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INFLUENCE OF NATURAL GUMS FOR EFFECTIVE COLON TARGETING OF METHOTREXATE FOR THE TREATMENT OF COLORECTAL CANCER

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ABSTRACT

Colon-specific drug delivery systems (CDDS) are developed to reduce side effects and to achieve high local drug concentration at the affected site in the colon, hence optimal therapeutic effectiveness and good patient compliance. The aim of the present investigation was to develop colon targeted drug delivery system for methotrexate using guar gum and pectin as a carriers in the treatment of crohn's disease, ulcerative colitis, colorectal cancer, etc. Fast-disintegrating methotrexate core tablets were compression-coated with different ratios of guar gum and pectin. All the formulations were evaluated for the hardness, drug content uniformity, and subjected to *in vitro* drug release studies with and without rat caecal contents. The *in vitro* studies concluded that, amongst the different formulations, the F10 containing polymers 50%(guar gum and pectin) in the ratio 1:1 showed better drug release and satisfactory results showing a release of 83.4±0.98% of methotrexate after degradation by colonic bacteria at the end of 24 hrs of the dissolution study and 49.4±0.9% in simulated colonic fluids. The statistical significance was tested by using Student's t-test and found statistically significant. Optimised F10 formulation was subjected to stability studies at 40ºC±2ºC/75%±5% RH for 3 months and observed no significant change either in physical appearance, drug content or dissolution pattern. The DSC study showed that methotrexate did not react with polymers (guar gum & pectin) or other excipients used in the study.

Keywords: Methotrexate, Guargum, Pectin, Colon targeting, In *vitro dissolution*.

INTRODUCTION

Until recently, colon was considered as a site for water re-absorption and residual carbohydrate fermentation. However, it is currently being viewed as a site for drug delivery. Oral ingestion has long been the most convenient and commonly employed route of drug delivery due to its ease of administration, high patient compliance, least sterility constraints and flexibility in the design of the dosage form. In conventional oral drug delivery, there is no control on drug release and large doses have to be given in order to achieve effective concentration at target site.

So, there is a need for newer type of drug delivery system such as controlled drug delivery system. This type of drug delivery can achieve uniform drug concentration over a prolonged period of time [1].

Among the controlled delivery systems, colon targeted drug delivery systems have been the focus of interest for the last decade, which is useful not only for local but also for systemic therapy. By definition, colonic delivery refers to targeted delivery of drugs into the lower GI tract, which occurs primarily in the large intestine (i.e. colon). The site-specific delivery of drugs to lower parts of the GI tract is advantageous for localized treatment of several colonic diseases, mainly inflammatory bowel disease (Crohn's disease and ulcerative colitis), irritable bowel syndrome, and colon cancer. Other potential applications of colonic delivery include chronotherapy, prophylaxis of colon cancer and treatment of nicotine addiction. It has also gained increased importance not just for the delivery of drugs for the treatment of local diseases, but also potential site for the systemic delivery of

therapeutic proteins and peptides which are being delivered by injections. These delivery systems when taken orally, allow drugs to release the drug from the delivery system once the delivery system arrives into the colon [2 ,3, 4].

The drug release in the colon of the gastrointestinal tract locally accumulates the drug in a high concentration without involving absorption in the small intestine, which leads to reduction of systemic side effects. Delivery to the colon would ensure direct treatment at the disease site, lower dose with fewer systemic side effects. In addition to local therapy, the colon can also be utilized as a portal for the entry of drugs into the systemic circulation[5,6].

