Research Article

Chitosan-eudragit Magnetic Microspheres of Sulfasalazine for Colon Drug Delivery

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Accepted on: 26-09-2016; **Finalized on:** 30-11-2016.

ABSTRACT

The objective of the present study is on the formulation of sulfasalazine loaded magnetic microsphere coated with eudragit L-100 in the treatment of Inflammatory Bowel Disease and also to evaluate the total amount of magnetite present in microsphere. Sulfasalazine loaded magnetic microsphere was prepared using simple cross linking method. The formulated magnetic microsphere was further evaluated for physiochemical property and was found to be within acceptable level. The magnetite solution was prepared using suitable method sulfasalazine microsphere was loaded with magnetite.. FTIR and DSC were evaluated for drug-excipient interaction, the morphological study was done with Scanning Electron Microscopy and the particles were found to be round, rough and discreet. Micrometric property revealed that all particles have better flow property. *In-vitro* study was carried out and it was found that the maximum drug release was found for formulation F4 which was 96.45±2.25% in 24hrs. Cell line study concluded that the optimized F4 formulation showed down regulation of COX -2as compared to control and marketed formulation. Stability study proved that F4 formulation was found to be stable for different temperature condition. The optimized formulation F4 showed diffusion controlled sustained drug release mechanism and therefore can have benefits such as reduction in total dose and frequency of administration. In future, magnetic studies and *in-vivo* studies are essential to prove the site specific delivery and magnetic targeting effect of microsphere.

Keywords: Sulfasalazine, Chitosan, Magnetite, Magnetic microsphere, Cell line study.

INTRODUCTION

Novel therapeutic delivery strategies have opened a new window for targeting of drug to the specific sites they have gained vast importance in recent times. The current conventional delivery system has major setback such as increased toxicity, low solubility, low permeability and stability problems that effects the competence and commercialization of the drug making them impact. The most conservative route of drug delivery is oral for patients. But due to certain drawbacks like poor absorption, inclination towards digestive enzyme in the gastro intestinal tract (GIT), and limitation of transport across the intestinal barrier.[1]

The drug delivery to colon has solved the problems regarding the delivery of drug in the management of ailment of colon like inflammatory bowel diseases.[2,3] To achieve better release of drug in the colon rather than in the stomach can be made by only targeting the drug directly to the by means of controlled release dosage form or superior coated dosage forms.[4,5]

Inflammatory bowel disease (IBD) is the chronic inflammation of intestinal mucosa and colon. They are further alienated into 2 major group: Crohn's disease in which inflammation of digestive tract including small intestine and large intestine and Ulcerative colitis include the inflammation of lining of large intestine and rectum. Both the disease is characterized with similar signs like abdominal pain, blood filled stool, fatigue, fever etc due to many factors.[6]

Microsphere plays significant role in the delivery of drug to site due to its size it can be administered through needle and different dosage forms. Magnetite has great importance in the field of electronics, biomedical and magnetically carried drug delivery.[7]Although microsphere has potential to deliver drug at specific site addition or loading of magnetite into microsphere would enhance the delivery of drug through microsphere by using an external magnetic field application from outside the body. Employing this method for delivering drug through this method helps to reduce the freely moving drug and loss of drug due to various other activities inside the body.[8]

Sulfasalazine comes under prodrugs, where cleavage takes place in position N^4 in large intestine to amino salicylic acid and sulfapyridine by using azoreductase enzyme. Sulfapyridine have antibacterial action and aminosalicylic acid have antinflammatory action in colon region.[9]

MATERIALS AND METHODS

Sulfasalazine pure drug of 20gm was purchased from Swapnaroop pharmaceuticals thane. Chitosan was obtained from Nita pharmaceuticals. Acetic acid (glacial) from Nice chemicals, Light liquid paraffin from Spectrum, Dimethyl sulfoxide from Merck, Span 60 from Lobachemi Eudragit L-100 from Nice, Ferric chloride from Spectrum, Ferric sulfate from, Spectrum Magnetite was prepared using suitable method.

