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EPITOMIZATION OF MINI-TABLETS BY USING NATURAL POLYMERS FOR COLON SPECIFIC CHRONOTHERAPEUTIC DRUG DELIVERY

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ABSTRACT

During the past decades research is going on developing the methods to target specific regions by multiparticulate drug delivery systems (i.e. Mini-tablets, beads, liposomes). In the present study a novel colon specific chronotherapeutic multiparticulate system (CSCMS) of mini-tablets for the treatment of airway resistance diseases (Bronchial asthma, chronic bronchitis and emphysema) was developed. Colon specific chronotherapeutic multiparticulate system of mini-tablet prepared by direct compression method and coating material is prepared by wet granulation method to protect the core mini-tablets from upper GI track with different proportions (F1 to F13) of formulations by using natural polymers (Guar gum, xanthan gum, chitosan). Finally the optimized (F12) compression coated mini-tablets shows best drug release around at the targeted region. Combination of microbial degraded natural polymers and time dependent HPMC K15M provided better protection, showing increased drug release at lag time. Dissolution studies in pH 6.8 phosphate buffer containing 4% w/v rat caecal contents have demonstrated the susceptibility of the natural polymers to the colonic bacterial enzymatic action with subsequent drug release. The FTIR data indicated no possible interaction between drug and excipients, In-vivo x-ray studies shows that the tablet successfully reached the targeted area, avoid the release in upper regions of gastrointestinal system.

KEYWORDS: Solbutamol sulphate, Colon specific chronotherapeutic multiparticulate system (CSCMS), HPMC K 15 M, Guar gum, Xanthan gum, Chitosan.

INTRODUCTION

Tablet dosage forms occupy the massive and most remarkable place among all pharmaceutical dosage forms, oral aspect is considered to be one of the most suitable, popular way for administration of drugs to patients. Research is going ahead in building up the techniques to focus on the drugs to specific regions such as brain, colon, stomach etc.

Colon specific drug delivery[1] refers to targeted delivery of drugs into the lower GI tract primarily in the large intestine i.e. colon, which includes chronotherapy, treatment of colon diseases and nicotine addiction. These dosage forms offers many advantages such as constant drug level at the site of action, increased bioavailability of poorly absorbable drugs, reduction in dose of drug, prevents the gastric irritation, reduced dosage frequency, avoidance of side effects, improved patient compliance.[2][3][4]

Circadian rhythms are self sustaining endogenous oscillations that occur with periodicity of about 24 hrs. The efficacy, toxicity of many drugs vary depending on the relationship between the dosing schedule and 24 hrs rhythms[5][6] of biochemical, physiological, behavioral process. Also several drugs cause alteration to 24 hrs rhythms leading to illness and altered homeostatic regulation. The alteration of biological rhythms is a new concept of adverse effects which can be minimized by optimizing the dosing schedule, they are predictable resonating dynamic systems whom requires different amount of the drug at predictable different time within the circadian cycle which will be maximize desired and minimize undesired drug effects. Hence, chronopharmacotherapy drug orchestration is concur with biological rhythms build maximal curative of drug in the burst at circadian timings correlated with specific pathological disorders to achieve maximum drug effect.

Asthma is a chronic disorder, symptoms of the disease are pronounced predominantly during the early hours of the morning. Hence, a colon specific chronotherapeutic[13] multiparticulate system (CSCMS) with a predetermined lag time may useful for such patients as the drug is released at a predetermined time and maximum concentration of the drug can be reached

when the symptoms of the diseases worse to fatal. It will be more helpful if medication before bed time to overcome a high level of discomfort in the morning. CSCMS may be a successful tool for effective chronopharmacotherapy because of their unique drug release profiles.

Thus the formulation of salbutamol sulphate of CSCMS to diminished its adverse symptoms and produce huge absorption in the colon. In the present study salbutamol sulphate was selected as a model drug for the development of compression coated multiparticulate[7] colon specific chronotherapeutic drug delivery system. salbutamol sulphate act as a β2 adrenergic receptor agonist widely used for the treatment of nocturnal asthma, chronic bronchitis and emphysema. The drug undergoes extensive first pass metabolism, this requires frequent administration by oral route. Oral bioavailability of salbutamol sulphate very low (~40%) due to expensive metabolism via intestinal sulphonation, high in liver and also degradation in colon. Drug release in the colon by matrix erosion or dissolution of polysaccharides which controls the drug release through their enzymatic degradation by colonic micro flora, association of two or more approaches have shown superior result regarding CSCMS namely negligible drug release in the stomach, small intestine and considerable amount of drug release in the colon.

Therefore the present study sought to prepare a novel colon specific chronotherapeutic[13] multiparticulate system of compression coated mini-tablet[9] based on the combination of time and enzymatic controlled polymers. Recently mini-tablets have received interest as a potential oral dosage forms for pediatric and geriatric patients. Mini-tablets are very small tablets whose diameter is equal to or smaller than 4 mm that can be either placed in the sachets or compressed into tablets or filled into a capsule shell for easy administration. They are having more benefits over single unit larger tablets such as accurate, consistent drug release, uniform clinical performance and more flexibility during the formulation development and maximum stability on storage. Also mini tablets are easier to prepare using direct compression method which involves very less number of steps using single simple equipments for their manufacture, regular shape, excellent size uniformity, thus the time and cost can be saved.

MATERIALS AND METHODS

MATERIALS

Salbutamol sulphate from Cipla, Hyd., Sodium starch glycolate from FMC bio polymers, HPMC K15 M from Dr.Reddy's labs, Hyd., PVP K30 (PH 102) from Hetero Drugs was obtained as a gift samples, Starch, Micro crystalline cellulose, Magnesium stearate, Talc purchased from S.D Fine Chemicals Ltd., Guar gum, Xanthan gum, Chitosan purchased from Zeal Chemicals.

METHODS

Analytical studies

a) Determination of absorption Maxima(λ max)

A solution of drug containing the concentration 10µg/ml was prepared in the different buffer solutions i.e. SGF(pH-1.2), SIF(pH-7.4, pH-6.8) respectively, the solution was scanned in range of 200 to 400 cm^{-1} by using double beam UV visible spectrophotometer.

b) Construction of calibration curve

100 mg of drug was accurately weighed and dissolved in 100 ml of methanol containing 100 ml volumetric flask to make(1000 µg/ml) standard stock solution(1). Then 10 ml of stock solution(1) was taken in another 100 ml volumetric flask make up to the mark(100 µg/ml) with different Buffer solutions SGF(pH-1.2), SIF(pH-7.4, pH-6.8) that is standard stock (2), again take 1,2,3,4,5 ml of standard stock (2) was taken in another 10 ml of volumetric flask to get concentration of 10,20,30,40,50 µg/ml with the same buffer. The absorbance of standard solutions was determined by using UV spectrophotometer at 276ηm. Linearity of the standard curve was assessed from the square of correlation co-efficient or square which determined by least square linear regression analysis.