Methotrexate is an antineoplastic, antimetabolite with immunosuppressant properties. It is an inhibitor of tetrahydrofolate dehydrogenase and prevents the formation of tetrahydrofolate, necessary for synthesis of thymidylate, an essential component of DNA. Methotrexate is used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis and in autoimmune diseases such as Crohn's disease, etc [7, 8]. Lynch syndrome, often called hereditary nonpolyposis colorectal cancer, is a type of inherited cancer of the digestive tract, particularly the colon and the rectum. 40% of lynch syndrome related colorectal cancers are caused by inherited mutations in the MSH2 gene. Methotrexate selectively destroys the cells lacking the MSH2 gene function and is excellent treatment for patients with genetic alteration [9]. Among the different approaches to achieve colon-specific drug delivery, the use of polymers (chitosan, pectin, Guar-gum, Dextran, Chondrotin sulphate, etc.) which are biodegraded by colonic bacteria, holds great promise. Hence methotrexate drug is used as a novel drug to target the colon by using different biodegradable polymers to treat the conditions like *Crohn's* disease, severe ulcerative colitis and *Lynch* syndrome-related colorectal cancers.

In the present research work, a model drug methotrexate core tablets were compression-coated with a mixture of naturally occurring, biodegradable, inexpensive and non-toxic polysaccharide polymers guar gum and pectin in combination with hydrophilic swellable polymer HPMC for colon–specific delivery. The effect Guar gum-pectin mixture: HPMC ratio present in the coat formulation on the two critical release properties, drug release in upper part of gastrointestinal tract and drug release in target area or colon was investigated. *In vitro* drug release studies were carried out on compression-coated tablets coated with different quantities of guar gum and pectin in simulated gastrointestinal (GI) fluids in the presence and absence of rat caecal contents.

MATERIALS AND METHODS

Materials: Methotrexate was kindly gifted by Strides Arcolab, and all other ingredients like guar gum, pectin and excipients were provided by Karnataka Antibiotics Private Limited, Bangalore.

Preparation of fast disintegrating Methotrexate core tablets: Rapidly disintegrating Methotrexate core tablets (average weight 75 mg) were prepared by direct compression technique. The composition of core tablet was given in table 1. A weighed quantity of drug, Croscarmellose sodium at 5% level to obtain methotrexate tablets with fast disintegration characteristics (disintegration time less than 1 min), M.C.C, talc and magnesium stearate required for 50 tablets of each batch was thoroughly mixed in a mortar and pestle and passed through the mesh (250μm) to ensure complete mixing. Quantity weighing 75 mg was taken and compressed into tablets using 6 mm round; flat and plain punches on a rotary tablet punching machine (Elit Jemkay engineers Pvt Ltd). The quality control tests such as thickness, weight variation, hardness, friability, drug content and disintegration were performed on the core tablets. After confirming compliance with these tests, the core tablets were compression-coated with different coat formulations.

***Preparation of Methotrexate compression-coated tablets* [10, 11, 12]:** The formulated core tablets were compression-coated with the different granular coat formulation of Guar gum-pectin mixture and HPMC in different ratios with a coat weight of 300mg. The composition of compression-coat granular material was shown in table 2. Mixture of talc- magnesium stearate (2:1) was used as lubricant. The coat formulation containing various proportions of guar gum was prepared by wet granulation technique since guar gum and pectin was found to have poor compressibility and flow properties. The guar gum and pectin granules were prepared using starch paste as binder. The compression-coated tablets were prepared by applying maximum compression force and the hardness of the tablets was found to be in the range of 4.7±0.17–5.5±0.17 kg/cm^2. Methotrexate core tablets were compression coated with a different coating mixture. Initially, 50% (150 mg) of coat

weight granular material was placed in a 10 mm die cavity of tablet compression machine followed by carefully centering the core tablet and addition of reminder of coat weight (150 mg). The coating material was compressed around the core tablet with high compression force using 10 mm round flat and plain punches.

Determination of drug content **[13]:** 20 Tablets were weighed and finely powdered. Methotrexate, equivalent to 2.5 mg from the powder was taken and dissolved in phosphate buffer saline (PBS) solution of pH 6.8 and volume made up to 100 ml in the volumetric flask. A 0.1 ml aliquot was taken out and volume made up to 10 ml with methanolic PBS (pH 6.8) solution and filtered through whatman No.1 filter paper.

