Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Special Issue [1]2022 : 1602-1610

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Design, Development and Evaluation of Colon Targeted Hydrogel of An Anti-Inflammatory Drug Sulfasalazine

Vishal S. More*[1],Deshraj S. Chumbhale[1], Nalanda T. Rangari[2], Pranali A. Mishra[3],Vaishnavi C. Sonar[4]

[1]Faculty of Pharmacy, Amrutvahini College of Pharmacy, Sangamner, Maharashtra, India
[2]HOD and Associate Professor, Dept. of Pharamaceutics, Alard College of Pharmacy, Marunje, Pune
[3]Research Scholar, L. N. University, Bhopal
[4]Dept. of Pharmaceutical Quality Assurance, R. C. Patel institute of Pharmaceutical Education and Research
Corresponding Author: vsmore@amrutpharm.co.in

ABSTRACT

Colonic drug delivery is intended not only for local treatment in inflammatory bowel disease (IBD) but also for systemic delivery of therapeutics. The purpose of this research was to develop and evaluate chitosan hydrogel for colon-targeted delivery of sulfasalazine. Chitosan hydrogel were prepared by the cross-linking method. All formulations were evaluated for particle size, zeta potential, polydispersity index, entrapment efficiency and drug loading, swellability, in vitro drug release stability study and hemolysis assay. The particle size and PDI the drug free hydrogel was found to be 201nm and 0.3 respectively. The obtained zeta potential of SSZ-loaded hydrogel was found to be 5.52. SSZ was entrapped into hydrogel with loading capacity of 5.66% and entrapment efficiency of 96.66%. The swelling ratio was found to be 0.5 at pH 6.4 and 0.2 at pH 7.2. The amount of the drug released after 11 hours from the formulation was found to be 100%. It is clear that with increase in concentration, hemolytic ratio also increased however, it is far below 5 %, the critical safe hemolytic ratio for bio-materials according to ISO/TR 7406. This indicated that these SSZ- hydrogel samples are hem compatible. From stability studies, the obtained results revealed that there was no significant change in the MPS, PDI and % EE indicating that they were found to be stable at 25 ± 2°C, 60 ± 5%, RH for a total period of 3 months. Studies demonstrated that orally administered chitosan hydrogel can be used effectively for the delivery of drug to the colon.

Keywords:*Chitosan, colon specific drug delivery, hydrogel, multiparticulate system, sulfasalazine*

Received 13.03.2022 Revised 26.03.2022 Accepted 29.04.2022

INTRODUCTION

Targeted drug delivery system to colonic region of gastrointestinal tract (GIT) has been explored extensively in the last two decades not only for local treatment of colon diseases such as colon cancer and inflammatory bowel diseases (ulcerative colitis, Crohn's disease) but also for systemic delivery of peptides and proteins (1). It has been demonstrated that insulin, vasopressin and calcitonin can be absorbed in the colonic region (1,2). Colon is the preferred site for delivery of therapeutics due to low level of enzyme activity, nearly neutral pH, and long transit time (2–4). Designing of the oral formulation intended for colon-specific drug targeting is a challenging task, as it requires that the incorporated drug should exclusively release in the colon without chemical or enzymatic degradation in upper GIT (stomach and small intestine). Different strategies based on employing various physiological parameters such as pH, enzyme activity, microbial flora, GI transit time, and pressure (5-7) have been attempted to design the formulation for colon-specific delivery. Natural biodegradable polymers have been extensively used for developing solid oral dosage forms designed for colon-specific delivery of therapeutics (8,9). Guar gum and pectin are linear polysaccharides used preferably for colon drug delivery formulations (10-12). These linear polysaccharides remain intact in the upper GIT and are degraded by the microbial flora of colon. This attribute makes these polymers as preferable carriers for site-specific delivery. Chitosan is a functional linear polymer derived from chitin, the most abundant natural polysaccharide on the earth after cellulose, and it is not digested in the upper GI tract by human digestive enzymes (13,14). Chitosan is a copolymer consisting of 2-amino-2-deoxy- D-glucose and 2-acetamido2-deoxy-D-glucose units linked with β-(1–4) bonds. It should be susceptible to glycosidic hydrolysis by microbial enzymes in the colon

