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STUDY OF ENTERIC COATED SODIUM ALGINATE MICROSPHERE OF AZATHIOPRINE FOR COLON TARGETING

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ABSTRACT

The increase in incidence and prevalence of IBD over the last 15 years and its prevalence in most of the developing countries give the tremendous opportunity for the researchers to develop a colon-specific delivery of drugs for local and continuous effect. Microparticiulate drug delivery system have developed and tested for colon targeting. The aim of the study was to prepare site specific drug delivery of Azathioprine using sodium alginate, Eudragit S-100 and Ethyl cellulose as a pH sensitive polymer, respectively and to study the effect of coating of different polymers at different concentration. Core alginate microspheres of were prepared by ionotropic gelation method followed by cross linking with CaCl2, which was further coated with the pH dependent polymer Eudragit S-100 and Ethyl cellulose to prevent drug release in the upper gastrointestinal environment. Core and coated microspheres were characterized by FTIR spectroscopy, DSC, SEM, particle size analysis, drug content, entrapment efficiency, and *In vitro* drug release study in different simulated gastric fluids. SEM images revealed that the surface morphology was changed from rough surface to smooth surface after coating. FTIR study confirmed the compatibility of core and coated polymer with drug. The release of drug from core alginate microsphere release were pH independent and released 93.45%, 84.65% and 80.01% better controlled release at the end of 12 hrs for the formulationFA1, FA2, and FA3 respectively, followed Higuchi release kinetic model. Whereas the coated microsphere, the drug release was achieved only in pH 7.4 and avoiding release of drug in lower GIT pH environment. The Eudragit S100 coated formulation FB1 and FB2 release the drug after pH 7.4 whereas the Ethyl cellulose coated formulation FC1 and FC2 start to release the drug at pH 6.8. The release of coated formulation follows first order kinetics.

KEYWORDS: Azathioprine, Sodium alginate, Ionic gelation , Ethyl cellulose, Eudragit S 100, Multiparticulate system.

INTRODUCTION

Since past 70 years, it has profound insights into the physiology, physical chemistry of organs, biology cells, membranes, compartments, cellular organelles and functional proteins related with the absorption processes of drugs in the gastrointestinal tract (GIT). Oral colon targeted drug delivery systems has increased, for treatment of local colonic disorders. Colonic delivery offers several potential therapeutic advantages as a site for drug delivery because colon is rich in lymphoid tissue, uptake of antigens into the mast cells of the colonic mucosa produces rapid local production of antibodies and this helps in efficient vaccine delivery, where poorly absorbed drug molecule may have an improved bioavailability, reduced proteolytic activity in the colon, longer retention time, reduced fluid motility and motility in the colon when compared with small intestine.[1]

Dosage forms that deliver drugs in the colon rather than upper GIT has number of advantages. Oral delivery of drugs in the colon is valuable in the treatment of diseases of colon where by high local concentration can be achieved while minimizing side effects. The colon is attracting interest as a site where poorly absorbed drug molecule may have an improved bioavailability because the colon has a long retention time and appears highly responsible to agents that enhance the absorption of poorly absorbed drugs. The simplest method for targeting of drugs to the colon is to obtain slower release rates or longer release periods by the application of thicker layers of conventional enteric coating or extremely slow releasing matrices. 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. Coating of the drugs with pH sensitive polymers provides simple approach for colon-specific drug delivery.[2]

Because of the high water absorption capacity of the colon, the colonic contents are considerably viscous and their mixing is not efficient, thus availability of most drugs to the absorptive membrane is low. 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 azo reduction and enzymatic cleavage i.e. glycosides.8 These metabolic processes may be responsible for the metabolism of many drugs and may also be applied to colon targeted delivery of peptide based macromolecules such as insulin by oral administration.[3]

Azathioprine, an immunosuppressive antimetabolite has been used in the treatment of inflammatory bowel disease, ulcerative colitis and Crohn's disease. Azathioprine is a prodrug of 6-mercaptopurine that is further metabolized by various enzymes present in the liver and gut have proven efficacy in the treatment of inflammatory bowel disease. These drugs may reduce the need for steroid treatment and their use may therefore lead to a lower incidence of steroid related side effects. Apart from its therapeutic benefit, Azathioprine has various toxic effects that include suppression of haemopoietic system, hepatotoxicity and teratogenicity. Its oral bioavailability is limited to an extent of 41-50%. Plasma half-life of Azathioprine ranges from 3 to 5 hrs and because of short biological half-life; it has to be administered frequently to maintain effective plasma concentration.[4,5]

Hence in the present investigation we are aimed to develop a colon-specific microsphere delivery system of Azathioprine using Ethyl cellulose and Eudragit S 100 polymers as a carrier and to develop the colon-specific delivery that has potential for use as an adjuvant therapy for inflammatory bowel disease.

MATERIALS AND METHODS

The Azathioprine was gifted sample by sturdies acro lab Ltd. (Bangalore, India); sodium alginate; Eudragit S-100 Calcium chloride and hydrochloric acid; n-hexane, Disodium hydrogen phosphate, Potassium di-hydrogen phosphate, Ethyl acetate Span 80 sodium chloride sodium Hydroxide Pellets were supplied by S D fine chemicals Ltd, Mumbai.

