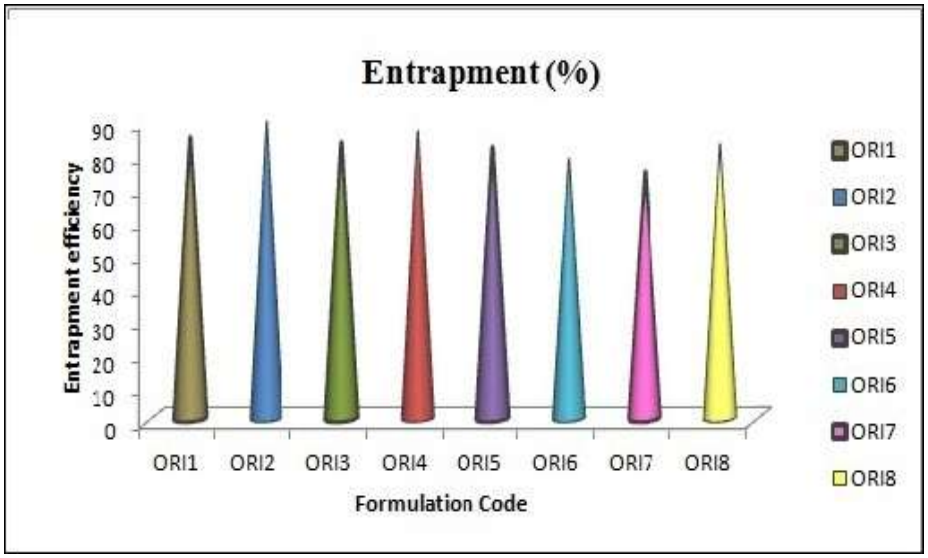

Figure 5: Entrapment efficiency of ornidazole microspheres with inulin

Entrapment efficiency decreased as concentration of crosslinker (glutaraldehyde) increased. Such decreasing trend is due to increasing cross link density. The microspheres became rigid thereby free volume space within polymer matrix reduced which resulted a reduction in encapsulation efficiency.

***In-vitro* dissolution study**

In vitro dissolution studies were performed to predict the dissolution profile during transit of dosage form from stomach through small intestine and finally reaching to colon environment. The dissolution studies were performed using USP II (basket type) dissolution apparatus in 250 mL of dissolution fluid. Use of small volume of buffer solution in this study help in better detection of drug concentration. Microspheres was encapsulated in hard gelatin capsule coated by double layer of Eudragit S100 which help in preventing leaching out of microspheres. The stirring speed was set 50 rpm and temperature was maintained 37±0.5°C throughout dissolution study. The pH of medium was kept as 1.2 pH for initial 2nd hr, 7.4 for next 3rd hr and pH 6.8 upto 12th hr. Samples were withdrawn every hr and analyzed for absorbance at UV spectrophotometer.

ORT batches (ornidazole-tragacanth gum microspheres)

The cumulative drug release of different prototype batches prepared with tragacanth gum are calculated by observing absorbance of different batches at different time intervals (upto 12 hrs.) and depicted in Table: 9.

Table 9: *In Vitro* % Cumulative drug release from microspheres of batches ORT_1 to ORT_8

Time (hr)	ORT_1	ORT_2	ORT_3	ORT_4	ORT_5	ORT_6	ORT_7	ORT_8
0	0	0	0	0	0	0	0	0
1	0.69	0.69	1.38	0.69	0.69	0.69	0.69	0.69
2	1.38	2.07	2.77	1.38	1.38	2.76	2.07	1.38
3	4.23	4.23	6.12	8.46	4.23	6.59	7.53	5.66
4	5.2	8.02	8.51	15.09	7.08	10.39	11.79	9.921
5	12.74	13.23	12.78	24.1	11.34	14.67	16.56	15.14
6	19.68	19.26	19.28	30.25	17.94	20.61	21.49	21.04
7	27.18	30.24	25.91	40.41	23.26	23.76	26.39	27.24
8	35.59	42.58	34.31	51.07	36.43	26.94	32.62	33.48
9	42.92	44.55	46.67	68.74	44.89	39.26	37.15	35.84
10	52.56	47.41	57.8	75.21	46.01	47.73	43.44	37.34
11	58.06	56.36	63.33	76.49	48.87	48.43	49.76	52.76
12	67.15	63.19	71.07	78.64	54.35	50.43	54.38	64.34

The graphical representation of dissolution profile of different batches prepared with tragacanth gum are shown in “Fig”: 6.

