INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR)
2(1): January –March: (2012), 301-307
ISSN: 2231-6078

Research Article

Design and Characterization of Eudragit Coated Chitosan Microspheres of Mesalazine for Irritable Bowel Disease

Vidhi Jain [1*], Shailesh Gupta[3], GK Pandey [1], BK Dubey [1], PK Jain [2], MS Saluja[1]

1. **Department of Pharmaceutics, TIT College of Pharmacy, Bhopal - 462002 (M.P.), India.**
2. **"SAPIENCE" Sapience bio-Analytical Research Laboratory, Indrapuri, Bhopal-462002 (M.P.), India.**
3. **Department of Pharmacy, Kalaniketan Polytechnic College, Jabalpur, 482002, (M.P.), India.**

Abstract

The purpose of this research work was to prepare and evaluate the colon-specific microspheres of Mesalazine for the treatment of irritable bowel disease. Core microspheres of chitosan were prepared by the emulsification method in liquid paraffin and by cross-linking with glutaraldehyde. The core microspheres were coated with EudragitS-100 by the solvent evaporation technique to prevent drug release in the stomach and small intestine. The microspheres were characterized by shape, size, surface morphology, size distribution, incorporation efficiency, and In-vitro drug release studies. The outer surfaces of the core and coated microspheres, which were spherical in shape, were rough and smooth; respectively.

The release profile of Mesalazine from Eudragit-coated pectin microspheres was pH dependent. In acidic medium, the release rate was much slower however the drug was released quickly at pH 7.4. It is concluded from the present work that Eudragit-coated chitosan microspheres are promising carriers for colon-targeted delivery of Mesalazine.

Key Words: Mesalazine, Colon-specific, Microspheres, Chitosan, Eudragit S-100.

Introduction

Colon specific diseases are often inefficiently managed by oral therapy, because most orally administered drugs are absorbed before arriving in the colon. Therefore, colon-specific drug delivery systems, which can deliver drugs to the lower gastrointestinal tract without releasing them in the upper GI-tract, can be expected to increase the quality of life for patients suffering from colon specific diseases. Treatment might be more effective if the drug substances were targeted directly on the site of action in the colon. Lower doses might be adequate and, if so, systemic side effects might be reduced.Number of serious diseases of the colon might be capable of being treated more effectively if drugs were targeted on the colon. Therefore, it appears that targeted drug delivery with an appropriate release pattern could be crucial providing effective therapy for these chronic diseases. In addition to providing more effective therapy of colon related diseases, colon specific delivery has the potential to address important unmet therapeutic needs including oral delivery of macromolecular drugs.

***Corresponding Author**
E-mail: Shailgpharma@yahoo.com
Mob. No. : +919893392221

The representatives of colon specific diseases are Inflammatory Bowel Disease (IBD), including Ulcerative Colitis and Crohn's disease, Irritable Bowel Syndromes (IBS), Constipation and Colorectal Carcinoma.on new system which combines specific biodegradability and pH-dependent release system. The system consists of chitosan (CS) microcores entrapped within acrylic microspheres. Sodium diclofenac (SD), used as a model drug, was efficiently entrapped within CS microcapsules using spray-drying and then microcapsules into Eudragit L-100. eudragit S-100 using an oil-in-oil solvent evaporation method. The size of the CS microcores was small (1.8-2.9µm) and they were efficiently encapsulated within Eudragit microspheres (size between 152 & 223µm) forming a multi reservoir system [1]. Glipizide microspheres containing chitosan were prepared by simple emulsification phase separation technique using glutaraldehyde as a cross-linking agent the colon specific microspheres of 5 FU for the treatment of colon cancer [2]. Chitosan are biocompatible, mucoadhesive, permeation enhancing effect, anticholesterolemic and antimicrobial agent [3]. Core microspheres of alginate were prepared by the modified emulsification method in liquid paraffin and by cross-linking with calcium chloride. The core microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the stomach and small intestine. The microspheres were characterized by shape, size, surface morphology, size distribution, incorporation efficiency and in-vitro drug release studies [4]. The properties of mucoadhesive microspheres, e.g., their surface characteristics, force of mucoadhesion, release pattern of the drug, and clearance, are influenced by the type of polymers used to prepare them. Suitable polymers that can be used to form mucoadhesive microspheres include soluble and insoluble, non-biodegradable and biodegradable polymers. These can be hydrogels or thermoplastics, homopolymers, copolymers or blends, natural or synthetic polymers [5,6]. Microspheres prepared by different method solvent evaporation [7], spray drying, chemical denaturation [8]. The objective of the present investigation was to design a multi particulate delivery system for site-specific delivery of mesalazine [9] using chitosan [10] and pH-sensitive polymer (Eudragit S100) for the treatment IBD. This system is anticipated to protect thedrug loss in the upper GI tract, which results from the inherentproperty of Eudragit S100 (ES) and deliver Mesalazine in the colon only. The use of enteric polymers