Design and Formulation of Multiparticulate System of Azathioprine

Preparation of drug loaded sodium alginate Microspheres

The Azathioprine was dispersed in an aqueous solution of 3%, 4%, and 5%.w/v (Table 1)sodium alginate with stirring to produce a viscous form. Then polymer drug solution was added drop wise by using syringe of 22 G in diameter from a height of about 5cm into a beaker containing 5% w/v solution of calcium chloride with continuous stirring by magnetic stirrer. Then the solution containing the gel formed microspheres were filtered by using

Whatman filter paper no-1. The microspheres were allowed to dry at about 30 to 40°C and stored in well-closed container for further use.[6]

Preparation of Eudragit S-100 coated sodium alginate microspheres

Sodium alginate microspheres were coated with Eudragit S100 using coacervation phase separation technique. Sodium alginate microspheres equivalent drug were dispersed in 10ml of coating solution prepared by dissolution of Eudragit S100 in ethanol: acetone (2:1) to give 1:3,1:6.(coat: core ratio) and containing 0.2%w/v Span 80.(Table 2) This mixture was agitated for 5min at 600rpm. Subsequently 50ml n-hexane (as the non-solvent) was poured into the polymeric solution containing the core material with the rate of 1ml/min. The medium was stirred for 60min to complete the process of microparticles coating. Coated microspheres were then washed with an excess of n-hexane, filtered and dried at room temperature.[7]

Preparation of Ethyl cellulose coated sodium alginate microspheres

Sodium alginate microspheres were coated with Ethyl cellulose using coacervation phase separation technique. Sodium alginate microspheres were dispersed in 10ml of coating solution prepared by dissolution of Ethyl cellulose in ethanol: acetone (2:1) to give 1:3,1:6.(coat: core ratio) and containing 0.2%w/v Span 80.This mixture was agitated for 5min at 600rpm. Subsequently 50ml n-hexane (as the non-solvent) was poured into the polymeric solution containing the core material with the rate of 1ml/min. The medium was stirred for 60min to complete the process of microparticles coating. Coated microspheres were then washed with an excess of n-hexane, filtered and dried at room temperature.[7]

Evaluation of Core and Coated Microsphere

The prepared core and coated microspheres were evaluated for various parameters like FTIR studies, particle size analysis, surface morphology, drug content, entrapment efficiency, DSC investigation and *in vitro* release studies are evaluated.[8.9.10]

Drug-excipients interactions studies by FTIR

Drug-excipients compatibility studies were carried out FTIR (Shimadzu IR affinity-1, Japan). Infrared spectrum of pure drug (Azathioprine) and optimized coated microspheres were carried out to investigate any changes in chemical composition of the drug after combination it with the excipients.

Surface morphology

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface. SEM studies were carried out by using JEOL JSMT-330A scanning microscope (Japan). The samples of SEM were prepared by lightly spinkling the microspheres powder on a double adhesive tape, which was stuck on an aluminum stub. The stubs were then coated with gold to thickness of about 300Å using a sputter coater. The photomicrographs were taken with the help of SEM analyzer.

In-vitro drug dissolution[11]

The drug release study was carried out in the United States of Pharmacopeia (USP) dissolution apparatus II for the core and coated formulation drug at 37± 5 ^{0}C. For simulation of physiological conditions, the study was carried out at three different pH conditions, namely, at pH 1.2and 6.8 and 7.4 initially, the drug release were determined in 900 mL of 0.1N (pH 1.2) hydrochloric acid containing 0.01% SLS for 2 hrs. After 2 hrs, 25.92 g disodiumhydrogen phosphate and 10.305 g di-hydrogen potassium phosphate were added to increase the pH to 6.8 and the drug release study was continued for another 6 hours. After the 6 hours, 2.142 g disodium hydrogen phosphate and 0.171 g sodium chloride were again added in order to increase the pH up to 7.4 and the study was continued for up to 80 % drug release. The samples were withdrawn at suitable intervals and replaced with fresh medium and analyzed UV spectrophotometrically at 279 nm. Drug release mechanism was determined by finding the best fit of the release data.

RESULT AND DISCUSSION

DSC thermograph of pure drug showed endothermic peak at 288.73℃(Fig- 1). The coated microsphere with Eudragit S 100 and Ethyl cellulose (Fig-2, and Fig-3) the endothermic peak was not observed because of the dilution effect of coating polymer and confirmed the sodium alginate microsphere is coated properly.

FTIR spectra of pure Azathioprine showed sharp characteristic peaks at 2919cm^{-1} 1077 cm^{-1}, 1018 cm^{-1}, and 1594 cm^{-1}. FTIR spectra of drug and coated formulation graphs are showed in (Fig-4, Fig-5, and Fig-6) showed the same characteristic peak with less intensity due to coating confirmed the compatibility.

The size analysis of prepared microsphere formulation was done by optical microscope for both core and coated microsphere. The effect of polymer concentration on the average

particle size of microsphere was studied. From the results we observed that for FA1 formulation maximum number of particles were in the range of 300 – 600 µm and on increasing the sodium alginate concentration, the particle size was found to be increased for FA2 formulation in which the maximum number of particle lies in the range of 600 – 900 µm and for FA3 formulation the maximum number particles were in the size range of 900 – 1200 µm as shown in (Fig-7) These results clearly stated that as the concentration of sodium alginate increases the size of the microsphere were also increased. In case of coated (Fig-8) microsphere we have observed that the particle size was increased than that of the core microsphere due to increase in coating thickness. The optimized core microsphere FA2 was coated with different concentration of Eudragit S100 and Ethyl cellulose. For the coated formulation the maximum number of particle size were observed in the range of 500 – 1000 µm for FB1 and FC1and increased polymer concentration the size range were shifted to 1000 – 1500 µm FB2 and FC2(Fig-10) The average particle sizes of different formulation were depicted in (Fig-10). From the result, we observed that, increase in the concentration sodium alginate in the formulation FA1 to FA3, the average particle size was also increased. The average particle size (Fig-9) of 482.83 µm was observed in FA1, 773.38 µm for FA2 and 1062.18 µm for FA3. The average particle size of Eudragit S100 coated microsphere were 925.23 µm and 1260.62 µm for the formulation FB1 and FB2 respectively. Similar results were observed for the Ethyl cellulosed coated formulation FC1 and FC2 with average particle size of 1390.34 µm and 1130.28 µm.

