DEVELOPMENT OF BIORESPONSIVE CHRONOPHARMACEUTICAL DRUG DELIVERY SYSTEM IN TREATMENT OF ASTHMA & COPD USING COLON TARGETED MUCOADHESIVE TABLETS IN CAPSULE SYSTEM

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Submitted by

KRISHNASWAMY R

261510256

Under the guidance of

Dr. V KAMALAKKANNAN M.Pharm, PhD.,

Department of pharmaceutics

J.K.K. NATTRAJA COLLEGE OF PHARMACY

KUMARAPALAYAM– 638 183

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EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **“ DEVELOPMENT OF BIORESPONSIVE CHRONOPHARMACEUTICAL DRUG DELIVERY SYSTEM IN TREATMENT OF ASTHMA & COPD USING COLON TARGETED MUCOADHESIVE TABLETS IN CAPSULE SYSTEM ”**, submitted by the Student bearing **Reg.no: 261510256** to **“ The Tamil Nadu Dr. M.G.R. Medical university – Chennai ”**, in partial fulfilment for the award of Degree of **Master of Pharmacy in Pharmaceutics** was evaluated by us during the examination held on ……..

Internal Examiner **External Examiner**

CERTIFICATE

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Place : Kumarapalayam

Date :

Dr. R. Sambathkumar M.pharm., phD.,

Professor & Principal,

Department of Pharmaceutics,

J.K.K.Nattraja college of Pharmacy,

Kumarapalayam – 638 183.

CERTIFICATE

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Place : Kumarapalayam

Date :

Dr. V. Kamalakkannan, M.pharm., phD.,

Associate Professor,

J.K.K.Nattraja college of pharmacy,

Kumarapalayam – 638183.

DECLARATION

I do hereby declare that the dissertation **"DEVELOPMENT OF BIORESPONSIVE CHRONOPHARMACEUTICAL DRUG DELIVERY SYSTEM IN TREATMENT OF ASTHMA & COPD USING COLON TARGETED MUCOADHESIVE TABLETS IN CAPSULE SYSTEM"**, submitted to **"The Tamil Nadu Dr.M.G.R. Medical university – Chennai"**, in partial fulfillment and requirement of university rules and regulation for the award of Degree of **Master of Pharmacy in Pharmaceutics**, is a bonafide research work has been carried out by me during the academic year 2016-2017, under the guidance and supervision of **Dr. V. Kamalakkannan, M.pharm., phD.,** Associate professor, Department of Pharmaceutics, J.K.K. Nattraja college of pharmacy, kumarapalayam.

I further declare that this research work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

Place : Kumarapalayam **KRISHNASWAMY R**

Date : **Reg.no: 261510256**

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KRISHNASWAMY R

Reg.No: 261510256

ABBREVIATIONS

S.no	Abbrevations	Meaning
1	ChrDDS	Chronopharmaceutical drug delivery system
2	CR	Controlled release
3	SR	Sustained release
4	ER	Extended release
5	IPEC	Interpolyelectrolyte complex
6	PEC	Polyelectrolyte complex
7	Mcc ph 102	Microcrystalline cellulose pH 102
8	HPMC	Hydroxypropyl methyl cellulose
9	SS	Salbutamol sulphate
10	pm	Physical mixture
11	CP	Carbopol 971 P
12	EE	Eudragit E po
13	EL	Eudragit L 100
14	SI	Swelling index
15	CI	Compressibility index
16	TD	Tapped density
17	BD	Bulk density
18	HR	Hausner ratio
19	kPa	kilopascals

CONTENTS

INTRODUCTION:

CONCEPT OF CHRONOPHARMACEUTICS

Chronopharmaceutics is a branch of pharmaceutics dealing with design and evaluation of drug delivery system that delivers the therapeutic agent in rhythm that ideally matches the therapeutic need of the biological system [1].

The biological systems require varying concentrations of drug within the circadian cycle at specified time. Hence, existence of such delivery system is necessary to match the circadian rhythm associated with certain diseases. This can be achieved with Chronotherapeutic drug delivery system. These systems deliver the required dose at time when it is needed [2].

The biological clock (24 hours rhythmic cycle) within our body drives various physiological body functions and pathophysiology of certain diseases. These diseases show up their symptoms at a peak level during a particular time period in a day. Such rhythms associated with disease symptoms are observed in bronchial asthma, ulcer, rheumatoid disease, hypertension and hyper cholesterolemia etc [3].

The possibility of deferring the drug release pattern within the system according to rhythmicity of diseases is a promising tool to treat certain diseases that emerge in circardian rhythm fashion.

Need for chronopharmaceutics drug delivery system (ChrDDS) [4]:

1.When possible variations in pharmacokinetic of drug responsible for time dependent variations in drug effects (e.g. some antimicrobial agents)

2.When drugs have a narrow therapeutic range.

3.When symptoms of certain diseases are circadian phase-dependent (e.g. Asthma, Angina pectoris, myocardial infarction).

Hurdles of ChrDDS:

1. Rhythmic bio-responsive materials and system design.

2. Rhythm delivery system engineering and modeling.

3. Regulatory guidance related to these types of modified dosage forms.

CHRONOMODULATED DRUG DELIVERY SYSTEMS [5-7] :

1. **Pulsatile drug delivery system:**

This system releases the drug suddenly after a well-defined lag time according to the circadian rhythm of diseases. Drug is not released from the device within this lag time. This method is advantageous for drugs with extensive first pass metabolism and those which can be targeted to specific sites in the intestinal tract. Thus by developing a pulsatile release system colonic drug delivery, the plasma peak can be obtained at specified time.

2. **Enteric-coated system**:

This system contains a core which is film coated with polymers such as HPMC and gastro-resistant polymer (Eudragit). In this system the lag time can be controlled by thickness of the coating layer. The disadvantage of this system is the unpredictability of gastric residence.

3. **Osmotic system**:

This system depends on osmotic pressure as a driving force for the pulsatile drug delivery. It consists of a semi-permeable membrane around the core containing osmotically active drug or a drug combined with an osmotic agent. The delivery orifice is drilled into the system with the help of laser. Lag time of 1-10 hours can be achieved based on the thickness, orifice diameter and concentration of osmotic agent.eg. port system.

4. **Swelling and erodible system**:

In this system the drug core is surrounded by polymeric barrier that swells and dissolves to release the drug after the lag time. The lag time of the system can be controlled by altering the thickness of the polymeric coating and its viscosity.

5. **Press coated system:**

It involves direct compression of coating layer over the core. The limitation of this system is that central positioning of the core within the coat cannot be assured. The lag time of this system can be adjusted by coating the tablet with semi-permeable polymer.

6. **Pulsincap:**

This system comprises a water insoluble capsule body enclosing the drug reservoir. The capsule is closed at one end with swellable hydrogel plug. When this capsule comes in contact with water it swells and pushes the plug after a lag time to release the drug. Rapid release of drugs can be achieved by inclusion of effervescent agents, super disintegrants and osmotic agent.

7. **Ultrasound drug delivery system**:

This system utilizes ultrasound effect that enhances the degradation of polymer in which the drug is incorporated. The drug released can be achieved by application of ultrasound.

8. **Multiparticulate system**:

The active agent is coated onto non-peril sugar pellets followed by coating with swellable polymer layer. The swelling agents may include super disintegrant, osmotic agent etc. upon ingress of water, the swellable layer swells and rupture the film resulting in rapid drug release.

CHRONOPHARMACEUTICAL TECHNOLOGIES:

1. **CONTIN technology**:

The Complex formed between the cellulose polymer and non polar solid aliphatic alcohol acts as a matrix system. This technology is used for more effective control of disease by reducing side effects. Eg: aminophylline, morphine etc [8,9] .

2. **CODAS technology**:

The chronotherapeutic oral drug absorption system (CODAS) is a multi-particulate system designed for bedtime drug dosing, with 4-5 h delay in drug delivery coated with non-enteric release-controlling polymer applied to drug loaded pellets. E.g. CODAS-verapamil extended release capsules [10,11] .

3. **CEFORM technology**:

This approach is based on meltspining which produces uniform shaped microspheres of pharmaceutical compounds. This technology is used to develop cardizemR LA, a one day diltiazem formulation based on ChrDDS [12,13].

4. **OROS technology**:

This delivery system reproducibly delivers a bolus drug dose within a specified time and in site specific manner to the gastrointestinal tract. This osmosis-based system is generally used in the designing of extended release tablet [14,15].

5. **DIFFUCAPS technology**:

This technology involves drug delivery from a capsule system in a circadian release fashion. It is a multiparticulate technology for chronotherapeutic delivery of combination of two drugs. This technology has been used to formulate propranolol containing ChrDDS for the management of hypertension (first & recently FDA approved) [16,17].

6. **EGALET**:

It is a delayed release dosage form consisting of impermeable shell with two lag plugs, enclosing a plug of active drug in the middle of its units.

7. **GEOCLOCK**:

The system is designed based on the concept of geomatrix technology. The active core is coated partially over the bases. Upon erosion, the surface of the active core is exposed with increasing time to the outer environment, which helps drug release [18].

8. **TIMERx**:

It is a hydrogel-based controlled drug delivery device. The drug release is controlled by rate of water penetration into the TIMERx gum matrix from the gastrointestinal tract. The system expands to form a gel and release the drug[19].

9. ***PORT® technology****:*

The Programmable Oral Release Technologies (PORT) system is a unique encapsulated system that provides multiple programmed release of drug [20]. It contains of polymeric core or may be a capsule which is coated with a semi-permeable, rate-controlling polymer. A blend of active medicament and osmotic agent is plugged inside the capsule shell and sealed with water insoluble plug. Immediate release compartment can also be incorporated based on the disease profile.

DISEASES WITH ESTABLISHED CIRCADIAN RHYTHMS IN THEIR PATHOGENESIS

There diseases which are influenced by biological rhythms require the development of chronopharmaceutical drug delivery system than conventional drug administration [21].

These include asthma, cancer, cardiovascular diseases, diabetes, etc.

Bronchial asthma:

It is characterized by airway inflammation which results in hyper responsiveness of lower respiratory tract in accordance with various environmental stimuli [22]. Airway resistance increases progressively at night in asthmatic subjects. Nocturnal asthma is an exacerbation of asthma at night with increase in symptoms, airway responsiveness

and lung function [23]. Antigen provokes the release of pro-inflammatory mediators from mast cells and eosinophils resulting in exacerbation of inflammation, smooth muscle bronchospasm and contraction. This can be best matched by targeting with chronotherapy because bronco constriction and exacerbation of symptoms vary on circadian fashion.

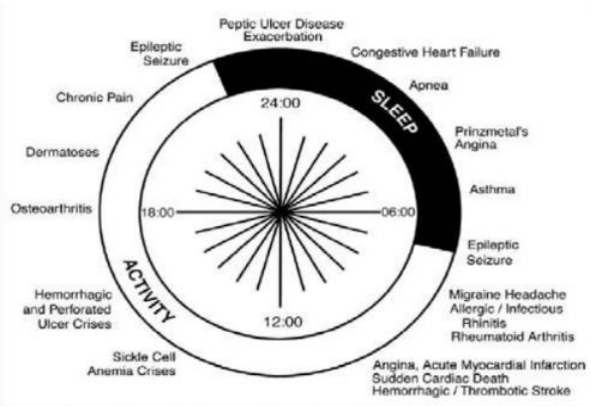


Figure 1: Circadian rhythm

***Arthritis**:*

Patients with rheumatoid arthritis, have pain that peaks in morning hours and decreases throughout the day. Circadian rhythm in the plasma concentration of c-reactive proteins and interlukin-6 has been reported [24]. Chronotherapy of arthritis should be timed to ensure that blood levels of the drugs should coincide with peak pain.

***Allergic rhinitis** [25]:*

The symptoms were found to occur most frequently in morning. The phases of allergic rhinitis include, early phase (developing within minutes) and late phase (manifesting after 12-16 h). The early phase occurs due to release of histamine, cytokines, prostaglandins, TNF-α, chemotactic factors etc. The late phase is due to distribution, adhesion and infiltration of circulating leukocytes, eosinophils, T cells causing nasal congestion, exacerbation of inflammation of upper airways.

Duodenal ulcer:

Generally gastric acid secretion is highest in the evening in subjects with duodenal ulcer and decreases in the early morning [26,27].A circadian rhythm has been found and duodenal perforations showed highest incidence in afternoon, which showed

a major peak around noon and secondary peak near midnight. Circadian pattern was characterized by 6-month rhythm, which significantly shows higher risk in May-June-July [28].

Epilepsy:

The circadian rhythm may also take part in epilepsy [29].The influence of the biological clock on seizure has been found in some experimental animal models. The methodology for measurement of the circadian rhythm in humans is also investigated. Behavioral chronobiology provides the detection of probable new regulation process concerning the central mechanisms of epilepsy [30].

Cardiovascular diseases*:*

Cardiovascular diseases involving several factors such as blood pressure, heart rate, stroke volume, cardiac output and blood flow were subject to circadian rhythms. capillary resistance and vascular reactivity are higher in morning whereas decrease later in the day; Platelet agreeability is increased and fibrinolytic activity is decreased in the morning, leading to a state of relative hypercoagulability of the blood [31-33].It was postulated that modification of these circadian triggers by pharmacologic agents may lead to the prevention of adverse cardiac events [34].

The circadian pattern of blood pressure is at its lowest during the sleep cycle and rises steeply during the early morning awakening period. Most patients with essential hypertension have a similar circadian rhythm of Blood pressure [35].

Diabetes:

In type I diabetes, circadian rhythms in insulin requirement and release involves pulsatile fashion.[36] Insulin release shows cyclic rhythmicity both in stimulated and inhibitory fashion based on requirement which induce a secondary feed-back signal on insulin release which can help to maintain blood glucose levels. The modulators of insulin release and action are secreted in a circadian fashion and secondarily impress the mode of insulin release.

CHRONOTHERAPY IN ASTHMA

In nocturnal asthma, the delivery of the drugs which could address the progressive increase in airway resistance during early morning hours could offer better symptom control. In chronopharmacotherapy of asthma drug administration is synchronized with circadian rhythms. If the symptoms occur at daytime a conventional dosage form can be administrated. If symptoms of the disease worse during night or in early morning hours the timing of drug delivery should be synchronized in such fashion which provides better disease control [37].

Most asthma attacks occur at 04:00 to 06:00 hours. Nocturnal asthma is a complex interaction of several coincident circadian rhythms e.g. secretion of hydrocortisone and adrenalin.

Enteric-coated formulations are used for site-specific delivery; they can also be used in time-controlled delivery systems when there is a necessity for lag time. Bogin and Ballard (1992) have successfully used salbutamol formulations for the treatment of nocturnal asthma. The polymers which dissolves in intestinal pH 6 were used [38].

Development of chronotherapy for nocturnal asthma, using theophylline, inhaled corticosteroid, inhaled anticholinergic agent and beta 2-agonist according to biological rhythm to maximize pharmacological effects and minimize side effects. The circadian rhythm of biological system is important in understanding the rhythmicity in lung function of asthmatics at night [39].

The circadian rhythm in peak expiratory flow (PEF) was altered according to severity of asthma occurring in midnight and early morning. A chronotherapy of evening dose of theophylline can be used for nocturnal asthma attacks which could effectively improve the values of PEF and symptoms responsible for nocturnal asthmatics [39].

Investigation of chronotherapeutically optimized, sustained-release theophylline formulation administered once daily in the evening at 8:00 P.M. is compared with a

conventional sustained-release theophylline administered twice daily at 8:00 A.M. and at 8:00 P.M. The improvement in PEF and FEV1 in once daily dose based on chronotherapy showed better symptom control than conventional sustained release formulation. Once-daily evening theophylline chronotherapy meets these goals, providing rising blood levels at night and in the early morning [40].

The theophylline levels remained practically constant for 24 hours under conventional theophylline treatment with twice-daily administration. In contrast, the variations of the theophylline serum levels and the night levels were higher after once-daily dosage of Euphylong providing better symptom control [40].

Timed administration of once-daily theophylline drug might provide maximum blood levels when needed and helps to stabilize 24-hour airflow. Chronotherapeutic potential of single-daily evening doses of a controlled-release theophylline preparation (Uniphyl 400-mg tablets) in nocturnal asthma was investigated. Nighttime blood concentrations with this regimen were higher compared to Theo-Dur tablets, B.I.D., in the same total daily doses or with once-daily morning Uniphyl administration.
In fed and fasted subjects, evening administration of Uniphyl 400-mg tablets was well tolerated and did not lead to 'dose dumping'[28].

MUCOADHESIVE DRUG DELIVERY SYSTEM IN COLON:

Gastrointestinal Mucoadhesive drug delivery system prolongs the residence time of dosage form at the site of absorption. It facilitates an intimate contact of dosage form with that of the underling absorption surface thus contributing to improved therapeutic performance of drugs [41].

The process of mucoadhesion involves a polymeric system that includes wetting, adsorption and interpenetration of polymer chains.

The problem frequently encountered with sustained release dosage forms is the inability to increase the residence time in the stomach and proximal portion of the small intestine.

Therefore it would be beneficial to develop a sustained release formulation which remains in contact with the absorption site for an extended period of time. This greatly enhances the pharmacotherapy of the GIT leading to high drug concentrations at the gastric or intestinal mucosa.

Mucosa of colon:

The GI tract consists of four concentric layers:

1. Mucosa
2. Submucosa
3. Muscularis externa (the external muscle layer),
4. Adventitia or serosa.

The mucosa is the innermost layer of GI tract that surrounds the lumen.
This layer comes in direct contact with food (or bolus), and is responsible for absorption, secretion and other important processes in digestion.

The mucosa can be divided into:

- Epithelium
- Lamina propria
- Muscularis mucosae

The mucosa are highly specialized parts of GI tract about 1mm thickness, facing low pH in stomach, absorbing a multitude of substances in small intestine, and absorbing specific quantities of water in the large intestine.

During fasting state an interdigestive series of electrical events take place, which cycle through both stomach and intestine every 2 to 3 hours. This is called the interdigestive myoelectric cycle or migrating myoelectric cycle (MMC), which is further divided into following 4 phases:

1.Phase I (basal phase)
2.Phase II (pre burst phase)

3.Phase III (burst phase)

4.Phase IV

During the fed state, onset of MMC is delayed.

Phase I - It is a quiescent period of about 30 to 60 min with no contractions.

Phase II - It consists of intermittent contractions that gradually increase in intensity as the phase progresses. It lasts about 20 to 40 min. Gastric discharge of fluid and other small particles begin later in this phase.

Phase III– It is a short period of intense distal and proximal gastric contractions (4–5contractions per min) lasting about 10 to 20 min; these contractions are also known as ‘‘house-keeper wave”.

Phase IV– It is a short transitory period of about 0 to 5 min. The contractions dissipate between last part of phase III and quiescence (phase I).

Mucoadhesion stages:

The stages of mucoadhesion involves,

1) An intimate contact between a bioadhesive and a membrane.
2) Penetration of the bioadhesive into the crevice of the tissue surface.
3) Mechanical interlocking between mucin and polymer.

Forces involved in mucoadhesion:

1) **Ionic bond:**

   Two oppositely charged ions attract each other via electrostatic interactions to form a strong bond (e.g. in a salt crystal).

2) **Covalent bond:**

   Electrons are shared in pairs, between the bonded atoms in order to fill the orbital. These are also strong bonds.

3) **Hydrogen bond:**

   Hydrogen atom covalently bonded to electronegative atoms such as oxygen, fluorine or nitrogen, carries a slight positive charge and is therefore attracted to other electronegative atoms. This bond is generally weaker than ionic or covalent bonds.

4) **Van-der-Waals bond:**

These are weakest forms of interaction that arise from dipole– dipole and dipole-induced attractions in polar molecules and dispersion forces with non-polar substances.

5) **Hydrophobic bond**:

These are indirect bonds that occur when non-polar groups are present in an aqueous solution. Water molecules adjacent to non-polar groups form hydrogen bonded structures.

Mucus: structure, function and Composition:

Mucus is a viscous adherent secretion which is secreted by specialized goblet cells. The thickness of this layer varies from 50-450 μm in humans.

Composition of mucus [42]:

Water	95.0 %
Glycoprotein & lipids	0.5 - 5.0 %
Mineral salts	1.0 %
Free proteins	0.5 - 1.0 %

Function of mucus layer [43]:

The primary functions of the mucus layer are: -

1. Protection- Resulting particularly from its hydrophobic nature.

2. Barrier- The role of mucus layer as barrier in tissue absorption of drugs and other Substances influence the bioavailibity of the drug.

3. Adhesion- Mucus has strong cohesional properties and firmly binds to the epithelial cells surface as continuous gel layer.

4. Lubrication- role of mucus layer is to keep the mucosal membrane moist by continuous secretion of mucus from the goblet cells. It is necessary due to removal of mucus layer due to digestion, bacterial degradation and solubilization of mucin molecules.

MUCOADHESIVE POLYMERS:

Mucoadhesive polymers may be water-insoluble and water-soluble polymers, which are swellable networks joined by cross-linking agents. These polymers possess optimal polarity to permit sufficient wetting by mucus secreations influencing interpenetration of polymer and mucus.

Classification of mucoadhesive polymer:

Natural /Semi-synthetic polymers:

Na^+ alginate, Agarose, Chitosan, Pectin, Tragacanth, Gelatin, Xanthan gum, Carragenan, Starch.

Synthetic polymers:

Poly vinyl alcohol, Polyamides, Polycarbonates, Poly alkylene glycols, Poly vinyl ethers, Esters and halides, Poly methacrylic acid, PMMA , Methyl cellulose,Ethyl cellulose, HPC, HPMC, Methyl cellulose, Sod. CMC.

Bicompatible polymers:

Esters of haluronic acid, Polyvinyl acetate, Ethylene glycol.

Biodegradable polymers:

Poly(lactides), Poly(lactide-coglycolides), Poly caprolactones, Poly alkyl cyanoacrylates, Poly orthoesters, Poly(glycolides), Poly phosphoesters, Poly anhydrides, Poly phosphazenes, Chitosan, Poly ethylene oxide.

FACTORS AFFECTING MUCOADHESION:

1) **Polymer Related Factors**[44]:

a) **Molecular weight**:

The interpenetration of polymer into the mucus layer is more for low molecular weight polymers than high molecular weight polymers since it favors entanglements.

b) **Concentration of active polymer**:

For solid dosage forms such as tablets, the higher the concentration of polymer, the stronger the bioadhesion force.

c) **Spatial Conformation**:

Bioadhesive force is also dependent on conformation of polymers, i.e., helical or linear. The helical conformation may shield many active groups which are primarily responsible for adhesion thus reducing the mucoadhesive strength of the polymer.

d) **Chain flexibility of polymer**:

It is important for interpenetration and enlargement. As water-soluble polymers become more and more cross linked, the mobility of the individual polymer chain decreases, cross linking density increases, effective length of the chain which penetrate into mucus decreases and mucoadhesive strength is reduced [45].

e) **Degree of Hydration**:

Another important factor which affects the mucoadhesive strength of polymeric components is the degree of hydration. Many polymers exhibit adhesive properties under conditions where the amount of water is limited. In such situation, adhesion is a result of combination of capillary attraction and osmotic forces between the polymer and mucosal surface. Hydration is essential for relaxation and interpenetration of polymer chains, excess hydration could lead to decreased mucoadhesion. Cross linked polymers that allows certain degree of hydration provides prolonged mucoadhesion [44].

f) **Functional Group Contribution**:

The attachment and bonding of bioadhesive polymers to the biological substrates occurs mainly through interpenetration which is followed by secondary non-covalent bonding (hydrogen bonding) between the substrates. Mucoadhesive polymers possessing hydrophilic functional groups such as, carboxyl(COOH), hydroxyl (OH), amide (NH_2) and sulphate groups (SO_4H) favours targeted drug delivery.

g**) Influence of pH**:

pH influences surface charge on mucus and polymers. Mucus will have different charge density depending on pH, because of difference in dissociation of functional

groups such as carbohydrate moiety and amino acids of polypeptide backbone, which may affect adhesion [46-49].

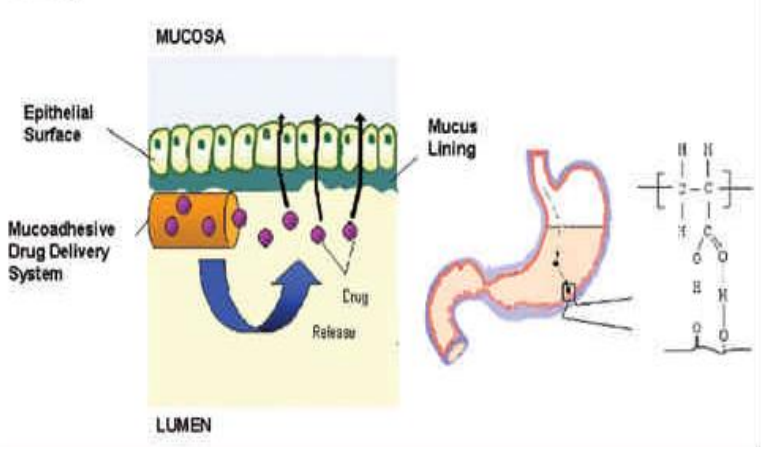


Figure 2: Mucosa of Colon

COLON TARGETING DRUG DELIVERY SYSTEM:

Site-specific delivery of drugs to the colon would additionally be valuable in treatment of chronic medical conditions like nocturnal asthma, which is reported to be circadian rhythm dependent [50].

Colon specific drug delivery can be achieved with suitable mechanism that triggers the drug release upon reaching colon. The physiological changes in the pH of the gastrointestinal tract and a pH-sensitive tablet in capsule system intended to match the chronobiology of nocturnal asthma is proposed for site specific release to the colon [51].

The delivery of dosage form to the colon via gastrointestinal (GI) tract requires protection of delivery system from being released in stomach and small intestine. Sustained release of drugs into colon can be useful in treatment of certain diseases for which systemic absorption of drugs to be achieved for prolonged time period.

The colon is most suitable site for absorption of peptides and protein drugs for the following reasons:

1. Less degradation by digestive enzymes.
2. The proteolytic activity of colon is less than that observed in small intestine, thus CDDS protects the drugs from hydrolysis, and enzymatic degradation which provides greater systemic bioavailability. The colon has a long residence time and hence it is highly responsible for enhancement of absorption.

ANATOMY AND PHYSIOLOGY OF COLON[52,53]:

The large intestine extends from the distal end of the ileum to the anus. Human large intestine is about 1.5 m long. The pathway is called the lumen and is about 2-3 inches in diameter. The cecum forms the first part of the colon followed by right colon, transverse colon, descending colon, sigmoid colon, rectum and the anal canal.

Table 1: Length of colon

Sr. no.	Large Intestine	Length (cm)
1	Cecum	6-9
2	Ascending colon	20-25
3	Descending colon	10-15
4	Transverse colon	40-45
5	Sigmoid colon	35-40
6	Rectum	12
7	Anal canal	3

pH in Colon :

The change in pH along the gastrointestinal tract has been used as a means for targeted colon drug delivery. The pH in colon may be influenced by carbohydrate rich diet due to fermentation of polysaccharides by colonic bacteria resulting in formation of short chain fatty acids. polysaccharide based drugs alter colonic pH. Laxatives drugs like lactulose are known to be fermented by colonic bacteria to produce lactic acid and reduce colonic pH.

Table 2 : pH of colon

Sr. no.	Location	pH
1	Stomach	1.5 - 2.0
2	Small intestine Jejunum Ileum	6.5 to 7.8
3	Large intestine	5.5 to 6.8

There is a fall in pH on the entry into the colon due to presence of short chain fatty acids due to bacterial fermentation of polysaccharides.

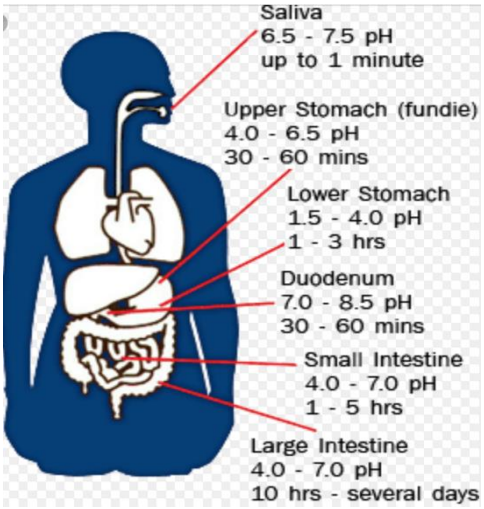


Figure 3: pH and transit time of GIT

Transit of material in the colon:

Gastric emptying of dosage forms is highly variable and depends on propertiesof the dosage form such as size and density. The arrival of an oral dosage form at the colon is determined by the rate of gastric emptying and the small intestinal transit time. The movement of materials through the colon is slow and dosage forms such as capsules and tablets pass through the colon in approximately 20-30 hours to more than 2 days can occur. Diseases state of colonic alters the transit time.

Table 3: Transit time of GIT

Organ	Transit time (hours)
Stomach	1-2
Small intestine	3-4
Large intestine	12 hours to > 2 days

Colonic Absorption:

Different factors affecting colonic absorption involves,

1. Passage through colonocytes (Transcellular transport).
2. Passage between adjacent colonocytes (Paracellular transport).

Transcellular absorption involves passage of lipophilic drugs through cells, where as paracellular absorption involves transport of Hydrophilic drugs through tight junctions between the cells.

Drugs that are well absorbed in colon -glibenclamide, diclofencac, theophylline, salbutamol, ibuprofen, metoprolol and oxyprenolol.

Drugs that are less absorbed in colon - furosemide, pyretanide, buflomedil, atenolol.

Factors affecting colonic absorption:

- Physical properties of drugs such as pKa and degree of ionization.
- Colonic residence time and transit through GIT
- Degradation by bacterial enzymes and metabolite products.
- Adherence to mucus.
- Diseased state of colon.

Colonic microflora [54,55]:

The presence of colon specific microflora formed basis for development of colon specific drug delivery system. Human colon is dynamic and ecologically diverse

environment, containing 400 distinct species of bacteria with a population of 1011 to 1012CFU/mL, which includes Bacteroides, Bifidobacterium, Eubacterium, Lactobacillus, etc.

Nitroreductase, azoreductase, N-oxide and sulfoxide reductase are the reductive enzymes, while glucosidase and glucronidase are the hydrolytic enzymes. The primary source of nutrition for these anaerobic bacteria is carbohydrates from the intestinal chime. Enzymes responsible for degradation of polysaccharides include α-Larabinofuranosidase, β-D-fucosidase, β-Dgalactosidase, β-Dglucosidase, β-xylosidase. A unique features of colon microflora is the growth and activity of certain specific species notably bifidobacteria and *lactobacilli*.

HYDROGELS

Hydrogels are cross linked polymers which have ability to swell in aqueous medium. Crosslinking in hydrogels occurs by chemical or physical means depending on the polymer properties. Intelligent hydrogels can able to respond to various environmental changes such as temperature, pH, and solvent composition, by changing their dimensions [56].

A cross linked hydrosol is called as hydrogel which can swell into certain swelling ratio, depending on the number of crosslinks, i.e., the crosslinking density.

Responsive Hydrogels:

Hydrogels can respond to environmental changes by changing its size or shape. Factors that triggers a hydrogel response includes pH, temperature, and swelling medium.

Nonionic hydrogels are almost insensitive to pH changes, while ionic hydrogels display a dramatic change in size with the pH change. Hydrogels containing hydrophobic groups susceptible to chain aggregation respond to temperature changes.

Hydrogels classified according to their charge,

1. Anionic polymers- carbopol, polyacrylates
2. Cationic polymers- chitosan, Eudragit E
3. Neural/ non ionic polymers- HPMC

pH-Responsive Hydrogels:

Polymers containing carboxyl groups or amino groups respond to the pH changes by changing their size in swollen state. At low pH values, the carboxyl-containing anionic polymers display minimum ionization and hence shows reduced hydration. Once the pH of the swelling medium rises above the pKa of the polymer, the carboxyl groups start to ionize and hydrate, which results in polymer expansion and hence higher swelling.

On contrary, cationic polymers containing amino groups(quaternary ammonium salts) display a stronger ionization and hence shows higher rate of swelling at low pH.

Eudragit ® L100-55, L30D-55, L100, or S100 are anionic polymers with methacrylic acid as functional groups. They dissolved at pH above 5.5, which provide drug protection at lowpH and drug release at high pH environment, which makes them suitable for drug delivery in intestine.

Eudragit ® E100 is, on contrary, a cationic polymer based on butyl and methyl methacrylate containing dimethylaminoethyl methacrylate providing pH-sensitive functionality. The polymer is soluble in low pH.

STIMULI RESPONSIVE SMART POLYMERS:

The Delivery systems that releases the drug based on the diseased state according to the physiological needs utilizes the advantages of ‘environmental-sensitive’ or ‘smart polymer’ systems [57]. These polymers experience rapid changes in their microstructure from a hydrophilic to hydrophobic state triggered by small environmental changes.

The changes are reversible; hence, the polymer is capable of returning to its initial state as soon as the trigger is removed. Stimuli may occur internally (e.g. a change in pH in certain organs or diseased states, a change in temperature or the presence of specific enzymes or antigens).

Smart polymeric drug delivery systems have been defined as “intelligent” drug delivery systems which are able to release bioactive agents at an appropriate time and site of action. These polymers releases the entrapped drugs in response to specific physiological triggers and exhibit a non-linear response to a small stimulus leading to a macroscopic alteration in their structure/properties [58].

STIMULI RESPONSIVE SMART POLYMERS:

Environmental stimulus Responsive polymers:

Temperature responsive:

Poloxamers, Poly(N-alkylacrylamide)s, Poly(N-vinylcaprolactam)s, Cellulose, xyloglucan, Chitosan.

pH responsives:

Poly(methacrylicacid)s, Poly(vinylpyridine)s, Poly(vinylimidazole)s.

Light Modified resposives:

Poly(acrylamide)s.

Electric field responsive:

Sulfonated polystyrenes, Poly(thiophene)s, Poly(ethyloxazoline)

Ultrasound responsives:

Ethylenevinylacetate

MECHANISM INVOLVED IN pH RESPONSIVE POLYMERS:

A stimuli-sensitive or smart polymer or intelligent polymers undergoes an abrupt change in its physical properties in response to a small environmental stimulus. They have the ability to return to their original shape after the trigger is removed [59-61].

These transitions are reversible and include changes in physical state, shape and solubility, solvent interactions, hydrophilic and lipophilic balances and conductivity.

The driving forces behind these transitions include neutralisation of charged groups by the addition of oppositely charged polymers or by pH shift, and change in the hydrophilic/lipophilic balance or changes in hydrogen bonding due to increase or decrease in temperature.

The major benefits of smart polymer-based drug delivery systems includes reduced dosing frequency, ease of preparation, maintenance of desired therapeutic concentration with single dose, prolonged release of incorporated drug, reduced side effects and improved stability[62-64]

MECHANISMS INVOLVED IN POLYELECTROLYTES:

All pH-sensitive polymers consist of pendant acidic or basic group that can either accept or release a proton in response to changes in environmental pH. Polymers with a large number of ionisable groups are known as polyelectrolytes.

Polyelectrolytes are classified into two types: weak polyacids and weak polybases. Weak polyacids accept protons at low pH and release protons at neutral and high pH [64]. Poly(acrylic acid) (PAAc) and poly(methacrylicacid)(PMAAc) are commonly used pH-responsive polyacids.

As the environmental pH changes, the pendant acidic group undergoes ionisation at specific pH called as pKa. This rapid change in net charge of the attached

group causes alteration in the molecular structure of the polymeric chain. This transition to expanded state is mediated by the osmotic pressure exerted by mobile counter ions neutralised by network charges [64].

STIMULUS HYDROGEL TYPE RELEASE MECHANISM:

A change in pH causes swelling of the hydrogel. Mechanisms capable of responding to these physiological variations can be used to design drug delivery systems in order to synchronize drug release profiles with changing physiological conditions. Ideally, a drug delivery system should respond to physiological requirements, sense the changes and alter the drug-release profile accordingly [65].

This paves the way for development of self regulated drug delivery which is adjusted to the staging of biological rhythms, since the onset of certain diseases exhibit strong circadian temporal dependence.

The macroscopic changes that occur are reversible; therefore the system is capable of returning to its initial state when the trigger is removed [65].

Responses to these stimuli may be manifested as changes in shape, surface characteristics, solubility, and formation of an intricate molecular assembly or sol-to-gel or gel-to-sol transition [65]

The swelling of pH-responsive hydrogels is governed by their degree of ionization i.e. protonation or deprotonation. On exposure to appropriate pH and ionic strength, the pendant groups ionize and develop fixed charges on the polymer network, causing electrostatic repulsive forces responsible for pH-dependent swelling or deswelling of the hydrogel, which ultimately controls & alters drug release profiles [65]

LITERATURE REVIEW

Literatures pertaining to incidence of circadian rhythm in asthma:

Calhoun., et al., (1992) [66] demonstrated that the patients with nocturnal asthma had increased proportion of low-density eosinophils at 4:00 A.M. as compared with 4:00 P.M.

Ballard., et al., (1989) [67] studied that the lower airway resistance to airflow rose progressively from 12 midnight to 6 AM in asleep asthmatic subjects.

D'Alonzo., et al.,[68] studied the sustained-release theophylline formulation administered once daily at evening 8:00 P.M in comparison with conventional sustained-release theophylline formulation administered twice daily at 8:00 A.M and at 8:00 P.M in same dose. However, between 2:00 and 6:00 A.M., PEF and FEV1 were significantly greater with Once daily dose.