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Preparation of Magnetite

Magnetic microsphere was prepared with some modification using acetic acid and 25% crosslinling agent.[10]

Preparation of sulfasalazine magnetic microsphere

Different chitosan concentration ie, 2%, 2.5%, 3%, 3.5%, 4%, 4.5% was dissolved in acetic acid (3%) and magnetite solution (3%) was added into chitosan acetic solution stirred until dispersed completely. The chitosan-magnetite solution was injected into the solution mixture containing SPAN 80 (5ml) and light liquid paraffin (60ml). Glutaraldehyde (2ml) was added drop wise. When microspheres were formed they were filtered washed to remove excess glutaraldehyde and soaked in Eudragit L-100 (150mg) coating solution (with solvent isopropyl alcohol and acetone in the ratio 2:3) for coating then the microsphere was separated and dried (Table1).

Table 1: Composition for Sulfasalazine loaded magnetic microspheres

S.no	Compositions	F1	F2	F3	F4	F5	F6
1	Sulfasalazine (mg)	100	100	100	100	100	100
2	Chitosan (%)	2	2.5	3	3.5	4	4.5
3	Magnetite (%)	3	3	3	3	3	3
5	Glacial Acetic Acid (%)	3	3	3	3	3	3
7	Dimethyl Sulfoxide (ml)	0.3	0.3	0.3	0.3	0.3	0.3
8	Light Liquid Paraffin (ml)	60	60	60	60	60	60
9	Span 80 (ml)	5	5	5	5	5	5
10	Eudragit L-100 (mg)	150	150	150	150	150	150
11	Glutaraldehyde (ml)	2	2	2	2	2	2

Evaluation of magnetic microsphere

Preformulation studies

Preformulation studies were done to deliver all obligatory data, like physiochemical, biopharmaceutical properties and compatibility of drug and excipients.

Melting point of drug

Melting point was of drug sample was done to indicates the purity of the sample. The impurity present in small amount was detected using capillary method.[11]

Lamda (λ) max of the drug

An absorption maximum of sulfasalazine was determined using methanol and phosphate buffer pH 6.8 using UV spectroscopy.[12]

Solubility studies

Solubility study of drug was done using different solvents such as water, ethanol, methanol, DMSO, phosphate buffer pH 6.8.[13]

Analytical methods using UV spectroscopy

Preparation of standard stock solution

100mg of sulfasalazine was weighed and transferred into 100ml volumetric flask and dissolved in pH 1.2. The flask was shaken and volume was made upto 100mL with pH 1.2 to give a solution of 1000µg/mL.

Preparation of standard calibration curve in pH 1.2

Aliquots of .1, .2, .3, .4, .5, .6, .7, .8, .9 and 1mL from above, were withdrawn into 10mL volumetric flask and diluted with pH 1.2 to achieve final concentration in the range 1-10µg/ml. The resulted solution's absorbance were taken at 359nm against pH 1.2 as blank.

Standard curve of drug in PBS pH 6.8

100mg of sulfasalazine was weighed and transferred into 100mL volumetric flask and dissolved in pH 6.8. The flask was shaken and made up to 100mL volume with pH 6.8 to give a 1000µg/mL solution.

Preparation of standard calibration curve in pH 6.8

From the standard stock solution, aliquots of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1mL were withdrawn into 10mL volumetric flask and diluted with pH 6.8 to get the concentration of 2-10µg/mL. The absorbance of all resulted solutions was measured at 359nm against pH 6.8 as blank.[14]

Fourier transforms infra-red spectrum

The spectrum of drug sulfasalazine, magnetite, chitosan, eudragit L-100, physical mixture and sulfasalazine loaded magnetic microspheres respectively were determined.[15]

Thermal stability studies

The stability studies using heat method for the drug sulfasalazine, magnetite and sulfasalzine magnetic microspheres by Differential scanning calorimetry instument.[16]