The SEM photographs of optimized microsphere formulation FA2 as shown in (Fig-11). The porous structure and rough surface were observed in uncoated microsphere. SEM photographs of coated microsphere formulation showed the smooth coating of Eudragit S 100 and Ethyl cellulose over the microsphere, the disappearance of rough surface due smooth coated surface confirmed the microsphere were well coated with Eudragit S 100 and Ethyl cellulose (Fig-12, Fig-13).

All the core and coated formulation showed higher % drug content(Fig.14) and % EE (Fig.15) and it was observed that these values are increased as the polymer concentration increased and coated microsphere showed higher values than the core microsphere. *In vitro* release study of Azathioprine from various uncoated microsphere formulations (Fig-16) FA1 to FA3 was conducted for 12 hrs by using USP basket type dissolution test apparatus, using phosphate buffer pH 7.4. The amount of drug release from formulation FA3 was showed

80.01% which was lower among the formulations FA1 to FA3. From the results we observed that the releases of drug from uncoated microsphere were varied according to concentration of sodium alginate content. It has been concluded that, if we increase the concentration of sodium alginate the release of drug also decreases. The drug release from core microsphere confirmed that drug was released within 10-12 hrs. This results suggested that not possible to colon region for targeting and also it was reported that sodium alginate could release the drug without pH dependency of GIT. Hence there is a need arises to coat the sodium alginate core microsphere with enteric polymer to make the release of drug only in the colon region. The optimized core microsphere FA2 was coated with different concentration of Eudragit S100 and Ethyl cellulose.

The in-vitro release profile of the coated microspheres (Fig-17) was carried out at different pH to mimic the GIT environment. Initially the release study was conducted in pH 1.2 and the results revealed the absence of drug release for the 2 hours tested. The pH was gradually increased from pH 1.2 to pH 5.5and release was determined for 2 to 4 hrs further the pH 5.5 is shifted to pH 6.8, the drug release was negligible. However, as the pH of the release medium was raised beyond 7, the drug was released into the medium. This was expected, as Eudragit S100, an enteric copolymer made of methacrylic acid-methyl methacrylate, dissolves at a pH 7.4. As the release medium pH was increased to 7.4, FB1 microspheres showed around 8% drug release in 2 hrs. against 4% drug release by FB2 in dissolution medium at pH 7.4.. Where as in Ethyl cellulose coated formulation FC1 and FC2 shows around 9% and 5% drug release in the period of 2 hrs. However, Ethyl cellulose coated microsphere showed drug release in pH 6.8. The amount of coating in FB2 and FC2 was larger, hence it took longer time to dissolve, as increased thickness of coating in microsphere.

The formulation with lower concentration of coating material FB1(82.16%) and FC1(84.24%) showed slightly higher drug release than that of higher one within 12-14 hrs period However the Eudragit S 100 coated could start release the drug only in colon region can improve the efficiency of drug in colon targeting. Release kinetic study (Table.4) revealed that all the core formulation follows Higuchi release model. Whereas coated microsphere sowed Zero order drug release pattern. The ‘n’ values for all the formulation were found to be more than 0.5. This indicates that the release approximates non-Fickian.

Table. 1: Formulation design of Uncoated Azathioprine microspheres.

Formulation code (Uncoated microspheres)	Drug: polymer ratio (w/w)	Polymer to cross linking agent- Ratio(w/w)	Time of cross Linking (min)

		(Calcium chloride)	
FA1	1:3	5%	10min
FA2	1:4	5%	10min
FA3	1:5	5%	10min

Table. 2: Formulation design of Eudragit S-100 coated Azathioprine microspheres.

Formulation code (coated microspheres)	Core: coat ratio (w/w)	Polymer to cross linking agent- Ratio (w/w) (Span 80%w/v)	Time of cross linking(min)
FB1	1:3	0.2%w/v	60min
FB2	1:6	0.2%w/v	60min

Table. 3: Formulation design of Ethyl cellulose coated Azathioprine microspheres.

Formulation code (coated microspheres)	Core: coat ratio (w/w)	Polymer to cross linking agent- Ratio (w/w) (Span 80%w/v)	Time of cross linking(min)
FC1	1:3	0.2%w/v	60min
FC2	1:6	0.2%w/v	60min

Table. 4: Release kinetics of core and coated formulation.

Formulation code	Zero order r^2	First order r^2	Higuchi plot r^2	Pappas plot	
				n	r^2
FA1	0.829	0.982	0.992	1.218	0.564
FA2	0.837	0.963	0.981	1.217	0.587
FA3	0.907	0.993	0.997	1.207	0.619
FB1	0.892	0.829	0.663	1.709	0.755
FB2	0.873	0.815	0.637	1.661	0.735
FC1	0.940	0.905	0.762	1.806	0.868
FC2	0.923	0.894	0.733	1.780	0.860

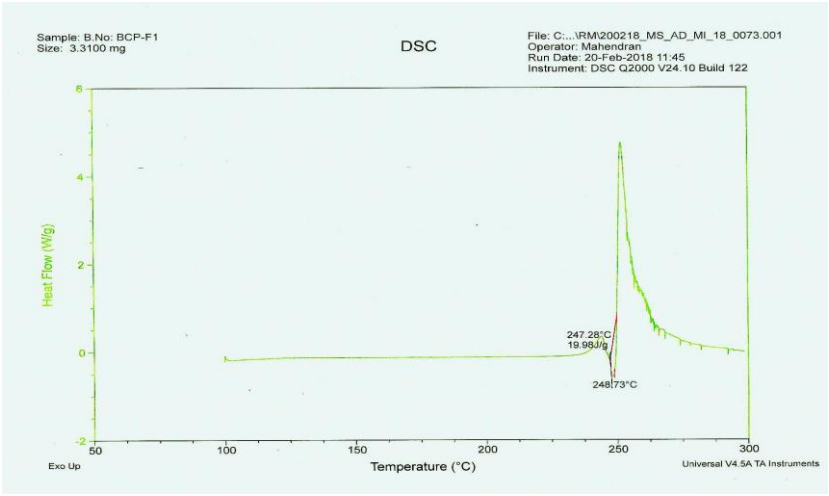

Fig. 1: DSC Thermograph of Azathioprine.