Rofstad., et al., (2006) [69] stated that an obvious pH changes occurs along gastrointestinal tract Chronic inflammation and in cancer tissue, hasbeen reported to be acidic extracellularly. The same is true for different cellular compartments of thebody. Since variations in pH occur within the body this property can be exploited to directa response and exploited for modulating drug release.

Ekbom et al., (2008) [70] showed higher risk of CD in COPD sufferersby relating the relationship between gut and lung. Specific intestinal manifestations of COPD include atrophic gastritis etc. The report also showed the prevalence of colonic inflammation was higher in COPD and in other respiratory diseases.

Black et al.[71] performed a literature survey that indicated 55 articles citing respiratory disorders in IBD patients which involves airway inflammation accounting for 39% of these associations. Three more specific studies inIBD patients showed

increased incidence rates of pulmonary organ involvement at about 50%. Pulmonary involvement was more likely in colonic diseases.

Kinoseet al.,(2011) [72]have recently identified increase in prevalence of NOD2single-nucleotide polymorphism (SNP) in COPD patients. NOD2 leads to factor-κB activation and enhances inflammatory cytokine response upon stimulation. Defect in NOD2 signaling leads to impaired epithelial barrier function which leads to increased IL-1β and TLR2 response, which increases serum IL-12. NOD2 mutations were found in CD population. Thus, NOD2 may therefore be a common link between COPD and CD.

Literatures pertaining to polymers:

Perez-Marcos et al., (1995) studied decrease in Atenolol release rate from tablets with increasing level of Carbopol ® polymer attributed to formation of thicker and stronger gel on tablet surface that controlled drug release in more efficient way.

Khan and Jiabi., (1998) studied drug release profile by Increasing Carbopol polymer in Ibuprofen tablets resulted in reduction of drug release rate and linearization of drug release curve was achieved (release profiles in pH=7.2 buffer shiftedfrom anomalous type of drug release towards swelling-controlled, Case II mechanism). This phenomenonwas considered due to reduction in regions of microviscosity and the closing of micropores in swollen tablets. Carbopol ® polymers have pKa of 6, so at pH 1.2 they are un-ionized; but ionize at above pH 4.5. At lower pH, polymer is not fully swollen, and there are larger regions of low microviscosity; hence solvent can penetrate fast and deep into the glassy core and drug is released faster. As the pH increases, ionization of carboxylic acid groups causesmaximum swelling, resulting in fewer regions of microviscosity. Rapid gel formation actsas barrier thus prolonging the drug release.

Efentakis., et al., (2000)studied formulations containing Carbopol ® 974P NF polymer in pH=5.8 bufferwhich attributed to polymer relaxation followed by diffusion of drug from surface ofthe tablet. This is due to strong entanglement of polymer molecules which delays movement of drug molecules from interior of polymer mass toward the surface.

Bulut-Oner., et al., (1989) showed that the release of Isoniazid (a water soluble drug) from carbomer tablets was faster in simulated gastric fluid and prolonged drug release in simulated intestinal fluid releasing 50 % of drug in seven hours.

Parojcic., et al., (2004) evaluated the rate ofdrug release from Acetaminophen tablets containing Carbopol ® polymer matrices in different buffered and unbuffered media, the results showed that most rapiddrug release was observed in unbuffered 0.1N HCl, where as drug release from phosphate buffers medium (pH=5.8, 6.8) showed slower rate of drug released 60 – 70 % for a period of 8 hours.

Tatavarti., et al., (2004) reported that the incorporation of Carbopol ® 71G NF polymer in matrix tablets resultedin enhanced release of drugin buffer pH 6.8. Verapamil HCl (solubility at pH=6.8 2.71 mg/ml) is used as model drug. The phenomenon was attributed to modulation of microenvironmental pH to acidic side and observed that there is an increased solubility of the active ingredients insidethe matrix which enhanced drug release.

Draganoiu., et al., (2004) studied that the anionic nature of Carbopol ® polymers that forms ionic complexes with cationic soluble drugs, which is advantageous for retarding drug release from the matrix. The release of Propranolol hydrochloride, a cationic drug with solubility over physiological range (220 mg/ml in 0.1N HCl and 254 mg/ml in pH=7.4 phosphate buffer) was extended by incorporating Carbopol ® 71G NF polymer in matrix attributed to drug – polymer ionic complex formation.

Khamanga and Walker., (2005) reported that when Carbopol ® 974P NF polymer was used in combinationwith other polymers, controlled-release performance can be enhanced as a result of interaction betweenthe polymers. Tablets prepared with the blends of HPMC (Methocel ® K100M) and Carbopol ® 974P NF polymer using Surelease ® E-7-19010 (ethyl cellulose) or Eudragit ® NE 30D as granulating agents sustained the drug release of Verapamil, better than when Carbopol ® 974P NF polymer or Eudragit ® RS were used alone (in direct compression).The authors finally concluded that combination of Carbopol ® 974P NF and Methocel ® K100M producedsynergistic increase in viscosity due to stronger hydrogen bonding between

carbomer and HPMC resulting in stronger cross-link forming more rigid structurethrough which drug diffusion occurs.

Samani., et al., (2003) evaluated the effect of polymer blends on in vitro release profile of diclofenacsodium. The author concluded that when an appropriate blend of carbomer and HPMC was used, the drug release was moreuniform, fluctuations were diminished and kinetics well fitted to zero order. They also concluded thatwith blend of polymersit was possible to reduce total amounts of polymer in formulation, thus reducing the size and weight of tablets.

Perez-Marcos., et al., (1996) investigated the release profile of Propranolol hydrochloride tablets containing Carbopol ® 974P NF polymer and HPMC K4M in various media such as 0.1N HCl or phosphate buffer at pH 4.5 or pH 7.5. At buffer pH 7.5, synergistic interaction of two polymers was observed, thus contributing to matrix integrity and controls drug release.

Sharma., et al., (2004) investigated the combination of Carbopol ® 971P NF and hydroxypropyl cellulose in sustained-release portion of Nitrofurantoin controlled-release dosage form, which alsohad an immediate release portion.

Singh and Ahuja (2002) formulated Diltiazem controlled-release buccoadhesive hydrophilic matrices with varying amount of carbomer and HPMC. Suitable combinations of two polymers gave adequate bioadhesive strength and drug release for prolonged period. Bioadhesive strength varied linearly with varying amount of each polymer. Drug release pattern for all combinations were found to be non-fickian, approaching zero-order kinetics. The values of permeation coefficient tends tovary non-linearly depicting possible interaction between two polymers.

Kenneth chibuzor., et al., (2013)[73]studied the formation of interpolyelectrolyte complexes (IPECs), formed between Eudragit RL100 (EL) and chitosan (CS) by wet granulation method were evaluatedfor oral CTDDSs for ibuprofen (IBF). The results showed that tablets formed by IPECs shows pH-dependent swelling properties and prolonged the in vitro release of IBF from the tablets. An electrostatic interaction between the carbonyl (–CO–) group of Eudragit and amino ($–NH_3^+$) group of Chitosan. Tablet formulated with IPECs was capable of preventing drug release. Kinetic analysis of drug release profiles showed that the systems predominantly released IBF in a zero-

order manner. IPECs based formulations can be successfully for colon-targeted delivery of IBF in the treatment of IBDs.

Mustafin., et al., (2005) [74] studied the swelling behavior of potential polymeric carriers which can be used as controlled release. They evaluated the swelling behavior of polycomplex matrices made from CS and EL 100 in simulated gastro-intestinal tract (GIT) pH 1.2 and in pH 6.8 medium. According to specifications of Degussa, dissolution of EL depends on copolymer structure and is regulated by the ratio between methyl methacrylate or ethyl acrylate and methacrylic acid. The swelling behavior of IPECs films is completely different from normal matrix systems. In these systems, the factor responsible for swelling is the electrostatic repulsion of free ionized amino groups. In case of IPEC made up of CS : EL 2 : 3 ratio, the degree of swelling was 150% at pH 1.2,and then two-fold increase in swelling at pH 6.8 could be observed.

Asghar LF., et al., (2016)[75] investigated the effect of incorporating pH-responsive polymers Eudragit (L100 or S100) in matrix bases of hydrophilic polymers like polycarbophil and carbopol to design controlled release formulations with sigmoidal release profile to target the dosage form to colon. Matrix tablets were prepared by wet granulation technique using indomethacin as model drug. The gastrointestinal (GI) transit of selected formulations was also investigated in human subjects using gamma scintigraphy. In vitro release studies, showed 10-15% drug release in 6 h followed by controlled release for next 8-10 h in simulated GI fluid pH (without enzymes). The presence of Eudragit L (alkaline soluble polymer) in hydrophilic matrix base retarded the rate of swelling in acidic to weakly acidic pH, but in alkaline pH, enhancement in drug release rate of 80-90% release in 14 h was observed due to dissolution of EudragitL from matrix base resulting in porous matrix structure. In vivo gamma scintigraphystudies in healthy human subjects proved that the formulations had acceptable matrix strength to withstandcolonic transit. The mean colonic residence time varied between 15 and 19 h. such matrix systems have application as pH dependent and time dependent release systems.

Monica RP Roa., et al., [76] studied the mucoadhesive properties of HPMC and carbopol in combination in matrix tablets .The formulations containing carbopol-HPMC combinations were found to have uniform thickness, weight, drug content and adequate

mucoadhesive strength and swelling index. Higher swelling index of Carbopol-HPMC matrix is relatively due to higher hydrophilicity of carbopol.

Mishra S.K., et al.,[77]developed once daily controlled release matrix tablets of Tramadol Hcl. Controlled release matrix tablets of Eudragit RS-100 and Carbopol 934P were formulated and showed satisfactory controlled release of drug for 24hours with maximum release of 95.73 %

Kim., et al.,used anionic hydrogels as delivery carriers due to their pH-responsive swelling behavior. The dynamicswelling behavior of poly(methacrylic acid-co-methacryloxyethylglucoside) and poly(methacrylic acid-g-ethylene glycol) hydrogels wasinvestigated to determine the mechanism of water transport throughthese anionic hydrogels. Mechanism of water transport was significantly affectedby pH of medium and became more relaxation controlled in medium of pH 7.0.

Cilurzo., et al.,developedlow swelling mucoadhesive dosage forms based on methacrylic copolymers of Eudragit L100 and Eudragit S100. The adhesion properties of these materials, measuredby texture analyzer, were similar to Carbopol 934P. When these polymers are used in combination they promote adequate bioadhesive strength with good patient compliance due to their low swelling properties.

Bravo., et al.,(2004) [78] evaluated therelease behavior of diclofenac sodium from swellable matrix tabletcontaining hydroxypropyl methylcellulose (HPMC) and Carbopolmade by wet granulation. Drug release was studied in terms of polymer content, polymer ratio, and pH. The overall releasewas found to be pH dependent and follows zero order kinetics. While a lowpH medium increases the drug release from carbopol, and retards drug release as pH increases.

Parthiban et al., (2015) [79] developed mucoadhesive tablets of cephalexin monohydrate and studied the effect of Carbopol and HPMC K100M to achieve desired mucoadhesion and controlled release. The selected formulation FC2 exhibited 99.51% of drug release over a period of 24 hours with mucoadhesive strength found to be 95.04 gm. Hence it has the ability to adhere on the mucosa for an extended period of time. Kinetic study follows zero order release and matches with Higuchi regression.

Prajapathi., et al., (2009)[80] developed floating matrix tablets of domperidone for prolong gastric residence time and thereby increasing drug bioavailability. The tablets were prepared by wet granulation method, using polymers such as hydroxypropylmethylcellulose K4M, carbopol 934P, and sodium alginate, alone and in combination. Tablets were evaluated for in vitro release study and was found to prolong the drug release for a period of 24 hours. Release mechanism followed linear regression analysis. Carbopol loading showed negative effect on floating properties but were found helpful to control the release rate of drug.

Literature pertaining to salbutamol sulphate:

Shahnooshijavad F., et al., (2014)[81] developed bucco adhesive tablets of salbutamol sulphate using bioadhesive polymers like carbopol 934P,Hydroxy Propyl Methyl Cellulose (HPMC K4M) and present study demonstrated that salbutamol sulphate can be successfully developed as buccal adhesive tablets and offers better route for oral drug delivery to by pass first pass metabolism.

Venkateswaran., et al., (2013)[82] formulated an Extended-Release (ER) tablets of Albuterol Sulphate which is simple in design and cost effective to patients. Matrix tablets were prepared by wet granulation process using Methocel K100M CR as release rate controlling polymer. The tablets were coated with Opadry Clear YS-1-7006 followed by 4% coating with ethyl cellulose to prevent initial burst release from matrix tablets. The drug release rate of 8 hours was achieved and comparable to marketed product of VOSPIRE® follows Higuchi‟s model of drug release kinetics indicating fickian diffusion.

Zahirul., et al., (1999)[83] stated that Eudragit L100 and Eudragit S100 which dissolves at pH 6.0, and 7.0,respectively, do not dissolve in stomach pH due to hydrogen bondingbetween hydroxyl groups of carboxylic moietyand carbonyl oxygen of ester groups in polymermolecules. Hence, they dissolves in colon due to ionization of their carboxyl functional groups and releases the drug. He also stated that by alteric polymer characteristics (using combination of polymers in varying ratio) drug delivery can be targeted at specified site in GIT.

Threveenchalla., et al., (2011)[84] stated that in comparison to other region of GIT,movement of material through the colon is slow. Total time required totransit tends to be highly variable and influenced by number offactors such as diet, stress, diseased condition and drugs. The colonic transit time ranges from 20 to 30 hours, and can be increase in presence of active disease upto 50 to 70 hours. Longer residence time and contact of dosage formwith micro flora in colon govern the release andabsorption of drug from its dosage form.

Rana., et al., (2013) [85] stated that intestinal-colonic transit time influences the performance of CDDS and colonic bioavailability of drugs. Transit times was also influenced by colonic disease and the study showed that in patients with IBD, the orocecal transit time was delayed.

Sarfaraz MD., et al., (2013)[86] developed immediate release solid dosage form of salbutamol sulphate. Immediate release dosage forms are fast growing drug delivery systems that improve the onset of action of drugs. The attempt was for the selection of superdisintegrants like croscarmellose sodium, crospovidone and sodium starch glycolate in different concentrations (2.5 – 7.5%w/w). Tablets were evaluated for their disintegration behavior. Microcrystalline cellulose andlactose were used as diluents. The tablets were prepared by direct compression method and evaluated for drug release. Formulation containing 7.5 % of croscarmellose sodium released 99.26% and 99.75% of drug in 12 minutes in pH 6.8 and pH 7.4 phosphate buffer.

Literature pertaining to tablets in capsule system & enteric coating of capsules:

Marilena Vlachou., (2017) [87]investigated the release behavior of multiple-unit modified release formulations (“tablets incapsule system”) of theophylline. Mono-layered and three-layered minitablets, filled into capsules, (flat mini tablets of 6.5 mm diameter, compressed at hardness of 8 – 10 kpa) were prepared using theophylline and dextran or pectin, as excipients.Their release behavior was compared with that of powder filled capsules and commercially available Theodur® 200 mg tablets. The Dissolution tests wasperformed in three different pH media (1.5, 7.4 and 6.0 pH) in presence and in absence of enzyme Pectinex® Ultra SP-L solution, which degrades polysaccharides.

Theresults indicated that the enteric-coated capsules showed no release of drugs in acidic media for a period of 2 hours. Dextran forms thicker gelled layer than pectinand therefore suitable forextended release formulation as “tablets in capsule” system. Drug is released from 3-layered mini tablets at slower rate compared to matrix mini tablets. Pectinex® Ultra SP-L, gives accurate dissolution results when used in dissolution medium, mimicking the large intestine/colon.

Asnani., et al., (2013)[88] investigated the enteric-coated capsules which played importantrole in initial drug release retardation. In all cases, release of drug enteric coated capsules was found to be 0% in acidic medium for a period of 2 hours.Hence this Enteric coating capsule system can be used to modify their release and to achieve targeted release into other regions of GIT tract.

Leopold., et al.,[89] studied the colonic pH in healthy individuals which is about 6.4 to 7.0 pH but can drop to pH 2.3 to 4.7 in UC. Developed dexamethasone mini tablets coated with acid soluble polymers (eudragit E) and found that eudragit E rapidly dissolves in buffer at pH range of 2.0 to 5.0 which releases drug within in 10 to 50 min.

Stubbs et al., studied the effect of dawn and dusk of dosage forms on mobility of colon. The results revealed that colonic transit of dosage form was delayed during sleep and larger dosage form. The study also showed that enteric coated capsule transited faster compared to enteric coated tablets and smaller dosage forms (dispersed particles or pellets).

Literature pertaining to relationship between gut and lung:

Seham Ahmed ali., et al., (2010)[90] investigated that significantly larger population (74.1%) of asthmatics had IBS. Higher proportion of females with IBS were observed (61.54%). Patients with known IBS 87% cases using inhalers, 13% with additional oral theophylline. As 66.6% cases, had IBS with relatively short duration of asthma. Predominant symptoms of IBS were found in asthmatics (64.8%). This study relatively confirmed that IBS is significantly higher in asthmatic patients. This in turn might be addressed in treatment of asthma, for better health care. The link in the

pathogenesis between asthma and IBS needs further studies to document the role of smooth muscle dysfunction.

Roussos., et al., (2003) [91] reported excess prevalence of bronchial hyper-responsiveness has been shown among patients with IBS (inflammatory bowel syndrome). Colonic inflammation was also investigated in asthmatic patient. Hence, reveals that both conditions coexist and they are related. Further to explore this association, studies have been conducted among asthma patients for IBS, and among IBS patients for asthma. They evaluated 150 patients with asthma, 130 patients with other pulmonary disorders and 120 healthy subjects. They final found that patients with bronchial asthma have an increased prevalence of IBS. An association of gastrointestinal symptoms like IBS in asthma has been reported in large case-control study conducted by Nick Powell.

White., et al., (1991)[92] reported an increased prevalence of bronchial hyper-responsiveness in IBS patients. To support his study, Yazar et al., found that there is anhigher rate of asthma symptoms in IBS patients and speculated that gastrointestinal system and respiratory system may reflex common symptoms.

Kennedy et al., (1998)[93] found that IBS, GERD and symptomatic bronchial hyper-reactivity occur more frequently in asthmatics and vice versa and these conditions are independently associated with each other. To support his study, BabakAmra et al., reported similar observations that symptoms of IBS and asthma occur more frequently together and are independently associated with each other.

Panicker., et al., (2008)[94] conducted study in larger population of women and reported that IBS was more common in asthmatic women. Higher prevalence of IBS among asthmatic women is well documented. Stress, anxiety and psychosomatic factors were implicated in asthmatics and IBS patients, and these may also influence the pathogenesis of IBS in asthma.

Ekici., et al.,(2005) [95] compared two groups of asthmatic patients ageing >60 and < 60 years with age-matched controls. The prevalence of IBS was high among asthmatics with age <60 years and this observation supports that most of the asthma patients with IBS (68.4%) were in age group of 31-50 years. This finding correlates the relationship between asthmatics and inflammation in colon.

Lodi et al., (1997)[96] stated that gastrointestinal symptoms might indicate common pathology of smooth muscle dysfunction in asthma and IBS which involves generalized disorders of bronchial, gastro-intestinal and other smooth muscles. In asthmatics with GERD, there is an evidence of primary autonomic dysfunction with hyper-vagal responsiveness.

Jan Fallingborg., et al.,[97] measured Intraluminal gastrointestinal pH using radiotelemetry capsules and its location was determined by fluoroscopy. pH levels were normal in stomach and small intestine but very low pH levels (2.3, 2.9, and 3.4 pH) were found in proximal parts of colon. Increased fecal concentrations of lactate occurs in active disease. The study demonstrated that very low intraluminal pH levels were found in colon. He also stated that this might be an indicator of severe activity of the disease.

Babakamra., (2003)[98] conducted study at iran in 4762 subjects with 86.7% response showedprevalence rates of IBS in asthmatics. Logistic regression showed independent associations between IBS and most asthma symptom categories.

Brassard paul., et al.,(2014) [99]resent study published in the European Respiratory Journal, that researchers found an increased rate of inflammatory bowel disease among asthmatics and COPD patients. The study involves data collected from health records of 136,178 people with asthma and 143,904 people with COPD. The result concluded that, compared to general population, people with asthma had 27% higher rate of ulcerative colitis where as COPD patients had 55% higher rate of Crohn's disease.

Hauptmann., et al., (1998) [100] The median gastric pH values in patient with Crohn's disease showed an average pH value of 2.4 (range 1.5–4.1 pH); in ulcerative colitis an average pH value of 1.95(range 1.55–4.4 pH) were significantly higher than those observed in the controls (1.55, range 0.95–2.6). In small bowel and in colonic segments, all pH values of colonic disease were comparable with controls. Patients with active disease had comparable gastrointestinal pH values to patients in remission.

Tzanakis N., et al., (1998)[101] reported that, in patients with inflammatory bowel disease (IBD), the airways are involved with number of clinical manifestations.

The study investigated the function of the small airways in IBD. 30 patients with IBD, 12 with Crohn's disease and 18 with ulcerative colitis, were studied and compared with a control group. Maximal expiratory flow-volume curves were performed. The differences of flows at 50% of FVC and volume of equal flows indicated as small airways function. In addition, spirometry, lung volumes, and diffusing capacity were measured. The result was significantly greater in patients with either CD or UC than in control subjects. Reduction in TL(CO), was noticed in active stage of the disease in both groups of patients. This indicates that lung parenchyma is also involved in active IBD.

Songur., et al., [102] studied pulmonary function abnormality was present in 21 out of 36 IBD patients. In IBD patients, DLCO were significantly lower, but RV/TLC was significantly higher than those of the controls. HRCT revealed air trapping, fibrosis, emphysema, bronchiectasis and alveolitis in 19 patients. About 80% of patients with pulmonary involvement had active bowel disease. Hence concluded, Pulmonary involvement is common in patients with IBD.

Ekbom A., et al., (2008) [103] investigated the occurrence of IBD among COPD patients, indicating common inflammatory pathways and shared vulnerability on genetic basis. Investigational study was designed as population-based cohort study. Peoples with COPD from 1987 to 2002 were identified in Swedish Inpatient Register (n=180,239) which is compared with discharges involving diagnosis of UC or CD. The Hazard ratios (HR) for IBD were determined by Cox proportional hazards regression analysis. COPD patients had a significantly higher risk of both UC and CD. Finally,results suggested that COPD and IBD may have inflammatory pathways in common, which may include genetic variants of genes predisposing for disease.

Keely S., et al., (2012) [104] stated that COPD and IBDs are chronic inflammatory diseases of mucosal tissues that affect respiratory and gastrointestinal tracts. They share many similarities in epidemiological and clinical characteristics, as well as in inflammatory pathologies.

Ceyhan BB., et al., (2003) [105]investigated the prevalence of abnormal pulmonary function tests, BHR and atopic status in patients with IBD. 30 patients with IBD (19 with ulcerative colitis and 11 with Crohn's disease) and 16 controls without any GI disease were included. Patients were questioned forpulmonary and allergic symptoms.Lung function tests, BHR, skin prick test positivity, peripheral eosinophilia and serum IgE levels were evaluated and compared with control subjects.IBD patients had significantly more associated respiratory symptoms in comparison with controls. Previous diagnosis of asthma and drug treatment were noted in 10% IBD patients. Allergic symptoms, respiratory symptoms, abnormal lung function tests and skin prick test positivity were common among IBD patients in comparison with controls.

Colby et al., (2007)[106] reported that bronchial hyper-reactivity occurred 48% of patients with UC and CD, even in absence of any clinical, radiological and functional evidence of airway disease. Bronchial hyper-reactivity occurred in 71% of CD subjects. Exaggerated immune response is trigerred by allergens, which can aggravate an immediately atopic allergic reaction. Combination of histological examination andHRCT revealed multiple centers of pulmonary inflammatory responses, which share similar characteristics with atopic allergic reactions.Lungsmay duplicates the “social” inflammatory reaction associated with intestine.

Abrahamsson TR et al, (2014) [107] stated that Chronic lung disorders such as asthma and chronic obstructive pulmonary disease (COPD) exhibit intestinal disease manifestation. Respiratory infections are often accompanied by intestinal symptoms.

Literatures pertaining to 72 hours drug delivery:

Javid Ali., et al., (2013)[108] Studied the drug loading and Drug Release Behavior ofPoly (N-Vinyl-2-Pyrrolidone) Gel evaluated using ketotifen as model drugs. The hydrogel was cut into small discs (3 mm thickness and diameter) and immersed in the solutions of the ketotifen for three days. The hydrogel immersed in 0.1 N HCl and phosphate buffers (pH 6.8) showed 31.82 % and 29.78% loading of ketotifen. Under acidic condition (0.1 N Hcl) only 10 % of drug was released in about 72 hours andfollowed Higuchi model and release follows Fickian diffusion.

Sreenivas SA., et al., [109] Formulated cefuroxime axetilmucoadhesive matrix tablets and microspheres exhibit prolonged controlled drug release.The cumulative % drug release of cefuroxime axetil matrix tablets was found to be 89.93% at the end of 72 hours whereas themicrospheres released 96.15% of drugs at the end of 72 hours.

Jaymin shah., et al., [110] developed an extended-release formulation of bupivacainethat after a single-dose administration could provide prolonged post surgery local analgesia up to 72 hours. SABER-Bupivacaine contains 132 mg bupivacaine base/mL. SABER-Bupivacaine is a sustained-release formulationin a controlled-release matrixcomposed of esterified sugar derivative such as sucrose acetate isobutyrate (SAIB) and benzyl alcohol, administered together as solution.

Omwoyo WN., et al., (2014) [111] designed primaquine (PQ)-loaded solid lipid nanoparticles (SLNs) (PQ-SLNs) as potential drug-delivery system. PQ-SLNs were prepared by modified solvent emulsification evaporation method (w/o/w) double emulsion. A spherical morphology of PQ-SLNs was seen in scanning electron microscope. In vitro drug release showed steady drug release over a period of 72 hours. DSC thermograms demonstrated the presence of drug-loaded nanoparticles suggesting stability of drugs in prepared formulations.

Das., et al,(2012) developed formulation based on nanotechnology which overcomes the poor bioavailability of drugs in posterior chamber of eyescompared to conventional ophthalmic dosage forms.nanoparticles based on albumin / xanthum gum prepared by coacervation and loaded with acetyl salicylic acid produced drug release in a sustained manner releasing 90 % of drugs over a period of 72 hours.

Literature pertaining to mucoadhesion:

Shweta Agarwal., et al.,(2015) studied different polymer concentration and its effect on drug release and as well as mucoadhesive gastro retentive properties. HPMC has mucoadhesive properties and also gives good controlled release. Since, it is a non-ionic polymer, its mucoadhesion is independent of pH of the medium. The mucoadhesive property of HPMC is attributed to hydrogen bond formation with mucus

components in intestine. It possess large number of hydroxyl bonds which are responsible for adhesion.

Banerjee., et al., compared the adhesion force between eudragit E po– porcine intestine and carbopol -porcine intestine. To measure the Mucoadhesive strength, the device was incubated in pH 7.4 phosphate buffer for 30 min in solution at 37° Calong with porcine intestine. The force required to completely detach the device from the intestine was noted. Eudragit E po (24.2 ± 0.95 mN) possess higher mucoadhesive force compared to carbopol devices (17.5 ± 1.3 mN) .

Ashwini madgukar., et al., (2008)[112] Formulated itraconazole sustained release mucoadhesive tablets using Eudragit E po spray dried and incorporated into hydrophilic matrix of carbopol 934 P and methocel k4M (HPMC). Finally reported that fairly regulated release profiles and Increased mucoadhesion strength was found polymers used in combination.

Robinson and bologna reported that polycarbophil (carbomer) has the ability to adhere to mucus membrane for 3 to 4 days and provides excellent drug delivery of progesterone.

Literature pertaining to interpolyelectrolyte complex:

Wasfy M., et al., (2015) [113] investigated the ability of polymer to modify the release rate of paracetamol (water soluble drug) by the influence of eudragit E 100 and carbopol 971 P NF prepared by direct compression method. Eudragit E in combination with carbopol 971 P NF was capable of sustaining the release properties of water soluble drugs. They do no dissolve in aqueous environment but swells to form 3-D hydrophilic gel structure called interpolyelectrolyte complex. At lower pH values, polymers are not fully swollen and drug is released faster from the matrix. At higher pH values, shows maximum swelling due to ionization of carboxylic acid groups, resulting in fewer/smaller regions of microviscosity, thus prolonging the drug release.

Kuldeepmalodia., et al., (2013) [114] developed extended release matrix tablets of Salbutamol sulphate, for the treatment of Chronic Obstructive Pulmonary Disease

(COPD). The matrix tablets were prepared by direct compression method using hydroxyl propyl methyl cellulose (HPMC K100M)in varying ratios. In vitro dissolution study was carried out by using type II dissolution apparatus for a period of 24 hours and F7 shows 96.49% of drug release at the end and hence capableof providing extended drug release.

Sabitha P., et al., (2010) [115] designed Chitosan coated alginate microcapsules as oral sustained delivery carriers for antitubercular drugs (rifampicin, isoniazide, pyrazinamide) to reduce dosing frequency and to improve patient compliance in management of tuberculosis (TB).Ionotropic/external gelation method is used to encapsulate anti-tuberculosis drugs (ATDs) within Alginate–chitosan microparticles.The formulation was designed in the ratio of 1:2:2 (drug: sodium alginate: chitosan). By In-vitro drug release studies, carried out in pH 1.2 (2 hour) and then in pH 7.4 (72 hours), microcapsules exhibited sustained release of drugs 95.33 % (isoniazide) , 96.46 % (rifampicin) and 97.27 % (isoniazide) over a period of 72 hours.

Sreenivas SA., et al., (2009) [116] designed of the formulation containing cefuroxime axetil mucoadhesive matrix tablets and microspheres using thiolated chitosan which exhibited controlled drug release for prolonged period.The cumulative drug release of cefuroxime axetil from matrix tablet was about 89.93% at the end of 72 hours while microspheres released 96.15% at the end of 72 hours.

Omwoyo WN., et al., (2014) [117] formulated dosage form containing primaquine which is an antimalarial drug entrapped into SLNs and exhibited sustained release of drug over a period of 72 hours and suppression of 94 % of plasmodium in mouse was achieved by SLNs.

Jagdishbidada., et al., (2011) [118] developed Ranolazine (anti-anginal drugs) matrix tablets containing different ratios of Carbopol 971 P(hydrophilic & pH dependent) and ethylcellulose (Ethocel N20/N50), water-insoluble and pH-independentpolymers. in vitro drug release was studied using USP Type II (Paddle) apparatus. The release kinetics indicates, drug release from matrix tablets depends on drug diffusion and

polymer relaxation. Hence , followed non-Fickian oranomalous release. The developed ER matrix tablets of Ranolazine provided drug release up to 12 hours.

Sobhitarani., et al., (2014)[119] formulated enteric coated capsules for site specific drug delivery of Satranidazole to colon. The capsules containing carbopol and drug is coated with HPMC & Eudragit S-100. Theenteric coated capsules shell was capable of delaying drug release for a period of 3-5 h in simulated upper gastrointestinal pH.

Literature pertaining to mucoadhesive colon targeting:

Ramesh Reddy., et al., (2015) [120] developed mucoadhesive tablets using PVP and Pectin inter polymer complexes containing Prednisolone. Mucoadhesive tables were prepared by direct compression method and enteric coated with polymers to target into colon.

Literatures pertaining to mucoadhesive tablets in capsule system:

Tapan kumar panda., et al., (2016) [121]formulated once daily multiple unit mucoadhesive sustain release (SR) mini tablets filled into capsule. The matrix tablets consists of bosentan, gelucire, hydroxypropyl methyl cellulose (HPMCK4M), sodium carboxymethyl cellulose (NaCMC) and chitosan in various proportions to sustain drug release for a period of 24 h. The release of bosentan from gelucire based SR mini tablets extended drug release over a period of 24 h with an initial burst release of 32 %. Incorporation of Na^+ CMC, HPMCK4M and chitosan into the mini tablets controlled initial burst release and produced adequate mucoadhesion.

Lu Z., et al.,(2010)[122] studied interpolyelectrolyte complex between chitosan and polycarbophil and its matrix forming ability to control the release of water-soluble drugs. Swelling, erosion, and drug release performance of matrix tablets containing chitosan-polycarbophil complex was compared with matrix tablets containing HPMC and mixture of chitosan and polycarbophil. IPECs complex showed good swelling, low erosion and slower drug release compared to polymeric matrix approaching zero-order kinetics. The mechanism of drug release followed diffusion from swollen systems.

BhusnureOmprkash G., et al., (2016)[123] investigated extended release tablet forming interpolyelectrolyte complex (IPEC) containing Eudragit E100 (cationic polymer) and Eudragit L100 (anionic polymer) polymers. Controlled drug delivery is can be achieved at location determined by needs of body or disease state over a specified period of time. The network formed between Eudragit E100 and L100 polymers had been coupled satisfactorily with controlled release of dugs. The results concluded that wetting forms interpolyelectrolyte complex (IPEC) and is suitable to formulate controlled release dosage form of Desvenlafaxine succinate.

Rosalia Rodriguez., et al., (2001) [124] reported the influence of structural properties and rheological behavior of Carbopol polymer. The results showed pH-sensitivity of Carbopol when used in the formulation. The rheological consistency of carbopol polymer increased dramatically when pH changed from 4.5 to 7.4 and vise versa. This structural property can be used to design pharmaceuticals with gelling system.

Sabar MH., et al., [125] investigatedthe formation of polyelectrolyte complex (PECs) using sustained release oral tablet of ketoprofen composed of chitosan (cationic polymer) and carbopol (anionic polymer).Using FT-IR spectra formation of Polyelectrolyte complex were analysed and found due to electrostatic interaction between the carboxyl group of Carbopol and amine group of Chitosan at pH5. Different factors influencing swelling index and release rate were studied and results showed that carbopol retarded drug release and also concluded pH dependent drug release characteristics of PECs complex containing carbopol.

Mustafin RI., (2011)[126] studied interpolyelectrolyte complex (IPEC) between Carbomer 940 and Eudragit® EPO using polycomplex matrix system (PMS) containing diclofenac sodium. Evaluation of pharmacokinetic parameters of PMS matrix revealed a close relationship with in vitro/in vivo correlation. By enteric coating of PMS matrix, it can be targeted to deliver the drug in colon region.

Hemant., et al.,[127] formulated theophylline extended release matrix tablets containing pH sensitive hydrogels like CMC and carbopol 934 which shows high swelling in basic pH and thus can delay the drug release in intestinal tract. In vivo studies exhibited controlled release of theophylline from hydrogel formulation for

prolonged period compared to marketed sustained release formulation. The study also stated that pH-sensitive hydrogel can be successfully used for extended release of theophylline in intestine in treating nocturnal asthma symptoms.

Parojcic J., et al.,[128] studied the pH dependent nature of Carbopol®971P NF which is a hydrophilic lightly cross-linked polymer of Carbomer series. Unlike other hydrophilic polymers, Carbopol® polymers do not dissolve, but swells in aqueous environments to forms 3-D gel structure. Due to anionic nature of Carbopol® polymers, drug release from matrices is pH-dependent. At low pH values, the drug is released faster. As pH increases, ionizationof carboxylic acid groups causes maximum swelling,resulting in fewer and smaller regions of microviscosity causing prolonged drug release.

Rouslan., et al.,[129] studied Eudragit®E100 based interpolyelectrolyte complex that shows solubility and drug release in weakly acidic buffer solutions up to pH 5.Presence of Eudragit®E100 modifies the swelling behavior of accompanying hydrophilic polymer. The pKa of eudragit E 100 is 7.0–7.3; hence it is partially protonated at pH close to 5, hence an electrostatic interaction with anotherionized polymers could contribute to drug release.

Lu., et al. (2007) [130] investigatedthe formation of separate chemical entity which is not just a simple mixture between chitosan and polycarbophil (oppositely charged polymers) was confirmedby differential scanning calorimetry. And the formation of interactions betweenthese two polymers was further confirmed byFT-IR spectroscopy. From FT-IR spectra, it is clear that a peak appeared at 1561 cm−1which maybe due to carboxylate groups of polycarbophil that formes ionic bond with the protonated amino groups of chitosan resulting in formation of interpolyelectrolyte complex. This was also studied in Eudragit E and Eudragit L (Moustafine., et al., 2005) where ionic bond is the primary binding force for formation of IPECcomplex.

AIM & OBJECTIVES

The main aim of the project was to develop a delivery system that claims the futuristic approach in the field of pharmaceutical science based on bioresponsiveness and timed delivery of drugs into the body.

The objective of the present study is to develop the delivery system and to evaluate its delivery profile based on triggered release, timed release and site targeting.

Salbutamol sulphate was chosen as the model drug for the present study to designa dosage form for treatment of asthma. Carbopol 971 P and eudragit E was chosen as key ingredients in the formulation to attain goal in this study.

The development of tablets-filled-capsule systems offer benefits for the production of sustained release multiple unit dosage form. This system has specific advantages over conventional single unit dosage forms. Advantages of multiple unit dosage forms such as Mini-tablet-in capsule systems include a lower risk of dose dumping, less inter and intra such variability and a high degree of dispersion in the digestive tract, thus minimizing the risks of high local drug concentrations.

Oral drug delivery represents by far the most common & convenient way of drug delivery. The gastro-Intestinal tract is still the route of choice for drug administration & absorption.

