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FORMULATION, DEVELOPMENT AND EVALUATION OF COLON TARGETING MICROSPHERES FOR EFFECTIVE TREATMENT OF COLONTITIS

Ms. Archana Pachore*; Dr. B. K. Dubey; Dr. Deepak Kumar Basediya; Mr. Anubhav Shrivastava

Technocrats Institute of Technology Pharmacy, Bhopal

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ABSTRACT:

Inflammatory bowel disease (IBD) is a commonly relapsing and relapsing seditious disease of the small and large intestine. IBD includes ulcerative colitis (UC) and Crohn's disease (CD) and is the leading cause of the spread of colon cancer known as cancer-associated colitis (CAC). A bead-based drug delivery system targeting the oral colon containing metronidazole-loaded chitosan beads coated with eudragit S-100 was prepared, optimized, and characterized. Metronidazole-loaded colon-targeted microspheres were prepared with a simple emulsion system followed by a cross-linking system. The variable drug polymer ratio was optimized for based on particle size, capture efficiency, further optimized F2 composition was coated with Eudragit S-100. The prepared microspheres were stable spherical particles and showed favorable release profiles in the simulated colonic fluid.

Keywords: Microspheres, Metronidazole, Inflammatory bowel disease, Colon-targeted, Chitosan

INTRODUCTION

The oral route of drug administration is the most convenient and important method of administering drugs for systemic effect. Nearly 50% of the drug delivery systems available in the market are oral D.D.S. and these systems have more advantages due to patient acceptance and ease of administration [1-2]. During the last decade there has been interest in developing site-specific formulations for targeting drug to the colon. Colonic drug

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delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon like Crohn's disease, ulcerative colitis, irritable bowel syndrome and constipation but also for the systemic delivery of proteins, therapeutic peptides, antiasthmatic drugs, antihypertensive drugs and antidiabetic agents [3-4]. There are various methods or techniques through which colon drug targeting can be achieved, for example, formation of prodrug, coating with pH sensitive polymers, coating with biodegradable polymers, designing formulations using polysaccharides, timed released systems, pressure-controlled drug delivery systems, osmotic pressure controlled systems [5-6]. Coating of the drugs with pH-sensitive polymers provides simple approach for colon-specific drug delivery [7].

- 5-fluorouracil
- 9-aminocamptothecin
- Capecitabine
- Cetuximab
- Trinotecan
- Levamisole hydrochloride
- Oxaliplatin
- Trimetrexate
- UFT (ftorafur and uracil)
- Bevacizumab Cisplatin

Advantages of colon targeting drug delivery system: [8-10]

- Colon is an ideal site for the delivery of agents to cure the local diseases of the colon.
- Local treatment has the advantage of requiring smaller drug quantities.
- Reduces dosage frequency. Hence, lower cost of expensive drugs.
- Possibly leading to a reduced incidence of side effects and drug interactions.
- The colon is an attractive site where poorly absorbed drug molecules may have an improved bioavailability.
- Reduce gastric irritation caused by many drugs (e.g. NSAIDS).

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- Bypass initial first pass metabolism.
- Extended daytime or nighttime activity.
- Improve patient compliance.
- Targeted drug delivery system.
- It has a longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs.
- It has low hostile environment, less peptidase activity so peptides, oral vaccines, insulin, growth hormones, can be given through this route.[11]

Limitations of colon targeting drug delivery system:

a) Multiple manufacturing steps

b) The resident microflora could also affect colonic performance via metabolic degradation of the drug
c) Incomplete release of drug

d) Bioavailability of drug may be low due to potentially binding of drug in a nonspecific way to dietary residues, intestinal secretions, mucus or faecal matter.
e) Drug should be in solution form before absorption and there for rate limiting step for poor soluble drugs.
f) Non availability of an appropriate dissolution testing method to evaluate the dosage form in-vitro [12]
g) An important limitation of the pH sensitive coating technique is the uncertainty of the location and environment in which the coating may start to dissolve. Normal in patients with ulcerative colitis[13-14]
h) Limitations of prodrug approach is that it is not very versatile approach as it's formulation depends upon the functional group available on the drug moiety for chemical linkage. Furthermore prodrugs are new chemical entities and need a lot of evaluation before being used as carriers[15-16]

Methods used for drug targetting to the colon

Formation of prodrugs: (Example: AzoProdrug, Glucuronide conjugate, etc.)

