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FORMULATION DEVELOPMENT AND EVALUATION OF COLON TARGETING MICROSPHERE

Yogendra Sikarwar, Dr. Kratika Daniel, Dr. Sudha Vengrulekar, Dr. Sachin K. Jain
Oriental College of Pharmacy and Research, Oriental University, Indore (M.P.)

***Correspondence Info:**
Yogendra Sikarwar
Oriental College of Pharmacy and Research, Oriental University, Indore (M.P.)
Email:
yogendrasikarwar724@gmail.com

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ABSTRACT

Targeted drug delivery into the colon is highly desirable for local treatment of a variety of bowel diseases such as ulcerative colitis, Crohn's disease, amebiosis, colonic cancer, local treatment of colonic pathologies, and systemic delivery of protein and peptide drugs Thus selective delivery of drugs to the colon could not only lower the required dose but also reduce the systemic side effects caused by high doses. Microspheres are defined as "Monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles". The aim of this study is to prepare microsphere loaded with Rifaximin for colon targeting. The result showed that the percentage yield of different formulation was in range of 69.85±0.26 – 79.85±0.25%. The maximum percentage yield and entrapment efficiency was found formulation F3. Results of zeta potential of optimized formulation F4 microspheres were found to be -30.50 mV respectively. The average particle size of microspheres was found 185.65, 186.32 and 182.25nm after 1, 2 and 3 month of storage at 4.0 ±0. 2°C while at 25-28±2°C the average vesicle size was found 196.25, 215.65 and 285.45 nm after 1, 2 and 3 month of storage. % EE in microspheres formulation was 73.32, 72.12 and 70.15% after 1, 2 and 3 month of storage at 25-28±2°C while there were no significant changes in % EE and physical appearance in microspheres formulation was observed after 3 month of storage at 4ºC.Thus, from the above results itt can be concluded that the prepared microsphere have all ideal characteristics parameters and can be used for colon targeted delivery.

Keywords: Microsphere, Rifaximin, Colon targeting, Targeted drug delivery

INTRODUCTION

Targeted drug delivery into the colon is highly desirable for local treatment of a variety of bowel diseases such as ulcerative colitis, Crohn's disease, amebiosis, colonic cancer, local treatment of colonic pathologies, and systemic delivery of protein and peptide drugs. The colon specific drug delivery system (CDDS) should be capable of protecting the drug en route to the colon i.e. drug release and absorption should not occur in the stomach as well as the small intestine, and neither the bioactive agent should be degraded in either of the dissolution sites but only released and absorbed once the system reaches the colon (Philip *et al.,* 2009; Oluwatoyin and John, 2005; Akala *et al.,* 2003).

Administration of glucocorticoids namely dexamethasone and methyl prednisolone by oral and intravenous routes produce systemic side effects including adenosuppression,

immunosuppression, cushinoid symptoms, and bone resorption. Thus selective delivery of drugs to the colon could not only lower the required dose but also reduce the systemic side effects caused by high doses. Microspheres are defined as "Monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles" (or) can be defined as structure made up of continuous phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level. The natural polymers include albumin and gelatin, the synthetic polymer include poly lactic acid and polyglycolic acid. Microspheres for oral use have been employed to sustain the drug release, and to reduce or eliminate gastrointestinal tract irritation. In addition, multiparticulate delivery systems spread out more uniformly in the gastrointestinal tract. This results in more reproducible drug absorption and reduces local irritation when compared to single-unit dosage forms such as no disintegrating, polymeric matrix tablets. Unwanted intestinal retention of the polymeric material, which may occur with matrix tablets on chronic dosing, can also be avoided (Kulkarni, 1999; McLeod *et al.*, 1994; Mathew *et al.*, 2008).

MATERIALS AND METHODS

Chemicals

Rifaximin was obtained as gift sample by Bioplus Life Science, Bangalore. Disodium Hydrogen Phosphate and Di potassium Hydrogen Orthophosphate was obtained from S. D. Fine Chem. Ltd., Mumbai. Chitosan used was from Himedia laboratories. Sodium tripolyphosphate was obtained from Loba chemie, Mumbai. Other chemicals used were of laboratory grade.

Organoleptic properties:

Organoleptic properties of the drug substance are very important for designing the dosage form. The colour, odour and tests of the drug are characterized.

Solubility analysis

For the determination of solubility of Rifaximin in various solvents that were methanol, ethanol, chloroform and distilled water etc. 5mg of Rifaximin was added to 10 ml of each solvent in a test tube and shaken for few minutes at room temperature (21.0 ± 1.5°C) (Indian pharmacopeia. 2007).

Loss on drying

Loss on drying was directly measured by IR moisture balance. Firstly calibrated the instrument by knob, then taken 5 gram of sample (powder) and fixed the temperature at 100°C to 105°C for 15 minutes and constant reading, and fixed the knob and check percent moisture (European Pharmacopoeia, 2004).

