Formulation and Optimization of Sustained Release Floating Matrix Tablets of Salbutamol Sulphate Using Xanthan Gum and Hydroxypropyl Methylcellulose Polymer Blend

By:

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This is to certify that the thesis prepared by Zewdu Yilma, entitled: *Formulation and Optimization of Sustained Release Floating Matrix Tablet of Salbutamol Sulphate Using Xanthan Gum and Hydroxypropyl Methylcellulose Polymer Blend* and submitted in partial fulfillment of the requirements for the Master of Science Degree in Pharmaceutics complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Salbutamol sulphate is a directly acting sympathomimetic agent with selective action on $ß_2$-receptors. It is used as bronchodilator in the management of disorders involving reversible airways obstruction and in chronic obstruction pulmonary diseases. Oral salbutamol sulphate has site-specific absorption in the stomach and upper part of the small intestine. Its bioavailability is about 40% due to extensive hepatic first pass metabolism, sulphonation in intestinal fluid, degradation in colon and narrow absorption window. The aim of this study was to formulate and optimize a sustained release floating tablet of salbutamol sulphate using xanthan gum (XG) and hydroxypropyl methylcellulose (HPMC) as release retarding agents and sodiumbicarbonate ($NaHCO_3$) as floating aid in order to improve bioavailability, reduce dosing frequency, and increase patient compliance.

The sustained release floating tablets of salbutamol sulphate was prepared by wet granulation technique using XG and HPMC as release retarding polymers. The effects of formulation variables: percentage of polymer (XG, HPMC, or XG/HPMC) and percentage of sodium bicarbonate on response variables: floating lag time, floating duration, cumulative release within 1 hr, and release rate were investigated. Preliminary studies revealed that the percentage of sodium bicarbonate, percentage of polymer (XG, HPMC, or XG/HPMC) significantly affected the floating lag time, cumulative release within 1 hr and release rate ($P < 0.05$), but not floating duration. Among the polymers used, the one with 1:3 (XG:HPMC) ratio was selected for further optimization purpose due to that it contains relatively high amount of HPMC, which has low hydration power than XG, that can release enough amount of drug in the first 1 hr which can be used as bolus dose for rapid relief of asthma. The effect of formulation variables on floating lag time was significant, but all formulations floated below 10 seconds and not considered during optimization. The effects of percentage of $NaHCO_3$ and percentage of XG/HPMC (1:3) were studied and optimized for maximum desired output of drug release rate and cumulative release within 1 hr using central composite design statistical approach. Design-Expert 8.0.7.1 software was employed to carry out the experimental design, statistical analysis, and numerical and graphical optimization.

Linear and quadratic models were developed as best fit models for release rate and cumulative release at 1 hr, respectively. The analysis of variance (ANOVA) of the models showed that the linear effects of both parameters were significant for the linear model of release rate; and all the linear, interaction and quadratic effects were significant for the quadratic model of cumulative release at 1 hr. The effect of percentage of XG/HPMC was more pronounced than the effect of percentage of $NaHCO_3$ on both models. Finally, simultaneous optimization of cumulative release at 1 hr and release rate was performed and the most desirable representative optimal point was obtained to have release rate of 28.49 $hr^{-1/2}$ and cumulative release at 1 hr of 24% at corresponding levels of 24.79% of XG/HPMC and 5% of $NaHCO_3$ with desirability of 0.756. The validity of this optimal point was confirmed by the low magnitude of percent prediction error. Evaluation of the optimized formulation showed successful formulation of the floating tablets with excellent granule and tablet property. Comparison of the release profiles of three different batches of the optimized formulation by dissolution efficiency revealed that there was no statistically significant difference ($p > 0.05$) in release profiles of the formulations. In addition, drug release kinetics and drug release mechanism studies indicated that the optimized formulation followed Higuchi square root kinetic model with non Fickian diffusion release mechanism. In conclusion, this study has come up with an optimum formulation for the development of floating tablet of salbutamol sulphate that could remain buoyant and release the drug over a period of 12 hr in a sustained manner *in vitro*.

***Keywords*:** GRDFs; Floating tablet; Salbutamol sulphate; Xanthan gum; HPMC; Optimization

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Acronyms

AHFS	American Hospital Formulary Service
ANOVA	Analysis of variance
CCD	Central composite design
CR	Controlled release
DDS	Drug delivery system
DE	Dissolution efficiency
FDDS	Floating drug delivery system
FTIR	Furior transform infrared
GET	Gastric emptying time
GIT	Gastrointestinal tract
GRDFs	Gastroretentive dosage forms
GRT	Gastric retention time
HPMC	Hydroxypropyl methyl cellulose
MCC	Microcrystalline cellulose
PVP	Polyvinyl pyrolidone
RSM	Response surface methodology
USP	United States Pharmacopoeia
XG	Xanthan gum
IMMC	Interdigestive myoelectric motor complex

1 INTRODUCTION

1.1 Gastroretentive drug delivery system

Oral route is the most convenient and preferred means of drug delivery to the systemic circulation due to its ease of administration, patient compliance, least sterility constraints and flexible design of dosage forms [Bajaj *et al*., 2011]. However, the development process is presented with several physiologic difficulties, such as an inability to restrain and localize the drug delivery system within desired regions of the gastrointestinal tract (GIT), an unpredictable gastric emptying rate that varies from person to person, a brief gastrointestinal transit time and the existence of an absorption window in the upper small intestine for several drugs [Badoni *et. al.,* 2012]. Depending upon the physiological state of the subject and the design of the pharmaceutical formulation, the emptying process can last from a few minutes up to 12 hr. This variability, in turn, may lead to unpredictable bioavailability and times to achieve peak plasma levels, since the majority of drugs are preferentially absorbed in the upper part of the small intestine. The relatively brief gastric emptying time (GET) in humans, which normally averages 2 to 3 hr through the major absorption zone (stomach or upper part of the intestine), can result in incomplete drug release from the drug delivery system (DDS) leading to diminished efficacy of the administered dose [Kumar *et al*., 2010; Parkash *et al.,* 2011].

In addition, some drugs display region specific absorption which is related to differential drug solubility and stability in different regions of GIT, as a result of changes in environmental pH, degradation by enzymes present in the lumen of the intestine or interaction with endogenous components such as bile. Active transport mechanisms for drugs involving carriers and pump systems have been also well

described [Viswanatha *et al.,* 2011]. These drugs show absorption window, which signifies the region of GIT where absorption primarily occurs [Nayak *et al*., 2010]. Drugs released from sustained/controlled release systems, after absorption window has been crossed, go waste with negligible absorption which indicates that absorption window can limit the bioavailability of orally administered compounds and can be a major obstacle to the development of sustained/controlled release drugs [Mamidala *et al*., 2009]. Therefore, it would be beneficial to develop sustained release formulations which remain at the absorption site for an extended period of time. One of the feasible approaches for achieving prolonged and predictable drug delivery profile in GIT is to control gastric retention time (GRT) of the formulation. Dosage forms with prolonged GRT, i.e., Gastro Retentive Dosage Forms (GRDFs), will overcome the problems of simple sustained release dosage forms [Gattani *et al*., 2009; Vinod *et al.,* 2010; Zate *et al.,* 2010].

GRDFs are dosage forms which prolong the retention time of a drug in the GIT, thereby improving the oral bioavailability of the drug and can also be used as sustained release device with a reduced frequency of administration and therefore, improve patient compliance [Kavitha and Mehaboob, 2011].

1.1.1 Biological aspects of GRDFS

1.1.1.1 The role of stomach

It is worth briefly reviewing the role of the stomach in terms of anatomical structure and physiological function to understand its implication in the development of GRDFs [Swarbrick, 2007].

The stomach is a J-shaped organ located in the upper left hand portion of the abdomen, just below the diaphragm. It occupies a portion of the epigastric and left hypochondriac region [Patel *et. al.,* 2013]. It is divided into three major regions: fundus, body, and antrum. Its fundus and body region are capable of displaying a large expansion to accommodate food without much increase in the intragastric pressure. Whereas, the antrum is the main site for mixing motions and act as a pump for gastric emptying by propelling actions [Touitou and Barry, 2007]. Following meals, food is stored in the upper part of the stomach, and approximately 5 to 10 min after food ingestion, the stomach motor activity starts and persists as long as the food exists in the stomach (2 to 4 hr). The peristaltic contractions of the proximal stomach slowly compress the food toward the pyloric sphincter, and the stomach contents are evacuated. The pyloric sphincter causes an appreciable constriction of the lumen at the gastroduodenal junction [Swarbrick, 2007].

Under physiological condition, the gastric absorption of most drugs is insignificant as a result of its limited surface area (0.1 - 0.2 m^2) covered by a thick layer of mucous coating, the lack of villi on the mucosal surface, and the short residence time of most drug in the stomach. Rapid gastric emptying, also called dumping syndrome, occurs when undigested food empties too quickly into the small intestine. Stomach emptying is a coordinated function by intense peristaltic contractions in the antrum. At the same time, the emptying is opposed by varying degrees of resistance to passage of chyme at the pylorus. The rate depends on pressure generated by antrum against pylorus resistance [Bhowmik *et al.,* 2009; Amit and Umashankar, 2011].

The motility of the stomach differs remarkably between the fasted and the fed state. The motoric activity in the fasting state, termed ‘‘interdigestive myoelectric motor

complex (IMMC),'' is a 2 to 3 hr cycle of peristaltic activity that is generated in the stomach and progresses toward the ileocecal junction. It aims to clear the stomach and the small intestine of indigested residues, swallowed saliva, and sloughed epithelial cells. The IMMC is composed of four phases: the first phase lasts for 30 to 60 min with a few or no contractions. The second phase (20 to 40 min) consists of intermittent irregular sweeping contractions and lasts until the intense peristaltic contractions of the third phase starts. The peristaltic waves of the third phase, also called the ''housekeeper phase,'' last for 10 to 20 min and decrease gradually in the 4^{th} phase to prepare the stomach for the next cycle [Svenberg *et. al.,* 1987; Paradkar, 2008; Romanski, 2009; Amit and Umashankar, 2011; Narang, 2011].

1.1.1.2 Factors controlling GRDFs

The stomach anatomy and physiology contain parameters to be considered in the development of gastroretentive dosage forms. For example, to pass through the pyloric valve into the small intestine, the particle size should be in the range of 1 to 2 mm. The most important parameters controlling the gastric retention time (GRT) of oral dosage forms include: density, size and shape of the dosage form; food intake and its nature; posture, gender, age, and sex of the patient; and others [Bhowmik *et al.,* 2009].

Density, shape and size of dosage forms

The density of a dosage form affects the gastric emptying rate and determines the location of the system in the stomach. Dosage forms having a density lower than the gastric contents can float to the surface, while high density systems sink to bottom of the stomach. Both positions may isolate the dosage system from the pylorus. A density of < 1.0 gm/cm^3 is required to exhibit floating property [Amit *et al.,* 2010].

Shape and size of the dosage forms are also important in designing indigestible single unit solid dosage forms. The mean gastric residence times of nonfloating dosage forms are highly variable and greatly dependent on their size, which may be large, medium and small units. In most cases, the larger the dosage form the greater will be the gastric retention time (GRT) due to the larger size of the dosage form would not allow this to quickly pass through the pyloric sphincter into the intestine. Ring-shaped and tetrahedron-shaped devices have a better gastric residence time as compared with other shapes [Kavitha *et al.,* 2010].

Food intake and its nature

Viscosity and volume of food, caloric value and frequency of feeding have a profound effect on the gastric retention of dosage forms. The presence or absence of food in the gastrointestinal tract (GIT) influences the gastric retention time (GRT) of the dosage form. Usually the presence of food in the GIT improves the GRT of the dosage form and thus, the drugs absorption increases by allowing its stay at the absorption site for a longer period [Ami *et al.,* 2012].

Effect of gender, posture and age

Generally females have slower gastric emptying rates than males. GRT can vary between supine and upright ambulatory states of the patient; the floating and non floating systems behaved differently [Goyal *et al.,* 2011]. In the upright position, the floating systems floated to the top of the gastric contents and remained for a longer time, showing prolonged GRT. But the non-floating units settled to the lower part of the stomach and underwent faster emptying as a result of peristaltic contractions. However, in supine position, the floating units are emptied faster than non-floating

units of similar size. In case of elderly persons, gastric emptying is slowed down [Badoni *et al.,* 2012].

1.1.2 Drug candidates for gastric retention

Various drugs have their greatest therapeutic effect when released in the stomach, particularly when the release is prolonged in a continuous and controlled manner. Potential drug candidates for gastroretentive drug delivery systems include: drugs that are locally active in the stomach (e.g. misoprostol, antacids etc.); drugs that have narrow absorption window in gastrointestinal tract (e.g. L-DOPA, para-aminobenzoic acid, furosemide, riboflavin, salbutamol [Swarbrick, 2007; Shinde *et al*, 2010]); drugs that are unstable in the intestinal or colonic environment (e.g. captopril, ranitidine HCl, metronidazole.); drugs that disturb normal colonic microbes (e.g. antibiotics against *Helicobacter pylori*) and drugs that exhibit low solubility at high pH values (e.g. diazepam, chlordiazepoxide, verapamil HCl) [Hoffman, 2004; Kavitha 2010; Nayak *et al*., 2010].

1.1.3 Approaches for gastric retention

Various approaches (Fig. 1.1) have been pursued over the last decades, to increase the retention of oral dosage forms in the stomach. The most common approaches used to increase the gastric residence time of pharmaceutical dosage forms include: high-density dosage forms, bio/mucoadhesive dosage forms, expandable or swellable dosage forms, and low density dosage forms [Dolas *et al*., 2004].