The absorbance and percent drug content of the filtrate was recorded with the help of double-beam UV-Spectrophotometer. The test was performed with formulations by assaying them individually according to USP limits.

In Vitro dissolution studies **[14, 15]:** Drug release studies were carried out using USP XXIII dissolution test apparatus (apparatus 1) at 100 rpm, 37.5°C. The formulations were tested under extreme conditions of GIT tract to account for individual variability by subjecting the formulations to prolong dissolution studies based on conditions mimicking from mouth to colon and Gastro intestinal transit time.

The ability of compression-coated tablets of methotrexate to remain intact in the physiological environment of stomach and small intestine was assessed by conducting in vitro drug release studies in 0.1N HCL for 2 hrs, as the average gastric emptying time is about 2 hrs and in sorenson's phosphate buffer pH 7.4 for 3 hrs, as the average small intestine time is about 3hrs and continued for another 19 hrs in phosphate buffer saline pH 6.8 with and without rat caecal content in dissolution medium to assess the ability of the compression-coated tablets to release drug in the physiological environment of colon target area.

At different time intervals, 1 ml of dissolution samples was obtained without pre-filter taken in 10 ml volumetric flask, made up to volume transfered to centrifuged tube and centrifuge at 2500 rpm for 15 min, supernatant liquid was filtered through, G-5 borosil filter and analyzed for methotrexate content by UV-spectrophotometric method.

The results obtained were compared in order to find out the drug release in the presence and absence of caecal content.

Dissolution studies in presence of 4% w/v rat caecal contents **[16]:** The animal ethical committee approved the experimental protocol under strict compliances of CPCSAE guidelines (Ref: KCP/IAEC-83/2010-11). In-vitro drug release testing was investigated in presence of rat caecal content medium. The susceptibility of guar gum coats to the enzymatic action of colonic bacteria was assessed by continuing the drug release studies in 100 ml of pH 6.8 phosphate buffered saline (PBS) containing 4%w/v of rat caecal contents.

The caecal contents were obtained from male albino rats, weighing (150–200 g) after pre-treatment for 7 days with guar gum dispersion. Earlier studies (Rama Prasad et al., 1998) have shown that the presence of 4% w/v rat caecal contents in pH 6.8 PBS obtained after 7 days of pre-treatment of rats with 1 ml of 2% w/v aqueous dispersion of guar gum provide the best conditions for in vitro evaluation of guar gum. 30 min before the commencement of drug release studies, five rats were killed by spinal traction. The abdomen were opened, the caecai were isolated, ligated at both ends, dissected and immediately transferred into pH 6.8 PBS, previously bubbled with CO2.

The caecal bags were opened, their contents were individually weighed, pooled and then suspended in PBS to give a final caecal dilution of 4% w/v. As the caecum is naturally anaerobic, all these operations were carried out under CO2.

The drug release studies were carried out using dissolution rate test apparatus (Apparatus 1, 100 rpm, 37ºC) with slight modifications. A beaker (capacity 200 ml) containing 100 ml of rat caecal content medium was immersed in the water maintained in the 1000ml vessel, which, in turn, was in the water bath of the apparatus. After completing the dissolution study in 0.1M HCl (2hrs) and pH 7.4 phosphate buffers (3hrs), the partially swollen guar gum formulations were placed in the baskets of the apparatus and immersed in the rat caecal content medium.

As the caecum is naturally anaerobic the experiment was carried out with continuous nitrogen gas supply into the medium to stimulate anaerobic environment of the caecum. At different time intervals 1 ml of the dissolution sample was taken in 10 ml volumetric flask and made up to volume with pH 6.8 PBS,

transferred to centrifuge tube, centrifuged at 2500 rpm for 15 min, supernatant liquid was filtered through G-5 borosil filter and analysed for methotrexate by UV-spectrophotometric method.

