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FORMULATION AND EVALUATION OF BIOPOLYMERS COATED PELLETS OF CYCLOPHOSPHAMIDE FOR COLON DRUG DELIVERY

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ABSTRACT
The present study objective was to develop novel colon specific drug delivery systems for cyclophospahmide using konjac glucomannan as a biodegradable polymeric carrier and to coat the optimized batches with a pH dependent polymeric coating solution containing zein. Pellets containing four proportions of KGM and zein were prepared. The pellets were evaluated for physicochemical properties, drug content, dissolution, water uptake & erosion characteristics, *in-vitro* drug release studies. The amount of cyclophospahmide released from the biopolymers coated pellets at different time intervals was estimated by UV spectrophotometric method at 260nm. Zein coated KGM pellets prevented release of the cyclophospahmide in the physiological environment of stomach and small intestine depending on the proportion of biopolymers used in the formulation. The dissolution profile and *in vitro* release kinetics showed that pectin pellets were promising for colon delivery of the drug. The findings of the present study conclusively state that biopolymers coated pellets are promising for colon delivery of cyclophospahmide to synchronize the chronobiological symptoms for effective treatment of colon cancer.

KEYWORDS: Cyclophospahmide, Konjac Glucomannan, Pellets, Zein, Colon Drug Delivery.

INTRODUCTION
The coating of pharmaceutical dosage form started in the early ninth century B.C., with Egyptian. The pills and hand shaped spherical mass containing drug, sugar and other diluents were the primary dosage form at that time. These dosage forms were coated with a variety of materials including talc, gelatin, sugar, gold and silver. Most of these coatings were ineffective due to a chemical attack in the digestive tract. The candy making industry was the first to develop and enhance the art of coating and later on the pharmaceutical industry adapted the art of sugar coating for its own purposes. The first sugar coated pills were developed in 1854 in Philadelphia, the USA. Enteric coatings were started in 1880. In 1953, the first compression coated tablets were introduced and in 1954 the foundation for the film coatings was developed.[1]

The pharmaceutical dosage forms are also coated to protect the incorporated active ingredients against light, oxygen, moisture, mechanical burden and the degradation in gastric juice. Economic and safety reasons have to be taken into consideration such as product appearance, identification, taste and odour masking of bitter drugs. A variety of release profiles and mechanisms can be adapted: enteric coating, colon targeting, pulsatile release, extended release or fast dissolving. In order to achieve the desired release profile, different polymers have to be selected, which are characterized by different solubility and swelling properties in water, gastric and intestinal fluids.

Colon drug delivery has the potential to deliver bioactive agents for the treatment of a variety of colonic diseases including colon cancer, inflammatory bowel disease (IBD) and rheumatoid arthritis and can be effectively treated by the local delivery of drugs to the large intestine. Targeting of drugs to the colon via the oral route can be achieved by different approaches including different formulation systems, for which the drug release is controlled by different pH conditions, transit time, and intestinal microbial flora.[2] Specific targeting of drugs to the colon is recognized to have several therapeutic advantages. The colon is an ideal site for both systemic and local delivery of drugs.[3] To reach the colon and absorb the drug there the dosage forms must be formulated taking into account the obstacles of the gastrointestinal tract (GIT). Various strategies have been developed to achieve this goal, such as, use of specific characteristics of the organ, for example, pH, microbial flora, enzymes, reducing medium, and transit time.[4] A number of serious diseases of the colon, for example,

colorectal cancer, ulcerative colitis, and other inflammatory conditions could be treated more effectively if drugs are targeted to the colon.[5,6] The colonic site is being investigated as a potential site for the delivery of proteins, peptides, vaccines, and other drugs such as nifedipine, theophylline, and isosorbide.[7,8] Due to a comparatively longer transit time than in the stomach, colonic absorption of poorly absorbed drugs can be improved.[9] Methods for drug delivery to the colon have recently been discussed.[10]

A new multi particulate dosage form consisting of a hydrophobic core, which is further coated with a pH dependent swelling polymer (acrylic polymer such as Eudragit S100), is proposed for colonic specific delivery of drugs. With respect to this a pelletization process[11], powder layering process has been adopted.[12]