1.1.3.1 High-density systems

These systems involve dosage forms that can be retained at the bottom of the stomach. Sedimentation has been employed as a retention mechanism for pellets that are small enough to be retained in the rugae or folds of the stomach body near the pyloric region, which is the part of the organ with the lowest position in an upright posture. Dense pellets (approximately 3g/cm^3) trapped in rugae also tend to withstand the peristaltic movements of the stomach wall. Commonly used excipients are heavy inert materials such as barium sulphate, zinc oxide, titanium dioxide and iron powder [Garg and Gupta, 2008; Kadam *et. al.,* 2011].

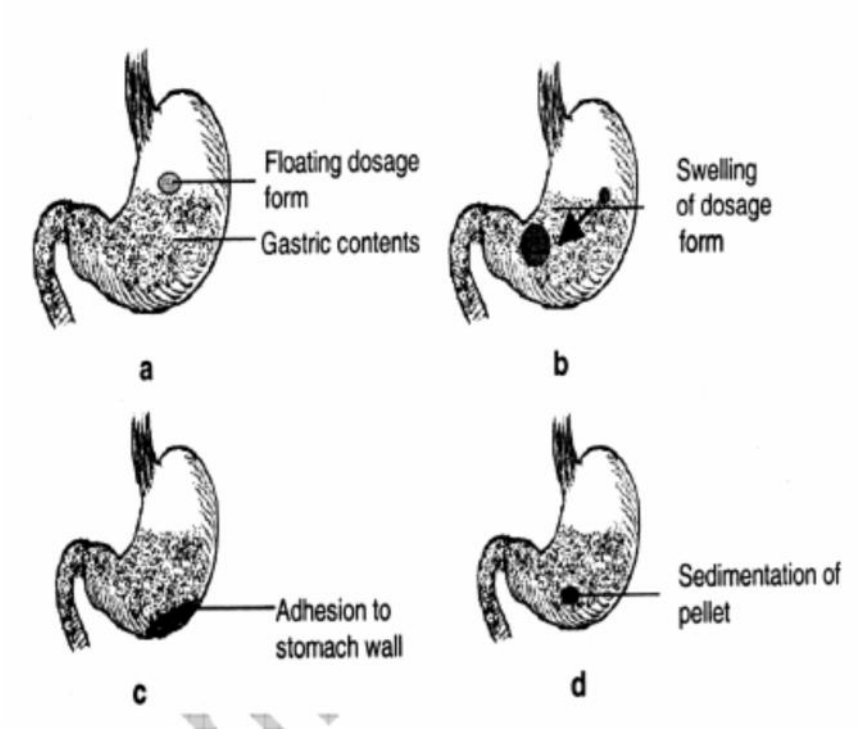

Figure 1.1: Various forms of gastroretentive systems: (a) Floating gastroretentive drug delivery systems; (b) Swelling gastro-retentive drug delivery systems; (c) Bioadhesive gastroretentive drug delivery systems; (d) High density gastro retentive drug delivery systems [Dolas *et al*., 2004].

1.1.3.2 Swelling and expandable systems

These are dosage forms which are capable of swelling to a size that prevents their passage through the pylorus; as a result, the dosage form is retained in the stomach for a longer period of time. Upon coming in contact with gastric fluid, the polymer imbibes water and swells [Ami *et al.,* 2012]**.** A key element of this formulation is the envelope composed of elastic, non dissolvable but water permeable polymer. The envelope contains an agent capable of swelling due to the entrance of water. Once sufficient moisture dissolves the drug in the reservoir, the envelope controls drug release. After drug release, their dimensions are minimized with subsequent evacuation from the stomach [Touitou and Barry, 2007; Kadam *et. al.,* 2011].

1.1.3.3 Bio/mucoadhesive systems

This approach is based on that bio/mucoadhesive systems bind to the gastric epithelial cell surface, or mucin, and extend the gastric retention time (GRT) by increasing the intimacy and duration of contact between the dosage form and the biological membrane. Once attached, it will remain there until mucus turnover sloughs it off [Washington, 2003]. However, the application of mucoadhesive systems is usually limited due to the rapid turnover of mucus, prevention of bond formation by the acidic environment and thick mucus present in the stomach and the difficulty to specifically target the gastric mucus with bioadhesive polymers. Some of the most promising excipients that have been used commonly in these systems include polycarbophil, carbopol, and chitosan [Garg and Gupta, 2008].

1.1.3.4 Floating systems

Floating systems, first described by Davis in 1968, are low-density systems that have sufficient buoyancy to float over the gastric contents and remain in the stomach for a prolonged period [Surana and Kotecha, 2010]. While the system floats over the gastric contents, the drug is released slowly at the desired rate, which results in increased gastro-retention time and reduces fluctuation in plasma drug concentration [Bhowmik *et. al.,* 2009; Putheti and Patil , 2009].

1.1.4 Technological developments in floating drug delivery system (FDDS)

Most of the floating systems reported in the literature are single-unit systems, such as floating drug delivery systems of capsules and tablets [Kawashima *et al*., 1991]. Floating drug delivery systems either float due to their low density than stomach contents or due to the gaseous phase formed inside the system after they come in contact with the gastric environment. Based on the mechanism of buoyancy, two distinctly different technologies have been utilized in the development of FDDS, Non-effervescent FDDS and effervescent FDDS [Joshi *et. al.,* 2012].

1.1.4.1 Non-effervescent FDDS

The FDDS belonging to this class are usually prepared from gel forming or highly swellable cellulose type hydrocolloids, polysaccharide or matrix forming polymers like polyacrylate, polycarbonate, polystyrene and polymethacrylate [Ami *et al.,* 2012]. When non-effervescent floating dosage forms come in contact with an aqueous medium, the hydrocolloids absorb water and start to hydrate, forming a gel at the surface and become less dense which leads them to float. The resultant gel layer

subsequently controls the trafficking of drug out and passage of solvent into the dosage form. The drug in the dosage form dissolves in and diffuses out with the diffusing solvent [Sarojini and Manavalan, 2012].

1.1.4.2 Effervescent FDDS

The buoyant delivery system utilizes matrices prepared with swellable polymers, such as HPMC and effervescent components, e.g. sodium bicarbonate [Ami *et al.,* 2012]. The matrices are fabricated in such a manner that when they come in contact with stomach fluid, carbon dioxide is generated and is entrapped in the hydrocolloid gel. This leads to an upward drift of the dosage form and maintains it in floating condition [Mayavanshi and Gajjar, 2008].

1.1.5 Polymers used in floating sustained release systems

Polymers are generally employed in floating drug delivery systems so as to target the delivery of drug to a specific region in the gastrointestinal tract i.e. stomach. Both semi-synthetic (eg., HPMC) and natural (eg., Xanthan gum) polymers have been explored for their promising potential in stomach-specific drug delivery [Pahwa *et al.,* 2010; Radhakrishna *et. al.,* 2012]. For example, Bomma *et al.,* (2009), Lende *et. al.,* (2012), and Shah *et. al.,* (2012) developed floating tablets of norfloxacin, stavudine, and metformine, respectively, using HPMC and XG as drug release sustaining agent.

Hydroxypropyl methylcellulose

Hydroxypropyl methylcellulose (HPMC) is a partly O-methylated and O-(2-hydroxypropylated) cellulose obtained by treating alkali cellulose with chloromethane and propylene oxide. It is available as odorless and tasteless granular or fibrous

powder which is creamy white or white in color [Cole, 2002]. Its structure (Fig. 1.2) is:

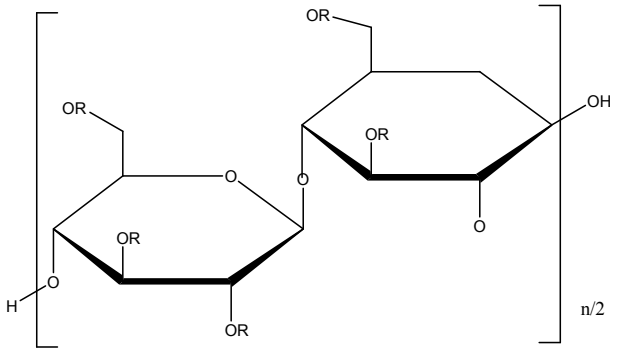

Figure 1.2: Structure of HPMC [Rowe *et al.,* 2009].

The substituent R represents either a –CH, or a –CHCH(CH)OH group, or a hydrogen atom (H). The physicochemical properties of this polymer are strongly affected by the methoxy group content, the hydroxypropoxy group content, and the molecular weight [Siepmann and Peppas, 2001]. The USP distinguishes four different types of HPMC, classified according to their relative –OCH and –OCHCH(CH)OH content: HPMC 1828, HPMC 2208, HPMC 2906 and HPMC 2910. The first two numbers indicate the percentage of methoxy-groups, the last two numbers indicate the percentage of hydroxypropoxy-groups, determined after drying at 105 ^{o}C for 2 hr.

HPMC is widely used in oral, ophthalmic, nasal, and topical pharmaceutical formulations. In oral products, HPMC is primarily used as a tablet binder, in film-coating, and as a matrix for use in extended release tablet formulations. High-viscosity grades may be used to retard the release of drugs from a matrix at levels of 10 – 80% w/w in tablets and capsules. HPMC is also used in liquid oral dosage forms as a suspending and/or thickening agent at concentrations ranging from 0.25 – 5.0% [Rowe *et al.,* 2009].

A number of studies explored it as a drug release retarding agent in floating dosage forms. For example, formulation and development of floating tablet of stavudine [Feleke *et al.,* 2007], famotidine [Kumar *et al.,* 2009], ofloxacin [Padmavathy *et al.,* 2010], atenolol [Manivannan and Chakole, 2011], gliclazide [Chowdary and Hussainy, 2012], cefuroxime axetil [Sandeep *et al.,* 2012], and voriconazole [Parthibarajan *et al.,* 2012] were done using HPMC.

Xanthan gum

Xanthan gum is a high molecular weight extracellular polysaccharide produced by pure culture aerobic fermentation of carbohydrate with *Xanthomonas campestris* bacteria [Vendruscolo *et al.,* 2005]. It is a long chained polysaccharide with large number of trisaccharide side chains. The main chain consists of β-(1, 4)-linked D-glucose units (Fig.1.3). The side chains are composed of two mannose units and one glucuronic acid unit attached with alternate glucose residues of the main chain. The terminal D-mannose residues may carry a pyruvate function, the distribution of which is dependent on the bacterial strain and the fermentation conditions. The non-terminal D-mannose unit in the side chain contains an acetyl function. The anionic character of this polymer is due to the presence of both glucuronic acid and pyruvic acid groups in the side chain [Mesnukul and Phaechamud, 2009; Tiwari and Kumar, 2009; Jackson and Ofoefule, 2011]. This gum develops a weak structure in water, which creates high viscosity solutions at low concentration. Viscosity remains fairly constant from 0 °C to 100 °C.

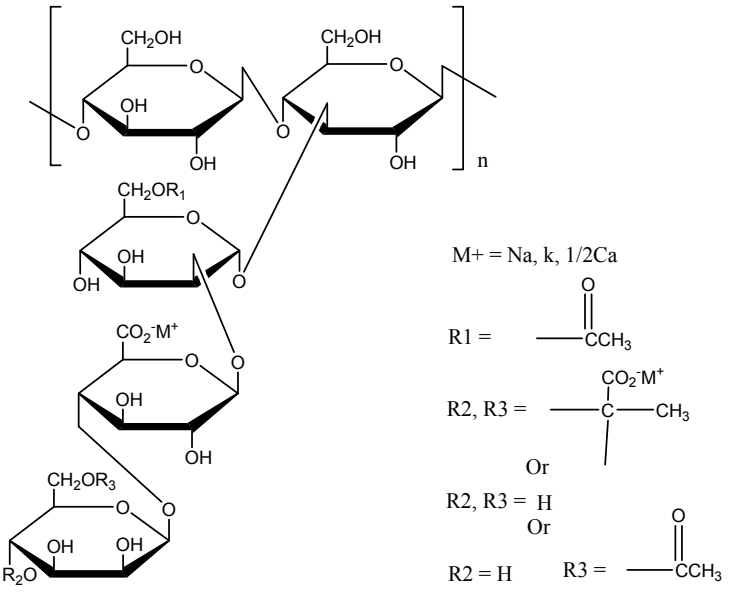

Figure 1.3: Structure of xanthan gum [Rowe et al., 2009].

It is a cream colored powder that is soluble in hot or cold water with high viscosity even at low concentrations. Xanthan gum has been extensively investigated as a possible polymeric material in diverse floating drug delivery technology in addition to being used as gelling agent; stabilizing agent; suspending agent; and viscosity-increasing agent [Rowe *et al.*, 2009]. For example, formulation and evaluation of rosiglitazone maleate [Kavitha *et al*., 2010], acyclovir [Akelesh *et al*., 2011], propranolol hydrochloride [Safhi, 2011], captopril [Teyade *et al*., 2011], and tapentadol hydrochloride [Jagdale *et al*., 2012] were done using XG.

Combining polymers is important in order to improve the drawbacks of one polymer with the other one. For example, HPMC shows an initial burst release, higher release rate (low drug retarding ability), low compressibility and time-dependent release when compared to xanthan gum [Talukdar *et al*., 1996]. To get a formulation at least with good sustaining ability, it requires an increase in the concentration of the polymer (HPMC). However, it increases the cost of the formulation. Therefore, to

modulate the burst release of HPMC and at the same time to have a good loading dose, it is better to combine HPMC and Xanthan gum in the formulation of soluble drugs like salbutamol sulphate.

1.2 Salbutamol sulphate

Salbutamol sulphate (RS)-1-(4-hydroxy-3-hydroxy-methyl-phenyl)-2-(tert-butylamino) ethanol sulphate is a directly acting sympathomimetic agent with selective action on β_2-receptors. It is used as bronchodilator in the management of disorders involving reversible airways obstruction and in chronic obstruction pulmonary diseases [Murtaza *et al.,* 2009; Sonar *et al*., 2009; Goudanavar *et al.,* 2010].