Statistical analysis: The cumulative percent of methotrexate released from F9, F10 compression-coated tablets (n = 3) in the dissolution medium at 24hrs with and without rat caecal contents (control study) was compared, and the statistical significance was tested by using Students t-test. A value of $P<0.05$ was considered statistically significant.

Differential scanning calorimetry **(DSC):** The possibility of any interaction between methotrexate and polymers during tablet processing was assessed by carrying out thermal analysis on pure drug (methotrexate), guar gum, pectin, powdered samples of optimized compression coat tablets (before storage) and powdered samples of optimized compression coat tablets (after storage) using DSC, Mettler, IISc. Samples were accurately weighed into aluminium pans and then hermetically sealed with aluminium lids. The thermograms of the samples were obtained at a scanning rate of 20ºC/min conducted over a temperature range of 10-250ºC.

Stability studies [17]**:** In the present study, short term stability studies were carried out at 40ºC±2ºC/75%±5% RH for a specific time period up to 3 months for optimised formulations. And the optimized formulation were evaluated periodically for the 1 st ,2 nd ,3 rd month for appearance, hardness, friability, drug content, *in vitro* release, differential scanning calorimetry.

RESULTS AND DISCUSSION

The present investigation was aimed to develop novel oral colon targeted compression-coated tablet formulations of methotrexate for safe and effective therapy of IBD and colon cancer by using guar gum, pectin and HPMC mixture as a coating materials. The rapidly disintegrating methotrexate core tablets were prepared by direct compression technique using croscarmellose sodium as a super disintegrant to aid fast disintegration of the core tablet and M.C.C. as a direct compression aid. The compressional force was adjusted to give core tablets with approximately 3.0 kg/cm^2 hardness.

The physical parameters for the core tablet formulations were found to be within the limits. Average weight of the core tablet was fixed at the lowest possible level (75 mg) to accommodate maximum amount of coat material over the core tablet and the average percentage deviation of core tablet was within the official limit. The core tablets were found to disintegrate within 1 min showing required fast disintegration characteristics. The core tablet formulations passed the test for friability (0.093±0.014%) and core tablets showed 97.99±0.24% of labeled amount of drug indicating uniformity of drug content in the core tablet formulation. All formulations showed drug content within the range of 94.82 to 105.42%. All formulations showed uniform thickness in a range of 4.16±0.01 to 4.26±0.01mm. In a weight variation test, the pharmacopoeial limit for the percentage deviation for the tablets of more than 250mg is ± 5%. Good uniformity in drug content was found among different batches of the tablets, and the percent of drug content was in the range of 94.82±0.04% to 105.42±0.62 %. All the formulations showed a hardness value in the range of 4.7±0.17 to 5.5±0.17 kg/cm^2.

In vitro release studies: The ability of compression-coated tablets of methotrexate to remain intact in the physiological environment of stomach and small intestine was assessed by conducting *in vitro* drug release studies in 0.1N HCL for 2hrs and in sorenson's phosphate buffer pH 7.4 for 3hrs and continued for another 19 hrs in phosphate buffer saline pH 6.8 with, and without rat caecal content in dissolution medium to assess the ability of the compression-coated tablets to release drug in the physiological environment of colon target area and results are shown in Table 3 and 4. Formulations F1, F3, F5 and F7 were prepared only with guar gum in the increased ratio from 40-70% of total coating composition.

Formulations F2, F4, F6 and F8 were prepared only with pectin in the increased ratio from 40-70% of total coating composition. Formulations F9-F12 were prepared with a combination of guar gum and pectin at 1:1 ratio, ranging from 40-70% of the total coating composition. All Formulations except F9 and F10 showed less drug release at the end of 24 hrs dissolution study. Hence these F9 and F10 formulations were taken for further release studies in rat caecal content.