SEM

SEM were studied for understanding the morphological parameters of microsphere. Optimized microsphere formulation were diluted with distilled water and placed

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on an aluminium platform and microscopic examinations were performed.[17]

Micrometric property

The micrometric property of the microspheres was studied by using graduated open cylinder method and the flow property was assessed.[18]

Entrapment efficiency

To the prepared microsphere, 10mL pH 6.8 PBS was added, sonicated, filtered and 1mL of the filtrate was pipette out and made up to the volume with phosphate buffer in 10mL standard flask. Absorbance was measured at 359nm.The total drug concentration in microspheres was calculated for entrapment efficiency.[19]

Drug content analysis

Weighed 100mg microsphere and extracted with 10mL methanol using mechanical stirrer. The volume was made up 100mL. Then filtered and diluted to get the concentration which was determined spectrophotometrically at 360nm.[20]

Determination of percent magnetite content

An accurately weighed amount of magnetic microspheres was dissolved in mixture of water (200 mL) and Conc. HCl (200mL) by heating it to the boiling point. The solution was boiled for 15sec and cooled rapidly. Then potassium iodide (3g) was added and kept in dark for 15min. The liberated iodine was then titrated with 0.1 N sodium thiosulphate using starch as indicator. A blank titration was carried out. The difference between titrations gave the amount of iodine liberated by ferric ion. The procedure was repeated thrice in order to get the concordant value.[21]

***In-vitro* drug release study**

Release studies of sulfasalazine loaded chitosan magnetic microspheres by USP Type II paddle dissolution apparatus for 24hr and stirring rate of 100rpm. 100mg of sulfasalazine were placed in the medium containing pH 1.2 for 2hr, then using pH 6.8 maintained at 37± 0.5ºC. 5mL of sample solution was withdrawn at regular interval of time and fresh dissolution medium was simultaneously used to replace the quantity withdrawn. The samples were assayed spectrophotometrically at 359nm to estimate the drug concentration. All experiments were performed in triplicate. [22]

***In-vitro* Cell line study on CaCo2 cells**

Caco2cell lines were procured from National Centre for Cell Science, India. Culturing of cells were done in Dulbecco's modified eagle media, adopted RT- PCR procedure and the cytotoxicity studies of formulations were carried out. Finally the levels of COX-2 inhibited in different formulation treated CaCO2 cell line were also evaluated.[23]

Stability study

The stability evaluation were done for 3 months in various temperature conditions like refrigeration condition (4°C±2°C), room temperature (30°C±2°C/ 65±5% Relative Humidity) and ambient condition (45°C±2°C/75±5% Relative Humidity) to determine physical and chemical stabilities as per ICH guidelines. Samples were withdrawn at regular intervals for assessing percentage drug content.[24]

RESULT AND DISCUSSION

Preformulation studies were undertaken to confirm the identity, purity and to establish a suitable drug profile

Melting point determination

Melting point of pure sulfasalazine was found to (240°C - 245°C) and it was equivalent with the monograph value.

λ-max of the drug

The λ max of the drug was found to be 359 nm (fig. 1) in equivalent with the official standard.

Solubility of the drug

The solubility of the pure drug was compared with reference and it was tabulated in (table. 2).

Analytical methods using UV spectroscopy

Standard curve of drug in PBS pH 1.2

The calibration curve for pH 1.2 was plotted and the absorbance of all resulted solutions was measured at 359nm against pH 1.2 as blank(fig.12).

Calibration curve of drug in Phosphate Buffer Solution pH 6.8

The calibration graphof drug in pH 6.8 was plotted and the absorbance of all resulted solutions was measured at 359nm against pH 6.8 as blank.

Solubility studies of the drug

Drug is having good solubility in methanol, Ph6.8 and dimethyl formamide and insoluble in distilled water and ethanol.

Drug compatibility with excipients

Drug-Excipients compatibility was carried out for drug, magnetite, physical mixture and formulation F4 sulfasalazine magnetic microsphere (fig.1).