Salbutamol sulphate has a molecular weight of 288.35 with a structural formula $C_{13}H_{21}NO_3$, ½ H_2SO_4 as shown below (Fig 1.4). It is a white, odorless powder, bitter in taste, with a melting point of 150 ^oC; soluble in four parts of water; slightly soluble in ethanol 96%, chloroform and ether; contains not less than 90% and not more than 110% of $C_{13}H_{21}NO_3$, ½ H_2SO_4 calculated with reference to the dried substance [USP 30/NF25].

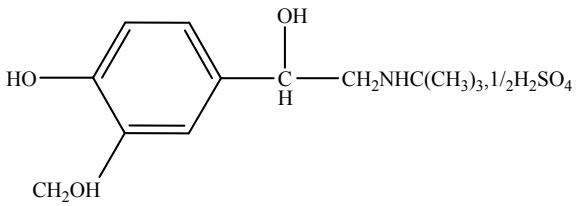

Figure 1.4: Structural formula of salbutamol sulphate

It is found in syrup, inhalation, aerosol, injection, and tablet dosage forms [AHFS, 2008].

In tablet form, a dose equivalent to 2 to 4 mg of salbutamol, 3 or 4 times per day is prescribed for adults [Chandiran *et. al.,* 2010]. After oral administration, it is rapidly absorbed, ~40% bioavailable, and has a plasma half-life of 2 - 7 hr [Ravikumar *et al*., 2012]. The drug is eliminated through urine and feces. It is metabolized by the intestine, liver and also degraded by colon [Shinde *et al.,* 2010]. The inactive metabolite is sulphate conjugate and about 25% of the administered dose is metabolized to the 4-O-sulphate ester [Özyazici and Sevg, 2003].

Salbutamol may give rise to some side effects (tremor of skeletal muscle, palpitations and muscle cramps, slight tachycardia, tenseness, headaches) [Özyazici and Sevg, 2003] and contraindicated in patients with hypertension, myocardial insufficiency and hyperthyroidism, in patients with diabetes mellitus, serious cardiovascular disorders, and in patients during the first and second trimester of pregnancy [AHFS, 2008].

1.3 The present study

Salbutamol sulphate is one of the widely used drugs for the treatment of bronchial asthma, chronic bronchitis and obstructive airway diseases [Pathan *et al.*, 2011]. The relatively short term acting injectables and aerosol dosage forms of salbutamol sulphate are recommended for instant relief in severe asthmatic attacks. The recommended dose of aerosols in adults and children is 2 – 3 inhalations every 4 – 6 hr [Fishwick *et al.*, 2001] and for conventional tablets, it is administered three to four times a day [AHFS, 2008] which causes poor patient compliance, multiple administration associated side effects, and plasma drug level fluctuation.

Its oral bioavailability is ~40% due to extensive metabolism via intestinal sulphonation, first pass metabolism in liver, narrow absorption window (site-specific

absorption in stomach and upper part of small intestine [Swarbrick, 2007]), and also degradation in colon [Mamidala *et al*., 2009; Ravikumar *et al*., 2012].

Asthma being a chronic disease, and as most of the patients suffer from nocturnal attacks, there is a need for drug delivery systems which maintains therapeutic concentrations for long duration [Shinde *et al*, 2010].

Thus, the present work attempts to develop and optimize a sustained release floating tablet, which releases salbutamol sulphate in the stomach and upper gastrointestinal tract that will increase its bioavailability, and improves patient compliance and therapeutic response of the drug.

1.4 Objectives

1.4.1 General objective

- To formulate and optimize a sustained release floating matrix tablet of salbutamol sulphate, using xanthan gum and HPMC as matrix forming polymers and $NaHCO_3$ as floating aid.

1.4.2 Specific objectives

- To prepare granules using xanthan gum and HPMC alone and in combination and determine their physical characteristics.
- To prepare floating tablets of salbutamol sulphate using xanthan gum and HPMC alone and in combination and determine their characteristics.
- To evaluate and compare the effects of HPMC, XG, combination of HPMC and XG, and $NaHCO_3$, on floating lag time, floating duration, cumulative release at 1 hr, and release rate of tablets.
- To optimize the formulation variables of the tablets using central composite design.
- To validate the optimized formulation.

2. EXPERIMENTAL

2.1. Materials

Salbutamol sulphate (Supriya Life Science Ltd., India), Xanthan gum, Povidone K-30, and Microcrystalline cellulose PH 101 (China Associate Co. Ltd, China) were supplied by Addis Pharmaceutical Factory (APF). HPMC K 4000cp (China Associate Co. Ltd., China) was donated by Ethiopian Pharmaceutical Manufacturing Sh. Co. (EPHARM). Sodium hexane-sulphonate (Merck, India) was supplied by East African Pharmaceuticals PLC. Sodium bicarbonate (UNI. CHEM., India), methanol (BDH Ltd., England), and hydrochloric acid (BDH Ltd., England) were all used as received.

2.2. Methods

2.2.1. Preparation of granules

Granulation was performed as per the method described by Shinde *et al.*(2010) with some modification. All the ingredients (Table 2.1), except the magnesium stearate, talc and PVP K-30, were weighed and mixed in geometrical dilution. Wet mass was formed by adding PVP K-30 isopropanol solution to the powder blend while mixing thoroughly. The wet mass was screened through a 1.6 mm mesh to form the granules. The wet granules were dried for 12 hr at 40 ^{o}C and were passed through a 1 mm mesh and then stored in air-tight containers.

2.2.2. Characterization of granules

Granules were evaluated as per the method described by Shahi *et al*.(2008).

2.2.2.1. Angle of repose

Thirty grams of granule were made to flow through stemless funnel, with an internal diameter of 10 mm at the bottom and 100 mm at the top, on to a graph paper from a height of 10 cm. The height (h) as well as the diameter (d) of the pile was measured. Then the angle of repose (θ) was calculated from h and radius (r = d/2) using Equation 2.1.

$$\theta = \tan^{-1}(h/r) \qquad \text{Eq. 2.1}$$

The experiment was done in triplicate

2.2.2.2. Density related properties

Bulk density

Thirty gram of granule was transferred into a 250 ml graduated cylinder and the volume of the granules was read after tapping the granule three times on a horizontal plane. Then, the mass (M) of the granule was divided by the volume obtained, which is the bulk volume (V_b), to obtain the bulk density (ρ_b), Equation 2.2.

$$\rho_b = M/V_b \qquad \text{Eq. 2.2}$$

The experiment was done in triplicate and mean and standard deviation were calculated.

Table 2.2: Composition of tablet for the preliminary experiment *.

Ingre.↓Formln.→	FH1	FH2	FX1	FX2	FM1	FM2	FM3	FM4	FM5	FM6	FS1	FS2	FS3	FS4
Drug (%)	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
Xanthan gum(%)	-	-	10	40	5	2.5	7.5	20	10	30	40	40	-	-
HPMC K4M(%)	10	40	-	-	5	7.5	2.5	20	30	10	-	-	40	40
$NaHCO_3$(%)	10	10	10	10	10	10	10	10	10	10	5	20	5	20
MCC(%)	69.8	39.8	69.8	39.8	69.8	69.8	69.8	39.8	39.8	39.8	44.8	29.8	44.8	29.8
MgSt(%)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Talc(%)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PVP K-30(%)	5	5	5	5	5	5	5	5	5	5	5	5	5	5

** FH: formulation that contains the polymer HPMC only*

FX: formulation that contains the polymer xanthan gum only

FM: formulation that contains the polymers both xanthan gum and HPMC

FSs: formulations that contain different concentrations of $NaHCO_3$

Each tablet weighs 300mg.

Tapped density

Thirty gram of granule was transferred into a 250 ml graduated cylinder and the volume of the granule was read after tapping the granule 250 times using Tap Densitometer (ERWEKA, SVM 20, Germany). Then, the mass (M) of the granule was divided by the volume obtained, which is the tapped volume (V_t), to obtain the tapped density (ρ_t), Equation 2.3.

$$\rho_t = M/V_t \qquad \text{Eq. 2.3}$$

The experiment was done in triplicate and mean and standard deviation were calculated.

2.2.2.3. Compressibility index and Hausner ratio

The compressibility index (CI) and Housner ratio were calculated from bulk and tapped densities, using Equation 2.4 and 2.5 [Shahi *et al.,* 2008].

$$\text{Compressibility Index (CI)} = \frac{\rho_t - \rho_b}{\rho_t} x100 \qquad \text{Eq. 2.4}$$

$$Hausner \quad ratio \ (H) = \frac{\rho_t}{\rho_b} \qquad \text{Eq. 2.5}$$

2.2.3. Preparation of floating tablets

Talc and magnesium stearate were added onto previously prepared and dried granules and blended for 3 min in a Turbula mixer (Willy A. Bachofen AG, Turbula 2TF, Basel, Switzerland) at 49 rpm. The blend was compressed into tablets, adjusting the hardness to be between 60N to 70 N, on eccentric tablet machine (EK0 Korsch, 8410-68, Berlin, Germany) which was fitted with 10 mm diameter flat-faced punches. The

tablets were kept for 24 hrs at room temperature in glass containers before their properties were evaluated.

2.2.4. Characterization of tablets

Tablets were characterized with respect to the following properties.

2.2.4.1. Drug content analysis

Twenty tablets were weighed and finely powdered and equivalent to about 50 mg of salbutamol, were transferred to a 2000-ml volumetric flask. Then 1200 ml of 1% acetic acid was added, shaked for 45 min by mechanical means, sonicated for 10 min, cooled to room temperature and was diluted with methanol to volume. It was then filtered with a 0.45 µm nylon filter. About 25 µl of this filtered solution was injected into the HPLC, the chromatogram was recorded, and the response for the major peak was measured. The quantity of $C_{13}H_{21}NO_3$ was calculated by comparing this peak response with the major peak response similarly obtained on chromatographing the standard preparation previously diluted with a mixture of water and methanol (6:4) and filtered [USP 30/NF 25, 2007]. It was done in triplicate for each batch and mean and standard deviation were calculated

2.2.4.2. Tablet hardness

The hardness of 10 tablets from each batch was determined using a hardness tester (CALIVA, THT2, England) and the average value was obtained.

2.2.4.3. Tablet thickness

The thickness of 10 tablets from each batch was measured by the hardness tester (CALIVA, THT2, England) putting the tablet with its side (in an upright position).

2.2.4.4. Tablet friability

The friability of the tablets was determined by placing 10 tablets in a friability tester (ERWEKA, TAR 20, Germany) and rotating them for 4 min at 25 rpm. The loss of tablet weight was calculated as a percentage of the initial weight after dedusting**.**

2.2.4.5. *In vitro* buoyancy studies

The time the tablets took to emerge on the fluid surface (floating lag time) and the time the tablets constantly float on the fluid surface (floating duration) in a USP type II apparatus, filled with 500 ml of 0.1N HCl solution (pH = 1.2) at 37 ± 0.5 ^{o}C were recorded by using stopwatch. Both of the variables was determined in triplicate and mean and standard deviation were calculated.

2.2.4.6. Matrix integrity

Matrix integrity was observed throughout *in vitro* dissolution studies and whether or not the swollen mass of the tablets remain intact was checked [Shinde *et al.,* 2010].

2.2.4.7. Calibration curve and system suitability tests

Stock solution of salbutamol sulphate reference standard was prepared by transferring 12 mg of salbutamol sulphate reference standard to a 100 ml volumetric flask, adding 60 ml of 1% acetic acid, sonicating it for 5 minutes, and diluting with methanol to volume. From this stock solution, six different volumes (125, 250, 500, 750, 1000 and 1250 µl) of the solution were transferred to 25 ml volumetric flasks and diluted with a mixture of water and methanol (6:4) to volume. The peak area readings of these solutions were measured at 276 nm using HPLC (LC-20AD, Shimadzu, Japan). The peak area versus concentration of solutions were plotted to obtain the calibration

curve. Each day, during analysis, the same step was repeated. The coefficients of determinations (R^2) were ≥ 0.997.

System suitability tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such [USP 30/NF 25, 2007]. So, in this study, in each day of analysis, five replicate injections of the standard preparation, required to demonstrate adequate system precision, was made before the injection of samples and the relative standard deviation was calculated ($\leq$ 2%).

2.2.4.8. *In vitro* drug release studies

The release rate of salbutamol sulphate from floating tablets was determined using Dissolution Testing Apparatus II (paddle method) (USP 30/NF 25, 2007). The dissolution test was performed using 500 ml of 0.1N HCl at 37 ± 0.5 °C and 50 rpm. A 5 ml sample of the dissolution medium was withdrawn at predetermined time intervals (0.5, 1, 2, 3, 4, 5, 6, 8, 10, and 12 hr) and the samples were replaced with fresh dissolution medium which is kept at 37 ± 0.5 ^{o}C. The samples were diluted to 20 ml and filtered through 0.45 µm nylon filter and were analyzed with HPLC (LC-20AD, Shimadzu, Japan) at 276.0 nm [USP 30/NF 25, 2007].

About 100 µl of a portion of the solution under test, previously passed through a 0.45 µm nylon filter, was injected into the HPLC, the chromatogram was recorded, and the response for the major peak was measured. The quantity of $C_{13}H_{21}NO_3$ dissolved was calculated by comparing this peak response with the major peak response similarly

obtained on chromatographing the standard preparation previously diluted with a mixture of water and methanol (6:4).

HPLC conditions

Assay of salbutamol sulphate using HPLC system was conducted as described in the United States Pharmacopeia (USP 30/NF 25, 2007). The following chromatographic conditions were employed:

Mobile phase—1.13 g of sodium 1-hexanesulfonate was dissolved in 1200 ml of water, 12 ml of glacial acetic acid was added, and mixed. A filtered and degassed mixture of this solution and methanol (6:4) was prepared and used.