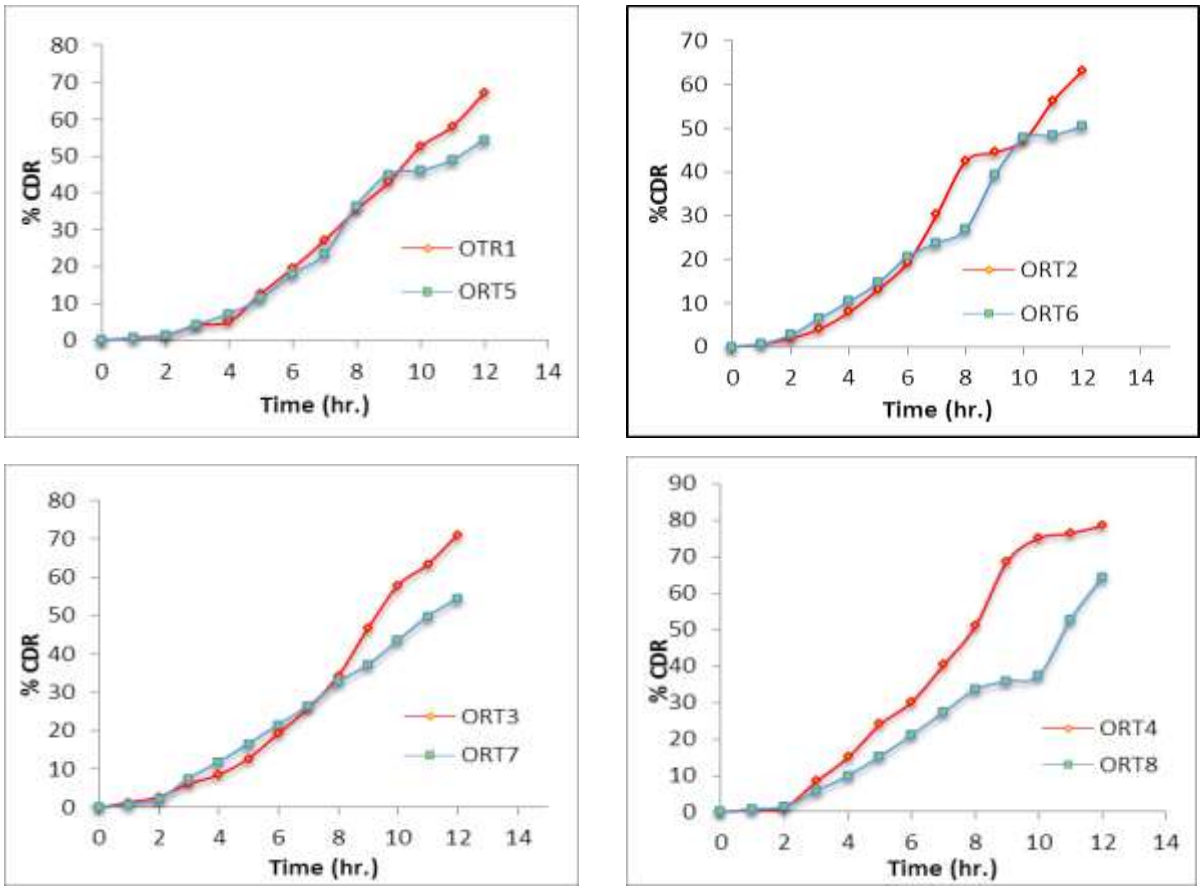

Figure 6: Graphical representation of dissolution profile of microspheres from formulation batches ORT_1-ORT_8 batches.

The dissolution profiles of ornidazole microspheres with polymer tragacanth gum in stimulated gastrointestinal fluids showed in different patterns and release in pH 7.4 and phosphate buffer 6.8 without enzyme.