The drug loaded magnetic microsphere formulation display a spectrum from 3500-3300cm^{-1} indicating the presence of amine group. The OH in-plane and out-plane bonds appears at 1666.50cm^{-1}, 1392.61$cm^{-1,}$ 927.96cm^{-1}, 684.74cm^{-1}. The sample display intense peak at 684.73cm^{-1} which is due to the stretching vibration that are associated with the metal NO_2 absorption band (Fe-O) bands in crystalline lattice of FeO_4.

The FTIR spectrum also shows the absorption band at 2099.26cm^{-1} which signify the presence of stretching

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vibration of carbonyl group (C=O).Absence of extra peaks indicates good compatibility between drug and the excipients.

Differential scanning colorimetry (DSC)

DSC spectrum of sulfasalazine magnetic microsphere shows endothermic peak at 82°C for magnetite, 105°C for chitosan and 152°C for eudragit L-100(fig.2). It concludes that the melting endotherm of sulfasalazine was not observed in drug loaded chitosan magnetic microsphere. This indicates that sulfasalazine was uniformly dispersed in amorphous state within polymeric matrix.

Analytical method by UV spectrophotometer

Scanning Electron Microscopy (SEM)

SEM images for sulfasalazine are shown in fig.3 Sulfasalazine loaded magnetic microsphere with eudragit polymer coating indicated that the particles are oval in shape, smooth and discrete. SEM image of sulfasalazine plain microsphere indicates that the particles are globular with rough surface and discrete features. Also 100-500 µm particle size was observed.

Micrometric properties

The values for micrometric property indicated that all the formulations had better flow properties for the microspheres.

PHYSICO- CHEMICAL CHARACTERISATION (figure: 4)

Percentage Entrapment Efficiency

The maximum entrapment efficiency was found to be 92.72 ± 3.03% for microsphere formulation F4. Entrapment efficiency of drug in microsphere increases with increase in polymer concentration. As the concentration of polymer increased, the drug entrapment efficiency also increased.