Chronopharmaceutics is a branch of pharmaceutics devoted to design and evaluation of drug delivery system that release a bioactive agent at a rhythm that ideally matches the biological requirement of a given disease therapy. Ideally chronopharmaceutical drug delivery system (ChrDDS) should embody time controlled and site specific drug delivery system.

A Bioresponsive drug delivery system is necessary since it works on the mechanisms capable of responding to physiological variations in the body, in order to synchronize drug- release profiles with changing physiological conditions.

PLAN OF WORK

1. literatue survey
2. procurement of drug , polymers and other excipients
3. Preformulation studies of mucoadhesive mini-tablets system (controlled release) by using different polymers
4. evaluation of preliminary batches of mucoadhesive mini tablets system (controlled release)
5. formulation of mucoadhesive mini tablets system (controlled release) using combination of polymers
6. evaluation of mucoadhesive mini tablets system (controlled release)
    A) Physical characterization of preliminary and Finalmucoadhesive mini tablets system (controlled release)
        a) weight variation
        b)Friability
        C)Hardness
        d)Thickness
        e) drug content
    B) In-vitro swelling study
    C) surface pH determination
    D) In-vitro bioadhesion study
    E) In-vitro drug release study
    F) In-Vitro permeation study
7. selection of capsule shell size for filling of mini tablets system (controlled release).

8. selection of polymers and other excipients for enteric coating (colon targeting) of mini tablets filled capsule system.
9. enteric coating (colon targeting) of mini tablets filled capsule system.
10. In-Vitro evaluation of enteric coated capsule system for colon targetting.
11.Selection and procurement of excipients for formulation of Immediate release tablets & pellets
12. formulation of immediate release tablets & pellets.

13. evaluation of physical parameters of immediate release tablets.

14. in-vitro drug release study of immediate release tablets.

15. selection of capsule shell for filling of enteric coated capsule system and an immediate release tablets.

16. Stability studies

DISEASE PROFILE

Asthma is common respiratory disease among adults and children whose prevalence is increasing worldwide; affecting 15 to 20 million Indians. Asthma leads to significant degrees of morbidity and mortality. It is characterized pathologically by lymphocytic and eosinophilic infiltration of the bronchial tree associated with airway narrowing. It is a syndrome characterized by symptoms such as (dyspnea, wheeze, chest tightness, and cough), airway dysfunction and airway inflammation with eosinophils and met achromatic cells [131].Causes of asthma include allergies, cold air, air pollutants, drugs, cigarette smoke, molds, exercise, and infections.

Asthma attacks (rapid worsening of symptoms) typically occur in episodes. Scientists now believe that asthma attacks vary according to the time of day.

The occurrence of asthma attacks is not random during day. Asthma symptoms frequently worse at night (nocturnal) for majority of asthma sufferers. The incidence of asthma attacks was more (100 times greater) during nighttime sleep, especially around 4 a.m., than it was during the middle of the day [132].

CIRCADIAN REGULATION:

Nocturnal asthma is variable exacerbation of underlying asthma condition associated with increase in symptoms, need for medication, airway responsiveness, and worsening of lung function. These changes are related to sleep and circadian events (24 hours) taking place at day time (diurnal) and at night time (nocturnal). The function of circadian regulation is to impose temporal organization on physiological processes and behavior. Disorders in circadian regulation may provoke exaggeration of disease symptoms.

Circadian rhythms follows two principal features: they run freely in absence of temporal cues particularly in light-dark cycle and under normal environmental circumstances entrained to light dark cycle [133].

These features indicate a neural system that expresses and regulates circadian function which involves circadian pacemaker(s); photoreceptors and visual pathways that transduce photic information into a neural information and transmit it to the pacemakers which output to the effector systems that expresses circadian function [134].

These effector systems then expresses physiological control mechanism over lung function and immunology. In asthma, there is a progressive increase in resistance across night, whether subjects is in sleep or not, the increase is much greater during sleep time [135].

CIRCADIAN CHANGES IN PULMONARY FUNCTION:

Normal and asthmatic subjects show circadian variation in pulmonary function as assessed by Spiro metric parameters such as forced expiratory volume(FEV1) and peak expiratory flow rate (PEFR), which lowest around 4 AM [136].

GUT- LUNG RELATIONSHIP

Research suggest that there's an apparent link between gut and the lungs. Scientist have come to realize that there is an increased rates of inflammatory bowel diseases in patients with asthma and COPD.

Chronic lung disorders such as asthma, chronic obstructive pulmonary diseases (COPD), cystic fibrosis, all exhibit a component of intestinal disease manifestations and vice versa.

Dr. talley sees relationship between the gut and lung, may be due to influx of cells called eosinophils. In his recent paper, he and his colleagues describe how patients with asthma and allergic rhinitis have abnormally high levels of eosinophils both

in their airways and in their large intestines. [137] Eosinophils are important immune system cells that are made in bone marrow and normally lines the mucous membrane in stomach, small intestine and colon.

The main purpose of these cells is to defend against bugs and toxins. But in peoples with asthma , the eosinophilic response is exaggerated, and exposure to allergens can trigger an excess of these cells in both gut and lungs.

Dr. talley said in an interview “I don’t think respiratory researchers have been terribly excited about gut problems, but I bet a lot of their asthma patients have gut problems if they ask them”

Asthma patients who quite commonly suffer from gastroesophageal reflux disease (GERD) or more severe conditions in colon cause gut-lung overlapping and eosinophilic excess was found both in gut and in lung.

Although eosinophils are normally protective in gut, but when in allergy, a glut in lungs and gastrointestinal tract reverses their function, causing damage to the mucous membranes. When this happens, the protective barrier in the gut is broken, allowing toxins to run into circulation causing inflammation. This increased leaky gut has been documented in asthma suffers.

While respiratory symptoms wax and wane with the seasons, gastrointestinal symptoms may wind up becoming chronic.

In the recent study published in the European respiratory journal, researches found an increased rate of inflammation in colon causing bowel diseases among patients with asthma and COPD. They finally concluded that peoples with asthma and COPD had a higher rate of colonic diseases [138].

Scientist believe that there is a connection between the lung and gut driven by number of factors including,

1. Shared risk factors – including smoking and family history
2. Prolonged inflammation- believed to be the root of many long term illnesses, including COPD and IBD

3. An abnormal immune system reaction to allergens and bacteria – in both respiratory and digestive tract.
4. Similarities in the mucus secreting membrane- lining the gut and lungs.

Pulmonary abnormalities, dysfunction or hyper responsiveness occurs in association with inflammatory bowel disease more frequently in colon. Emerging evidence suggests that subtle inflammation exist in colon among asthma patients and vice versa. Growing number of case reports shows that asthmatic patients develop rapidly progressive colonic inflammation such as IBD [139].

According to Chinese medicines, the lung and the intestine are pair of related organ systems (Biao-Li). IBD and COPD share many similarities in epidemiological and clinical characteristics, as well as in inflammatory pathology [140].

Figure 4: Related organ system

In a population-based cohort study, patients with COPD had significantly higher risk of IBD. Exposure to air pollution is an important environmental factor that directly in association with both asthma and IBD [141].

Local mucosal inflammation in airways may also be responsible for intestinal inflammation and vice versa. In view of the above phenomena, the lungs and intestine mainly colon are a pair of interacting organ systems[142-145]. Colby et al reported that bronchial hyper-reactivity occurred in 84 % of patients with colonic diseases[106]. Bronchial hyper-reactivity occurred in 71% of children and adolescents with CD[146].

The epithelial cells are considered not only the frontier sentinels and barriers, but also the central participants in innate and adaptive immune responses during mucosal inflammation. The airway and gut epithelial cells upon activation release large quantities of proinflammatory cytokines, growth factors and chemokines that attract inflammatory cells to initiate and sustain the inflammatory reaction[147-150].

Lung may duplicate atopic inflammation of the bowel and vice versa. Combined HRCT and histological examination have shown that there are multiple centers of airway epithelial (including goblet cells) responses and mucoid secretion in IBD [151-156].The interaction of lung and large intestine is mutually affected by internal and external relationships. It means that the lung diseases often have colon syndromes and colon diseases have lung syndromes [157-164]. Hence, Airways intrinsically accompany the main inflammation in the bowel and vice versa.

MECHANISM INVOLVED IN GUT LUNG RELATIONSHIP

Common physiology of the respiratory and gastrointestinal tracts

Respiratory and gastrointestinal epithelia share common embryonic origin in primitive foregut [165,166] which may account for similar inflammatory and immune components of these organs that are the cause of the overlap in pathological changes in respiratory and intestinal mucosal diseases.

Pattern recognition receptors:

Pattern recognition receptors highly conserved proteins that are expressed by cells of innate immune system. They recognize components termed “pattern-associated molecular patterns” of cellular stress signals, and damaged tissues. when activated, they induce production and secretion of inflammatory mediators and signalling molecules.

Two pattern recognition receptors families were involved in mucosal inflammatory response,

1. cytoplasmic NOD family of receptors
2. membrane-bound Toll-like receptor (TLR) family.[167-169]

Increases in prevalence of *NOD2* single-nucleotide polymorphism (SNP) was found in COPD and in colonic diseases. This SNP causes conformational change leading to activation of nuclear factor-κB which enhance inflammatory cytokine response upon stimulation [170].

Defects in NOD2 signaling leads to impaired epithelial barrier function, increased IL-1β, overcompensating TLR2 response, and promotes increases in serum IL-12 in both lung and in colon [171].

This inflammatory response was driven by IL-1β secretion from macrophages and neutrophil recruitment to lung tissue and in colon.

Smoke exposure study also drives TLR4 activation-dependent IL-8 production in monocyte-derived macrophages found in lung [172].

Inflammatory cytokine signalling results in increased TLR4 expression on macrophages from intestinal epithelium and lamina propria in colon [173,174].

Thus TLR4 activation have a common role in gut and lung resulting in hypersensitivity and exaggerated immune response in epithelial tissues and mucus lining of lung and intestine.

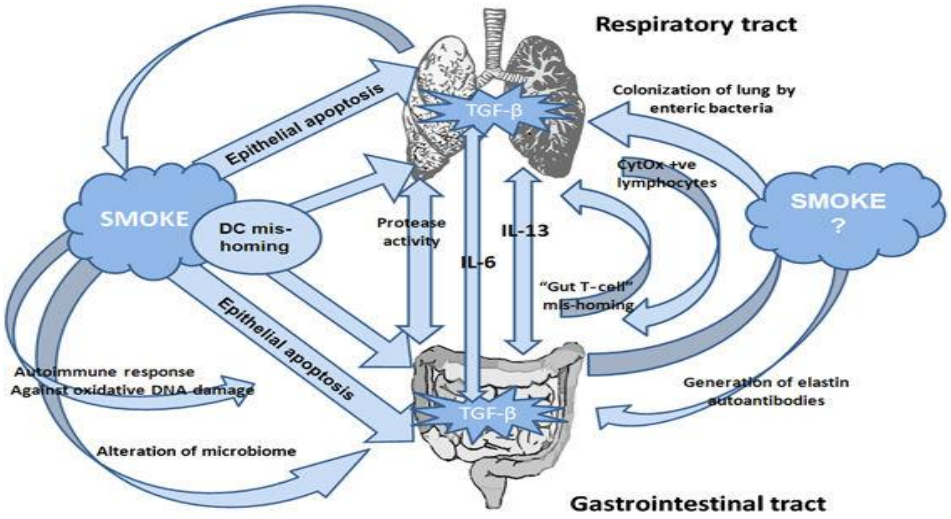


Figure 5: Factors involved in Gut – Lung relationship

Protease regulation in Lung and colon reflection:

There is evidence that mal-regulation of protease activity have a role in both COPD and IBD. Increased levels of proteases break down connective tissue components which have been identified in COPD & IBD patients and modeled in animals [175].

Increased level of epithelial and leukocyte MMP-2, MMP-9, and MMP-12 associated with the pathogenesis of COPD and IBD, which contributes to “runaway remodelling” process [176,177].

Immune cell homing and systemic factors:

Both COPD and IBD are considered as correlated systemic inflammatory diseases and peripheral lymphocytic activity may contribute to pathogenesis [178 -181].

During inflammation, bronchus-associated lymphoid tissue regulates the lymphocyte trafficking from lung tissue through general circulation [182].

This is mirrored by gut-associated lymphoid tissue. Both lung and intestinal lymphocytes migrate to other mucosal sites as a part of common mucosal immune response [183]. This trafficking is responsible for extra-organ inflammation associated with COPD and IBD.

Lymphocytes migrate through circulatory system in response to antigen exposure. They express unique homing receptors, which are specific for their target tissues. There is an evidence of abnormal function in peripheral lymphocytes that contribute to extrapulmonary disease in COPD patients.

Sauleda *et al.*, [184 showed increased cytochrome oxidase (CytOx) activity in circulating lymphocytes of COPD patients, which correlated with the study conducted by Salmi *et al.*, [185] with increased CytOx in IBD.

Analysis of sputum of IBD patients showed increased CD4/CD8 T-cell ratio in the lung tissue[186]. This represents lymphocyte mis-homing involved in pulmonary manifestations of IBD. Hence there relationship exist between gut and the lung.

IL-6 Plays role in acute phase response to inflammation and has been implicated in pathogenesis of both COPD [187,188] and IBD [189,190]. High levels of IL-6 and associated cytokines were identified in mucosa of both colon and lung in COPD patients.

IL-13 contribute to COPD progression and mutations in IL-13 promote pathogenesis in IBD. This activates macrophages, which in turn causes further IL-13 production.[191-196] This leads to STAT (signal-transducer and activator of transcription)6-dependent goblet cell hyperplasia, causing smooth muscle hyper-responsiveness in airway and in colon. [197,198].

GUT LUNG RELATIONSHIP AND CHANGE IN COLONIC pH IN ASTHMA

Intraluminal gastrointestinal pH was measured in patients. A radio-telemetry capsule was used and its location in GIT was determined by fluoroscopy. pH of the gastrointestinal tract was measured with small, pH sensitive, radiotransmitting capsule. The transmission frequency of the capsule is modulated by the pH of its surroundings and the signals from it were detected by a receiver. pH values were found normal in stomach and in small intestine. And low pH values were found in proximal part of colon in patients with IBD and respiratory diseases like asthma and COPD [199].

The colonic contents have longer residence time upto 5 days and the colonic mucosa is known to facilitate the absorption of several drugs, making this organ an ideal site for drug delivery.

Colonic pH is typically 5.5 to 7.0 for healthy persons but can drop to 4.7 and less in patients with asthma and colonic diseases which shows changes during attack. This can be ideally used for triggering drug release in colon using pH sensitive polymer technology [199].

OXIDATIVE STRESS IN ASTHMA

Asthma is aninflammatory illness with bronchial hyper reactivity with increasing number of inflammatory cells such as eosinophils, macrophages and lymphocytes found in bronchoalveolar lavage fluid in asthmatic patients (Caramori and Papi, 2004).

Measuring the exhaled levels of NO concentration provides useful noninvasive method to predict the bronchial hyper-responsiveness, airway obstruction and inflammation in asthma (Nogami, Shoji and Nishima, 2003).

Epidemiologic studies shows release of large number of inflammatory mediators associated with activation of lgE molecules, basophils and mast cells which involves synthesis of several proinflammatory mediators such as interleukin (IL)-4 and IL-13 (Schroederand MacGlashan, 1997). Histamine and leukotrienes are released from mast cells where as IL-4 and IL-13 are released from basophils.

Role of oxidative stress as a potential contributor to pathophysiology of asthma has been well explored.

An increased oxidative stress in asthma patients is associated with increased amounts of reactive oxygen species (ROS) like superoxide radial, hydroxyl radical and hydrogen peroxide generated by several inflammatory, immune and structural cells of the airways(Rahman, 2002). ROS play an important role in modulation of airway inflammation.

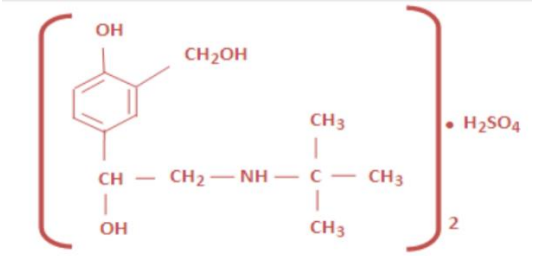
This concept is supported by the presence of high levels of ROS and oxidatively modified proteins in airways of asthma patients.

DRUG PROFILE

SALBUTAMOL SULPHATE

Salbutamol sulphate is a short-acting, selective β2-adrenergic receptor agonist used in the treatment of bronchial asthma and chronic obstructive pulmonary disease (COPD). Salbutamol sulphate is a racemic mixture of R- and S- isomer. R- isomer has 150 times greater affinity towards β2 – receptors.

Structural formula:



Synonym : Albuterol sulphate

Chemical name : 1-(4-hydroxy-3-hydroxymethylphenyl)–2–(t-butylamino)-ethanol sulphate.

Molecular weight : 288.35

Molecular formula : $C_{13}H_{21}NO_3$, H_2SO_4

Description : Odourless, slightly bitter in taste, white or almostwhite powder.

Melting point : 150 ºC

Pharmacology :

Mechanism of action:

Salbutamol stimulates β2-adrenergic receptors (predominant receptors in bronchial smooth muscles) of lungs.

Stimulation of β2-receptors causes activation of adenyl cyclase enzyme that forms cyclic adenosine-mono-phosphate (AMP) from adenosine-tri-phosphate (ATP). High level of cyclic AMP lowers intercellular ionic calcium concentrations which inturn relaxes bronchial smooth muscles and reduces airway resistence.

High level of cyclic AMP also inhibits the release of histamine, leukotriene (bronchoconstrictor mediators) from the mast cells in the airways.

Absorption:

On oral administration, approximately 50 % of salbutamol sulphate is absorbed from the intestinal tract with slower onset of action reaching peak concentration at about 2 hours. Through inhalation, only 10 – 20 % of salbutamol reaches lungs and the rest stays in mouth, stomach or on apparatus. The duration of action of salbutamol is 4 to 6 hours.

Distribution:

Salbutamol is not bound to plasma proteins and does not cross blood brain barrier to any significant extent. Salbutamol cross placental barrier, evidenced by increase in heart rate of fetus.

Metabolism:

Salbutamol is metabolized in liver by the process of conjugation with sulphate.

Excretion:

Salbutamol is excreted primarily via urine. Approximately 50 % is excreted as metabolites via urine and about 30 % is excreted as unchanged salbutamol in urine and rest in feces.

Dose:

Oral Immediate-releasetablets:

Initial dose: 2 mg or 4 mg orally three or four times a day. Dosage may be increased stepwise up to a maximum of 8 mg four times a day as tolerated (maximum of 32 mg/day).

Extended-release tablets:

Initial dose: 4 mg or 8 mg orally every 12 hours. The dosage can be increased stepwise under the control of physician to a maximum of 32 mg/day in divided doses (e.g., every 12 hours).

Use in pregnancy:

Salbutamol is pregnancy category C drugs. It can be used in pregnancy only if absolutely essential.

Use in nursing mothers:

No adverse effects have been reported in breast fed babies of mother receiving salbutamol sulphate.

Pediatric dose:

Not recommended in children below 2 years of age.

Precautions:

To be used in caution in patients with hypertension, hyperthyroidism, diabetes mellitus, and cardiac arrhythmia.

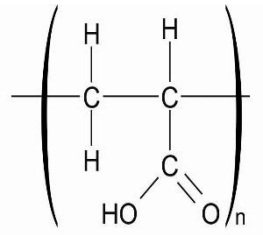
To be used in extreme caution with patients taking tricyclic antidepressant, monoamine oxidase inhibitors, thiazide diuretics**.**

Overdose:

Common symptoms include tremor, palpitation and tachycardia. It may also produce arrhythmia, seizures, fatigue, malaise, headache, dizziness and dry mouth.

EXCIPIENT PROFILE

1. CARBOPOL 971 P



Chemical name : Carboxy polymethelene

Empirical formula : (C3H4O2) x (-C3H5-sucrose) y

Viscosity : 4,000 to 11,000 cps at 2°C (0.5% neutralized aqueous solution).

Stability : It is unaffected by hydrolysis or oxidation and is resistant to bacterial growth.

Applications : Excellent thickening, emulsifying, suspending and gelling agent. It is used as tablet binder in sustained release formulations affording zero-order release. It is used as bioadhesive component in mucoadhesive tablets.

Safety : No primary irritation or any evidence of allergic reactions observed in humans following topical application.

2. EUDRAGIT E PO:

Aminoalkyl methacrylate copolymer

Solubility : Dissolves in aqueous isopropyl alcohol, methanol, ethanol and acetone. Practically insoluble in ethyl acetate, methylene chloride, petroleum ether and water.

Particle size : At least 95%, less than 0.25 mm.

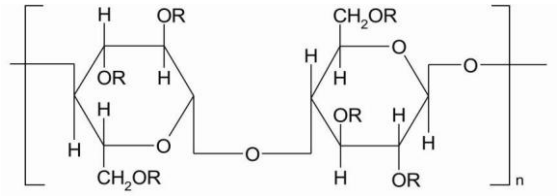
Film formation : When poured onto a glass plate, a clear film forms upon evaporation of the solvent.

Applications : Eudragit E po is an acid soluble polymer and hence used to mask bitter taste of formulations through film coating of tablets and pellets**.**

Storage : Protect from warm temperatures and against moisture.

Safety : Polymethacrylate co-polymers are widely used as film coating agent in oral pharmaceutical formulations. They are also used in lesser extent in topical formulations and are generally regard as nontoxic and non-irritant materials.

3. HYDROXYPROPYL METHYLCELLULOSE (Methocel K 100 M LVCR)



Molecular weight: Approximately 10,000 - 1,500,000.

Applications : HPMC is used as tablet binder, extended release tablet matrix, suspending and thickening agent. It is used as wetting agent for hard contact lenses and as adhesive in plastic bandages. HPMC shows temperature dependent change in viscosity. Raise in temperature reduces the viscosity of HPMC. It undergoes reversible gel-sol transformation depending on temperature of the surrounding environment.

Acidity / Alkalinity : pH = 5.5 - 8.0 (1% w/w aqueous solution).

Density (tapped) : 0.50 - 0.70 g / cm3

Melting point : 190-200 ^{o}C. Glass Transition Temperature170 - 180 ^{o}C.

Solubility : Soluble in cold water and practically insoluble in ethanol (95%), ether and chloroform. But soluble in mixtures of ethanol and dichloromethane.

Stability : It is stable in pH 3.0 to 11.0

Incompatibilities : Incompatible with some oxidizing agents and will not complex with metallic salts and ionic organics.

Safety : Extensively used in cosmetics, food products and widely used as an excipient in oral and topical formulations.

4. PROTANAL CR 8133 (Sodium alginate)

Description : Sodium alginate occurs as an odorless and tasteless, white to pale yellowish-brown coloured powder.

Sodium alginate CAS registry no. is [9005-38-3].

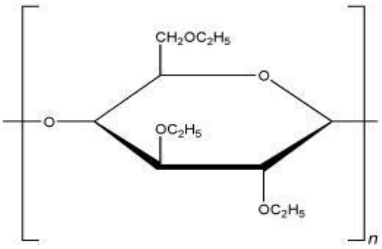
Application: Sodium alginate is the sodium salt of alginic acid. It is used in pharmaceutical formulations both as binders and disintegrants. It is also used in preparation of sustained release oral formulations as it delays the dissolution of drug from tablets.

Solubility& Visocity: Sodium alginate is practically insoluble in ethanol and other organic solvents but soluble in water slowly forming a viscous colloidal solution. Various

grades of sodium alginate are commercially available ranging from 20-400 cps. Its viscosity decreases above pH 8.0.

Stability: Sodium alginate is hygroscopic material. Stable if stored at low relative humidity's and cool temperature. It is susceptible on storage to microbial spoilage which may affect the viscosity. The bulk storage in air tight container in cool, dry place.

5. ETHYL CELLULOSE N 50



Chemical name: Cellulose ethyl ether (CAS no. 9004-57-3).

Nature of polymer : It is a derivative of cellulose in which some of the hydroxyl groups in repeating glucose units were converted into ethyl ether groups.

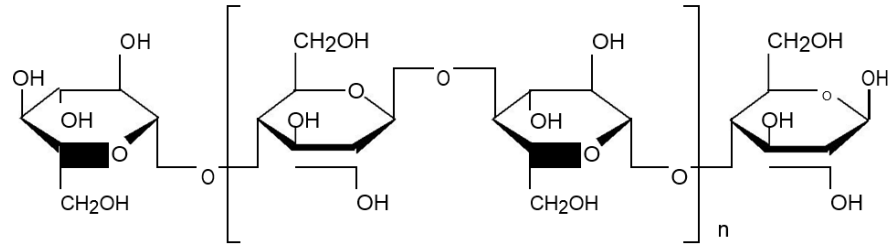
Typical characteristics: It is available as free flowing powder, white to off-white in color with a density of 0.4g/cm^2. It is practically insoluble in water, glycerol and propane-1-2-diol, but soluble in organic solvents. Ethyl cellulose containing 46-48% of ethoxyl group is freely soluble in ethanol, methanol, chloroform and ethyl acetate. Neutral to litmus.

Applications: It is mainly used as thin-film coating material. It is used as matrix in extended release matrix systems and provides improved lipophilic properties by increasing surface area. This flexibility is enhanced to modify release profiles when ethyl cellulose is used in combination with water-soluble excipients such as HPMC.

Stability : Ethyl cellulose is stable, slightly hygroscopic material. It is chemically resistant to alkalis.

Storage : Ethyl cellulose is prone to oxidative degradation in presence of UV light. Ethyl cellulose should be stored at a temperature not exceeding 32°C in a dry area and away from heat.

6. MICROCRYSTALLINECELLULOSE pH 102



Synonyms
Avicel PH; Celex; cellulose gel; Celphere; Ceolus KG; crystalline cellulose; E460; Emcocel; Ethispheres ; Fibrocel; Pharmacel; Tabulose;

Chemical Name and CAS Registry Number : Cellulose [9004-34-6]

Empirical Formula and Molecular Weight : $(C_6HO_{105})n$ = 36 000,where *n* =220.

Functional Category : Adsorbent, suspending agent, tablet and capsule diluent and tablet disintegrant.

Applications in Pharmaceutical Formulation or Technology:

Microcrystalline cellulose is widely used in pharmaceuticals, primarily as a binder/diluent in oral tablet and capsule formulations by direct compression processes. In addition to this, Microcrystalline cellulose is also used for its lubricant and disintegrant properties that make it useful in tableting. Microcrystalline cellulose is also used in cosmetics and food products.

Uses of Microcrystalline cellulose.
1. Adsorbent 20–90 %
2. Antiadherent 5–20%
3. Capsule binder/diluents 20–90%
4. Tablet disintegrant 5–15%
5. Tablet binder/diluents 20–90%

Description

Microcrystalline cellulose is a purified, partially depolymerized cellulose that occurs as white, odorless, tasteless, crystalline powder with porous particles.

Typical Properties
Density (bulk) : 0.32 g/cm
Density (tapped) : 0.45 g/cm

Density (true) : 1 .512–1.668 g/cm
Melting point : Chars at 260–270°C.

Moisture content: Typically less than 5% w/w. However, different grades may contain varying amounts of water. Microcrystalline cellulose is hygroscopic.

Particle size distribution: Typical mean particle size is 20–200µm. Different grades may have a different nominal mean particle size.

Solubility: Slightly soluble in 5% w/v sodium hydroxide solution; practically insoluble in water, dilute acids, and most organic solvents.

Stability and Storage Conditions:

Microcrystalline cellulose is a stable though hygroscopic material. The bulk material should be stored in well-closed container in a cool, dry place.

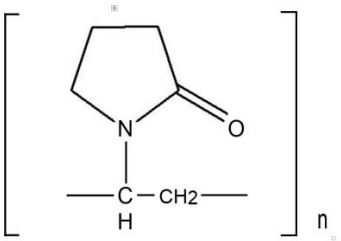
Incompatibilities

Microcrystalline cellulose is incompatible with strong oxidizing agents.

Safety

Microcrystalline cellulose is widely used in oral pharmaceutical formulations and regarded nontoxic and non irritant. Microcrystalline cellulose is not absorbed systemically following oral administration. Consumption of large quantities of cellulose may have a laxative effect.

7. CROSPOVIDONE XL-10



Synonyms:

Crosslinked povidone, E1202, Kollidon CL,Kollidon CL-M, Polyplasdone XL, PolyplasdoneXL-10, polyvinylpolypyrrolidone, 1-vinyl-2-pyrrolidinone homopolymer.

Chemical Name and CAS Registry Number : 1-Ethenyl-2-pyrrolidinone homopolymer [9003-39-8]

Empirical Formula and Molecular Weight : $(C_6H_9NO)n$ >1 000000. The USPNF 23 describes Crospovidone as a water-insoluble synthetic crosslinked homopolymer of *N*-vinyl-2-pyrrolidinone. An exact determination of the molecular weight has not been established due its insolubility.

Functional Category: Tablet disintegrant

Applications in Pharmaceutical Formulation or Technology

Crospovidone is water-insoluble tablet disintegrant and dissolution agent used at 2–5% concentration in tablets prepared by direct compression or wet- and dry-granulation methods. It rapidly exhibits high capillary activity and pronounced hydration capacity, with little tendency to form gels. Studies suggest that the particle size of Crospovidone strongly influences disintegration of tablets. Larger particles provide a faster disintegration than smaller particles. Crospovidone can also be used as solubility enhancer. With the technique of co-evaporation, Crospovidone can be used to enhance the solubility of poorly soluble drugs. The drug is adsorbed on to Crospovidone in the presence of a suitable solvent and the solvent is then evaporated. This technique results in faster dissolution rate.

Description

Crospovidone is white to creamy-white, finely divided, free flowing, tasteless, odorless or nearly odorless, hygroscopic powder.

Typical Properties

Acidity/alkalinity : pH = 5.0–8.0 (1% w/v aqueous slurry)
Density : 1.22 g/cm3
Density (bulk) : 0.35 g/cm3
Density (tapped) : 0.45 g/cm3
Moisture content : Maximum moisture sorption is approximately 60%.Particle size distribution : 50% greater than 50 m and maximum of 3% greater than 250 µm
Solubility : Practically insoluble in water and most common organic solvents.
Specific surface area : 1.0 m2/g
Storage Conditions : Crospovidone is hygroscopic, it should be stored in an airtight container in acool, dry place.

Incompatibilities

Crospovidone is compatible with most organic and inorganic pharmaceutical ingredients. When exposed to a high water level, Crospovidone may form molecular adducts with some materials.

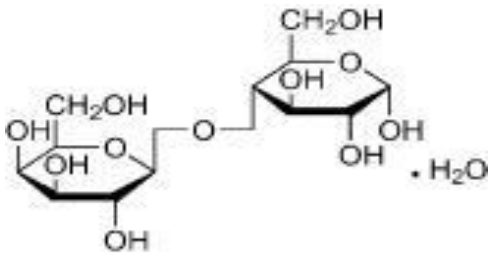
Safety

Crospovidone is used in oral pharmaceutical formulations and is generally regarded as nontoxic and nonirritant material. Short-term animal toxicity studies have shown no adverse effects associated with Crospovidone. However, owing to the lack of available data, an acceptable daily intake in humans has not been specified by the WHO.
LD50 (mouse, IP): 12 g/kg.

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection, gloves and dust mask are recommended.

8. LACTOSE MONOHYDRATE DCL-11



Description: Lactose monohydrate occurs as white, odourless, free flowing powder slightly sweet in taste. It is a natural disaccharide, obtained from milk, which consists of one glucose and one galactose moiety.

Application : Lactose monohydrate, spray dried lactose and anhydrous lactose are widely used as diluent in tablets and capsule formulations. It produces a hard tablet and the tablet hardness increases on storage. Disintegrant is usually needed in lactose containing tablets. Drug release rate is usually not affected.

Stability: It is usually non reactive, except for discoloration when formulated with amines and alkaline materials due to maillard reaction. It contains approximately 5% water.

Processing: It needs high compression pressures in order to produce hard tablets. Lactose monohydrate (SuperTab® 30) is produced by fluid bed granulation and has good flow properties. It shows consistent compaction over a wide range of humidity.

Storage: Mould growth may occur under humid conditions. Lactose should be stored in a well closed container and stored in cool dry place.

9. Croscarmellose sodium:

Chemical name : Cellulose carboxymethyl ether sodium salt

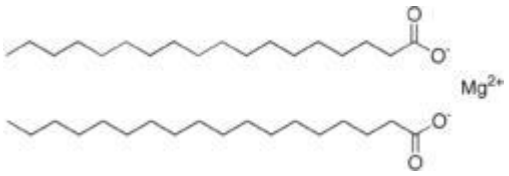
Category: Tablets and capsule disintegrant

Application in Pharmaceutical formulations:

Croscarmellose sodium is used in oral pharmaceutical formulation as disintegrant for capsules, tablets and granules. In tablet formulation, croscarmellose sodium can be used in both direct compression and wet-granulation process. 10 - 25 % can be used in capsules, 0.5 to 5.0 % used in tablets.

Solubility : insoluble in water, swells rapidly 4 – 8 times its volume on contact with water. Practically, insoluble in acetone and ethanol.

10. MAGNESIUM STEARATE



Magnesium stearate [CAS no. 557-04-0] is official in Ph.Eur., USPNF, BP and JP.

Description : Magnesium stearate is white and solid at room temperature.

Chemical formula: Mg $(C_{18}H_{35}O_2)_2$. It is the salt containing two equivalents of stearate (the anion of stearic acid) and one magnesium cation (Mg++).

Safety : Magnesium stearate melts at about 88°C, is not soluble in water and is generally considered safe for human consumption at levels below 2500 mg/kg.

Pharmaceutical application:

Magnesium stearate is used for its lubricating properties in tablets, capsule and powder, producing good flow properties of powder blend and preventing ingredients from sticking to manufacturing equipment during compression into tablets. Studies have shown that that magnesium stearate may affect release of active ingredients from tablets. Magnesium stearate is manufactured from both animals and vegetables.

11. COLLOIDAL SILICON DIOXIDE(Aerosil)

Colloidal anhydrous silica available under the trade name Aerosil 200 sourced from Evonik. Aerosil® 200 is hydrophilic fumed silica with a specific surface area of $200m^2/g$ with a particle size of about 15 nm.

Molecular formula : SiO_2
CAS registry no: [7631-86-9]
Molecular weight: 60.08.

Descripton: It is loose, bluish-white colored, odorless, tasteless, non gritty amorphous powder.

Pharmaceutical application: It is frequently used in pharmaceutical formulation to improve flow properties of powder blend in tablet and capsule manufacturing. Aerosil 200 is intended specifically for pharmaceutical industry, tested in accordance with

European, American and Japanese pharmacopeia. Addition to 0.2 - 1.0% by weight of Aerosil® colloidal silica. In many formulations, hydrophilic Aerosil® colloidal silicon dioxide increases the rate of tablet disintegration and release of active ingredients.

12. PURIFIED TALC:

Emperical formula : $Mg_6 (Si_2O_5)_4(OH)_4$

Category: Glidant, tablet and capsule lubricant.

Description: Very fine, white to grayish white, odourless powder. It adheres readily to skin and is free from grittiness.

Solubility: Insoluble in dilute acids, alkalis, organic solvents and water.

Stability and storage: Stable material and can be sterilized by heating at 160 °C. It can be sterilized by exposure to ethylene oxide and gamma irradiation and should be stored in well-closed container in cool place.

Incompatibilities: Incompatinble with quarternary ammonium compounds.

Applicaton: Used as lubricant in tablet formulations, In novel powder coating for extended release pellets and as adsorbent.

Stability: croscarmellose sodium is stable.

13. EUDRAGIT - L100

Molecular weight : approximately 135,000.

Solubility : Dissolves in aqueous isopropyl alcohol,methanol, ethanol and acetone. Practically insoluble in ethyl acetate, methylene chloride, petroleum ether and water.

Particle size : At least 95%, less than 0.25 mm.

Film formation : When poured onto a glass plate, a clear film forms upon evaporation of the solvent.

Applications : Polymethacrylates are primarily used in oral capsule and tablet formulations as film coating agents. Depending on the type of polymer used, films of different solubility characteristics can be produced. Used in enteric coating formulations to target drug solubility at pH above 6 in GIT.

Storage : Protect from warm temperatures and against moisture.

Safety : Polymethacrylate co-polymers are widely used as film coating agents in oral pharmaceutical formulations. They are also used to a lesser extent intopical formulations and are generally regard as nontoxic and non-irritant materials.

MATERIALS & INSTRUMENTS

8.1 Materials used for formulation of Mucoadhesive tablet:

s.no	Name of the chemicals	Mfg./ gifted by	Control / lot number
1	Salbutamol sulphate	Supriya life science ltd	S/114/15-16
2	Carbopol 971 P (acrypol)	Corel pharma chem	44215008
3	Eudragit E PO	Evonik industries	B131203014
4	Methocel K 100 M LVCR (ID2964)	Colorcon	GA448695
5	Protanal CR 8133	FMC health & Nutrition	GQB0204703
6	Ethyl cellulose EC-N 50	TaianRuitai cellulose	N502012100288
7	Microcrystalline cellulose ph 102	DFE pharma	170425W2
8	Magnesium stearate	Amishi drugs & chemicals	270417
9	Aerosil	Wacker	YA46966

8.2 Materials used for formulation of Immediate release tablets:

s.no	Name of the chemicals	Mfg./ Gifted by	Control / lot number
1	Salbutamol suphate	Supriya life science ltd	S/114/15-16
2	Lactose monohydrate DCL-11	DFE pharma	1016ZD5
3	Microcrystalline cellulose ph 102	DFE pharma	170425W2
4	Magnesium stearate	Amishi drugs & chemicals	270417
5	Crospovidone XL-10	Huangshan Bonsun pharmaceuticals	BP20170303
6	Croscarmellose sodium	Amishi drugs & chemicals	ACRMC/03-0/240317

8.3 materials used for Enteric coating of capsule shell:

s.no	Name of the chemicals	Mfg./ Gifted by	Control / lot number
1	Eudragit E PO	Evonik industries	B131203014
2	Opadry enteric coating system (white) 940580000 - eudragit L100	colorcon	TNL59653
3	Lactulose	Lactose india limited	L15009
4	Triethyl citrate	Indo-GSP	1702TEC028
5	Isopropyl alcohol	Hazchem	1219

Empty hard gelatin capsule shell “2” size and “0 el” size was purchased from natural capsules ltd.