Only 0.69% to 2.76% release in upper gastrointestinal tract environment at the end of 2 h. 4.23% to 24.10 % drug release was observed at pH 7.4 at the end of 5 h. In phosphate buffer 6.8 without enzyme the drug release was found to be 19.26% to 78.64 %. The increase in release pattern of drug from microspheres occurred due to high swelling of microspheres as they passes from pH 1.2 to pH 7.4. The dissolution profile showed that by increasing the concentration of glutaraldehyde (cross linker) in formulation gradually decrease in cumulative drug release.

Data obtained from *in- vitro* drug release analysis were represented in table-25 and figure-29, a comparison can be conducted between formulation codes ORT_1 and ORT_5, ORT_2 and ORT_6, ORT_3 and ORT_7, ORT_4 and ORT_8 on the basis of immediate release profile and sustained release drug profile. ORT_1, ORT_2, ORT_3 and ORT_4 shows %CDR of 67.15%, 63.19%, 71.07% and 78.64% respectively with fast release pattern while ORT_5, ORT_6, ORT_7 and ORT_8 microspheres were found with %CDR as 54.35%, 50.43%, 54.38% and 64.34% respectively.

Although latter one have lesser % CDR with respect to their compared one but all follows drug release pattern with sustained profile. This effect is due to increased amount of glutaraldehyde in later one batches. There was not a very big difference in drug retention pattern due to increased glutaraldehyde amount but release pattern was significantly affected leading to sustained release profile from immediate one.

ORI batches (ornidazole-inulin microspheres)

The cumulative drug release of different prototype batches prepared with inulin are calculated by observing absorbance of different batches at different time intervals (upto 12 hrs.) and depicted in Table: 10.

Table 10: *In Vitro* % Cumulative drug release from microspheres of batches ORI_1 to ORI_8

Time (hr)	ORI_1	ORI_2	ORI_3	ORI_4	ORI_5	ORI_6	ORI_7	ORI_8
0	0	0	0	0	0	0	0	0
1	0.69	1.38	0.69	0.69	0.69	0	0.69	2.07
2	1.38	2.08	1.38	2.08	1.38	0.69	2.08	5.55
3	5.17	6.12	6.58	5.65	4.70	3.29	6.12	7.07
4	9.43	8.03	7.56	9.91	6.61	6.6	11.32	11.34
5	11.36	9.02	10.4	13.72	9.93	9.92	13.26	15.63
6	14.48	11.43	14.91	15.81	13.59	10.53	18.42	25.41
7	17.61	21.06	23.25	25.02	17.14	19.29	20.26	29.47
8	24.66	32.48	35.56	35.16	25.06	23.74	24.29	33.11
9	36.1	34.4	40.1	45.79	32.59	31.26	34.42	36.33
10	42.38	41.11	49.88	59.08	33.21	32.3	43.3	46.53
11	50.01	54.37	52.33	60.71	36.86	37.26	45.71	51.57
12	58.54	56.41	61.75	63.22	45.33	42.68	46.4	53.58

The graphical representation of dissolution profile of different batches prepared with inulin are shown in “Fig” 7.