The drug delivery systems targeted to the colon should not only protect the drug from being released in the physiological environment of stomach and small intestine, but also release the drug in colon after enzymatic degradation by colonic bacteria. Hence, in vitro drug release studies were carried out for F9 and F10 in pH 6.8 PBS containing 4% w/v of

rat caecal contents. Formulations of methotrexate F9, F10 retained their physical integrity up to 24 hrs in dissolution study conducted with and without rat caecal contents in the dissolution medium (control). The percentage of methotrexate released from the F9 compression-coated tablets at the end of 24 hrs with rat caecal contents was found to be 73.4±1.34%, where as in control study (without rat caecal contents in the dissolution medium) only 52.8±0.9% of methotrexate was released. The percentage of methotrexate released from the F10 compression-coated tablets at the end of 24 hrs with rat caecal contents was found to be 83.4±0.98%, where as in control study (without rat caecal contents in the dissolution medium) only 49.4±0.9% of methotrexate was released as shown in Figure 1.

Formulations F11 and F12 with higher guar gum and pectin at 1:1 with 60% and 70% of coating formulations showed complete retardation of drug release, indicating 50% guar gum and pectin combination is high enough for colonic enzymes to act upon formulation and degrade it. The study shows that the release of methotrexate in the physiological environment of colon is due to the microbial degradation of polymers (guar gum and pectin) in the presence of rat caecal contents. The dissolution study was conducted without rat caecal contents (control study) to ensure that the drug release was not due to the mechanical erosion which is likely to occur because of bowel movements in humans. The study showed formulation F10 with guar gum and pectin at 60% of coating composition showed higher drug release in the physiological environment of colon and found optimal for selective delivery of methotrexate to the colon. The cumulative percent of methotrexate released from F9, F10 compression-coated tablets (*n* = 3) in the dissolution medium at 24hrs with and without rat caecal contents (control study) was compared, and the statistical significance was tested by using Student's t-test. A value of $P<0.05$ was considered statistically significant.

Stability studies: In view of the potential utility of F10 formulation for targeting of methotrexate to colon, stability studies were carried out at 40ºC±2ºC/75%±5% RH for 3 months. After the storage the formulation (optimized) was subjected to hardness, friability, drug content, *in vitro* drug release studies as seen in Table 5 and DSC studies. When compression coated formulation F10 was stored at 40ºC±2ºC/75%±5% RH for 3 months there appeared no much difference in physical appearance or in drug content, friability and hardness. As shown in Figure 2, when the dissolution study was conducted in the simulated physiological environment of stomach, small intestine and colon as described, no significant difference was observed in the cumulative percent of methotrexate released from F10 stored at 40ºC±2ºC/75%±5% RH for 3 months.

Differential Scanning Calorimetry: Interaction between the drug, guargum, pectin or other excipients in F10 formulation was predicted by differential scanning colorimetric studies. DSC of methotrexate is shown in Figure 3 and formulation F10 before and after storage at 40^{0} C at 75% RH were shown in Figures 4 and 5. A sharp Endothermic peak corresponding to the melting point of methotrexate was found at 199.05ºC for the drug sample.

The endothermic peak corresponding to the melting point of methotrexate in the powdered sample of compression coat tablet of (F10) formulation lightly shifted to 198.45ºC. Even after storing at 40ºC±2ºC/75%±5% RH for 3 months, the thermogram of the powdered sample of the compression coat tablet in F10 formulation did not show any significant shifting the endothermic peak. The results of the DSC study indicate the absence of possible interactions between methotrexate and polymers (guar gum, pectin) or other formulation excipients.

