ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Design and Evaluation of Microporous Colon Osmotic Drug Delivery System of Methylprednisolone A Central Composite Design for Optimization of Enteric Coating.

Krishna Reddy KV, Sathish D, Himabindu S, and Shayeda*.

Department of Pharmaceutics, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana, India.

ABSTRACT

The objective of this study was to develop a suitable drug delivery system for the delivery of Methylprednisolone to colon by using central composite design software. Methylprednisolone core tablets were formulated with HPMC K15M, Mannitol: SLS in four different drug: polymer: osmogen ratios and coated with cellulose acetate polymer having three different ratios of pore forming agent i.e., pectin. The formulation C3 with semi permeable coating having 20% pectin was found to be optimal and further coated with EudragitL100. The formulated semi permeable coated core tablets were evaluated for physicochemical parameters. From the *in vitro* dissolution data of tablets coated with semi permeable coating with 20% pectin as a pore former showed 98.5% release (C3b). This coated tablets were further subjected to the enteric coating. The enteric coating was done by using central composite design. The independent factors chosen were polymer percentage and weight gain. The dependent factors chosen are lag time, hardness, process time. From *in vivo* x-ray studies in humans,it was concluded that all the coated tablets were invariably found to be present in colon after 6 hours of ingestion. No tablet was observed after 24 hours. It may indicate complete dissolution before 24 hours.

Keywords: Methylprednisolone, *in vivo* x-ray studies, pore forming agent, osmogen.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Due to the lack of digestive enzymes, colon is considered as suitable site for the absorption of various drugs. Over the past two decades the major challenge for scientist is to target the drugs specifically to the colonic region of GIT. Colon targeting is used to treat constipation, diarrhoea, inflammatory bowel disease (Ulcerative colitis & Crohn's disease) and colon carcinoma. Colon having the lower level of luminal & mucosal digestive enzymes as compared with the small intestine reduces the chances of drug degradation like facilitating absorption of acid and enzymatically labile materials, especially proteins and peptides. [1,2]. Colon delivery also a mean of achieving chronotherapy of disease that is sensitive to circadian rhythm such as asthma & arthritis [3,4].

Methyl prednisolone is a potent and commercially available non-steroidal anti-inflammatory drug (NSAID) that is widely used in the treatment of chronic inflammatory states and showed a promising activity for prevention and treatment of colitis and colon cancers [5,6]. Delivery of Methylprednisolone to colon was limited because of its undesirable gastrointestinal toxicity such as gastrointestinal intolerance and ulceration and is itself rapidly and completely absorbed from the GI tract with an *in vivo* bioavailability of 95% when given orally [7,8]. As such the drug from a conventional dosage form does not reach the site of action i.e., colon in sufficient quantities and/or larger doses are needed for effective concentration levels in the colon.

The present study has been taken up so as to achieve the therapeutic concentration of the drug in the colon, decrease in dose and dosing frequency and decrease the incidence of side effects by localizing drug in colon.

MATERIALS AND METHODS

Materials

Methylprednisolone was a gift sample from Aurobindo Pharma Ltd, Medak, Telangana, India. Cellulose acetate, Hydroxy Propyl Methylcellulose (HPMC K15M) were procured from Loba chemicals Pvt Ltd., India. All other excipients and reagents used were of analytical grade.

Methods

Formulation development

Preparation of Methylprednisolone core tablets

Core tablets of methylprednisolone were formulated using HPMC K15M in four different polymer:osmogen ratios by wet granulation technique. Then semipermeable coating was done with different levels of pore forming agent. Accurately weighed quantities of drug (methylprednisolone), polymer (HPMC K15M), osmogen (mannitol) and diluent MCC (Avicel pH 101) were mixed in a mortar. Required quantity of binder (PVP K30 in Iso propyl alcohol (IPA) as 5% solution) was added and the same was mixed thoroughly to form a mass suitable for granulation. The dough mass was passed through sieve # 20 to form granules which were dried in an oven at 50^0C for 30 minutes. The granules were mixed with required quantities of lubricant (talc) and glidant (Magnesium stearate) and were compressed to form tablets in a 16 station rotary tablet machine (Riddi, Ahmedabad) using 10mm round concave punches. Three formulations of 50 tablets each were prepared with varying polymer and osmogen concentrations. The total weight of each tablet was 350mg and containing 40mg of methylprednisolone. Different core formulations of methylprednisolone are shown in table 1.

Coating

The coating solution was prepared by taking the solvent in a glass beaker and adding the pre-weighed quantities of polymer cellulose acetate (in semipermeable coating), Eudragit L 100/ Eudragit S 100 (in enteric coating) in small quantities at a time. Mixing was ensured by means of a mechanical stirrer. After complete solubilisation of the polymer then plasticizer, pore forming agent (in case of semipermeable coating i.e., pectin) and presolubilised color was incorporated into the solution and kept for overnight stirring [9].

Initially, pan was rotated at low speed and hot air was passed and when the pan gets heated, core tablets were placed in the coating pan along with filler tablets (tablets made using 6mm round deep concave punches and containing microcrystalline cellulose/dibasic calcium phosphate, magnesium stearate and talc). Hot air was passed through the tablet bed and pan speed was increased to 20-30 rpm and coating solution was sprayed. (Table 2)

Evaluation of the developed formulations

Determination of flow properties of granules

The quality of tablet, once formulated by rule, is generally dictated by the quality of physicochemical properties of blends. There are many formulations and process variables involved in mixing and all these can affect the characteristics of blends produced. The various characteristic properties of blends were evaluated by using Angle of repose, Bulk density, Tapped density, Carr's Index and Hausner's Ratio.

Characterization of semipermeable membrane coated tablets

The prepared methylprednisolone tablets were studied for their physicochemical properties like weight variation, hardness, thickness, friability and drug content.

***In vitro* drug release studies**

Drug release studies of semi permeable membrane coated tablets were carried out by USP type I (basket) apparatus. The test was carried over a period of 16 hours using phosphate buffer pH 7.4.