Prodrug is defined as an inert drug that becomes active only after it is transformed or metabolized by the body.[17] Covalent linkage is formed between drug and carrier, which upon oral administration reaches colon without being absorbed from upper part of GIT. In the colon drug release is triggered by high activity of certain

enzymes in comparison to stomach and small intestine.

Azo bond conjugate: Sulfasalazine is mainly used for the treatment of inflammatory bowl diseases. It is 5- Amino

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Salicylic Acid (5-ASA) prodrug. 85% of oral dose of sulfasalazine reaches to the colon unabsorbed, where it is reduced by the anaerobic environment into 5ASA and sulphapyridine [18]

Various studies are conducted on sulphapyridine which lead to the formation of other prodrug like Olsalazine, Balsalazine, 4-amino benzoyl-βalanine [19] Intestinal microflora produces glycosidase, one of prominent group of enzyme. Colon specific formulation of flurbiprofen had been evaluated by using azo-aromatic and pHsensitive polymer and it was concluded that azoaromatic polymer (poly- methylmethacrylate- hydroxy rthylmethacrylate: 1:5) and pH sensitive polymer eudragit S can successfully be used for colonic drug delivery [20] Pulsincap drug delivery of salbutamol sulphate had been investigated. An empty gelatin capsule was coated with ethyl cellulose keeping the cap portion as such. A hydrogel plug made of gelatin was suitably coated with cellulose acetate phthalate in such a way that it was fixed to the body under the cap.

Eudragit microspheres containing the salbutamol sulphate were prepared by emulsion solvent evaporation method and were incorporated into this specialized capsule shell. In vitro dissolution results indicated that the onset of drug release was after 7 to 8 hr of the experiment started [21]. Mutual azo prodrug of 5-aminosalicyic acid with histidine, was synthesized by coupling L-histidine with salicylic acid, for targeted drug delivery to the inflamed gut tissue [22].

Glucuronide conjugate: Glucuronide and sulphate conjugation is the major mechanisms for the inactivation and preparation for clearance of a variety of drugs. Bacteria of the lower gastrointestinal tract secrete glucuronidase that glucouronidate a variety of drugs in the intestine. Since the glucuronidation process results in the release of active drug and enables its reabsorption, glucuronide prodrugs would be expected to be superior for colon targeted drug delivery [23]

Cyclodextrin conjugates: The hydrophilic and ionisable Cyclodextrins can serve as potent drug carriers in the immediate release and delayed release-formulations, while hydrophobic Cyclodextrins can retard the release rate of water. Moreover, the most desirable attribute for the drug carrier is its ability to deliver a drug to a targeted site. Conjugates of a drug with Cyclodextrins can be a versatile means of constructing a new class of colon targeting prodrugs soluble drugs [24]. Ibuprofen prodrugs of α- , β-and γ-Cyclodextrins were investigated [25]. Methotrexate prodrugs of α- and γ-Cyclodextrins were also synthesized and result established the primary aim of masking the

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ulcerogenic potential of free drug, by using 12-fold dose of the normal dose of methotrexate and equivalent doses of the esters [26].

Dextran conjugates: Dextran ester prodrugs of metronidazole have been prepared and characterized. Dextran ester prodrugs of dexamethasone and methyl prednisolone was synthesized and proved the efficacy of the prodrugs for delivering drugs to the colon. Methyl prednisolone and dexamethasone were covalently attached to the dextran by the use of a succinate linker [27].