Konjac glucomannan is a high-molecular weight, water soluble, non-ionic, highly viscous, fermentable dietary fiber extracted from the tuber (or) root of the elephant yam, also known as Kojac (*Amorphophallus konjac* or *Amorphophallus rivieri*) which is native to Asia. KGM, is a naturally occurring biopolymer that is finding increasing applications in the pharmaceutical and biotechnology industry. Irrespective of its origin, Glucomannan consists of a polysaccharide chain of β-D-glucose and β-D-mannaose with attached acetyl groups in a molar ratio of 1.6:1 with β-1, 4 linkages. Because human salivary and pancreatic amylase cannot split β-1,4 linkages, the degree of solubility is controlled by the presence of acetyl groups.

Zein belongs to the class of proteins known as prolamines, which occur in most of the cereals. Commercial production of zein began in 1939 from corn gluten meal (contains 62-74% protein on dry basis), a coproduct of corn wet mills. "Freeman Industries, USA and Showa Sangyo Corp., Japan" are the only two companies which currently produce zein commercially. Its price depends on its purity, because of its high price and manufacturing cost its utilization is limited.[13]

Multiparticulate approaches tried for colonic delivery include formulations in the form of pellets, granules, microparticles and nanoparticles. The multiparticulate drug delivery systems are used in preference to single unit dosage forms for colon targeting. The multiparticulate systems enabled the drug to reach the colon quickly .The purpose of designing multiparticulate dosage form is to develop a reliable formulation that has all the advantages of a single unit formulations.[14]

This is particularly important for anticancer drugs, such as cyclophosphamide, temozolomide, curcumin and quercetin, which are the standard drug for the local treatment of colorectal cancer (S.S.Agrawal and Pallavi Sharma 2017[17], Gulfam M *et al.*, 2012[15], Antonio Di Martino *et al.*, 2017.[16]

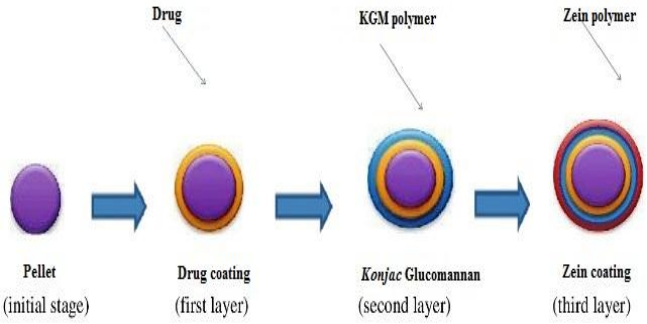

Figure. 1: Schematic representation of the principle of the investigated colon targeting approach.

Basically, two polymers have been investigated and drug loaded pellets coated with biopolymer i.e. *Konjac* glucomannan (a hydrophilic molecule, high-molecular weight, non-ionic polysaccharide extracted from the tuber or root of the elephant yam, also known as *konjac* (Amorphophallus konjac). Due to the presence of α-1, 6 linkages and non digestible glycoside linkages (e.g., α-1, 2 and α-1, 3), KGM is only incompletely hydrolyzed and absorbed in the small intestine (approximately 10-15 %). But this polysaccharide derivative is progressively fermented to about 85 % in the colon.

Subsequently functional membrane ER (second layer) coated with zein was shown to be particularly promising. Furthermore, zein is water-insoluble and avoids premature film dissolution in the upper GIT known to exhibit a significant pre-biotic activity, normalizing the microflora and enzyme patterns in the colon of the patients. This is of major clinical benefit for this type of colorectal disease. Thus, the investigated polymeric networks consist of two compounds: (i) Konjac glucomannan, which should be preferentially degraded by the enzymes present in the colorectal cancer disease patients, and (ii) Zein, assuring that the film coatings do not spontaneously dissolve in the contents of the stomach and small intestine.

MATERIALS AND METHODS

Materials: Neutral pellets were obtained as a gift sample from Micropellets, Nashik India. Cyclophosphamide was obtained as gift sample from Biophore Pharmaceutical Pvt. Ltd. Hyderabad, India, and *Konjac* glucomannan was obtained from Dalian Ruishengda International Trade Co., Ltd., Western Hills Village Shahekou District Dalian, China. Zein (MW 220,000) was procured from Central Drug House, New Delhi, India. Other excipients used were of analytical grade and purchased from Loba Chemie, Mumbai, India.