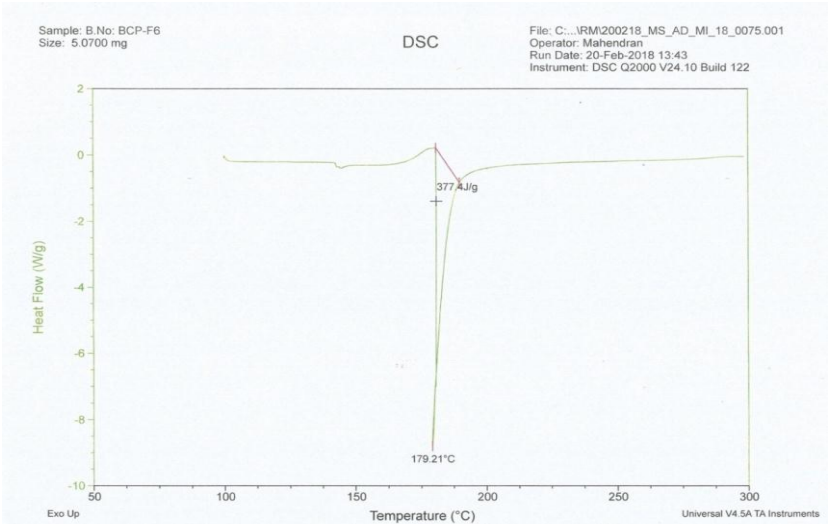

Fig. 2: DSC thermograph of Eudragit coated microsphere.

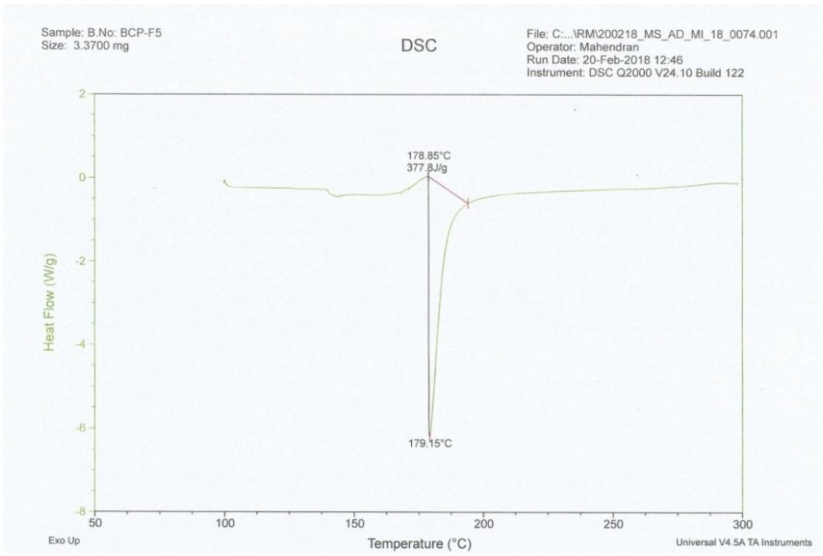

Fig. 3: DSC thermograph of Ethyl cellulose coated microsphere.

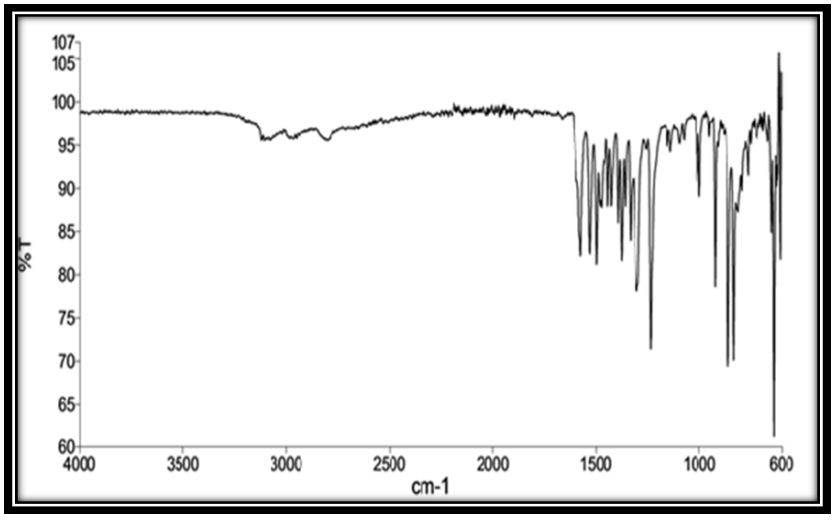

Fig. 4: FTIR spectrum of Azathioprine.