Amino-acid conjugates: Due to the hydrophilic nature of polar groups like NH2 and COOH, that is present in the proteins and their basic units (i.e. the amino acids), they reduce the membrane permeability of amino acids and proteins. Various prodrugs have been prepared by the conjugation of drug molecules to these polar amino acids. Non-essential amino acids such as tyrosine, glycine, methionine and glutamic acid were conjugated to Salicylic acid [28-29].

Coating with pH dependent polymers:

The pH in the terminal ileum and colon in higher than in any other region of the gastrointestinal tract and thus dosage forms which disintegrate at high pH ranges can be target into the region. A level of pH is higher in the terminal ileum region then in the cecum. Dosage forms are often delayed at the ileocecal junction, careful selection of enteric coat composition and thickness is needed to ensure that disintegration does not occur until the dosage from moves through the ileocecal junction from the terminal ileum into the cecum. Synonyms for eudragit are Eastacryl.

Microspheres for Drug Delivery

Microspheres can encapsulate many types of drugs including small molecules proteins, and nucleic acids and are easily administered through a syringe needle. They are generally biocompatible, can provide high bioavailability, and are capable of sustained release for long periods of time. Several commercial products are based on polymer microspheres including Lupron Depotr and Nutropin Depotr. Disadvantages of microspheres include difficulty of large-scale manufacturing, inactivation of drug during fabrication, and poor control of drug release rates.

For example, Nutropin Depot, comprising Genentech's recombinant human growth hormone (rhGH) encapsulated within poly (d,llactide-co-glycolide) (PLG) microspheres using Alkermes' proprietary ProLeaser encapsulation

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technology, was recently pulled from the market because manufacturing and production costs were too high.

Fabrication of polymer micro- and nanoparticles

Microsphere drug delivery systems have been fabricated by a variety of techniques including combinations of phasese parationor precipitation, emulsion/solvent evaporation, and/or spraying methods Variations of the fabrication parameters generally allow control of the particle size and size distribution. Drugs may be incorporated into the particles in several different ways depending on the properties of the drug. Hydrophobic therapeutics may be co-dissolved with the polymer in a solvent such as methylene chloride or ethyl acetate. Hydrophilic therapeutics, including. Alternatively, an aqueous solution of a hydrophilic therapeutic may be mixed with the organic polymer solution to form a water-in-oil emulsion.

Techniques for fabricating uniform microspheres

As pointed out in the preceding sections, an important limitation in the development of biodegradable polymer microspheres for controlled-release drug delivery applications has been the difficulty of specifically designing systems exhibiting precisely controlled release rates. Because microparticle size is a primary determinant of drug release [30-31] it is worthwhile to develop a methodology for controlling release kinetics employing monodisperse microspheres.

Uniform polymer microspheres produced by PPF technology

Uniform microspheres of controlled sizes, both solid and hollow, were previously fabricated from a variety of non-polymeric materials using acoustic excitation. More recently, we have demonstrated the PPF technology for fabricating monodisperse microspheres of various polymers such as PLG, polyanhydrides, EC, chitosan, gelatin hydrogel, and hetastarch, with average diameters from ~4 μm to >500 μm.

These micrographs clearly demonstrate that the PPF technology: (a) can produce uniform polymeric microspheres with precisely controlled sizes.

Precision core-shell micro particle fabrication

Following previous work reporting production of core/shell particles made from a variety of inorganic materials, the uniform solid micro sphere fabrication methodology described in the preceding sections has been further extended to

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produce uniform double-walled polymeric microspheres with controllable size and shell thickness. As before, the non-solvent carrier stream surrounding the coaxial jet accelerates and makes it thinner before its breakup. The orientation of the jets, material flow rates, and rate of solvent extraction are controlled to vary the shell thickness.