Effect of pH and agitational intensity on drug release

To study the effect of pH and to assure a reliable performance of the developed formulations independent of pH, release studies of the optimized formulations were conducted in different pH buffers. The release media used were 900ml of buffer (pH 7.4) and 900ml of 0.1N HCl (pH 1.2), 900ml of buffer (pH 6.8). The samples (5ml) were withdrawn at predetermined intervals and analyzed using UV-Visible spectrophotometer (Elico, India) at 243 nm. The in-vitro drug release studies at various agitation rates of 25, 50 and 100 rpm were carried out. The results obtained (Fig 11) showed that there was no significant difference in the cumulative percentage drug release form osmotic systems.

Effect of agitational intensity on drug release

The in-vitro drug release profiles at various agitation rates of 25, 50 and 100 rpm are presented in Fig 12. It showed that a change in agitational intensity did not significantly affect the drug release. The cumulative percentage drug release at 25, 50 and 100rpm were found to be 85.31%, 87.20% and 91.04% respectively. Therefore, the variations in peristaltic movements of the gastrointestinal tract might not affect the drug release

Optimization of Enteric coating by Central Composite Design

The coating solution was prepared by taking the solvent (IPA) in a glass beaker and adding the pre-weighed quantities of polymer Eudragit L100 in small quantities at a time. Mixing was ensured by means of a mechanical stirrer. After complete solubilisation of the polymer then plasticizer and presolubilised color was incorporated into the solution and kept for overnight stirring.

Experimental Design for Enteric Coating Formulations.

A full 3^2 factorial design was used for optimization of coating solutions. The concentration of Eudragit L100 and weight gain was selected by using central composite design (CCD) under Design Expert Software

(version 8.0). The independent variables were concentration of enteric polymer (X1), weight gain (X2). The dependent variables selected for the study include lag time (Y1), hardness (Y2), process time (Y3).

Statistical Analysis of Data and Coating Optimization

The response values (lag time in hour, hardness and process time) of coated tablets based on 3^2 factorial design were subjected to analysis by response surface reduced quadratic model with the help of Design Expert software (Version 8.0). Statistical validity of the polynomial was established on the basis of ANOVA provision in the design expert software and significant terms were chosen for final equations. Response surface plots and 3D contour plots were constructed using the output files generated. Preliminary experiments and evaluations of runs are presented in table

Characterization of Enteric coated tablets

Percentage increase in weight of the coated tablets

Twenty coated tablets were taken and their weight was determined individually and collectively on a digital weighing balance. The average weight of one coated tablet was determined from the collective weight of a batch. The percentage increase in weight from that of the weight of uncoated tablet was determined using the following equation.

$$\text{Total Weight Gain TWG} = \frac{\text{Final weight of tablet after coating}}{\text{weight of core tablet}} \times 100$$

Hardness and Thickness

The coated tablets must be hard enough to maintain the tablet integrity during the dissolution process. From each formulation, the hardness of 6 tablets was determined using Pfizer hardness tester. Twenty coated tablets were taken and their thickness was recorded using digital micrometer (Digital caliper, Aerospace, India). and the average is calculated and presented with standard deviation (Table 20, 26).

Determination of drug content

Ten tablets were finely powdered, quantities of the powder equivalent to 50mg of methylprednisolone were accurately weighed, transferred to a 100mL volumetric flask containing 50mL of phosphate buffer (pH 7.4) and allowed to stand for 5h with intermittent sonication to ensure complete solubility of the drug. The mixture was made up to volume with pH 7.4 phosphate buffer. The solution was suitably diluted and the absorption was determined by UV-Visible spectrophotometer at 243 nm. The drug concentration was calculated from the calibration curve (Table 20, 26).

***In vitro* drug release studies**

Drug release studies of core tablets

In vitro evaluation was carried out by USP type I (basket) apparatus. The test was carried over a period of 20 hours using phosphate buffer pH 7.4.

Drug release studies of enteric coated tablets

The release of methylprednisolone from coated tablets was carried out using USP type I dissolution apparatus (Electro lab, TDT-08L) at a rotation speed of 50 rpm, and a temperature of 37±0.5 °C.

For tablets, simulation of gastrointestinal transit conditions was achieved by using different dissolution media. Thus, drug release studies were conducted in simulated gastric fluid without pepsin (SGF, pH 1.2) for the first 2h as the average gastric emptying time is about 2h. Then, the dissolution medium was replaced with enzyme-free simulated intestinal fluid (SIF, pH 4.5, pH 6.8) and tested for drug release for 3h, as

the average small intestinal transit time is about 3h and finally simulated colonic fluid (SCF, pH 7.4) with pectinase was used for 16h to mimic colonic pH conditions.

Drug release was measured from coated tablets, added to 900 mL of dissolution medium. Samples withdrawn at various time intervals were analyzed spectrophotometrically at 243 nm. All dissolution runs were performed in triplicate.

***In vivo* X-ray studies**

X-ray imaging technique or Roentgenography was used to monitor tablets throughout the GI system. The inclusion of radio opaque material into the solid dosage form enables it to be visualized by the use of X-rays. By incorporating barium sulphate into the pharmaceutical dosage forms, it is possible to follow the movement, location and integrity of the dosage form after oral administration by placing the subject under a fluoroscope and taking a series of X-rays at various time points.

Preparation of tablets for X-ray studies

Half the quantity of the drug in the core tablet was replaced with a radiopaque substance i.e., $BaSO_4$. Coating was done like normal tablets till a desired weight gain was obtained. The composition of core tablet used for X-ray studies given in table 3. The study was conducted with prior approval of "Institutional Human Ethical Committee" **(File no. UCPSc/KU/BA/2013-02).** Three healthy human volunteers, male, with an age limit of 22-30 years and 50-70 kg body weight, were participated in the study. They were non-alcoholics, non-smokers and have not taken any drugs. The purpose of the study was fully explained and volunteers had given their written consent. Each subject ingested barium sulphate containing tablets orally with 200mL water, after an overnight fast. The tablets were visualized using X-ray. Abdominal radiographs were taken after 3, 6, 12 and 24 h.

RESULTS AND DISCUSSIONS

Formulation development

Preparation of core tablets

Core tablets of methylprednisolone were prepared by wet granulation method using the formula listed in table 1. Granules were prepared, dried, mixed with glidants and lubricants and were successfully punched into tablets using 10mm concave punches.