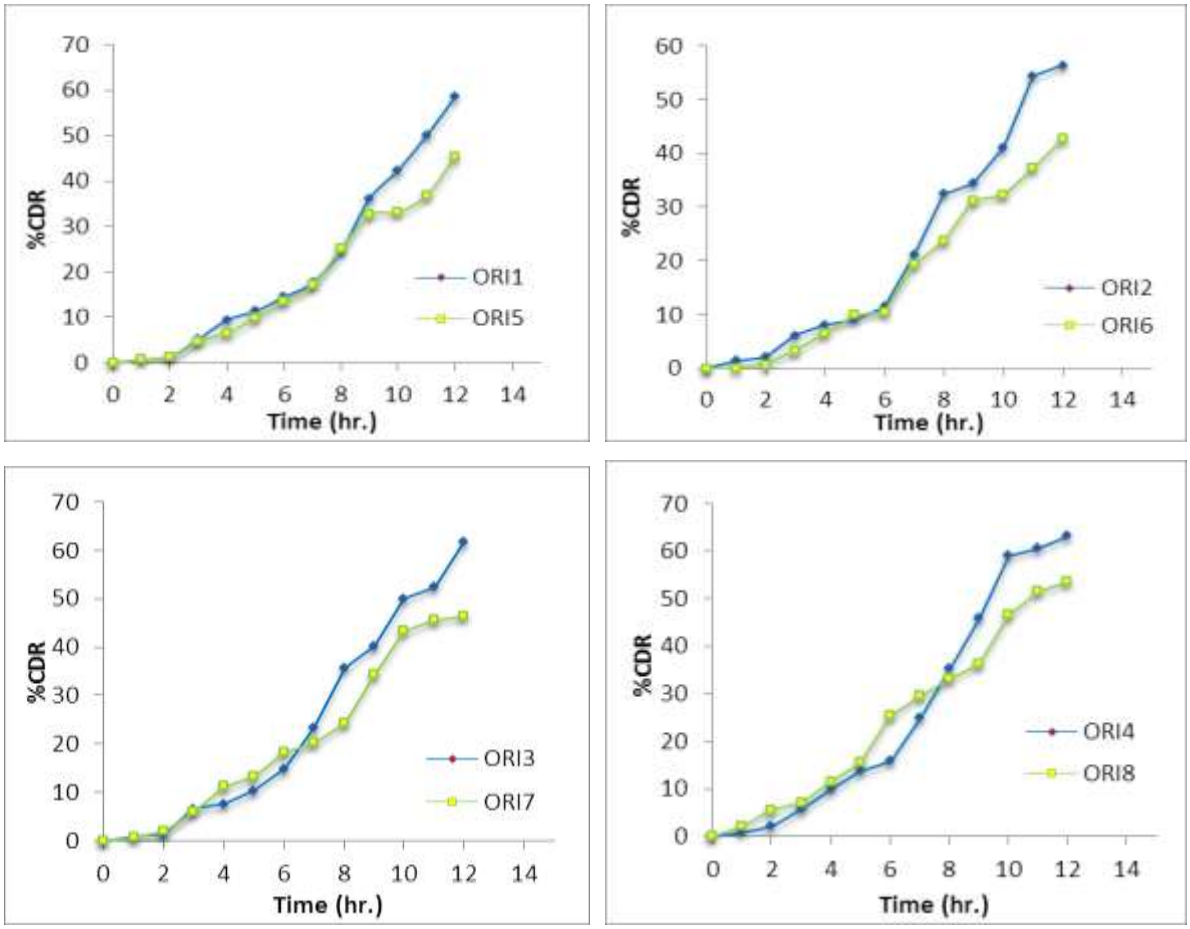

Figure 7: Graphical representation of dissolution profile of microspheres from formulation batches ORI_1-ORI_8 batches.

The in vitro dissolution profiles of ornidazole microspheres with polymer inulin in stimulated gastrointestinal fluids showed in different patterns and release in pH 7.4 and phosphate buffer 6.8 without enzyme. Only 0.01% to 5.55% release in upper gastrointestinal tract environment at the end of 2 h. 3.29% to 15.63 % drug release was observed at pH 7.4 at the end of 5 h. In phosphate buffer 6.8 without enzyme the drug release was found to be 10.53% to 63.22 %. The increase in release pattern of drug from microspheres occurred due to high swelling of microspheres as they passes from pH 1.2 followed to pH 7.4. The dissolution profile showed that by increasing the concentration of glutaraldehyde (cross linker) in formulation gradually decrease in cumulative drug release.

Data obtained from *in- vitro* drug release analysis were represented in table-26 and figure-30, a comparison can be conducted between formulation codes ORI_1 and ORI_5, ORI_2 and ORI_6, ORI_3 and ORI_7, ORI_4 and ORI_8 on the basis of immediate release profile and sustained release drug profile. ORI_1, ORI_2, ORI_3 and ORI_4 shows %CDR of 58.54%, 56.41%, 61.75% and 63.55% respectively with fast release pattern while ORI_5, ORI_6, ORI_7 and ORI_8 microspheres were found with %CDR as 45.33%, 42.68%, 46.40% and 53.58% respectively. Although latter one have lesser % CDR with respect to their compared one but all follows drug release pattern with sustained profile. This effect is due to increased amount of glutaraldehyde in later one batches. There was not a very big difference in drug retention pattern due to increased glutaraldehyde amount but release pattern was significantly affected leading to sustained release profile from immediate one.