Drug-excipient compatibility studies

Fourier Transform Infrared (FT-IR) spectroscopy

The physical properties of the physical mixture were compared with those of plain drug. By using Fourier transform infrared (FT-IR) spectroscopy **(**Bruker, India) IR spectrum were recorded from 3500 cm^{-1} to 500 cm^{-1}. The resultant spectrum was compared for any spectrum changes.

Evaluation of Precompression studies

Angle of repose

It is defined as maximum angle possible between surface of the pile of the powder to the horizontal plane. The angle of repose of granules was determined by the funnel method. The accurately weighed granules were taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the granules. The granules were allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation:

$$\tan\theta = h/r,$$

where θ = angle of repose, h = height, and r = radius.

Bulk density

Bulk density is defined as ratio of total mass of powder to the bulk volume of powder. Bulk volume is the volume occupied by a certain mass of powder when gently poured into a measuring cylinder. It was measured by pouring initially weighed powder into a measuring cylinder and the volume (bulk volume) was noted. From this, the bulk density was calculated according to the formula mentioned below. It is expressed in g/ml and is given by

$$Db = M/Vb,$$

where M is the mass of powder and Vb is the bulk volume of the powder.

Tapped density

It is the ratio of total mass of the powder to the tapped volume of the powder. Volume was measured by tapping the powder for 100 times and then the tapped volume was noted. It is expressed in g/ml and is given by

$$Dt = M/Vt,$$

where M is the mass of powder and Vt is the tapped volume of the powder.

Compressibility index

It indicates powder flow properties. It is expressed in percentage and is given by

$$I = (Dt - Db) \times 100,$$

where Dt is the tapped density of the powder and Db is the bulk density of the powder.

Hausner ratio

The Hausner ratio is a number that is correlated to the flowability of powder. It is calculated by the following formula:

$$\text{Hausner ratio} = Dt/Db,$$

where Dt is the tapped density and Db is the bulk density.

A Hausner ratio greater than 1.25 is considered to be an indicator of poor flowability.

Formulation of compression coated tablets

Preparation of fast dissolving core mini-tablets

Salbutamol sulphate was chosen as a model drug to extensive metabolism via intestinal sulphonation microbial degradation in the colon, optimize the different proportions of natural polymers as a coating material i.e. Guar gum, Xanthan gum, Chitosan to target the colon. Colon specific chronotherapeutic drug delivery system were prepared by using compression coating initially internal core mini-tablets prepared by incorporating different proportions of super disintegrant according to table: 1 (disintegrant time <1 min), PVP K30 (Binder), MCC (Diluent), Magnesium stearate (Lubricant), Talc (Glidant). The core tablet excipients were accurately weighed, mixed and passed through the No. #60(250µm) mesh screen to ensure complete mixing. Core mini-tablets compressed by direct compression method using 3 mm round, flat, plane punches on 16 station tablet punching machine (Cadmach, India).

Table 1: Composition of salbutamol core mini tablets.

Ingredients Name	C1	C2	C3	C4
Salbutamol Sulphate	2	2	2	2
Sodium starch glycolate	2.5	5	7.5	10
PVP K30	1	1	1	1
Micro crystalline cellulose(PH-102)	18.75	16.25	13.75	11.25
Magnesium stearate	0.25	0.25	0.25	0.25
Talc	0.50	0.50	0.50	0.50
Total Weight	25mg	25mg	25mg	25mg

Preparation of compression coated mini-tablets

The produced core mini-tablets from the previous part were subjected to compression coating is shown in table 2. All the ingredients of each coating layer were accurately weight, taken into a motor and pestle by passing through a No. #40 mesh screen, mixed manually for 5 min. Then the blend was granulated with starch paste (10%) as a granulating agent then mass was passed through the No. #22 mesh screen, dried in a hot air oven at 50ºC for 2 hr, and dried granules passed through the No. #44 mesh screen. Finally talc, magnesium stearate was added to the sieved granules and mix it for about 5 min in a poly bag. Compression coating of tablets was performed by using 8mm round, flat, plane punches on 16 station tablet punching machine (Cadmach, India). Half amount required for the coat was placed in the die then two core mini-tablets are carefully positioned in the center of the die and then the other half was added, the powder were compressed around the core mini-tablets using constant compression force 5kg/cm^2.

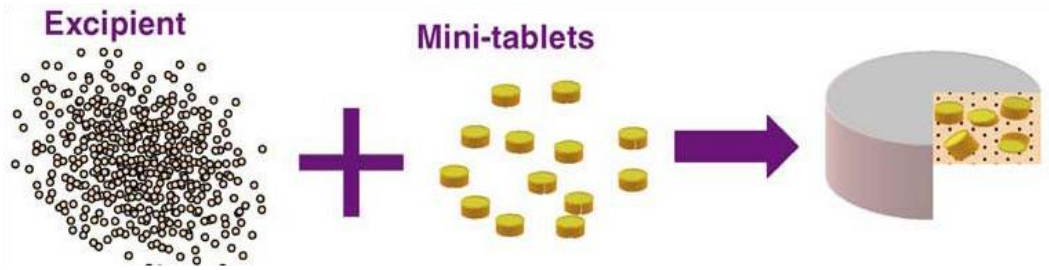


Fig. 1: Epitomization of mini-tablets.