because it possesses glycosidic linkages similar to those of other enzymatically depolymerized polysaccharides. The polysaccharide, on reaching the colon, undergoes assimilation by microorganisms or degradation by enzymes or break down of the polymer backbone leading to a subsequent reduction in molecular weight and thereby loss of mechanical strength and is unable to hold the drug any longer(15). Chitosan has drawn attention for its potential to achieve site-specific delivery to the colon. Few reports have been published on the investigation of the application of chitosan in colon targeting(16,17). Hydrogel is a network of polymer chains that are water-insoluble, sometimes found as a colloidal gel in which water is the dispersion medium 4. Hydrogels are superabsorbent (they can contain over 99% water) natural or synthetic polymers. Hydrogels also possess a degree of flexibility very similar to natural tissue, due to their significant water content. Hydrogels are crosslinked polymer networks that absorb substantial amounts of aqueous solutions. Hydrogels have played a key role in is drug delivery technology. Investigation of sulfasalazine metabolism suggests that 5- aminosalicylic (5-ASA) acid is the therapeutically active portion of the drug (18,19) in inflammatory bowel diseases (IBD). However, it has been reported that 5-ASA is extensively absorbed and metabolized in the upper gastrointestinal tract by first pass metabolism and is not made available to the desired site, i.e., colon (20). To improve the site availability of the drug, several approaches have been utilized (21, 22). In present work, attempt was made to formulate and evaluate hydrogel drug delivery systems. Attempts were made to enhance drug absorption and exposure to improve therapy by controlling the rate of drug release from dosage forms. Rate of drug release was modified using cross-linking agents, gelling or thickening agents. The ultimate aim was to improve bioavailability of the drug and to improve the market formulation by the use of combination of hydrophilic polymers.

MATERIAL AND METHODS

Materials

Sulfasalazine was kindly provided as a gift sample from Valens molecules Pvt Ltd, Hyderabad, India. HCl, acetic acid, ethanol, DMSO were purchased from Central Drug House (P) Ltd, New Delhi, India. Chitosan, carboxy methyl cellulose was purchased from Hi-Media laboratories Mumbai, India. Double distilled water was prepared freshly and used whenever required. All other ingredients and chemicals used were of analytical grade.

Formulation development

Preparation of Hydrogel

Chitosan in acetic acid 1% (W/V) solution and carboxy methyl cellulose aqueous solution was prepared and both the solution was ready. Then chitosan solution added slowly to aqueous carboxy methyl cellulose under Probe ultra sonicator was stirred for 2h. The pH of the resultant solution about 4.5and adjusts it up to 6.8. The mixture was warmly heated at 40˚C for 5 min to produce homogeneously dispersed hydrogels.

Preparation of Hydrogel formulation

The Hydrogel was prepared by using Carboxymethyl cellulose as gelling agent. The required amount Sulfasalazine drug was added to 2% of DMSO to get 1% drug in hydrogel. Carboxymethyl cellulose was dispersed to previously prepared Sulfasalazinestirred using magnetic stirrer at 500rpm. Stirring was continued till complete dispersion of the carboxy methyl cellulose to obtain homogeneous hydrogel.

Isolation of Hydrogel

Centrifugation of optimized hydrogel dispersion was done for the separation of nanoparticles by using Optima “MAX-XP” ultracentrifuge at 45,000 rpm for 35 minutes. Deposited particulate was dispersed in minimum quantity of water with appropriate concentration of mannitol.

Table 1 Formulation batches of SSZ hydrogel.

Batches	% Chitosan acetic acid solution (w/v) g	% TWEEN 80 (w/v) g	CMC (w/v) g
F-1	0.04	0.06	0.7
F-2	0.04	0.03	0.5
F-3	0.02	0.06	0.7
F-4	0.04	0.03	0.7
F-5	0.04	0.06	0.5
F-6	0.02	0.03	0.7
F-7	0.02	0.03	0.5
F-8	0.02	0.06	0.5

Characterization

Mean particle size and polydispersity index

The MPS and PDI were determined by PCS with a Malvern Zetasizer (Nano ZS 90, Malvern ltd., and UK). The measurement using PCS is based on the light scattering phenomena in which the statistical intensity fluctuations of the scattered light from the particles in the measuring cell are measured. Prior to the measurements, all samples were diluted with double distilled water to produce a suitable scattering intensity. The z-average and PDI values were obtained at an angle of 90° using disposable polystyrene cells having 10 mm diameter cells at 25°C, which were equilibrating for 120 seconds. All measurements were performed in triplicate at 25°C

Zeta potential

The zeta potential (ZP), reflecting the electric charge on the particle surface and indicating the physical stability of colloidal systems, was measured by determining the electrophoretic mobility using the Malvern Zetasizer (Nano ZS 90, Malvern ltd., UK). The measurements were performed with diluting in double-distilled water. It was measured using Dip cell with applying field strength 20 V/cm and the average of the zeta potential was given from 30runs. The production yield of hydrogel formulation was calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of hydrogel.