All chemicals and reagent used were of AR grade.

INSTRUMENTS USED

s.no	Instrument name	Company	Equipment number
1	Weighing balance	Vibra – Essae teraoka	FD/INS/039
2	UV spectrophotometer (UV-1800)	Shimadzu	A114550
3	FT-IR spectrometer	Perkin elmer	AD/INS/066
4	Differential scanning calorimetry	-	FD/INS/005
5	pH meter MP-1 plus	Susima	AD/INS/086
6	Roche friabilator EF-2	Electrolabs	AD/INS/011
7	Monsanto Hardness tester	Shankar	FD/INS/021
8	Digital vernier caliper	Mitutoyo	FD/INS/022
9	Mini Tableting machine MRT-8	Kambert	FD/INS/006
10	Octagonal blender 10L	Sams techno mech	FD/INS/007
11	Capsule filling machine MFD-418	ACG-pam pharma tech	FD/INS/014
12	Coating machine	Sams techno mech	FD/INS/017
13	Dissolution apparatus	Electrolabs	ETC15LX
14	Syringe pump	Electrolabs	ESP-124(B)
15	Sample collector	Electrolabs	ESC-12DX
16	DT apparatus ED-2L	Electrolabs	FD/INS/018
17	Tray dryer	Rays scientific instruments	FD/INS/011
18	Multi mill	Sams techno mech / croma	FD/INS/010
19	Packing machine (Ezee blist)	Mechtek	FD/INS/035
20	Leak test apparatus	Labpro	FD/INS/020
21	Tap density tester	Electrolabs	FD/INS/013
22	Electromagnetic sieve shaker	Electrolabs	FD/INS/036
23	Lab scale stirrer	Remi motors	FD/INS/024
24	Stability chamber	Thermolabs	FD/INS/038

PUNCHES & TOOLING:

S.no	Punch description	Tooling	Mfg by
1	5.5 mm circular standard concave plain punch	B tooling	Eliza tools
2	6.35 mm circular standard concave plain punch	B tooling	Eliza tools

PREFORMULATION

Preformulation is the first step in rational development of dosage forms which involves investigation of physical and chemical properties of drug substances alone and in combination with excipients.

Preformulation supports pharmaceutical and analytical investigation with efforts formulation and development of the dosage form.

9.1 Organoleptic properties:

9.1.1 Color and nature:

A small quantity of the sample was spreaded on a piece of paper and examined visually

9.2 Physical characteristics:

9.2.1 Flow properties:

The flow property of the powder blend is crucial for uniform filling of blended drug-excipient powder into die cavity for effective compression of tablets.

Angle of Repose:

Angle of repose is the maximum angle between the surface of pile of powder and the horizontal plane. The angle of repose was determined by the funnel-method. The accurately weighed Quantity of powder blend was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the pile formed. The powder blend were allowed to flow through the funnel freely onto the surface of the graph paper. The diameter and height of the pile formed is measured and angle of repose was calculated using the equation.

$\theta = \tan^{-1} h/r$
θ = Angle of repose
h = height of the pile
r = radius of the pile

Table 4: Angle of repose

Angle of repose	Flowability
< 25	Excellent
25 - 30	Good
30 - 40	Passable
>40	Very poor

Determination of Bulk Density:

Bulk density is the ratio of mass of the powder to the bulk volume. Bulk density is determined by measuring the volume of known mass of the powder blend using a graduated cylinder.

An accurately weighed quantity of the granules/ powder (m) was carefully poured into the graduated measuring cylinder and the volume (V_b) was measured. The bulk density of the powder blend is denoted by gram perml. it is calculated using the formula,

$$\rho_{b = m / V_b}$$

ρ_b= bulk density

m = mass of the powder

V_b = initial / bulk volume

Determination of Tapped Density:

Tap density is the ratio of mass of the powder to tapped volume.The graduated cylinder which holds known initial volume and mass of the powder blend was closed with lid and set into the tap density tester (USP). The density apparatus was set for 100 tabs and after that the volume (V_t) was measured.

Tapped density was calculated using the following formula,

$$\rho_{t =} m / V_t$$

Compressibility Index (Carr's Index):

Compressibility index is the measure of propensity of powder blend to be compressed. It measures relative importance of inter particulate interactions. In case of free flowing powder, such interactions are relatively less and greater interaction was found in poor flowing materials. This difference can be observed in bulk and tapped density and reflected when compressibility index is calculated

$$\% \text{ compressibility} = \frac{\text{Tapped density – initial bulk density}}{\text{Tapped density}} \times 100$$

Table 5 : compressibility index

% Compressibility	Flow ability
5 – 12	Excellent
12 – 16	Good
18 – 21	Fair
23 – 25	Poor
33 – 38	Very poor
More than 40	Very very poor

Hausner's Ratio:

It is the ratio of tapped density and bulk density.

$$\text{Hausner ratio} = \rho_t / \rho_b$$

Hausner found that this ratio was related to Interparticle friction and, as such, could be used to predict powder flow properties). Generally a value less than 1.27 indicates good flow properties, which is equivalent to 20% of Carr's index.

COMPATIBILITY STUDIES:

The compatibility of the drug with excipients was determined by assay. The study was done by filling 1:1 ratio of powder blend into the vial and loading it into accelerated stability chamber 40° C / 75% RH for a period of 30 days and analysed at Initial, 15th day and 30th day for Physical appearance (color change) and Assay of salbutamol sulphate.

The assay was determined by UV absorption spectroscopy against freshly prepared standard solution.

Table 6 : Compatibility Studies of Drug and Excipients

Ingredients	Sample No.	Condition	Initial %	40°C/75% RH condition	
		Vial		15days	30days
Salbutamol Sulphate (A)	A-1	Open	99.98	99.93	99.89
	A-2	Closed		99.95	99.88
Carbopol 971 P (B)	B-1	Open	-	-	-
	B-2	Closed		-	-
Salbutamol sulphate + Carbopol 971 P	AB-1	Open	98.93	98.89	98.87
	AB-2	Closed		98.91	98.86
Eudragit E po	C-1	Open	-	-	-
	C-2	Closed		-	-
Salbutamol sulphate + Eudragit E po	AC-1	Open	98.95	98.90	98.86
	AC-2	Closed		98.85	98.79
Salbutamol sulphate+ Carbopol 971 P +	ABC-1	Open	98.89	98.81	98.76

Eudragit E po	ABC-2	Closed		98.84	98.80
Ethyl cellulose N 50	D-1	Open	-	-	-
	D-2	Closed		-	-
Salbutamol sulphate + Ethyl cellulose N 50	AD-1	Open	98.92	98.89	98.77
	AD-2	Closed		98.90	98.85
Protanal CR 8133	E-1	Open	-	-	-
	E-2	Closed		-	-
Salbutamol sulphate + Protanal CR 8133	AE-1	Open	98.94	98.84	98.72
	AE-2	Closed		98.81	98.76
Hydroxypropyl methyl cellulose K 100 M LVCR	F-1	Open	-	-	-
	F-2	Closed		-	-
Salbutamol sulphate +HPMC K 100 M LVCR	AF-1	Open	98.91	98.83	98.75
	AF-2	Closed		98.86	98.74
Mcc ph 102	G-1	Open	-	-	-
	G-2	Closed		-	-
Salbutamol sulphate + Mcc ph 102	AG-1	Open	99.46	99.26	98.87
	AG-2	Closed		99.27	98.92
Lactose monohydrate DCL-11	H-1	Open	-	-	-
	H-2	Closed		-	-
Salbutamol sulphate + Lactose monohydrate DCL-11	AH-1	Open	99.64	99.43	99.19
	AH-2	Closed		99.52	99.24
Crospovidone XL 10	I-1	Open	-	-	-
	I-2	Closed		-	-
Salbutamol sulphate + crospovidone XL 10	AI-1	Open	99.71	99.62	99.51
	AI-2	Closed		99.63	99.45
Croscarmellose sodium	J-1	Open	-	-	-
	J-2	Closed		-	-
Salbutamol sulphate + croscarmellose sodium	AJ-1	Open	99.73	99.46	99.25
	AJ-2	Closed		99.51	99.30
Magnesium stearate	K-1	Open	-	-	-
	K-2	Closed		-	-

Salbutamol sulphate +Magnesium	AK-1	Open	98.84	98.49	98.38
stearate	AK-2	Closed		98.53	98.45
Aerosil	L-1	Open	-	-	-
	L-2	Closed		-	-
Salbutamol sulphate + aerosil	AL-1	Open	99.61	99.40	99.31
	AL-2	Closed		99.47	99.39
Carbopol 971 P + Eudragit E po + mcc ph 102 + magnesium stearate+ Aerosil	M-1	Open	-	-	-
	M-2	Closed		-	-
Salbutamol sulphate + Carbopol 971 P + Eudragit E po + mcc ph 102 + magnesium stearate+ Aerosil	AM-1	Open	98.69	98.51	98.36
	AM-2	Closed		98.72	98.63
Carbopol 971 P + Eudragit E po + ethyl cellulose + mcc ph 102 + magnesium stearate+ Aerosil	N-1	Open	-	-	-
	N-2	Closed		-	-
Salbutamol sulphate+ Carbopol 971 P + Eudragit E po + ethyl cellulose + mcc ph 102 + magnesium stearate+ Aerosil	AN-1	Open	98.71	98.50	98.47
	AN-2	Closed		98.56	98.49
Carbopol 971 P + Eudragit E po + Protanal CR 8133 + mcc ph 102 + magnesium stearate+ Aerosil	O-1	Open	-	-	-
	O-2	Closed		-	-
Salbutamol sulphate+ Carbopol 971 P + Eudragit E po + Protanal CR 8133 + mcc ph 102 + magnesium stearate+ Aerosil	AO-1	Open	98.67	98.42	98.36
	AO-2	Closed		98.45	98.31
Carbopol 971 P + Eudragit E po + HPMC K100M LVCR + mcc ph 102 + magnesium stearate+ Aerosil	P-1	Open	-	-	-
	P-2	Closed		-	-
Salbutamol sulphate+ Carbopol 971 P + Eudragit E po + HPMC K100M LVCR + mcc ph 102 + magnesium stearate+ Aerosil	AP-1	Open	98.66	98.44	98.30
	AP-2	Closed		98.49	98.38
Lactose DCL - 11 + mcc ph 102 + crospovidone XL 10 + magnesium stearate	Q-1	Open	-	-	-
	Q-2	Closed		-	-
Salbutamol sulphate+ Lactose DCL - 11 + mcc ph 102 + crospovidone XL 10 + magnesium stearate	AQ-1	Open	98.91	98.65	98.54
	AQ-2	Closed		98.71	98.67
Lactose DCL - 11 + mcc ph 102 +	R-1	Open	-	-	-

croscarmellose sodium + magnesium stearate	R-2	Closed		-	-
Salbutamol sulphate + Lactose DCL - 11 + mcc ph 102 + croscarmellose sodium + magnesium stearate	AR-1	Open	98.87	98.75	98.61
	AR-2	Closed		98.76	98.63

SPECTROSCOPIC STUDIES:

FTIR studies:

Characterization of salbutamol sulphate:

The infrared spectrum of pure salbutamol sulphate was recorded and spectral analysis was done. The dry sample of the drug was thoroughly mixed with potassium bromide and pressed into pellets and then directly placed in sample holder and analysed under FTIR.

Characterisation of Interpolyelectrolyte complex:

The infrared spectrum of Carbopol 971 P and Eudragit E po was recorded and spectral analysis was done to identify the formation of "Interpolyelectrolyte complex".The dry sample of carbopol 971 P and Eudragit E po was thoroughly mixed with potassium bromide and pressed into pellets and then directly placed in sample holder and analysed under FTIR.Similarly, IR spectrums of salbutamol sulphate and polymers (1:1 ratio) was analysed.

UV spectroscopy (Determination of λ max):

UV spectrum of salbutamol sulphate was carried out in 0.1 N Hcl, pH 6.8 phosphate buffer and pH 7.4 Phosphate buffer. Salbutamol sulphate was weighed accurately and transferred to 100 ml volumetric flask. This is dissolved and volume made up with distilled water. This solution was treated as stock solution and contains 100 µg/ml of salbutamol sulphate. The solution was appropriately diluted with distilled water to obtain a concentration range of 10 µg/ml of salbutamol sulphate. The UV spectrum was recorded at 276 nm.

Preparation of standard curve:

Salbutamol sulphate (10 mg) was weighed accurately and weighed accurately and transferred to 100 ml volumetric flask. This is dissolved and volume made up with distilled water. This solution was treated as stock solution and contains 100 µg/ml of salbutamol sulphate. From this stock solution 0.2 , 0.4 , 0.6 , 0.8 , 1.0 , 1.2 ml were withdrawn and taken in separate 10 ml volumetric flask. The volume was made with buffer solution to obtain concentrations of 2,4,6,8,10,12 µg/ml respectively. Absorbance of these solutions were measure at 276 nm for preparation of standard curve. Using absorbance concentration data, lamberts and beers graph was plotted. This is used for estimating the release of drug from its dosage form.

METHOD FOR TESTING SOLUTION STABILITY:

Since dissolution was to be carried out for a period of 72 hours (3 days) the stability of the dissolution medium is important.

The test is conducted by comparing the freshly prepared standard solution with that of the simulated dissolution medium which is priorly prepared and studied for a period of 75 hours.The analysis was carried out every 12 hours.

Preparation of Fresh Standard solution:

Standard solution of 10 µg/ml of salbutamol sulphate was prepared freshly in 6.8 pH phosphate buffer medium whenever the study is being carried out.

Preparation of dissolution medium for analyzing solution stability:

Medium A :10 mg of salbutamol sulphate was accurately weighed and dissolved in 100 ml of 6.8 pH phosphate buffer such that its concentration ranges 100 µg/ml. The medium was stored in 1000 ml beaker and closed with lid. Samples where withdrawn every 12 hours interval and diluted to attain concentration of 10 µg/ml and analyzed against freshly prepared standard solution under spectroscopy at 276 nm.

Medium B : 10 mg of salbutamol sulphate was accurately weighed and dissolved in 100 ml of 6.8 pH phosphate buffer containing 5 % of goat's caecal content (100 µg/ml of salbutamol sulphate) . The medium was stored in 1000 ml beaker and closed with lid. Samples where withdrawn every 12 hours interval and diluted to attain concentration of 10 µg/ml and analyzed against freshly prepared standard solution under spectroscopy at 276 nm.

Table 7: Method to test solution stability

S.no	Time interval (hours)	Fresh Standard (10 µg/ml)	Medium A		Medium B	
			Blank	Test	Blank	Test
1	Initial	100.00 %	0 %	99.93%	0 %	99.90%
2	12^{th} hour	100.00 %	0 %	99.84%	0 %	99.72%
3	24^{th} hour	100.00 %	0 %	99.79%	0 %	99.51%
4	36^{th} hour	100.00 %	0 %	99.81%	0 %	99.35%
5	48^{th} hour	100.00 %	0 %	99.73%	0 %	99.27%
6	60^{th} hour	100.00 %	0 %	99.68%	0 %	98.79%
7	72^{th} hour	100.00 %	0 %	99.57%	0 %	98.14%
8	84^{th} hour	100.00 %	0 %	99.36%	0 %	97.83%

PREFORMULATION STUDY FOR COATING:

Eudragit E po:

Eudragit E po is dissolved in IPA: methylene dichloride 50:50 ratio and triethyl citrate was used in various proportion to confirm film formation using a glass slide. 10 % triethyl citrate is sufficient to form better film.

Eudragit E po dissolves completely in methylene dichloride but web like formation was found during automization from nozzle.

Hence, IPA: water 90:10 ratio was used as recommended in literature published by Evonik.

Talc was added as anti-tacking agent to prevent stickiness during coating process. 8 % talc is sufficient to prevent excess stickiness.

Eudragit L 100:

Eudragit L 100 is dissolved in IPA and triethyl citrate was used in various proportion was used to confirm film formation using a glass slide. 10 % triethyl citrate is sufficient to form better film.

Eudragit L 100 dissolves completely in methylene dichloride but web like formation was found during automization from nozzle.

Hence, IPA: water 90:10 ratio was used as recommended in literature published by Evonik.

Talc was added as anti-tacking agent to prevent stickiness during coating process. 8 % talc is sufficient to prevent stickiness.

FORMULATION

FORMULATION OF MUCOADHESIVE TABLETS

The main aim of formulation is to develop a mucoadhesive tablets as drug delivery system in colon which forms interpolyelectrolyte complex (IPEC) forming hydrogel in contact with water which delivers controlled release of drug as well as triggered release as per chronobiological aspects.

The main theme of forming interpolyelectrolyte complex is advantageous such that the system regains its initial state when applied stress condition in removed.

Through IPEC complex , initial burst release can be prevented, dose dumping can be prevented.

The developed formulation should also be simple for ease of manufacturing and reproducibility such that cost effective dosage form can be delivered to patients. Hence, tableting process is adopted for this novel drug delivery system which is simple and easy to manufacture compared to complicated systems like microencapsulation and nanotechnology which involves high cost of production.

Eudragit E po (Aminoalkyl methacrylate co-polymer) an anionic polymer and carbopol (cationic) polymer (containing carboxylate backbone) was chosen for the formation of “interpolyelectrolyte” complex. Due to opposite charge of polymers, they attract each other and forms complex which reduces swelling and erosion.

Carbopol 971 P is selected because of its good mucoadhesive property and recent report by *Lubrizol* that carbopol 971 P has the ability to form reversible gel to sol transformation inpresence of hydroxyl radicals and when pH is reduced.

Salbutamol sulphate was chosen as the model drug since it is highly soluble and stable. Since it is potent drug, formulation of mini tablets system is merely possible.

To develop a 3 days once dosage form, 24 mg of salbutamol sulphate is to be delivered as mucoadhesive mini tablets in capsule system.Each mini tablets containing 6 mg of salbutamol sulphate. The dosage was aimed to delivereach 4 mg of salbutamol sulphate in colon for 12 hours in controlled manner as specified in USP.

In the preformulation study it is known that combination of eudragit E po & carbopol 971 P possed poor flow. Hence direct compression method becomes impossible.

The drug : polymer was prepared in various ratio’s and analysed under FTIR for determination interpolyelectrolyte complex forming hydrogel system.

Table 8 : Initial Formulation involving Wet granulation method:

Formula trial	Drug:polymerratio	Salbutamol sulphate	Eudragit E po	Carbopol 971 P	Purified water	Isopropyl alcohol	Mccph 102	Mg. stearate	Avg. wt
FA1	1:1:1	6 mg	6 mg	6 mg	Qs	-	60 mg	2 mg	80 mg
FA2	1:1:1	6 mg	6 mg	6 mg	-	qs	60 mg	2 mg	80 mg

The blend was analysed in FTIR for the formation of hydrogel forming interpolyelectrolyte complex. FA1 batch which is granulated with purified water shows formation of interpolyelectrolyte complex hydrogel system. FA2 batch granulated with isopropyl alchohol, the complex formation was not clear.

Hence, water is regarded as the mediator for formation of such hydrogel IPEC system.

In wet granulation process a problem is faced during granulation. On addition of water during granulation it forms a sticky mass which is elastic in nature due to hydrogel complex formation. Since colon contains water content, and the system is capable of forming hydrogel complex in presence of water the development was focused on dry granulation (slugging process) to improve the flow property of the powder blend.

In slugging process, carbopol 971 P and eudragit E po was blended in 1:1 ratio and various proportion of magnesium stearate was added to the blend to improve the flow property. But there is no improvement in flow property. Hence, combination of colloidal silicon dioxide (aerosil) and magnesium stearate was used to improve flow property and results are satisfactory.

1:1:1 ratio of salbutamol sulphate ,carbopol 971 P and eudragit E po was blended and analysed under FTIR. The blend showed no such complex formation. But when the same blend was analysed by wetting it with small proportion of purified water it showed complex formation. Thus it is clear that combination of carbopol 971 P and eudragit E po forms hydrogel complex structure (peak appeared at 1561 cm-1 on FT-IR spectrum of carbopol-Eudragit E po IPEC which may be assigned to the carboxylate group of carbopol which formed ionic bonds with protonated amino group of eudragit E po). Hence, it has the capacity to form complex in colon by observing fluids from the environment of large intestine.

Slugging process:

The powder blends are passed through 40# and blended. slugged using 16 mm circular flat punch at about 10 to 12 kg/cm^2 of hardness. Deslugged in multi-mill and passed through 24 # mesh. Lubricated and compressed into mini tablets of 5.5 mm circular standard concave punch at about 12 kg/cm^2 of hardness.

Table 9: Formulation containing Carbopol 971 P and Eudragit E po

Formula trial	Drug:polymer ratio	Salbutamol sulphate	Eudragit E po	Carbopol 971 P	Mccph 102	Aerosil	Mg. stearate	Avg. wt
FA3	1:1:1	6 mg	6 mg	6 mg	70 mg	1 mg	1 mg	90 mg
FA4	1:2:2	6 mg	12 mg	12 mg	58 mg	1 mg	1 mg	90 mg
FA5	1:3:3	6 mg	18 mg	18 mg	46 mg	1 mg	1 mg	90 mg
FA6	1:3:4	6 mg	18 mg	24 mg	40 mg	1 mg	1 mg	90 mg
FA7	1:4:5	6 mg	24 mg	30 mg	28 mg	1 mg	1 mg	90 mg
FA8	1:4:6	6 mg	24 mg	36 mg	22 mg	1 mg	1 mg	90 mg
FA9	1:4:7	6 mg	24 mg	42 mg	16 mg	1 mg	1 mg	90 mg
FA10	1:4:8	6 mg	24 mg	48 mg	10 mg	1 mg	1 mg	90 mg
FA11	1:5:8	6 mg	30 mg	48 mg	4 mg	1 mg	1 mg	90 mg
FA12	1:4:9	6 mg	24 mg	54 mg	4 mg	1 mg	1 mg	90 mg

From the mucoadhesion strength of the tablets, it is observed that increasing the ratio of carbopol 971 P gave good mucoadhesion but its effiency to retard drug release was reduced. And at the same time increasing the concentration of Eudragit E po reduced the mucoadhesive strength of the tablets.

Eudragit E po controls swelling. IPEC prevents intial burst release and gives controlled release.

Increasing the concentration of Eudragit E po gave good controlled release and also gave triggered release when pH is lowered to resemble chronobiology. Hence, concentration of eudragit E po and Carbopol has to be optimized to get good mucoadhesion, controlled release and triggered release.

Increase in concentration of Mcc ph102 , causes erosion type of swelling.

From the study, 42 mg of carbopol 971 P in 90 mg of tablet is suffient to cause good mucoadhesion. Carbopol 971 p ratio is fixed 42 mg per tablet. By reducing the concentration of Eudragit E po good mucoadhesion was achived but the system fails to retard drug release for prolonged period but also gave triggered release when pH is lowered. By reducing the concentration of Eudragit E po, IPEC complex formation was not affected.

Formulation involving Ethyl cellulose N 50:

Hence attempt was made to include release retarding polymer like ethyl cellulose.

Table 10: Formulation containing Carbopol, Eudragit and Ethyl cellulose N 50

Formula trial	Drug:polymer ratio	Salbutamol sulphate	Carbopol 971 P	Eudragit E po	Ethyl cellulose N 50	Mcc ph 102	Aerosil	Mg. stearate	Avg.wt
FA13	1:7:1:1	6 mg	42 mg	6 mg	6 mg	28 mg	1 mg	1 mg	90 mg
FA14	1:7:2:2	6 mg	42 mg	12 mg	12 mg	16 mg	1 mg	1 mg	90 mg
FA15	1:7:2:4	6 mg	42 mg	12 mg	24 mg	4 mg	1 mg	1 mg	90 mg

FA16	1:7:2:5	6 mg	42 mg	12 mg	30 mg	-	1 mg	1 mg	92 mg

Ethyl cellulose was added to the formulation and was found its ability to retard drug release. Since 72 hours drug release to be achieved, concentration of ethyl cellulose was increased in the formulation. By increasing the concentration of ethyl cellulose drug release was retarded, but mucoadhesion property was also reduced.

Formulation involving sodium alginate:

Hence , study was concentrated on hydrophilic polymers like sodium alginate and hydroxy propyl methyl cellulose.

This hydrophilic polymers helps in formation of hydrogel complex that readily absorbs water from the environment of colon and has the capacity to hold up water in it remaining in gel state. Thus providing, controlled release of drug from the dosage form. Since they are hydrophilic polymers, they also possess mucoadhesive property.

Table 11: Formulation containing Carbopol, Eudragit and Protonal CR 8133

Formula trial	Drug: polymer ratio	Salbutamol sulphate	Carbopol 971 P	Eudragit E po	Protonal CR 8133	Mcc ph 102	Aerosil	Mg. stearate	Avg.wt
FA17	1:7:1:2	6 mg	42 mg	6 mg	6 mg	28 mg	1 mg	1 mg	90 mg
FA18	1:7:2:4	6 mg	42 mg	12 mg	24 mg	4 mg	1 mg	1 mg	90 mg
FA19	1:7:3:4	6 mg	42 mg	18 mg	24 mg	-	1 mg	1 mg	92 mg
FA20	1:7:2:5	6 mg	42 mg	12 mg	30mg	-	1 mg	1 mg	92 mg
FA21	1:6:3:5	6 mg	36 mg	18 mg	30 mg		1 mg	1 mg	92 mg
FA22	1:5:2:7	6 mg	30 mg	12 mg	42 mg	-	1 mg	1 mg	92 mg
FA23	1:4:2:8	6 mg	24 mg	12 mg	48 mg	-	1 mg	1 mg	92 mg

Protonal CR 8133 (Sodium alginate) retarded the delivery of drugs and provided drug release in controlled manner in pH 6.8 buffer medium. But in presence of goat's caecal content the drug release was faster compared to normal pH 6.8 buffer medium. Its drug retarding property is not sufficient to provide drug delivery for 72 hours. Since, sodium alginate is also degraded by colonic microbiota drug release may be higher in colon. And also sodium alginate is an anionic polymer , it releases drug in pH independent manner when used in combination with Interpolyelectrolyte complex. Hence it lags in chronobiological based release profile.

Formulation involving HPMC K 100 M LVCR:

Hydroxy propyl methyl cellulose is a non-ionic hydrophilic polymer that in combination with interpolyelectrolyte complex forms an hydrogel system. Hydroxypropyl methyl cellulose is not degraded by colonic microbiota.

In recent study it has shown temperature dependent release and it was reported. Hence, use of Hydroxyl propyl methyl cellulose is also advantageous in colon delivery of drug based on chronobiology since there exist an acute inflammatory reaction in colon

during asthma attacks which raises temperature and lowers pH in the lumen of the colon.

Table 12: Formulation containing Carbopol, Eudragit and HPMC K 100 M LVCR

Formula trial	Drug:polymer ratio	Salbutamol sulphate	Carbopol 971 P	Eudragit E po	HPMC K 100 M LVCR	Mcc ph 102	Aerosil	Mg. stearate	Avg.wt
FA24	1:7:2:2	6 mg	42 mg	12 mg	12 mg	16 mg	1 mg	1 mg	90 mg
FA25	1:7:2:4	6 mg	42 mg	12 mg	24 mg	4 mg	1 mg	1 mg	90 mg
FA26	1:7:2:5	6 mg	42 mg	12 mg	30 mg	-	1 mg	1 mg	92 mg
FA27	1:7:3:4	6 mg	42 mg	18 mg	24 mg	-	1 mg	1 mg	92 mg
FA28	1:6:3:5	6 mg	36 mg	18 mg	30 mg	-	1 mg	1 mg	92 mg

Presence of HPMC also increases the mucoadhesive property of the mini tablets. Since its mucoadhesion property is independent of pH and formation of good hydrogel system is taken into consideration. Controlled delivery of the drug from the dosage form is achieved and also provides effective drug release in combination with interpolyelectrolyte complex when triggered.

Higher viscosity polymers like HPMC k 100 M may cause initial burst release from the dosage form. but presence of carbopol , a low viscosity grade polymer prevents such burst release.

Final formula (1:7:3:4) provided control release of drug over a period of 72 hours. Releasing 1 mg of salbutamol sulphate in every 3 hours. (Resembling 12 hours dose of 4 mg SR tablets as recommended in USP) . And also provided excellent drug triggered release when pH of dissolution medium is lowered (5 pH) by addition of 5 N Hcl. And the system had the capacity to regain its initial property of retarding drug release when condition is taken back to initial (6.8 pH) by addition of 5 N NaOH.

Note: The addition of 5 N Hcl and 5 N NaOH is involved to adjust the pH in dissolution medium within few drops. Because, addition of diluted 1 N Hcl and 1 N NaOH should not affect the volume of dissolution medium which may alternatively affect the result during UV absorbance study.

Trigerring release based on severity of asthma:

pH of the dissolution medium was lowered to various pH levels. Since colon may have the pH of less than 4.2 during asthma attack. The pH of the dissolution medium was changed to 4.5 and 4.0 pH. The release of drug from the dosage form was noted. It is observed that as the pH lowers, drug release is triggered more. And the system had capacity to regain its original state when pH is reversed. Hence it is concluded that, drug release is achieved based on severity of asthma attack.

Filling of mini tablets into capsules:

“2 size” empty transparent hard gelatin capsule was chosen as it can fit 4 mini-tablets of 5.5 mm diameter into it. Filling was done in semi-automatic capsule filling

machine. Transparent capsule shell was chosen to ensure that all capsules are filled by 4 mini-tablets and there is no empty space left.

FORMULATION OF IMMEDIATE RELEASE TABLETS:

Immediate release tablet of salbutamol sulphate 2 mg was prepared to give initial plasma concentration of drug.

The immediate release tablet is to be designed in such a way that it dissolves completely within few seconds in stomach. Hence, tablet formulation is based of fast disintegrating property of the tablets in 0.1 N Hcl.

To make the manufacturing process more easier direct compression method was adopted.

Table 13: Formula for Immediate release Tablets

Formula trial	Salbutamol sulphate	Mccph 102	Lactose Monohydrate DCL-11	Croscar mellose sodium	Crospovidone XL 10	Mg. stearate	Avg.wt
FB1	2 mg	35 mg	68 mg	4.0 mg	-	1 mg	110 mg
FB2	2 mg	35 mg	62 mg	5.0 mg	-	1 mg	105 mg
FB3	2 mg	40 mg	52 mg	-	5.0 mg	1 mg	100 mg
FB4	2 mg	45 mg	46 mg	-	6.0 mg	1 mg	100 mg
FB5	2 mg	50 mg	40 mg	-	7.0 mg	1 mg	100 mg

Tablet was compressed using 6.35 mm standard concave punches at 5 kg/cm^2 hardness.

Formula FB5 showed fast disintegration < 15 sec and complete drug release was achieved within 15 min in 0.1 N Hcl dissolution medium.

Crosspovidone XL 10 have larger particle size compared to normal crospovidone and croscarmellose sodium, hence higher rate of wicking caused faster disintegration , thus resulting in faster drug release.

Increase in concentration of Mcc ph 102 and decrease in concentration of lactose DCL -11 showed significant improvement in disintegration and faster rate of drug release.

Colon targeted coating:

Targeting Mucoadhesive mini tablets to colon have immense advantage such that it remains in colon for prolonged period of time release drug in a controlled manner. Mucoadhesive Mini tablets can reside in colon for longer duration compared to other Larger dosage forms as it can withstand colonic force.

Since capsule is to be enteric coated, it reaches the colon faster than other dosage form due to its slippery nature and density.

Since pH of the GIT varies from regions, coating by pH sensive polymers like Eudragit L100: S 100 combination alone is not suffient for colon targeting.

There is a possibility that this may release the dosage form in small intestine where the pH is above 7.hence colon targeting cannot be achieved.

Systems utilizing formaldehyde treatment of capsule for colon targeting may fail to release the drug in required site since transit time of GIT varies from individual to individual and also in diseased state.

These things have to be taken into consideration while developing a colon targeted drug delivery system.

Coating the gelatin capsule shell with lactulose solution. Followed by coating with acid soluble polymer Eudragit E po and subsequent coating with Eudragit L 100.

Eudragit L 100 protects the delivery system in acidic pH of stomach for 2 to 3 hours. When it reaches the small intestine, Eudragit L 100 coating dissolves in alkaline pH of small intestine (6.8 to 7.9). Eudragit E po which is acid soluble polymer protects the delivery system in alkaline pH of small intestine and delivers the drug to the colon.

After reaching the colon, Eudragit E po starts swelling due to slight acidic pH of colon (5.5 to 6.8 pH). Due to this swelling, fluid, enzymes and microbiota influx causing digestion of lactulose which creates acidic environment and starts dissolving the thin layer of Eudragit E po. Due to digestion of lactulose, water content in colon increases which is taken up by gelatin shell for its swelling and ruptures to release the mini tablets into the system.

These tablets adheres to mucus membrane of the colon and releases the drug in controlled manner.

The capsule is enteric coated and is small, dense and slippery , so that it reaches the colon soon compared to transit time due to density of the tablets in capsule system and its slippery nature compared to other dosage forms.

Weight of tablet filled capsule:

Average weight of 1 tablets = 92 mg

Total weight of 4 tablets = 92 x 4 mg = 368 mg

Weight of “2” size empty hard gelatin capsule shell = 68 mg

Final weight of tablets filled capsules = 436 mg

Lactulose coating

Thin layer of 70 % lactulose solution is coated on to the delivery system. Minimum of 2 % to 3 % weight build up is suffient to cause complete film formation.

3 % weight build up = [(436/100) x 3]+ 436 = 449.08 mg

Eudragit E po coating:

Various weight build up 3%, 4 %, 5 % was made and the dissolution was checked in 7.4 pH phosphate buffer. It was found that 4 % coating is sufficient to prevent drug release in simulated small intestinal fluid.

Table 14: Eudragit E po Coating solution

Ingredients	Percentage used
Eudragit E po	85 %
Triethyl citrate	10 %
Talc	8 %
Isopropyl alcohol	qs
water	qs

Coating solution prepared with 15 % solid content and 85 % of solvent. Isopropyl alcohol : water was used in the ratio of 90:10 proportion.

Weight of lactulose coated capsule = 449.08 mg

3 % weight build up = [(449.08/100) x3] + 449.08 = 462.55 mg

4 % weight build up = [(449.08/100) x4] + 449.08 = 467.04 mg

5 % weight build up = [(449.08/100) x5] + 449.08 = 471.53 mg

Eudragit L 100 coating:

Various weight build up 8 % , 10 % was made and the dissolution was checked in 0.1 N Hcl. It was found that 10 % weight build up is sufficient to prevent drug release in simulated gastric fluid.

Table 15: Eudragit L 100 Coating solution

Ingredients	Percentage used
Eudragit L 100	85 %
Triethyl citrate	10 %
Talc	8 %
Titanium dioxide	2 %
Isopropyl alcohol	qs
water	qs

Coating solution prepared with 15 % solid content and 85 % of solvent. Isopropyl alcohol : water was used in the ratio of 90:10 proportion.

8 % weight build up = [(467.04/100) x 8] + 467.04 = 504.40 mg

10 % weight build up = [(467.04/100) x 10] + 467.04 = 513.74 mg

Filling of “Tablets in capsule system” and immediate release tablets :

The enteric coated capsule and immediate release tablet is filled into “0 el” size empty hard gelatin capsule.

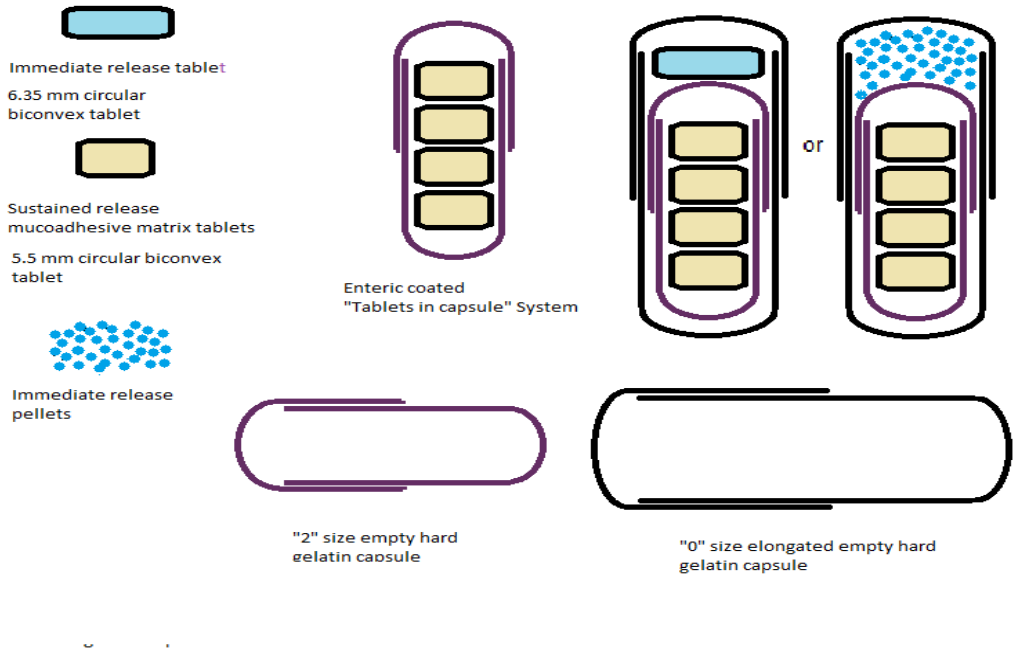


Figure 6 : Mucoadhesive tablets in capsule system

EVALUATION OF TABLETS

WEIGHT VARIATION TEST:

20 tablets from each formulation were individually weighed and average weight was calculated. Individual tablet weight was then compared with the average value to find out the deviation in weight. % weight variation is also calculated.