Table 2: Composition of compression coated salbutamol core mini-tablets

Formulation	Guar gum	Xanthum gum	Chitosan	HPMC K15M	PVP K30	Starh	MCC	Mg stearate	Talc	Coat weight	Core weight	Total weight
F1	100	10	20	-	8	20	36	2	4	200	25	250
F2	100	15	15	-	8	20	36	2	4	200	25	250
F3	100	20	10	-	8	20	36	2	4	200	25	250
F4	125	12.5	25	-	10	25	45	2.5	5	250	25	300
F5	125	18.5	18.5	-	10	25	45	2.5	5	250	25	300
F6	125	25	12.5	-	10	25	45	2.5	5	250	25	300
F7	150	15	30	-	12	30	54	3	6	300	25	350
F8	150	22.5	22.5	-	12	30	54	3	6	300	25	350
F9	150	30	15	-	12	30	54	3	6	300	25	350
F10	150	22.5	22.5	4	12	30	50	3	6	300	25	350
F11	150	22.5	22.5	8	12	30	46	3	6	300	25	350
F12	150	22.5	22.5	12	12	30	42	3	6	300	25	350
F13	150	22.5	22.5	16	12	30	38	3	6	300	25	350

Evaluation of Postcompression studies for prepared Tablets

Weight variation

For estimating weight variation, 20 tablets of each formulation were weighed using an electronic balance (Veego, India) and the test was performed according to the official test.

% Deviation = (Individual weight – Average weight / Average weight) × 100

Thickness

The thickness of the tablet was measured using a Digital Vernier Calliper (Digimatic Calliper, Japan).

Hardness

The crushing strength of ten tablets was measured using monsanto tablet hardness tester (Interlabs, Ambala, India). A tablet hardness of about 5-7 kg/cm2 is considered adequate for mechanical stability.

Friability

The friability of the tablets was determined in Roche Friabilator (Veego, India). Six tablets were weighed accurately from each batch of tablets and placed in the tumbling chamber and rotated at 25 rpm for a period of 4 min. Tablets were taken and again weighed. The percentage loss was determined by using the formula:

$$\% \text{ Friability} = [(W_1 - W_2) / W_1] \times 100$$

Where W_1 = Initial weight of 20 tablets W2 = Weight of the 20 tablets after testing

Drug content

Tablets were finely powdered and quantity of the powder equivalent to 25 mg was accurately weighed and transferred to a volumetric flask containing 50 ml of phosphate buffer (pH-6.8). The flask was shaken to solubilize the drug and the volume was made up to 100 ml with phosphate buffer. The solution was filtered through a membrane filter (0.22 μm) and analyzed for drug content using UV/Visible spectrophotometer (SL 210, ELICO, India) at 276 ηm.

Wetting time

Wetting time is closely related to the inner structure of the tablets and to the hydrophilicity of the excipient. According to the following equation proposed by Washburn E.W (1921), the water penetration rate into the powder bed is proportional to the pore radius and is affected by the hydrophilicity of the powders.

$$dl/dt = r \gamma \cos \theta / (4 \eta l)$$

Where I is the length of penetration, r is the capillary radius, γ is the surface tension, η is the liquid viscosity, t is the time, and θ is the contact angle. It is obvious that pores size becomes smaller and wetting time increases with an increase in compression force or a decrease in porosity. A linear relationship exists between wetting time and disintegration time. Thus wetting is the important step for disintegration process to take place.

The wetting time of tablet was measured by the method described by Bi et al. (1996). The method is as follows. A piece of tissue paper folded twice was placed in a small culture dish (72.39 cm^2) containing 6 ml of purified water. A tablet was placed in the centre of dish time for complete wetting is measured. The test was done in triplicate.

***In vitro* drug release studies**

Drug release studies of salbutamol core mini-tablets

The core mini-tablets containing 25mg salbutamol of were tested in different Buffer solutions SGF(pH-1.2), SIF(pH-7.4, pH-6.8) for their dissolution rates. Dissolution studies were performed using USP dissolution test apparatus (Apparatus 2, 50 rpm, 37±0.5 ^{0}C). At various time intervals, a sample of 5 ml was withdrawn and replaced with equal volume of fresh medium. The samples were analyzed spectrophotometrically at 276 ηm.

Drug release studies of compression coated salbutamol tablets

The release of salbutamol from compression coated mini-tablets was carried out using USP basket-type dissolution apparatus (Labindia DS 8000, India) at a rotation speed of 100 rpm, temperature of 37±0.5^{0}C. Thus drug release studies were conducted in simulated gastric fluid (SGF, pH 1.2) for the first 2 hr as the average gastric emptying time is about 2 hr. Then the dissolution medium was replaced with enzyme-free simulated intestinal fluid (SIF, pH 7.4) tested for drug release for 3 hr, as the average small intestinal transit time is about 3 hr, and finally enzyme-free simulated intestinal fluid (SIF, pH 6.8) was used for 19 hr to mimic colonic pH conditions. Drug release was measured from compression coated salbutamol mini-tablets; a sample of 5 ml was withdrawn and replaced with equal volume of fresh medium, analyzed spectrophotometrically at 276 ηm. All dissolution runs were performed in triplicate.

Drug release studies in presence of rat caecal contents

Preparation of rat caecal contents

The susceptibility of natural polymer coat to the enzymatic action of colonic bacteria was assessed by continuing the drug release studies in 100 ml of SIF (pH 6.8) containing 4% w/v of rat caecal contents. The caecal contents were obtained from male albino rats after pre-treatment for 7 days with natural polymer dispersion. Presence of 4% w/v rat caecal contents in SIF (pH 6.8) obtained after 7 days of pre-treatment of rats with 1 ml of 2% w/v aqueous dispersion of natural polymer provide the best conditions for *in vitro* evaluation of natural polymer.

Thirty minutes before the commencement of drug release studies, rats were killed by spinal traction. The abdomen was opened, the caecal were isolated, ligated at both ends, dissected and immediately transferred into SIF (pH 6.8), previously bubbled with CO_2. The caecal bags were opened their contents were individually weighed, pooled and then suspended in SIF (pH 6.8) to give a final caecal dilution of 4% w/v. As the caecum is naturally anaerobic, all these operations were carried out under CO_2.

Dissolution studies in artificial rat caecal contents

The in vitro drug release studies were carried out using USP dissolution rate test Apparatus 1, 100 rpm, 37^{0}C with slight modifications. A beaker (capacity 250ml) containing 100 ml of 4% rat ceacal content medium was immersed in the phosphate buffer pH 6.8 maintained in 1000-ml vessel which in turn was in the water bath of the apparatus. The tablet formulation after completing the dissolution studies in 0.1M HCl (2 hr) and Phosphate buffer pH 7.4 (3 hr) were placed in the basket of the apparatus and immersed in the rat ceacal content medium contained in 250 ml beaker. The drug release studies were carried out for 19 hr (usual colonic transit time is 20–30 hr) and 1 ml samples were taken at different time intervals without a prefilter and replaced with 1 ml of fresh SIF (pH 6.8) bubbled with CO_2. To the samples, 1 ml of ethanol was added to ensure solubility of finely suspended drug particles released due to break down of the coat by the caecal enzymes. The volume was made up to 10 ml with SIF (pH 6.8), centrifuged and the supernatant was filtered through a bacteria-proof filter and the filtrate was analyzed for salbutamol content at 276 ηm as described above. The above study was carried out on F10, F11, F12 and F13 formulations.