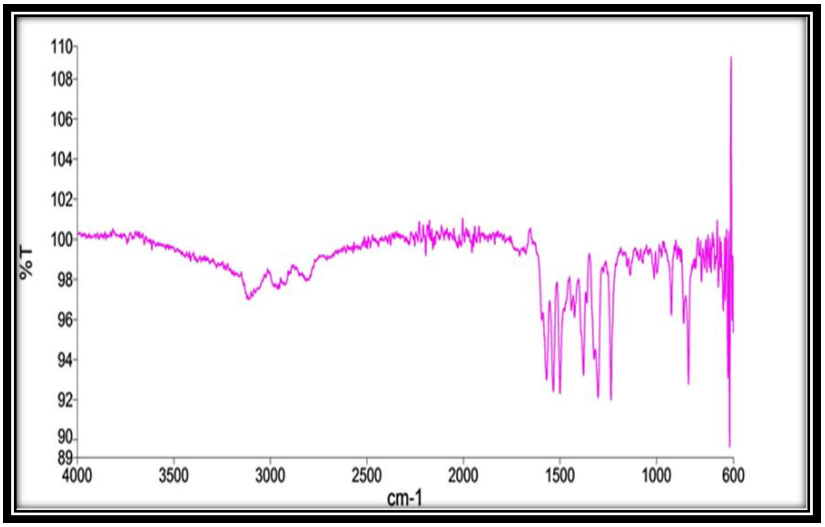

Fig. 5: FTIR spectrum of Eudragit coated microsphere.

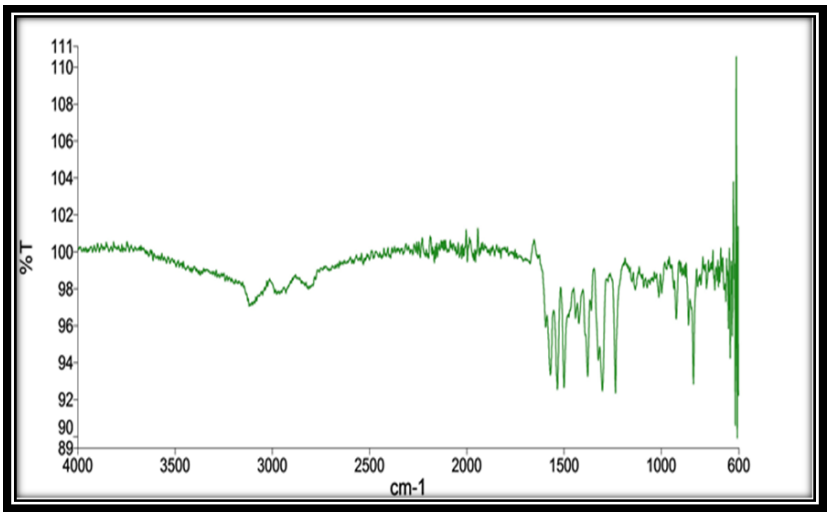

Fig. 6: FTIR spectrum of Ethyl cellulose coated microsphere.

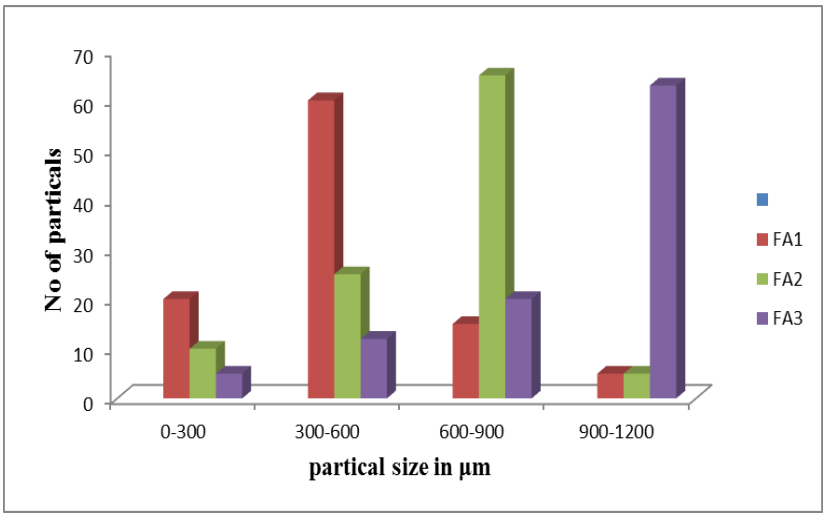

Fig. 7: Particle size distribution of uncoated microsphere.

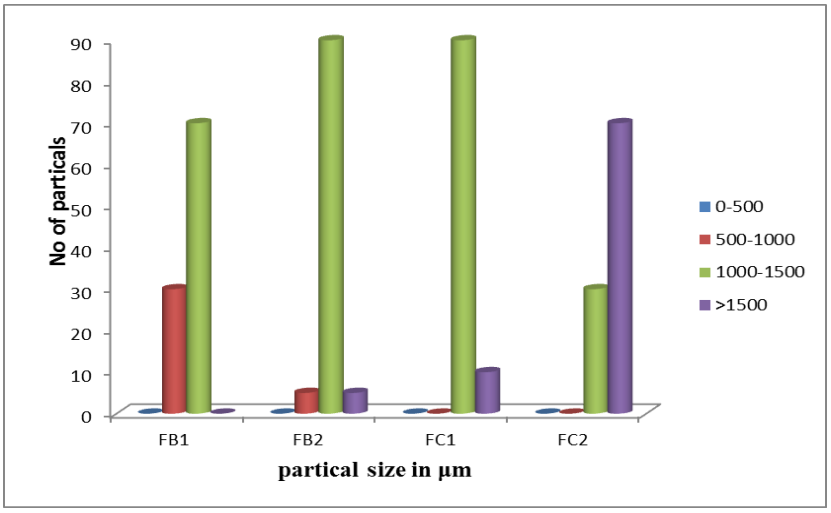

Fig. 8: Particle size distribution of coated microsphere.