Table 16: Specifications of weight variations for tablets as per IP

s.no	Average weight of tablets	% Deviation
1	80 mg or less	10%
2	>80 mg but <250 mg	7.5%
3	250 mg or more	5%

THICKNESS:

The thickness of the tablets was determined by using digital vernier caliper. Five tablets from each formulation was evaluated and average values were calculated. The readings were recorded as mm.

HARDNESS:

Hardness of 5 tablets from each of the formulation was determined using Monsanto hardness tester. The readings were recorded as kg/cm2.

FRIABILITY:

Friability test for each of the formulation was carried out in Roche friabilator. About 6.5 gram of mini tablets/immediate release tablets were weighed and placed in the friabilator which was operated at 100 revolutions (25 rpm per min). The percent friability was determined using the formula,

$$\% \text{ Friability} = \frac{\text{Initial weight of the sample} - \text{final weight}}{\text{Initial weight of the sample}} \times 100$$

Limits for friability should be less than 1 %

UNIFORMITY OF CONTENT:

STANDARD SOLUTION:

Weigh accurately about 10 mg of salbutamol sulphate standard and transfer it into 100 ml volumetric flask. Dissolve it in distilled water and make up the volume to 100 ml with distilled water. The solution was filtered through 0.45µ nylon filter. 5 ml of filtrate was diluted distilled water in volumetric flask so as to get final concentration of 24 µg/ml.

SURFACE pH OF THE TABLET:

The surface pH of the tablet is measure by placing the tablet in petridish containing distilled water for 2 hours. The tablet was collected and surface ph of the tablet was determined by pH paper.

***In-vitro* swelling index studies:**

The swelling index of mucoadhesive tablets was determined using phosphate buffer pH 6.8. The tablets were weighed individually and placed separately in watch glass containing 4 ml of phosphate buffer pH 6.8. The weight of the watch glass was taken as tare weight (w1). At regular 3 hours of intervals, the excess phosphate buffer in watch glass is removed using filter paper carefully. The weight of the watch glass along with tablet was noted (w2) and is subtracted from the tare value. Thus water uptake by the tablet is determined without disturbing the tablet. Fresh 4 ml of phosphate buffer was replaced resembling sink condition in colon.

weight of swellen tablet = w2-w1

Note: This method is advantageous over older methods of removing tablets from watch glass and studying swelling index, which may have the possibility of damaging the swellen tablets and affecting the study. This method also resembles maintaining sink condition in colon by replacing with fresh buffer.

Swelling index was calculated using the formula,

$$\text{Swelling Index (SI)} = \frac{\text{weight ofswellen tablet} - \text{initialweight of tablet}}{\text{Initial weight of tablet}} \times 100$$

***In-vitro* bioadhesion study:**

Fabrication of test assembly:

The weighing balance formed the basis for fabrication of *In-vitro* bioadhesion test apparatus. The apparatus consists of glass vial which is fixed to one side of the balance. To bottom surface of this glass vial, mucoadhesive mini tablet was made to adhere using adhesives. A glass bottle is placed in lower side of this assembly and Goat's intestinal mucosal membrane is tied to mouth of this glass bottle which is filled with 6.8 pH phosphate buffer to maintain goat's intestinal mucosal membrane in moist condition during the study.

To another side of the balance, it carries a specific gravity bottle and the balance was adjusted to maintain equilibrium. Tare weight of specific gravity bottle was noted.

The mini tablet which is attached to bottom of the vial was moistened with little amount of 6.8 pH phosphate buffer. And the tablet is made to adhere to the goat's intestine.

Water was slowly added to the specific gravity bottle little by little. The quantity of water required to detach mini tablets from the goat's mucosal membrane was weighed.

Measurement of adhesion force:

The weight required to detach mini tablets from the goat's mucosal membrane was measured in grams. This is then converted to kilopascals (kPa).

The peristaltic force experienced in colon is 2.1 to 2.8 kilopascals. Hence, the tablet should withstand peristalsis in colon.

1 kilopascals (kPa)= 10.197 gram force / cm2

$$\text{Bioadhesive force (kPa)} = \frac{\text{bioadhesive strength in grams}}{10.197}$$

Bioadhesive force can also be measured in newton force (N) which is calculated by following equation.

Bioadhesive force (N) = Bioadhesion strength in grams x 9.81

100

***In-vitro* drug release studies for "mucoadhesive tablets in capsule system":**

The in-vitro drug release study of salbutamol sulphate from mucoadhesive mini tablets in capsule system was carried out in USP Type II apparatus (paddle method) at 50 rpm using shinker. Medium used for drug release study was 500 ml of 6.8 pH phosphate buffer. During the course of study the medium was maintained at 37*c ± 0.5*c. 5 ml of sample were withdrawn at regular 3 hours time intervals and replaced with fresh buffer. The amount of drug release from the dosage form was determined spectrophotometrically at 276 nm.

***In-vitro* drug release studies for "Enteric coated tablets in capsule sytem":**

The in-vitro drug release study of salbutamol sulphate from "enteric coated tablets in capsule system" was carried out in USP Type II apparatus (paddle method) at 50 rpm using shinker. The drug release study was carried out in 500 ml of 0.1 N Hcl for a period of 2 hours (average gastric transit time is 2 hours), then the dissolution medium was replaced with 7.4 pH phosphate buffer for a period of 3 hours (average small intestinal transit time is about 3 – 4 hours), finally study was continued with 6.8 pH phosphate buffer. During the course of study the medium was maintained at 37*c ± 0.5*c. 5 ml of sample were withdrawn at regular time intervals and replaced with fresh buffer.

***In-vitro* drug release studies for "Enteric coated tablets in capsule sytem" in presence of goat's caecal contents:**

The in-vitro drug release study of salbutamol sulphate from "enteric coated tablets in capsule system" was carried out in USP Type II apparatus (paddle method) at 50 rpm using shinker. The drug release study was carried out in 500 ml of 0.1 N Hcl for a period of 2 hours (average gastric transit time is 2 hours), then the dissolution medium was replaced with 7.4 pH phosphate buffer and the drug release was tested for a period of 3 hours (average small intestinal transit time is about 3 – 4 hours), then finally the study was continued with dissolution mediumcontaining 5 % of goat's caecal content. During the course of study the medium was maintained at 37*c ± 0.5*c and

with continuous supply of CO_2 into the beaker to simulate anaerobic environment of the colon. 5 ml of sample were withdrawn at regular 3 hours time intervals and replaced with fresh buffer.

Note: goat's caecal content weighing 250 grams was added to 5000 ml of 6.8 pH phosphate buffer medium.

In-vitro drug release based on chronopharmaceutics:

The in-vitro drug release study was carried out using 6 jars in USP Type II apparatus (paddle method) at 50 rpm using shinker. Medium used for drug release study was 500 ml of 6.8 pH phosphate buffer containing 5 % goat's caecal content. During the course of study the medium was maintained at 37*c ± 0.5*c and with continuous supply of CO_2 into the beaker to simulate anaerobic environment of the colon. 5 ml of sample were withdrawn at regular 3 hours time intervals and replaced with fresh buffer.

Since, the drug release profile must match chronobiological aspects the pH of the dissolution medium is lowered upto5.0 , 4.5 and 4.0 pH in between the study by addition of 5 N Hcldrop by drop with the help of pH meter, without disturbing the dissolution apparatus. Resembling the changes in pH of the colonic lumina during asthma attack in early morning hours.

The pH of the dissolution medium was lowered at 24^{th}hours.At 24^{th} hour, pH was lowered to pH 5.0 in Jar : 1 and 2 by drop wise addition of 5 N Hcl into the dissolution medium.

In the same manner, pH was lowered to pH 4.5 in Jar : 3 and 4, followed by pH 4.0 in Jar : 5 and 6.The temperature of the medium was maintained at 39*c ± 0.2*c

After regular 3 hours interval, 5 ml of sample was withdrawn and 5 ml was replaced with fresh buffer. Then it is analysed for drug content under spectroscopy at 276 nm.

Then the pH of the dissolution medium was taken back to normal state of 6.8 pH by addition of 5 N NaOH drop by drop into the dissolution medium without disturbing the dissolution apparatus. The temperature of the medium was also brought back to 37*c ± 0.5*c

Note: 5 N Hcl and 5 N NaOH was used instead of dilute solution to change the pH of the medium without affecting the volume of the dissolution medium.

In-vitro drug release study for immediate release tablets:

The in-vitro drug release study of Immediate release tablets of salbutamol sulphate was carried out in USP Type II apparatus (paddle method) at 50 rpm for a period of 15 min. The medium used for drug release study was 900 ml of 0.1 N Hcl. During the course of study the medium was maintained at 37*C ± 0.5*c. 5 ml of sample were withdrawn and analysed for the drug content spectrophotometrically at 276 nm.

In-vitro **Drug permeation:**

The in-vitro Intestinal drug permeation study of salbutamol sulphate through goat's intestinal mucosal membrane (colon) was performed using Keshary-chien type glass diffusion cell at 37*c ± 0.2*c.Freshly slaughtered colon of goat was mounted between donor and receptor compartment. 4 mucoadhesive mini tablets was placed on to the mucosal membrane and the compartments are clamped together. The donor compartment was filled with 1 ml of phosphate buffer pH 6.8. The receptor compartment (15 ml capacity) was filled with phosphate buffer of pH 7.4 resembling pH in serosal side. Hydrodynamics in receptor compartment was maintained by stirring with magnetic bead at 50 rpm. A 1 ml sample was withdrawn at predetermined time intervals and analyzed for the drug content at 276 nm using UV spectrophotometer.

In-vitro residence time of mucoadhesive mini tablets:

The in-vitro residence time for mucoadhesive mini tablets containing salbutamol sulphate was determined using modified USP dissolution apparatus. The dissolution medium composed of 500 ml of pH 6.8 phosphate buffer maintained at 37*C ± 0.5*c. A segment of the Goat's intestinal mucosal membrane was glued to the surface of each glass slide. 4 mucoadhesive mini tablets were wetted with phosphate buffer containing erythrosine supra dye and made to adhere to the surface of intestinal mucosa. The slide was immersed in dissolution apparatus and height of the paddle to that of the tablet surface was adjusted at a distance of 5 cm and rotated at 50 rpm. The time required for complete erosion or detachment of the tablets from the mucosal membrane was recorded.

MECHANISM OF DRUG RELEASE:

Various models were tested for explaining the kinetics of drug release.

To investigate the mechanism of drug release rate kinetics from the dosage form, the obtained data were fitted with zero-order, first-order, Higuchi and korsmeyer-peppas release model.

Zero order release rate kinetics:

To investigate zero-order release kinetics, the drug release rate was fitted to the equation,

$$F = Ko.t$$

Where,

F = drug release, K= release rate constant and t = time taken for drug release. Plot of % drug release versus time is linear.

First order release rate kinetics:

To investigate first-order release kinetics, the drug release rate data was fitted to the equation,

$$Log\ (100 – F) = Kt$$

A plot of Log % drug release versus time is linear.

Higuchi release model:

To investigate Higuchi release kinetics, the release rate data were fitted to the following equation.

$$F = K\ t^{1/2}$$

Where K = Higuchi constant.

In Higuchi model, plot of % drug release verses square root of time is linear.

Korsmeyer – peppas model:

To investigate Korsmeyer – peppas release kinetics, the release rate data were fitted to the following equation,

$$M_t/M_\infty = K.t^n$$

Where, M_t/M_∞ = fraction of drug released.

K = release constant.

t= time taken for release.

n= diffusion exponent.

If n is equal to 0.89, the release is zero order.

In this model, a plot of log (M_t/M_∞) versus log time is linear.

RESULTS & DISCUSSION

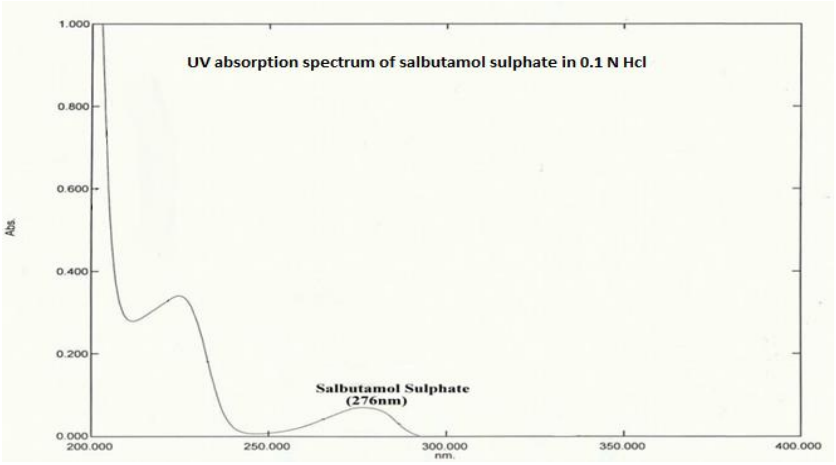
SPECTROSCOPIC STUDIES:

UV spectroscopy (Determination of λmax):

The λmax for salbutamol sulphate was determined in different medium like 0.1 Hcl, pH 7.4 phosphate buffer, pH 6.8 phosphate buffer & pH 6.8 phosphate buffer containing 5 % of goat's caecal content.

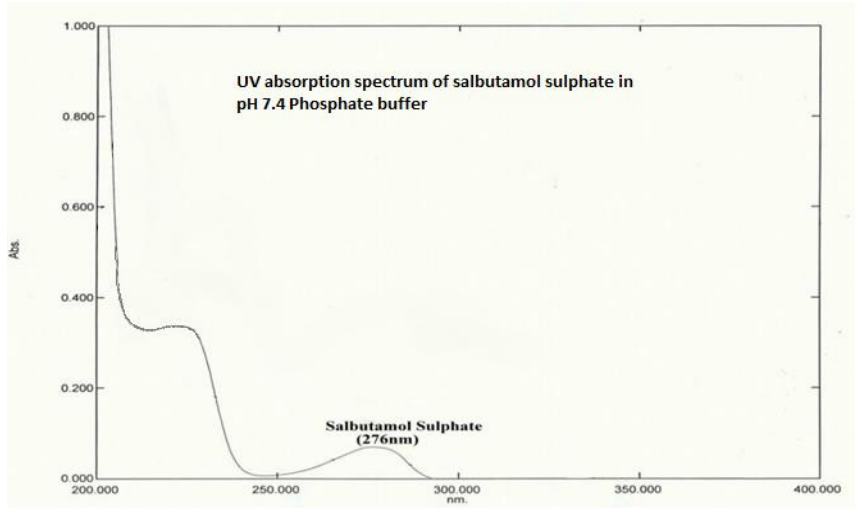
The wavelength of maximum absorbance was found to be 276 nm.

UV absorption spectrum of salbutamol sulphate in 0.1 N Hcl:



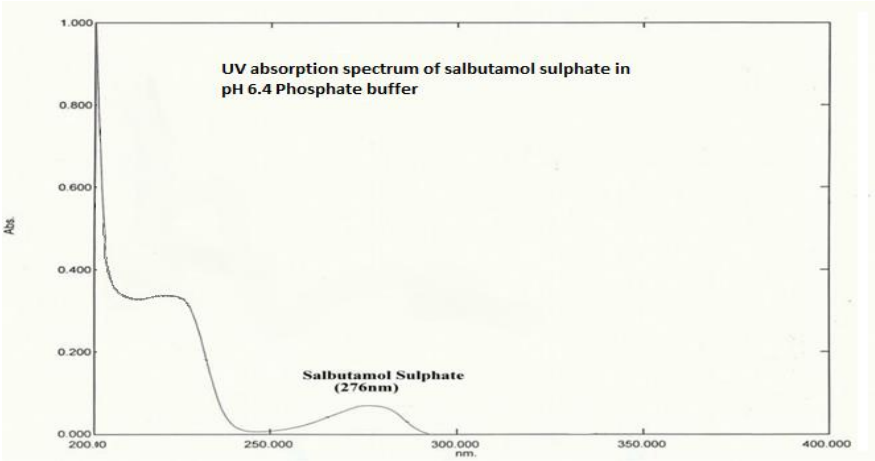
Graph 1 : UV absorption spectrum of SS in 0.1 N Hcl

UV absorption spectrum of salbutamol sulphate in 7.4 pH phosphate buffer:



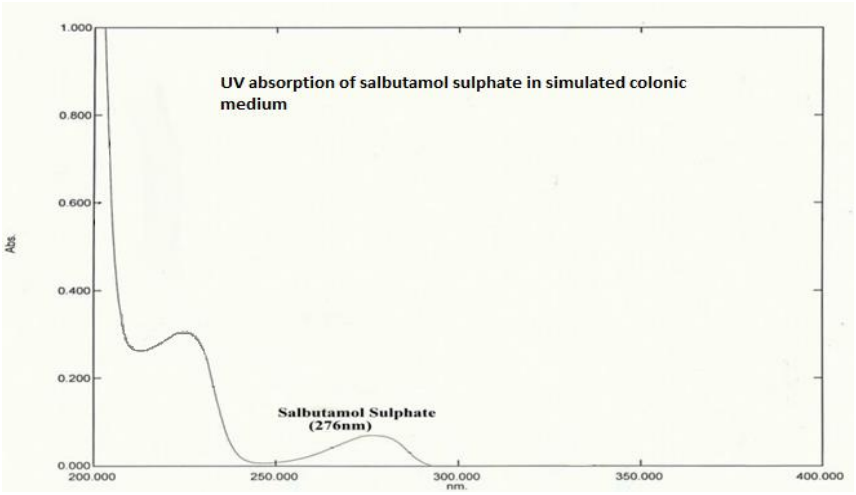
Graph 2 : UV absorption spectrum of SS in pH 7.4 phosphate buffer

UV absorption spectrum of salbutamol sulphate in 6.8 pH phosphate buffer:



Graph 3: UV absorption spectrum of SS in 6.8 pH phosphate buffer

UV absorption spectrum of salbutamol sulphate in pH 6.8 phosphate buffer containing 5 % Goat's caecal content at 276 nm (colon simulated medium):



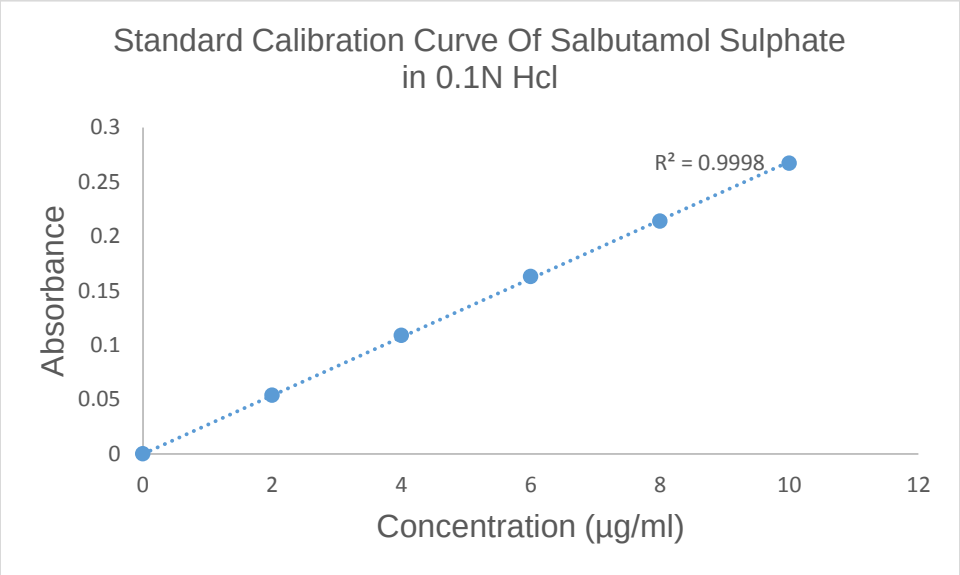
Graph 4 : UV absorption spectrum of SS in colon simulated medium

Standard calibration curve of salbutamol sulphate:

Calibration of salbutamol sulphate in 0.1 N Hcl at 276 nm:

Table 17: Calibration curve of SS in 0.1 N Hcl

s.no	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.054
3	4	0.109
4	6	0.163
5	8	0.214
6	10	0.267

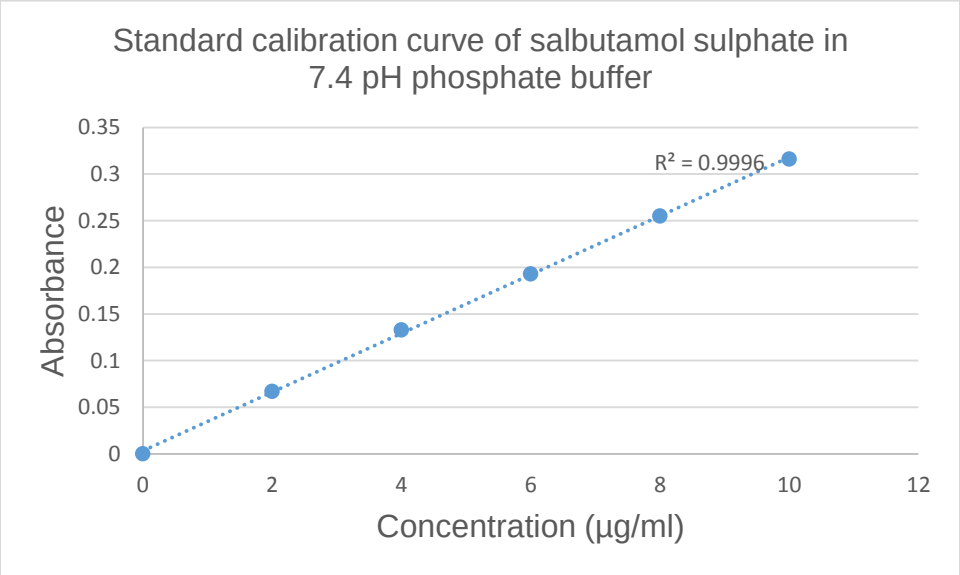


Graph 5: standard calibration curve of SS in 0.1 N Hcl

Calibration of salbutamol sulphate in pH 7.4 phosphate buffer at 276 nm:

Table 18: Calibration curve of SS in 7.4 pH phosphate buffer

s.no	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.067
3	4	0.133
4	6	0.193
5	8	0.245
6	10	0.316

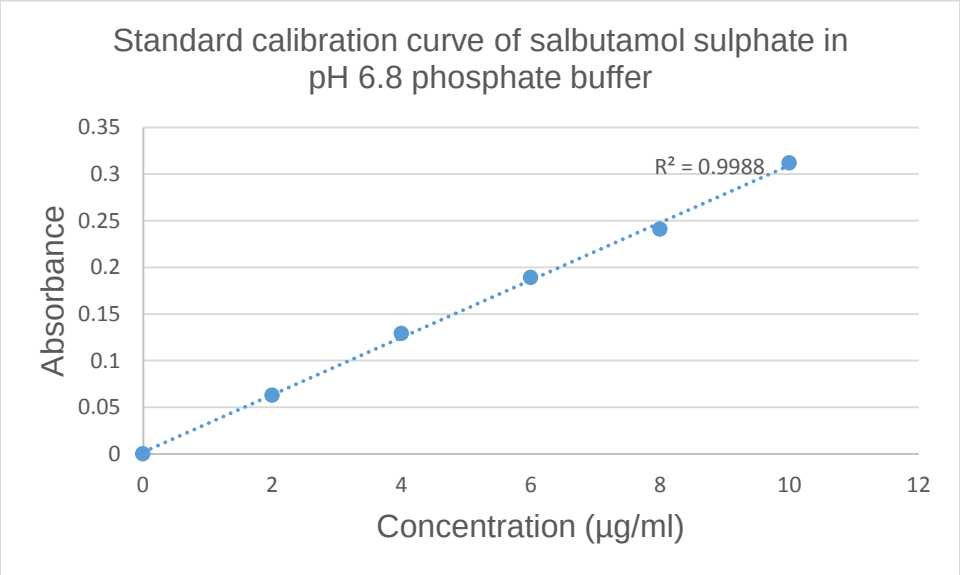


Graph 6: standard calibration curve of SS in 7.4 pH phosphate buffer

Calibration of salbutamol sulphate in pH 6.8 phosphate buffer at 276 nm:

Table 19: Calibration curve of SS in 6.8 pH phosphate buffer

s.no	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.063
3	4	0.129
4	6	0.189
5	8	0.241
6	10	0.312

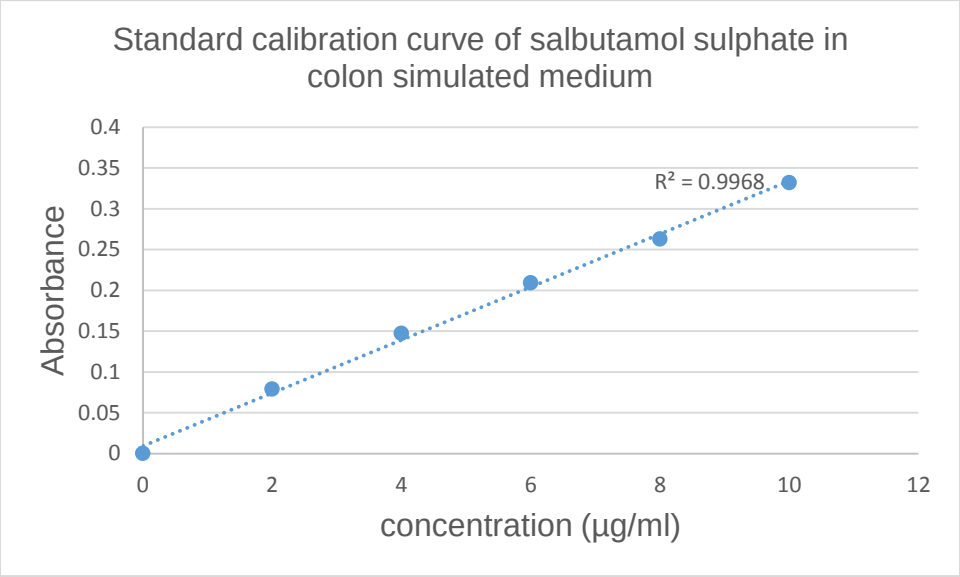


Graph 7: standard calibration curve of SS in 6.8 Phosphate buffer

Calibration of salbutamol sulphate in pH 6.8 phosphate buffer containing 5 % Goat's caecal content at 276 nm (colon simulated medium):

Table 20: Calibration curve of SS in colon simulated medium

s.no	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.079
3	4	0.147
4	6	0.209
5	8	0.263
6	10	0.332



Graph 8: standard calibration curve of SS in colon simulated medium

IR spectrum interpretation:

IR spectrum of salbutamol sulphate:

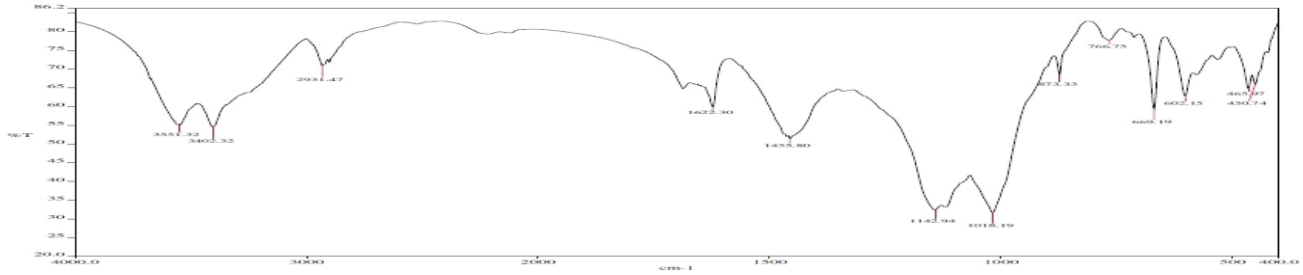
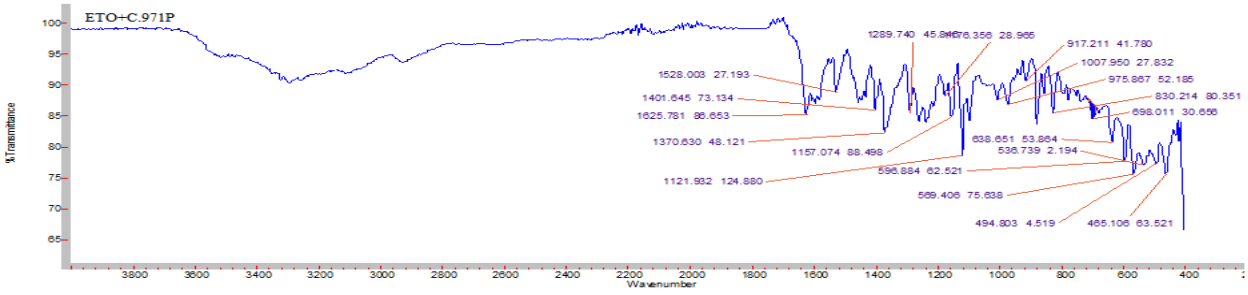


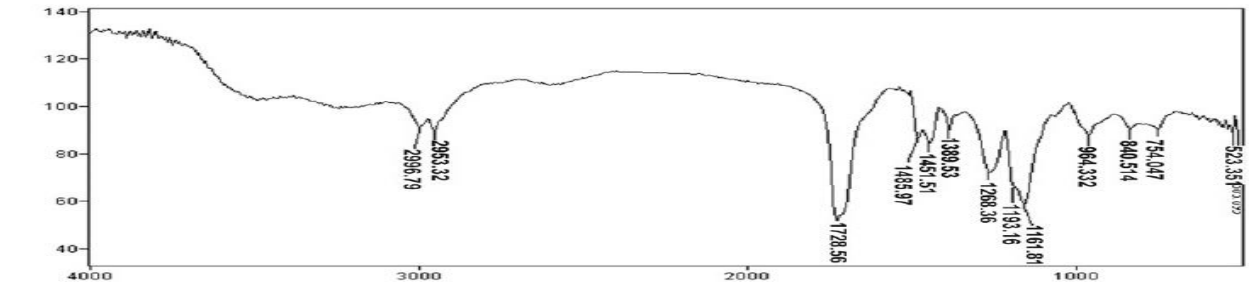
Table 21: IR spectrum of salbutamol sulphate

Salbutamol sulphate	
Wave Number in cm-1	Characteristic bands
3551.32	O – H stretching
3402.32	N – H stretching
2931.47	C – H stretching
1622.30	C = C stretching
1455.80	C – H Bending
1142.94	C – O stretching Ether
1018.19	C – O stretching
873.33, 766.75, 669.19, 602.15	C – H (OOP) For Aromatic rings
465.97, 450.74, 444.88, 419.45	C – X stretching

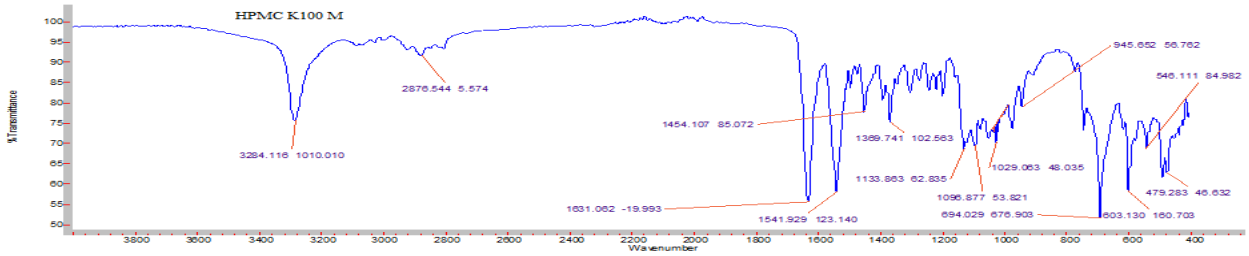
IR spectrum of carbopol 971 P:



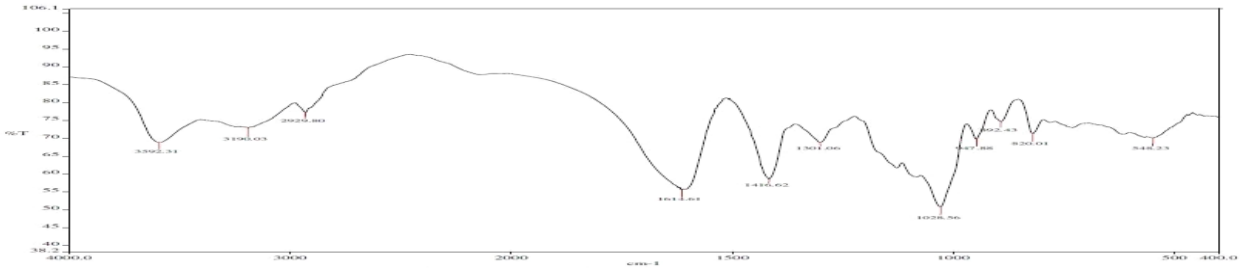
IR spectrum of Eudragit E po:



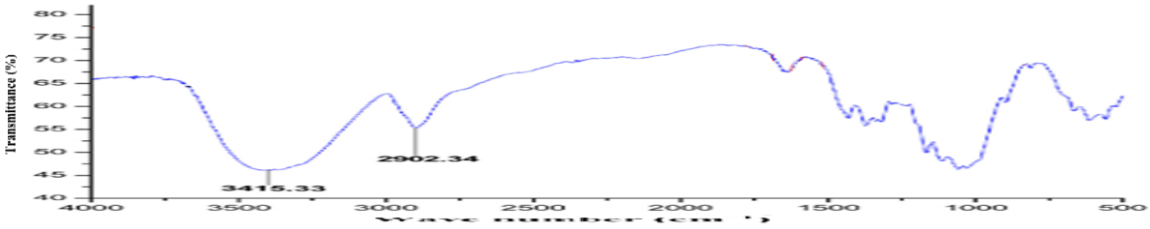
IR spectrum of HPMC K 100 M LVCR



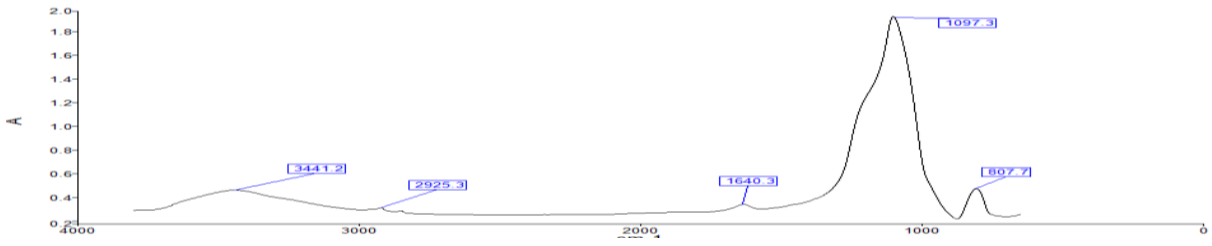
IR spectrum of Protanal CR 8133 (sodium alginate):



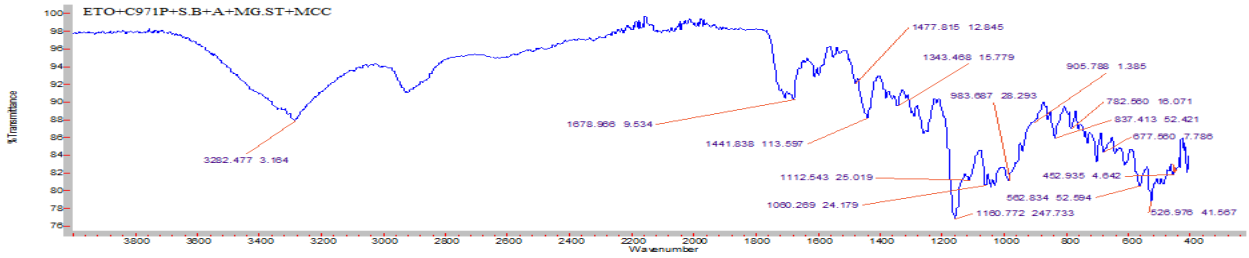
IR spectrum of Magnesium stearate:



IR spectrum of colloidal silicon dioxide:



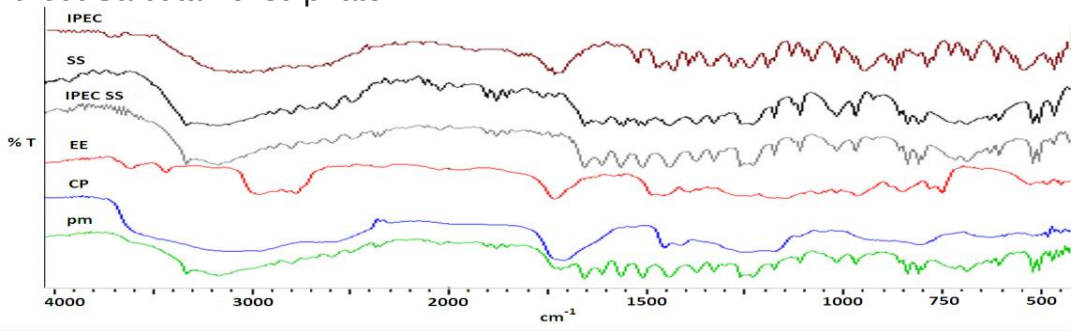
IR spectrum of Drug with excipients:



Formation of Interpolyelectrolyte complex by FTIR:

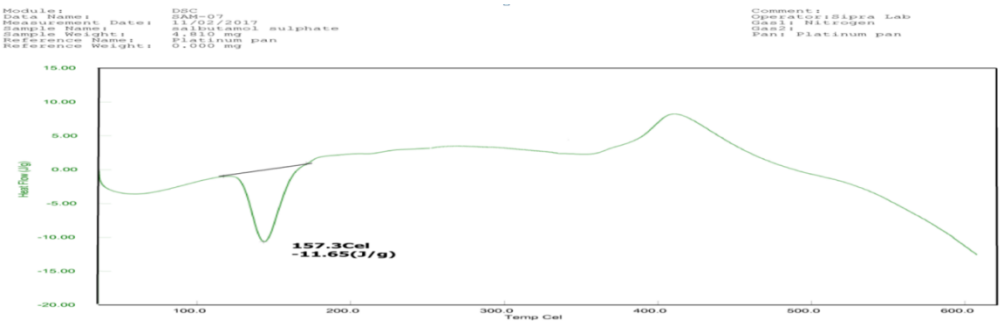
Eudragit E po showed characteristic band at 1,730 cm−1 which corresponds to absorbance of ester groups. The absorbance bands at 2,770 and 2,820 cm−1 can be assigned to non-ionized dimethyl amino groups.