Methods

Preparation of coating solution for drug loading:

Preparation of drug-layered pellets

Drug loaded pellets were prepared by spraying drug solution over non-pareil seeds by fluidized bed coating technique. Curcumin was homogeneously dispersed in an organic solution of PVP K30 and HPMC E5 as

plasticizer while stirring with a magnetic stirrer. The drug dispersion was passed through a 100 mesh sieve.

Coating of Konjac glucomannan over drug layered pellets (protective coating/sub-coating)

In order to bring the rupture of the outer functional coat, a layer of swelling agent *Konjac* glucomannan was applied over the drug layered pellets by fluidized bed coating technique. *Konjac* glucomannan coating solution was prepared by mixing required amount of PVP K30 and PEG 400 as plasticizer in aqueous medium.

Application of outer enteric functional coat of Zein (Enteric coating/Functional membrane): Zein coating solution preparation requires addition of Zein to the mixture of solvents acetone, isopropyl alcohol and purified water which is mixed together properly stirring with a magnetic stirrer. This was followed by the addition of stated amount of triethyl citrate as plasticizer and stirred the solution for few minutes.

Evaluation of coated pellets

1. *Appearance:* The pellets were visually observed for physical appearance of pellets.

2. *Particle size:* Particle size distribution of micropellets was determined by optical microscopy using calibrated ocular eye piece. Fifty micropellets were evaluated and the experiment was performed. Geometric mean diameter was then calculated using the equation.[18]

Xg=10 X [(niXlogXi)/N]

Xg is geometric mean diameter, ni isnumber of particles in the range, Xi is the midpoint of range, N is total number of particles analyzed.

3. *Angle of repose*

The dried micropellets were allowed to fall freely through a funnel fixed at 1 cm on a horizontal surface and the angle of repose (θ) was measured.

$\theta = \tan^{-1} h/r$.

Where h is the height of the heap, r is the radius.

4. *Drug content:* 200mg pellets were weighed and powdered, a quantity of powder equivalent to 10 mg of each formulation was transferred to a 25 ml volumetric flask and 15 ml water is added. The drug is extracted in water by vigorously shaking the stoppered flask for 15 minutes. Then the volume is adjusted to the mark with distilled water and the liquid is filtered. The drug content was determined by measuring the absorbance at 260 nm after appropriate dilution. The drug content was calculated using the standard calibration curve. The mean percent drug content was calculated.[19]

Table. 1: Formulation for pellets coating.

Ingredients (g)	CPM1	CPM2	CPM3	CPM4	CPM5	CPM6	CPM7	CPM8	CPM9	CPM10
Sugar spheres (18/20#)	100	100	100	100	100	100	100	100	100	100
DRUG LOADING										
Cyclophosphamide	8	8	8	8	8	8	8	8	8	8
PVP K30	1	1	1	1	1	1	1	1	1	1
HPMC E5	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Talc	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85
Isopropyl alcohol	50	50	50	50	50	50	50	50	50	50
Purified water	50	50	50	50	50	50	50	50	50	50
Amaranth red	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BARRIER COATING/PROTECTIVE COATING										
KGM	1	2	3	4	5	6	7	8	9	10
PEG 400	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Talc	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Isopropyl alcohol	100	100	100	100	100	100	100	100	100	100
Distilled water	50	50	50	50	50	50	50	50	50	50
Sunset yellow	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
FUNCTIONAL MEMBRANE/ENTERIC COATING										
Zein	2.5	5	7.5	10	12.5	15	17.5	20	22.5	25
PEG 400	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Talc	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
triethyl citrate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
aceton	20	20	20	20	20	20	20	20	20	20
Isopropyl alcohol	100	100	100	100	100	100	100	100	100	100
Purified water	50	50	50	50	50	50	50	50	50	50

5. *Scanning electron microscopy:* Morphological examination of the surface and internal structure of the dried beads was performed by using a scanning electron microscope (SEM). Coated pellets before dissolution only subjected to SEM study since, after dissolution the pellets become swollen palpable mass. Photographs were taken within the range of 50-500 magnification.