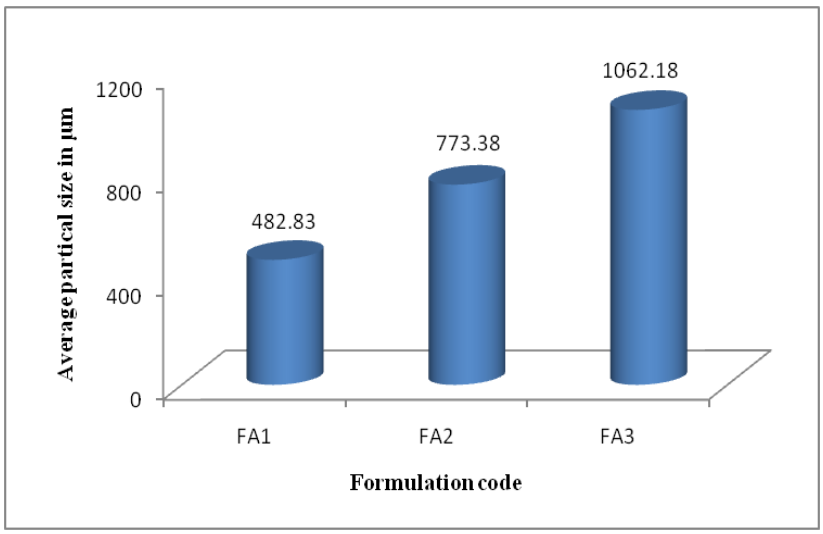

Fig. 9: Average particle size of uncoated microsphere.

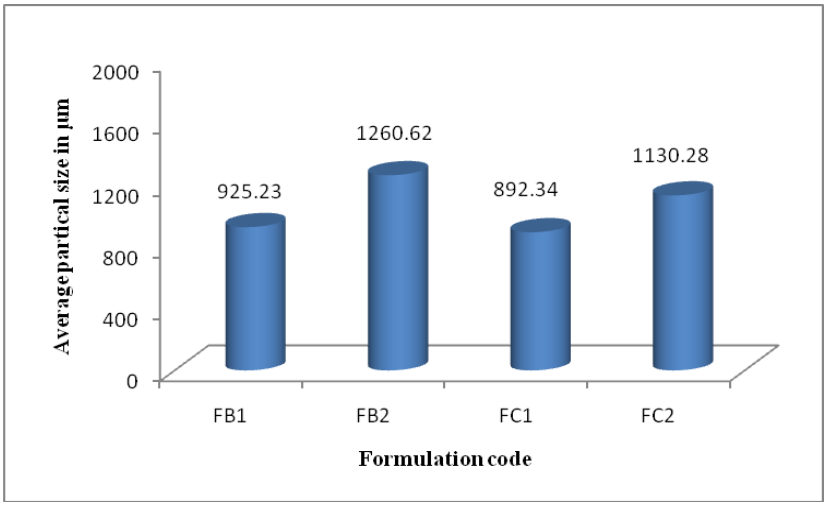

Fig. 10: Average particle size of coated microsphere.

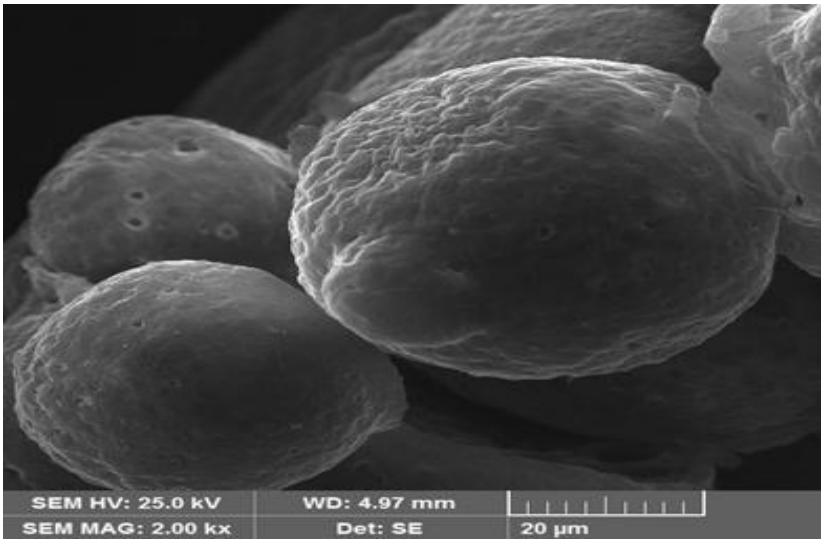

Fig. 11: Scanning electron micrograph of uncoated Microsphere FA2.

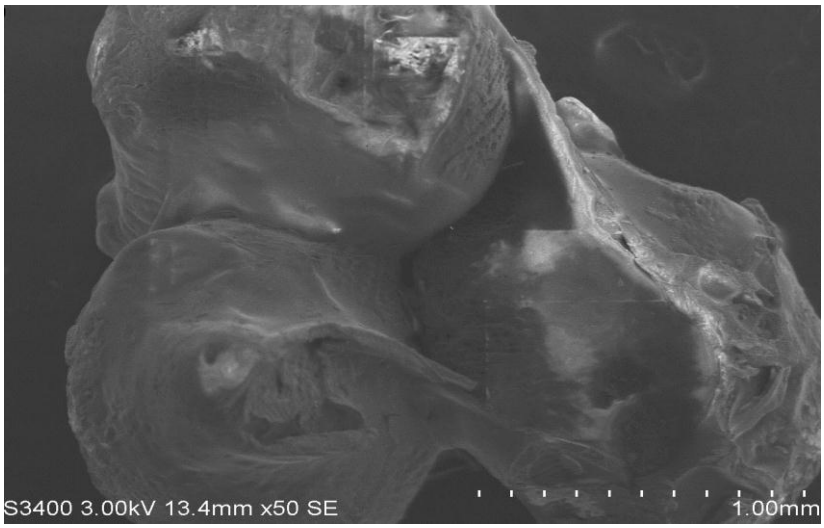

Fig. 12: Scanning electron micrograph of Eudragit coated microsphere FB1.

