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RESEARCH ARTICLE

FORMULATION AND EVALUATION OF COLON TARGETED DELAYED RELEASE MICROSPHERIC CAPSULES OF ALBENDAZOLE

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ABSTRACT

The aim of the present work was to prepare and evaluate the colon targeted delayed release microspheric capsules of albendazole. The preparation of albendazole microspheres by ionic gelation method avoids the usage of toxic organic solvents and considered to be relatively economical method. Calcium chloride was used as a counter ion and two polysaccharides namely sodium alginate and chitosan were used as a polymers that restrict the drug release in stomach and upper GIT and thus delayed the drug release. Different formulations were formulated by varying the polymers and counter ion concentration. These formulations were subjected to various evaluation parameters like percentage yield, particle size, surface morphology, drug entrapment efficiency, DSC study, flow property and *in vitro* drug release studies. The results of all parameters are tabulated and depicted graphically in the result and discussion section. The experimental results demonstrated that the prepared microspheres of albendazole for colon targeting may reduce the side effects of the drug caused by its absorption from the upper part of GIT when given in conventional dosage forms. Thus the albendazole microspheres have the potential to be used as a drug carrier for an effective colon targeted drug delivery system.

KEYWORDS: Colon targeting**,** Albendazole, Ionic gelation, Microspheres, Capsule, Colon.

INTRODUCTION:

The soil-transmitted helminths are one of the world's most important causes of physical and intellectual growth retardation. Yet, despite their educational, economic, and public-health importance, they remain largely neglected by the medical and international community[1]. Over the past 5 years, however, the worldwide community has begun to recognize the importance of these infections after revised estimates showed that their combined disease burden might be as great as those of malaria, tuberculosis or HIV infection[2].

It is now recognized that STH infection causes significant morbidity worldwide with 39 million disability adjusted life years (DALYs) lost each year-more than those lost to tuberculosis (36 million yearly) and approaching those lost to malaria (46.5 million yearly)[1]. Hookworm infection alone causes the loss of 22 million DALYs[3].

Overall, it is believed that, together with schistosomiasis, the soil-transmitted helminth infections account for 40% of the global morbidity caused by all infectious diseases, exclusive of malaria[4].

In some of the less developed regions of the world, helminthic intestinal infections may impair ocular, muscle or blood systemic circulation[5]. The low cost, good tolerance and broad spectrum of activity of albendazole make it typically the drug of choice for these cases.

To the date, oral delivery is still the most favorable route of drug administration, especially for chronic therapies where repeated administration of drugs is required. Oral administration offers less pain, good patient convenience and reduced risk of cross infection and needle stick injuries[6]. Thus, formulations of oral drug delivery continue to dominate more than half of the drug delivery market share[7]. Despite these advantages, the oral route is not applicable to the administration of protein and polypeptide drugs, due to their high susceptibility to digestive enzymes in the gastrointestinal tract (GIT), and poor absorption. As a result, new strategies of drug delivery have been developed to overcome obstacles encountered by oral delivery. Among these strategies, oral colon-specific delivery has been extensively studied from the last two decades[6].

The colonic region of the GIT is one area that would benefit from the development and use of such modified release technologies. Although it has simple functions in the form of water and electrolyte absorption, the colon is vulnerable to a number of disorders including ulcerative colitis, crohn's disease, helminthes, irritable bowel syndrome and carcinomas[7]. Targeted drug delivery to the colon, by means of combination of one or more controlled release mechanisms, hardly releases drug in the upper part of the

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GIT but releases in the colon following oral administration.

Specifically delivering drug to the colon, a lot of benefits would be acquired in terms of improving safety and reducing toxicity when treating local or systemic chronic diseases[8].

Most of the conventional drug delivery systems for treating the colon disorders are failing as the drugs do not reach the site of action in appropriate concentrations. Thus, an effective and safe therapy of these colonic disorders, using site specific drug delivery system is a challenging task to the pharmaceutical technologists[9]. The therapeutic advantages of targeting drug to the diseased organ include

- Delivery of drug in its intact form as close as possible to the target site.
- The ability to cut down the conventional dose.
- Reduced incidence of adverse side effects.
- Low hostile environment, the colonic transit time is long (20-30 h). The longer residence time, less peptidase activity, natural absorptive characteristics and high response to absorption enhancers make the colon a promising site for the delivery of most of drugs for systemic absorption.[9]

In the present study the microsphereic capsules had formulated using sodium alginate and chitosan as polymers and calcium chloride as a counter ion by ionic gelation technique.