6. *In-vitro drug release studies*

The release of drug from the developed formulation in the GIT was determined using USP dissolution type II apparatus. The drug release studies were carried out in pH 1.2 for 2 hrs, in Ph 6.8 for next 3 hrs and in pH 7.4 for up to 24 hrs at 37± 0.50°C and 100 rpm. At regular time interval, 5 ml of sample was withdrawn from the dissolution medium and replaced with equal volume of fresh medium. After filtration and appropriate dilution, the samples were analyzed at 260 nm for cyclophosphamide against blank using UV-Visible spectrophotometer. The amount of drug present in the samples was calculated using standard curve.[20]

7. *Release drug data model fitting*

The suitability of several equation that are reported in the literature to identify the mechanisms for the release of drug was tested with respect to the release data up to the first 50% drug release. The data were evaluated according to the following equations:

Zero order model

$Mt = M0 + K0t$

Higuchi model11

$Mt = M0 + KH\ t\ 0.5$

Korsmeyer-Peppas model12

$Mt = M0 + KKtn$

Where Mt is the amount of drug dissolved in time t. M0 is the initial amount of the drug. K0 is the Zero order release constant, KH is the Higuchi rate constant, K is a release constant and n is the release exponent that characterizes the mechanism of drug release.

RESULTS AND DICUSSION

The coated pellets were prepared by powder layering technique using different polymers did show significant results during their evaluation. The appearance shows the pellets being spheroid in shape and showing smooth surface of pellets.

The size of coated pellets found to be in the range of 1695 µm to 1793 µm and it was observed that increase in concentration of coating polymer particle size of the pellets significantly increased. The average particle size is highest for CPM7. The particle size distribution is uniform and narrow. Results were shown in table 2. Angle of repose ranged from 24.83 ± 0.128 to 26.08 ± 0.612. The flow properties of coated pellets in all formulations exhibit good flow. Results were shown in table 2.

The scanning electron microscope shows the pellets being spheroid in shape. Surface depression was noted at the point of contact on the drying paper. On comparison of the coated pellets from polymer s in high concentrations more roughness was observed with zein polymers. Results FT-IR and DSC were shown in Figure 4-5.

The *in-vitro* drug release data of all the formulations were fitted in zero order, first order and peppas model and the rate constant (k), correlation coefficient (R2) and n values were compared to know the mechanism of drug release from the micropellets. Comparing the R2 values of all formulations, it is evident that CPM1, CPM2, CPM3, CPM4, CPM5and CPM6 formulations following peppas release, CPM7, CPM8 formulations following zero order release and CPM9 and CPM10 formulations following first order release. The formulation CPM10 showing high cumulative % drug release after 24 hrs was found to be 58.16 %, which contains KGM (10%) and zein (25%). The drug gets released by passive diffusion. Results were described in table 3-4 and Figure 3-4. The dissolution profile and *in vitro* release kinetics showed that pectin pellets were promising for colon delivery of the drug. The findings of the present study conclusively state that biopolymers coated pellets are promising for colon delivery of cyclophospahmide to synchronize the chronobiological symptoms for effective treatment of colon cancer.

Table. 2: Physical and chemical parameters of cyclophosphamide colon drug release pellets.