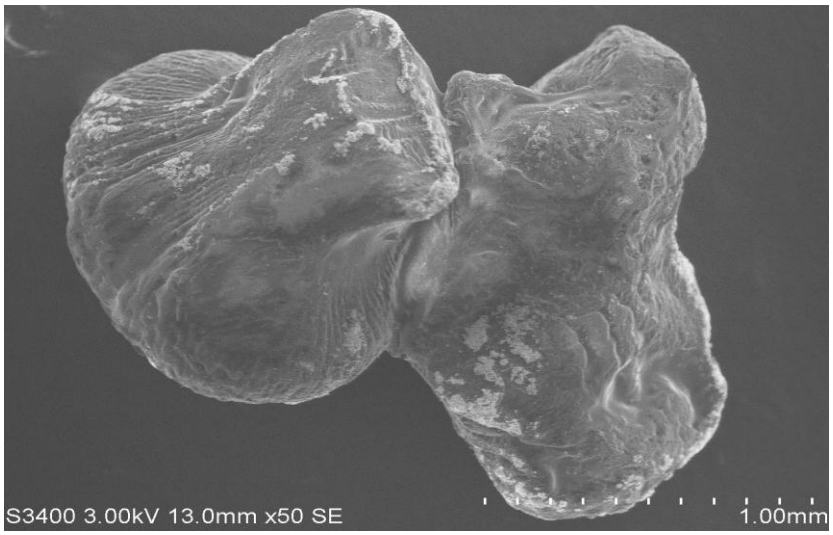

Fig. 13: Scanning electron micrograph of Ethyl cellulose coated microsphere FC1.

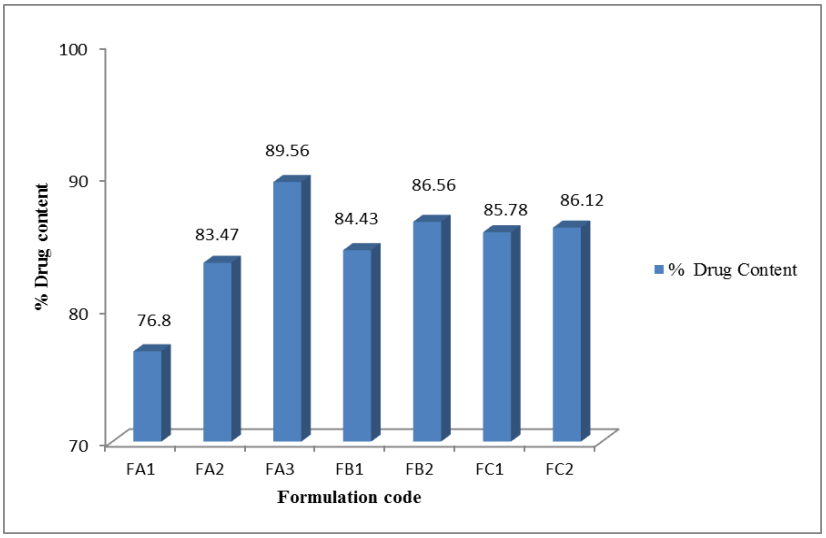

Fig. 14: percentage Drug content of formulation from FA1-FC2.

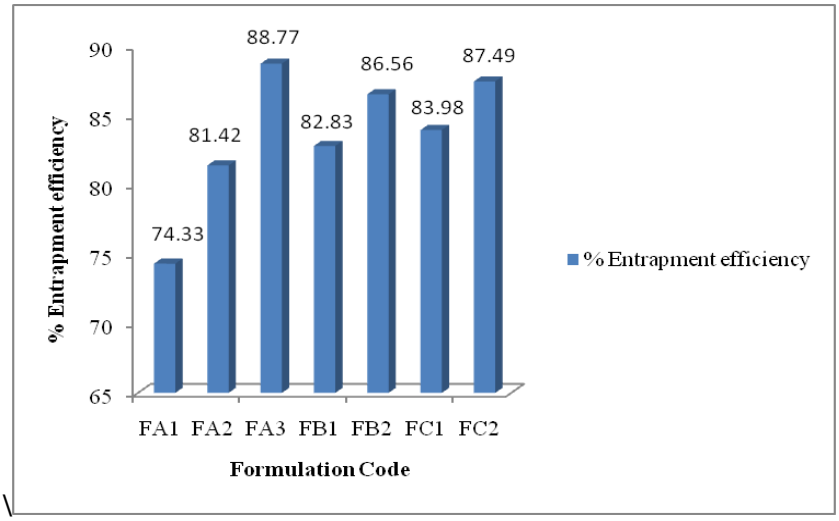

Fig. 15: Percentage Entrapment efficiency of formulation from FA1-FC2.