MATERIALS AND METHODS:

MATERIALS:

Albendazole was received as gift samples from Maan pharmaceuticals pvt. Ltd. Gujarat, India. Chitosan was purchased from OZONE® International, Mumbai. Sodium alginate and Glacial acetic acid were purchased from Central Drug House, New Delhi. All the materials used in this research study comply with the pharmaceutical and analytical standards, respectively. Whole research work was carried out at Maharishi Arvind Institute of Pharmacy, Jaipur during year 2012-2013.

METHOD:

METHODS OF PREPARATION:[10,11]

Ionic gelation method was used for the preparation of albendazole microspheres using chitosan and sodium alginate as polymers. The formulations were prepared using 40ml of sodium alginate solution containing albendazole and 100ml of Chitosan solution (prepared in 0.5% v/v glacial acetic acid) containing calcium chloride dihydrate. PH of chitosan solution was adjusted to 5.5 with 10% sodium hydroxide solution. Sodium alginate and drug solution was loaded into a syringe fitted with 23G needle. 100ml of chitosan-calcium chloride solution was taken in a beaker and stirred at 400 rpm. Alginate and drug solution was added at a constant rate to chitosan-calcium chloride solution with constant stirring. A reaction time of 10 minutes was used. After forming microspheres, it was filtered, washes with distilled water and hardened with acetone. The microspheres were dried overnight and kept in an airtight container for further studies. Drug to Polymer ratio of 1:1 was selected for preliminary trials. Different batches were prepared by varying the drug to polymer ratios and counter ion concentration.

After the microspheres were produced, they were weighed and filled in to the empty gelatin capsules.

Table 1: Formulation Plan

Batch Code	Drug (mg)	Sodium alginate (mg)	Chitosan (mg)	RPM	Calcium chloride (%w/v)
F1	200	400	-	400	1
F2	200	600	-	400	1
F3	200	700	-	400	1
F4	200	800	-	400	1
F5	200	900	-	400	1
F6	200	1000	-	400	1
F7	200	800	-	400	2
F8	200	800	-	400	3
F9	200	800	-	400	4
F10	200	800	-	400	5
F11	200	800	-	400	6
F12	200	800	100	400	2
F13	200	800	200	400	2
F14	200	800	300	400	2
F15	200	800	400	400	2

CHARACTERIZATION:

FOURIER TRANSFORM INFRARED (FTIR):

Fourier transform infrared (FTIR) spectra were obtained on an IR spectrophotometer (Maharishi Arvind Institute of Pharmacy, Jaipur) from 3500 to 600 cm^{-1}.

PARTICLE SIZE DETERMINATION OF NANOCRYSTALS:[12]

Particle size was measured by light Microscope (Quasmo, India) with stage micrometer and eye- piece. Size of 100 particles of pure albendazole and different batches were determined and averages of all batches were calculated.

% YIELD DETERMINATION:[13]

The yield of each batch was calculated in terms of percentage yield as per following formula.

% Yield= (practically obtained yield/theoretical yield) ×100

Theoretical yield was determined by calculation assuming the entire drug and polymer present in the prepared solution used for microencapsulation and no loss occurs at any stage of preparation. Practically obtained yield was determined by weighing all microspheres which were obtained by ionic gelation method.

SURFACE MORPHOLOGY -SCANNING ELECTRON MICROSCOPY (SEM):[14]

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). A small amount of microspheres was spread on aluminium stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope chamber. Scanning electron photomicrograph was taken at the acceleration voltage of 20 KV, chamber pressure of 0.6 mm Hg, at different magnification. Microspheres of the various batches were characterized in terms of sphericity and clumping of microspheres.

FLOW PROPERTIES:[15]

Flow properties of microspheres were studied by measuring the angle of repose of the formulation by employing fixed funnel method. Albendadazole microspheres were weighed and passed through the funnel, which was kept at a height of 'h' from the horizontal surface. The passed microspheres formed a pile of a height 'h' above the horizontal surface and the radius 'r' of the pile was measured and the angle of repose was determined for all the batches by using the formula.

$\tan\theta = h / r$,

Where, θ=angle of repose

h= height of the pile

r = radius of the pile

PERCENTAGE DRUG ENTRAPMENT EFFICIENCY:[16]

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula

PDE = (practical drug entrapped / theoretical drug entrapped) ×100

Theoretical drug entrapment was determined by calculation assuming that the entire drug present in the microspheres and no loss occurs at any stage of preparation of microspheres. Practical drug entrapment was determined by taking a calculated mg of accurately weighed microspheres were crushed in a glass mortar-pastel and the powdered microspheres were dissolved in 10 ml of DMF. The solution was analyzed using UV spectrophotometer (Shimadzu 1800, Japan) for the drug entrapment.