Parameters	CPM1	CPM2	CPM 3	CPM 4	CPM 5	CPM 6	CPM 7	CPM 8	CPM 9	CPM 10
Particle Size (µm)	1763 ±18	1742 ±20	1789 ±32	1695 ±41	1753 ±26	1760 ±21	1793 ±48	1786 ±07	1725 ±24	1782 ±37
Angle of repose (θ) ± SD*	25.27 ±0.302	25.60 ± 0.246	25.52 ± 0.416	24.86 ± 0.147	25.79 ±0.439	26.08 ± 0.612	25.31 ± 0.462	24.83 ± 0.128	25.44 ± 0.632	26.06 ± 0.231
Bulk Density (D_B) $(gm/cm)^3$± SD*	0.821 ±0.002	0.8352 ± 0.006	0.8245 ± 0.004	0.8147 ± 0.001	0.8246 ± 0.004	0.8238 ± 0.012	0.8305 ± 0.012	0.8252 ± 0.004	0.8108 ± 0.012	0.823 ± 0.009
Tapped Density (D_T) $(gm/cm)^3$± SD	0.8726± 0.016	0.8432± 0.010	0.8831 ± 0.024	0.8625 ± 0.014	0.8934 ± 0.016	0.8856 ± 0.023	0.8862 ± 0.012	0.8958 ± 0.010	0.8547 ± 0.12	0.8836 ± 0.16
Hausner Ratio (HR) ± SD*	1.06 ±0.022	1.08 ± 0.036	1.05 ± 0.022	1.08 ± 0.034	1.07 ± 0.008	1.04 ± 0.027	1.05 ± 0.035	1.08 ± 0.042	1.08 ± 0.053	1.07 ± 0.068
Carr's Index (%CI) ± SD	4.28 ±0.012	4.86 ± 0.036	5.17 ± 0.016	4.48 ± 0.025	4.60 ± 0.019	4.86 ± 0.026	5.12 ± 0.030	4.57 ± 0.010	4.62 ± 0.023	4.52 ± 0.031
Moisture content (%)± SD*	1.42 ±0.025	1.56 ± 0.018	1.48 ± 0.060	1.52 ± 0.072	1.46 ± 0.043	1.72 ± 0.038	1.58 ± 0.052	1.80 ± 0.083	1.76 ± 0.024	1.65 ± 0.022
Drug content (%)	99.16 ±0.026	99.05 ± 0.017	98.52 ± 0.031	98.86 ± 0.028	99.10 ± 0.034	99.53 ± 0.049	99.34 ± 0.065	99.18 ± 0.042	98.47 ± 0.050	99.64 ± 0.058

Table. 3: %Cumulative *in vitro* drug release from CPM1 to CPM5 (S.D. n=3).

Time (hr)	% Cumulative drug release				
	CPM1 (1:2.5)	CPM2 (2:5)	CPM3 (3:7.5)	CPM4 (4:10)	CPM5 (5:12.5)
1	12.11±0.18	10.22±0.16	07.12±0.21	6.34±0.14	4.10±0.12
2	17.27±0.21	14.21±0.12	11.51±0.31	9.14±0.18	7.12±0.41
3	23.31±0.24	20.51±0.14	18.22±0.53	14.19±0.34	10.34±0.33
5	26.23±0.61	23.58±0.32	20.11±0.61	17.27±0.57	13.23±0.88
6	32.14±0.43	29.46±0.13	27.11±0.27	25.56±0.51	22.52±0.44
8	38.31±0.23	34.62±0.14	32.26±0.62	31.14±0.17	29.23±0.32
10	44.34±0.55	41.76±0.45	40.43±0.54	38.12±0.67	35.56±0.76
12	49.67±0.66	46.23±0.34	44.32±0.34	41.10±0.89	39.25±0.30
14	60.34±0.13	55.56±0.57	53.98±0.53	51.90±0.66	48.12±0.26
16	74.19±0.27	72.21±0.11	68.34±0.65	64.47±0.35	59.34±0.15
18	79.42±0.34	75.27±0.33	73.65±0.43	71.41±0.43	66.15±0.39
20	85.18±0.23	82.16±0.41	78.67±0.28	75.21±0.11	72.85±0.23
24	94.78±0.48	92.20±2.40	88.46±2.06	84.05±1.25	82.47±2.56

Table. 4: %Cumulative *in vitro* drug release from CPM6 to CPM10 (S.D. n=3).

Time (hr)	% Cumulative drug release				
	CPM6 (6:15)	CPM7 (7:17.5)	CPM8 (8:20)	CPM9 (9:22.5)	CPM10 (10:25)
1	3.05±0.04	2.11±0.03	0	0	0
2	6.24±0.12	4.22±0.28	1.76±0.08	0	0
3	07.34±0.23	6.10±0.62	2.3±0.07	0.56±0.06	0.12±0.02
5	11.24±0.28	9.36±0.58	4.27±0.64	1.45±0.08	0.86±0.07
6	18.52±0.44	15.18±0.22	8.32±0.35	3.84±0.07	1.54±0.05
8	24.23±0.32	22.02±0.19	13.64±0.18	6.43±0.19	3.82±0.04
10	32.56±0.76	30.12±0.37	22.63±0.45	9.82±0.32	6.81±0.28
12	41.25±0.30	38.32±0.48	28.27±0.19	16.24±0.75	12.50±0.71
14	49.11±0.25	42.03±0.51	36.45±0.67	24.86±0.42	18.45±0.48
16	56.34±0.15	51.19±0.63	42.24±0.14	32.65±0.37	24.52±0.35
18	62.15±0.39	57.12±0.23	48.35±0.53	38.51±0.41	30.62±0.16
20	67.25±0.38	62.10±0.28	54.09±0.22	46.39±0.12	38.45±0.64
24	78.52±0.26	75.34±0.18	71.82±0.35	64.24±0.30	58.16±0.34