Standard preparation—about 12 mg of reference salbutamol sulphate, accurately weighed, was transferred to a 100 ml volumetric flask. Then 60 ml of 1% acetic acid was added, and sonicated for 5 min, and diluted with methanol to volume, and mixed. About 125 µl to 1250 µl of this solution was pipetted into a 25 ml volumetric flask, diluted with a mixture of water and methanol (6:4) to volume, and used.

Chromatographic system — the liquid chromatograph was equipped with a 276 nm detector and a 4.6 mm × 15 cm column (MOS-1 Hypersil). The flow rate was about 1.5 ml per min. The standard preparation was chromatographed, and the peak responses were recorded following the standard procedure (USP 30 NF 25, 2007), i.e., the column efficiency determined from the analyte peak should not be less than 800 theoretical plates; the tailing factor for the analyte peak should not be more than 2.5; and the relative standard deviation for replicate injections should not be more than 2.0%.

2.2.4.9. Release profiles comparison

Dissolution efficiency (DE) is one of the common model independent approaches used for comparing drug release profiles. In this study, DE was determined from the *in vitro* dissolution data of the various formulations to compare their release profiles using Equation 2.6.

$$DE\ (\%) = \frac{\int_{t_1}^{t_2} y.dt}{y_{100}\ (t_2 - t_1)} \times 100 \qquad \text{Eq. 2.6}$$

where y is the percentage of dissolved product at any time t, y_{100} denotes 100% dissolution, and the integral represents the area under dissolution curve between time points t_1 and t_2 [Sadray *et al*., 2010].

DE could be defined for every sampling time. In this study, DE was calculated for the first 12 hr release, setting t_1 at 0 hr and t_2 at 12 hr. One way ANOVA was applied to determine whether the existing differences were significant or not.

2.2.5. Kinetics and mechanism of drug release

In order to assess and describe the release kinetics of the drug from the tablets under study, the drug release data were fitted to the following release kinetic models:

Zero order release model

$$Q = Q_o - k_o.t \qquad \text{Eq. 2.7}$$

Where, Q_o is the initial amount of drug, Q is amount of drug remaining at time t, K_0 is zero-order rate constant expressed in units of concentration/time and t is the time.

First order release kinetic model

$$\text{Log } Q = \text{Log } Q_o - k_1 t / 2.303 \qquad \text{Eq. 2.8}$$

Where, Q_o is the initial amount of drug, Q is amount of drug released at time t and K_1 is first order constant.

Higuchi square root model

$$Q = K.t^{1/2} \qquad \text{Eq. 2.9}$$

Where, Q is amount of drug released at time t and K is the constant reflecting the design variables of the system.

Hixson-Crowell cube root model

$$Q_o^{1/3} - Q_t^{1/3} = K_{HC.}\, t \qquad \text{Eq. 2.10}$$

Where, Q_t is the amount of drug released in time t, Q_0 is the initial amount of the drug in the tablets and K_{HC} is the rate constant for Hixson-Crowell rate equation.

In order to find out the mechanism of drug release from the polymeric tablets, drug release data were fitted in Korsmeyer–Peppas model:

$$M_t / M_\infty = Kt^n \qquad \text{Eq. 2.11}$$

Where M_t / M_∞ is fraction of drug released at time *t*, *K* is the rate constant and *n* is the release exponent. The *n* value is used to characterize different release mechanisms as given in Table 2.2 for cylindrical shaped matrices [Shoaib *et al.,* 2006].

Table 2.3: Diffusion exponent and solute release mechanism for cylinderical shape.

Exponent (n)	Release mechanism
≤ 0.45	Fickian diffusion
0.45 < n < 0.89	Anomalous (non-Fickian) transport
0.89	Case-II transport
> 0.89	Supper case-II transport

2.2.6. Formulation optimization

Since the traditional one-factor at a time approach for optimization of variables requires determination of the dependent variable at each and every combination of independent variables, just varying only one at a time and keeping all others constant in batch studies, causes the requirement to carry out a large number of experiments and the uncertainty of actually finding the optimal conditions (because the interactions between factors are not taken into account), the use of advanced techniques of optimization such as response surface methodology (RSM) is applied in this study. RSM avoids problems of the traditional approach in that it provides best possible optimized solution and works efficiently at very few number of experiments to be performed that minimizes time, as well as cost. [Takahara *et al*., 1997; Bas and Boyaci, 2007; Dasari *et al*., 2009; Giordano *et al*., 2010].

RSM is a combination of mathematical and statistical techniques for designing experiments, building models, evaluating the effects of several factors, and searching optimum conditions for desirable responses [Raissi, 2009; Khayet *et al*., 2010]. The relationship among the significant variables is usually expressed in a second-order equation:

$$Y = \beta_o + \sum \beta_i X_i + \sum \beta_j X_j + \sum \beta_{ii} X_i^2 + \sum \beta_{jj} X_j^2 + \sum \sum \beta_{ij} X_i X_j + \varepsilon \qquad \text{Eq. 2.12}$$

where Y is the predicted (estimated) response, β_0 is the constant, β_i and β_j are the linear effects, β_{ii} and β_{jj} are the quadratic effects, β_{ij} ($i \neq j$) is the interaction effect, ε is an experimental error. X_i and X_j represent the independent variables in the form of coded values transformed from the actual values using Equation 2.13.

$$x_i = \frac{(X_i - X)}{\Delta X_i} \qquad \text{Eq. 2.13}$$

where x_i, X_i and X are the transformed, actual and actual at the center point values of the independent variable i, respectively, and ΔX_i is the step change of X_i corresponding to a unit variation of the transformed value [Liu and Tang, 2010].

Central composite design (CCD), one of the RSM designs and which is a powerful statistical approach to model and optimize the response of interest influenced by several independent variables, was used in this study. It is efficient for experiments with few numbers of factors ($2 \leq n \leq 6$) [Takayama *et al*., 2003]. The total number of experiments to be performed in this type of design are generally given as sum of the 2^n factorial runs, 2n axial runs, and n_c center runs ($2^n + 2n + n_c$), where n is the number of independent variables. The factorial design portion is used for estimating main effects (linear terms) and two-factor interactions; 2n axial points for estimating the second-order terms, and n_c repeated center points for the curvature and experimental error of the model [Singh *et al*., 2010].

Among different methods for dealing with the multiple response problems, graphic method and desirability function were used in this study. The first method developed for optimizing multiple responses was to overlay contour plots (graphic method), that work well when there are only a few factor variables. The second common approach

of simultaneous multi-response optimization, the desirability function, involves a transformation of each response variable to individual desirability functions (d_i) by inserting optimum factor levels obtained from software solver to their respective fitted models, and finally calculating the overall desirability function (D) as a geometric mean of all the individual desirability functions. The desirability function ranges from 0 to 1 [Raissi and Farsani, 2009; Zadbood and Noghondaian, 2011].

2.3. Experimental design

On the basis of the preliminary experiments, the amount of sodium bicarbonate (X_1) and one of the XG/HPMC ratios (1:3) (X_2) were identified as the two most important independent formulation variables to be considered in the optimization study. Cumulative release in the first 1 hr and drug release rate in 12 hr period were selected as two of the most important response variables. Thus, CCD was employed in the optimization study. The selected formulation variables with their limits, units and notations are given in Table 2.3.

Table 2.4: Independent variables and their limits.

Variables	Limits				
	-α	-1	0	+1	+α
$NaHCO_3$, X_1 (%)	1.89	5	12.5	20	23.11
XG/HPMC, X_2 (%)	3.79	10	25	40	46.21

α = 1.414

CCD was chosen as it can detect any non-linearity in factor-response relationship [Singh *et al*., 2010]. According to the CCD matrix for two independent variables (n = 2), the total number of experiments (N) was determined as: $N = (2^n + 2n + n_c) = 2^2 +$

(2×2) + 5 = 13. The 13 experimental runs of the CCD matrix were carried out and the observations were analyzed using Design-Expert 8.0.7.1 software to find the optimum area at which the desired responses are achieved, and to construct the response surface plots and contour plots for the fitted polynomial equations of the responses.

2.4. Drug-excipient interaction study

Drug-excipient interaction was studied with Fourier transformed infrared (FT-IR) spectroscopy. FT-IR spectra for pure salbutamol sulphate and optimized salbutamol tablet formulation were acquired at room temperature using FT-IR spectrophotometer (FTIR-8400S, Shimadzu, Japan) in transmittance mode. The samples were first ground in a mortar to reduce the average particle size. About 8 mg of finely ground samples were mixed with an oily mulling agent (Nujol) in a mortar and pestle. The sample mixture was then placed onto the face of a potassium bromide (KBr) plate and the second window was placed on top of the first salt plate to form a thin film of the mull by compression between the two plates. The sandwiched plates were placed in the IR spectrometer and the spectra were obtained. Each IR spectrum was collected with 20 scans and spectral resolution of 4 cm^{-1}. Scanning was performed between wave numbers 4000 - 400 cm^{-1}. Background spectrum was collected before running each sample. IR Solution Software was used for data treatment.

2.5. Statistical analysis

The statistical analysis of all batches was performed with Microsoft Excel and plots of drug release profiles were constructed using Origin 8 Software (OriginLab Corporation, MA, and USA). One way analysis of variance (ANOVA) was applied for comparison of all results. To demonstrate graphically the influence of each factor on responses and to indicate the optimum level of factors, the contour and response surface plots were generated using Design-Expert 8.0.7.1 software (Stat-ease, Corp. Australia). $p < 0.05$ was considered significant.

3. RESULTS and DISCUSSION

3.1. Preliminary studies

Before applying the experimental design for optimization, preliminary studies were conducted in order to compare the release characteristics of the polymers used and identify the most critical factor variables. Factors that could possibly have significant effects on the response variables, according to literature, were considered in the preliminary studies. These include polymer type, polymer concentration, polymers ratio and percentage of floating agent. The response variables considered in the preliminary studies include cumulative drug release at 1 hr, drug release rate, floating lag time, and floating duration.

The polymers' percentage range as drug sustaining agent were from 10% - 80% for HPMC [Rowe *et al*., 2009] and from 5% - 40% for xanthan gum [Asghar *et al*., 2009; Hamza and Aburahma, 2009]. To compare the sustaining ability of the polymers, the low level and high level of the polymers used in the current study were 10% and 40% for both HPMC and XG.

Sodium bicarbonate is reported to be used as floating agent from 5 to 80% [Salve, 2011; Sungthongjeen *et al*., 2008], but it is incompatible with xanthan gum above 30%, therefore, it is used in concentrations ranging between 5% to 20% [Rowe *et al*., 2009].

3.1.1. Granule and tablet characteristics of the preliminary formulations

3.1.1.1. Physical evaluation

As displayed in Table 3.1, all formulations exhibited excellent flow property except for formulations FH1, FH2, FS3 and FS4, which were prepared with HPMC alone and one of the formulations with combination of XG and HPMC (FM5). Angle of repose ranged from 26.46 to 30.05^{o}, Hausner ratio ranged from 1.05 to 1.10 and the compressibility index ranged from 4.43 to 9.24. The bulk density and tapped density of the prepared granules ranged from 0.30 to 0.36 and 0.32 to 0.39, respectively. The results of angle of repose indicated excellent flow property of the granules and the values of compressibility index further support for the excellent flow property. Since HPMC, relative to xanthan gum, has poor flow property [Talukdar *et al.,* 1996], those formulations with only HPMC and higher proportion of HPMC (in the case of the combination formulations) showed fair to good flow property.

As shown in Table 3.2, the thickness of the tablets ranged between 3.35 to 3.61 mm. The hardness of the tablets was between 6 kg/cm^2 and 7.2 kg/cm^2 (60 N - 72 N). The friability was found to be 0.12 to 0.35%, which is an indication of satisfactory mechanical strength of the tablets. The drug content estimations showed values in the range of 95.42 to 102.09% reflecting good uniformity among different formulations. Except FH1 and FM2, all the formulations kept their matrix integrity for more than 12 hr. All the formulations showed values within the prescribed limits for tests of hardness, friability and drug content indicating that the prepared tablets were of standard quality.

Table 3.1 Granule properties of salbutamol sulphate in the preliminary formulations*.

Formulation Code	Flow rate (g/sec)	Bulk density(g/ml)	Tapped density(g/ml)	Compressibility index (CI)	Housner ratio (HR)	Angle of repose(^{o})
FH1	1.70 ± 0.24	0.23 ± 0.01	0.28 ± 0.00	15.60 ± 0.61	1.19 ± 0.01	35.62 ± 1.51
FH2	1.97 ± 0.15	0.20 ± 0.00	0.25 ± 0.00	17.79 ± 0.07	1.22 ± 0.07	36.62 ± 0.66
FX1	6.24 ± 0.12	0.34 ± 0.00	0.36 ± 0.00	5.69 ± 0.00	1.06 ± 0.00	26.46 ± 1.33
FX2	7.02 ± 0.03	0.36 ± 0.00	0.39 ± 0.00	5.87 ± 0.84	1.06 ± 0.01	27.32 ± 1.52
FM1	3.21 ± 0.31	0.31 ± 0.00	0.34 ± 0.00	9.21 ± 1.05	1.10 ± 0.02	29.41 ± 1.42
FM2	1.86 ± 0.14	0.30 ± 0.00	0.33 ± 0.00	7.66 ± 0.00	1.08 ± 0.00	30.05 ± 0.45
FM3	5.22 ± 0.05	0.32 ± 0.00	0.33 ± 0.00	4.51 ± 0.94	1.05 ± 0.01	29.96 ± 1.44
FM4	5.15 ± 0.23	0.32 ± 0.00	0.35 ± 0.00	6.46 ± 1.93	1.07 ± 0.07	29.61 ± 1.46
FM5	1.92 ± 0.06	0.28 ± 0.00	0.30 ± 0.00	8.38 ± 1.05	1.09 ± 0.03	34.50 ± 1.03
FM6	6.04 ± 0.23	0.32 ± 0.00	0.34 ± 0.00	5.94 ± 0.00	1.06 ± 0.02	28.42 ± 1.32
FS1	7.04 ± 0.08	0.30 ± 0.00	0.32 ± 0.00	4.43 ± 0.00	1.05 ± 0.00	25.88 ± 3.23
FS2	7.01 ± 0.12	0.32 ± 0.00	0.35 ± 0.00	6.35 ± 0.58	1.07 ± 0.00	28.56 ± 1.87
FS3	1.53 ± 0.52	0.21 ± 0.00	0.24 ± 0.00	12.35 ± 0.00	1.14 ± 0.01	34.26 ± 1.25
FS4	1.59 ± 0.45	0.22 ± 0.00	0.25 ± 0.00	12.04 ± 0.23	1.14 ± 0.00	35.43 ± 1.32

* **Values are in the form of Mean ± SD.**

Table 3.2: Tablet properties of Salbutamol sulphate in the preliminary formulations*.