Table 1: Various formulations tried for optimization of core tablets

Ingredients	C1	C2	C3	C4
Methylprednisolone(mg)	40	40	40	40
HPMC K15M(mg)	0	10.5	28	35
Mannitol : SLS(1:1)	70	70	52.5	45.5
MCC 101 (mg)	218	207.5	207.5	207.5
Magnesium stearate(mg)	12	10	10	10
Talc (mg)	10	12	12	12
Total tablet weight(mg)	350	350	350	350

Formulations of cellulose acetate coated core tablets of methylprednisolone

The core compartment is surrounded by a membrane consisting of a semi-permeable membrane-forming polymer and a plasticizer capable of improving film-forming properties of the polymer. Cellulose acetate used as semi-permeable membrane forming polymers. PEG-400 was used as a water soluble plasticizer.

ISSN: 0975-8585

Table 2: Pan coating specifications

Parameter	Value
Atomizing air pressure	10-15 psi
Temperature	
1)Inlet air	55–60°C
2)Tablet bed	50–55°C
3)Exhaust air	48–52°C
Pan speed	20–30 rpm
Flow rate	1-4mL/min
Pan capacity	50 g
Pan diameter	5 inches
Spray to bed distance	8 cm

Table 3: Composition of core tablet used for X-ray studies

S.NO	Ingredient	Amount (mg/tablet)
1	Methylprednisolone	20
2	Barium sulphate	20
3	HPMC K15M	28
4	Mnnitol:SLS	52.5
5	Talc	12
6	Magnesium stearate	10
7	MCC 101	207.5

Optimization of semipermeable coating formulations

Three coating solutions of cellulose acetate in acetone containing different levels of pore-forming agent i.e., pectin (10% w/v, 20% w/v and 30% w/v) were prepared for semipermeable membrane coating. The compositions of semipermeable coating solution are given in Table 4. Dibutylphthalate (1% w/w of total weight of coating materials) was added as plasticizer. The coating was carried out by pan coater (V.J Instruments, Mumbai), having diameter of 50 cm. The rotating speed was kept at 23 rpm. The coating solution was sprayed with the help of low pressure air-atomized spray gun at a fixed rate of 6 ml/min. The coated tablets were dried at 50^0C for 4 h. The average thickness and average weight gain of the tablet after Microporous semipermeable membrane coating were found to be 5.000 ± 0.0372 mm and 7.11 ± 0.0488%, respectively.

Table 4: Semipermeable coating formulations

S.NO	Ingredients	A	B	C
1.	**cellulose acetate**	1.8	2.2	2.6
2.	**Pectin (pore former)**	1.2(30%)	0.8(20%)	0.4(10%)
3.	**PEG400**	1mL	1mL	1mL
4.	**Acetone:water (60:40)**	100	100	100

Evaluation of the developed formulations

Determination of flow properties of granules

Various properties of granules such as bulk density, tapped density, carr's index, hausner's ratio and angle of repose were determined and the results are shown in the table 5. The angle of repose for all the formulations was found to be $<30^0$ indicating free flowing of the material and Carr's index values were found to be in the range of 12-16 indicating good flow properties.

ISSN: 0975-8585

Table 5: Flow properties of granules of various formulations

Formulation code	Angle of repose	Bulk density	Tapped density	Carr's index	Hausner's ratio
C1	28.02±1.10	0.388	0.443	12.41	1.41
C2	29.13±1.26	0.354	0.422	16.11	1.19
C3	27.01±0.84	0.342	0.412	16.99	1.20
C4	27.45±	0.345	0.409	16.21	1.21

Evaluation of process parameters of core tablets

All the four formulations were tested for physical parameters like hardness, thickness, weight variation, friability and found to be within the pharmacopoeial limits. The results of the tests were tabulated (table 6).

Table 6: Process parameters of various formulations

Formulation Code	Thickness(mm)	Wt gain (%)	Hardness (Kg/cm^2)	Weight variation(mg)	Friability (%)	%Drug content
C1	5±0.05	7.11± 0.0398%	6.5±0.15	374±1.6	0.05	101.3
C2	5±0.08	7.24± 0.0488%	6.8±0.12	378±1.5	0.04	99.98
C3	5±0.05	7.18 ± 0.046%	6.7±0.14	380±1.2	0.08	99.48
C4	5±0.05	7.28 ± 0.045%	6.7±0.14	376±1.2	0.08	99.48

The results of the physical tests of the formulations were within the limits and comply with the standards. The weights of the tablets ranged from 374 to 380mg; the weights being with in ±5% of the average weight. The thickness was found to be 5mm. Hardness of the tablets was in the range of 6.5 to 6.8 kg/cm^2 and friability was in the range 0.04-0.08%, indicating that the tablets were hard enough to withstand the tumbling action in the coating pan. The drug content on an average was found to be 99%. All these parameters were within acceptable limits.

***In vitro* drug release profile of core tablets**

The cumulative percentage drug release profiles from various core tablet formulations were represented in table 7

Table 7: *In Vitro* drug release of various core tablet (C1, C2 and C3) formulations

Time (h)	C1A	C1B	C1C	C2A	C2B	C2C	C3A	C3B	C3C
0	0	0	0	0	0	0	0	0	0
0.5	8.44± 0.17	15.1± 0.66	21.9± 0.33	7.22± 0.57	8.44± 0.87	9.11± 2.14	4.66± 1.5	5.54± 2.21	8.44± 2.6
1	15.3± 0.14	38.6± 0.82	41.7± 0.47	12.8± 0.49	18.8± 1.4	25.6± 1.18	7.43± 2.9	8.44± 1.32	14.36±2 .1
2	36.2± 0.44	53.4± 0.73	60.5± 0.52	29.39± 0.43	37.2± 1.43	48.05± 2.45	13.6± 3.1	16.65± 1.45	21.3± 3.1
4	54.04± 0.56	73.6± 0.31	84.4± 0.51	41.78± 1.17	53.2± 1.17	60.71± 3.23	20.09± 3.2	31.41± 3.76	34.5± 0.9
6	76.32± 0.41	96.5± 0.45	99.2± 0.32	53.03± 0.72	66.4± 1.89	75.65± 2.21	33.83± 3.5	45.7± 4.21	53.9± 2.3
8	91.81± 0.23			63.94± 0.49	84.4± 1.46	85.75± 2.12	47.31± 3.1	54.7± 2.78	60.5±3. 3
10				85.75±0 .71	95.8±1.0 7	98.5±1.6 7	60.37±3. 5	72.9± 3.14	84.4±3. 2
12				98.5±0. 57			71.6±2.5	89.7± 4.13	91.81± 2.3
16							83.73±3. 2	98.5± 2.12	99.22± 3.2

Values are expressed as mean cumulative percentage release± SD with n=3

It is evident from the drug release studies, the core tablets C3b showed 98.55% of drug release in 16 h. Since colon residence time is approximately 16-18h. From the *in vitro* drug release studies formulation C3b has shown approximately 100% drug release in 16h. The release kinetics showed that C3b is 0.982 which follows perfect zero order drug release. Hence C3b was considered optimal and used in further experimentation.