Production yield

The production yield of hydrogel formulation was calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of hydrogel.

$$\text{Production Yield} = \frac{\text{Amount of freeze dried powder}}{\text{Amount of SSZ and copolyer infeed}}$$

Entrapment efficiency and drug loading

Percent entrapment efficiency (EE) is defined as the percentage of drug incorporated into the polymeric hydrogel relative to the total drug added. It specifies how much percent of drug is included in the particles and how much percent of free drug are still present in the dispersion medium. For this Sulfasalazine hydrogel dispersion was centrifuge at 45,000 rpm for 35 min; 1.0 mL of the supernatant collected after centrifugation was diluted with 3.0 mL of DMSO and methanol and then make up volume up to 10 ml in 10ml volumetric flask and measured spectrophotometrically at 359nm using UV-Visible spectrophotometer (UV 1700, Shimadzu, Japan). The entrapment efficiency of the NPs and standard deviation was calculated for optimized batch of hydrogel Loading capacity / Drug loading (DL) refers to the percentage of drug incorporated into the polymeric hydrogel relative to the total weight of the hydrogel (i.e. polymer + drug). For this, SSZ from Lyophilized flakes was extracted by triturating 10mg powder with DMSO in mortar pestle and diluted up to 10 ml in volumetric flask.Sulfasalazine content in the DMSO extract was analyzed spectrophotometrically (UV 1700, Shimadzu, Japan) at 359nm, against the standard DMSO and methanolic solution of CRM.

$$\text{Entrapment efficiency (\%)} = \text{Total amount of drug} - \text{unentrapped drug} / \text{Total amount of drug} \times 100$$

$$\text{Drug loading (\%)} = \text{Actual amount drug in hydrogel} / \text{Total amount of drug} \times 100$$

Swelling study

The degree of swelling was calculated by finding out weight of swollen hydrogels. The swelling behavior of the hydrogels was studied at two different pH conditions (pH 1.2, 6.8, 7.2). The swelling ratio was calculated using the following formula after determining the dry as well as wet weight of the lyophilized, hydrogel after sufficient exposure to the corresponding pH solution. The swelling at each pH was studied in triplicate.

Accelerated stability study

Variations to MPS, PDI and % EE was observed during 3 months of storage. Stability profile of optimized batch.

***In vitro* drug release study**

In vitro diffusion study of hydrogel optimized batch was carried out by Franz diffusion cell having 2.0 cm diameter and 25 ml capacity. Dialysis membrane (Himedia) having molecular weight cut off range 12000 – 14000 kDa was used as Diffusion membrane. Pieces of dialysis membrane were soaked in Phosphate buffer solution (PBS) for 24 h prior to experiment. Diffusion cell was filled with PBS and dialysis membrane was mounted on cell. The temperature was maintained at 37°C. After a pre-incubation time of 20 minutes, the lyophilized powder equivalent to 10mg of was dispersed in 3ml of PBS and was placed in the donor chamber. Samples were withdrawn half hour from the receptor compartment for 5 hours and

replaced with the same amount of fresh PBS, and assayed by a UV spectrophotometer at 359nm. In this type of hydrogel drug release through stimuli responsive if alteration in pH then drug release start.