Formulation Code	Hardness (N)	Friability (%)	Thickness (mm)	Assay (%)	Matrix Integrity
FH1	65.67 ± 0.06	0.33	3.45 ± 0.04	98.46 ± 0.00	Not intact
FH2	65.67 ± 0.05	0.23	3.61 ± 0.04	97.15 ± 0.00	Intact
FX1	64.67 ± 0.09	0.12	3.51 ± 0.05	100.17 ± 0.00	Intact
FX2	69.67 ± 0.06	0.25	3.44 ± 0.03	99.45 ± 0.00	Intact
FM1	69.00 ± 0.01	0.24	3.62 ± 0.06	96.54 ± 0.01	Intact
FM2	66.00 ± 0.08	0.32	3.45 ± 0.03	95.42 ± 0.00	Not intact
FM3	67.00 ± 0.04	0.23	3.39 ± 0.04	98.23 ± 0.00	Intact
FM4	71.67 ± 0.06	0.32	3.55 ± 0.03	99.89 ± 0.00	Intact
FM5	60.33 ± 0.03	0.28	3.52 ± 0.04	100.64 ± 0.01	Intact
FM6	64.33 ± 0.0	0.32	3.43 ± 0.04	97.86 ± 0.01	Intact
FS1	72.33 ± 0.04	0.13	3.35 ± 0.03	102.09 ± 0.00	Intact
FS2	66.67 ± 0.05	0.25	3.51 ± 0.02	99.37 ± 0.00	Intact
FS3	66.00 ± 0.06	0.23	3.46 ± 0.05	98.31 ± 0.00	Intact
FS4	60.00 ± 0.02	0.35	3.37 ± 0.03	101.34 ± 0.01	Intact

* **Values are in the form of Mean ± SD.**

3.1.2. Effect of polymer type

The effect of polymer type (HPMC K4M or XG) on the various response variables of the tablets such as floating lag time, floating duration, cumulative drug release in the first 1 hr, and release rate are presented in Table 3.3. The effect on drug release profile is also shown in Figure 3.1. In order to investigate the effect of the polymer type, formulations were prepared at 10% and 40% using the two polymers. The level of sodium bicarbonate was kept constant at 10%.

At 10% concentration, HPMC couldn't retain its physical integrity and released its whole content within 1 hr. But XG retained its physical integrity for over 12 hr. When the polymer changed from HPMC to XG, the cumulative release in the first 1 hr was decreased from 100.35% to 25.90%. This is due to the fact that xanthan gum has a rapid hydration power than HPMC, which can prevent initial burst release of soluble drugs [Tiwari and Rajabi-Siahboomi, 2009]. The floating lag time also changed from 1.2 ± 0.1 sec to 1.5 ± 0.1 sec. In addition, only XG floated for more than 12 hr.

At 40% concentration, both HPMC and XG retained their physical integrity for a period of 12 hr and there was no significant change ($p > 0.05$) in cumulative release within the first 1 hr, but there was a significant change ($p < 0.05$) in release rate over 12 hr period when the polymer type changed from HPMC to XG. The floating lag time changed from 2.3 ± 0.42 sec to 4.8 ± 0.26 sec, but there was no change in floating duration, i.e., both formulations floated for more than 12 hr.

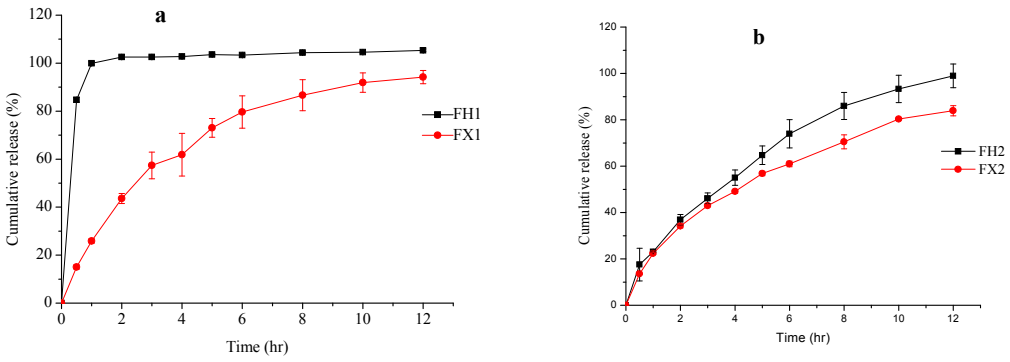

Figure 3.1: Effect of Polymer type at 10% [a] and 40% [b] on cumulative release of salbutamol sulphate.

Table 3.3: Effects of formulation parameters on floating lag time, floating duration, cumulative release in the first 1 hr, and release rate.

Factors	Response variables				
	Floating lag time (Sec)	***Floating duration(hr)***	***Cumulative release in 1 hr (%)***	***Cumulative release in 12 hrs (%)***	***Release rate ($h^{-1/2}$)***
Xanthan gum *[10% $NaHCO_3$]*					
10% (FX1)	1.5 ± 0.20	>12	25.90	94.21	29.73
40% (FX2)	4.8 ± 0.21	>12	22.34	83.92	25.97
HPMC K 4M *[10% $NaHCO_3$]*					
10% (FH1)	1.2 ± 0.19	Not intact	100.35	-	-
40% (FH2)	2.6 ± 0.17	>12	23.09	93.95	29.59
XG/HPMC K 4M ratio (10%) *[10% $NaHCO_3$]*					
(1:1) (FM1)	1.3 ± 0.13	>12	28.98	96.60	28.92
(1:3) (FM2)	0.8 ± 0.21	2	66.60	105.47	33.12
(3:1) (FM3)	1.4 ± 0.21	>12	26.88	93.09	28.47
XG/HPMC K 4Mratio (40%) *[10% $NaHCO_3$]*					
(1:1) (FM4)	3.3 ± 0.22	>12	21.49	89.05	27.89
(1:3) (FM5)	2 ± 0.23	>12	25.04	89.56	28.15
(3:1) (FM6)	4.6 ± 0.32	>12	21.45	86.54	27.63

$NaHCO_3$ (%)										
	40% XG	**40% HPMC**	**40% XG**	**40% HPMC**	**40% XG**	**40% HPMC**	**40% XG**	**40% HPMC**	**40% XG**	**40% HPMC**
5	10.4 ±0.21	3.96 ±0.15	>12	>12	21.35	15.99	73.91	80.03	22.27	27.24
10	4.8 ± 0.21	2.6 ± 0.17	>12	>12	22.34	23.09	83.92	93.95	25.97	29.59
20	3.7 ± 0.35	2.3 ±0.32	>12	>12	16.35	31.93	86.32	101.74	28.93	29.81

3.1.3. Effect of polymer concentration

The effect of polymer concentration on the various response variables is presented in Table 3.3.

In the case of XG, when the concentration increased from 10% to 40%, the cumulative release decreased from 25.90% to 22.34% ($p < 0.05$) in the first 1 hr, the release rate changed from 29.73 to 25.97 $hr^{-1/2}$ ($p < 0.05$) in 12 hr (Fig. 3.2), and the floating lag time increased significantly from 1.5 ± 0.1 sec to 4.8 ± 0.26 sec ($p < 0.0001$) due to the fact that as the concentration increases the dosage form becomes more dense and needs time to become swell and float. In the case of HPMC, the cumulative release in the first 1 hr decreased significantly from 100.35% to 23.09% ($p < 0.0001$) and the floating lag time changed significantly from 1.2 ± 0.1sec to 2.3 ± 0.42 sec ($p < 0.05$) as the concentration increased from 10% to 40%. This observation was in agreement with other reports [Hu *et al*., 2011]. An increase in the polymer concentration causes increase in the viscosity of the gel and leads to formation of gel layer with a longer diffusion path. This leads to a decrease in the diffusion of the drug and therefore a reduction in the drug release rate [Rajukar *et al*., 2011]**.**

Within the range studied, the polymer concentration didn't show any significant difference in floating duration (> 12 hr) in the case of XG, but in the case of HPMC, it showed a great difference when the concentration increased from 10% (it disintegrated after few minutes) to 40% (> 12 hr).

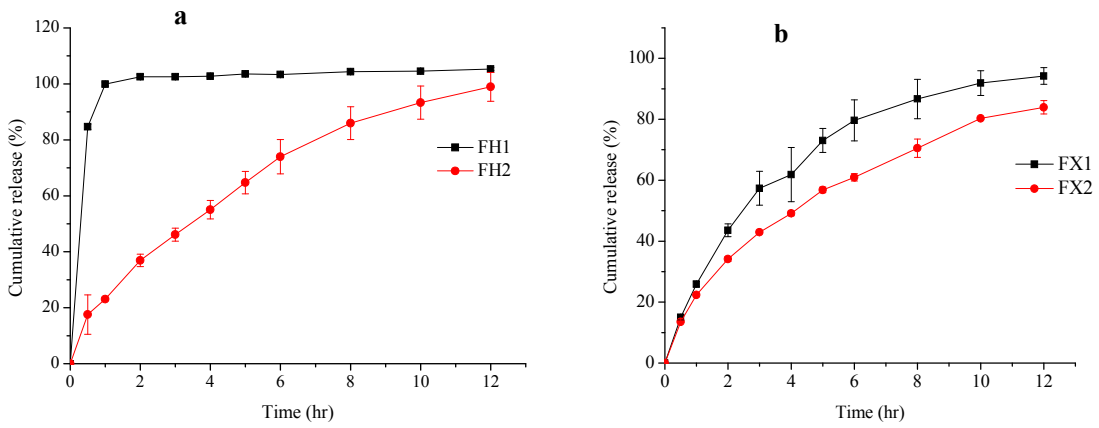

Figure 3.2: Effect of polymer concentration: FH1 (10%) and FH2 (40%) of HPMC [a]; FX1(10%) and FX2(40%) of XG [b] on cumulative release of salbutamol sulphate.

3.1.4. Effect of polymers ratio

The effect of XG/HPMC polymers ratio on the response variables is presented in Table 3.3.

Upon changing the XG/HPMC ratio from 1:1 to 1:3 (at 10% of the total polymer), the cumulative release increased from 28.98% to 66.59% ($p < 0.05$) in the first 1 hr and the release rate changed significantly from 28.92 to 33.12 $hr^{-1/2}$ ($p < 0.05$) over the period of 12 hr. On the other hand, when the XG/HPMC proportion changed from 1:1 to 3:1, the cumulative release decreased from 28.98% to 26.88% in the first 1 hr and the release rate changed from 28.92 to 28.47 $hr^{-1/2}$ over a period of 12 hr. This was because at lower concentration, HPMC showed burst release due to its low hydration power. However, xanthann gum, which has rapid hydration power to form a gel, can control the initial burst release of the water soluble drug [Tiwari and Rajabi-Siahboomi, 2009].

At 40% of the total polymer concentration, the cumulative release in 1 hr was increased from 21.49% to 25.04% when the XG/HPMC ratio changed from 1:1 to 1:3, but the change in release rate in 12 hr was not significant ($p > 0.05$). When the XG/HPMC ratio changed from 1:1 to 3:1, the cumulative release in the first 1 hr and the release rate in 12 hr didn't change at all ($p > 0.05$) because the total polymer was at higher concentration. As the percentage of polymer increases, it produces a greater cross-linking of polymer chains, which results in decreased porosity, and increased tortuosity of the gel retarding the release of drug through the gel [Maderuelo *et al*., 2011].

When the total polymer concentration increased from 10% to 40%, the cumulative release in the first 1 hr and in 12 hr changed significantly in general ($p < 0.0001$, and $p < 0.05$, respectively).

3.1.5. Effect of sodium bicarbonate

The effect of sodium bicarbonate on the various response variables of the floating tablets such as floating lag time, floating duration, release rate, and cumulative drug release in the first 1 hr and in 12 hr are also presented in Table 3.3. The effect on drug release profile is also shown in Fig. 3.3. In order to investigate the effect of sodium bicarbonate, formulations were prepared at 5%, 10%, and 20%. The levels of all other factors were kept constant at specified values (the total polymer was set at 40%).

It was observed that as the concentration of $NaHCO_3$ increased from 5% to 20%, the cumulative release in the first 1 hr increased from 16.00% to 31.93% ($p < 0.0001$) and the release rate changed from 27.24 to 29.81 $hr^{-1/2}$ ($p < 0.05$) in the case of the tablets formulated with HPMC. For tablets formulated with XG, a regular pattern was not shown

in the cumulative release in the first 1 hr, but it showed a significant increase in release rate from 22.27 to 28.93 $hr^{-1/2}$ ($p < 0.0001$) when the concentration of $NaHCO_3$ increased from 5% to 20%. As the concentration of gas-forming agent increases, it would generate larger amounts of effervescence leading to an increase in the rate of pore formation, rapid hydration of the tablets' matrices and consequently a faster drug release rate [Tadros, 2010]. The floating lag time decreased ($p < 0.05$) from 3.82 to 2.30 sec in the case of HPMC and from 10.10 to 3.27 sec ($p < 0.05$) in the case of xanthan gum when the concentration of $NaHCO_3$ increased from 5 to 20%. But the floating duration didn't show any change in both polymers, both of them floated for more than 12 hr.