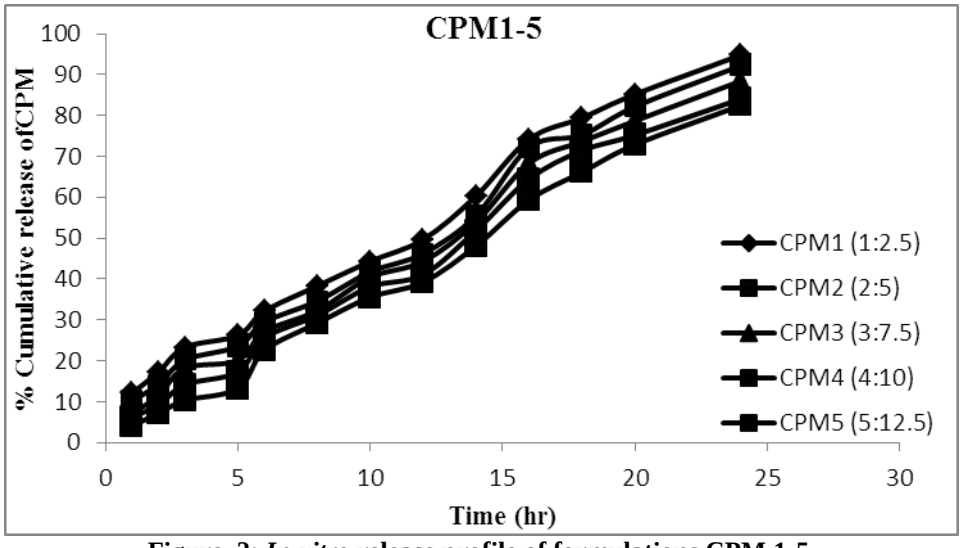

Figure. 2: *In-vitro* release profile of formulations CPM 1-5.

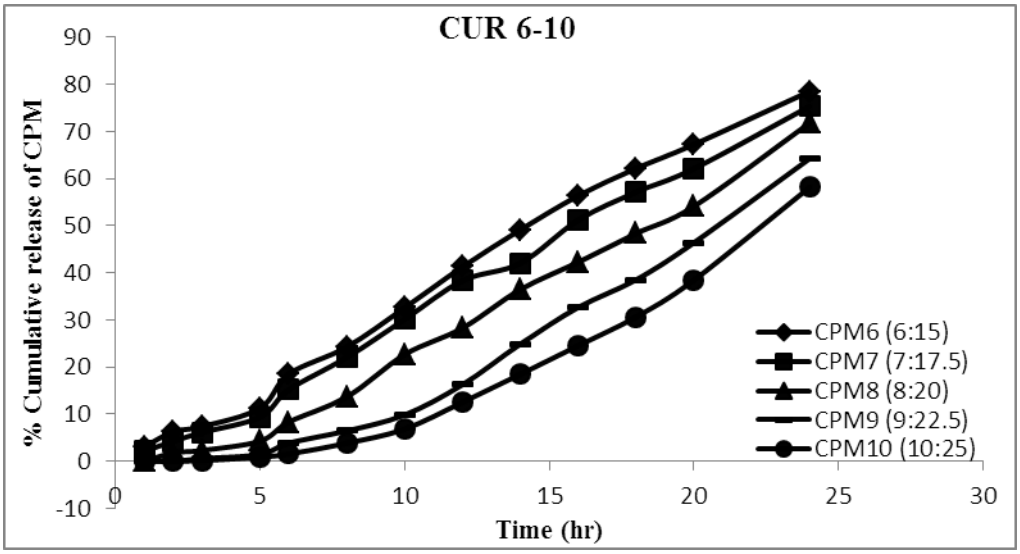

Figure. 3: *In-vitro* release profile of formulations CPM 6-10.