(ES) as protective coating on the microspheres makes them able to release the drug at the particular pH of colonic fluid.

Material and Methods

Mesalazine as a gift sample from torrent pharmaceutical, India**,** Chitosan was obtained from Hi Media Laboratories Ltd, Mumbai, India. Eudragit S-100 was obtained from Ranbaxy Laboratory Ltd (Haryana, India). Span 80, glutaraldehyde, toluene, acetone, hexane, and light liquid paraffin were purchased from Central Drug House Pvt Ltd, Mumbai, India. All other reagents and chemicals used were of analytical grade.

Method of Preparation:

Preparation of chitosan microspheres Cross linked Chitosan microspheres were prepared using emulsion method employing glutaraldehyde as cross linker [11]. Chitosan solution (4%w/v) was prepared in 5% aqueous acetic acid solution in which the drug was previously dissolved and dispersed in liquid paraffin (1:1 mixture of light and heavy) containing span 80 (1%w/v). The dispersion was stirred using a specially fabricated stainless steel half-moon paddle stirrer and glutaraldehyde saturated toluene solution (1ml to 3ml) was added with stirring. The stirring was continued further 4hr, then microspheres were centrifuged, washed two times with hexane and acetone and dried in vacuum dessicator for 48 hrs. Preparation of cross linked chitosan microspheres shown in Table 1.

Coating of chitosan microspheres:

Chitosan microspheres were coated with ES using oil-in-oil solvent evaporation method. Chitosan microspheres (50 mg)were dispersed in 10 mL of coating solution prepared by dissolution of 500 mg of ES 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% w/v 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 dessicator.

Characterization of Microspheres

Morphological Study of Microspheres:

The shape and surface morphology of chitosan microspheres were investigated by using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300 A^0 under an argon atmosphere using a gold sputter module is a high vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope [12].

***In Vitro* Drug Release from Microspheres:**

In vitro drug release studies of both coated and uncoated microspheres were carried out in gastrointestinal fluid at different ph. extraction technique using USP dissolution test apparatus. The dissolution studies were carried out in 100 ml dissolution medium, which was stirred at 100rpm at 37±0.1°C (apparatus 2). The scheme of using the simulated fluids at different pH 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 and 5th hour:Simulated intestinal fluid (SIF) of pH 6.8

6th hour: SIF pH 7.5.Cross linked chitosan microspheres and Eudragit (S-100) coated chitosan Microspheres bearing drugs were suspended in dissolution media (100ml) at 37±0.1°C. Samples were withdrawn periodically and compensated with same amount of fresh dissolution media. The samples were analyzed for drug content by measuring absorbance using UV Spectrophotometer, Shimadzu 1700 [13].

Stability Study:

Stability of microspheres formulation on storage is of great concern as it is the major restraint in their development as marketed preparation. Eudragit (S 100) coated chitosan microspheres (Optimized formulation) was subjected to exhaustive stability testing during which they were stored in amber colored bottles at 4˚C, at Room Temperature and at 45±2˚C for 30 days period. Samples were withdrawn and observed for any change in particle size and encapsulation efficiency.

Particle Size:

Particle size analysis of microsphere was determined by using optical microscopy method. Approximately 500 microspheres were counted for particle size using a calibrated compound microscope.