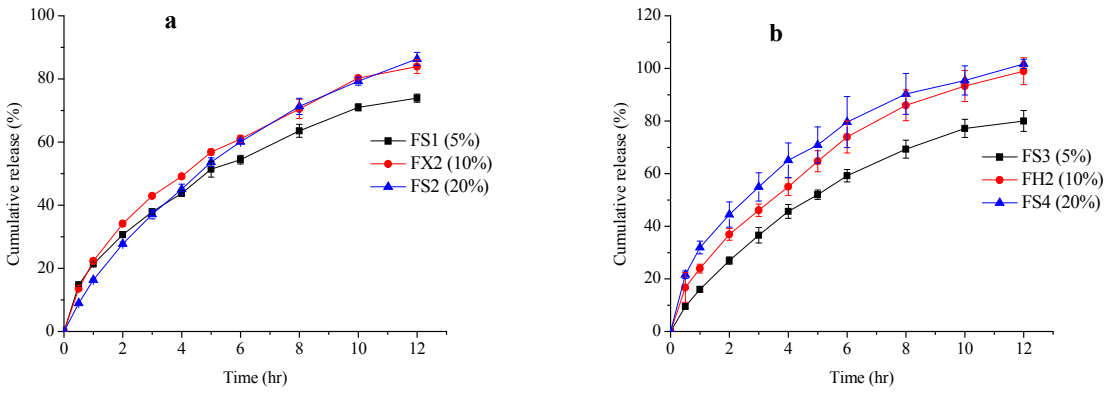

Figure 3.3: Effect of sodium bicarbonate at 5%(FS1, FS3), 10%(FX2, FH2), and 20%(FS2, FS4) of 40% XG[a] and 40% HPMC [b], respectively, on cumulative release of salbutamol sulphate.

3.1.6. Comparison and selection of formulations

Three types of floating tablets were formulated. These were formulations with only XG, HPMC, and with the combination of XG and HPMC as release sustaining agents. Their cumulative release pattern is shown in Fig. 3.4.

Formulations with HPMC alone sustained the drug release for 12 hr at 40% and the cumulative release in the first 1 hr and in 12 hr were 23.09% and 93.95%, respectively. But at 10%, the whole content was released in the first 1 hr. The one with 40% polymer floated for more than 12 hr, but at 10%, it disintegrated in the first few minutes.

Formulations with only XG sustained the drug release for 12 hr at both levels (10% and 40%). The cumulative releases in the first 1 hr and in 12 hr were 25.90% and 94.21% at 10%, and 22.34% and 83.92% at 40%, respectively. All the formulations floated for more than 12 hr.

Formulations with the combination of XG and HPMC showed a good release pattern in general. Those formulations at 40% of the total polymer (XG/HPMC; 1:1, 1:3, & 3:1) sustained the drug release for 12 hr and floated for more than 12 hr. The cumulative release at the first 1 hr and 12 hr were 21.49%, 25.04%, & 21.45% and 89.05%, 89.56%, & 86.54%, respectively. Two of the formulations at 10% of total polymer (XG/HPMC; 1:1 & 3:1) floated for 12 hr and showed a cumulative release of 28.98% and 26.88% at the first 1 hr and 96.60% & 93.09% in 12 hr period, respectively. The one with XG/HPMC ratio of 1:3 (at 10% of total polymer) showed a burst release of 66.59% at the first 1 hr; it released the whole content over a period of 8 hr.

Table 3.4 shows their significant change, at 95% confidence interval, in cumulative release in the first 1 hr and release rate in 12 hr period when the total polymer changes from 10% to 40%.

Table 3.4: P values in cumulative release in the first 1 hr and release rate in 12 hr period when the total polymer concentration increased from 10% to 40%.

		P value for CR at 1 hr	*P value for release rate*
XG		0.05098	0.04284
HPMC		0.000006	-
XG/HPMC	1:1	0.00575	0.27569
	1:3	0.0000008	0.03243
	3:1	0.02924	0.55075

As shown in Fig. 3.5, formulations from XG and from combinations of HPMC and XG, except FM2 which released more than 60% within the first 1 hr, showed good release pattern. Among these formulations, the combination with 1:3 polymer ratio (XG/HPMC) was selected for further optimization because it contains relatively high amount of HPMC, which has low hydration power than XG, that can release enough amount of drug in the first 1 hr which can be used as bolus dose for rapid relief of asthma. In addition, this formulation also showed significant difference in cumulative release at 1 hr and release rate, relative to the other formulations, when the polymer concentration increases from 10% to 40%.

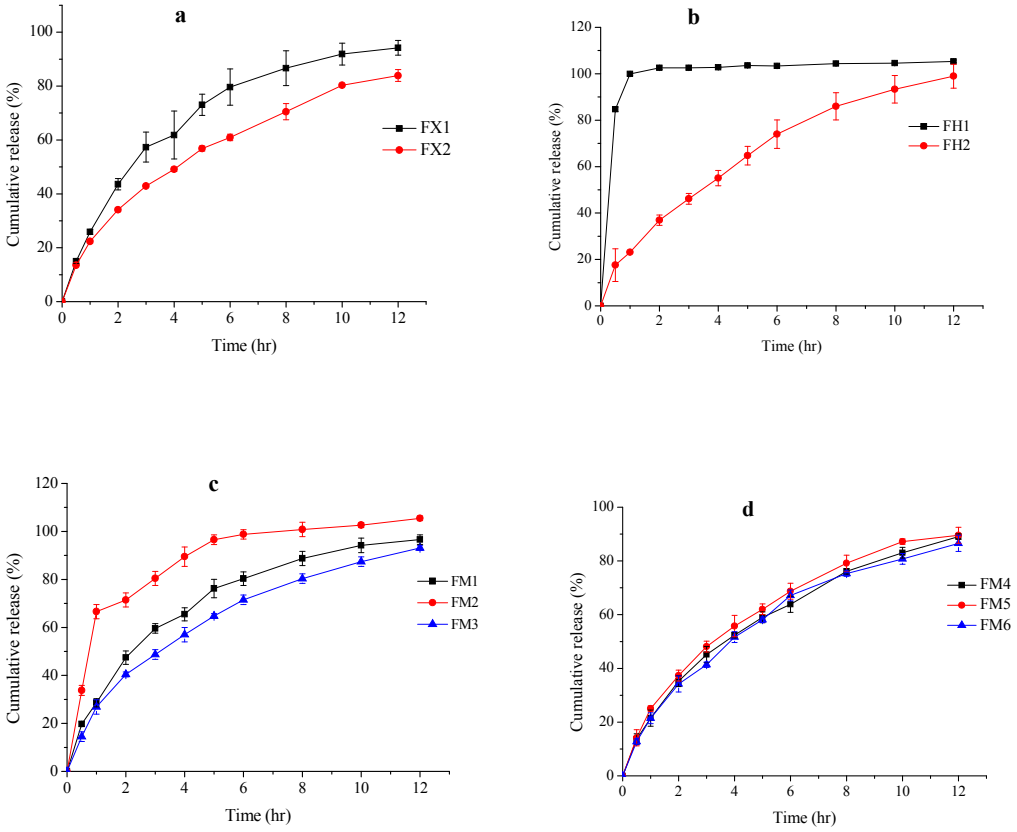

Figure 3.4: Effect of polymer concentration and polymer ratio on cumulative release of salbutamol sulphate: XG at 10% (FX1) and at 40% (FX2) [a]; HPMC at 10% (FH1) and at 40% (FH2) [b]; XG/HPMC at 10% {1:1(FM1), 1:3(FM2), and 3:1(FM3)} [c]; XG/HPMC at 40% {1:1(FM4), 1:3(FM5), and 3:1(FM6)}.

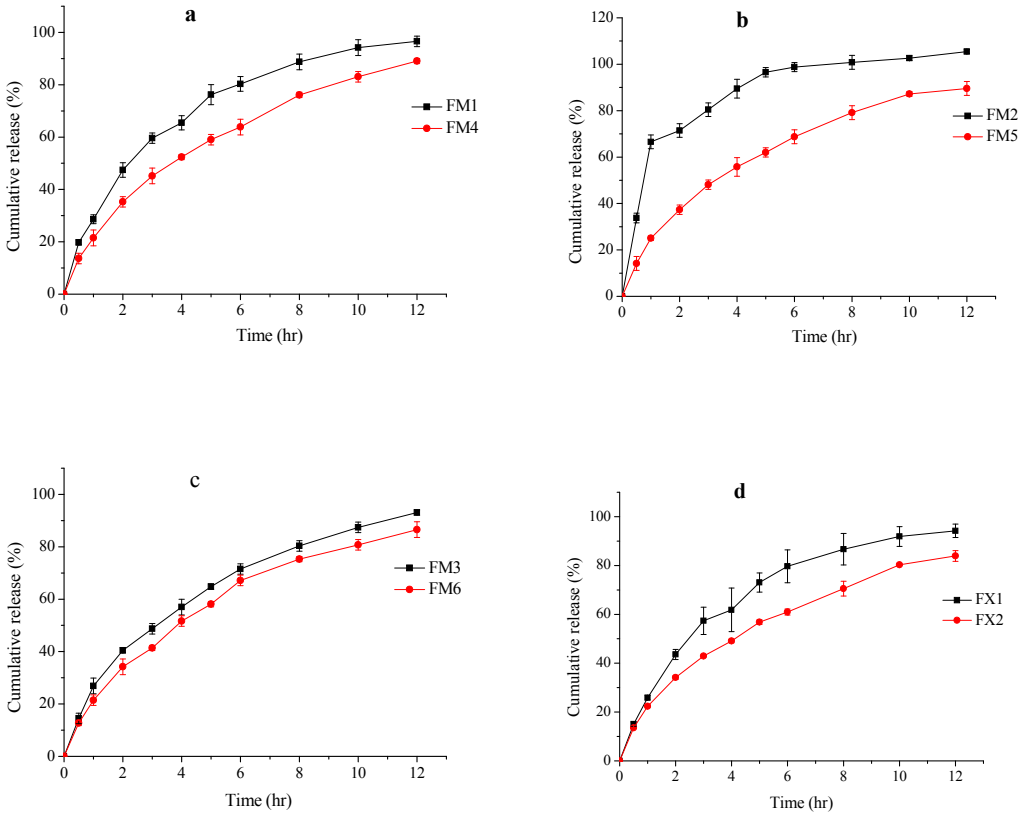

Figure 3.5: Effect of polymer concentration on cumulative release of salbutamol sulphate: XG/HPMC (1:1) at 10% (FM1) and 40% (FM4) [a], XG/HPMC (1:3) at 10% (FM2) and 40% (FM5) [b], XG/HPMC (3:1) at 10% (FM3) and 40% (FM6) [c], XG at 10% (FX1) and 40% (FX2) [d].

3.2. Optimization

From the preliminary experiments on the floating sustained release matrix tablets of salbutamol sulphate formulations, formulation that contains 1:3(XG/HPMC) ratio of the polymers was selected for further optimization. Hence the percentage of XG/HPMC (1:3) and the percentage of $NaHCO_3$ were considered as the independent variables and their effects on the characteristics of sustained release oral floating salbutamol sulphate tablets were further studied and optimized using RSM. To calculate quadratic regression model coefficients, each design variable must be studied at least at three distinct levels [Freiberg and Zhu, 2004], and consequently a CCD was used in the optimization study as it considers five distinct levels. The key characteristics selected as response variables for the optimization purpose were cumulative release in the first 1 hr and drug release rate. Even if the factors showed significant difference on floating lag time, it was not taken as a response because all formulations took less than 10 seconds to float on the surface of the media.

On the basis of the preliminary experiments, the range of the factors 10% to 40% for the polymer (XG/HPMC; 1:3) and 5% to 20% for $NaHCO_3$ were considered. To provide a CCD for two factors, a full 2^2 factorial design was combined with five replicates of the centre points and 2×2 axial points (Table 3.5). Experiments were performed in random order to minimize the effects of uncontrolled variables that may introduce bias into the measurements.

Table 3.5: Composition of the thirteen formulations.

Formulation code	Point type	Factors	
		XG/HPMC (%)	$NaHCO_3$ (%)
F1	factorial	10(-1)	5(-1)
F2	factorial	40(+1)	5(-1)
F3	factorial	10(-1)	20(+1)
F4	factorial	40(+1)	20(+1)
F5	axial	3.79(-α)	12.50(0)
F6	axial	46.21(+α)	12.50(0)
F7	axial	25(0)	1.89(-α)
F8	axial	25(0)	23.11(+α)
F9	center point	25(0)	12.50(0)
F10	center point	25(0)	12.50(0)
F11	center point	25(0)	12.50(0)
F12	center point	25(0)	12.50(0)
F13	center point	25(0)	12.50(0)

3.2.1. Granule characteristics

The physical properties of the granules (bulk density, tapped density, compressibility index, Hausner ratio, and angle of repose) of all the 13 formulations, which were obtained from Design Expert 8.0.7.1 software, were determined and are presented in Table 3.6.

The bulk density of the formulations ranged from 0.28 to 0.34 and the tapped density ranged from 0.29 to 0.38.

The blend indicated excellent to good flow properties for all formulations with the angle of repose values ranging from 29.26^{o} to 35.62^{0} except for formulation F9 with angle of repose value of 36.50^{o} according to fixed funnel method. The values of compressibility index ranged between 5.46 and 9.43, while the Hausner's ratios were between 1.06 and 1.10; indicating excellent flow properties.

Table 3.6: Granule characteristics of the formulations of salbutamol sulphate*.