The development of PPF technology has allowed the production of uniform microspheres and double-wall microspheres capable of efficiently encapsulating model drugs .Of primary importance was the ability of monodisperse microsphere formulations to eliminate initial drug burst while modulating the onset of steady drug release. Modified PPF technology has also been established as a single-step method for producing uniform polymeric microcapsules of controllable size and shell thickness. Monodisperse or precisely defined particle size distributions can be achieved while maintaining the desired polymeric shell thickness. Exact control of the volumetric flow-rates of the core and shell materials also allows the formation of particle populations exhibiting discretely or incrementally increasing shell thickness. Controlled release systems, especially those comprising biodegradable polymer microparticles, have been heavily studied and have even reached the clinic in several cases. However, notable limitations remain, especially in controlling delivery rates. Monodisperse PPF microspheres and core-shell microparticles offer advantages in reproducibility, control, and consistency that may provide valuable assistance in designing advanced drug delivery systems. The FFESS technique is capable of producing nanometer-scale solid particles as small as 10 nm or even smaller, and may be applicable to fabrication of nanocapsules. However, to achieve precise control of the particle size and reproducibly fabricate nanocapsules the technology needs to be further refined and developed.

PREPARATION AND CHARACTERIZATION

Preparation of colon targeting microspheres of Metronidazole

Chitosan microspheres were prepared by ionotropic gelation method [32]. Chitosan stock solution (1% w/v) was prepared by dissolving chitosan in acetic acid (1% v/v) at room temperature. The drug (2-5 mg) was dissolved in chitosan solution. 1% Sodium tripolyphosphate solution was prepared in water. Sodium tripolyphosphate solution was added drop wise with a syringe to chitosan solution while stirring. The solution was magnetically stirred for half an hour followed by filtration and rinsing with distilled water. Microspheres were obtained which was air dried for twenty four hours followed by oven drying for six hours at 40˚C.

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Formulations of chitosan microspheres prepared

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 [33]

Evaluation of microspheres

There are many formulations and process variables involved in mixing step and all these can affect characteristics of blend produced, bulk density, true density and percent compressibility index have been measured which are given in table.

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.

Procedure:-

A known quantity of powder was poured into the measuring cylinder carefully level the powder without compacting, if necessary and read the unsettled apparent volume, Vo, to the nearest graduated unit. Calculate the bulk density, in gm per ml gm/ml, by the formula

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Bulk density = Bulk Mass/ 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.

It can be calculated as per given formula:

$$C.I = \frac{\text{Tapped density- Bulk density}}{\text{Tapped density}} \times 100$$

Hausner ratio:

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

Hausner ratio = Tapped density / Bulk Density

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.

***In-vitro* Release Studies**

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

The prepared microspheres were evaluated for *in vitro* drug release. The drug release studies were carried out using USP I Basket type dissolution test apparatus. The dissolution study was carried out in 900 ml dissolution medium which was stirred at 100 rpm maintained at 37±0.2□C. The scheme of using the simulated fluids at different timing was as follows:

1st hour: Simulated gastric fluid (SGF) of pH 1.2.

2nd and 3rd hour: Mixture of simulated gastric and Intestinal fluid of pH 4.5.

4th to 5th hour: Simulated intestinal fluid (SIF) of pH 6.8.

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6^{th} hour and onward: SIF pH 7.4

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 242.0 nm for percent of release
Metronidazole using UV visible spectrophotometer. The release of Metronidazole was calculated with the help of Standard curve of Metronidazole.

RESULTS AND DISCUSSION

Evaluation of Metronidazole microspheres

Results of flow properties of prepared Metronidazole microspheres

The loose bulk density (LBD) and Tapped bulk density (TBD) of the microspheres of different formulations were evaluated before the compression of powders in to tablets. The bulk density and the tapped density for all the formulations varied from
0.355 to 0.385gm/cm^3 and 0.456 to 0.485gm/cm^3 respectively.
The values obtained lies within the acceptable range. The difference exists between the bulk density and tapped density found to be very few. This result helps in calculating the % compressibility of the powder.

The result of Hausner's ratio of all formulations ranges from 1.260 to 1.304. Results of Hausner's ratio of all formulations were shown in Table no 8.1 which indicates that the flow ability of all the formulation.

The results of the Compressibility index of all the formulations ranges from 20.619% to 23.305%. Results of Compressibility index of all the formulations were shown in the Table no 8.1. Results clearly showed that the flow ability of all the formulations was good and also the powder had good compressibility.