Percent Entrapment Efficiency:

Percent Entrapment Efficiency of chitosan microspheres and Eudragit (S-100) coated chitosan microspheres was determined after removal of surface adsorbed drug. The surface adsorbed drug was removed by dispersing accurately weighed amount of microspheres in 10ml PBS pH 7.4 for 10min with occasional shaking. The suspension was then centrifuged at 3000rpm for 5min and the supernatant was kept aside. The sediment microspheres were retreated in the same manner and supernatant of this centrifuge was mixed with first supernatant and then these microspheres were then incubated for 48 hrs with PBS pH 7.4 and the drug concentration was determined spectrophotometrically by UV at 230nm. The Percent Entrapment Efficiency of Eudragit coated chitosan microspheres was determined in the same manner replacing PBS pH 7.4 with 0.1N HCl for removal of surface adsorbed.

Drug –Excipients Compatibility Study By Differential scanning calorimetry:

In order to determine the physical state of drug, i.e. amorphous or crystalline, before and after final formulation and to evaluate any possible drug-polymer, drug-other components interaction, DSC examination was conducted for the optimized formulation, pure drug, the

polymer, the coupling agents using a DSC instrument. Samples of 2-6 mg were placed in aluminium pans and sealed. The probes were heated from 25 ^{0}C to 400 ^{0}C at a rate of 10^{0}C/min under nitrogen atmosphere.

Results and Discussion

Preparation of Eudragit-Coated Chitosan Microspheres:

Chitosan microspheres of Mesalazine were successfully prepared by emulsion technique. The chitosan microspheres were coated with Eudragit S100 by oil-in-oil solvent evaporation method, using coat:core ratio 5:1. The method was optimized using different stirring rate and emulsifier concentration to produce microspheres of smallsize and narrow size distribution, high drug loading efficiency and controlled drug release at the colonic pH. Uniform, surface cross linked and spherical microspheres were obtained as shown in Figure 1 A.

Morphological Study of Microspheres:

Photomicrographs of the chitosan microspheres were taken from a digital optical microscope and were characterized in terms of sphericity and clumping of microspheres, as observed from the photomicrograph. Microspheres prepared from chitosan solution having concentration 4% were perfectly spherical, smaller clumping was found at 1000 rpm.

In-Vitro Drug Release Study:

In vitro dissolution study was conducted to understand in-vitro drug release profile of uncoated and coated microspheres. The purpose of this formulation was to avoid release of drug in gastric and upper intestinal region but to release the drug slowly in the lower part of the intestine maximizing drug concentration in the colon. Accordingly, the in-vitro drug release study was conducted in pH change method as per USP protocol. percent drug release rate shown in Tables 2 and 3. Release Rate from Chitosan Microspheres presented in Figures 2, 3 and 4 respectively.

Stability Studies:

Stability studies were carried out with optimized formulation which was stored for a period of 45 days at 4±1°C, RT and 40±1°C. The particle size of formulation was determined by optical microscopy using a calibrated ocular micrometer. The particle size of the microspheres was found to increase at RT, which may be attributed to the aggregation of microspheres at higher temperature. At 45±2°C the microspheres aggregate i.e. these microspheres were unstable at higher temperature like 45±2°C. Percent efficiency of microspheres also decreases at higher temperature Like 45±2°C.

Particle Size and Entrapment Efficiency:

Several batches of chitosan microspheres prepared using different drug: polymer ratios yielded microspheres in the size range between 61 and 110µm. The percentage entrapment varied between 54 to 82 % depending on drug: polymer ratio and the emulsifier concentration. With increase in drug: polymer ratio, an increase in the entrapment efficiency and particle size. D:P ratio of 1:2 and 1:3 showed marginal change in the entrapment efficiency (54-66%) and particle size (61 – 80) while, D:P ratio of 1:4 and 1:5 recorded entrapment efficiencies in the range between 71-82 % and particle size between 90 – 110 microns. These figures demonstrate the requirement of D:P ratio in this range for satisfactory entrapment efficiency. Hence, D: P ratio of 1:5 and emulsifier concentration of 1 ml was optimized. effect of storage on particle size and % entrapment efficiency of chitosan microspheres were shown in Table 4.