DIFFERENTIAL SCANNING CALORIMETRY (DSC) STUDIES:[14]

DSC study was carried out using DSC-60 instrument (Shimadzu, japan) to check the matrix formation as well as the compatibility of ingredients. DSC thermograms of pure drug and polymers were taken for their identical endothermic reaction. Finally physical mixture of drug-polymers and microspheres was scanned for DSC. The entire samples were run at a scanning rate of 10°C/min from 50-300°C.

***IN VITRO* DRUG RELEASE**[17,18]

In vitro release study of microspheres was carried out using USP Type 1 dissolution apparatus (Electro lab, TDT-06T, India) at 37°C ± 0.5°C and at 50 rpm using 900 ml of dissolution medium. Microspheres equivalent to 20 mg of albendazole were used & they were placed into the empty gelatin capsule and it was placed into the basket. First 2 hrs. dissolution was carried out in acidic medium ph 1.2, then 18 hrs in phosphate buffer ph 7.4 containing enzymes (enzyme pectinase & dextranase). Ten ml of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45µm membrane filter, diluted suitably and analyzed using UV spectrophotometer (Shimadzu 1800, Japan). Equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percentage drug dissolved at different time intervals was calculated.

RESULTS AND DISCUSSION:

FTIR STUDY:

Complex of ABZ and polymers showed superimposed spectra of ABZ and both polymers which prove the compatibility of excipients with the ABZ.