RESULTS AND DISCUSSION

Hydrogel prepared by simple cross linking of cationic and anionic polymer. In which chitosan was cationic and CMC in anionic in nature. In this type of cross linking two oppositely charge molecules associate readily as a result of electrostatic attractions which non-covalently bonded. Van der wall forces play vital role in cross linking. So, by adjusting the ratio between both the polymers, hydrogel was prepared.Isolated SSZ loaded hydrogel was successfully freeze dried using the bench top freeze dryer. The obtained lyophilized powder was found to be dry, porous and friable after 72h. The particle size and PDI the drug free hydrogel was found to be 201nm and 0.3 respectively. After then drug loading partical size of SSZ loaded hydrogel was increase 226 nm there was no significant change in PDI. Partical size of hydrogel a crucial factor because it determines the rate and extent of drug release as well as drug absorption. The smaller droplet size provides a larger interfacial surface area for drug absorption. The particles having average diameter up to 300 nm could be easily transported Parental route. In addition, it was suggested that the smaller droplet size permit a faster release rate. Also, it has been reported that the smaller particle size may lead to more rapid absorption and improve the bioavailability. Also, PDI measures the width of particle size distribution. If PDI is lower than 0.1, it might be associated with a high homogeneity in the particle population, whereas high PDI values suggest a broad size distribution Figure 1.The obtained zeta potential of SSZ-loaded hydrogel was found to be 5.52. The Zeta potential represents the electrical charge to the hydrogel surface. The greater the ZP value, more likely the suspension is to be stable because the charged particles repel one another and thus overcome the natural tendency to aggregate. It is currently admitted that higher ZP values, either positively or negatively charged, indicates that the dispersion having long term stability Figure 2.The production yield of hydrogel formulation was found to be 77.52%. The production yield of hydrogel formulation were calculated using the weight of final product after drying (690 mg) with respect to the initial total weight of the drug and polymer (890 mg) used for preparation of hydrogel.SSZ was entrapped into hydrogel with loading capacity of 5.66% and entrapment efficiency of 96.66%. The swelling ratio was found to be 0.5 at pH 6.4 and 0.2 at pH 7.2.Figure 3, shows comparison of release between pure drug and hydrogel. The drug is released via diffusion. Drug release hydrogel via exhibiting a phase transition in responsive to change in external conditions such as pH. There was decrease in drug release of hydrogel as compared to that of pure drug. SSZ–hydrogel show sustains release pattern.The corresponding plot of (log cumulative percent drug release Vs log time) of the Korsmeyer-Peppa's equation indicated a good linearity of regression coefficient (R^2) and K value 0.913as shown in Table 2.The drug was released during in vitro dissolution studies by a specific transport mechanism which was identified by using certain kinetic models. The value of R^2 indicates the linearity and optimized constant drug release during in vitro dissolution studies. The value of 'n' which is a diffusion coefficient as mentioned in Korsmeyer Pappa's equation represents the transport mechanism. In the given study the value of 'n' for the F-7 were greater than 0.5 and less than 1 indicating Anomalous (Non-Fickian) transport mechanism. That indicates chitosan, CMC polymers swell and drug get diffused through polymer matrix Figure 4-7. From Figure 8, it is clear that with increase in concentration, hemolytic ratio also increased however, it is far below 5 %, the critical safe hemolytic ratio for bio-materials according to ISO/TR 7406. This indicated that these SSZ- hydrogel samples are hem compatible.From stability studies, it was observed that particle size was slightly increased from 226.2 ± 0.027 nm to 227 ± 1.80 nm and % EE was decreased to 87.01 ± 1.35 % during storage. Additionally, there was not much change in PDI means, initially it was 0.189 ± 0.89 and changed to 0.262 ± 1.045. Minimum loss of % EE indicates that the drug was retained within the matrix carriers during the stability period and minimum loss of drug was occurred. From stability studies, The obtained results revealed that there was no significant change in the MPS, PDI and % EE indicating that they were found to be stable at 25 ± 2°C, 60 ± 5% RH for a total period of 3 months Table 3.