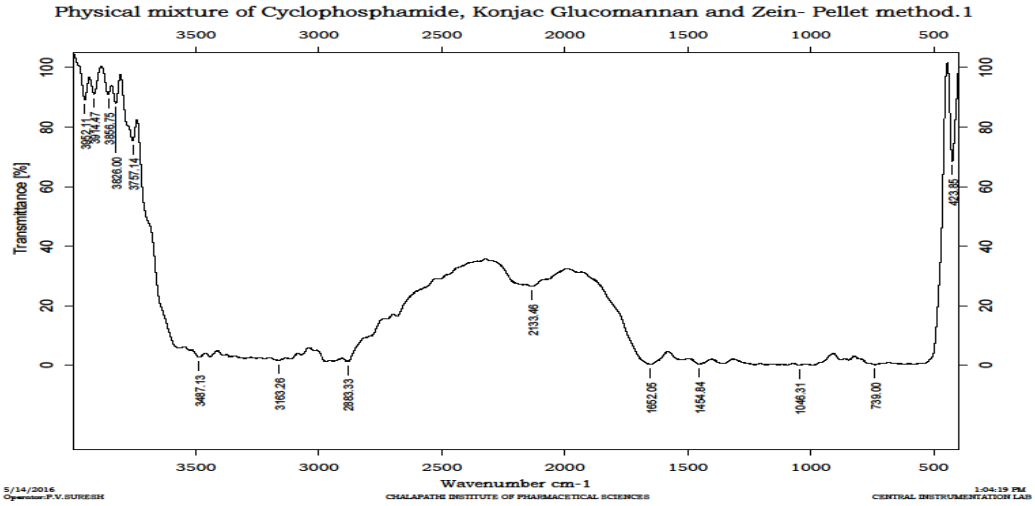

Figure. 4: FT-IR graph of physical mixture of Cyclophosphamide, *Konjac* Glucomannan and Zein.

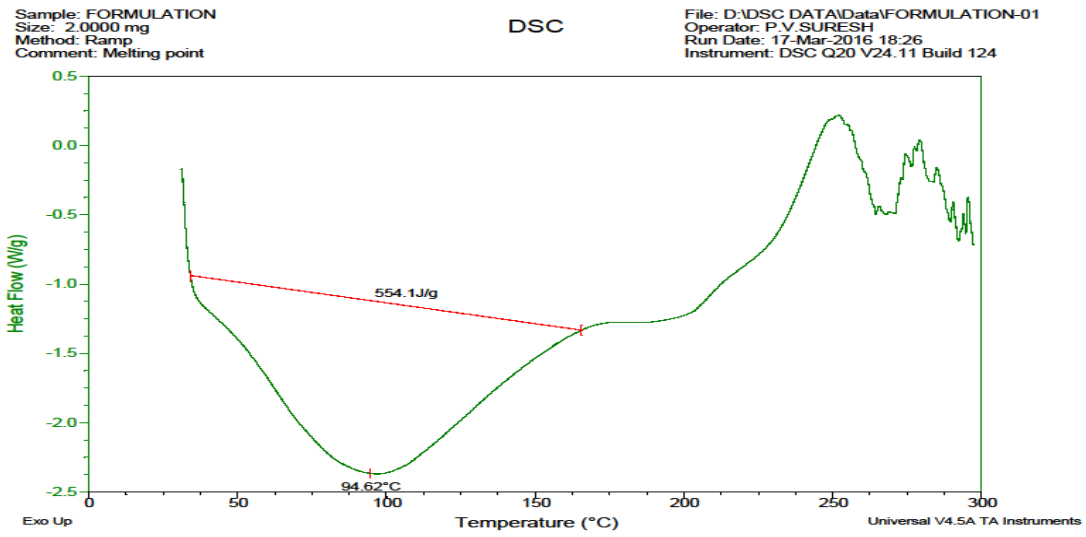

Figure. 5: DSC thermogram of optimized formulation.

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