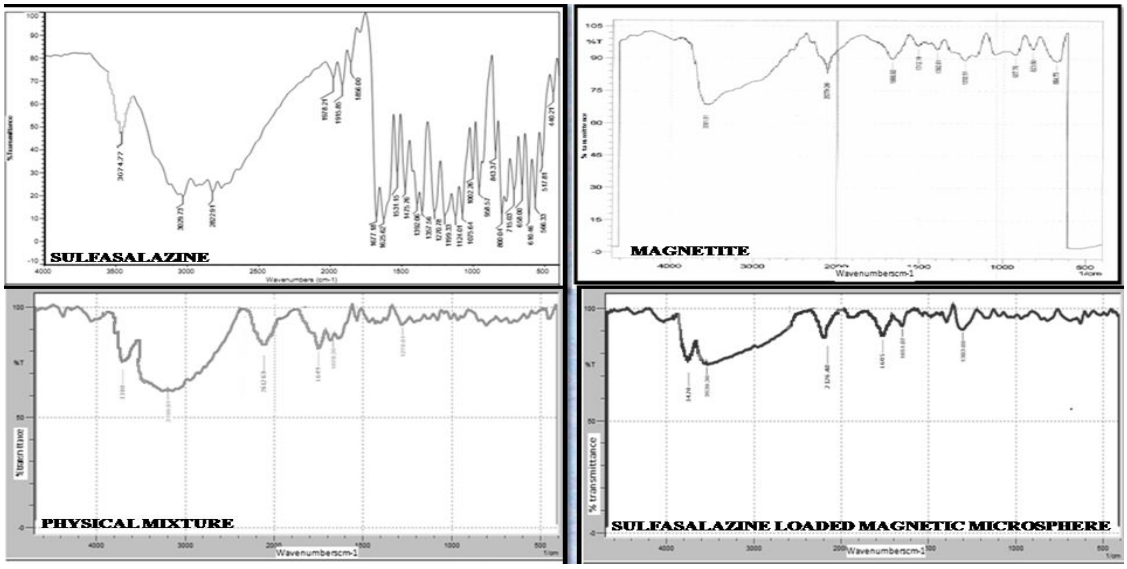

Figure 1: FTIR Spectra of different samples

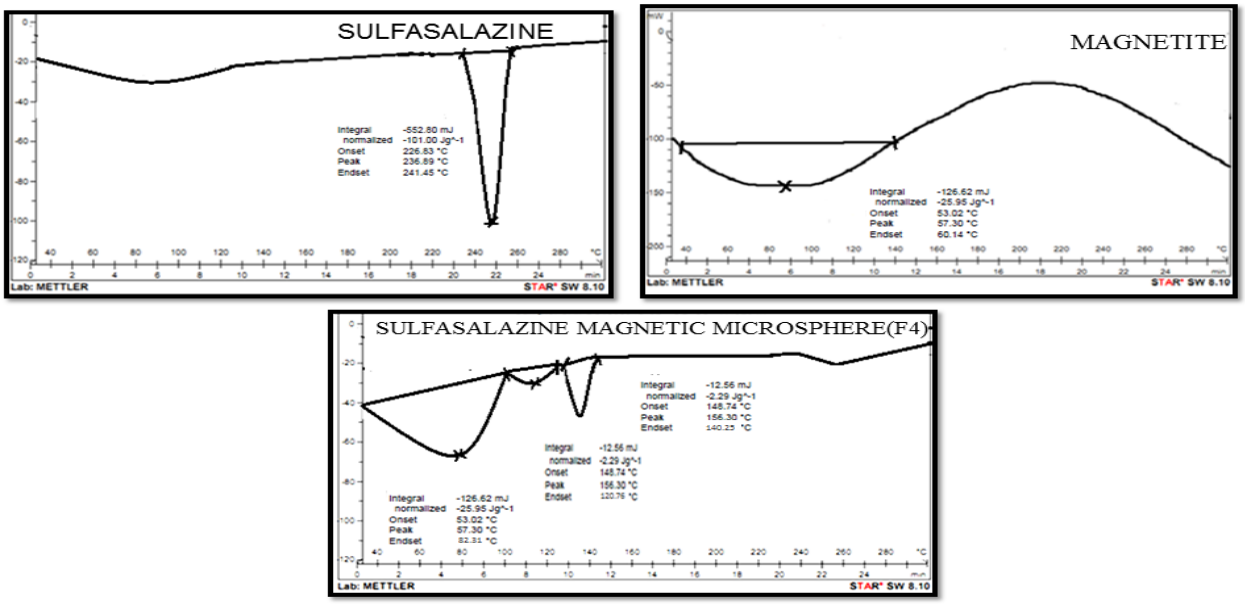

Figure 2: DSC spectrum of Samples

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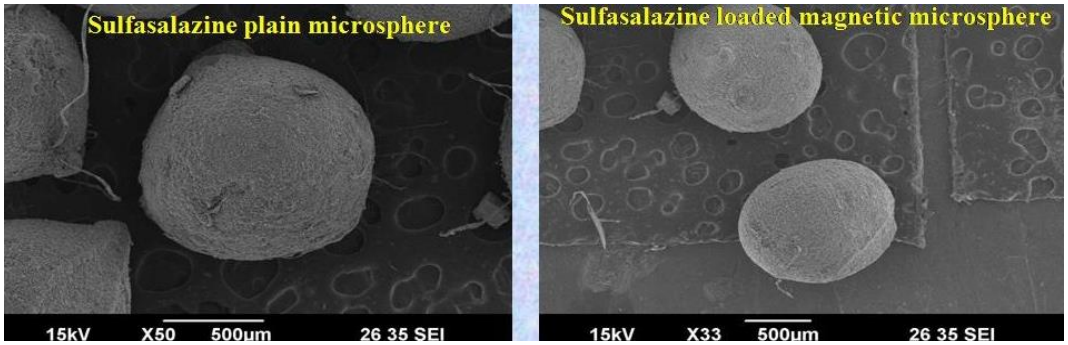

Figure 3: SEM images of prepared microspheres

Drug Content Analysis

The maximum drug content was found to be 90.33 ± 0.36% for microsphere formulation F4. Drug content increases with increase in polymer concentration.