The IR spectrum of Carbopol 971P is characterized by principal absorption peaks at 3,110 (O–H stretching) and 1,720 cm−1 (carboxyl group). The FT-IR spectra indicated that drug bands were preserved within the tablets (mixed powders) and within IPEC without Salbutamol sulphate.

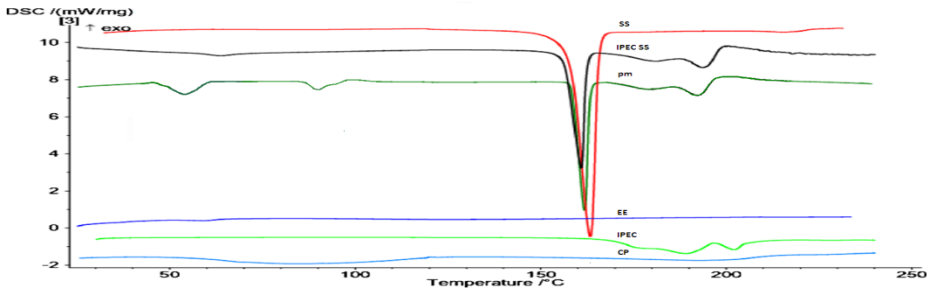


In IPEC complex , Eudragit E po peak at 2,820 cm−1 was greatly minimized, and the peak at 1,730 cm−1 disappeared, in case of Carbopol 971P, the peak at 1,720 cm−1 disappeared in IPEC containing Salbutamol sulphate. This can be attributed to the existence of Salbutamol sulphate as the major constituent.

DSC of Salbutamol sulphate:



DSC of interpolyelectrolyte complex:



These results were also confirmed by DSC thermograms, which indicated that salbutamol sulphate was preserved in crystalline state in the formulation. The transition that appeared at temperature 195 °C in IPEC SS could be attributed to IPEC itself. This was further confirmed by existence of same transition in DSC trace of IPEC alone (without Salbutamol sulphate). Hence, formation of IPEC complex without affecting salbutamol sulphate in formulation was confirmed by FTIR and DSC.

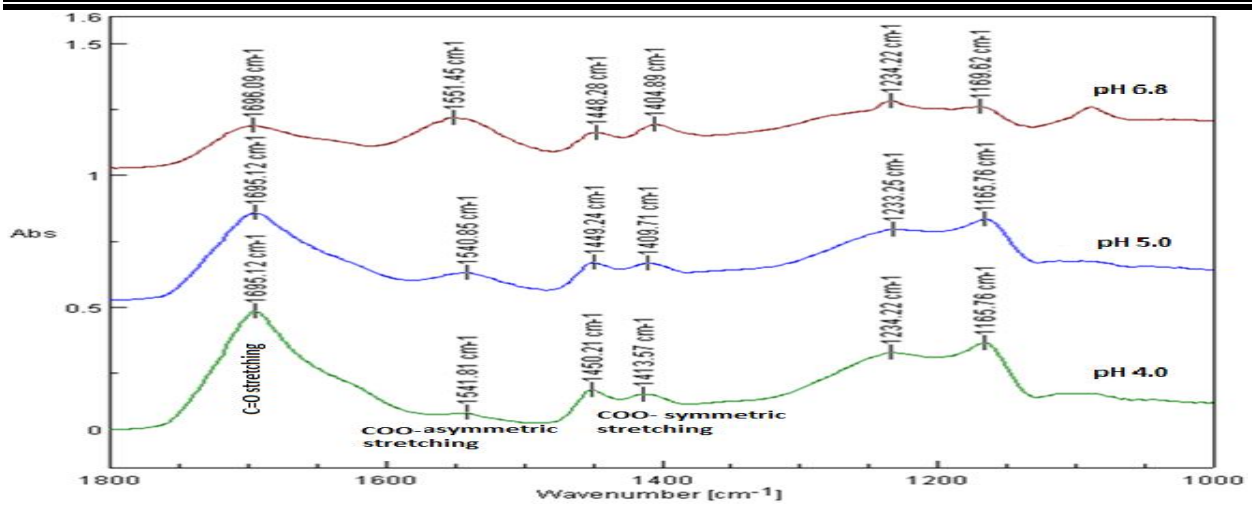
Reason for trigerred release in different pH:

Carbopol has three characteristic peaks when determined by IR spectroscopy between 1800 - 600 cm-1.

1. C=O stretch band appears around 1695 cm-1, which corresponds to free carbonyl group.
2. Hydrogen bonded C=O stretch band appears at lower 100 wavenumbers, usually as a shoulder of free C=O stretch band.
3. The deprotonated carboxyl (COO-) group has absorption bands around 1560 cm-1 due to its asymmetric stretching (COO- stretch asymmetric) and 1410 cm-1 due to symmetric stretching (COO- stretch symmetric) (Pretsch et al., 2009).

IR spectra of IPEC complex, correspond to differently protonated state of carbopol molecule in different pH values. It can be noticed that there is a changes in intensity of COO- stretching bands when pH levels are altered. IR spectroscopy is able to detect increase or decrease in amount of deprotonated carboxylic groups.

On other hand, asymmetric COO- stretching band shifted significantly to higher wave numbers at basic conditions and vice versa when pH is lowered, which indicates changes in chemical environment of molecule. These changes can be associated with swelling and rheological characteristics such as gel-sol transformation of polymers due to electrostatic repulsion between the groups, which in turn causes development of adjacent negatively charged carboxyl groups.



C=O stretching band of Carbopol was easily detectable at lower pH values (1699 cm-1) and asymmetric COO- stretching of Carbopol at higher values pH 6.8 (1557 cm-1). The progressive decrease of C=O stretching and increase of asymmertic COO- stretching as a function of pH indicated that IPEC complex responded to changes in pH of environment.

It is important to note that drug release was higher at lower pH values 4.0 and release rate was lowered at pH 6.8. Some state of swelling and de-swelling of matrix was also observed when pH is altered. Tan et al. (2001).

Evaluation of tablets:

Tablets containing Eudragit E po & Carbopol 971 P:

Table 22: Blend parameters of FA1 – FA12

Formula trial	Bulk density (gm/ml)	Tapped density (gm/ml)	Compressibility index %	Hausner ratio	% drug content
FA1	0.412	0.489	15.746	1.187	99.87 %
FA2	0.401	0.481	16.632	1.200	99.73 %
FA3	0.398	0.453	12.141	1.138	99.56 %
FA4	0.391	0.447	12.528	1.143	99.23 %
FA5	0.387	0.442	12.443	1.142	100.01 %
FA6	0.382	0.436	12.385	1.141	99.99 %
FA7	0.376	0.429	12.354	1.141	100.08 %

FA8	0.37	0.424	12.736	1.146	99.25 %
FA9	0.363	0.42	13.571	1.157	99.87 %
FA10	0.356	0.418	14.833	1.174	99.42 %
FA11	0.35	0.411	14.842	1.174	100.06 %
FA12	0.349	0.409	14.670	1.172	99.65 %

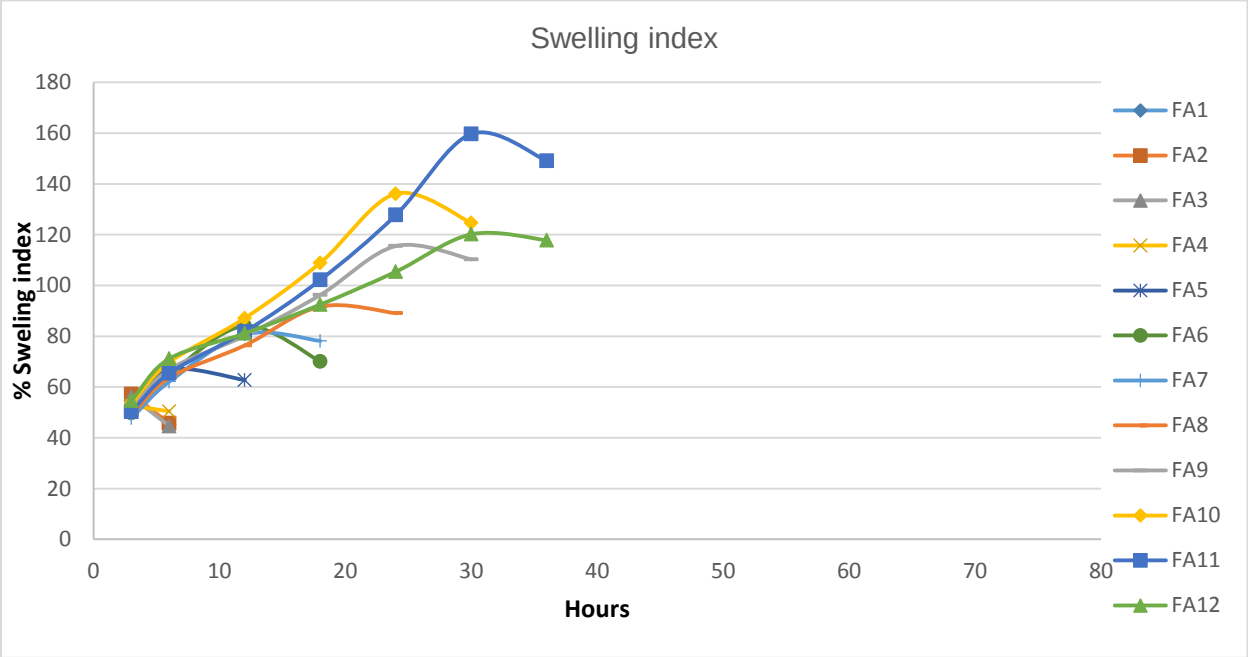
Table 23: Tablet Physical parameters FA1 – FA12

Formula trial	Thickness (mm)	Hardness kg/cm^2	% Friability	Content uniformity	Weight variation	Surface pH
FA1	3.3 mm ± 0.3 mm	10 kg/cm^2	0.04 %	99.83 %	complies	6.46
FA2	3.4 mm ± 0.2 mm	10 kg/cm^2	0.05 %	99.70 %	complies	6.49
FA3	3.2 mm ± 0.1 mm	10 kg/cm^2	0.02 %	99.52 %	complies	6.53
FA4	3.3 mm ± 0.3 mm	12 kg/cm^2	nil	99.21 %	complies	6.56
FA5	3.5 mm ± 0.1 mm	13 kg/cm^2	nil	99.98 %	complies	6.60
FA6	3.3 mm ± 0.2 mm	13 kg/cm^2	nil	99.89 %	complies	6.63
FA7	3.6 mm ± 0.1 mm	13 kg/cm^2	nil	100.01 %	complies	6.65
FA8	3.4 mm ± 0.2 mm	13 kg/cm^2	nil	99.15 %	complies	6.70
FA9	3.3 mm ± 0.1 mm	13 kg/cm^2	nil	99.78 %	complies	6.74
FA10	3.3 mm ± 0.2 mm	13 kg/cm^2	nil	99.41 %	complies	6.77
FA11	3.2 mm ± 0.2 mm	13 kg/cm^2	nil	100.02 %	complies	6.81
FA12	3.3 mm ± 0.3 mm	13 kg/cm^2	nil	99.61 %	complies	6.84

Table 24: swelling index of FA1 – FA12

Trial	FA1	FA2	FA3	FA4	FA5	FA6	FA7	FA8	FA9
3 h	56.73	57.24	56.21	52.72	51.43	49.74	47.76	48.98	51.43
6 h	45.19	45.70	44.67	60.41	66.86	64.66	62.09	63.67	66.86
12 h				50.21	62.76	84.06	80.71	76.41	80.23
18 h						70.04	78.16	91.69	96.28
24 h								89.07	115.53
30 h									110.27

Trial	FA10	FA11	FA12
3 h	53.62	50.32	54.74
6 h	69.71	65.42	71.16
12 h	87.13	81.77	81.12
18 h	108.92	102.21	92.48
24 h	136.14	127.77	105.43
30 h	124.63	159.71	120.19
36 h		149.03	117.79

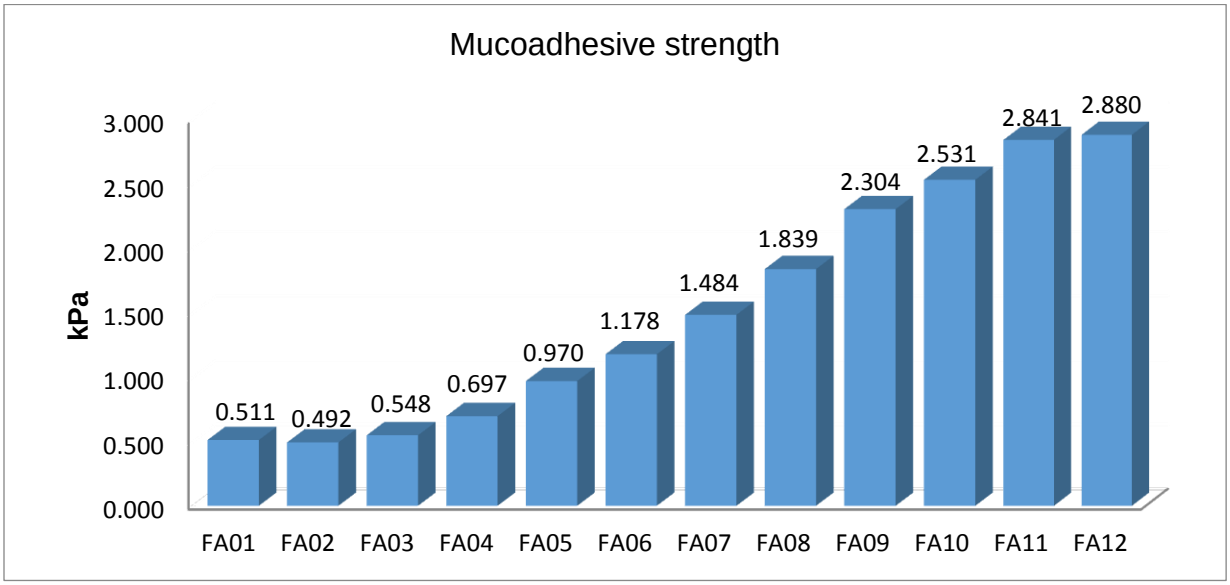


Graph 8: Swelling index of tablets containing Carbopol & Eudragit

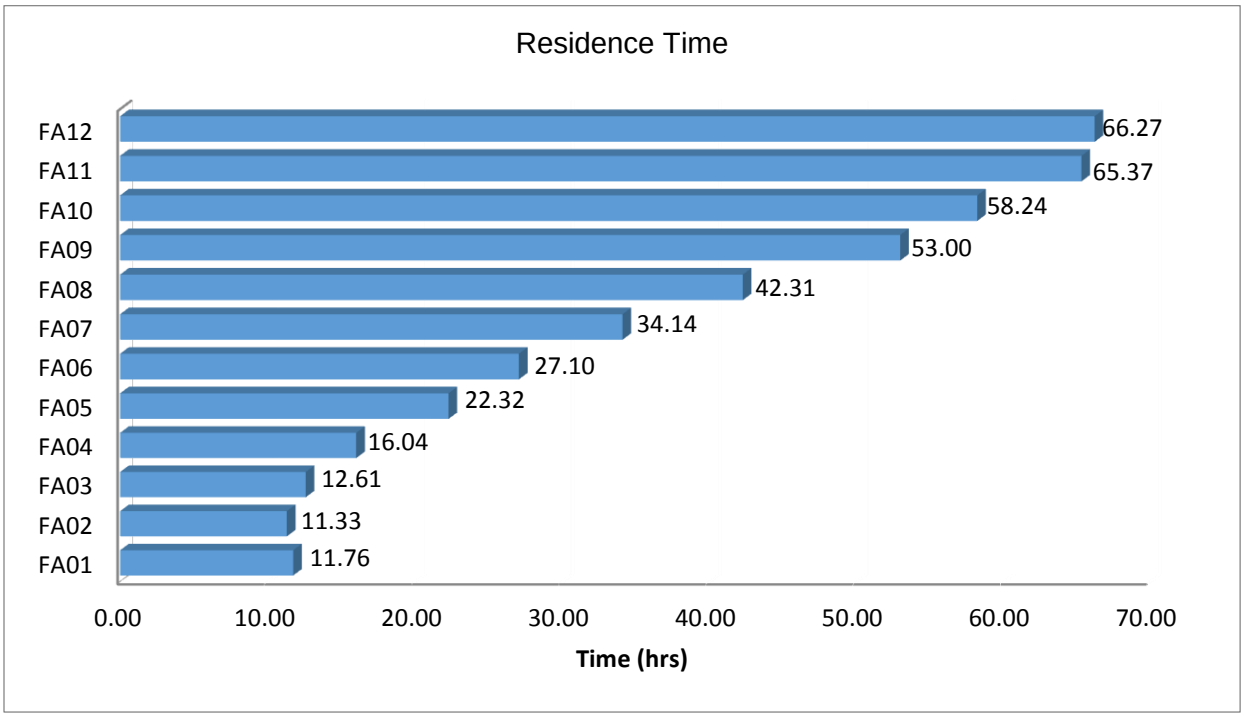
Table 25: Mucoadhesive strength & residence time of tablets FA1 – FA12

formula trial	Mucoadhesion strength (gm)	Mucoadhesive force (kPa)	Residence time (hours)
FA1	5.21	0.511	11.76
FA2	5.02	0.492	11.33
FA3	5.59	0.548	12.61
FA4	7.11	0.697	16.04
FA5	9.89	0.970	22.32
FA6	12.01	1.178	27.10
FA7	15.13	1.484	34.14
FA8	18.75	1.839	42.31

FA9	23.49	2.304	53.00
FA10	25.81	2.531	58.24
FA11	28.97	2.841	65.37
FA12	29.37	2.880	66.27



Graph 9: Mucoadhesive strength of formulation FA1 – FA12

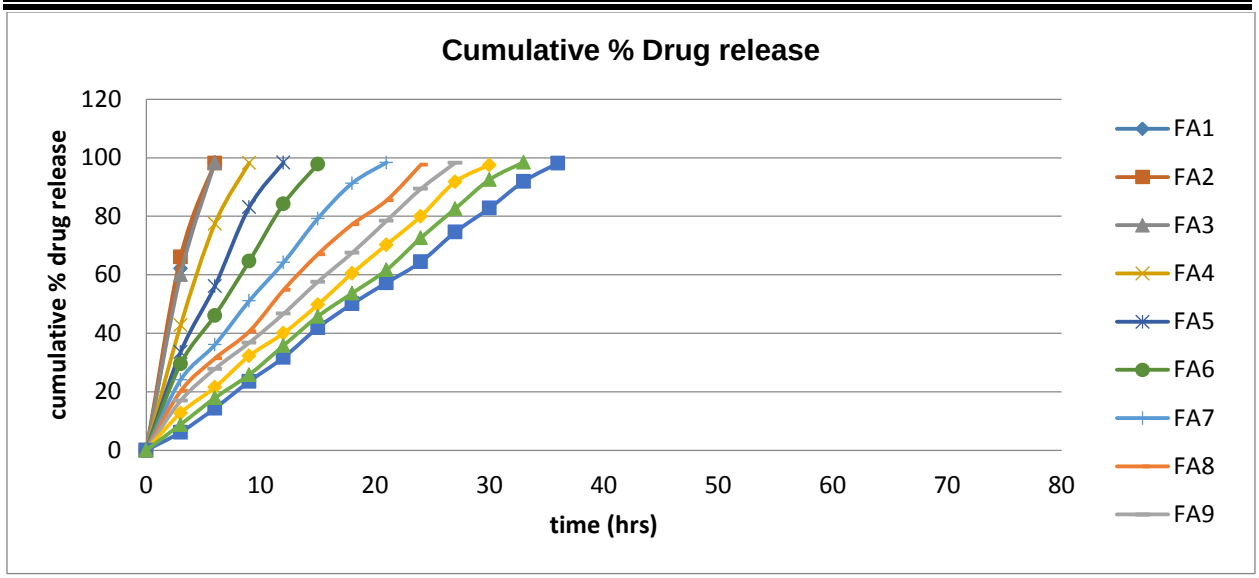


Graph 10: Residence time of Formulation FA1 - FA12

Table 26: Cumulative % Drug release - Dissolution test in 6.8 pH phosphate buffer medium:

Trial	FA1	FA2	FA3	FA4	FA5	FA6	FA7	FA8	FA9
3 h	62.21	66.11	60.12	42.75	33.59	29.56	24.05	20.21	16.92
6 h	98.42	98.21	98.39	77.51	56.18	46.11	36.11	31.41	27.84
9 h				98.26	83.13	64.67	51.06	40.62	36.76
12 h					98.35	84.22	64.21	54.82	46.68
15 h						97.78	79.26	67.02	57.61
18 h							91.32	77.23	67.53
21 h							98.37	85.44	78.45
24 h								97.64	89.37
27 h									98.29

Trial	FA10	FA11	FA12
3 h	12.75	6.18	8.81
6 h	21.51	14.36	17.91
9 h	32.26	23.54	25.86
12 h	40.02	31.72	35.81
15 h	49.77	41.90	45.77
18 h	60.52	50.08	53.72
21 h	70.28	57.25	61.68
24 h	80.03	64.43	72.63
27 h	91.79	74.61	82.58
30 h	97.54	82.79	92.54
33 h		91.97	98.49
36 h		98.15	



Graph 11: Cumulative % drug release of formulation FA1 – FA12

From the mucoadhesion strength of the tablets, it is observed that increasing the ratio of carbopol 971 P gave good mucoadhesion but its effiency to retard drug release was reduced. And at the same time increasing the concentration of Eudragit E po reduced the mucoadhesive strength of the mini - tablets.

Eudragit E po controls swelling. IPEC prevents intial burst release and gives controlled release.

Increasing the concentration of Eudragit E po gave good controlled release and also gave triggered release when pH is lowered to resemble chronobiology. Hence, concentration of eudragit E po and Carbopol has to be optimized to get good mucoadhesion, controlled release and triggered release.

Increase in concentration of Mcc ph102 , causes erosion type of swelling.

From the study, 42 mg of carbopol 971 P in 90 mg of tablet is suffient to cause good mucoadhesion. Carbopol 971 p ratio is fixed 42 mg per tablet. By reducing the concentration of Eudragit E po good mucoadhesion was achived but the system fails to retard drug release for prolonged period but also gave triggered release when pH is lowered. By reducing the concentration of Eudragit E po, IPEC complex formation was not affected.

Tablets containing Eudragit E po, Carbopol 971 P & ethyl cellulose:

Table 27 :Blend parameters of FA13 – FA16

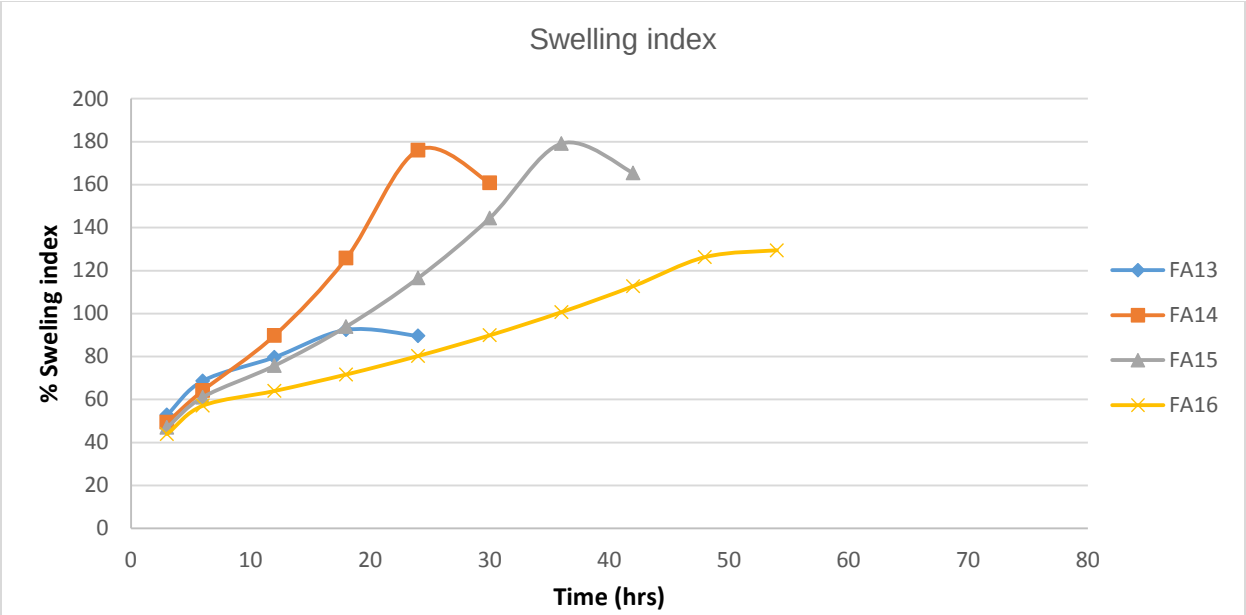
Formula trial	Bulk density (gm/ml)	Tapped density (gm/ml)	Compressibility index %	Hausner ratio	% drug content
FA13	0.359	0.414	13.285	1.153	99.23%
FA14	0.341	0.423	19.385	1.240	99.26%
FA15	0.338	0.422	19.905	1.249	99.56%
FA16	0.326	0.418	22.010	1.282	99.48%

Tables 28 : Tablet Physical parameters of FA13 – FA16

formula trial	Thickness (mm)	Hardness kg/cm^2	% Friability	Content uniformity	Weight variation	Surface pH
FA13	3.4 mm ± 0.1 mm	13 kg/cm^2	nil	99.19%	complies	6.81
FA14	3.3 mm ± 0.2 mm	13 kg/cm^2	nil	99.21%	complies	6.84
FA15	3.3 mm ± 0.1 mm	14 kg/cm^2	nil	99.50%	complies	6.88
FA16	3.2 mm ± 0.2 mm	14 kg/cm^2	nil	99.43%	complies	6.91

Tablets 29: Swelling index of FA13 – FA16

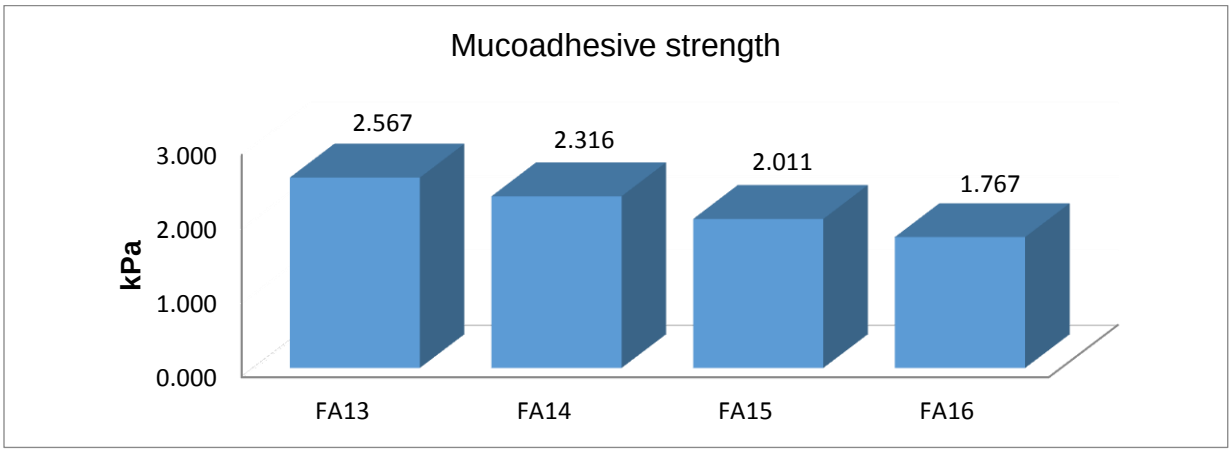
Trial	FA13	FA14	FA15	FA16
3 h	52.85	49.36	47.01	43.95
6 h	68.71	64.17	61.11	57.14
12 h	79.70	89.84	75.78	63.99
18 h	92.45	125.77	93.97	71.67
24 h	89.57	176.08	116.52	80.27
30 h		160.76	144.48	89.90
36 h			179.16	100.69
42 h			165.39	112.77
48 h				126.31
54 h				129.46



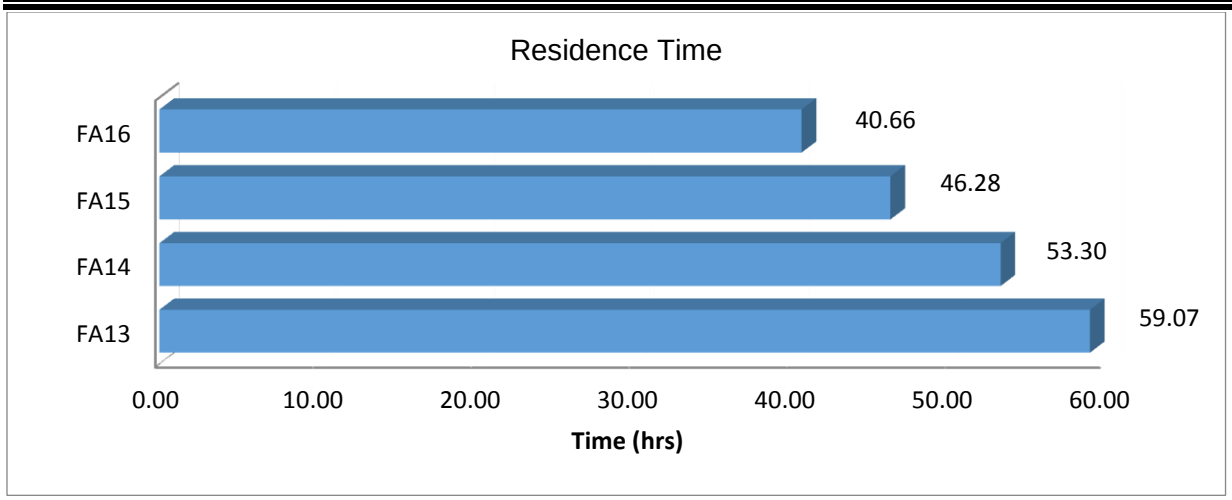
Graph 12: Swelling index of formulation FA13 – FA16

Table 30: Mucoadhesive strength & residence time of tablets

formula trial	Mucoadhesion strength (gm)	Mucoadhesive force (kPa)	Residence time (hours)
FA13	26.18	2.567	59.07
FA14	23.62	2.316	53.30
FA15	20.51	2.011	46.28
FA16	18.02	1.767	40.66



Graph 13 :Mucoadhesive strength of formulation FA13 – FA16

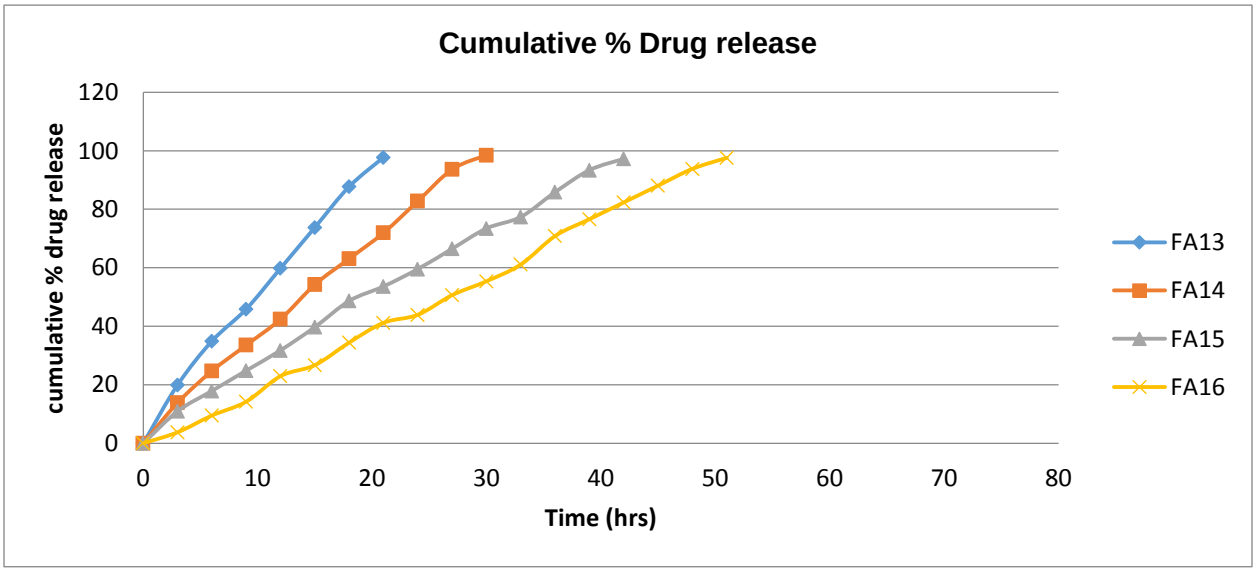


Graph 14 : Residence time of formulation FA13 – FA16

Table 31: Cumulative % Drug release - Dissolution test in 6.8 pH phosphate buffer medium:

Trial	FA13	FA14	FA15	FA16
3 h	19.96	13.85	10.95	3.74
6 h	34.93	24.70	17.89	9.48
9 h	45.89	33.55	24.84	14.22
12 h	59.85	42.43	31.78	22.96
15 h	73.81	54.26	39.73	26.70
18 h	87.78	63.11	48.67	34.44
21 h	97.74	71.96	53.62	41.18
24 h		82.81	59.57	43.92
27 h		93.66	66.51	50.65
30 h		98.51	73.46	55.39
33 h			77.40	61.13
36 h			85.87	70.87
39 h			93.40	76.61
42 h			97.24	82.35
45 h				88.09
48 h				93.83

51 h				97.57



Graph 15 : Cumulative % Drug release of formulation FA13 – FA16

Ethyl cellulose was added to the formulation and was found its ability to retard drug release. Since 72 hours drug release to be achieved, concentration of ethyl cellulose was increased in the formulation. By increasing the concentration of ethyl cellulose drug release was retarded, but mucoadhesion property was also reduced.

Hence , study was concentrated on hydrophilic polymers like sodium alginate and hydroxy propyl methyl cellulose.

This hydrophilic polymers helps in formation of hydrogel complex that readily absorbs water from the environment of colon and has the capacity to hold up water in it remaining in gel state. Thus providing, controlled release of drug from the dosage form. Since they are hydrophilic polymers, they also possess mucoadhesive property.