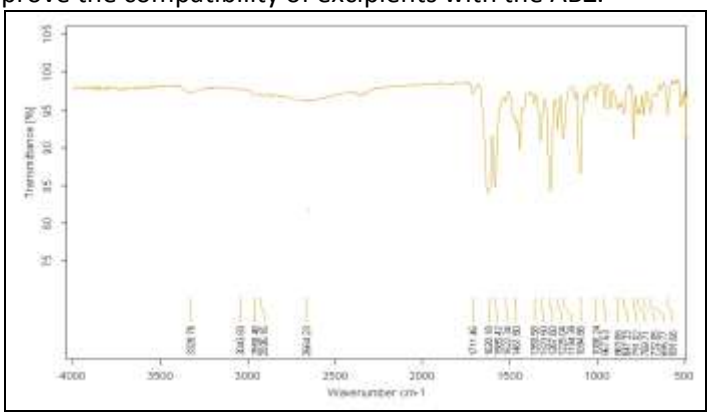

Figure 1: IR Spectra of pure drug

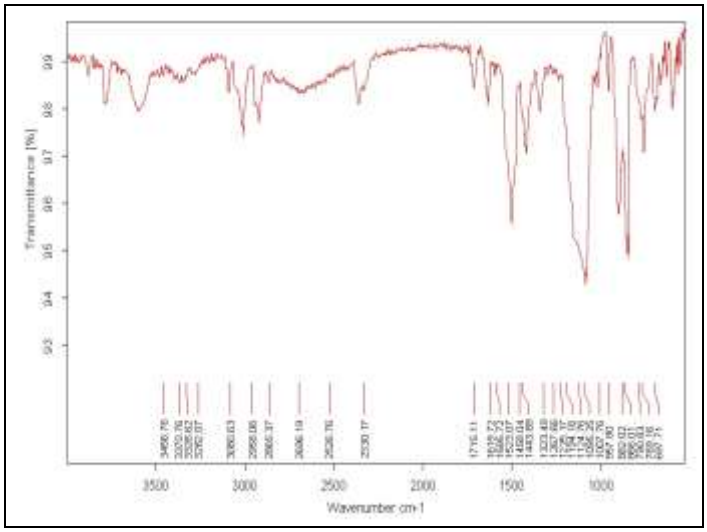

Figure 2: IR Spectra of Sodium alginate

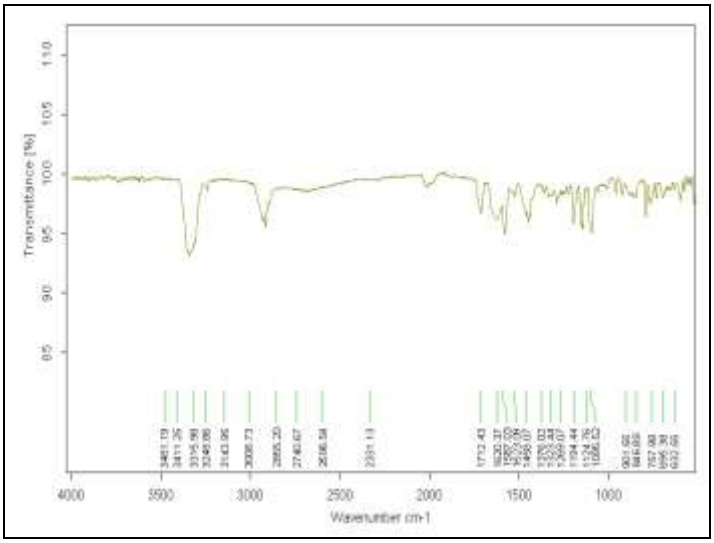

Figure 3: IR Spectra of Chitosan

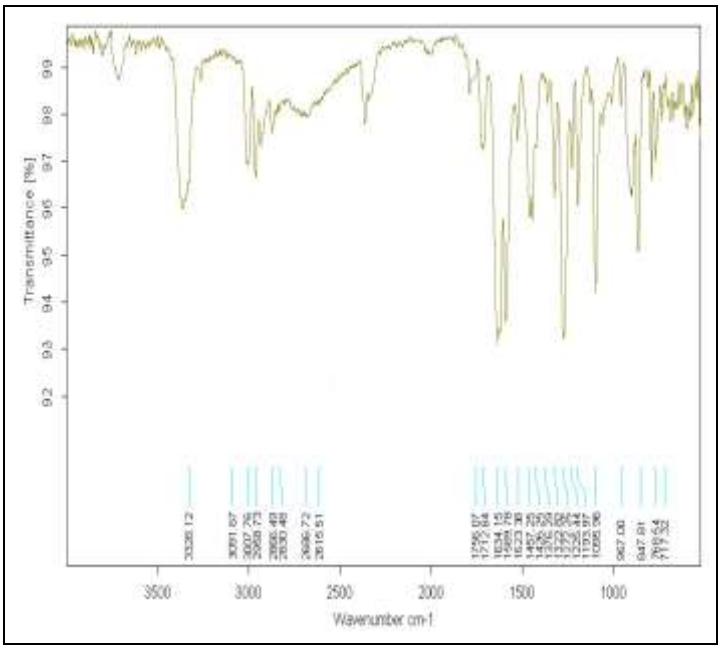

Figure 4: IR Spectra of Physical mixture

PARTICLE SIZE ANALYSIS:

Particle size distribution of microspheres was determined by optical microscope fitted with an ocular micrometer and stage micrometer. The particle sizes of the microspheres were found between 104.28 µm & 212.85 µm. The particle sizes of all the fifteen formulations were shown in the Table 3.

It was observed that with increase in polymer concentration in the microspheres from F1 to F6, the particle size of the microspheres increases. This was due to the increase in relative viscosity, which led to increase in particle size.

PERCENTAGE YIELD:

Percentage yield of different formulations were calculated and the % yield was found between 63.6% and 86.5%. The same was showed in Table 3. The results indicated that optimum concentration of polymer and counter ion yields better percentage of albendazole microspheres.

SHAPE AND SURFACE MORPHOLOGY:

Surface morphology of the microspheres was investigated with a scanning electron microscope. Different magnifications were used while taking these photomicrographs. Particles surface of all formulations was slightly rough surfaced but spherical and discrete.

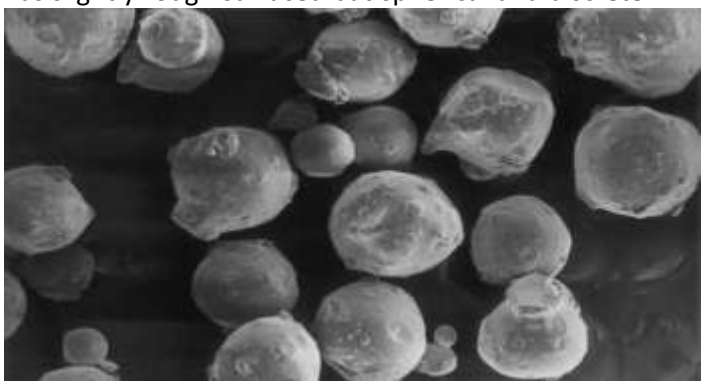

Figure 5: SEM photograph (100X) of microspheres (batch F14)