Content of magnetite

The amount of magnetic content in microsphere was carried out using conventional titrimetric method using thiosulphate and potassium iodide for quantitative analysis. The maximum amount of magnetite was found in formulation F4 with 31.9±0.67% (fig.4). The maximum reference amount of magnetite used is 20-60% to avoid systemic toxicity of excess magnetite in the body.

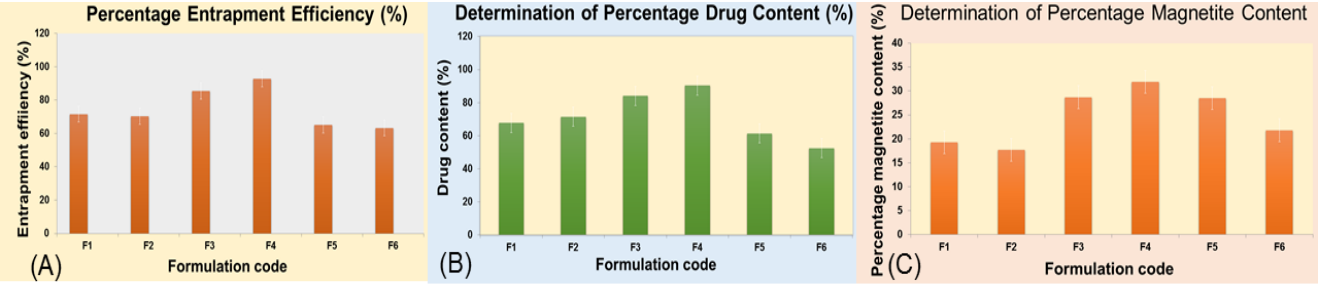

Figure 4: (A) Determination of Percentage Entrapment Efficiency B) Determination of Drug Content (C) Determination of Percentage Magnetite Content

***In-vitro* drug release studies**

The drug release is also affected by particle size, entrapment efficiency and concentration of polymer. The drug release was found to be 62.73± 3.20%, 74.47±1.56%, 86.67±2.56%, 96.45±2.25%, 57.72±0.85%, 59.38±1.57% for formulation F1 to F6 in 24hrs.The polymer concentration in formulation F1 and F2 is low; hence the particle size is very small. So the amount of drug entrapment in formulation F1 and F2 is less and it affects the drug release. The concentration of polymer is relatively high in formulation F5 and F6 hence the particle size of microsphere was relatively large, viscosity of polymer and solution was high. The higher concentration of polymer increases thickness of microsphere which slows down the release of drug from microsphere with respect to time. The formulation F3 and F4 have shown significant increase in diffusion rate as compared to other formulations. The concentration of polymer in F3 and F4 were acceptable because the amount of drug loading was good for those formulations and drug release rate was excellent compared to the rest of formulations, hence it was concluded that F4 had maximum drug release as mentioned in (fig.5).

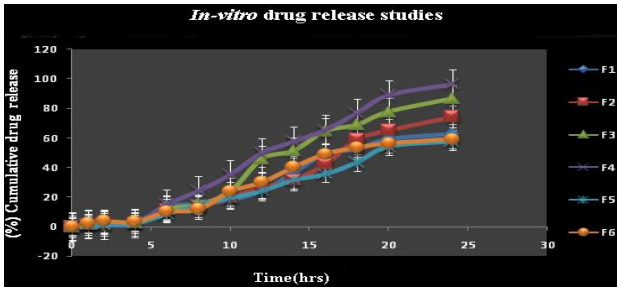

Figure 5: *In-vitro* drug release studies

***In-vitro* cell line study on caco$_2$ cells.**

It was found that the level of COX-2 was inhibited in different formulation treated CaCo2 cell lines.F4 formulation, marketed formulation and control drug solution showed regulation on the level of COX-2 (Figure: 6). Sulfaslazine magnetic microsphere (F4) formulation showed maximum COX-2 inhibition within 24hrs of treatment compared to other formulations whereas the normal saline and the culture media showed no COX-2 inhibition.