Drug Exciepient Compatability:

DSC is very useful in the investigation of the thermal properties of microspheres in the present investigation, DSC thermo grams of pure drug, blank Eudragit S-100, chitosan microspheres. Eudragit coated chitosan microspheres shown drug and polymers are compatible with each other. Figures 5, 6, 7, 8 and 9 presents DSC of Chitosan, Mesalazine, Eudragit S-100, Chitosan microspheres, Eudragit coated chitosan microspheres respectively.

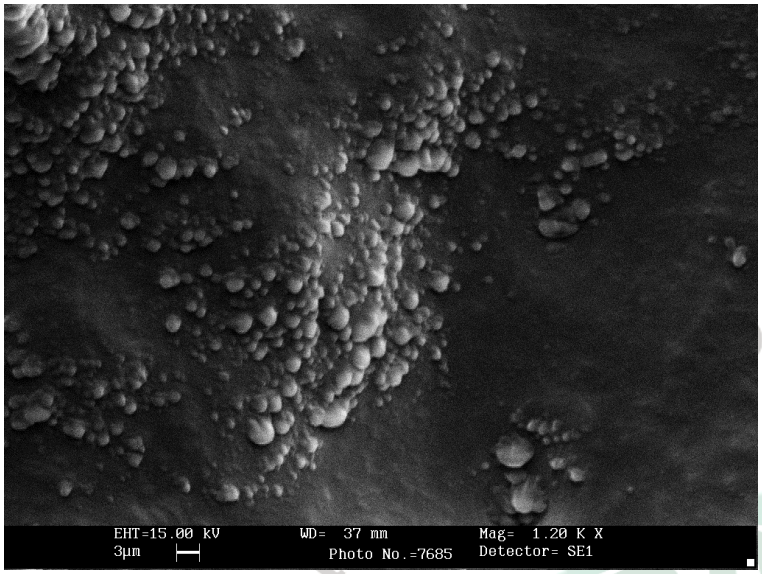
The results of the present study indicate the potential of Eudragit S – 100 coated cross linked chitosan microspheres in designing site-specific delivery for treating colon related disorders for Mesalazine. The investigations of different parameters involved in preparation of coated system proved that the method is simple and reproducible. Eudragit S–100 coated microspheres showed no release in SGF, negligible release in SIF and maximum release in colonic media. Thus, the designed formulation was found suitable for colon delivery which resists drug release in stomach and in upper small intestine but can release maximum amount of drug in colon.

Conclusion

The results of the present study indicate the potential of Eudragit S – 100 coated cross linked chitosan microspheres in designing site-specific delivery for treating colon related disorders for Mesalazine. The investigations of different parameters involved in preparation of coated system proved that the method is simple and reproducible. Eudragit S–100 coated microspheres showed no release in SGF, negligible release in SIF and maximum release in colonic media. Thus, the designed formulation was found suitable for colon delivery which resists drug release in stomach and in upper small intestine but can release maximum amount of drug in colon.

Acknowledgement

Authors are thankful to Mr. Subhendu Sekhar Mishra, Mr. Prabhat kumar Jain, SAPIENCE Bio-Analytical Lab, Bhopal, India providing all the facilities for this research work.