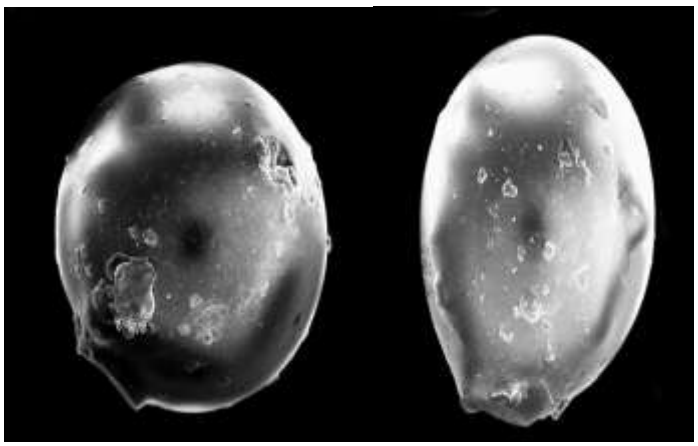

Figure 6: SEM photograph (500X) of microsphere (batch F14)

FLOW PROPERTIES:

Flow properties of the prepared microspheres were characterized by measuring the angle of repose. All the formulations showed improved flow properties, as compared to the pure drug. The values of angle of repose were found between 17° & 34° indicates optimum flow and all the batches of microspheres were found to fit in respect of flowability and thus it was possible to fill the microspheres in to the capsules. It was found that increase in polymer content will increase in angle of repose. The results were shown in Table 2.

Table 2: Flow Properties of albendazole Microspheres

Formulation	Angle of Repose	description
Pure drug	39° 69"	Poor
F1	17° 21"	Excellent
F2	18° 36"	Excellent
F3	20° 16"	Good
F4	23° 29"	Good
F5	28° 45"	Good
F6	33° 39"	Passable
F7	21° 18"	Good
F8	23° 27"	Good
F9	22° 77"	Good
F10	27° 62"	Good
F11	24° 07"	Good
F12	22° 08"	Good
F13	21° 42"	Good
F14	26° 85"	Good
F15	30° 37"	Passable

DRUG ENTRAPMENT EFFICIENCY:

The drug entrapment efficiency was determined and the results were listed in Table 3. The values of drug entrapment efficiency were found between 43% and 68.25%. As the polymer concentration was increased the entrapment efficiency was also increased. The results indicated the polymer concentration plays a major role in drug entrapment efficiency.

Table 3: Particle size, percentage yield and drug entrapment efficiency of albendazole Microspheres

Formulation	Particle Size (µm)	% Yield (%)	Entrapment fficiency (%)
F1	104.28	63.6	64.5
F2	109.71	64.1	52.7
F3	111.71	80.2	56.75
F4	116. 0	82.3	59.5
F5	123.31	69.3	60.75
F6	157.25	78.3	64.25
F7	109.5	83.4	67.0
F8	105.88	84.2	49.5
F9	115.14	83.7	53.0
F10	121.71	84.8	49.0
F11	113.14	72.8	43.0
F12	196.57	78.2	62.75
F13	212.85	83.4	67.5
F14	179.14	86.5	68.25
F15	171.71	76.3	60.0

DIFFERENTIAL SCANNING CALORIMETRY (DSC) STUDIES:

DSC thermograms of pure drug and polymers as well as mixture and microsphere were taken for their identical endothermic reaction.