Dissolution profile modeling:

Dissolution data of the optimized formulations were fit into various mathematical models (zero-order, first-order, Higuchi and Korsmeyer-Peppas) to describe the kinetics of drug release. An ideal osmotic system should be able to release a high percentage of drug content with a constant release rate (zero order kinetics) during dissolution. Goodness-of-fit test (R^2) was taken as a criterion for selecting the most appropriate model.

SEM studies of core tablet before and after dissolution

To investigate the changes in the membrane structure, surface of coated tablets was studied using SEM. Figure 1,2 showed SEM micrographs of membrane surface of optimized formulation containing 20% pectin as a pore former before and after dissolution studies. After dissolution studies, coating was intact without any cracks. However, there was formation of channels/pores in the membrane, which possibly acted as exit ports for the drug.

Table 8: Correlation coefficient (R^2) of different kinetic models for various formulations

Formulation	%drug release	Time (hrs)	R^2 value				n value
			Zero-order	First order	Higuchi	Koresmeyer-Peppas	
C1A	91.89	8	0.9809	0.9577	0.9643	0.9883	0.8678
C1B	96.534	6	0.9292	0.9267	0.918	9.9402	0.6882
C1C	99.229	6	0.9031	0.9181	0.9917	0.9127	0.5909
C2A	98.555	12	0.9845	0.7851	0.9601	0.9876	0.7959
C2B	95.861	12	0.9666	0.9257	0.9819	0.9778	0.7758
C2C	98.555	10	0.92	0.8752	0.9826	0.9332	0.7253
C3A	83.735	16	0.9896	0.967	0.9359	0.9901	0.9901
C3B	98.555	16	0.982	0.929	0.9504	0.9902	0.9932
C3C	99.229	16	0.962	0.899	0.9639	0.9927	0.9639

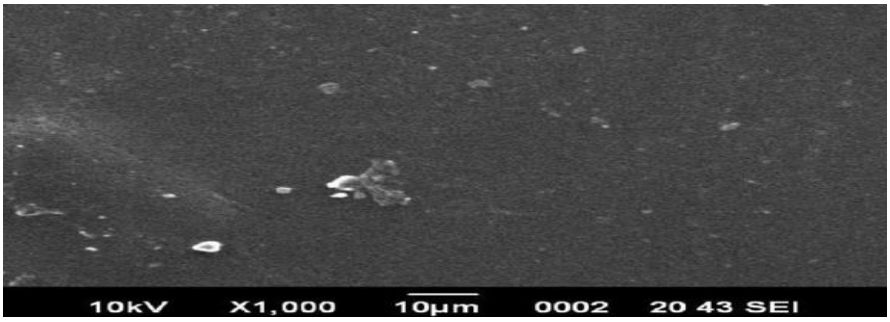


Figure 1*:* SEM of optimized tablet before dissolution

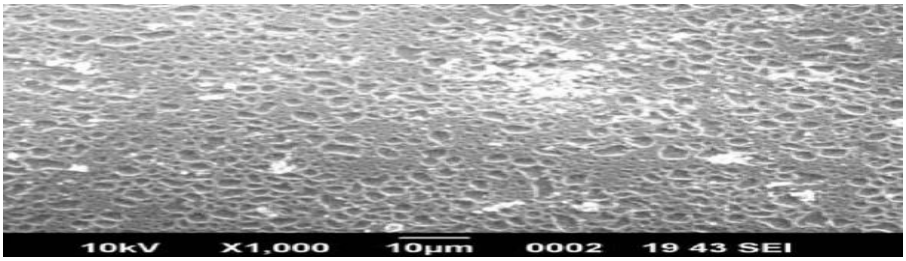


Figure 2: SEM of optimized tablet after 16hr dissolution

***In vitro* drug release profile of semipermeable membrane coated core tablets**

Effect of pH

In order to study the effect of pH and to assure a reliable *in vivo* performance, release studies of the optimized formulations were conducted in media of different pH. The release media are 0.1N HCl, pH 6.8, pH 7.4. Release profile of the drug from the target formulation in these media are reported in Table 9.

Table 9: *In vitro* Drug release profile of optimized core tablet in different buffers

Time (h)	Release (pH7.4)	Release (pH 6.8)	Release (pH 1.2)
0	0	0	0±
0.5	5.54±2.21	4.34±1.21	4.78±1.45
1	8.44±1.32	6.92±1.23	7.89±1.98
2	16.65±1.45	14.21±1.89	15.56±2.34
4	31.41±3.76	26.98±2.34	28.9±2.45
6	45.76±4.21	40.09±2.56	42.87±1.9
8	54.78±2.78	50.09±1.89	52.67±2.34
10	72.95±3.14	64.21±2.98	67.98±3.12
12	89.79±4.13	84.65±2.34	86.98±2.78
16	98.55±2.12	94.98±2.46	97.56±2.34

Effect of agitational intensity

Drug release from osmotic pumps, to a large extent, is independent of agitational intensity of the release media. In order to verify effect of agitational intensity, release studies were carried out in USP-II dissolution apparatus at varying rotational speed (50, 100, and 150 rpm). The data of the drug release profile of the tablets at different rpm conditions are shown in the Table 10. The cumulative percentage drug release in 24 h was found to be 100, 99, and 98% at 50, 100, and 150 rpm respectively. There was no drastic change in the drug release i.e., the drug release from the CPOP is independent of agitational intensity.