Control Study: The drug release studies were also conducted without pretreatment of gum in SIF (pH 6.8) by following the same experimental conditions as mentioned above.

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

X-ray imaging technique or Roentgenography was used to monitor tablet, 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 form, 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.

Three healthy human volunteers, male, with an age limit of 22-30 years and 50-70 kg body weight, were participated in *in vivo* studies. 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 natural polymer and HPMC K15 M compression coated (F12 formulation) tablets orally with 200 ml water, after an overnight fast. The tablets were visualized using X-ray. Abdominal radiographs were taken after 30 min, 3, 6, 8 and 24 h in all subjects. The volunteers were served with food; 2 h (breakfast) and 4 h (lunch) after the administration of the tablet.

Release Rate Kinetics Studies

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first order, Higuchi and Korsmeyer-Peppas release model.

The data were evaluated according to the following equations.

Zero order: $Mt = Mo + Kot$

First order: $\ln Mt = \ln Mo + K1t$

Higuchi model: $Mt = KH \sqrt{t}$

Korsmeyer –Peppas model: $Mt/Mo = Kktn$

Where, Mt is the amount of drug dissolved in time t,

Mo is the initial amount of drug, K1is the first order release constant,

K0 the zero order release constant,

KH the Higuchi rate constant,

Kk the release constant and

n is the diffusional release exponent indicative of the operating release mechanism. The value of n for a tablet, n = 0.45 for Fickian (Case I) release, > 0.45 but < 0.89 for non Fickian (anomalous) release and 0.89 for case II (zero-order) release and > 0.89 for super case II type of release.

The correlation coefficient R^2 was used as an indicator of the best fitting, for each of the models considered.

Stability studies

The stability studies were carried out according to ICH to assess the drug formulation stability. Optimized formulation was sealed in aluminum packaging laminated with polyethylene. Sample were kept at 40 ^{0}C and 75% RH for 3 months. At the end of the study period, the formulation was observed for change in physical appearance, color, drug content and drug release characteristics.

RESULTS AND DISCUSSION

The present research was aimed to develop novel colon specific chronotherapeutic drug delivery system of salbutamol for safe and effective therapy of asthma by using natural polymers, HPMC K15M by using wet granulation method as a coating material, an a active pharmaceutical agent formulated as a core mini-tablet, which is compression coated by non-interacting materials. The terms "compression coated solid dosage form" as used herein refer to a solid core mini-tablet comprising the active ingredient, which solid core mini-tablet is substantially covered with a compression coating.

The ability of compression coated tablets of salbutamol 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 2hr, phosphate buffer pH 7.4 for 3hr and continued in phosphate buffer pH 6.8 for another 19 hr to assess the ability of the compression coated tablets to release drug in the physiological environment of colon.

Analytical studies

Constructions of calibration curve

The standard graph of salbutamol in SGF (pH 1.2) showed good linearity with R^2 value of 0.999, which suggest that it obeys the "Beer – lambert" law. The standard graphs in SIF (pH 7.4) and SIF (pH 6.8) had R^2 values of 0.999 and 0.998 respectively. Standard calibration curve values were shown in Table 3. Calibration curves were shown in Figure 2, 3, and 4.

Table 3: Standard graph of salbutamol in SGF and SIF.

Concentration (µg/ml)	SGF (0.1N HCL)	SIF (pH 7.4)	SIF (pH 6.8)
0	0	0	0
10	0.164	0.160	0.140
20	0.309	0.321	0.292
30	0.470	0.477	0.458
40	0.624	0.647	0.628
50	0.789	0.804	0.799

SGF = simulated gastric fluid; SIF = simulated intestinal fluid

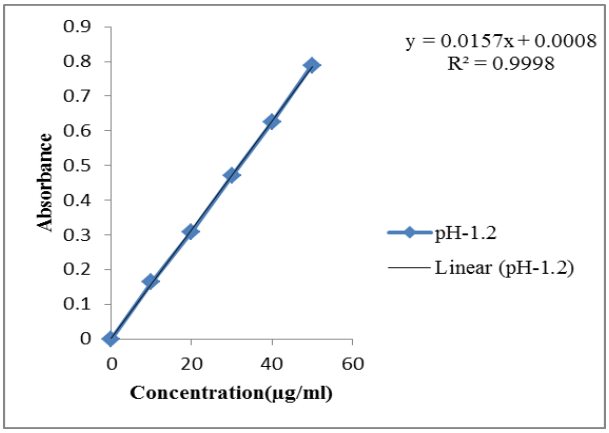


Fig. 2: Standard graph of salbutamol in SGF (0.1N HCL).

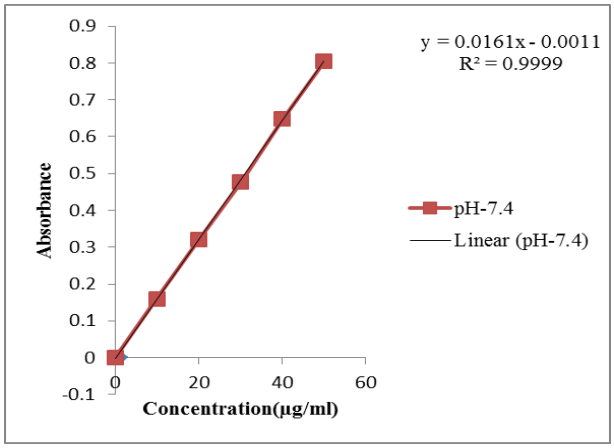


Fig. 3: Standard graph of salbutamol in SIF (pH 7.4).

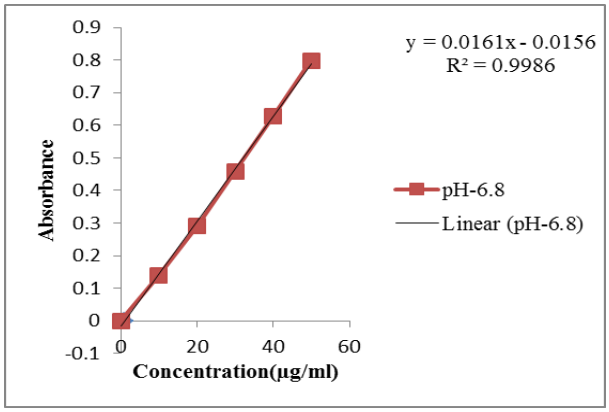


Fig. 4: Standard graph of salbutamol in SIF (pH 6.8).