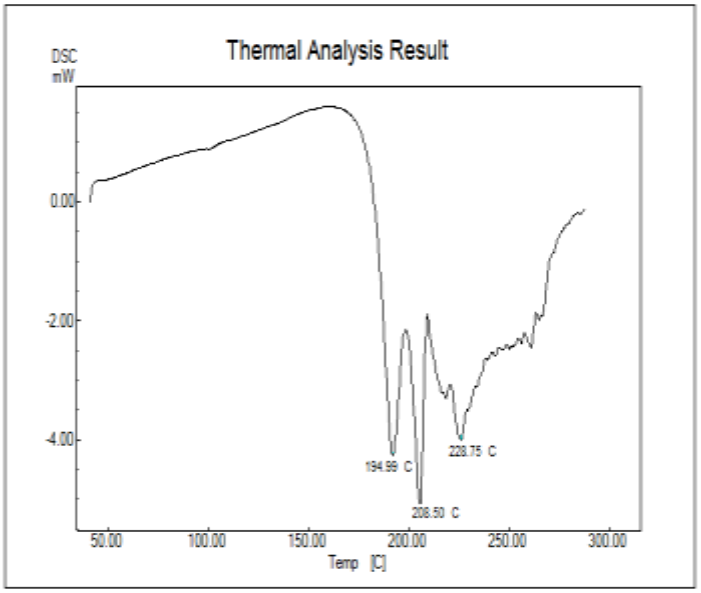

Figure 7: DSC graph of albendazole

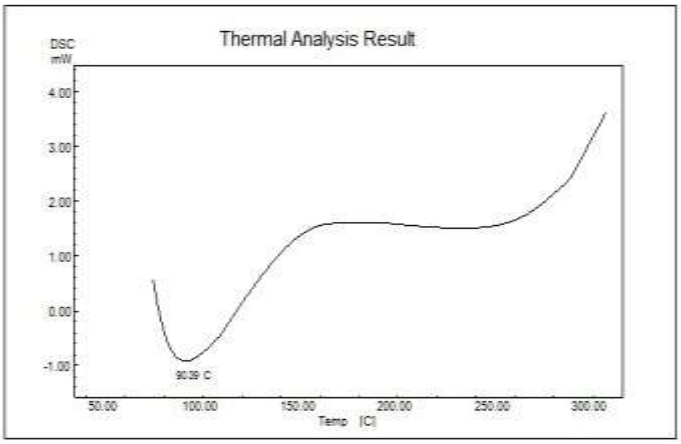

Figure 8 DSC graph of chitosan

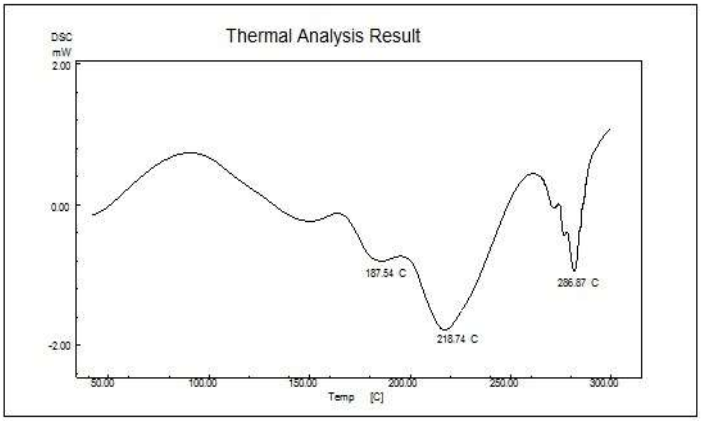

Figure 9: DSC graph of sodium alginate

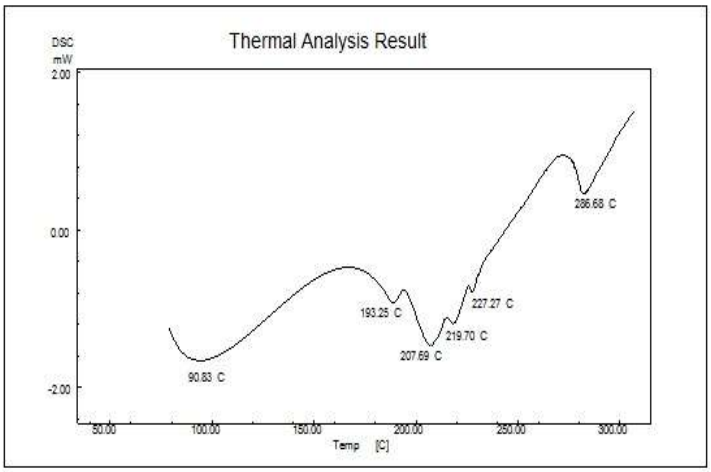

Figure 10: DSC graph of physical mixture

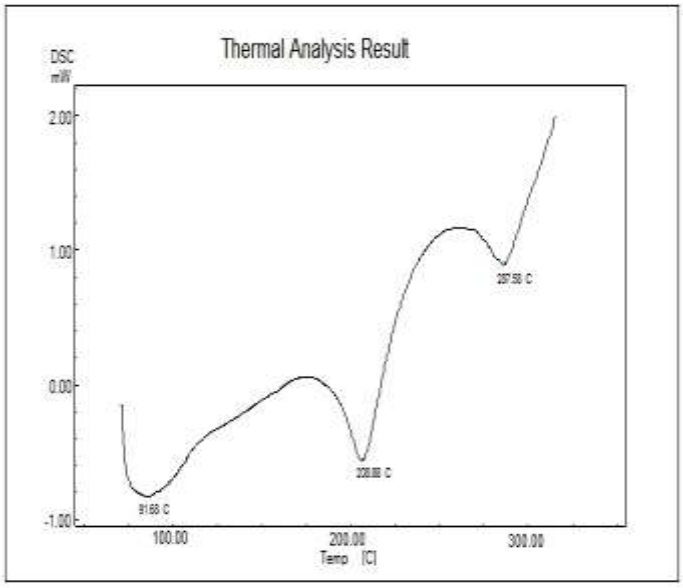

Figure 11: DSC graph of microsphere (batch F14)

IN VITRO DRUG RELEASE STUDIES:

In vitro release study of albendazole microspheres were performed in pH progression medium (pH 1.2 to pH 7.4) at 37ºC ± 0.5ºC. The *in vitro* release profile obtained for all formulations, F1 to F15, were shown in Table 4 and 5.