Table 10: *In Vitro* Drug release profile of optimized core tablet at different RPM

Time	100 RPM	50 RPM	25 RPM
0	0	0	0
0.5	5.54±1.32	4.22±2.22	3.98±1.45
1	8.44±2.22	6.78±3.21	5.92±1.98
2	16.65±1.34	13.21±2.23	12.26±2.34
4	31.41±2.2	24.87±2.12	22.54±1.23
6	45.76±3.34	41.98±2.09	39.43±1.9
8	54.78±3.21	51.09±2.4	50.08±2.34
10	72.95±3.21	70.98±3.4	68.93±2.6
12	89.79±2.98	85.98±4.3	81.09±2.78
16	98.55±2.21	93.34±2.3	90.67±2.34

Effect of osmotic pressure of the dissolution medium

The release of drug from the osmotic system is dependent on the osmotic pressure. Difference in the osmotic pressure of the solution on either side of the semipermeable membrane can act as driving force for drug release. Hence release studies of the optimized formulation were conducted in media of different osmotic pressure (table 11)

Table 11: *In Vitro* Drug release profile of optimized core tablet in different osmotic pressures

Time (h)	O.P.1	0.P.2	O.P.3
0	0	0	0
0.5	5.54±1.23	4.09±1.43	2.98±1.54
1	8.44±1.34	5.91±1.21	4.09±1.65
2	16.65±2.21	12.98±3.21	11.09±1.76
4	31.41±1.45	26.81±2.89	20.9±1.76
6	45.76±1.34	40.09±2.45	35.98±1.87
8	54.78±2.21	49.21±3.2	47.65±1.97
10	72.95±1.43	68.79±2.4	66.09±1.45
12	89.79±1.21	82.78±2.1	77.09±1.56
16	98.55±1.56	91.89±1.2	86.09±2.21

Optimization of the composition of the polymeric coating solution

Coating solution needed to be optimized with regard to the selection of suitable plasticizers and the quantities in which they were used. The parameters on which this was done included the total weight gain (TWG) of coating achieved with the solution and the ability of the coat to maintain integrity of the tablet core in 0.1N HCl, also pH 4.5 buffer and pH 6.8 buffer.

Coating was carried out on the core tablets (C3B) that were optimised in the earlier step. For the purpose of optimisation of coating solution composition, a uniform coating was applied on the core tablets and was visually inspected for characters such as lustre of film, appearance, elegance etc.

Table 12: Optimization of Eudragit L100 Coating solution compositions

S.NO	Ingredient
1	Eudragit L100
2	Dibutyl phthalate
3	Isopropyl alcohol
4	Acetone
5	Talc

Optimisation was done based on the conclusions drawn from various coating solution compositions. When acetone was used alone rapid drying of the solution was noticed. The polymer dried up even before reaching the tablet bed. It also resulted in significant blocking of the atomizer head. It was decided that the replacement of acetone with a solvent of higher boiling point would ease the problem. IPA was substituted for acetone. As such the total content of IPA in the coating solution was increased to 100mL. The coating solution exhibited better properties suitable for pan coating. The polymer remained in the liquid state until it reached the tablet bed. Since the tablets still posed sticking problem it was inferred that the use of anti-adherent in the coating solution would remedy the problem. Addition of talc in the coating solution alleviated the problem of sticking. Tablets showed a marked decrease in sticking tendency [10,11].

Preliminary experiments to determine design space:

Preliminary experiments were conducted by varying the Eudragit L100 and weight gain and the results are presented in Table 13

From the trail formulations, design space was determined i.e. Eudragit L100 concentration (4 to 8%) and weight gain (7 to 15%). Below 4% Eudragit L100 and 7% weight gain polymeric film formed was not elegant and not covered the tablet uniformly. Above 8% spray properties were severelyaffected.The spray gun was blocked above 8% Eudragit L100.This design space was subjected to the central composite design by using design expert software which has given the following runs. The runs along with results are presented table 13.

ISSN: 0975-8585

Table 13: Preliminary experiments:

polymer concentration	Weight gain	Lag time (hrs.)	Hardness(kg/cm^2)	process time (min)
	7%	2	6.14	35
	12%	3	12.33	50
4%	16%	6	13.23	90
	7%	4	8.09	50
6%	12%	5	10.09	60
	16%	6	12.45	90
	7%	5	11.23	50
	12%	5	12.89	70
8%	16%	7	16.09	100
	7%	6	16.45	70
	12%	8	17.05	100
10%	16%	11	18.06	120

Optimization of enteric coating formulation using central composite design

After successful preparation and characterization, the responses were then analyzed using Design-Expert ©software for evaluation and optimization of above formulations. The optimized enteric coated formula was selected based on criteria of attaining the maximum value of lagtime, within range hardness and a within the range process time by applying of Design-Expert©Soft ware. Then an optimized formulation and its predicted responses were obtained from the software. The given formulation was prepared and then the actual and predicted responses were compared. It was found that there was no significant difference between the latter and former one.

The response layout and results of central composite design batches are shown in Table 14, 15 respectively, which clearly indicates that both the independent variables are dependent on lag time, hardness and process time as they showed distinct variation among the thirteen batches.

Table 14: Independent factors and there levels given by the software are

Independent variables	coded values	actual values (X1)	actual values (X2)
X1-eudragit L100 concentration	-1.41	3.17	5.34
X2-wt gain	-1	4	6
Dependant variables	0	6	11
Y1-lag time	1	8	15
Y2-hardness	1.41	8.83	16.66
Y3-process time			

Table 15: Evaluation of enteric coated tablets

FORMULATION CODE	Run	Eudragit L100	wt. gain (%)	lag time (h)	Hardness (kg/cm^2)	process time (min)
C2bE1	1	3.17	11	5	12	60
C2bE2	2	4	15	6	13	90
C2bE3	3	6	11	5	10	60
C2bE4	4	6	11	5	10	60
C2bE5	5	6	16.66	6.5	13	120
C2bE6	6	8.83	11	6.5	12	90
C2bE7	7	6	11	5	10	60
C2bE8	8	6	11	5	10	60
C2Be9	9	6	11	5	10	60
C2bE10	10	8	7	5	11	50
C2bE11	11	4	7	3	9	30
C2bE12	12	6	5.34	4	9	30
C2bE13	13	8	15	6	16	100

ISSN: 0975-8585

The selection of model for analyzing the responses i.e. *lag time, hardness and process time* by design expert software was done based on comparisons of parameters that include R^2, Predicted residual sum of square (PRESS) and S.D. The model chosen should have high R^2 and low value of PRESS. Linear model was chosen to analyze the responses *lag time, hardness and process time* because the increase in the response with increase in levels of independent factors was linear.