CONCLUSIONS

Twelve formulations of methotrexate compressed coated tablet were formulated with different ratios of guar gum and pectin. Among all formulations, formulation F10 containing 1:1 ratio of guar gum and pectin as a coat material applied over the core tablet was capable of protecting the drug from being released in physiological environment of stomach and small intestine and was susceptible to colonic bacterial enzymatic actions with resultant drug release in colon. The optimized formulation F10 released only 0.1±0.0% and 1.2±0.4% of drug in the physiological environment of stomach and small intestine respectively and released 49.4±0.9% (control) and 83.4±0.98% (with rat caecal content) of the drug in the target area i.e. physiological environment of colon. From this study it can be concluded that the compression coated tablet is an unique approach for colonic delivery of drug having appropriate site specificity and feasibility and controlled release of methotrexate. Thus the efforts to formulate compression coated tablet of methotrexate to release specifically in the colon was found successful in treating colorectal cancer.

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Table 1: Composition of fast disintegrating Methotrexate core tablets

Ingredients	Quantity mg per each tablet
Methotrexate	2.5
MCC	64
Cross carmalose sodium	4
Talc	2.5
Mg Stearate	2
Total	74

Table 2: Composition of granular coat formulation for compression over methotrexate core tablets

Ingredients	Formulation Code											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Gaur Gum	120	-	150		180		210		60	75	90	105
Pectin		120		150		180		210	60	75	90	105
HPMC K4M	126	126	96	96	66	66	36	36	126	96	66	36
MCC	15	15	15	15	15	15	15	15	15	15	15	15
Starch (paste)	30	30	30	30	30	30	30	30	30	30	30	30
Talc	6	6	6	6	6	6	6	6	6	6	6	6
Mg Stearate	3	3	3	3	3	3	3	3	3	3	3	3
Total	300	300	300	300	300	300	300	300	300	300	300	300

Table 3: % CDR of compressed coated tablet (F1 to F6) in 0.1 M HCl, pH 7.4 Sorenson's phosphate buffer, pH 6.8 Phosphate buffer saline

Time in Hrs	F1	F2	F3	F4	F5	F6
			0.1 M HCl			
2	1.0±0.1	0.1±0.0	1.2±0.2	1.3±0.0	1.2±0.0	2.9±0.1
			pH 7.4 Sorenson's phosphate buffer			
5	3.9±0.3	2.9±0.4	2.1±0.3	3.5±0.3	3.4±0.3	3.6±0.5
			pH 6.8 Phosphate buffer saline			
6	5.2±0.1	10.5±0.2	8.8±0.1	11.8±0.2	15.5±0.1	6.3±0.1
8	7.1±0.7	11.4±0.3	15.0±0.1	19.0±0.3	17.7±0.1	12.5±0.2
10	13.8±0.1	17.3±0.1	15.4±0.0	22.9± 0.0	23.6±0.5	20.8±0.6
12	22.2±0.2	17.9±0.1	19.3±0.9	28.2±0.7	26.5±0.4	21.0±0.9
20	38.6±0.2	35.3±0.4	32.3±0.3	39.4±0.4	29.3±0.5	26.3±0.3
24	38.6±0.9	35.5±0.4	39.9±0.1	41.0±0.9	32.1±0.8	26.5±0.7

Value shown in table indicates mean±S.D

Table 4: % CDR of compressed coated tablet (F7 to F12) in 0.1 M HCl, pH 7.4 Sorenson's phosphate buffer, pH 6.8 Phosphate buffer saline

Time in Hrs	F7	F8	F9	F10	F11	F12
			0.1 M HCl			
2	1.8±0.6	2.5±3.5	1.0±0.1	0.1±0.0	1.2±0.2	1.3±0.2
			pH 7.4 Sorenson's phosphate buffer			
5	2.3±0.3	3.9±0.4	2.9±0.3	1.2±0.3	2.1±0.3	3.5±0.5
			pH 6.8 Phosphate buffer saline			
6	12.9±0.1	13.1±0.2	11.8±0.1	8.5±0.2	11.8±0.1	18.5±0.1