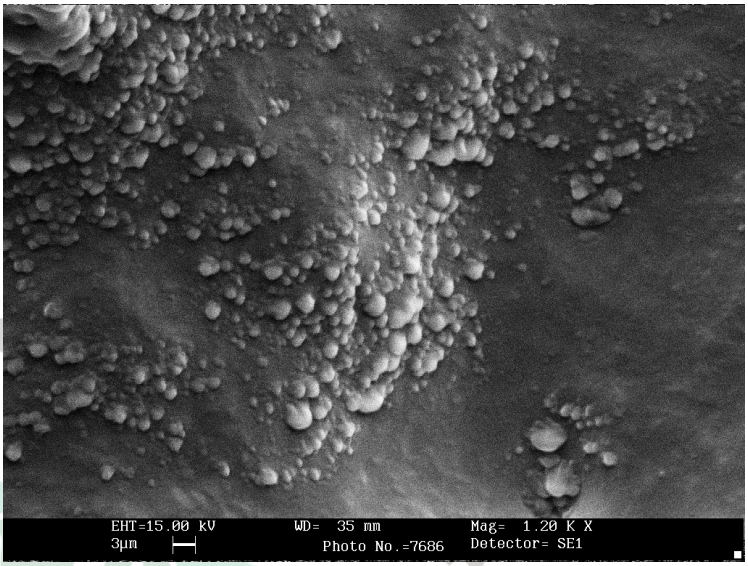


Figure 1A: Chitosan Microsphere

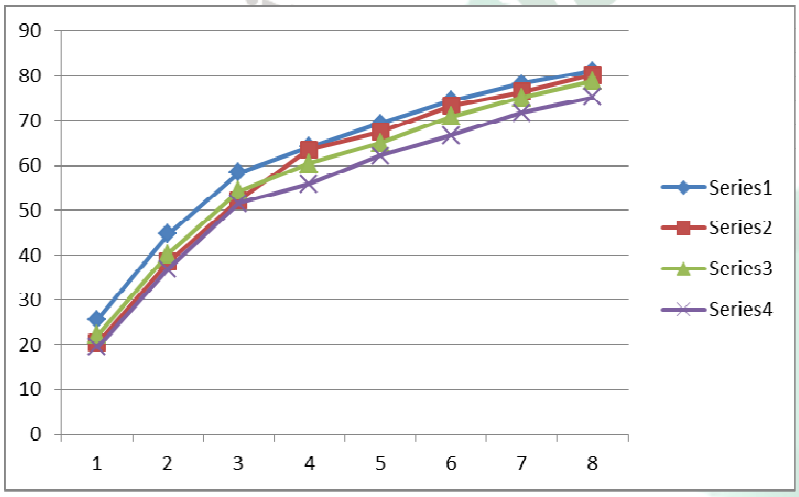


Figure 2: Release Rate from Chitosan Microspheres

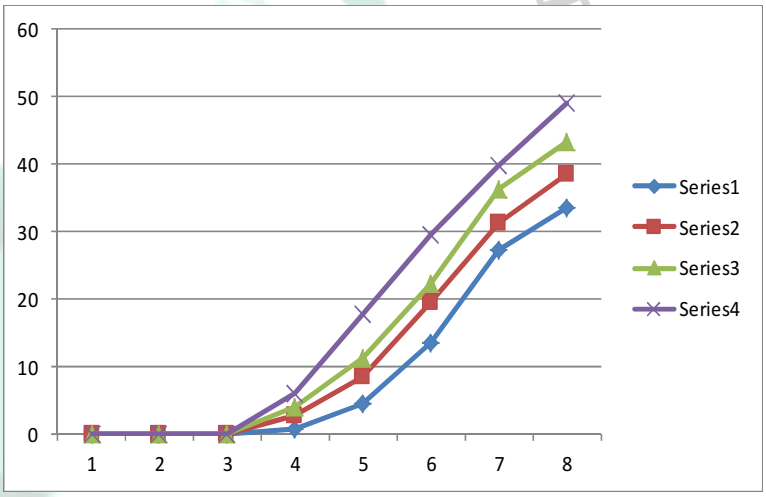


Figure 5: Release Rate from Eudragit Coated Chitosan Microspheres

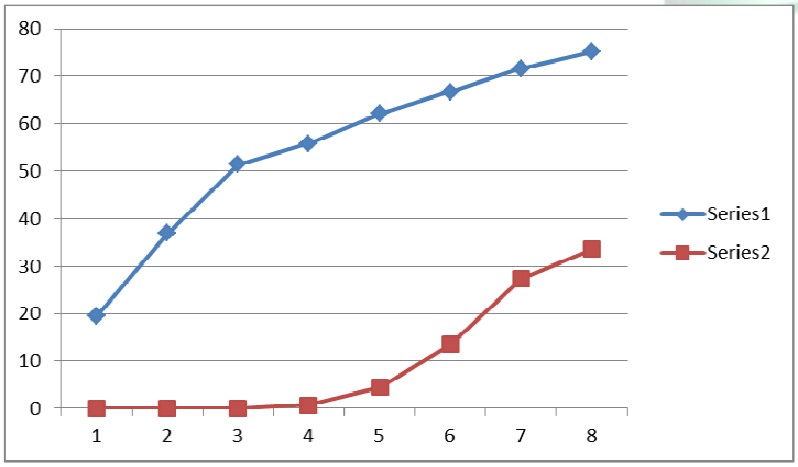


Figure 3: Drug release from chitosan and Eudragit Coated Chitosan Microspheres

Table 1: Preparation of cross linked chitosan Microspheres

Batch No.	Drug:Polymer w/w	Emulsifier conc. (ml)	Particle size (µm)	Entrapment efficiency
CC 1 A	1:2	0.75	72.21 ± 1.93	57.18 ± 0.65
CC 1 B	1:2	1.00	65.22 ± 0.98	56.08 ± 1.17
CC 1 C	1:2	1.25	61.22 ± 1.28	54.22 ± 1.07
CC 2 A	1:3	0.75	80.02 ± 1.80	66.50 ± 0.82
CC 2 B	1:3	1.00	74.15 ± 0.84	64.19 ± 0.97
CC 2 C	1:3	1.25	72.05 ± 1.12	63.29 ± 0.97
CC 3 A	1:4	0.75	98.91 ± 1.20	76.70 ± 1.14
CC 3 B	1:4	1.00	93.41 ± 1.43	75.40 ± 1.34
CC 3 C	1:4	1.25	90.41 ± 1.83	71.44 ± 1.34
CC 4 A	1:5	0.75	110.68 ± 1.41	82.45 ± 0.97
CC 4 B	1:5	1.00	105.51 ± 0.98	81.04 ± 1.15
CC 4 C	1:5	1.25	101.21 ± 1.87	80.00 ± 0.11

Table 1: % Drug release rate