Tablets containing Eudragit E po, Carbopol 971 P & Protanal CR 8133:

Table 32: Blend parameters of FA17 – FA23

Formula trial	Bulk density (gm/ml)	Tapped density (gm/ml)	Compressibility index %	Hausner ratio	% drug content
FA17	0.382	0.456	16.228	1.194	99.67%
FA18	0.365	0.443	17.607	1.214	99.85%
FA19	0.346	0.440	21.364	1.272	99.59%
FA20	0.378	0.432	12.500	1.143	98.20%
FA21	0.362	0.421	14.014	1.163	100.00 %
FA22	0.36	0.418	13.876	1.161	98.66%
FA23	0.358	0.420	14.762	1.173	99.47%

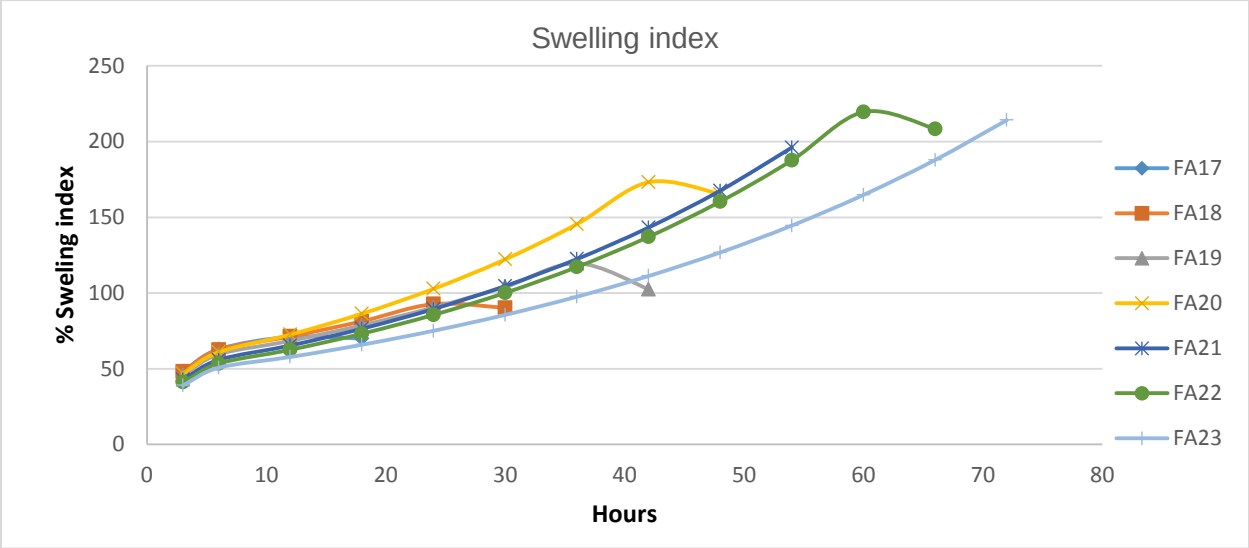
Table 33: Tablet Physical parameters of FA17 – FA23

formula trial	thickness (mm)	hardness kg/cm^2	% friability	content uniformity	weight variation	Surface pH
FA17	3.2 mm ± 0.2 mm	12 kg/cm^2	nil	99.60%	complies	6.86
FA18	3.3 mm ± 0.1 mm	12 kg/cm^2	nil	99.79%	complies	6.88
FA19	3.3 mm ± 0.2 mm	12 kg/cm^2	nil	99.43%	complies	6.91
FA20	3.4 mm ± 0.1 mm	13 kg/cm^2	nil	98.03%	complies	6.94
FA21	3.5 mm ± 0.1 mm	13 kg/cm^2	nil	99.86 %	complies	6.99
FA22	3.4 mm ± 0.2 mm	13 kg/cm^2	nil	98.52%	complies	7.01
FA23	3.3 mm ± 0.2 mm	14 kg/cm^2	nil	99.37%	complies	7.04

Tables 34: Swelling index of FA17 – FA23

Trial	FA17	FA18	FA19	FA20	FA21	FA22	FA23
3 h	48.39	48.14	45.77	46.92	42.97	41.12	38.99
6 h	62.91	62.58	59.50	61.00	55.86	53.46	50.69
12 h	71.08	71.34	68.43	72.59	65.36	62.54	57.78
18 h	69.87	81.33	78.69	86.38	76.47	73.18	65.87
24 h		92.72	90.49	102.79	89.47	85.62	75.10
30 h		90.17	104.07	122.32	104.68	100.17	85.61
36 h			119.68	145.56	122.47	117.20	97.59

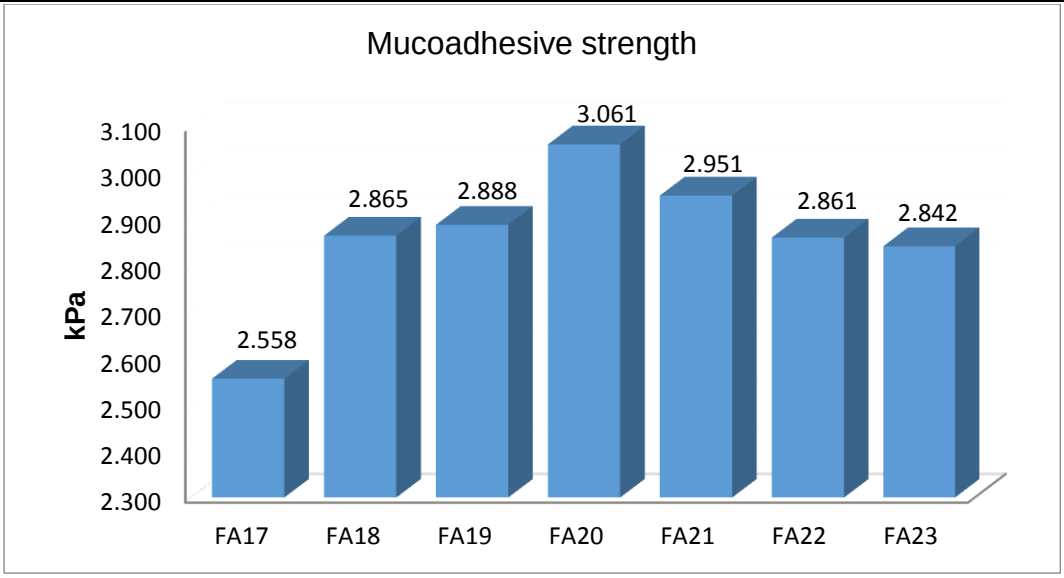
42 h			102.53	173.21	143.29	137.12	111.26
48 h				165.34	167.65	160.43	126.83
54 h					196.15	187.71	144.59
60 h						219.62	164.83
66 h						208.42	187.91
72 h							214.22



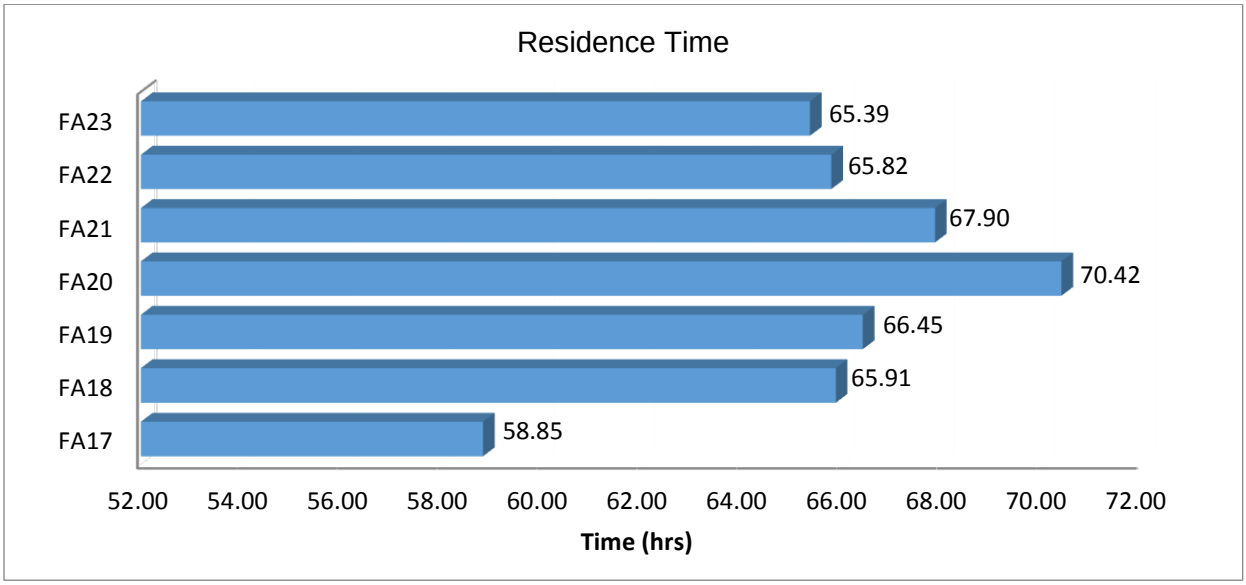
Graph 16: Swelling index of formulation FA17 – FA23

Table 35: Mucoadhesive strength & residence time of tablets

formula trial	Mucoadhesion strength (gm)	Mucoadhesive force (kPa)	Residence time (hours)
FA17	26.08	2.558	58.85
FA18	29.21	2.865	65.91
FA19	29.45	2.888	66.45
FA20	31.21	3.061	70.42
FA21	30.09	2.951	67.90
FA22	29.17	2.861	65.82
FA23	28.98	2.842	65.39



Graph 17: Mucoadhesive strength of formulation FA17 – FA23

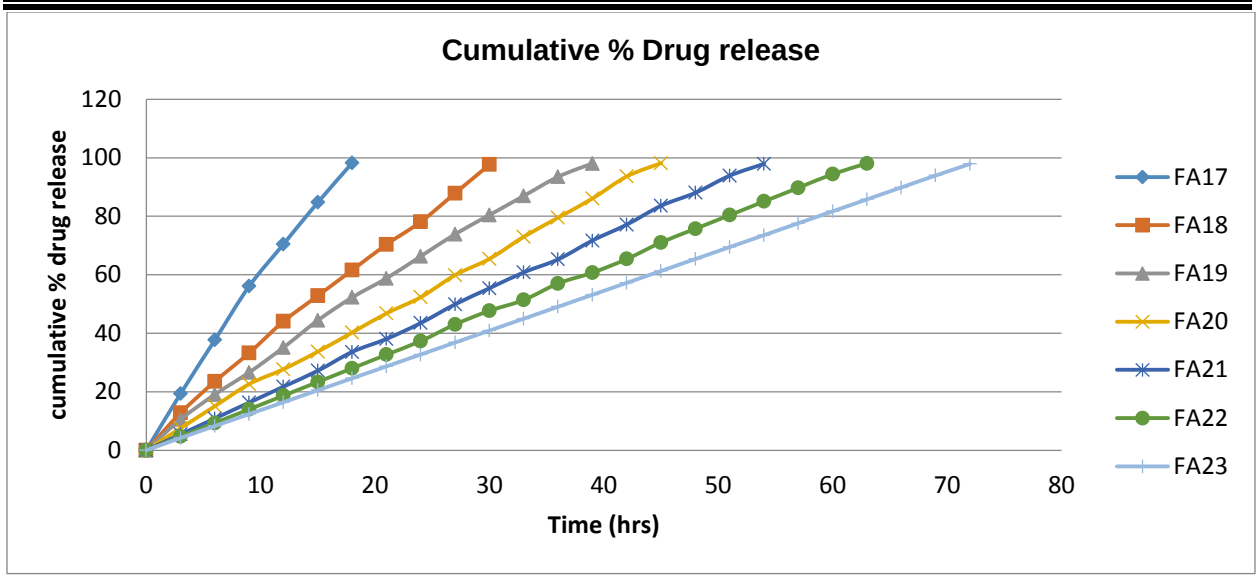


Graph 18: Residence time of formulation FA17 – FA23

Table 36: Cumulative % Drug release - Dissolution test in 6.8 pH phosphate buffer medium

Trial	FA17	FA18	FA19	FA20	FA21	FA22	FA23
3 h	19.38	12.77	10.55	7.55	5.44	4.67	4.21
6 h	37.75	23.54	19.09	15.09	10.88	9.34	8.29
9 h	56.14	33.31	26.64	22.64	16.32	14.01	12.37
12 h	70.51	44.08	35.18	27.73	21.76	18.69	16.45

15 h	84.80	52.85	44.43	33.73	27.20	23.36	20.53
18 h	98.26	61.61	52.27	40.28	33.64	28.03	24.61
21 h		70.39	58.82	46.82	38.08	32.70	28.69
24 h		78.16	66.36	52.37	43.52	37.37	32.77
27 h		87.93	73.91	59.91	49.91	43.04	36.85
30 h		97.70	80.45	65.46	55.41	47.71	40.93
33 h			87.00	73.01	60.85	51.39	45.01
36 h			93.54	79.55	65.29	57.06	49.09
39 h			98.09	86.10	71.73	60.73	53.16
42 h				93.64	77.17	65.40	57.24
45 h				98.19	83.61	71.07	61.32
48 h					88.05	75.74	65.40
51 h					93.90	80.41	69.48
54 h					97.93	85.09	73.56
57 h						89.76	77.64
60 h						94.43	81.72
63 h						98.10	85.80
66 h							89.88
69 h							93.96
72 h							97.91



Graph 19: Cumulative % drug release of formulation FA17 – FA23

Tablets containing Eudragit E po, Carbopol 971 P & HPMC K 100 M LVCR:

Table 37: Blend parameters of FA24 – FA28

Formula trial	Bulk density (gm/ml)	Tapped density (gm/ml)	Compressibility index %	Hausner ratio	% drug content
FA24	0.397	0.473	16.068	1.191	99.66%
FA25	0.376	0.467	19.486	1.242	99.84%
FA26	0.364	0.451	19.290	1.239	99.64%
FA27	0.348	0.44	20.909	1.264	98.61%
FA28	0.333	0.422	21.090	1.267	99.36%

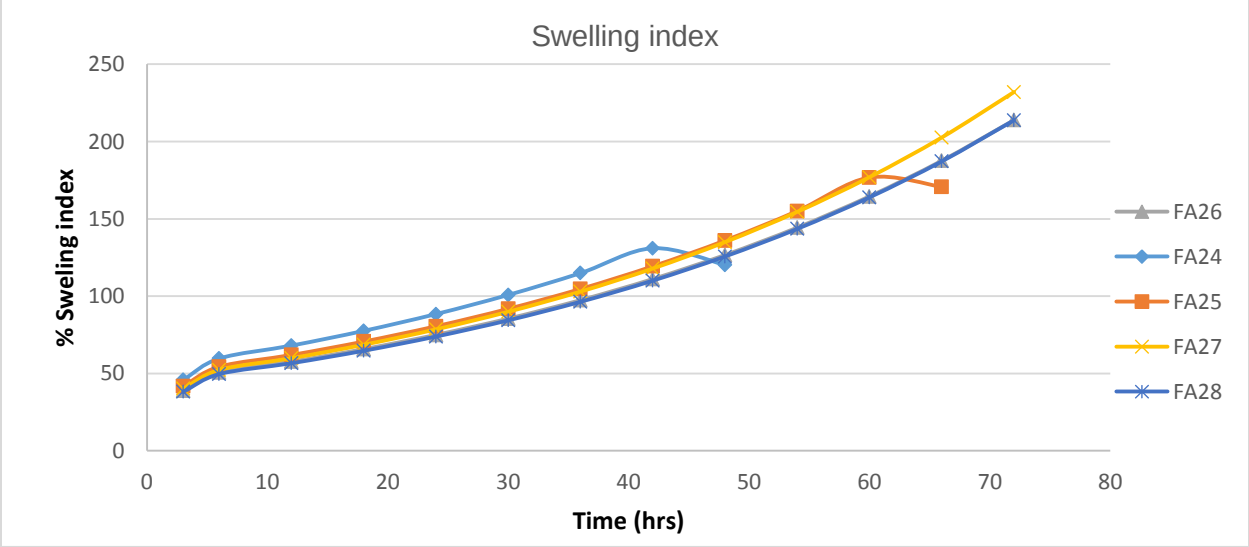
Table 38: Tablet Physical parameters of FA24 – FA28

formula trial	thickness (mm)	Hardness kg/cm^2	% Friability	Content uniformity	Weight variation	Surface pH
FA24	3.2 mm ± 0.1 mm	12 kg/cm^2	nil	99.53%	complies	6.97
FA25	3.3 mm ± 0.1 mm	12 kg/cm^2	nil	99.54%	complies	7.01
FA26	3.3 mm ± 0.2 mm	13 kg/cm^2	nil	99.24%	complies	7.04
FA27	3.2 mm ± 0.2 mm	14 kg/cm^2	nil	98.21%	complies	7.08

FA28	3.3 mm ± 0.1 mm	14 kg/cm^2	nil	99.16%	complies	7.11

Table 39: Swelling index of FA24 – FA28

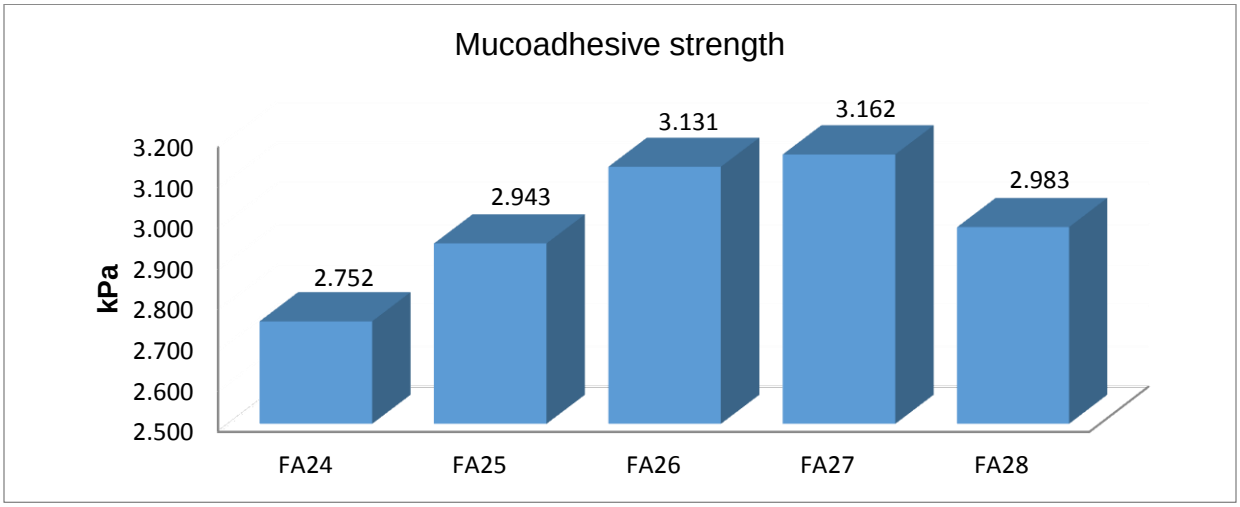
Trial	FA24	FA25	FA26	FA27	FA28
3 h	45.92	41.82	40.25	38.93	38.17
6 h	59.70	54.37	52.33	50.61	49.62
12 h	68.05	61.98	59.91	57.69	56.67
18 h	77.58	70.65	68.60	65.77	64.71
24 h	88.44	80.55	78.55	74.98	73.90
30 h	100.82	91.82	89.94	85.48	84.40
36 h	114.94	104.68	102.98	97.44	96.38
42 h	131.03	119.33	117.91	111.09	110.07
48 h	120.14	136.04	135.00	126.64	125.70
54 h		155.08	154.58	144.37	143.55
60 h		176.80	176.99	164.58	163.93
66 h		170.62	202.66	187.62	187.21
72 h			232.04	213.89	213.79
78 h			230.40		244.15
84 h					242.24



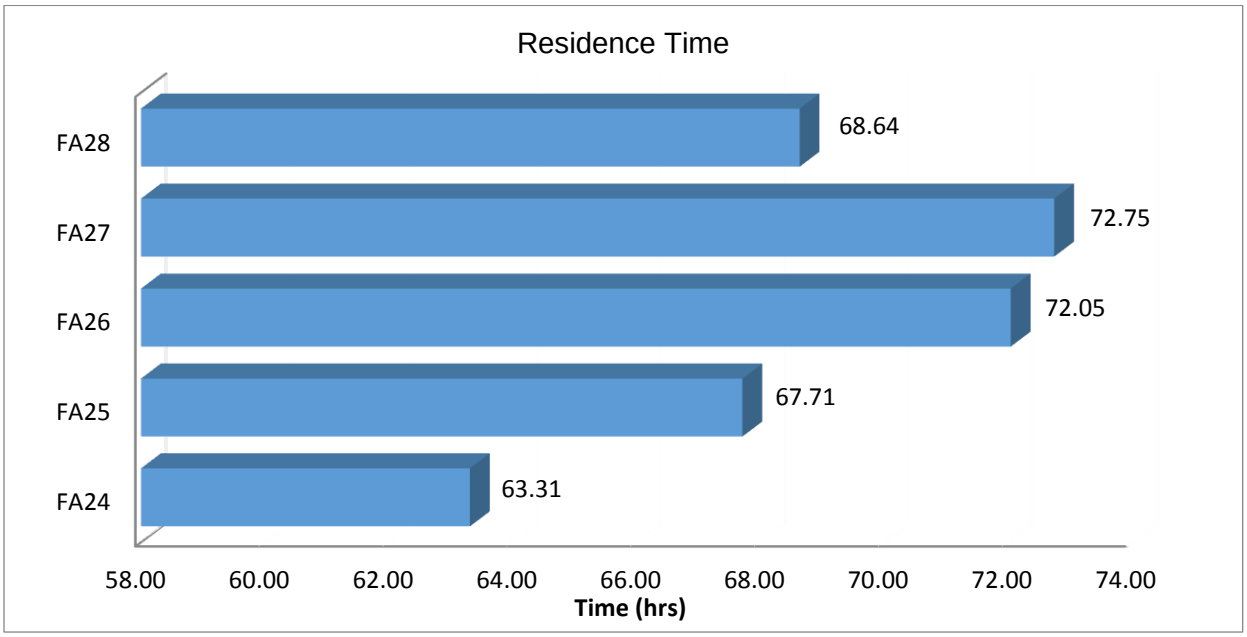
Graph 20: swelling index of formulation FA24 – FA28

Table 40 : Mucoadhesive strength & residence time of tablets FA24 – FA28

Formula Trial	Mucoadhesion strength (gm)	Mucoadhesive force (kPa)	Residence time (hours)
FA24	28.06	2.752	63.31
FA25	30.01	2.943	67.71
FA26	31.93	3.131	72.05
FA27	32.24	3.162	72.75
FA28	30.42	2.983	68.64



Graph 21: Mucoadhesive strength of formulation FA24 – FA28

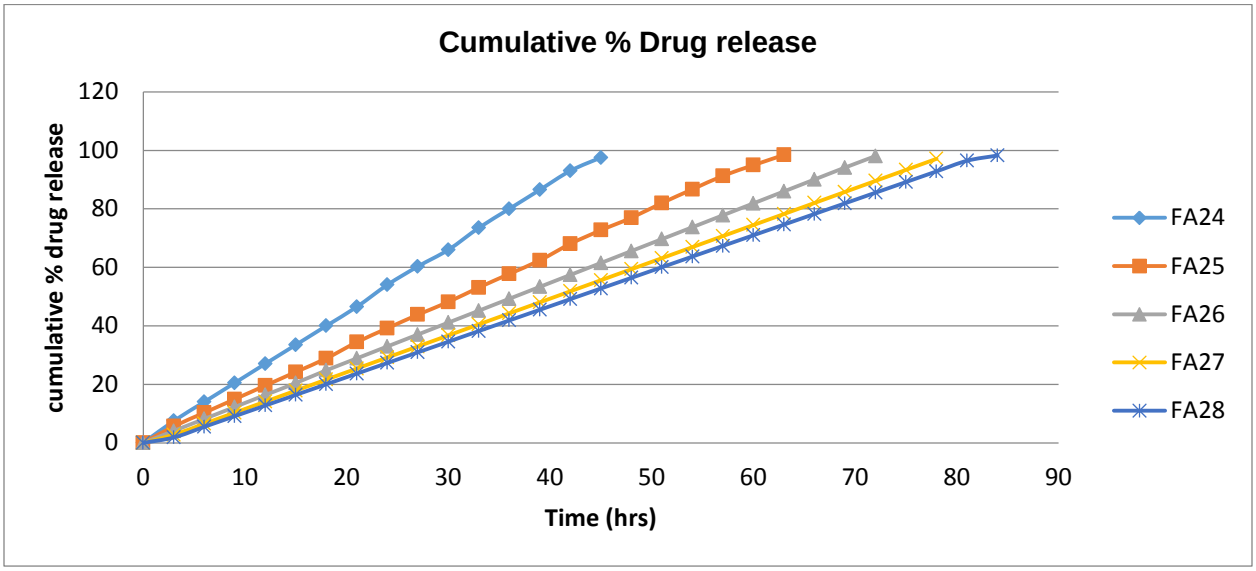


Graph 22: Residence time of formulation FA24 – FA28

Table 41: Cumulative % Drug release - Dissolution test in 6.8 pH phosphate buffer medium:

Trial	FA24	FA25	FA26	FA27	FA28
3 h	7.50	5.65	2.77	4.09	1.84
6 h	14.01	10.30	6.55	8.19	5.48
9 h	20.51	14.94	10.32	12.28	9.12
12 h	27.05	19.59	14.10	16.37	12.76
15 h	33.52	24.24	17.87	20.46	16.40
18 h	40.02	28.89	21.65	24.81	20.04
21 h	46.52	34.43	25.42	28.89	23.68
24 h	54.03	39.18	29.19	32.97	27.32
27 h	60.33	43.83	32.97	37.05	30.96
30 h	66.03	48.18	36.74	41.12	34.60
33 h	73.54	53.13	40.52	45.20	38.24
36 h	80.04	57.78	44.29	49.28	41.88
39 h	86.54	62.43	48.07	53.36	45.52
42 h	93.05	68.07	51.84	57.44	49.15
45 h	97.55	72.72	55.61	61.52	52.79
48 h		76.98	59.39	65.59	56.43
51 h		82.02	63.16	69.67	60.07
54 h		86.71	66.94	73.75	63.71
57 h		91.31	70.71	77.83	67.35
60 h		94.96	74.48	81.91	70.99
63 h		98.46	78.26	85.99	74.63
66 h			82.03	90.06	78.27
69 h			85.81	94.14	81.91
72 h			89.58	98.07	85.55

75 h			93.36		89.19
78 h			97.13		92.83
81 h					96.47
84 h					98.27



Graph 23: Cumulative % drug release of formulation FA24 – FA28

Dissolution test for enteric coated capsule:

Table 42: % Drug release of enteric coated capsule system - dissolution test in 0.1 N Hcl for 2 hours

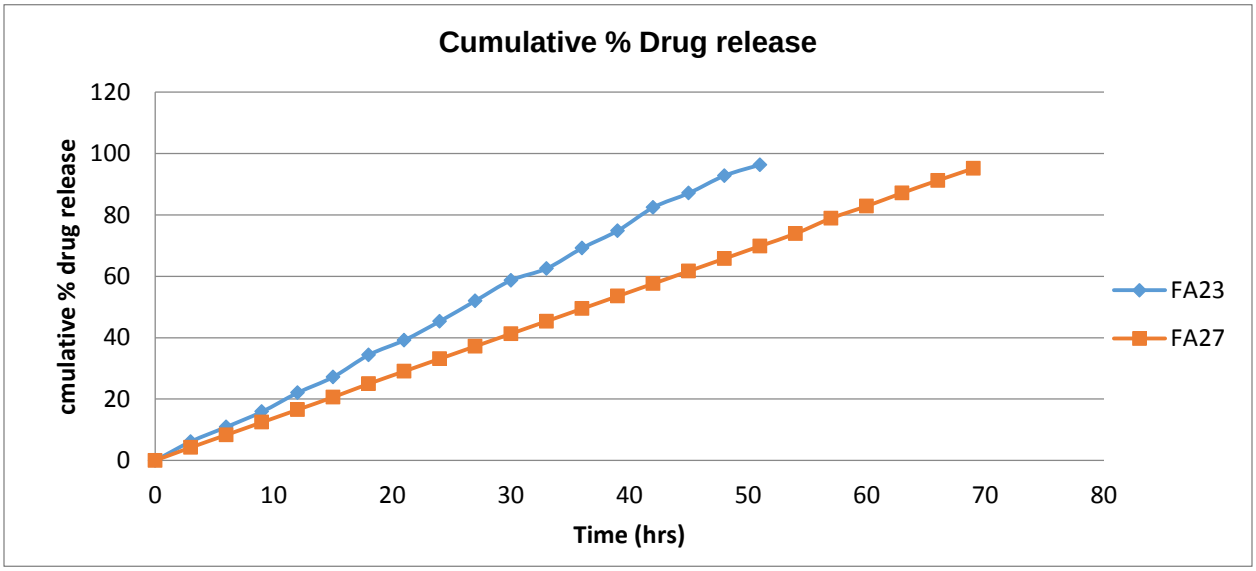
Eudragit L 100 coating	FA23	FA27
8 %	6.17%	5.76%
10 %	-	-
12 %	-	-

Table 43: % Drug release of enteric coated capsule system - dissolution test in 7.4 pH phosphate buffer for 3 hours:

Eudragit E po coating	FA23	FA27
3 %	3.96%	3.55%
4 %	-	-
5 %	-	-

Table 44: Cumulative % Drug release of enteric coated capsule system - dissolution test in 6.8 pH phosphate buffer containing 5 % goat's caecal content:

Trial	FA23			FA27			
hours	% drug release	hours	% drug release	hours	% drug release	hours	% drug release
3 h	6.15	39 h	74.85	3 h	4.25	39 h	53.52
6 h	10.92	42 h	82.42	6 h	8.35	42 h	57.60
9 h	15.89	45 h	87.09	9 h	12.44	45 h	61.68
12 h	22.04	48 h	92.76	12 h	16.53	48 h	65.75
15 h	27.19	51 h	96.30	15 h	20.62	51 h	69.83
18 h	34.38	54 h	-	18 h	24.97	54 h	73.91
21 h	39.18	57 h	-	21 h	29.05	57 h	78.89
24 h	45.35	60 h	-	24 h	33.13	60 h	82.86
27 h	52.02	63 h	-	27 h	37.21	63 h	87.15
30 h	58.69	66 h	-	30 h	41.28	66 h	91.22
33 h	62.51	69 h	-	33 h	45.36	69 h	95.13
36 h	69.18	72 h	-	36 h	49.44	72 h	-



Graph 24: Cumulative % drug release of formulation FA23 and FA27 in colon simulated medium

Protonal CR 8133 (Sodium alginate) retarded the delivery of drugs and provided drug release in controlled manner in pH 6.8 buffer medium. But in presence of goat's caecal content the drug release was faster compared to normal pH 6.8 buffer medium. Its drug retarding property is not sufficient to provide drug delivery for 72 hours. Since, sodium alginate is also degraded by colonic microbiota drug release may be higher in colon. And also sodium alginate is an anionic polymer , it releases drug in pH independent manner when used in combination with Interpolyelectrolyte complex. Hence it lags in chronobiological based release profile.

Hydroxy propyl methyl cellulose is a non-ionic hydrophilic polymer that in combination with interpolyelectrolyte complex forms an hydrogel system. Hydroxypropyl methyl cellulose is not degraded by colonic microbiota.

In recent study it has shown temperature dependent release and it was reported. Hence, use of Hydroxyl propyl methyl cellulose is also advantageous in colon delivery of drug based on chronobiology since there exist an acute inflammatory reaction in colon during asthma attacks which raises temperature and lowers pH in the lumen of the colon.

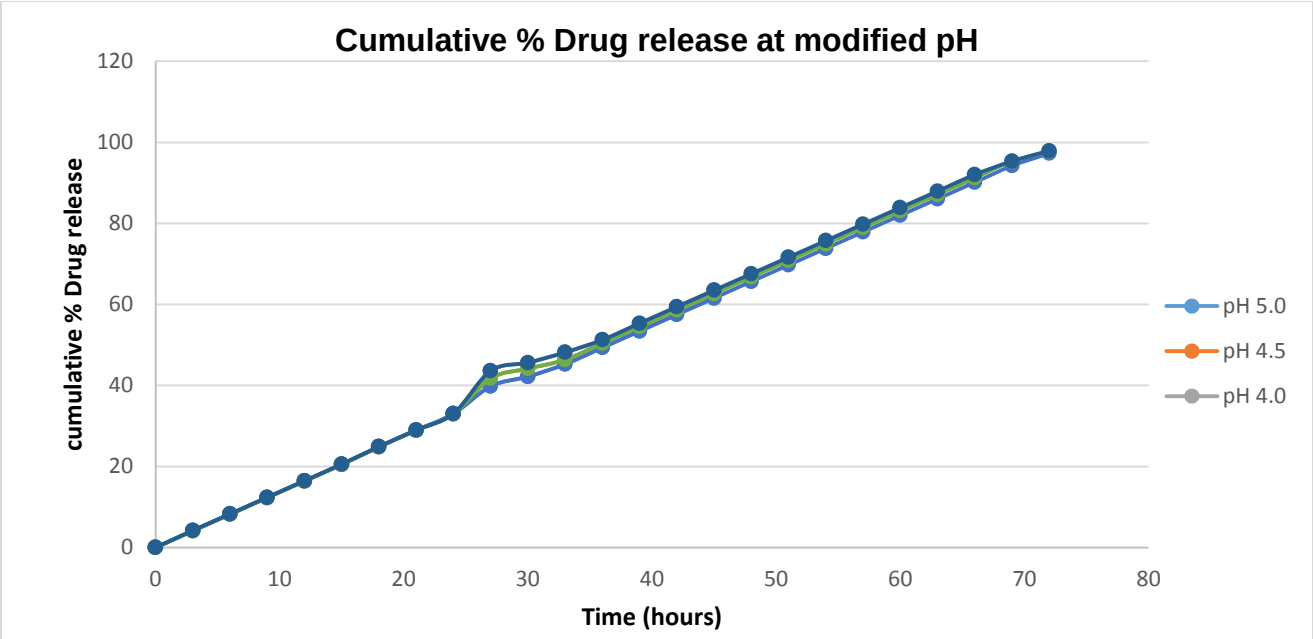
Presence of HPMC also increases the mucoadhesive property of the mini tablets. Since its mucoadhesion property is independent of pH and formation of good hydrogel system is taken into consideration. Controlled delivery of the drug from the dosage form is achieved and also provides effective drug release in combination with interpolyelectrolyte complex when triggered.

Higher viscosity polymers like HPMC k 100 M may cause initial burst release from the dosage form. but presence of carbopol , a low viscosity grade polymer prevents such burst release.

Table 45: Dissolution test based on chronobiology (pH modification) in 6.8 pH phosphate buffer containing 5 % goats caecal content (FA27):

Trial	**FA27 a**			**FA27 b**				**FA27 c**			
Time	**% drug release**	**Time**	**% drug release**	**Time**	**% drug release**	**Time**	**% drug release**	**Time**	**% drug release**	**Time**	**% drug release**
3 h	4.16	**36 h**	49.35	**3 h**	4.12	**36 h**	50.45	**3 h**	4.20	**36 h**	51.26
6 h	8.26	**39 h**	53.43	**6 h**	8.22	**39 h**	54.53	**6 h**	8.30	**39 h**	55.34
9 h	12.35	**42 h**	57.51	**9 h**	12.31	**42 h**	58.61	**9 h**	12.39	**42 h**	59.42

12 h	16.44	**45 h**	61.59	**12 h**	16.40	**45 h**	62.69	**12 h**	16.48	**45 h**	63.50
15 h	20.53	**48 h**	65.66	**15 h**	20.49	**48 h**	66.76	**15 h**	20.57	**48 h**	67.57
18 h	24.88	**51 h**	69.74	**18 h**	24.84	**51 h**	70.84	**18 h**	24.92	**51 h**	71.65
21 h	28.96	**54 h**	73.82	**21 h**	28.92	**54 h**	74.92	**21 h**	29.00	**54 h**	75.73
24 h	33.04	**57 h**	77.90	**24 h**	33.00	**57 h**	79.00	**24 h**	33.08	**57 h**	79.81
5.0 pH		**60 h**	81.98	**4.5 pH**		**60 h**	83.08	**4.0 pH**		**60 h**	83.89
27 h	39.82	**63 h**	86.06	**27 h**	41.78	**63 h**	87.16	**27 h**	43.61	**63 h**	87.97
6.8 pH		**66 h**	90.13	**6.8 pH**		**66 h**	91.23	**6.8 pH**		**66 h**	92.04
30 h	42.19	**69 h**	94.21	**30 h**	44.15	**69 h**	95.31	**30 h**	45.63	**69 h**	95.34
33 h	45.27	**72 h**	95.73	**33 h**	46.37	**72 h**	-	**33 h**	48.18	**72 h**	-



Graph 25: Cumulative % drug release of formulation FA27 in modified pH conditions

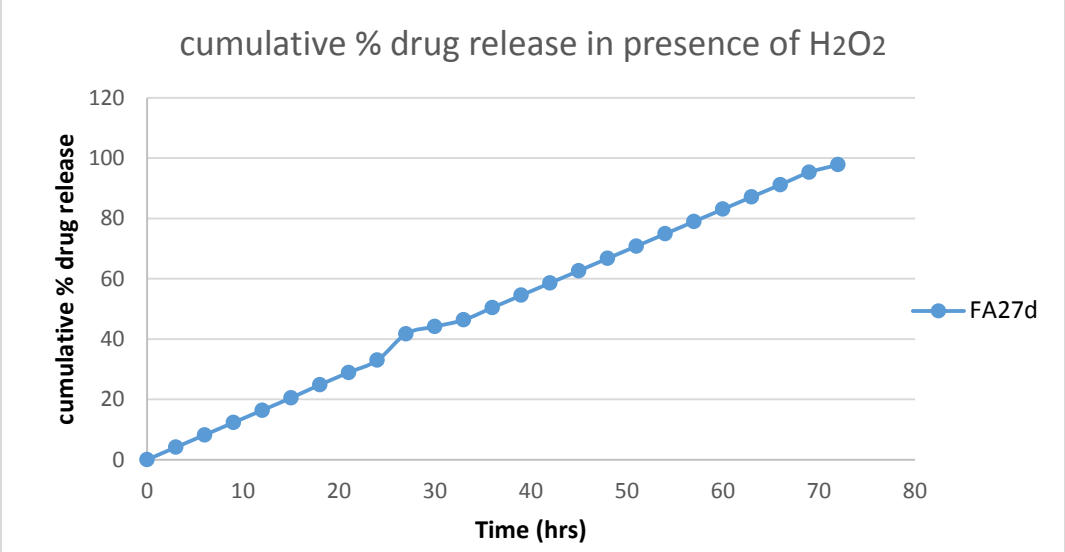
Final formula (1:7:3:4) provided control release of drug over a period of 72 hours. Releasing 1 mg of salbutamol sulphate in every 3 hours. (Resembling 12 hours dose of 4 mg SR tablets as recommended in USP). And also provided excellent drug triggered release when pH of dissolution medium is lowered (5 pH) by addition of 5 N Hcl. And the system had the capacity to regain its initial property of retarding drug release when condition is taken back to initial (6.8 pH) by addition of 5 N NaoH.