The mathematical relationship in the form of a polynomial equation generated by Design-Expert 8.0 software for the measured responses is:

Lag time= -3.16099+0.94508*Eudragit L100+0.61049*weight gain –
0.062500*Eudragit L100*weight gain

Hardness= +21.03593 – 3.59375*Eudragit L100-0.67479*weight

gain+0.031250 *weight gain+0.29688*Eudragit $L100^2$+

0.04269*$weight\ gain^2$

Process time = -41.80156+4.52665*Eudragit L100+7.41498*weight gain

The above equations represent the quantitative effect of independent variables and their interactions on the responses. A positive sign indicates synergetic effect and a negative sign indicates antagonist effect.The predicted values can be obtained by substituting the values of A,B,C into the given equations, the following table gives regression analysis for the responses. The relationship between the responses and independent factors were further elucidated using response surface plots. Three dimension surface plots (3-D) for the measured responses were formed, based on the model polynomial functions to assess the change of response surface. The lag time showed linear pattern with Eudragit L100 and weight gain, whereas the concentration of Eudragit L100 and weight gain was increased the lag time was also increased (Fig 3).

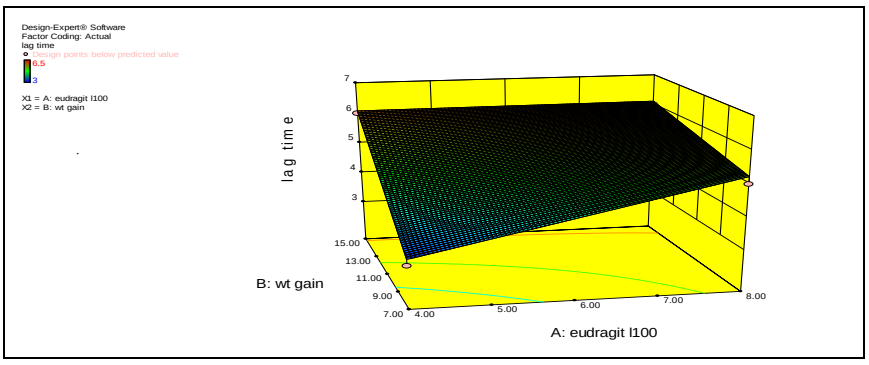


Figure 3: The effect of variable Eudragit L100 and weight gain on the response, lag time.

The hardness showed linear pattern with Eudragit L100 and weight gain, whereas the concentration of Eudragit L100 and weight gain was increased the hardness was also increased (Fig 4).

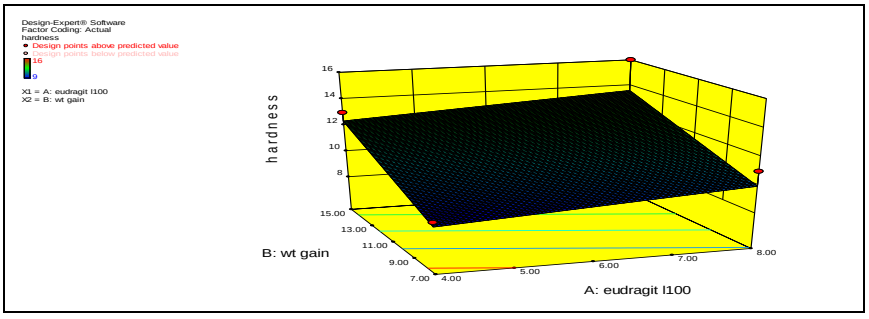


Figure 4: The effect of variable Eudragit L100 and weight gain on the response, hardness

ISSN: 0975-8585

The process time showed linear pattern with Eudragit L100 and weight gain, whereas the concentration of Eudragit L100 and weight gain was increased the process time was also increased (Fig 5). The overlay plot and degree of desirability of the formulation obtained from the **Design-Expert©software** are shown below in Fig 6

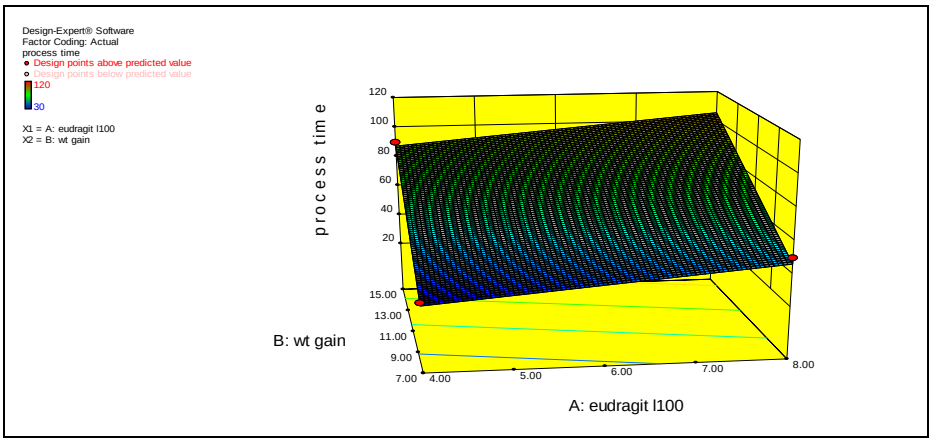


Figure 5: The effect of variable Eudragit L100 and weight gain on the response, process time.