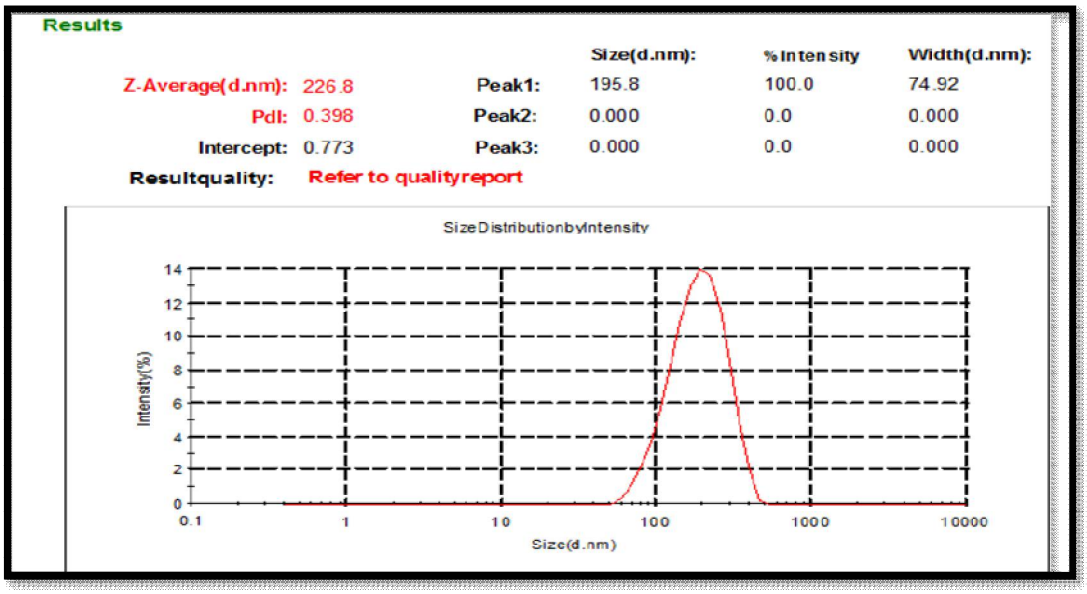

Figure 1 Particle size analysis of hydrogel

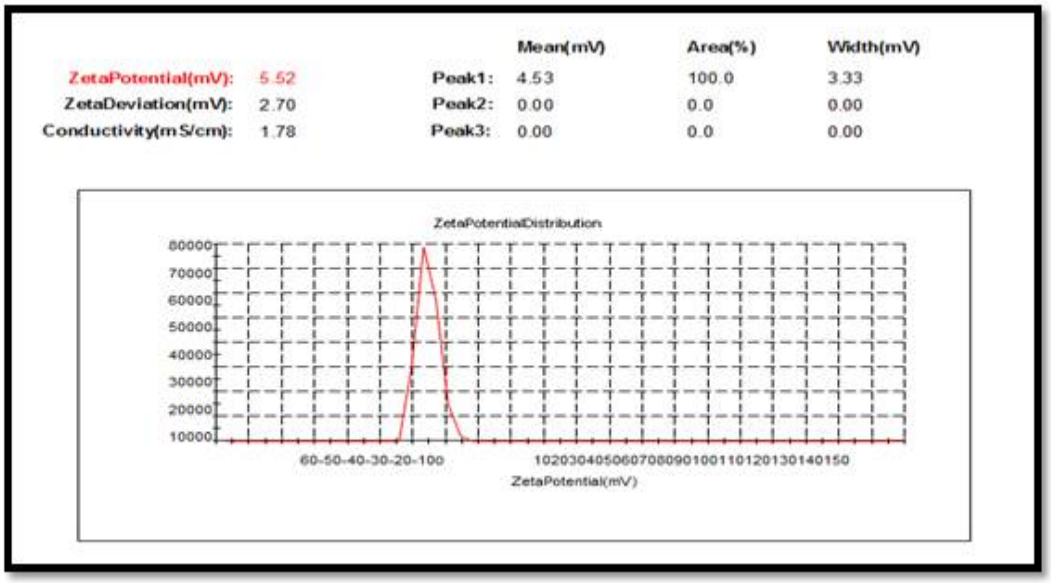

Figure 2 Zeta potential distribution of hydrogel

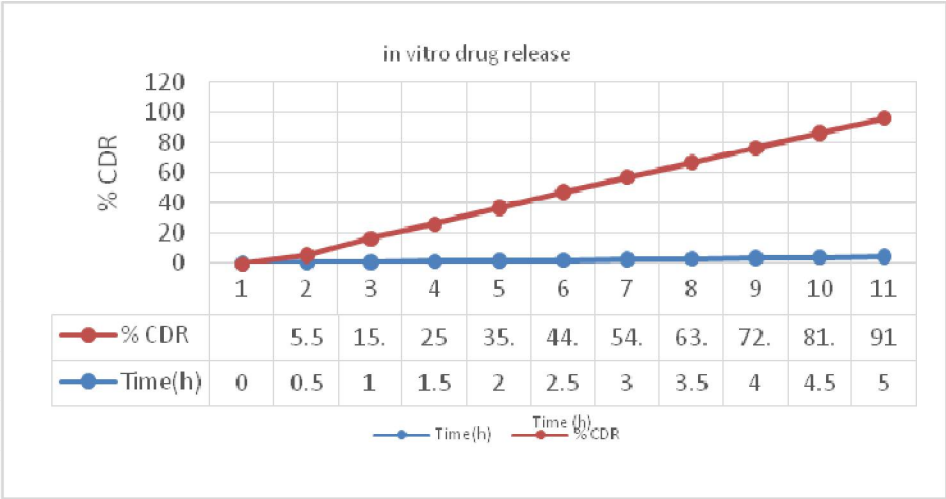

Figure 3 In *vitro* drug releases of SSZ- solution and SSZ–hydrogel

Table 2 Model fitting of the release profile of optimized formulation

Batch	Kinetic Models (R^2)			
	Zero Order	**First Order**	**Higuchi**	**Korsmeyer Peppa's**
F-7	0.716	0.716	0.869	0.913