Evaluation of Precompression studies

The powder mixtures of different formulations were evaluated for angle of repose, bulk density, compressibility index, Hausners Ratio and their values were shown in Table 4.

Table 4: Characterization of powder mixture.

Formulation code	Angle of Repose(ºC)	Bulk density	Tapped bulk density	Compressibility Index (%)	Hausners Ratio
Core	26.15±1.21	0.429	0.469	8.53	1.09
F1	26.54±1.34	0.435	0.462	5.84	1.06
F2	25.18±1.17	0.436	0.478	8.79	1.10
F3	26.74±1.12	0.444	0.466	4.72	1.05
F4	27.14±1.23	0.421	0.491	14.26	1.17
F5	27.19±1.56	0.436	0.487	10.47	1.12
F6	28.64±1.39	0.425	0.472	9.96	1.11
F7	27.02±1.14	0.421	0.468	10.04	1.11
F8	26.65±1.53	0.435	0.484	10.12	1.11
F9	25.16±1.98	0.427	0.472	9.53	1.11
F10	26.65±1.39	0.432	0.465	7.10	1.08
F11	25.97±1.43	0.423	0.474	10.76	1.12
F12	26.14±1.34	0.437	0.468	6.62	1.07
F13	25.19±1.98	0.431	0.466	7.10	1.08

The apparent bulk density and tapped bulk density values ranged from 0.421 to 0.444 and 0.465 to 0.491 respectively. The results of angle of repose, compressibility index (%) and Hausner ratio ranged from 25.16±1.98 to 27.02±1.14, 5.84 to 14.26 and 1.05 to 1.17. The results of angle of repose (<35), compressibility index (<15) and Hausner ratio (<1.25) indicates good flow properties of the powder mixture.

Evaluation of Postcompression studies

Physical characterization of salbutamol core mini-tablets

The rapidly disintegrating salbutamol core mini tablets were prepared by taking different proportions of superdisintegrant were shown in table 1 by direct compression technique, the physical parameters for the mini-tablet formulations were within the limits. Compression force approximately 2.64±0.16kg/cm2 hardness, 2.19±0.07mm thickness, friability (<1) and showed 100.05% of labeled amount of drug indicating uniformity of drug content, disintegrates within 58sec showing the required fast disintegration characteristics.

Wetting time

Wetting time is closely related to the inner structure of the tablet and to the hydrophilicity of the excipients. Wetting time for all the tablets was found in the range of 18.33 ± 0.58 (C2) sec.

Physical characterization of salbutamol compression coated Tablets

The compression-coated tablet formulations were prepared according to the formula table 2; different formulations were subjected to various evaluation tests such as uniformity of weight, drug content, hardness, friability and *in vitro* dissolution.

Table 5: Physical properties of salbutamol core and compression coated tablets.

Formulation Code	Hardness (Kg/cm^2)	Deviation in Weight variation (mg)	Thickness of Tablets(mm)	Friability (%)	Drug Content (%)
Core	2.64±0.16	25.9±1.61	2.19±0.07	0.55	100.05
F1	4.61±0.18	250.82±0.57	4.12±0.16	0.46	99.94
F2	4.68±0.22	250.60±0.74	4.08±0.20	0.52	99.26
F3	4.49±0.32	250.61±0.21	4.08±0.11	0.48	100.2
F4	5.22±0.17	300.98±0.34	4.20±0.11	0.49	98.58
F5	5.27±0.14	300.87±0.40	4.18±0.10	0.61	99.45
F6	5.21±0.08	301.21±0.60	4.17±0.09	0.59	100.01
F7	5.55±0.09	350.85±0.43	4.24±0.12	0.45	100.98
F8	5.71±0.18	350.51±0.23	4.20±0.21	0.52	101.01
F9	5.51±0.16	351.18±0.51	4.28±0.06	0.53	99.98
F10	5.60±0.03	350.84±0.70	4.39±0.27	0.45	99.85
F11	5.41±0.19	351.04±0.58	4.21±0.14	0.60	99.87
F12	5.50±0.51	350.45±0.04	4.29±0.16	0.46	101.23
F13	5.30±0.03	350.04±0.58	4.28±0.06	0.48	100.01

Data represents mean ± SD, n = 3

The physical properties of compression coated tablets are given in Table 5. Weight variation was found to be 250.60±0.74 to 351.18±0.51, Pharmacopoeial limit for the percentage deviation for the tablets of more than 250 mg is ± 5%, Thickness of the coat of compression-coated salbutamol tablets 250, 300, 350 mg coat weights over the core tablets (diameter 8 mm) was measured using a digital caliper, mean thickness of the compression coated tablets was found to be 4.08±0.11 to 4.39±0.27, friability lose less than 1%, hardness value in the range of 4.49±0.32 to 5.71±0.18 kg/cm^2. It was found that crushing strength of compression coated tablets was dependent on amount of guar gum, xanthan gum, chitosan, HPMC polymers. When HPMC in polymer mixture increased the crushing strength of coated tablets increased (India Pharmacopoeia, 1996).

Drug release studies of compression coated salbutamol mini-tablets

The prepared salbutamol compression coated mini-tablets were subjected to in-vitro dissolution testing to identify a suitable formulation which immediately release solbutamol after lag time (<10%) if minimum 5hr. So, we prepared nine formulations of compression coated mini-tablets of different coat weights (200, 250, 300 mg) with different ratios of natural polymers (Guar gum 50%, Xanthan gum: Chitosan-5:10, 7.5:7.5, 10:5 %) as shown in the table 2. F1 to F6 (200, 250 mg coat) fail to protect the drug release in the upper regions of gastro intestinal tract (figure 5, 6), Cumulative amount of drug released from 300mg coat weight contains F7, F8, F9 was found to be 4 to 10% in SGF (2hr), 10 to 14% in SGF (5hr), 26 to 27% in SIF (24hr) as shown in the table 5. It was found that as the concentration of gum polymer was increasing, the release rate of salbutamol from compression coated mini-tablets was decreasing. This is due to hydrophilic nature of gum polymer and its rate of hydration has increased by the rise its concentration resulting in decreased dissolution rate. After that we prepared from the optimized formulation (F8) of F10, F11, F12 and F13 with time dependent polymer (HPMC K15M) in the different ratios 2%, 4%, 6%, 8% of four formulations further retard the drug release. Cumulative percent of drug release from the above formulations was found to vary from 0 to 2% in SGF (2hr), 10 to 14% in SGF (5hr), 25 to 27% in SIF (24hr) respectively.