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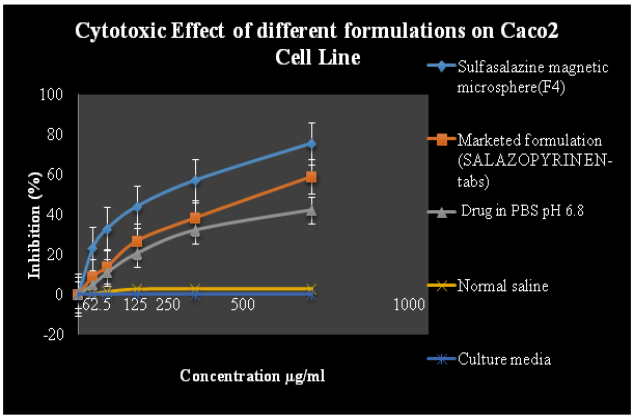

Figure 6: *In-vitro* cell line study on Caco2 cells

Stability study

Stability study of all the formulations were carried out and from the above observation, it was found that F4 formulation (Figure: 7) is the best since it is stable at three different conditions of refrigeration, humidity control and ambient temperature compared with other formulations.

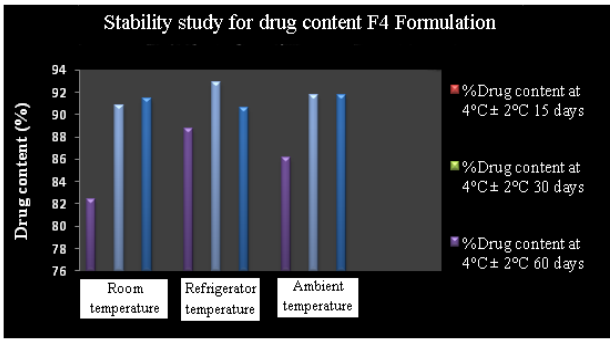

Figure 7: Stability study for drug content F4 Formulation

CONCLUSION

In the current research work, sulfasalazine loaded magnetic microspheres were prepared by ion gelation modified technique with chitosan polymer and eudragit-L-100 for coating of sulfasalazine magnetic microsphere for site specific colon treatment mode for inflammatory bowel disease. The FT-IR studies showed significant compatibility between the drug and the polymer. The optimized F4 formulation showed down regulation of *COX -2* as compared to control and marketed formulation. The formulation F4 showed no evidence of hemolysis (<0.5%) after 2hr of incubation. The rate of sulfasalazine release from chitosan magnetic microsphere decreased with increase in the amount of polymer. The study results proved that sulfasalazine magnetic microspheres can be used for colon drug delivery due to the coating of eudragit L-100. The optimized formulation F4 showed diffusion controlled sustained drug release mechanism and therefore can have benefits such as reduction in total dose and frequency of administration. In future, magnetic studies and *in-vivo* studies are essential to prove the site specific delivery and magnetic targeting effect of microsphere.