8	13.3±0.1	19.4±0.1	24.2±0.0	9.1± 0.0	15.0±0.5	15.8±0.6
10	20.5±0.2	21.9±0.1	29.9±0.9	14.8±0.7	18.4±0.4	19.4±0.9
12	21.1±0.2	22.8±0.1	34.2±0.9	29.2±0.7	19.3±0.4	20.3±0.9
20	24.9±0.2	31.7±0.4	47.4±0.3	44.6±0.4	25.3±0.5	26.3±0.3
24	25.8±0.9	32.8±0.4	52.8±0.1	49.4±0.9	27.9±0.8	28.9±0.7

Value shown in table indicates mean±S.D

Table 5: Characteristics of methotrexate compression coated tablets (F10) before and after storage at 40ºC±2ºC/75%±5% RH for 3 months

Duration in months	Physical appearance	Hardness	% Drug content
0	+++	5.1±0.17	94.97±0.55
1	+++	5.0±0.46	95.52±1.73
2	+++	4.9±0.76	93.92±0.15
3	+++	5.0±0.57	94.31±0.57

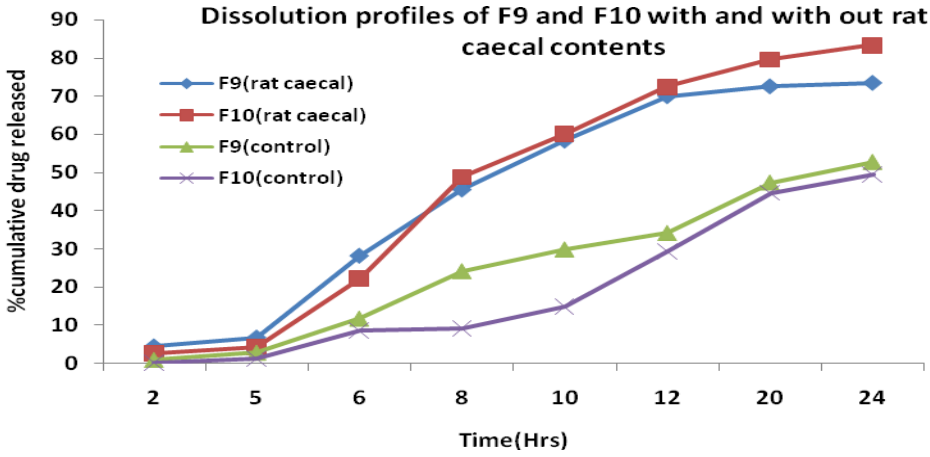

Figure 1: % CDR of formulations F9 and F10 in 0.1 M HCl, pH 7.4 Sorensons phosphate buffer, pH 6.8 Phosphate buffer saline with and without rat caecal content

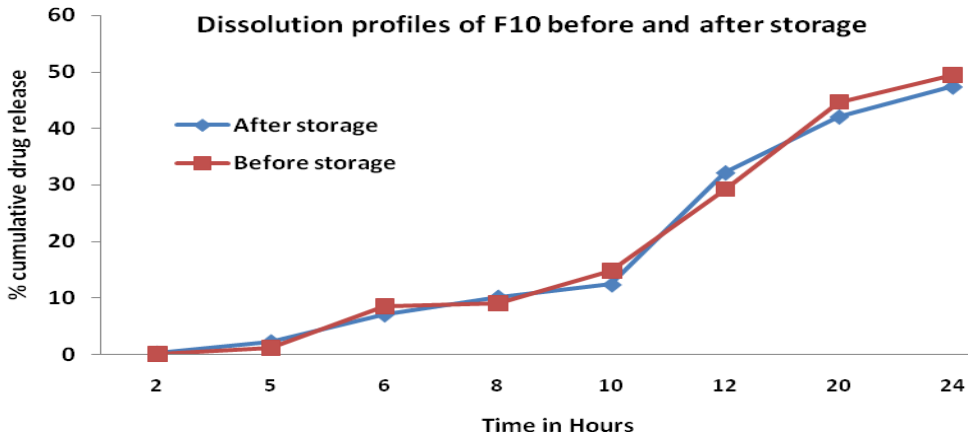

Figure 2: Release profile of optimised formulation (F10) before and after storage