Thus the F1 to F9 were not studied further in rat caecal contents. Even though F10, F11, F12 and F13 formulation releasing small amount of drug after 24 hr, it was further studied in 4% caecal contents to know the effect of coat thickness (300 mg coat weight) compared with 2% caecal contents.

Table 5: Cumulative percentage drug release of F1-F13 with different coat weights.

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	14.55	11.10	12.68	8.25	5.10	4.80	3.23	2.10	1.20	0	0	0	0
2	18.98	17.33	17.63	12.38	12.68	8.85	5.40	3.00	2.18	1.20	1.43	0	0
3	31.57	29.81	30.52	23.41	17.51	12.09	10.13	6.75	4.29	3.94	2.33	0.90	1.80
4	35.44	33.05	34.88	30.09	22.92	16.38	12.59	10.27	5.63	9.91	9.84	5.84	7.03
5	39.59	34.31	38.32	36.49	23.77	21.52	14.13	11.39	10.48	14.20	12.80	10.83	11.81
6	58.15	49.57	52.73	49.85	40.08	37.05	22.57	22.99	22.50	22.22	22.57	21.23	21.23
8	61.10	56.11	58.36	51.26	42.33	40.92	23.34	24.26	24.05	23.13	24.05	22.99	23.34
10	62.23	62.30	61.52	57.45	46.34	44.09	24.54	24.68	25.17	24.40	24.33	23.84	24.40
12	72.42	69.47	67.57	58.22	49.08	45.77	25.52	25.31	25.66	25.03	25.10	25.17	25.52
24	82.20	78.75	71.72	59.34	50.55	47.11	27.14	26.23	26.44	26.93	26.09	25.66	25.24

Data represents mean ± SD, n = 3

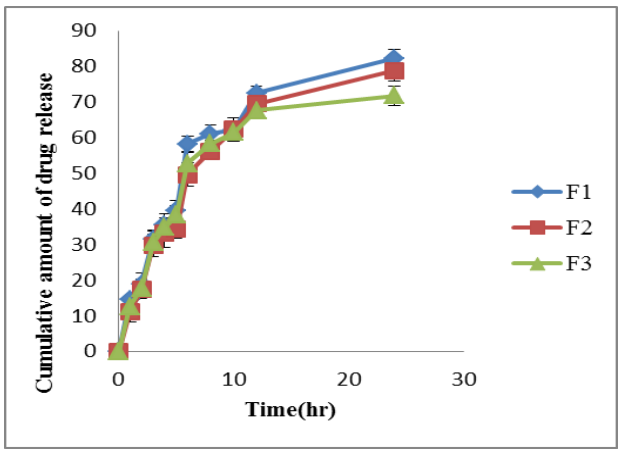


Fig. 5: Dissolution profiles of F1-F3 formulations containing 200 mg coat weight.

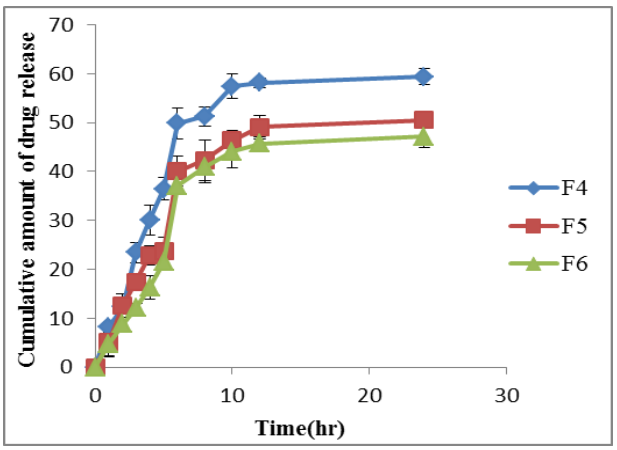


Fig. 6: Dissolution profiles of F4-F6 formulations containing 250 mg coat weight.

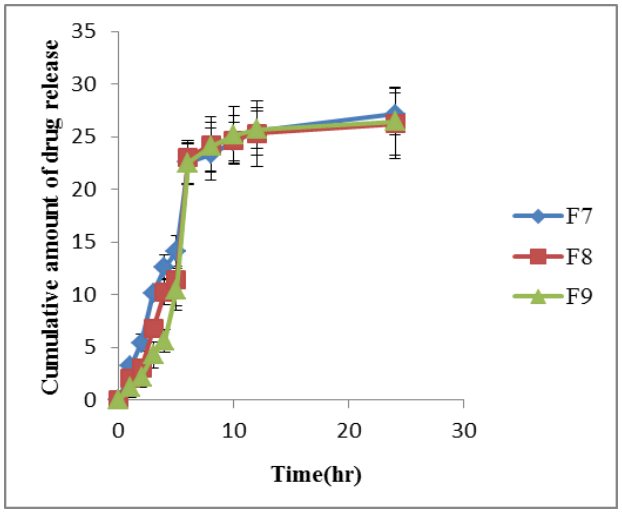


Fig. 7: Dissolution profiles of F7-F9 formulations containing 300 mg coat weight.

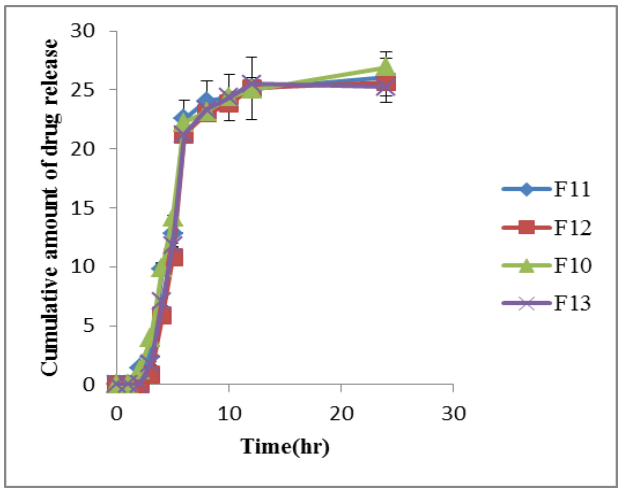


Fig. 8: Dissolution profiles of F10-F13 formulations containing combination of Natural polymers and HPMC K15M 300 mg coat weight.