Melting point:

Melting point of Rifaximin was determined using open capillary method by melting point apparatus. Fine powder of the drug was filled in glass capillary tube which was sealed at one end. The capillary tube was tied to the thermometer and thermometer was kept in theils tube apparatus and then slowly increased the temperature of the apparatus and recorded the temperature at which drug was completely melted. The observed melting point of the drug was compared with melting point given in literature.

Determination of UV visible absorption maxima

A standard stock solution of Rifaximin was prepared by dissolving 10 mg (accurately

weighed) of the standard Rifaximin in 10 ml of methanol. This stock solution was further diluted to get working standard solutions of 10µg/ml. Aliquots (0.05, 0.1, 0.15, 0.2, 0.25ml) of working standard solution were transferred into a series of 10 ml volumetric flasks to get the desired concentration range for calibration curve. The volumes were made up with 7.2 pH phosphate buffer. This solution was perused in UV-Visible Spectrophotometer. The absorbance of these solutions was restrained at 222nm and a graph of concentration versus absorbance was plotted.

FTIR spectroscopy

The purity of pure drug was determined by I.R. Approximately 10 mg of Rifaximin was triturated with 100 mg of dried potassium bromide (KBr) in agatte mortar. Pellet was prepared by using KBr press pellet method. Pellet was scanned between the ranges of 400 to 2000 cm^{-1} with background correction. The spectrum was recorded and major peaks were determined.

Formulation of Chitosan microsphere

Coating of chitosan microspheres

Microspheres were coated with Eudragil S-100 (ES) using solvent evaporation method. Microspheres (50 mg) were dispersed in 10 mL of coating solution prepared by dissolving 500 mg of eudragit S-100 in ethanol: acetone (2:1) to give 5:1 (coat: core ratio). This organic phase was then poured in 70 mL of light liquid paraffin containing 1% wt/vol Span 80. The system was maintained under agitation speed of 1000 rpm at room temperature for 3 hours to allow for the evaporation of solvent. Finally, the coated microspheres were filtered, washed with n-hexane, and dried in desiccators (Priyadarshini *et al.,* 2014).

Table 1: Formultion of chitosan microsphere

Sr. No	Formulation Code	Rifaximin (mg)	Chitosan (mg)	STPP (mg)
1.	F1	50	250	500
2.	F2	50	250	750
3.	F3	50	250	1000
4.	F4	50	500	500
5.	F5	50	500	750
6.	F6	50	500	1000

Evaluation of microspheres

Bulk density

Bulk density is determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder or through a volumetric measuring apparatus into a cup. Bulk density can be calculated by dividing bulk mass by bulk volume.

Compressibility index (Carr's index):

Compressibility index (C.I.) is an important measure that can be obtained from the bulk and tapped densities. Carr's index a material having values of less than 20% to 30% is defined as the free flowing material.

Hausner ratio:

It indicates the flow properties of the powder and it can be measured by the ratio of tapped density to bulk density.

Percentage Yield

The prepared microspheres F1-F6 were collected and weighed from each formulation.

Entrapment efficiency

10 mg of chitosan microspheres from each batch were accurately weighed. The powder of chitosan microspheres were dissolved in 10 ml 7.4 pH Phosphate Buffer and centrifuge at 1000 rpm. This supernatant solution is than

filtered through whatmann filter paper No. 44. After filtration, from this solution 0.1 ml was taken out and diluted up to 10 ml with 7.4 pH Phosphate Buffer. The supernant was analyzed for drug content by measuring the absorbance at 222.0nm.

Measurement of mean particle size

The mean particle size of the nanoparticle was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer (Malvern particle size analyser) at a scattering angle of 90°. A sample (0.5mg) of the nanoparticle suspended in 5 ml of distilled water was used for the measurement (Dhanaraju *et al.,* 2009).

Determination of zeta potential

The zeta potential of the drug-loaded microspheress was measured on a zeta sizer (Malvern particle size analyser) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate (Thejeswini *et al.,* 2014).

***In vitro* drug release in gastrointestinal fluids of different pH**

A weighed quantity of formulation (equivalent to 30mg) was filled in capsule and kept in basket of dissolution apparatus with dissolution media (900 ml) at 37±0.2 C.

Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 5ml by media. The samples withdrawn were assayed spectrophotometrically at 222.0 nm for percent of release Rifaximin using UV visible spectrophotometer. The release of Rifaximin was calculated with the help of Standard curve of Rifaximin.

Drug release kinetic data analysis

Several kinetic models have been proposed to describe the release characteristics of a drug from matrix. The following three equations are commonly used, because of their simplicity and applicability. Equation 1, the zero-order model equation (Plotted as cumulative percentage of drug released vs time); Equation 2, Higuchi's squareroot equation (Plotted as cumulative percentage of drug released vs square root of time); and Equation 3, the Korsemeyer-Peppas equation (Plotted as Log cumulative percentage of drug released vs Log time).