The result shows that the cumulative percent drug release of batch F1 to F15 was between 53.85 & 73.86.

The cumulative percentage drug release from alginate-chitosan based microspheres (batch F12 to F15) showed the desired rate, as there was no measurable drug release observed up to 2 h in SGF (pH 1.2) and desirable colonic drug release was observed up to 16 hours

Table 4: *In Vitro* Release Profile of albendazole microspheres (F1-F6)

Time (hr.)	Percentage drug release					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	7.72	7.08	5.25	6.65	5.86	2.94
2	16.29	17.82	11.8	12.92	10.59	8.257
3	28.35	26.65	19.45	17.44	16.34	14.95
4	39.42	34.19	27.37	24.28	22.58	25.35
5	47.11	49.41	36.43	33.16	30.35	29.48

6	56.37	58.67	48.79	47.49	44.14	37.46
7	66.84	69.58	51.91	59.36	56.37	43.31
8	73.86	71.24	59.23	66.78	62.66	47.27
9	73.86	71.25	59.23	66.78	62.66	47.27

Table 5: *In Vitro* Release Profile of albendazole microspheres (F7-F15)

Time (hr.)	Percentage drug release (%)								
	F7	F8	F9	F10	F11	F12	F13	F14	F15
0	0	0	0	0	0	0	0	0	0
1	4.27	5.35	6.56	5.34	5.74	2.48	1.95	1.05	0.68
2	11.39	11.28	13.34	10.63	12.58	5.41	3.54	2.84	2.64
3	19.54	20.34	17.29	15.37	17.94	13.34	11.61	15.68	12.51
4	27.23	29.65	25.69	24.21	26.33	23.44	19.37	19.48	17.28
5	36.65	37.67	33.74	31.26	33.24	31.28	25.56	27.27	23.34
6	42.87	40.19	38.55	39.31	41.48	39.76	32.48	29.38	28.72
7	49.55	47.86	43.61	44.39	47.28	43.55	38.67	34.76	34.88
8	57.46	59.93	57.14	59.84	54.38	48.42	45.34	37.43	38.94
9	60.12	65.36	62.2	64.42	60.14	52.29	48.15	40.28	41.86
10	60.13	65.36	62.2	64.42	60.14	57.46	50.87	46.19	44.38
11						59.75	55.47	50.47	48.39
12						61.94	59.31	54.24	52.47
13						62.33	62.74	59.34	57.33
14						62.88	64.85	61.22	59.20
15						63.67	65.74	63.45	60.24
16						63.67	65.74	63.45	60.24

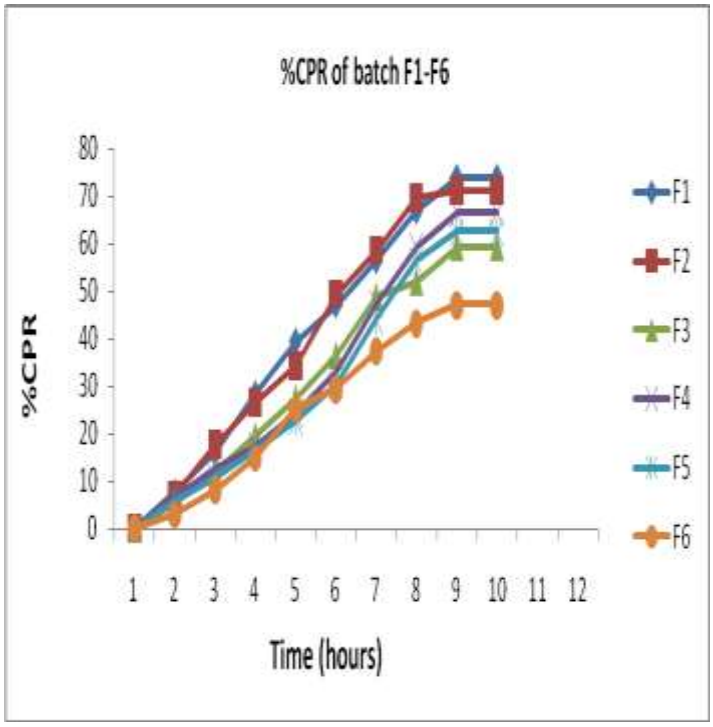

Figure 12: Plots of % Cumulative Drug Release Vs Time (batch F1 – F6)