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:eudragit l10(	maximize	4	8	1	1	3
B:wt gain	is in range	7	15	1	1	3
lag time	maximize	3	6.5	1	1	3
hardness	is in range	9	16	1	1	3
process time	is in range	30	120	1	1	3

Solutions

Number	eudragit l100	wt gain	lag time	hardness	process time	Desirability	
1	8.00	15.00	6.11095	13.611	105.636	0.943	Selected
2	8.00	14.41	6.04588	13.3412	101.269	0.933	
3	8.00	14.17	6.01941	13.2315	99.4928	0.929	

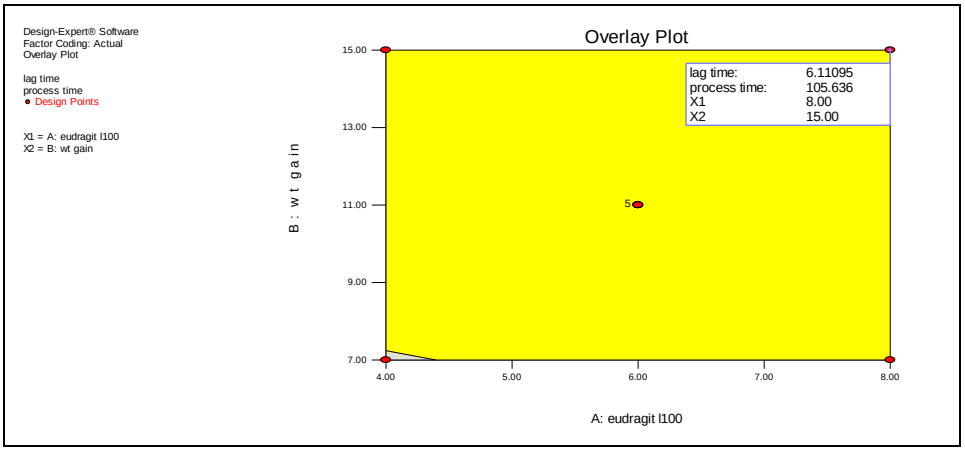


Figure 6: Overlay Plot with optimized levels of the 2 factors & predicted responses

The optimized semipermeable coated tablets (C3b) are further coated with optimized enteric formula given by the design expert software (Tables 16,17). The results are represented below (table 18).

ISSN: 0975-8585

Table 16: Regression analysis for response

	Lag time	hardness	Process time
Model chosen	Linear	Linear	Linear
R^2	0.8239	0.6286	0.9295
Adjusted R^2	0.7887	0.5543	0.9154
Predicted R^2	0.622	0.3244	0.8743
PRESS	4.23	32.22	104..36

Table 17: Optimized formula given by design expert software.

S.no	Ingredient	Quantity
1	**Eudragit L100***	**8**
2	Dibutyl phthalate	1.8
3	Isopropyl alcohol	100
4	Talc	1
5	Tio_2	1.2
6	**wt. gain**	**15%**

Table 18: Responses

S.no	Responses	predicted value	actual value
1	**lag time(hr.)**	6.11	6
2	**hardness(kgs)**	14.58	15±0.81
3	**process time(mints)**	107.463	105±0.67

***In vitro* drug release profile of optimized enteric coated tablets**

The cumulative percentage drug release profiles from enteric coated tablet formulation are represented in table 19. From the dissolution data it was observed that the formulations showed little or no significant release at pH 1.2, pH 4.5 (i.e., < 1% drug release). Release started in pH 6.8 buffer for the formulation. This may be attributed to the fact that the threshold pH (pH at which dissolution occurs) of Eudragit L100 is 6.0. The lag time was found to be 6 h. The lag time of 5 h is mainly due to the erosion of enteric coating. In the next 1 hr also it didn't release the drug due to semipermeable coating. During this time pectin was attacked by pectinase enzyme present in colonic media. Morphological changes were given in Figure 7

Table 19: *In vitro* drug release profile data for tablets coated with Eudragit L100

pH	Time (h)	Cum amount drug release ± SD
1.2	0	0±0
	0.5	0.03±0
	1	0.06±0
	2	0.08±0
	3	0.12±0
4.5	4	0.14±0
6.8	5	0.16±0.39
	6	0.26±0.12
	8	28.23±02.57
7.4	10	48.33±3.12
	12	62±4.67
	16	85.3±5.98
	24	99.9±3.85