S. No.	Time(hrs)	A1	A2	A3	A4	B1	B2	B3	B4
1	1	25.6	22.1	20.4	19.5	0	0	0	0
2	2	44.7	40.2	38.5	36.9	0	0	0	0
3	3	58.4	54.5	52.2	51.5	0	0	0	0
4	4	64.1	60.4	57.8	59.9	6	4	2.8	0.8
5	5	69.3	65.0	63.6	62.1	17.8	11.3	8.5	4.5
6	6	74.5	70.9	67.5	66.7	29.5	22.2	19.5	13.5
7	7	78.2	75.1	73.3	71.7	39.8	36.2	31.3	27.3
8	8	81.1	78.8	76.4	75.2	49.0	43.2	38.5	33.5

a: A1, A2, A3, A4 % drug release from chitosan microspheres (1:2,1:3,1:4,1:5)
b: B1, B2, B3, B4 % drug release from Eudragit coated chitosan microspheres (1:2, 1:3, 1:4,1:5)

Table 2: drug release from chitosan and Eudragit Coated Chitosan Microspheres

S. No.	Time (hrs)	Percent Drug Release	
		Chitosan Microspheres	Eudragit Coated Chitosan Microspheres
1	1	19.5%	0
2	2	36.9%	0
3	3	51.5%	0
4	4	59.9%	0.8%
5	5	62.1%	4.5%
6	6	66.7%	13.5%
7	7	71.7%	27.3%
8	8	75.2%	33.5%

Table 3: Effect of Storage on Particle Size and Percent Entrapment Efficiency of Chitosan Microspheres

Parameters	Initial Observation	After 30 days		
		At 4ºC	At RT	At 45±2 ºC
Particle Size (µm)	105.5	104.8	105.4	107.3
Percent Entrapment Efficiency	80.4%	79.8%	80.1%	76.2%