Formulation code	Flow rate (g/sec)	Bulk density(g/ml)	Tapped density(g/ml)	Compressibility index (CI)	Housner ratio (HR)	Angle of repose(^{o})
F1	1.97 ± 0.12	0.34 ± 0.00	0.38 ± 0.00	9.09 ± 0.61	1.10 ± 0.01	35.62 ± 1.51
F2	1.89 ± 0.04	0.32 ± 0.04	0.35 ± 0.01	7.53 ± 0.07	1.08 ± 0.07	32.62 ± 0.66
F3	1.70 ± 0.01	0.28 ± 0.00	0.31 ± 0.00	9.43 ± 0.00	1.10 ± 0.00	34.72 ± 1.33
F4	1.97 ± 0.14	0.33 ± 0.00	0.35 ± 0.00	6.52 ± 0.84	1.07 ± 0.01	32.32 ± 1.52
F5	1.83 ± 0.24	0.31 ± 0.00	0.33 ± 0.00	6.25 ± 1.05	1.07 ± 0.02	29.41 ± 1.42
F6	1.86 ± 0.09	0.30 ± 0.02	0.32 ± 0.00	6.00 ± 0.00	1.06 ± 0.00	29.45 ± 0.45
F7	1.79 ± 0.06	0.31 ± 0.00	0.33 ± 0.00	6.19 ± 0.94	1.07 ± 0.01	30.96 ± 1.44
F8	1.82 ± 0.07	0.31 ± 0.02	0.33 ± 0.00	6.15 ± 1.93	1.07 ± 0.03	29.61 ± 1.46
F9	1.62 ± 0.21	0.28 ± 0.01	0.30 ± 0.00	7.41 ± 1.05	1.08 ± 0.02	36.50 ± 1.03
F10	1.85 ± 0.06	0.32 ± 0.13	0.34 ± 0.00	7.08 ± 0.00	1.08 ± 0.00	29.42 ± 1.32
F11	1.74 ± 0.03	0.30 ± 0.00	0.32 ± 0.00	6.00 ± 0.00	1.06 ± 0.00	32.88 ± 3.23
F12	1.69 ± 0.31	0.28 ± 0.01	0.29 ± 0.00	6.42 ± 0.58	1.07 ± 0.01	30.56 ± 1.87
F13	1.86 ± 0.27	0.31 ± 0.04	0.33 ± 0.00	5.46 ± 0.00	1.06 ± 0.00	29.26 ± 1.25

* **Values are in the form of Mean ±SD**

3.2.2. Characteristics of tablets

The tablets of 13 different batches were evaluated for hardness, thickness, friability, and drug content. The tablets mean thickness values ranged from 3.25 mm to 3.66 mm. The hardness of the tablets ranged between 61.33 to 73.33 N. As shown in Table 3.7, the loss in friability was in a range of 0.22 to 0.41% and the percentage drug content of tablets was between 98.46% to 102.64%, which were within the limits of < 1% and 90% to 110%, respectively. Except for formulations F1, F3, and F5, which contain low percentage of the polymers, ≤ 10%, all other formulations kept their matrix integrity.

Table 3.7: Tablet characteristics of the formulations of salbutamol sulphate*.

Formulation Code	Hardness (N)	Friability (%)	Thickness (mm)	Assay (%)	Matrix Integrity
F1	61.33 ± 2.31	0.34	3.48 ± 0.04	98.46 ± 0.00	Not intact
F2	63.67 ± 3.51	0.41	3.61 ± 0.04	100.15 ± 0.00	Intact
F3	66.00 ± 7.94	0.22	3.30 ± 0.05	101.17 ± 0.00	Not intact
F4	71.67 ± 7.23	0.27	3.24 ± 0.04	99.66 ± 0.00	Intact
F5	67.67 ± 2.52	0.32	3.56 ± 0.07	100.54 ± 0.01	Not intact
F6	65.33 ± 2.52	0.32	3.56 ± 0.07	99.42 ± 0.00	Intact
F7	68.33 ± 5.51	0.23	3.25 ± 0.04	101.23 ± 0.00	Intact
F8	67.67 ± 2.52	0.32	3.56 ± 0.07	100.89 ± 0.00	Intact
F9	66.33 ± 4.73	0.28	3.47 ± 0.05	102.64 ± 0.01	Intact
F10	67.67 ± 2.08	0.32	3.56 ± 0.07	101.86 ± 0.01	Intact
F11	73.33 ± 2.08	0.21	3.56 ± 0.03	101.09 ± 0.00	Intact
F12	63.67 ± 2.08	0.27	3.66 ± 0.03	102.37 ± 0.00	Intact
F13	68.00 ± 3.61	0.32	3.56 ± 0.07	99.31 ± 0.00	Intact

* **Values are in the form of Mean ± SD**

3.2.2.1. *In vitro* drug release

The drug release profiles of the 13 different formulations are shown in Fig. 3.6. As shown in the figure, the percentage of polymer appears to influence the drug release pattern remarkably. For example, formulations F1 and F3 (10% polymer), and F5 (3.79% Polymer) showed an initial burst release of 62.32%, 87.79% and 99.79%, respectively, in the first 1 hr. This is due to the lower percentage of the polymer concentration that can't keep the physical integrity of the tablets when CO_2 is released from the formulation when in contact with the acidic dissolution media. Except for formulations F2 and F6, all other formulations having > 10% polymer, showed similar trend with no significant difference in their release pattern over a period of 12 hr. The lower release from F2 and F6 were due to their higher percentage of polymer concentration (40% and 46.21%, respectively) and lower percentage of sodium bicarbonate for F2.

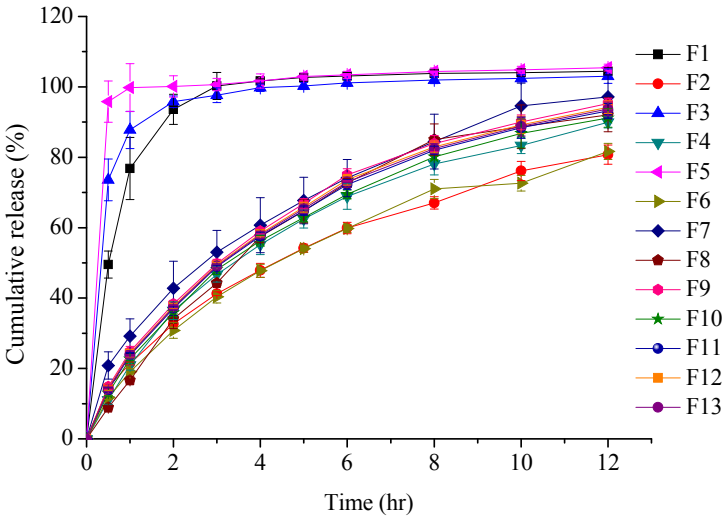

Figure 3.6: Effect of percentage of XG/HPMC and $NaHCO_3$ on the in vitro drug release of salbutamol sulphate.

The evaluation of the dissolution profiles is a useful tool in the development of formulations where it is possible to select those which present better performance with respect to the drug liberation [Ferraz *et al*., 2007]. Comparison of dissolution profiles of several formulations is analyzed simultaneously using several special measures including the dissolution efficiency (DE). In this study, DE after 12 hr of release was used to compare the results of release profiles of different formulations. The DE of all formulations is depicted in Fig. 3.7. Results from ANOVA indicated the presence of significant difference ($p < 0.0001$) among the formulations in their release profiles. These differences in release profiles evidenced that changes in values of the investigated formulation variables had significant influence on pattern of release and thus optimization was required to have an optimum release over a fixed period of time.

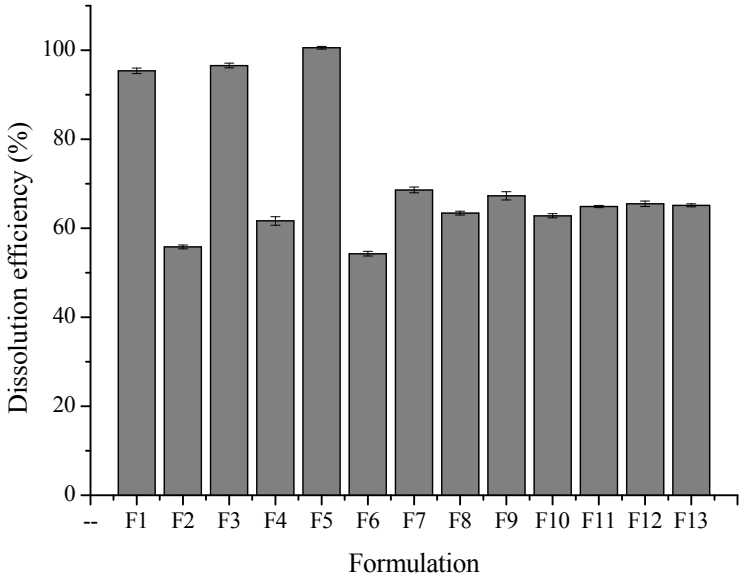

Figure 3.7: The dissolution efficiency values for formulations used in the optimization study.

3.2.3. Drug release kinetics

A linear regression analysis of the drug release data can be used as indicator for the release mechanism from matrix delivery systems. In this study, the *in vitro* drug release data were fitted to five commonly employed release kinetic models, namely; zero order, first order, Hixson-Crowell cube root model, and Higuchi model equations to analyze the *in vitro* drug release kinetics, and Korsmeyer-Peppas model up to the first 60% drug release to describe the drug release mechanism from a polymeric system. The results are shown in Table 3.8 and 3.9, respectively. The tables present the linear regression results of the model fitting tests of the formulations. Coefficients of determination (R^2) were used to evaluate the goodness of the model fit.

Except for three of the formulations (F1, F3, and F5), which released their whole content within the first 3 hr, all the formulations were subjected to the four kinetic models described. These formulations (F1, F3, & and F5) composed of small percentage of the polymers, at 3.79% (F5) and at 10% (F1 and F3), were not evaluated at all because of their initial complete burst release. As shown in Table 3.8, Higuchi square root model showed the best fit with high linearity (R^2 ranging: 0.976 - 0.997) for all formulations.

Table 3.9 shows the drug release model obtained by fitting the first 60% drug release to the Korsmeyer-Peppas model. In this model, the value of *n* illustrates the type of release mechanism. For cylindrical tablets; $n \leq 0.45$ corresponds to a Fickian diffusion mechanism, $0.45 < n < 0.89$ to non-Fickian transport, $n = 0.89$ to Case II (relaxational) transport, and $n > 0.89$ to super case II transport [Shoaib *et al*., 2006; Ali *et al*., 2007; Sinha *et al*., 2011].

Table 3.8: Rate constants and correlation coefficient fits of different kinetic equations for salbutamol sulphate tablets.

Formulation	Zero-order release model			First-order release model			Higuchi square-root release model			Hixson-Crowell cube root model		
	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2
F1	NA*	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F2	5.61	20.67	0.943	-0.13	1.49	0.796	24.51	-1.89	0.997	-0.15	1.82	0.855
F3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F4	6.53	21.67	0.905	-0.15	1.51	0.708	28.93	-5.44	0.985	-0.17	1.82	0.786
F5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F6	5.85	18.74	0.928	-0.14	1.60	0.762	25.68	-5.04	0.991	-0.16	1.92	0.828
F7	6.58	28.58	0.934	-0.12	1.18	0.801	28.81	1.95	0.994	-0.15	1.52	0.853
F8	7.28	18.97	0.893	-0.17	1.65	0.697	32.32	-11.39	0.976	-0.19	1.94	0.776
F9	6.82	24.61	0.911	-0.14	1.35	0.747	30.15	-3.55	0.986	-0.16	1.68	0.812
F10	6.63	22.51	0.916	-0.14	-0.86	0.739	29.24	-4.77	0.989	-0.17	1.77	0.809
F11	6.72	23.56	0.914	-0.14	1.40	0.744	29.70	-4.16	0.988	-0.16	1.72	0.811
F12	6.77	24.09	0.913	-0.14	1.37	0.745	29.92	-3.85	0.987	-0.16	1.70	0.811
F13	6.75	23.82	0.913	-0.14	1.39	0.744	29.81	-4.01	0.987	-0.16	1.71	0.811

*NA:- Not analyzed

Table 3.9: Drug release mechanism for floating salbutamol sulphate tablets evaluated by Korsmeyer-Peppas model.

Formulation	Intercept	K	Exponent (n)	R^2
F1	NA	NA	NA	NA
F2	-1.53	0.22	0.57	0.999
F3	NA	NA	NA	NA
F4	-1.61	0.20	0.75	0.991
F5	NA	NA	NA	NA
F6	-1.66	0.24	0.66	0.998
F7	-1.22	0.30	0.52	0.999
F8	-1.78	0.17	0.88	0.995
F9	-1.43	0.19	0.66	0.998
F10	-1.52	0.22	0.69	0.996
F11	-1.47	0.23	0.68	0.998
F12	-1.45	0.23	0.67	0.998
F13	-1.46	0.23	0.68	0.998

As shown in Table 3.9, the *n* value for all the formulations were found to be in the range of 0.52 to 0.88, which indicated that the drug release from these formulations generally followed non Fickian (anomalous) transport mechanism.

Higuchi square root model was selected for drug release rate analysis from each of the formulations in search of the optimum in optimization analysis because it showed better linear fitting trend for all the formulations.

With the goal of sustaining the release of the drug from floating tablets for 12 hr period, the Higuchi square root model was used to calculate the release rate on the assumption that greater than 90% of the drug would be released in 12 hr. Substituting 12 hr for time,

and the percentage release to be greater than 90% for cumulative drug released, the release rate (K) was found to range between 26 to 30 $hr^{-1/2}$. This range of release rate was used in the optimization study to obtain an optimum formulation that releases its drug content slowly and ultimately achieve greater than 90% release over a period of 12 hr. According to Bomma *et al.,* (2009), the theoretical cumulative drug release profile within 1 hr is between 20 and 25% and Pasa *et al.,* (2012) obtained 23 to 32% cumulative release within 1 hr from the said good formulations of sustained release matrix tablets of didanosine. In this study the cumulative release in the first 1 hr was assumed to be between 24 and 30% in order not to compromise the release rate needed to have.