Values are expressed as mean cumulative percentage release± SD with n=3

ISSN: 0975-8585

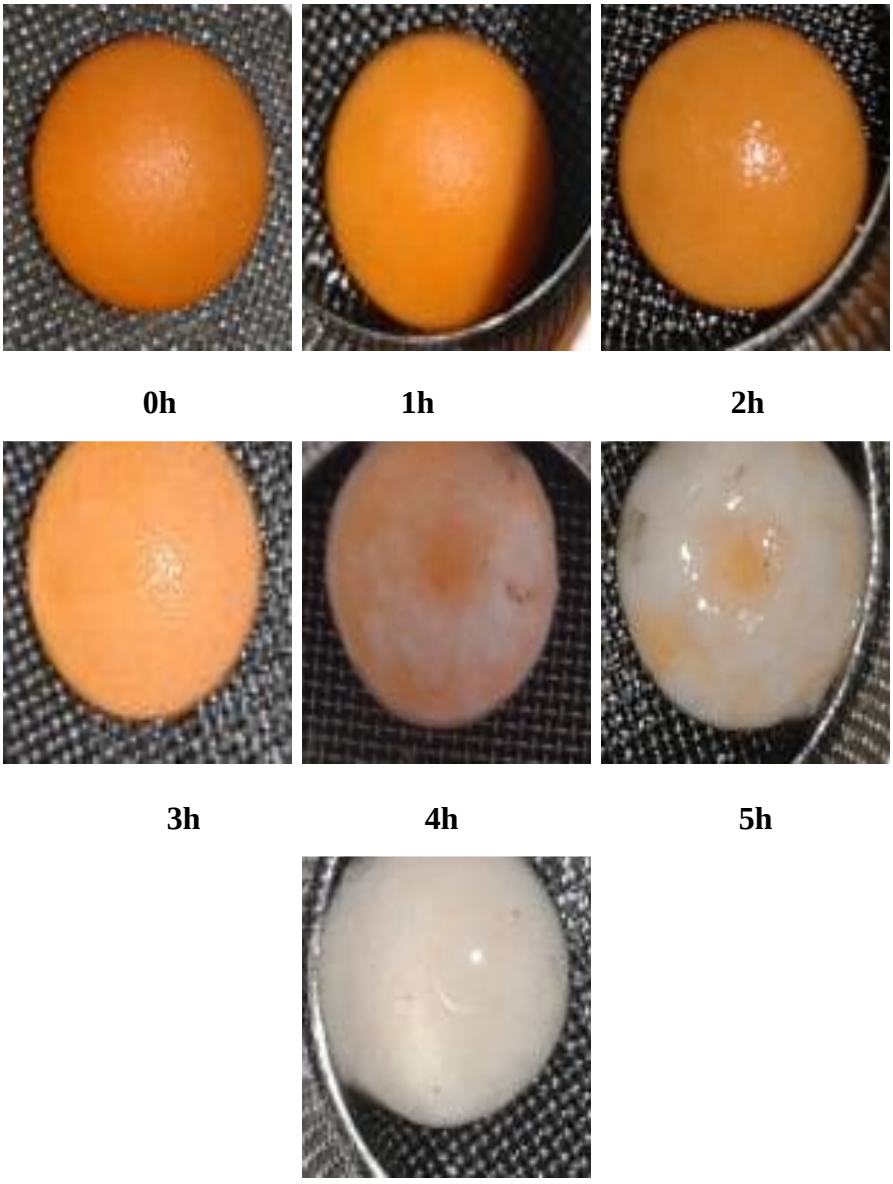
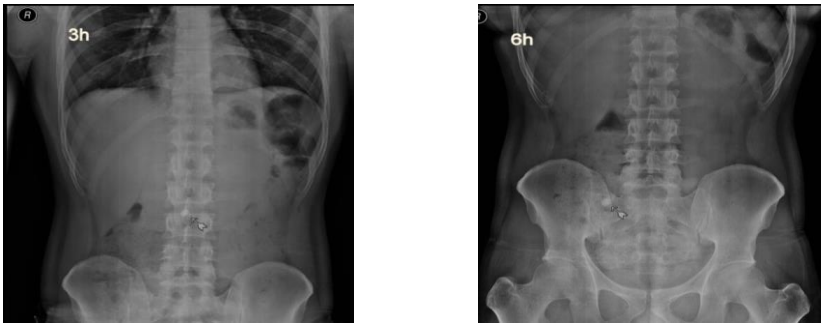


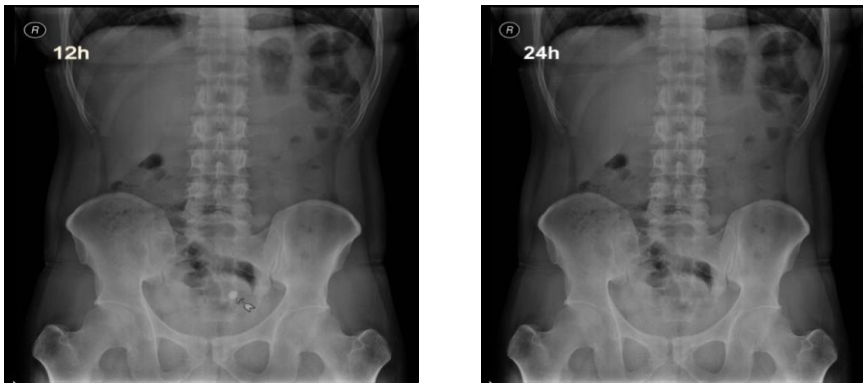
Figure 7: Morphological changes noticed during dissolution of optimized Eudragit L100 coated tablets.

The semipermeable membrane coated core tablets used for *in vivo* x-ray studies were coated with optimized coating solution to a total weight gain of 15.5%. The photographs were given in the following figures.



(a) **(b)**

ISSN: 0975-8585



(c) (d)

Figure 8: X ray photos of volunteer 1 after 3 ingestion of tablet showing the location of the delivery system in the A. small intestine; B. transverse colon; C. descending colon; D. abdomen.

CONCLUSION

The current research work demonstrates the successful development of Microporous colon osmotic tablets of Methylprednisolone for inflammatory bowel disease. Methylprednisolone core tablets were formulated with polymer HPMC K15M, Mannitol: SLS in four different drugs: polymer: osmogen ratios and coated with cellulose acetate polymer having three different ratios of pore forming agent i.e., pectin. The formulation C3 with semipermeable coating having 20% pectin was found to be optimal and further coated with pH dependent polymers EudragitL100. The pH dependent polymer and its weight gain was determined by central composite design.

From the *in vitro* dissolution data of tablets coated with semipermeable coating with 20%pectin as a pore former showed the 98.5% release (C3b). These coated tablets were further subjected to the enteric coating.

In vivo x-ray studies were conducted in three healthy human male volunteers at time points 3, 6, 12 and 24h, from these studies it was concluded that all the coated tablets were invariably found to be present in colon after 6 hours of ingestion. No tablet was observed after 24 hours. It may indicate complete dissolution before 24 hours.

REFERENCES

[1] Rubinstein A, Radai R. Eur J Pharm Biopharm 1995; 41: 291-295.
[2] Luppi B, Bigucci F, Cerchiara T, Mandrioli R, Pietra AM, Zecchi V. Int J Pharm 2008; 358: 44–9.
[3] Jose S, Prema MT, Chacko AJ, Thomas AC, Souto EB. Biointerfaces 2011; 83: 277–83.
[4] Mastiholimath VS, Dandagi PM, Jain SS, Gadad AP, Kulkarni AR. Int J Pharm 2007; 328: 49–56.
[5] Kamel A, Alaa M, Amal JF, Hussein IES. Int J Pharm 2008; 358: 248-255.
[6] Lee BJ, Zong ZP, Kyung ML. Int J Pharm 2008; 350: 205- 211.
[7] Modasiya MK, Patel VM. Journal of Pharmacy Research 2012; 5: 2253-2258.
[8] Nimkulrat S, Krisda S, Pranee P, Satit P. Int J Pharm 2004; 287: 27–37.
[9] Remon JP, Huyghebaert N, Vermeire. Int J Pharm 2005; 298: 26- 37.
[10] Nitesh S, Mayur P, Tejal S, Avani A. J Pharm Edu Res 2011; 2.
[11] Ravi V, Pramod TM, Siddaramaiah. Indian J Pharm Sci 2008;70: 111-113.