REFERENCES

1. Jain N K, Textbook of Controlled and Novel Drug Delivery, 1st edn, 3, 2002, 236-237.
2. Krishnaiah YSR, Bhaskar RPR, SatyanarayanaV, Studies on the development of oral colon targeted drug delivery systems for metronidazole in the treatment of amoebiasis, Int J Pharm. 236, 2002, 43-55.
3. Blumberg RS, Strober W, Prospects for research in inflammatory bowel disease, J Am Med Ass, 285(5), 2001, 643-647.
4. Kett K, Rognum TO, Brandtzaeg P, Mucosal subclass distribution of immunoglobulin g-producing cells is different in ulcerative colitis crohn's disease of the colon. J Gastro, 93, 1987, 919-924.
5. Vyas SP, Khar RK, Targeted and controlled drug delivery, 7, 2003, 418.
6. Manish KC, Colon targeting microsphere: A review, Asia J Pharm Edu Res, 1(2), 2012, 16-30.
7. Andreas S, Lubbe, Alexiou C, Bergemann C, Clinical applications of magnetic drug targeting, J Surg Res,95,2001,200–206.
8. Jawed A, Chaturvedi R, Sharma J, Mittal D,Pardhan P. Magnetized carrier as novel drug delivery system. Int J Drug Dev Tech, 1(1), 2009, 28-35.
9. Ilango K, Valentina P, Text Book of Medicinal Chemistry, 1, 2007, 250.
10. Mohamed KN,Moustafa MM, Mohamed AE, Preparation of biocompatible magnetic microspheres with chitosan, J Biomater Nanobiotechnol, 2, 2011, 194-200.
11. Raghavendra RNG, Ram P, Bussetti SS, Formulation and evaluation of gas powered systems of Cefixime tablets for controlled release, Int J Pharm Bio Sci, 1(2), 2010, 1-15.
12. The Indian pharmacopoeia, Controller of Publications, Ministry of health and family welfare,Published by The Indian pharmacopoeia commission, Ghaziabad, 2(6), 2010, 1012-1014.
13. Vinoth Kumar G, AnandBabu K,Ramasamy C, Formulation and evaluation of bilayered tablets of cefiximetrihydrate and dicloxacillin sodium,Int J Pharm Tech Res, 3(2), 2011, 613-618.
14. Balraj S, Gulshan B, Degradation study on sulfasalazine and a validated HPLC-UV method for its stability testing, Sci Pharm. 82, 2014, 295–306.
15. Garud N, Garud A, Jain N, Formulation, design and *in-vitro* evaluation of metformin microspheres using ionotropic gelation technique, J Pharm Res, 4(7), 2011, 2103-2106.
16. Jacob JS, Characterization of delivery systems, Microscopy, Encyclopedia of Controlled Drug Delivery, John Wiley and Sons Inc, New York, 1, 1999, 242-3.
17. Sreeja CN, Anoop KR, Local antimicrobial delivery of satranidazole loaded cross linked periodontal chips using bio degradable polymers,Int J Pharm Pharm Sci, 5(3), 2013, 839-847.

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18. Ramesh DV, Medlicott N, Razzak M, Tucker IG,Microencapsulation of FITC-BSA into Poly (β-Caprolactone) by a water-in-oil-in-oil solvent evaporation technique trends, BiomaterArtif Organs, 15(2), 2002, 31-36.

19. 19.Aswathy SN, Vidhya K M, Saranya TR, Sreelakshmy KR, Sreeja CN, Mucoadhesive Buccal Patch of CefiximeTrihydrate using Biodegradable Natural Polymer, Int J Pharm PharmSci, 6(6), 2014, 366-371.

20. Avivi S, Felner I, Novik I, Gedanken A, The preparation of magnetic proteinaceous microspheres using the sonochemical method, Biochimica EtBiophysica Acta, 1527, 2001, 123-129. Qadry JS, Qadry SZ, A Textbook of Inorganic Pharmaceutical and Medical Chemistry, B S Shah Prakashan Ahmedabad, 5, 1992,271.

21. Santhi K, Dhanraj S, Ponnusankar S, Suresh B.Study on formulation and targeting efficiency of amphotericin bnanospheres. Indian J Pharm Sci, 6, 2000, 421-3.

22. 23.Lamprecht A, Schafer U, Lehr CM. Size-dependent bioadhesion of micro- and nano particulate carriers to the inflamed colonic mucosa. Pharm Res, 6, 2001, 788-793.

23. The European Agency for the Evaluation of Medicinal Products, Stability Testing Guidelines: Stability Testing of New Drug Substances and Products, 2003.

Source of Support: Nil, **Conflict of Interest:** None.

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