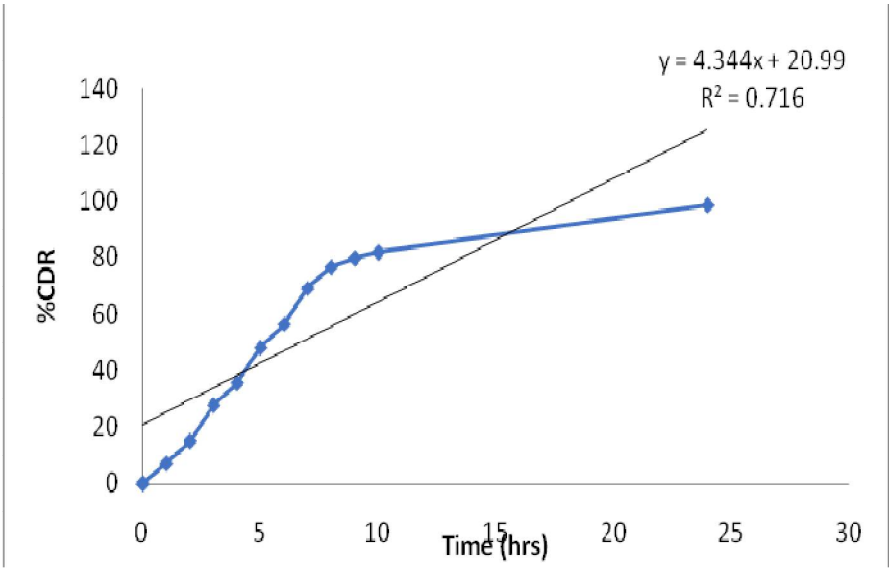

Figure 4 Zero order kinetic

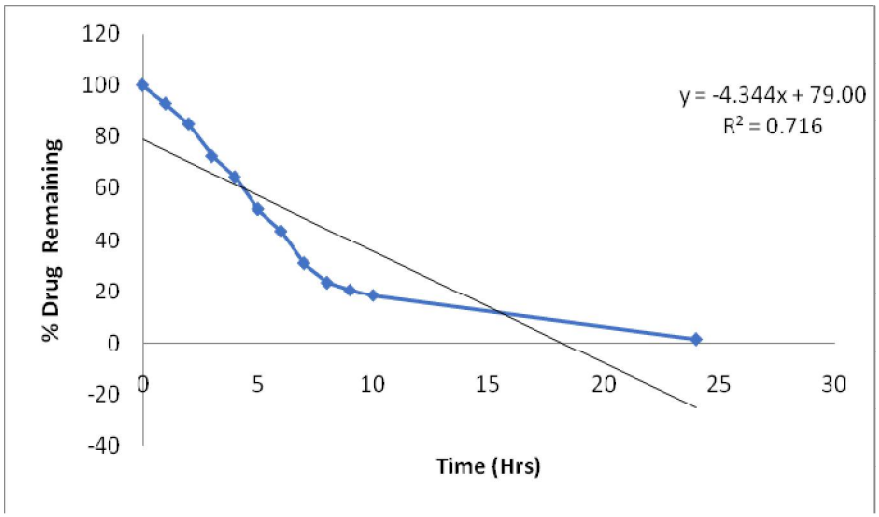

Figure 5 First order kinetic

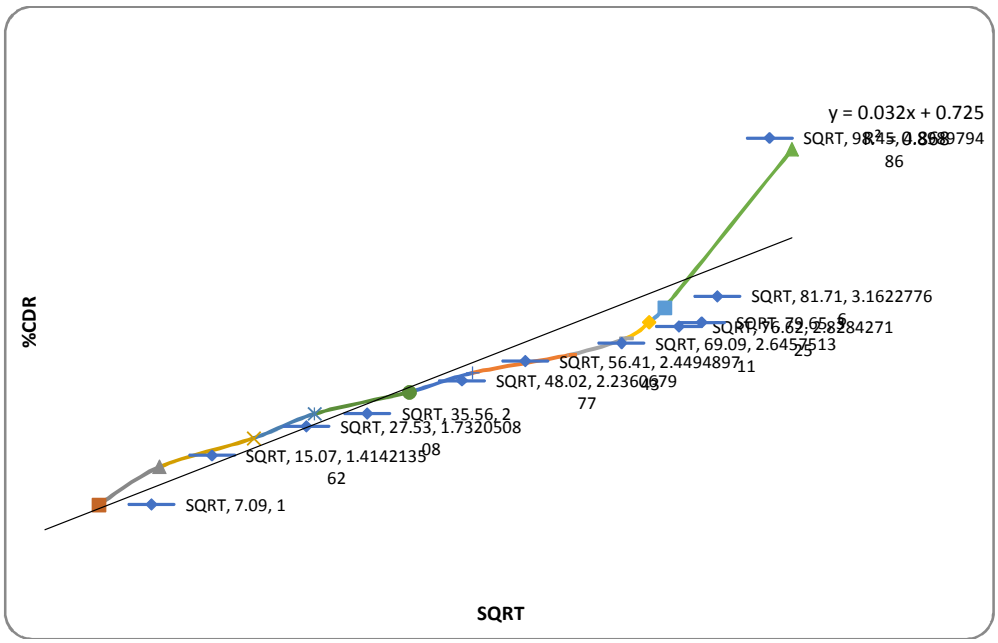

Figure 6 Higuchi model

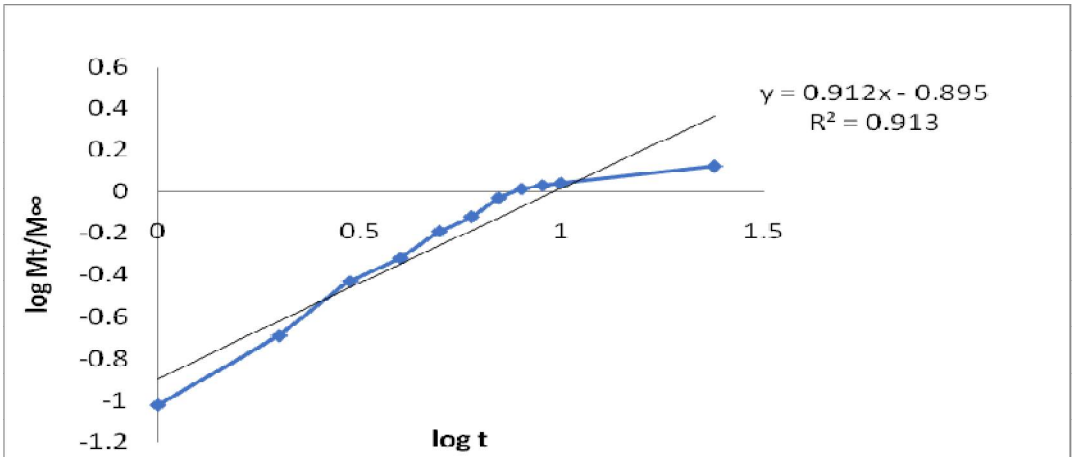

Figure 7 Korsmeyer peppa's model

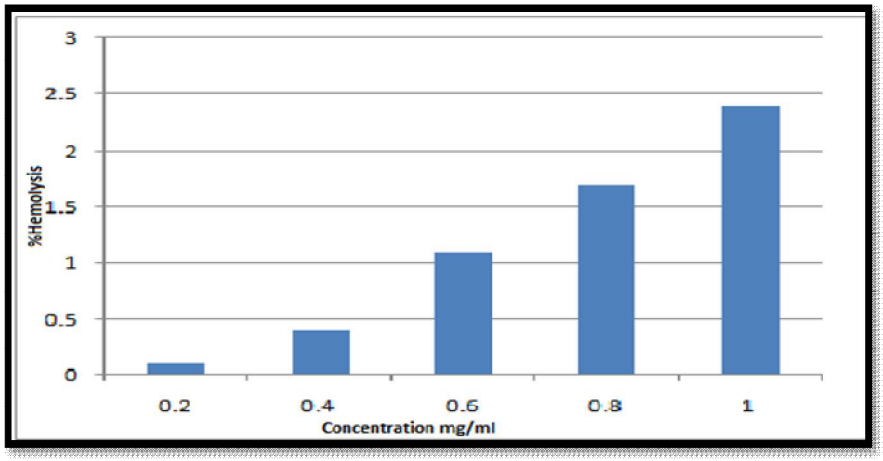

Figure 8. Hemolysis assay

Table 3 Stability studies

Stability parameter	Test period			
	0 Days	**30 Days**	**60 Days**	**90 Days**
MPS (nm)	226.2 ± 0.027	227.2 ± 1.80	229.9 ± 0.03	230.1 ±0.013
PDI	0.3 ± 0.19	0.3 ± 0.53	0.3 ± 0.57	0.3 ± 0.96
% EE	96.66 ± 1.18	94.02 ± 0.02	90.98 ± 1.05	87.01 ± 1.35

CONCLUSION

In the present study, attempts were made to develop and evaluate chitosan hydrogel for colon-targeted delivery of sulfasalazine by cross-linking method.After the formulation of hydrogel, they were evaluated to estimate their particle size, zeta potential, polydispersity index, entrapment efficiency and drug loading, swellability, in vitro drug release stability study and hemolysis assay. The F-7 batch showed most promising results compared to other. Though, long-term stability study and a clinical trial are required for future development of this formulation. From the experimental results it was evidenced that the controlled release of sulfasalazine hydrogel was successfully formulated with less side effects.