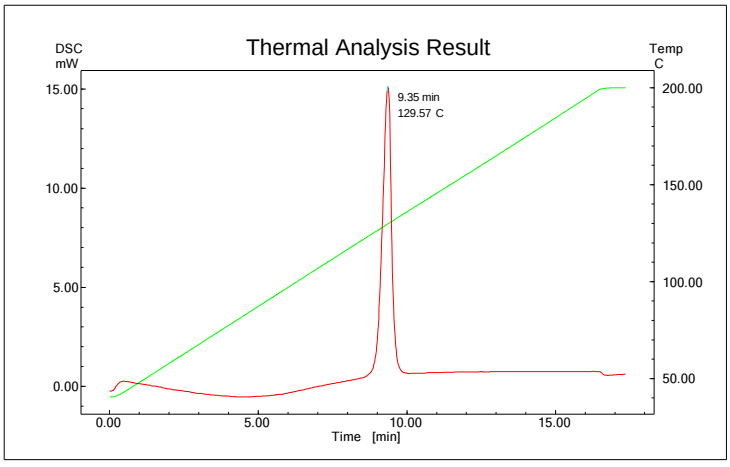


Figure 6: DSC of Chitosan

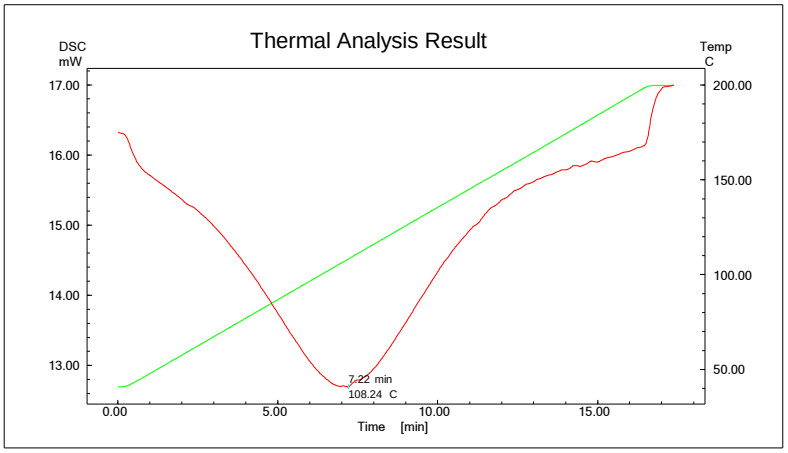


Figure 7: DSC of Mesalazine

References

1) Lorenzo LML, Remunan LC, Vila JJL, Alonso MJ, Design of microencapsulated chitosan microspheres for colonic drug delivery, J. Control. Release 1998; 52: 109-118.
2) Patel JK, Patel RP, Amin AF, Formulation and evaluation of mucoadhesive Glipzide microspheres, AAPS Pharm. Sci. Tech. 2005; 06 (1): E 49-E50.
3) Aranaz I, Mengibar M, Harris R, Heras A, Functional characterization of chitin & chitosan, Current Chemical Biology 2009; 3: 203-230.
4) Rahman Z, kohli K, Khar RK, Ali M, Characterization of 5-FU microspheres for colon delivery, AAPS Pharm. Sci. Tech. 2006; 7 (2): 47-53.
5) Vasir JP, Tambwekar K, Garg S, Bioadhesive microspheres as a controlled drug delivery system, Int. J. Pharm. 2003; 255: 13-32.
6) Vyas SP, Khar RK, Targeted and controlled drug delivery, 1st edition, CBS Publishers and Distributors, New Delhi 2002; 417-450.
7) Anande NM, Jain SK, Jain NK, Con-A conjugated mucoadhesive microspheres for the colonic delivery of diloxanide furoate, Int. J. Pharm. 2008; 359(1-2): 182-189.
8) Dubey RR, Parikh RH, Two stage optimization process for formulation of chitosan microspheres, AAPS Pharm. Sci. Tech. 2004; 7(2): E1-E9.
9) Florey K, Harry G, Analytical profile of drug substances and excipient, 25th Edition, Elsevier: 212-213.
10) Rowe RC, Shenskey PJ, Owen S, Handbook of Pharmaceutical excipient.5th edition, Published by pharmaceutical press; 2001: 159-161.
11) Thanoo et al; Eudragit coated chitosan microspheres of 5-FU for cancer. Int. j. Pharm, 1992.
12) Dodov MG, Calis S, Crcarevska MS, Geskovski N, PetrovskaV, Wheat germ agglutinin conjugated Chitosan-Ca-alginate microparticles for local colon delivery of 5–Fu: development and in vitro characterization. Int. J. Pharm. 2009; 381 (2): 116-175.
13) Indian Pharmacopoeia. Published by the Controller of Publications, Ministry of Health and Govt. of India, New Delhi. Vol 2; 2007: 133-1134.