Electron microscopy (SEM)

From the formulated batches of microspheres, formulations (F2) which showed an appropriate balance between the percentage drug releases was examined for surface morphology and shape using scanning electron microscope Jeol Japan 6000. Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 10KV during scanning. Microphotographs were taken on different magnification and higher magnification (200X) was used for surface morphology.

Stability studies for optimized formulation

Stability study data was revealed that the optimized nanoparticle formulation (F2) stable after 3 month of storage at 4ºC while at 25-28±2°C, the formulation was found unstable. Stability of formulation was observed on the basis of % EE, average particle size and physical appearance.

Table 2: Result of flow properties of prepared Rifaximin microspheres

F. Code	Bulk density(gm/cm^3)	Tapped density(gm/cm^3)	Compressibility index	Hausner ratio
F1	0.325	0.412	21.117	1.268
F2	0.347	0.425	18.353	1.225
F3	0.342	0.432	20.833	1.263
F4	0.352	0.462	23.810	1.313
F5	0.341	0.452	24.558	1.326
F6	0.338	0.448	24.554	1.325

Table 3: Percentage Yield for Different Formulation

Formulation	Percentage Yield
F1	68.89±0.25
F2	70.23±0.32
F3	75.56±0.14
F4	65.85±0.22
F5	71.12±0.15
F6	69.95±0.18

Table 4: Entrapment Efficiency for Different Formulation

Formulation	Entrapment Efficiency of prepared microspheres
F1	68.52±0.25
F2	73.32±0.32
F3	79.98±0.15
F4	75.45±0.16
F5	69.98±0.17
F6	68.12±0.32

Table 5: Cumulative % drug release of Rifaximin from plain and Eudragit

S. No.	Dissolution medium	Time (hrs)	% Cumulative Drug Release	
			Chitosan Microspheres	Eudragit S100 Coated Microspheres
1	SGF (pH 1.2)	1	12.23	1.12
2		2	26.65	2.36
3	SGF+SIF(pH 4.5)	3	35.45	3.12
4		4	43.32	6.45
5		5	56.65	10.25
6	SIF (pH 6.8)	6	69.98	18.85
7		7	72.23	23.32
8	SIF (pH 7.4)	8	75.65	45.65
9		9	88.85	58.89
10		10	92.23	63.32
11		12	98.78	79.98

Table 6: *In-vitro* Drug Release Data for Coated formulation

S. No.	Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release± SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
1	1	1	0	1.12	0.049	98.88	1.995
2	2	1.414	0.301	2.36	0.373	97.64	1.990
3	3	1.732	0.477	3.12	0.494	96.88	1.986
4	4	2	0.602	6.45	0.810	93.55	1.971
5	5	2.236	0.699	10.25	1.011	89.75	1.953
6	6	2.449	0.778	18.85	1.275	81.15	1.909
7	7	2.646	0.845	23.32	1.368	76.68	1.885
8	8	2.828	0.903	45.65	1.659	54.35	1.735
9	9	3	0.954	58.89	1.770	41.11	1.614
10	10	3.162	1	63.32	1.802	36.68	1.564
11	12	3.464	1.079	79.98	1.903	20.02	1.301

Table 7: Regression analysis data of microspheres formulation

Formulation	Zero order	First order	Higuchi plot	Pappas plot
F3 (r^2)	0.881	0.826	0.782	0.943

RESULTS AND DISCUSSION

The bulk density and the tapped density for all the formulations varied from 0.325 to 0.352gm/cm^3 and 0.412 to 0.462gm/cm^3 respectively. The result of Hausner's ratio of all formulations ranges from 1.225 to 1.326. The results of the Compressibility index of all the formulations ranges from 18.353% to 24.558%. The percentage yield of different formulation was in range of 69.85±0.26 – 79.85±0.25%. The maximum percentage yield and entrapment efficiency was found formulation F3. Results of zeta potential of optimized formulation F4 microspheres was found to be -30.50 mV respectively. The average particle size of microspheres was found 185.65, 186.32 and 182.25nm after 1, 2 and 3 month of storage at 4.0 ±0. 2°C while at 25-28±2°C the average vesicle size was found 196.25, 215.65 and 285.45 nm after 1, 2 and 3 month of storage. % EE in microspheres formulation was 73.32, 72.12 and 70.15% after 1, 2 and 3 month of storage at 25-28±2°C while there were no significant changes in % EE and physical appearance in microspheres formulation was observed after 3 month of storage at 4ºC.

CONCLUSION

Microspheres loaded Rifaximin have been prepared by easy emulsification method followed by cross-linking . The variables such as drug: polymer ratio and concentration of glutaraldehyde were optimized on the basis of particle size, entrapment efficiency. The prepared microspheres were stable, spherical particles and showed favourable release profiles in simulated colonic fluid. However, additional evaluation of these carriers can be performed for their probable to treat colonic diseases, as a future scope.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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