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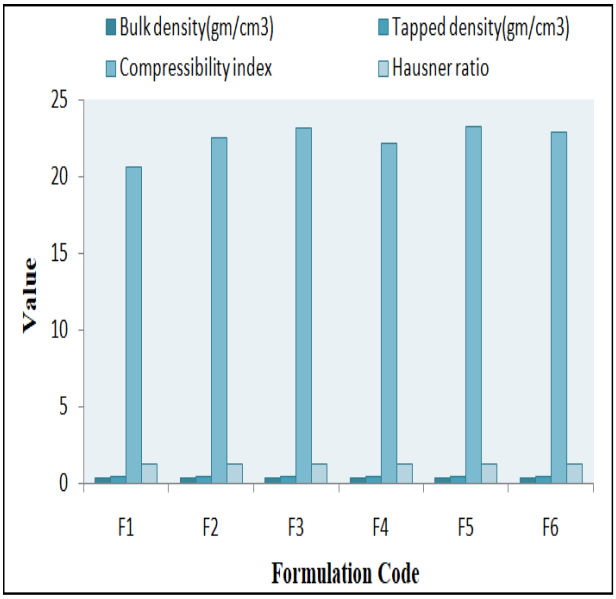
Result of flow properties of prepared Metronidazole



F. Code	Bulk density(gm/cm^3)	Tapped density(gm/cm^3)	Compressibility index	Hausner ratio
F1	0.385	0.485	20.619	1.260
F2	0.374	0.483	22.567	1.291
F3	0.365	0.475	23.158	1.301
F4	0.355	0.456	22.149	1.285
F5	0.362	0.472	23.305	1.304
F6	0.374	0.485	22.887	1.297

Result of pre-compression properties of Metronidazole microspheres Percentage Yield Percentage yield of different formulation was determined by weighing the microspheres after drying. The percentage yield of different formulation was in range of 67.75±0.16 – 76.65±0.15

Percentage Yield for Different Formulation

Formulation	Percentage Yeild
F1	73.32±0.25
F2	76.65±0.15
F3	68.85±0.32
F4	74.45±0.14
F5	67.75±0.16
F6	68.85±0.20

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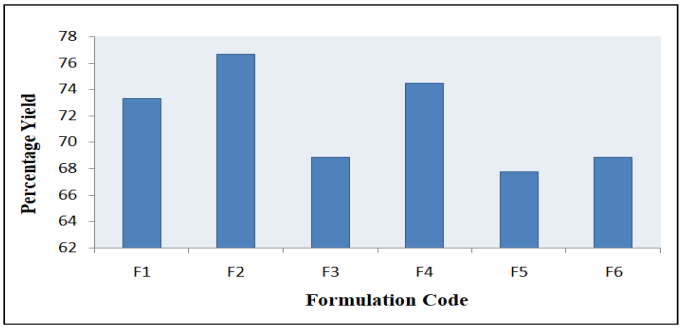
Percentage yield for different formulation



Entrapment efficiency

The drug entrapment of different formulations was in range of 67.75±0.14 to 76.65±0.25% w/w. This is due to the mucoadhesion characteristics of chitosan that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of Metronidazole microspheres.

Formulation	Entrapment Efficiency of prepared microspheres
F1	73.32±0.32
F2	76.65±0.25
F3	68.85±0.15
F4	74.45±0.36
F5	67.75±0.14
F6	68.85±0.26