Note: The addition of 5 N Hcl and 5 N NaoH is involved to adjust the pH in dissolution medium within few drops. Because, addition of diluted 1 N Hcl and 1 N NaOH should not affect the volume of dissolution medium which may alternatively affect the result during UV absorbance study.

pH of the dissolution medium was lowered to various pH levels. Since colon may have the pH of less than 4.2 during asthma attack. The pH of the dissolution medium was changed to 4.5 and 4.0 pH. The release of drug from the dosage form was noted. It is observed that as the pH lowers, drug release is triggered more. And the system had capacity to regain its original state when pH is reversed. Hence it is concluded that, drug release is achieved based on severity of asthma attack.

Table 46: Dissolution test based on chronobiology (Free radical induced) in 6.8 pH phosphate buffer containing 5 % goats caecal content (FA27):

Trial	**FA27 d**		
hours	**% drug release**	**hours**	**% drug release**
3 h	4.12	**39 h**	54.53
6 h	8.22	**42 h**	58.61
9 h	12.31	**45 h**	62.69
12 h	16.40	**48 h**	66.76
15 h	20.49	**51 h**	70.84
18 h	24.84	**54 h**	74.92
21 h	28.92	**57 h**	79.00
24 h	33.00	**60 h**	83.08
H2O2 addition		**63 h**	87.16
27 h	41.78	**66 h**	91.23
30 h	44.15	**69 h**	95.31
33 h	46.37	**72 h**	-
36 h	50.45		



Graph 25: Cumulative % drug release of formulation FA27 in presence of Hydroxyl radicals

Invitro Drug permeation study

Table 47 : Invitro drug permeation of FA27

Time (hr)	% Drug release	% Drug permeation
3 h	4.09	3.14
6 h	8.19	6.89
9 h	12.28	10.64
12 h	16.37	14.38
15 h	20.46	18.12
18 h	24.81	22.10
21 h	28.89	25.83
24 h	32.97	29.57
27 h	37.05	33.30
30 h	41.12	37.02
33 h	45.20	40.76
36 h	49.28	44.49
39 h	53.36	48.22

42 h	57.44	51.96
45 h	61.52	55.69
48 h	65.59	59.41
51 h	69.67	63.15
54 h	73.75	66.88
57 h	77.83	70.61
60 h	81.91	74.35
63 h	85.99	78.08
66 h	90.06	81.80
69 h	94.14	85.54
72 h	98.07	89.13

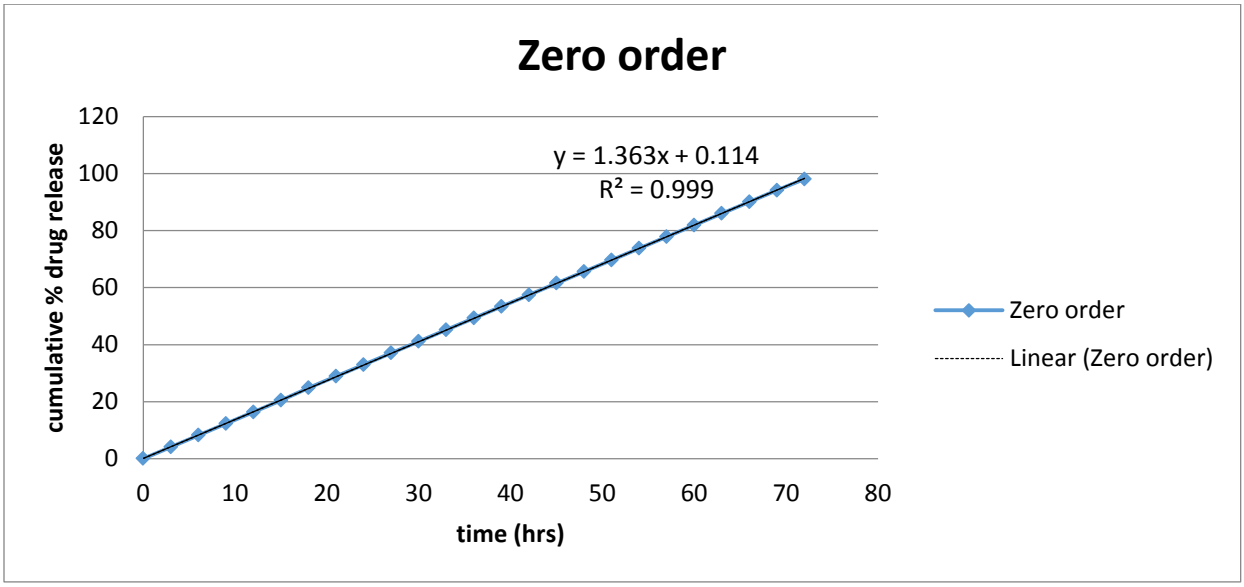
DRUG RELEASE KINETICS:

Table 48: Drug release kinetics of FA27

Time (hrs)	Cumulative % drug release	% drug remaining	Square root of time	Log % drug remaining	Log time	Log cumulative % drug released	% drug released	Cube root of % drug remaining (Wt)	Wo - Wt
0	0	100	0.000	2.000	0.000	0.000	100	4.642	0.000
3	4.09	95.91	1.732	1.982	0.477	0.612	4.09	4.577	0.065
6	8.19	91.81	2.449	1.963	0.778	0.913	4.09	4.511	0.131
9	12.28	87.72	3.000	1.943	0.954	1.089	4.10	4.443	0.199
12	16.37	83.63	3.464	1.922	1.079	1.214	4.09	4.373	0.269
15	20.46	79.54	3.873	1.901	1.176	1.311	4.09	4.301	0.341
18	24.81	75.19	4.243	1.876	1.255	1.395	4.09	4.221	0.421
21	28.89	71.11	4.583	1.852	1.322	1.461	4.35	4.143	0.499
24	32.97	67.03	4.899	1.826	1.380	1.518	4.08	4.062	0.580
27	37.05	62.96	5.196	1.799	1.431	1.569	4.08	3.978	0.664

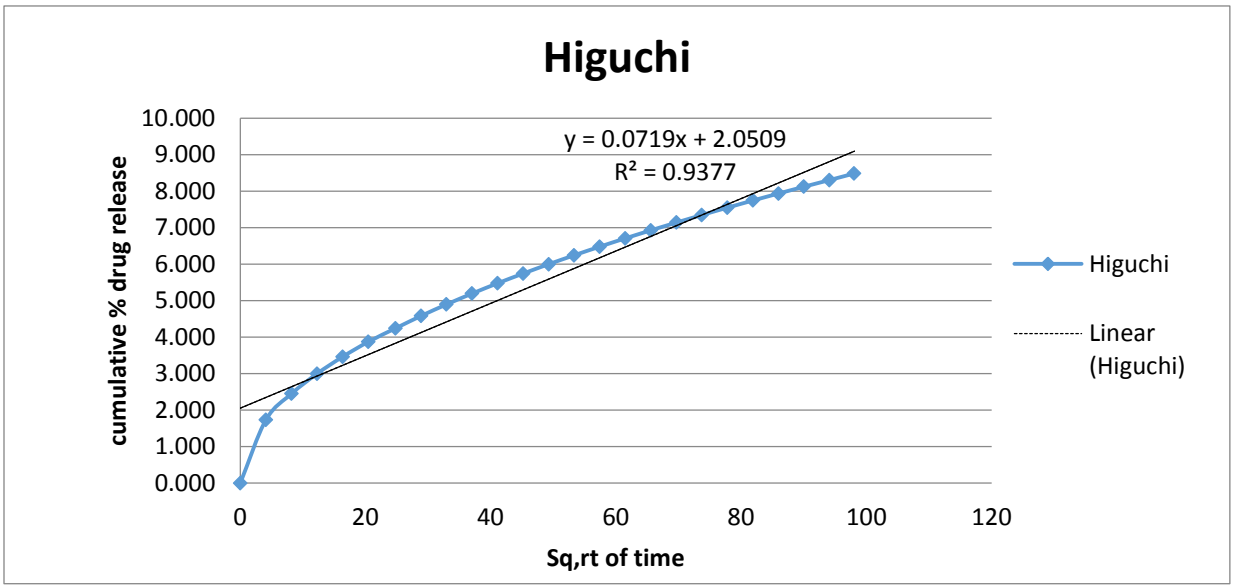
30	41.12	58.88	5.477	1.770	1.477	1.614	4.08	3.890	0.752
33	45.20	54.80	5.745	1.739	1.519	1.655	4.08	3.798	0.844
36	49.28	50.72	6.000	1.705	1.556	1.693	4.08	3.702	0.940
39	53.36	46.64	6.245	1.669	1.591	1.727	4.08	3.600	1.042
42	57.44	42.56	6.481	1.629	1.623	1.759	4.08	3.491	1.151
45	61.52	38.49	6.708	1.585	1.653	1.789	4.08	3.376	1.266
48	65.59	34.41	6.928	1.537	1.681	1.817	4.08	3.252	1.390
51	69.67	30.33	7.141	1.482	1.708	1.843	4.08	3.119	1.523
54	73.75	26.25	7.348	1.419	1.732	1.868	4.08	2.972	1.670
57	77.83	22.17	7.550	1.346	1.756	1.891	4.08	2.809	1.833
60	81.91	18.09	7.746	1.258	1.778	1.913	4.08	2.625	2.017
63	85.99	14.02	7.937	1.147	1.799	1.934	4.08	2.411	2.231
66	90.06	9.94	8.124	0.997	1.820	1.955	4.08	2.150	2.492
69	94.14	5.86	8.307	0.768	1.839	1.974	4.08	1.803	2.839
72	98.07	1.93	8.485	0.286	1.857	1.992	4.08	1.245	3.397

Zero order Kinetics:



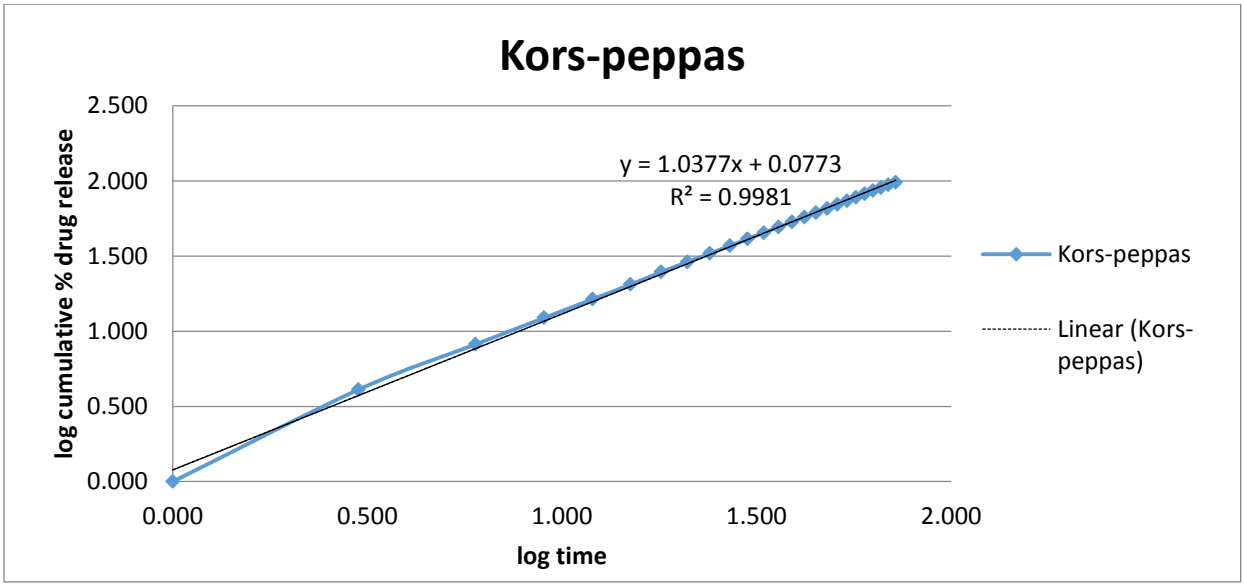
Graph 26: Zero order kinetics of formulation FA27 in colon simulated medium

Higuchi kinetics:



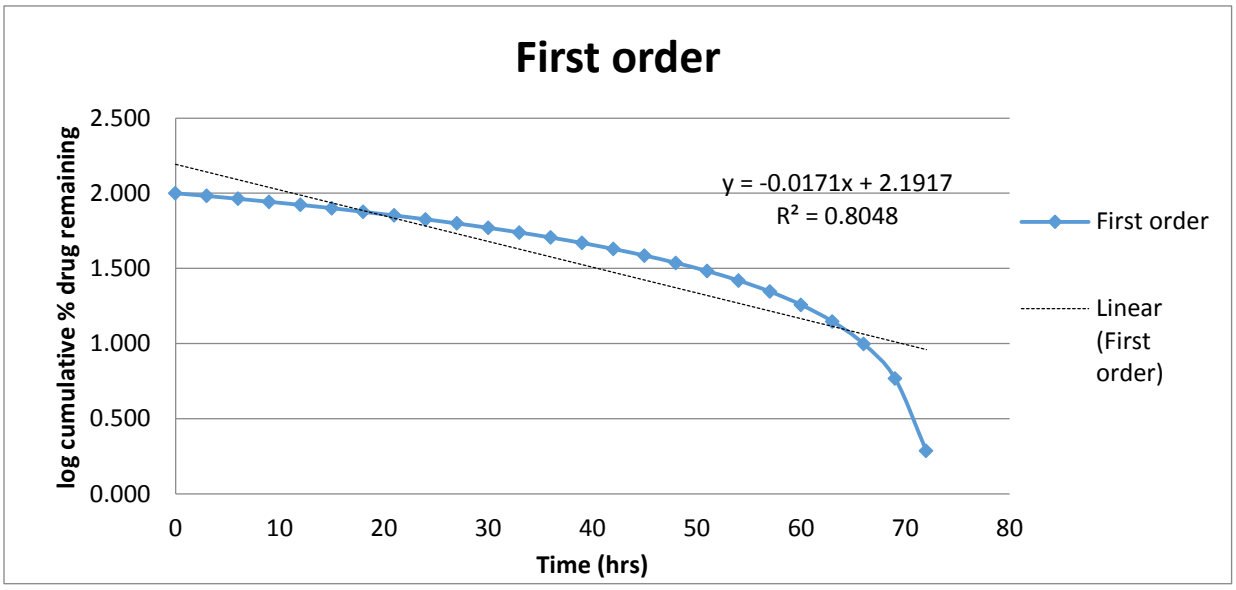
Graph 27: Higuchi kinetics of formulation FA27 in colon simulated medium

Kors-peppas kinetics:



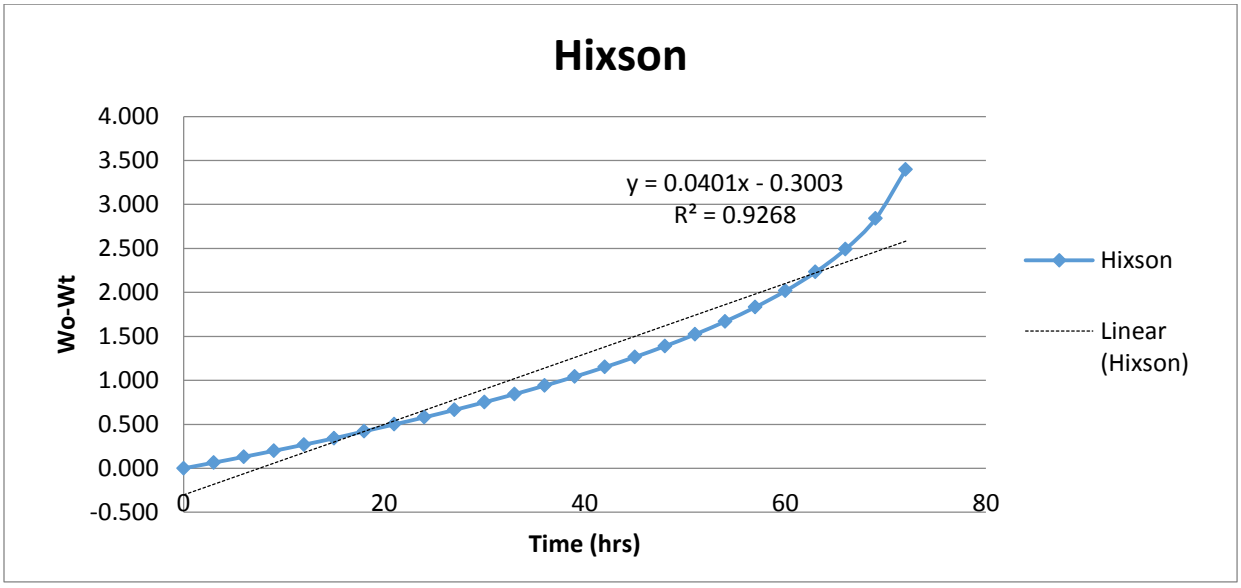
Graph 28: Kors-peppas kinetics of formulation FA27 in colon simulated medium

First order kinetics:



Graph 29: First order kinetics of formulation FA27 in colon simulated medium

Hixson kinetics



Graph 29: Hixson kinetics of formulation FA27 in colon simulated medium

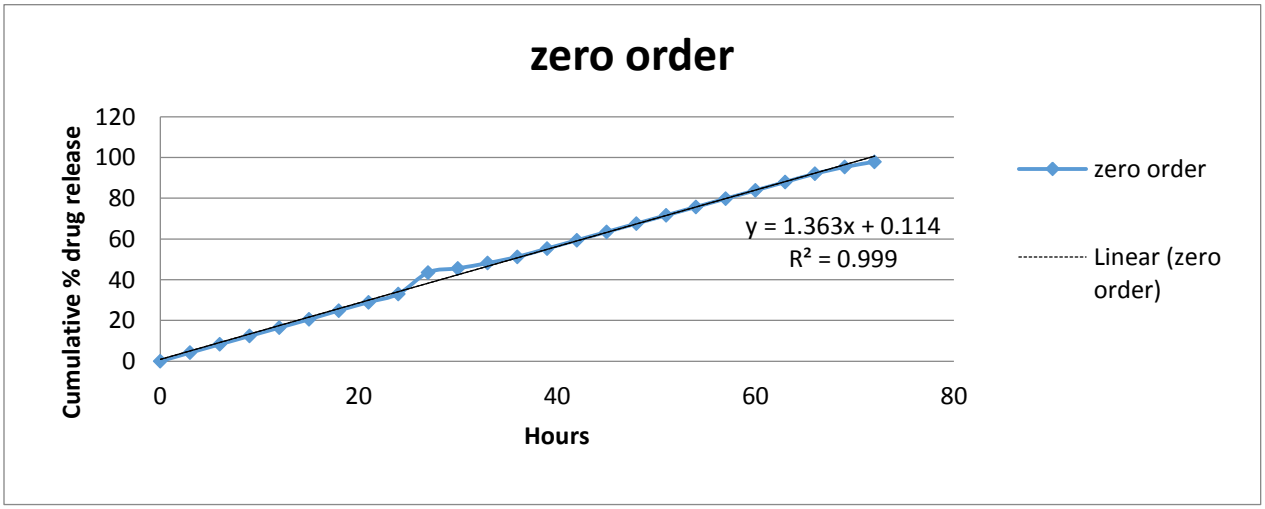
DRUG RELEASE KINETICS BASED ON CHRONOLOGICAL BEHAVIOUR:

Table 49: Drug release kinetics of FA27 based on chronological behavior.

Time (hrs)	Cumulative % drug release	% drug remaining	Square root of time	Log % drug remaining	Log time	Log cumulative % drug released	% drug released	Cube root of % drug remaining (Wt)	Wo - Wt
0	0	100	0.000	2.000	0.000	0.000	100	4.642	0.000
3	4.20	95.80	1.732	1.981	0.477	0.624	4.20	4.576	0.066
6	8.30	91.71	2.449	1.962	0.778	0.919	4.20	4.510	0.132
9	12.39	87.61	3.000	1.943	0.954	1.093	4.09	4.441	0.201
12	16.48	83.52	3.464	1.922	1.079	1.217	4.09	4.371	0.271
15	20.57	79.43	3.873	1.900	1.176	1.313	4.09	4.299	0.343
18	24.92	75.08	4.243	1.876	1.255	1.397	4.09	4.219	0.423
21	29.00	71.00	4.583	1.851	1.322	1.462	4.35	4.141	0.501
24	33.08	66.92	4.899	1.826	1.380	1.520	4.08	4.060	0.582
27	43.61	56.39	5.196	1.751	1.431	1.640	4.08	3.835	0.807
30	45.63	54.37	5.477	1.735	1.477	1.659	10.53	3.788	0.854
33	48.18	51.82	5.745	1.714	1.519	1.683	2.02	3.728	0.914
36	51.26	48.74	6.000	1.688	1.556	1.710	2.55	3.653	0.989
39	55.34	44.66	6.245	1.650	1.591	1.743	3.08	3.548	1.094
42	59.42	40.58	6.481	1.608	1.623	1.774	4.08	3.436	1.206
45	63.50	36.51	6.708	1.562	1.653	1.803	4.08	3.317	1.325
48	67.57	32.43	6.928	1.511	1.681	1.830	4.08	3.189	1.453
51	71.65	28.35	7.141	1.453	1.708	1.855	4.08	3.049	1.593
54	75.73	24.27	7.348	1.385	1.732	1.879	4.08	2.895	1.747
57	79.81	20.19	7.550	1.305	1.756	1.902	4.08	2.723	1.919
60	83.89	16.11	7.746	1.207	1.778	1.924	4.08	2.526	2.116
63	87.97	12.04	7.937	1.080	1.799	1.944	4.08	2.292	2.350

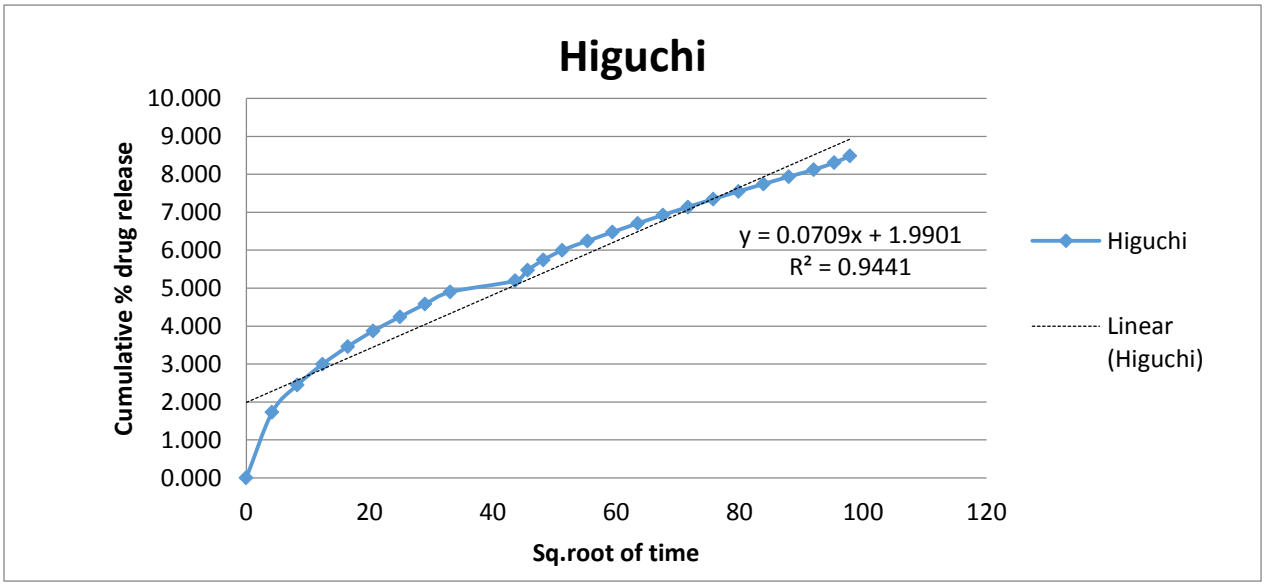
66	92.04	7.96	8.124	0.901	1.820	1.964	4.08	1.996	2.646
69	95.34	4.66	8.307	0.668	1.839	1.979	4.08	1.670	2.972
72	97.91	2.09	8.485	0.320	1.857	1.991	3.30	1.279	3.363

Zero order Kinetics:



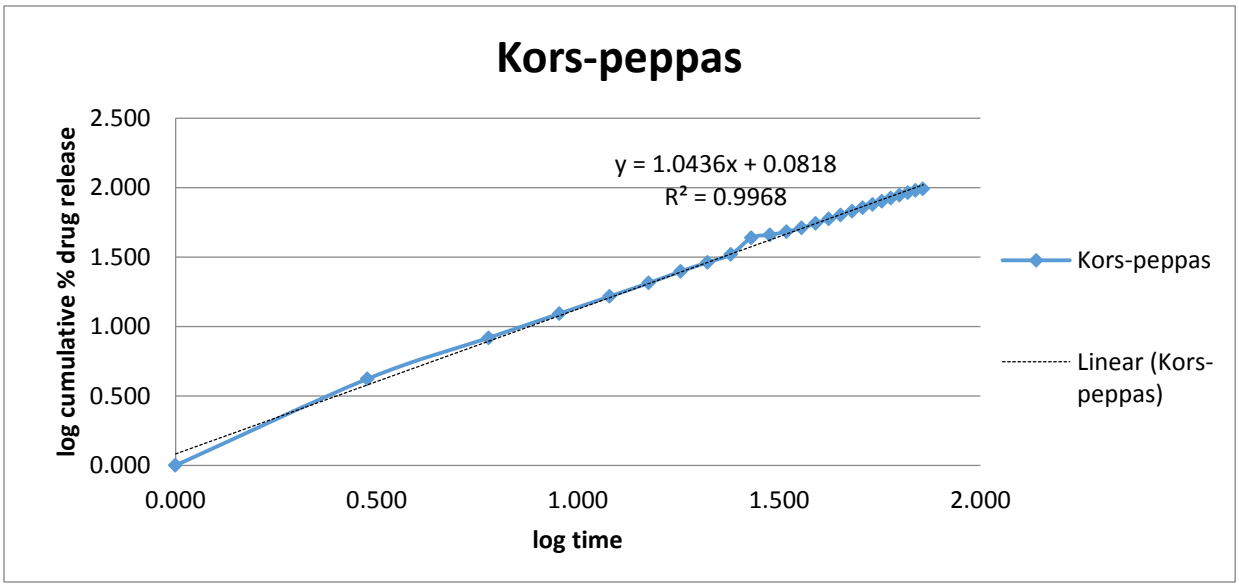
Graph 30: Zero order kinetics of formulation FA27 in colon simulated medium when triggered

Higuchi kinetics:



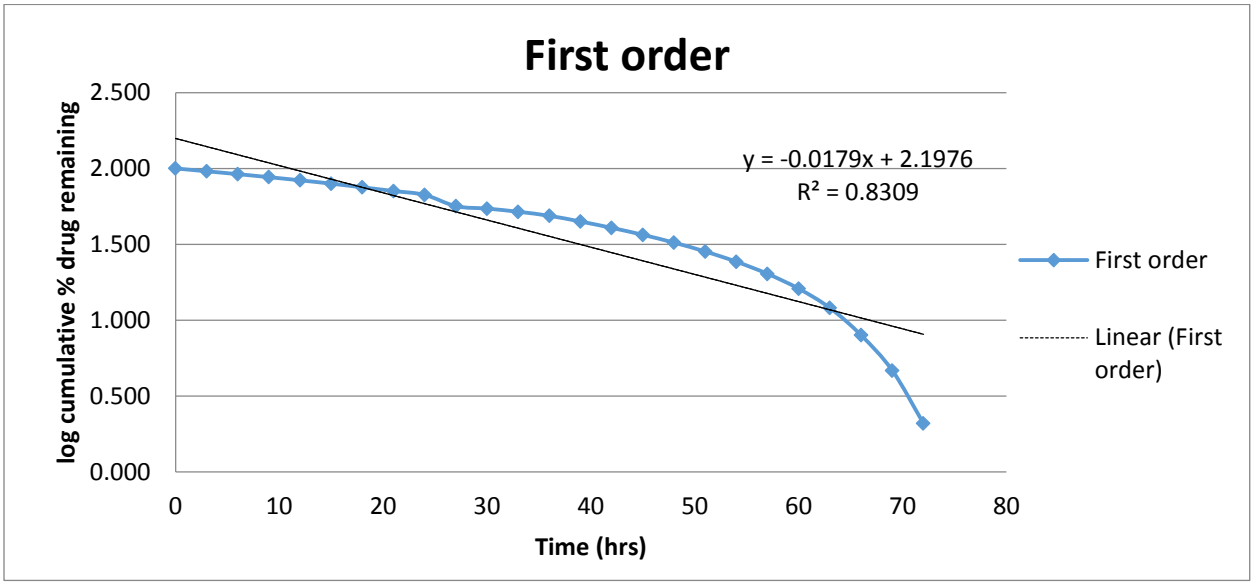
Graph 31: Higuchi kinetics of formulation FA27 in colon simulated medium when triggered

Kors-peppas kinetics



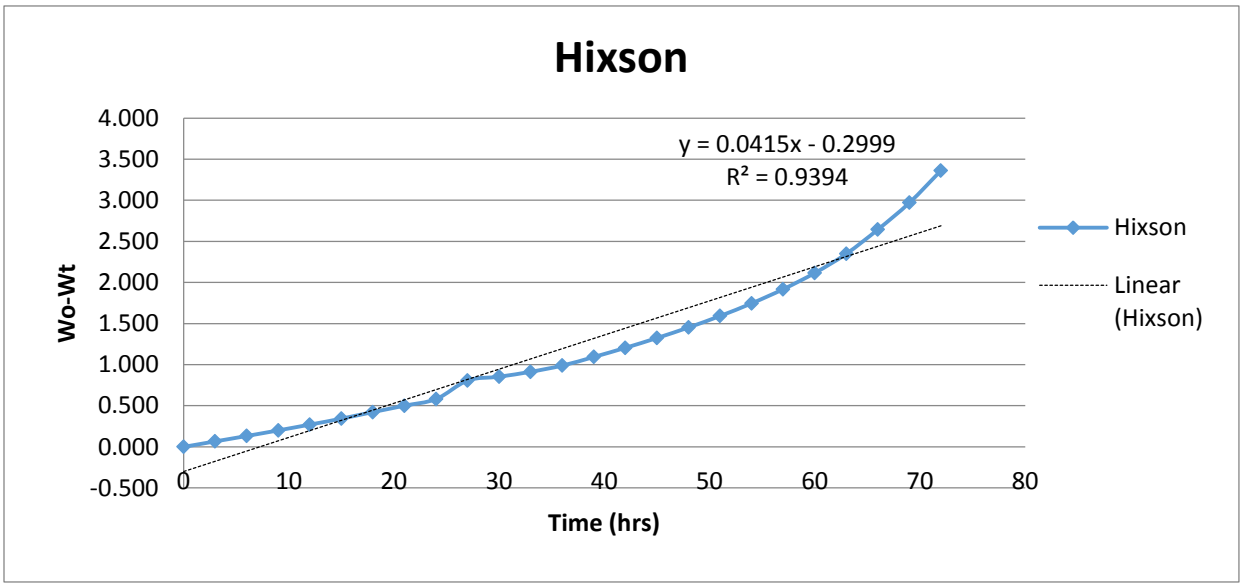
Graph 32: Kors-Peppas kinetics of formulation FA27 in colon simulated medium when triggered

First order kinetics:



Graph 33: First order kinetics of formulation FA27 in colon simulated medium when triggered

Hixson kinetics



Graph 34: Hixson kinetics of formulation FA27 in colon simulated medium when triggered

EVALUATION OF IMMEDIATE RELEASE TABLETS:

Table 50: Blend parameters of FB1 – FB5

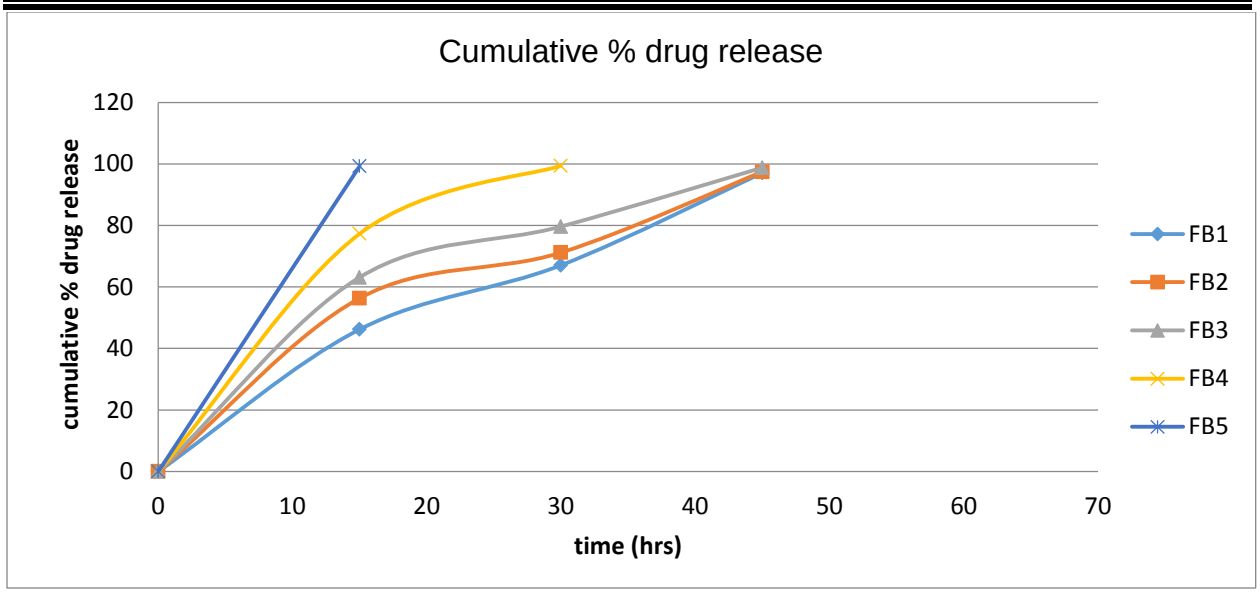
Formula trial	Bulk density (gm/ml)	Tapped density (gm/ml)	Compressibility index %	Hausner ratio	% drug content
FB1	0.421	0.523	19.503	1.242	99.52%
FB2	0.426	0.527	19.165	1.237	99.64%
FB3	0.432	0.531	18.644	1.229	99.35%
FB4	0.429	0.519	17.341	1.210	98.76%
FB5	0.418	0.521	19.770	1.246	99.50%

Table 51: Tablet Physical parameters of FB1 – FB5

Formula Trial	Thickness (mm)	Hardness kg/cm^2	% Friability	Disintegration time	Content uniformity
FB1	2.9 mm ± 0.3 mm	4 kg/cm^2	0.51 %	2 min 11 sec	99.45%
FB2	2.8 mm ± 0.3 mm	4 kg/cm^2	0.45 %	1 min 56 sec	99.51%
FB3	2.9 mm ± 0.2 mm	4 kg/cm^2	0.32 %	1 min 05 sec	99.26%
FB4	3.0 mm ± 0.2 mm	5 kg/cm^2	0.24 %	46 sec	98.58%
FB5	3.0 mm ± 0.1 mm	5 kg/cm^2	0.11 %	14 sec	99.23%

Table 52: Cumulative % Drug release - Dissolution test in 0.1 N Hcl

Trial	FB1	FB2	FB3	FB4	FB5
15 min	46.21	56.34	63.14	77.31	99.27
30 min	67.07	71.18	79.65	99.43	-
45 min	97.13	97.47	98.79	-	-



Graph 35: Cumulative % Drug release of formulation FB1 – FB5

Formula FB5 showed fast disintegration < 15 sec and complete drug release was achieved within 15 min in 0.1 N Hcl dissolution medium.

Crosspovidone XL 10 have larger particle size compared to normal crospovidone and croscarmellose sodium, hence higher rate of wicking caused faster disintegration , thus resulting in faster drug release.

Increase in concentration of Mcc ph 102 and decrease in concentration of lactose DCL -11 showed significant improvement in disintegration and faster rate of drug release.

STABILITY STUDIES :

Stability studies was carried out in accelerated stability chamber **40°C / 75% RH** for a period of 3 months and the results were found satisfactory.

Table 53: Stability studies of Mucoadhesive tablets:

FA27	**1st month**	**2nd month**	**3rd month**
Result	complies	complies	Complies

Table 54: Stability studies of Immediate release tablets:

FB5	**1st month**	**2nd month**	**3rd month**
Result	complies	complies	Complies

CONCLUSION

Chronopharmaceutical drug delivery can be used in case of diseases which shows circadian rhythm in their patho physiology which can tackle the problems as it is modulated to release the drug according to the biological clock.

Hence, a smarter drug delivery system is needed which alters drug release profile itself based on severity of asthma attacks and COPD. This benefits the maintenance of drugs in plasma and also releases more of the drug it holds when condition is severe based on the needs of the physiological system.

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