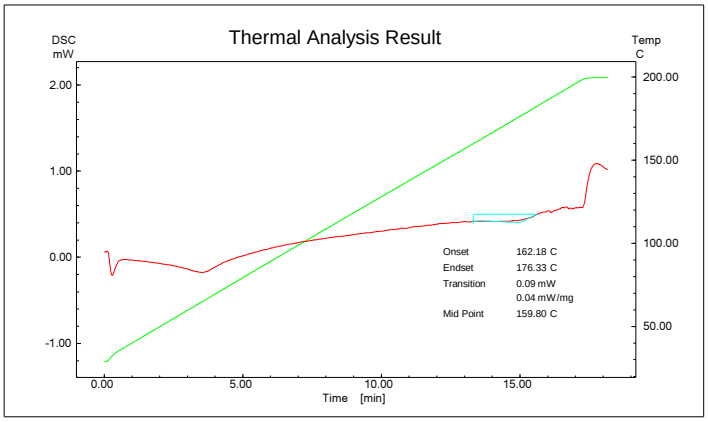


Figure 8: DSC of Eudragit S-100

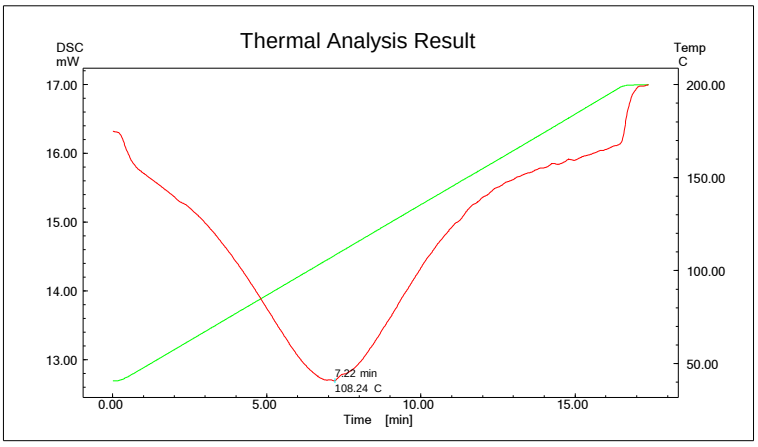


Figure 9: DSC of Chitosan microspheres

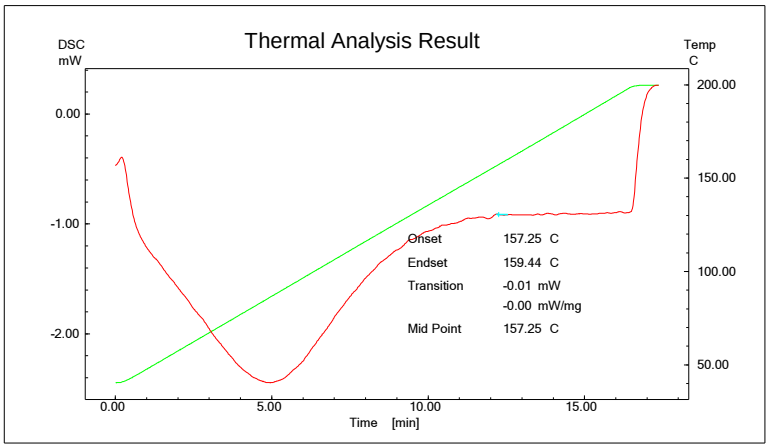


Figure 10: DSC of Eudragit coated chitosan microspheres