REFERENCES

1. Antonin KH et al. (1992). Colonic absorption of human calcitonin in man. Clin Sci (Lond). 83(5):627–31.
2. Saffran M et al. (1986). A new approach to the oral administration of insulin and other peptide drugs. Science. ;233(4768):1081–4.
3. Gibson SA et al. (1989). Significance of microflora in proteolysis in the colon. Appl Environ Microbiol. ;55(3):679–83.
4. Youan BB. (2004). Chronopharmaceutics: gimmick or clinically relevant approach to drug delivery? J Control Release. ;98(3):337–53.
5. Kinget R et al. Colonic drug targeting. J Drug Target. 1998;6(2):129–49.
6. Yang L, Chu JS, Fix JA. Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation. Int J Pharm. 2002;235(1-2):1–15.
7. Rubinstein A. (1990). Microbially controlled drug delivery to the colon. Biopharm Drug Dispos. ;11(6):465–75.
8. Brondsted H, Kopecek J. (1992). Hydrogels for site-specific drug delivery to the colon: in vitro and in vivo degradation. Pharm Res. ;9(12):1540–5.
9. Shah N, Shah T, Amin A.(2011). Polysaccharides: a targeting strategy for colonic drug delivery. Expert Opin Drug Deliv. 8(6):779–96.
10. Wakerly Z et al. (1996). Pectin/ethylcellulose film coating formulations for colonic drug delivery. Pharm Res. ;13(8):1210–2.
11. Krishnaiah YS et al. (2001). Guar gum as a carrier for colon specific delivery; influence of metronidazole and tinidazole on in vitro release of albendazole from guar gum matrix tablets. J Pharm Pharm Sci. 4(3):235–43.
12. Prasad YV, Krishnaiah YS, Satyanarayana S. (1998). In vitro evaluation of guar gum as a carrier for colon-specific drug delivery. J Control Release.;51(2-3):281–7.
13. Furda I. (1983). Aminopolysaccharides—their potential as dietary fiber. In: Furda I, ed. Unconventional Sources of Dietary Fiber, Physiological and In vitro Functional Properties. Washington, DC: American Chemical Society; :105Y122.
14. Ormrod DJ, Holmes CC, Miller TE.(1998). Dietary chitosan inhibits hypercholesterolaemia and atherogenesis in the apolipoprotein E-deficient mouse model of atherosclerosis. Atherosclerosis. 138: 329Y334.
15. Yamamoto A, Tozaki H, Okada N, Fujita T.(2000). Colon specific delivery of peptide drugs and anti-inflammatory drugs using chitosan capsules. STP Pharma Sciences. 10:23Y43.
16. Tozaki H, Odoriba T, Okada N, et al. (2002). Chitosan capsules for colon-specific drug delivery: enhanced localization of 5-aminosalicylic acid in the large intestine accelerates healing of TNBS-induced colitis in rats. J Control Release. ;82:51Y61.
17. Fernandez-Hervas MJ, Fell JT. (1998). Pectin/chitosan mixtures as coatings for colon-specific drug delivery: an in vitro evaluation. Int J Pharm. 169:115Y119.
18. Azad Khan AK, Piris J, Truelove SC. (1977). An experiment to determine the active therapeutic moiety of sulphasalazine. Lancet. 2(8044):892–5.
19. Chourasia MK, Jain SK. (2003). Pharmaceutical approaches to colon targeted drug delivery systems. J Pharm Pharm Sci. 6(1):33–66.
20. Bondesen S. (1997). Intestinal fate of 5-aminosalicylic acid: regional and systemic kinetic studies in relation to inflammatory bowel disease. Pharmacol Toxicol. 81 Suppl 2:1–28.
21. Friedman G. (1986). Sulfasalazine and new analogues. Am J Gastroenterol. 81(2):141–4.

22. Klotz U. (1985). Clinical pharmacokinetics of sulphasalazine, its metabolites and other prodrugs of 5-aminosalicylic acid. Clin Pharmacokinet. 10(4):285–302.

CITATION OF THIS ARTICLE

V S. More, D S. Chumbhale, N T. Rangari, P A. Mishra, V C. Sonar. Design, Development and Evaluation of Colon Targeted Hydrogel of An Anti-Inflammatory Drug Sulfasalazine. Bull. Env.Pharmacol. Life Sci., Spl Issue [1] 2022 : 1602-1610