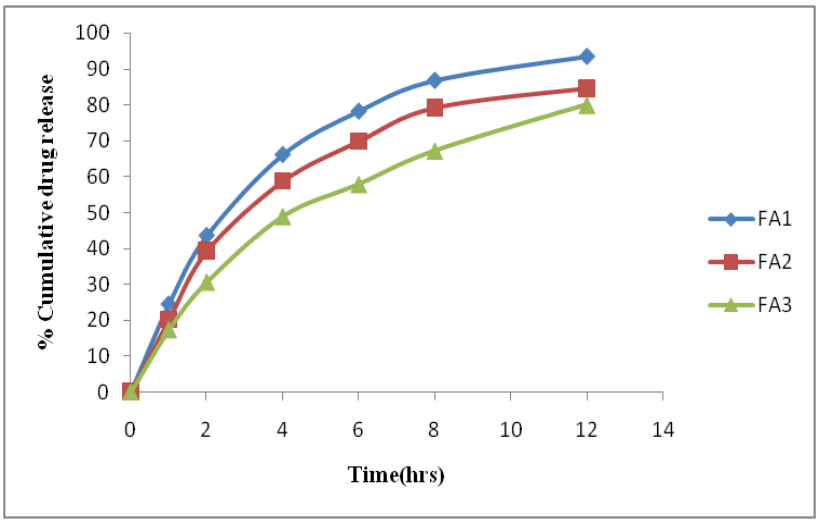

Fig. 16: *Invitro* dissolution profile of uncoated microsphere formulation.

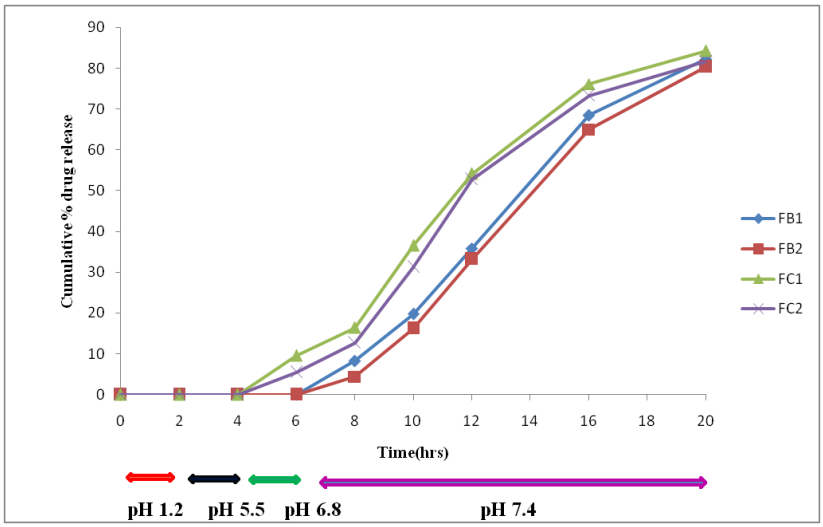

Fig. 17: *Invitro* dissolution profile of coated microsphere formulation.

CONCLUSION

Azathioprine, an immunosuppressive antimetabolite has been used in the treatment of inflammatory bowel disease, ulcerative colitis and Crohn's disease. Azathioprine has various toxic effects that include suppression of haemopoietic system, hepatotoxicity and teratogenicity. Its oral bioavailability is limited to an extent of 41-50% and need to be administered frequently to maintain effective plasma concentration. The present investigation concluded that microsphere coated with enteric polymers like Eudragit S 100 and Ethyl cellulose could potentially target the drug release in the colon region and sodium alginate was reported that better mucoadhesive property that increases the residence time of microsphere in colon region with controlled release makes the formulation suitable for colon targeting of Azathioprine to reduce the side effects.

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REFERENCE

1. Kataoka K, Harada A, Nagasaki Y. Block copolymer micelles for drug delivery: design, characterization and biological significance. Adv Drug Deliv Rev., 2001; 47(1): 113-31.
2. Singh A, Sharma A. Novel approaches for colon targeted drug delivery system. Int j res dev pharm life Sci., 2014; 3(2): 877-86.

3. Philip AK, Philip B. Colon targeted drug delivery systems; A Review on Primary and Novel Approaches. Oman Med J., 2010; 25(2): 70-78.
4. Girotra P, Singh SK. Formulation optimization for colon targeted delivery of katira gum matrix tablets containing Azathioprine. Int J Pharm Sci Drug Res., 2013; 5(4): 133-40.
5. Arunkumar PD, Saravanan M. Magnetic and compartmental targeting of Azathioprine using gelatin microspheres: formulation, physicochemical characterization and in vitro release studies. J Appl Pharm Sci., 2013; 3(10): 17-22. DOI: 10.7324/JAPS.2013.31004
6. Mazumder R, Nath LK, Haque A, Maity T, Choudhury PK, Shrestha B, Chakraborty M, Pal RN. Formulation and in vitro evaluation of natural polymers based microspheres for colonic drug delivery. Int J Pharma Pharm Sci., 2010; 2(1): 211-219.
7. Atyabi F, Vahabzadeh R, Dinarvand R. Preparation of ethylcellulose coated gelatin microspheres as a multiparticulate colonic delivery system for 5-aminosalicilic acid. Ira J Pharm Res., 2010; 2: 81-86.
8. Kumara BC, Parthiban S, Kumar GS, Mani TT. Formulation and evaluation of proliposomal gel containing repaglinide using mannitol as water soluble carrier. IJIR. Apr 1, 2016; 2(5): 1777-86.
9. Dashora A, Jain CP. Development and characterization of pectin Prednisolone microspheres for colon targeting. Int J Chem Technol Res., 2009; 1(3): 751-57.
10. Anusha K, Krishna SA. Preparation and evaluation of Mefenamic acid loaded microspheres using synthetic and natural polymers. Pharm Lett. 2016; 8(1): 197-05.
11. Gaur PK, Mishra S, Bajpai M. Formulation and evaluation of controlled-release of Telmisartan microspheres: In vitro/in vivo study. J Food Drug Anal, 2014; 22(4): 542-48.