Drug release studies in artificial rat caecal contents

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, the in-vitro drug release studies were carried out in pH 6.8 Phosphate buffer containing 4% and 2% w/v of rat caecal contents.

The presence of rat caecal contents in the dissolution medium resulted in improved drug release after 5hr when compared to control. The percent drug released after 24h of testing was 60 to 70% with 2%w/v caecal contents whereas it was only 25 to 27% in the absence of caecal contents indicating that polysaccharide are present in the caecal matter that metabolize polymers was shown in Figure 10. As complete drug release was not achieved with 2%w/v. Hence, the level of caecal matter in the dissolution medium was increased to 4%w/v and the percent drug released after 24h of testing was found to be 100%.

The amount of drug released in the study was found to increase with an increase in the quantity of rat caecal contents in the dissolution medium. Drug release studies were also conducted in 2%w/v rat caecal contents without natural polymer gum (Guar gum, Xanthan gum, Chitosan) pre-treatment and were compared with 2%w/v caecal contents which followed pre-treatment of natural polymer gum for 7 days. The percent drug released after 24h testing was 65 to 68% without pre-treatment and was 101 % with pre-treatment of natural polymer gum was shown in Figure 10. This is because of absence of gum for metabolism with the caecal enzymes of colonic bacteria.

In-vitro drug release studies and in-vivo studies using the formulation F12 clearly indicated that the combination of natural polymers as a coat material applied over core mini-tablets was capable of protecting the drug from being released in the physiological environment of stomach, small intestine and susceptible to colonic bacterial enzymatic action with resultant drug release in the colon. Thus, the study clearly indicated that the combination of natural polymers was a potential colon specific drug delivery carrier.

Table 6: Cumulative percentage drug release of F10, F11, F12 and F13 formulations in presence of 2%, 4% rat caecal contents and Control.

Time (hr)	2 % rat caecal contents				4 % Rat caecal Contents				Control			
	F10	F11	F12	F13	F10	F11	F12	F13	F10	F11	F12	F13
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
2	1.05	1.65	0	0	1.35	1.35	0	0	1.35	1.35	1.35	1.35
3	3.73	2.55	1.20	1.95	5.01	3.58	1.80	2.40	4.13	4.05	5.01	4.28
4	6.61	6.96	10.83	11.18	8.10	9.15	10.76	11.25	12.11	13.09	8.03	9.01
5	9.63	11.11	12.16	12.38	11.48	11.83	14.06	11.93	14.36	15.55	10.98	12.88
6	25.10	24.54	27.21	27.98	32.78	33.90	36.84	21.00	35.59	38.40	34.82	37.49
8	34.10	30.66	32.48	32.70	40.87	43.26	49.92	32.40	46.21	48.53	40.30	43.82
10	38.32	41.34	44.58	43.45	47.55	50.50	68.77	47.63	53.52	55.77	49.65	53.24
12	45.42	53.65	59.77	54.01	59.08	61.61	83.04	67.13	57.67	59.71	56.12	59.43
24	60.33	65.04	70.17	67.01	75.53	81.15	101.39	85.73	66.04	68.57	65.69	66.53

Data represents mean ± SD, n = 3

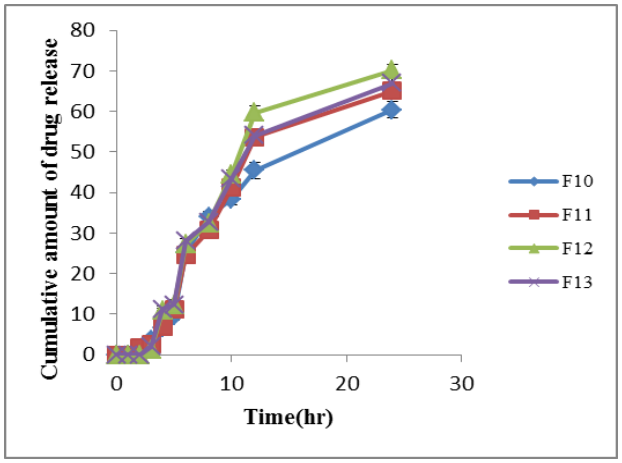


Fig. 9: Dissolution profiles of F10, F11, F12 and F13 formulations in presence of 2%rat caecal contents.

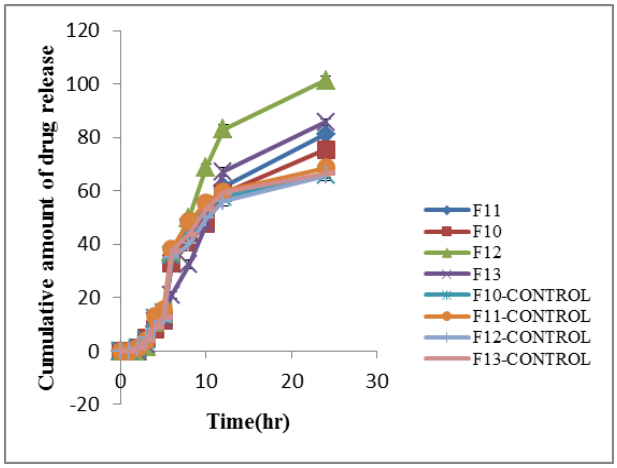
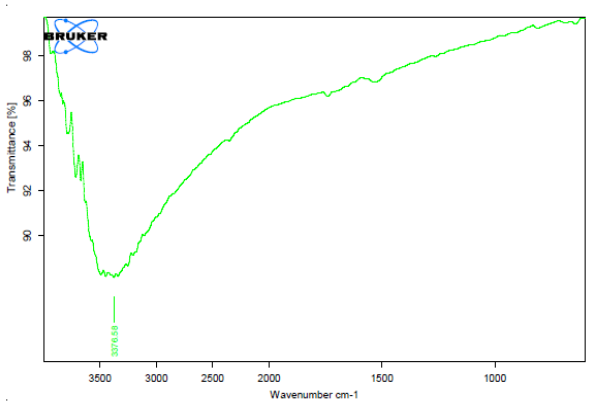


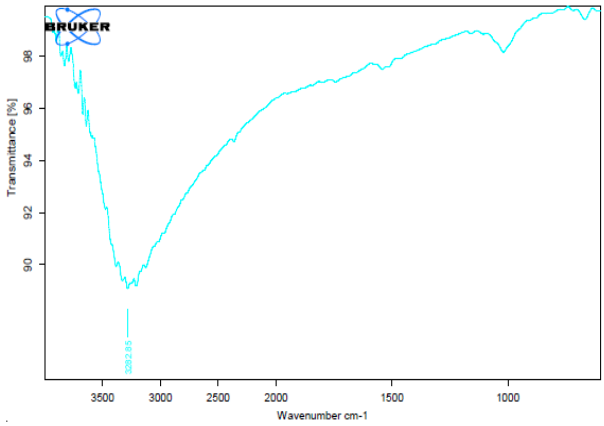
Fig. 10: Dissolution profiles of F10, F11, F12 and F13 formulations in presence of 4%rat caecal contents vs control.