The % CDR was overall less in microspheres prepared with inulin compared to microspheres prepared with tragacanth gum. This effect is credited to formation of a complex between the two polysaccharides might at a neutral Ph due to the hydrogen bonding, hydrophobic forces and formation of an interjunction zone with conformational changes of polysaccharides.

Selection of best batch from different Prototype formulations

Various prototype formulations were developed by using different concentration of tragacanth gum, sodium alginate and glutaraldehyde. Amount of drug with varying concentration of excipients in mg were weighed accurately and processed. Different responses are observed like %CDR & %EE which are based upon different concentration of sodium alginate, tragacanth and glutaraldehyde in colonic environment.

Table 11: Prototype formula for microspheres of ornidazole with tragacanth gum

Formulation Code	Tragacanth Gum (mg)	Sodium alginate (mg)	Glutaraldehyde (ml)	Entrapment Efficiency (%)	%CDR
ORT_1	240	680	0.5	91.62	67.15
ORT_2	280	680	0.5	88.87	63.19
ORT_3	240	720	0.5	92.80	71.07
ORT_4	280	720	0.5	94.99	78.64
ORT_5	240	680	1.5	85.82	54.35
ORT_6	280	680	1.5	75.07	50.43
ORT_7	240	720	1.5	77.13	54.38
ORT_8	280	720	1.5	91.62	64.34

The results concluded from Table: 11 that ORT_3 and ORT_4 showed max % CDR & maximum % EE but the batch ORT_4 showed max % cumulative drug release (78.64%) and % entrapment (94.99%).

Table 12: Prototype formula for microspheres of ornidazole with inulin

Formulation Code	Tragacanth Gum (mg)	Sodium alginate (mg)	Glutaraldehyde (ml)	Entrapment Efficiency (%)	%CDR
ORI_1	240	680	0.5	84.60	58.54
ORI_2	280	680	0.5	89.44	56.41
ORI_3	240	720	0.5	83.02	61.75
ORI_4	280	720	0.5	86.47	63.22
ORI_5	240	680	1.5	81.60	45.33
ORI_6	280	680	1.5	78.10	42.68
ORI_7	240	720	1.5	74.18	46.40
ORI_8	280	720	1.5	82.58	53.58

In the next formulation batch shown in Table: 12, the results concluded that the batch ORI_4 showed maximum % cumulative drug release (63.22%) and ORI_2 showed maximum % entrapment efficiency (94.99%).

On the basis of above observations, ORT_4 and ORI_4 are concluded to be the best prototype formulation.

Characterization of best batches ORT_4 and ORI_4

Swelling studies

The extent of swelling was determined by swelling behaviour of dried microspheres during GI passage; by measuring of water uptake in 1.2 pH for 2 hr and in phosphate buffer of pH 7.4 for 3hr and pH 6.8 until weight equilibrium has attained, maintained at physiological

temperature of 37°C, means in terms of percentage weight gained by microspheres. It was found that swelling of microspheres was occurred in stomach and it was gradually increasing when microspheres were transferred to intestine. As microspheres are prevented from acidic 1.2 pH, the swelling studies are also performed by escaping this medium. This study was mentioned in table form and it is given below. As microspheres are prevented from 0.1N HCl medium in final dosage form, swelling studies are also performed by escaping this medium as shown in Table: 13 given below. Chosen formulation ORT_4 and ORI_4 for swelling studies because the release rate is better as compare to other formulations.

Table 13: % Swelling Index of microspheres at different physiological pH.

Formulation Code	% Swelling Index		
	at 1.2 pH	At 7.4 pH	at 6.8 pH
ORT_4	13.5	16.8	19.2
	-	11.4	15.3
ORI_4	9.7	12.1	16.4
	-	10.4	12.8

CONCLUSION

From the conclusions of the results, the research study showed that the microspheres of ORT_4 batch was selected as colon targeted drug delivery as it shows no release of drug in stomach and upper git and shows the sustained release effect in the colon. This is due to swelling nature of the tragacanth gum. Microspheres prepared with inulin polymer, which is hydrophilic polymer, and shows the instant release of drug when reaches in the colon as compared to the tragacanth gum microspheres.

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