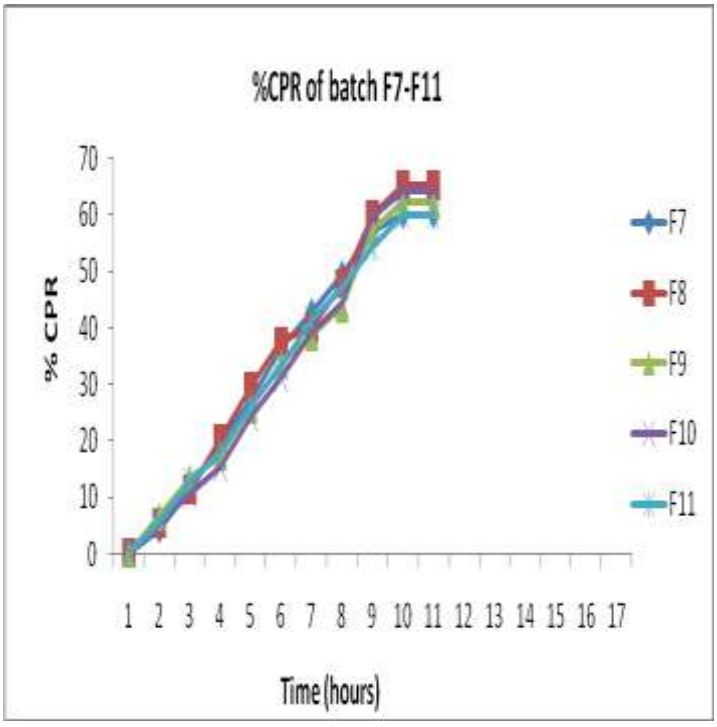

Figure 13: Plots of % Cumulative Drug Release Vs Time (batch F7- F11)

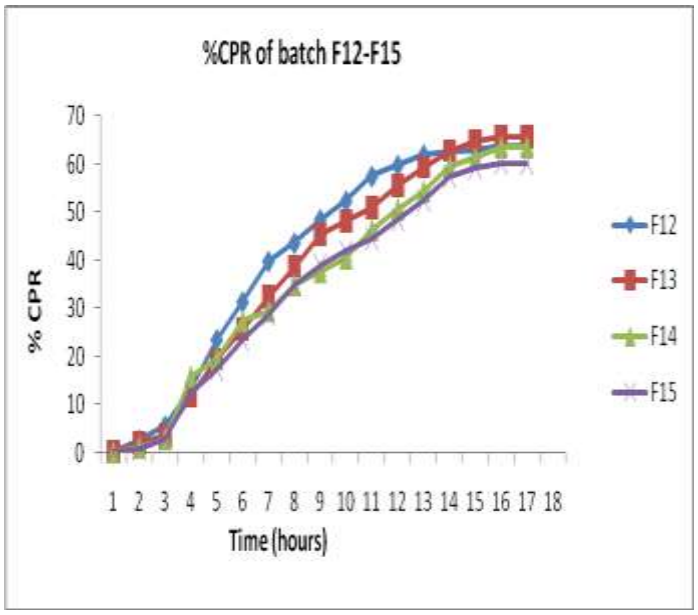

Figure 14: Plots of % Cumulative Drug Release Vs Time (batch F12-F15)

CONCLUSION:

In the present study, an attempt was made to prepare and characterize albendazole microspheres for colon specific drug delivery in order to target the drug to the colon. Prior to formulation, Preformulation studies were carried out in order to establish compatibility between drug and polymers by IR spectroscopy and DSC study. The results revealed that the drug and polymers were satisfactorily compatible, without any significant changes in the chemical nature of the drug. Fifteen formulations of albendazole microsphere were prepared by ionic gelation technique. After the formulation of microspheres it was filled in to the empty gelatin capsule for oral dose formulation. These formulations were subjected to various evaluation parameters like percentage yield, particle size, surface morphology, drug entrapment efficiency, flow property and *in vitro* drug release studies. The results of all parameters are tabulated and depicted graphically in the result and discussion section. The experimental results demonstrated that the prepared microspheres of albendazole for colon targeting may reduce the side effects of the drug caused by its absorption from the upper part of GIT when given in conventional dosage forms. Thus the albendazole microspheres have the potential to be used as a drug carrier for an effective colon targeted drug delivery system.

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