Fourier Transforms Spectroscopy Studies

The FT-IR spectrum of pure drug salbutamol sulphate and the physical mixture of formulation F12 showed the characteristic absorption peaks in the IR region with negligible variation in the band position of different functional groups and various bonds in comparison to FT-IR of pure drug. As there was no much variation in the nature of IR spectrum, it was concluded that there was no interaction of the drug with the excipients used for the study and the drug retained its identity without undergoing any type of interaction with the excipients. The FT-IR spectrums are given in Figure 11.



a) Salbutamol pure drug.



b) Physical mixture optimized formulation (F12).

Fig. 11: FT-IR Spectra's of salbutamol pure drug and Physical mixture optimized formulation (F12).

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

X-ray studies were carried out on the F12 formulation tablets, in order to see the compression coated tablets throughout the GI system. Barium sulphate was used as the marker. The position of the tablets in the body was monitored at different time points. The abdominal radiographs showed that, the tablets remained intact in the stomach in all subjects. The transit time of the tablets throughout the GI system was variable. The position of tablets at different time points is shown in Table 7 and the X-ray images of tablet throughout the GI system are shown in Figure 13.

The in-vivo results showed that the tablets (F12 formulation) reached the colon without disintegrating in the upper region of the GI system in all subjects. From the abdominal radiographs at different time points, tablets entered the colon varying between 5-6 h for all volunteers after tablet administration. The X-ray images showed that the tablets slowly disintegrated throughout the colon after reaching it.

Table 7: The position of the tablets throughout the GI tract at physical certain time points.

Subjects	30 min	3h	6h	8h	24h
1	Stomach	Caecum	Ascending colon	Ascending colon	Not observed

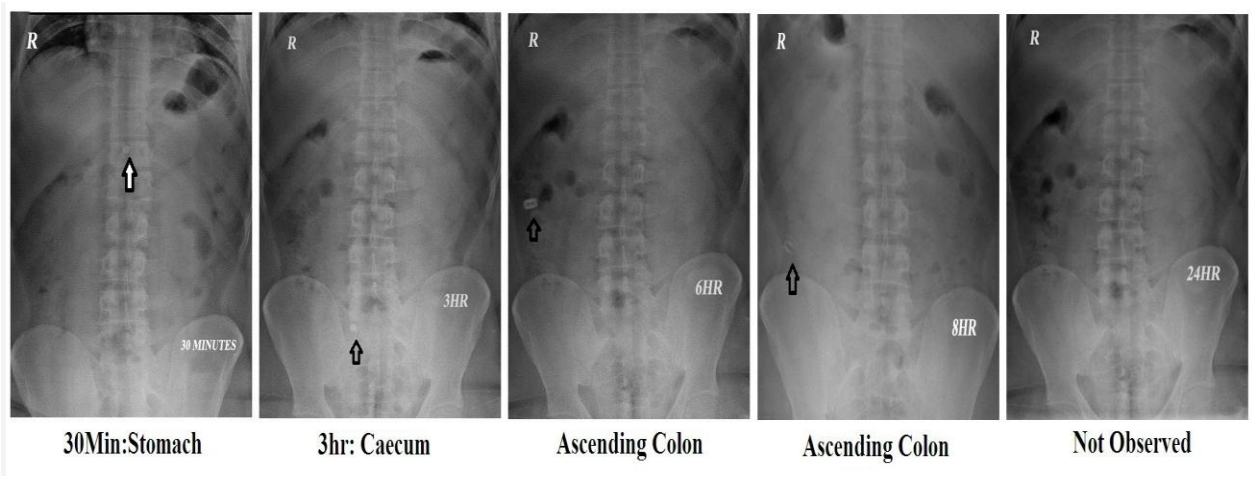


Fig. 13: The localization of the tablet in the gastrointestinal tract in subject.

Release Rate Kinetics Studies

The mechanism and kinetics of drug release of salbutamol is determined by the application of korsmeyer-peppas model, higuchi's model, zero order and first order kinetics as shown in Table 8. Since they fitted well with Korsmeyer–Peppas models as their R^2 values in the range of 0.843–0.895 with n value above 1.

Table 8: Drug release kinetics.

Formulation code	Zero order	First order	Higuchi	Korsmeyer & Peppas	Peppas (n)
F12	$R^2 = 0.865$	$R^2 = 0.631$	$R^2 = 0.843$	$R^2 = 0.895$	1.231

Stability studies

The stability studies indicated that after storing the optimized formulation (F12) for 3 months at 40°c and 75% RH, the percent degradation of the drug was 1 to 2 %, indicating stability of the formulation.

CONCLUSION

It was concluded that formulation F12 was better as it passed all specifications in limits for angle of repose, hardness, friability, content uniformity tests as well as weight variation test. The core mini-tablet are compression-coated with combination of natural polymers(Guar gum, Xanthan gum, Chitosan) and HPMC K15M as a coating material, released less than 2% of drug in the physiological environment of upper GI tract and released 100% of the drug in the target area i.e. physiological environment of colon. The in-vitro drug release studies, in-vivo x-ray studies indicated that formulation F12 was a promising system to provide targeting of salbutamol to the colon and release pattern of the above formulations was best fitted to all the possible kinetic models. The presence of combination of natural polymers in the coat reduces the initial swelling of HPMC K15M which retards the drug release in physiological environment of upper part of gastrointestinal tract and ensures complete release of drug in the colon due to its microbial degradation. FT-IR spectral studies showed that there is no interaction between the drug and the excipients. Thus the tablets containing optimum proportion of natural polymers and HPMC K15M is most likely to target salbutamol to the colon without being released significantly in stomach and small intestine.

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