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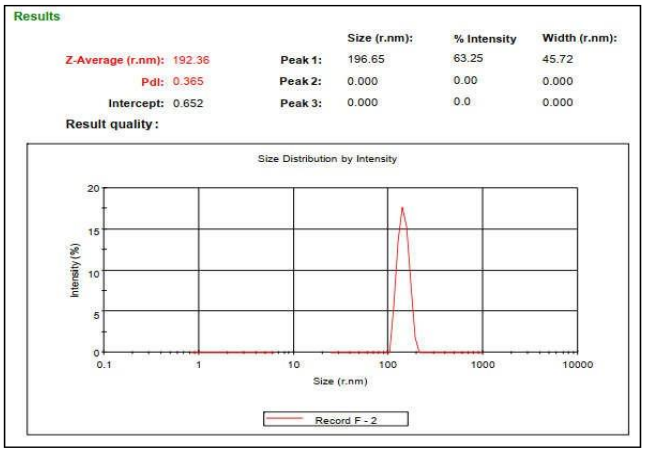
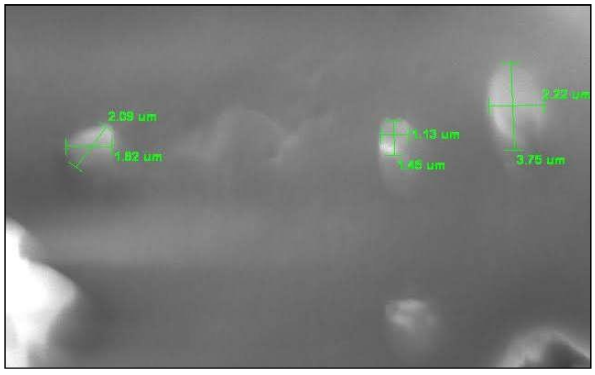
Particle size data of chitosan microspheres (F2)



Scanning Electronic Microscopy of optimized formulation (F2)



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***In vitro* drug release**

Cumulative % drug release of Metronidazole from plain and Eudragit S100 coated microspheres at different pH

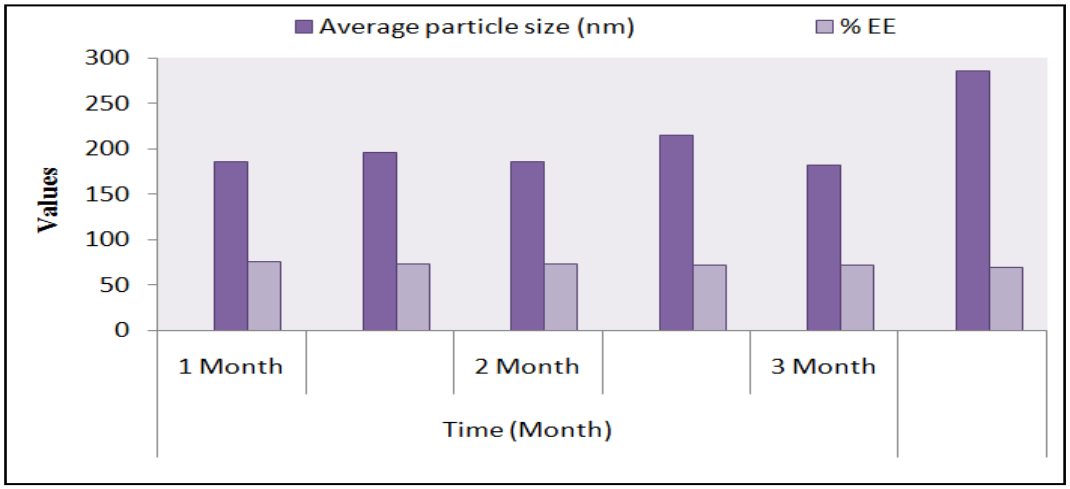
Regression Analysis Data of microspheres Formulation

Formulation	Zero order	First order	Higuchi plot	Pappas plot
F2	R^2 = 0.907	R^2 = 0.737	R^2 = 0.803	R^2 = 0.944

Stability studies

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.

Graph of stability study of Optimized formulation of microspheres F2



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Colon targeting Microspheres loaded Metronidazole have been prepared by easy emulsification method followed by cross-linking method. The variables such as drug: Polymer ratios were optimized on the basis of particle size, entrapment efficiency, further optimized formulation F2 was coated with Eudragit S-100. The prepared microspheres were stable, spherical particles and showed favorable release profiles in simulated colonic fluid.

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