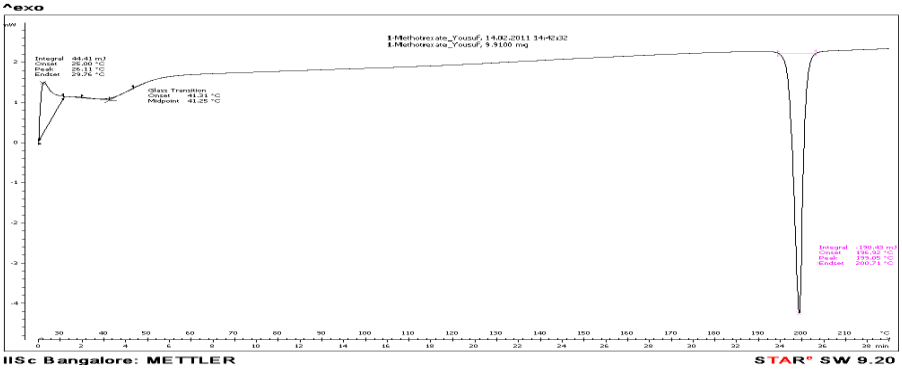

Figure 3: DSC of Methotrexate

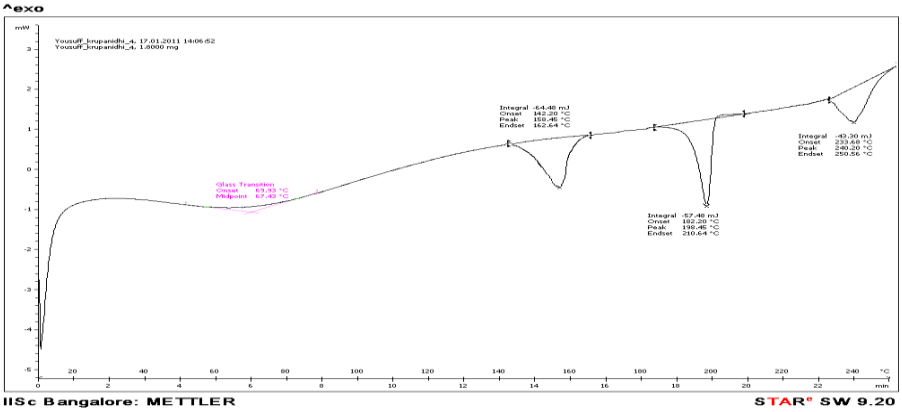

Figure 4: DSC thermogram of compression coat tablet (F10) before storage

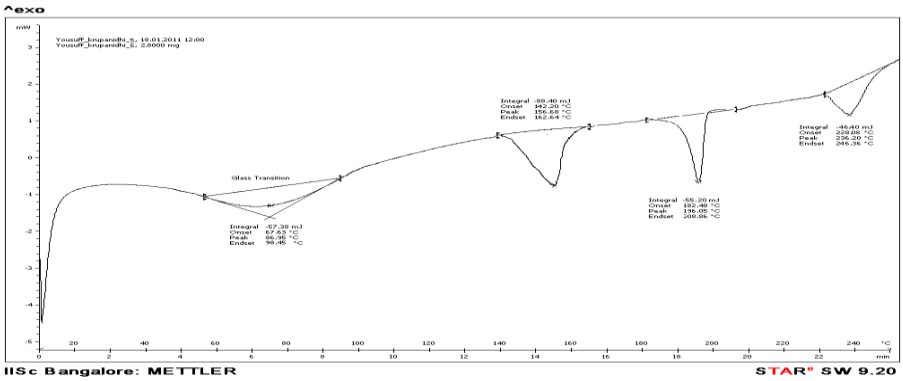

Figure 5: DSC thermogram of compression coat tablet (F10) after storage

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