The cumulative release in the first 1 hr and release rates obtained from the 13 formulations are shown in Table 3.10. These results were used as input in the Design-Expert 8.0.7.1 software for the optimization analysis.

Table 3.10: CCD matrix in terms of both actual and coded factor levels and summary of experimental measurements of cumulative release in the first 1 hr and release rate.

Formulation code	Responses	
	Cumulative release in the 1st hr (%)	Release rate ($hr^{-1/2}$)
F1	62.32	-
F2	21.42	24.51
F3	87.79	-
F4	22.94	28.77
F5	99.79	-
F6	20.08	25.68
F7	22.15	28.81
F8	35.07	32.32
F9	24.24	29.92
F10	22.68	29.24
F11	23.98	29.81
F12	24.77	30.15
F13	23.72	29.70

3.2.4. Response model selection

Suitable response models for the responses were selected based on the fit summaries. Table 3.11 provides the fit summaries output of both responses. The fit summary displays statistics such as lack of fit p-values and R-squared values for comparing models for each response. The program selects and suggests for further use the highest order polynomial where the additional terms are significant, the model is not aliased and Adjusted R-squared and Predicted R-squared are in a reasonable agreement (within 0.20 of each other). A model is considered if the model term's p-value is less than 0.05 and the lack of fit p-value is greater than 0.05. From the model term's p-value and the lack of fit p-values presented in Table 3.11, it can be stated that the linear contribution is suggested ($p <$

0.0001, p = 0.0573, respectively) for release rate and the quadratic contribution is suggested (p < 0.0001, p = 0.0615, respectively) for cumulative release at 1 hr. Therefore, with evidence of the fit summary output (Table 3.11), linear model and quadratic model were selected as best fit models for release rate and cumulative release at 1 hr, respectively, as suggested by the software analysis. In this study, the fit summary output was analyzed without performing any transformation (logarithmic, power, inverse, etc.) on the response data. Transformation is needed to meet the assumptions that make the ANOVA valid.

Table 3.11: Fit summary statistics for release rate and cumulative release at 1 hr.

Response	Source	R-Squared	Adjusted R-Squared	Predicted R-Squared	p-value	Lack of fit p-value	Remark
Release rate	Linear	0.9448	0.9290	0.8445	<0.0001	0.0573	suggested
	2FI	0.9679	0.9519	0.8428	0.0827	0.0965	
	Quadratic	0.9900	0.9776	0.2762	0.0965	0.0034	
	Cubic	0.9925	0.98813	-	-	-	aliased
Cumulative Release at 1hr	Linear	0.6209	0.5451	0.3199	0.0078	<0.0001	
	2FI	0.6561	0.5334	0.2363	0.4089	<0.0001	
	Quadratic	0.9985	0.9974	0.9909	<0.0001	0.0615	suggested
	Cubic	0.9995	0.9989	0.9881	0.0519	0.1862	aliased

3.2.5. Model adequacy checking

Regression analysis is the general approach to fit an empirical model with the collected response variable data [Aziz *et al*., 2008]. The ANOVA is the most important tool for the evaluation of significance and goodness of fit of the regressional model and significance of individual model coefficients [Noordin *et al*., 2004; Kusic *et al*., 2010]. Hence, in this

study, ANOVA was applied to test the significance of the selected models and model coefficients for the respective responses (linear for release rate and quadratic for cumulative release at 1 hr). The regression model and the terms in the model are considered to be significant when a p-value is less than 0.05. Summaries of the ANOVA results for both responses are given in Table 3.12.

Table 3.12: Summary of ANOVA results of response surface linear model for drug release rate and response surface quadratic model for cumulative release at 1 hr.

Response	Source	Sum of squares	df	Mean Square	F-value	p-value	Remark
Release rate	Model	43.03	2	21.52	59.88	<0.0001	Significant
	$NaHCO_3$ (X_1)	14.18	1	14.18	39.46	0.004	Significant
	XG/HPMC(X_2)	28.85	1	28.85	80.29	<0.0001	Significant
	Residual	2.52	7	0.36			
	Lack of fit	2.06	3	0.69	6.05	0.0573	Insignificant
	Pure error	0.45	4	0.11			
	Core total	45.55	9				
Cumulative Release at 1hr	Model	8487.30	5	1697.46	928.01	<0.0001	Significant
	$NaHCO_3$ (X_1)	266.88	1	266.88	145.91	<0.0001	Significant
	XG/HPMC(X_2)	5010.76	1	5010.76	2739.42	<0.0001	Significant
	(X_1X_2)	247.91	1	247.91	135.53	<0.0001	Significant
	$NaHCO_3{}^2$ *(X_1^2)*	221.90	1	221.90	121.32	<0.0001	Significant
	$XG/HPMC^2$ *(X_2^2)*	2898.68	1	2898.68	1584.73	<0.0001	Significant
	Residual	12.80	7	1.83			
	Lack of fit	10.41	3	3.47	5.79	0.0615	Insignificant
	Pure error	2.40	4	0.60			
	Core total	8500.10	12				

As shown in the table, models of both responses were significant ($p < 0.0001$ for linear model of release rate and $p < 0.0001$ for the quadratic model of cumulative release at 1 hr). Model significance is desirable as it indicates that the terms in the model have a

significant effect on the response. ANOVA results in the table also revealed that the main effects of both parameters, sodium bicarbonate percentage ($p = 0.004$) and percentage of XG/HPMC ($p < 0.0001$), were significant model terms for the linear model of release rate. For the quadratic model of cumulative release at 1 hr, the main effects of both parameters, the interaction effect (X_1X_2) ($p < 0.0001$), and the quadratic effects, X_1^2 ($p < 0.0001$) and X_2^2 ($p < 0.0001$) were significant model terms.

Table 3.13 presents the values of coefficients of determination (R^2), adjusted R^2, and predicted R^2 to indicate the adequacy of the models to the respective responses. As shown in the table, the value of R^2 for the linear model of release rate and quadratic model of cumulative release at 1 hr were 0.9448 and 0.9985, respectively. These values indicate the degree of correlation between the experimental and the predicted values. Table 3.13 also indicates, the adjusted R^2 and predicted R^2 values of release rate (0.9290 and 0.8445, respectively) and cumulative release at 1 hr (0.9974 and 0.9909, respectively) were in reasonable aggreement. The value of adequate precision (signal to noise ratio) of 21.021 for release rate and 84.11 for cumulative release at 1 hr obtained were very high compared to the desirable value of greater than 4 [Aziz *et al*., 2008], indicating that the model can be used to navigate the design space.

Table 3.13: Numerical test results of model adequacy checking for the linear model of release rate and quadratic model of cumulative release at 1 hr.

	Release rate	Cumulative release at 1hr
R-Squared	0.9448	0.9985
Adjusted R-squared	0.9290	0.9974
Predicted R-squared	0.8445	0.9909
Adequate precision	21.021	84.111

The normal probability plots of the residuals and the plots of the residuals versus the predicted response for cumulative release at 1 hr and release rate are shown in Fig. 3.8 to Fig. 3.11. The important information on the model performance is summarized in residuals (i.e. difference between observed and predicted values) providing a clear view for any discrepancy in fit to the model. Hence, two plots related to residuals: the normal probability plot of residuals and the plot of internally studentized residuals versus predicted values are considered as additional tests of model adequacy checking tools [Kusic *et al*., 2010]. A check on the plots in Fig. 3.8 and Fig. 3.10 showed that points or point clusters are placed closely to the diagonal line implying that the errors are distributed normally for both responses. Fig. 3.9 and 3.11 indicate that the points are randomly scattered, with no obvious pattern or structure, and all values lie within the recommended range of −3 and +3 (values between −3 and +3 are considered as the top and bottom outlier detection limits).

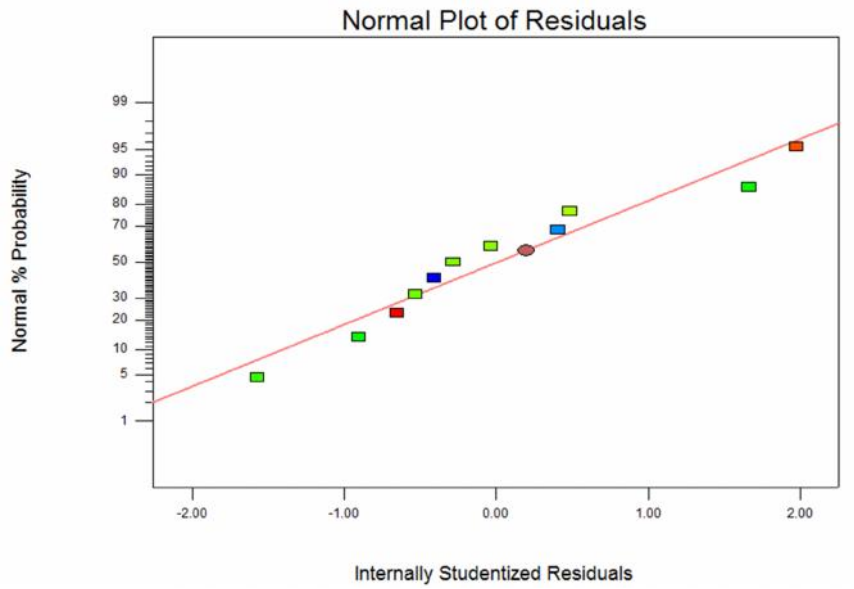

Figure 3.8: Normal probability plot of residuals for release rate.

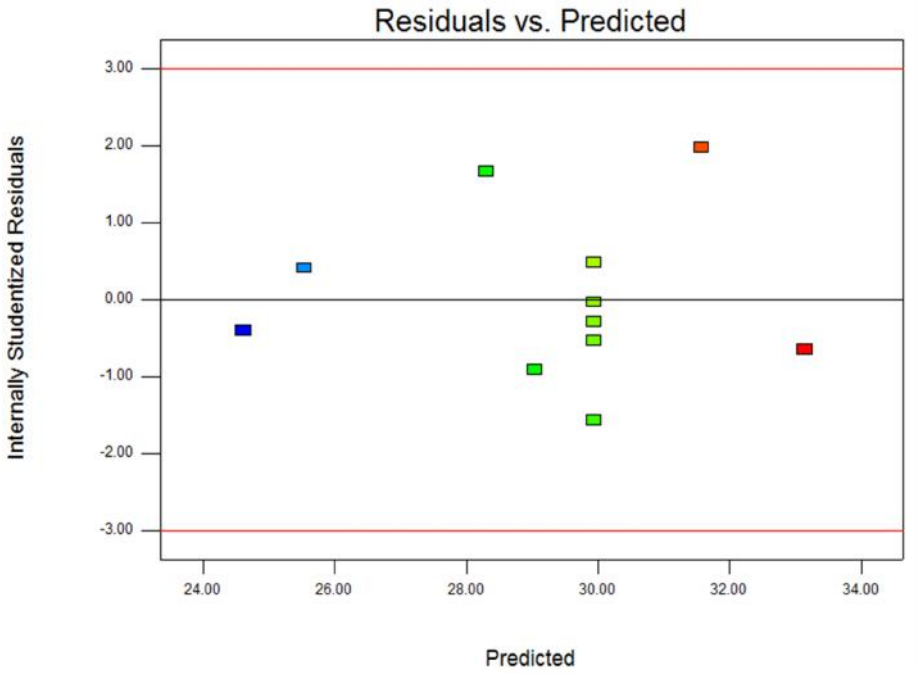

Figure 3.9: Plots of the residuals against predicted response for release rate.

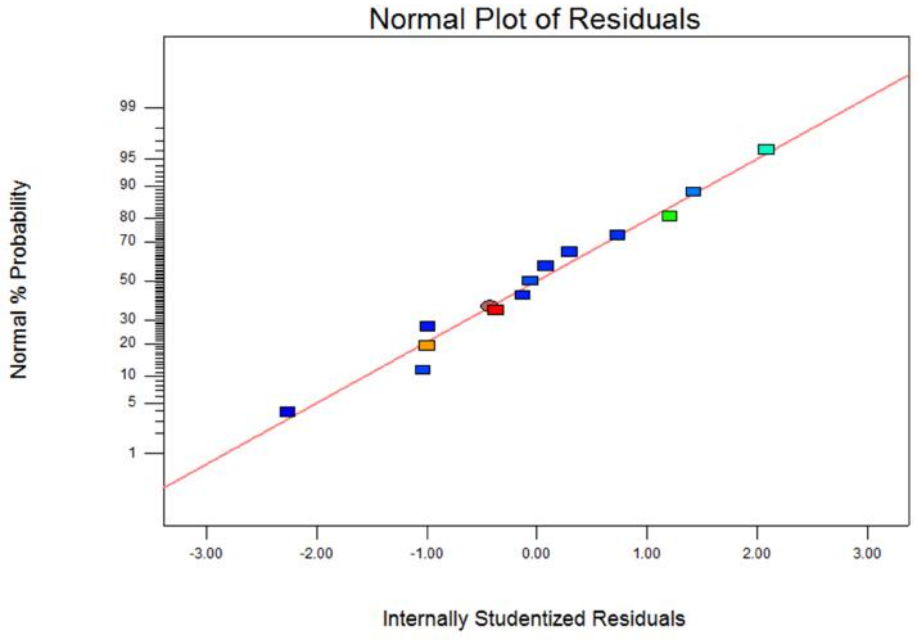

Figure 3.10: Normal probability plot of residuals for cumulative release at 1 hr.

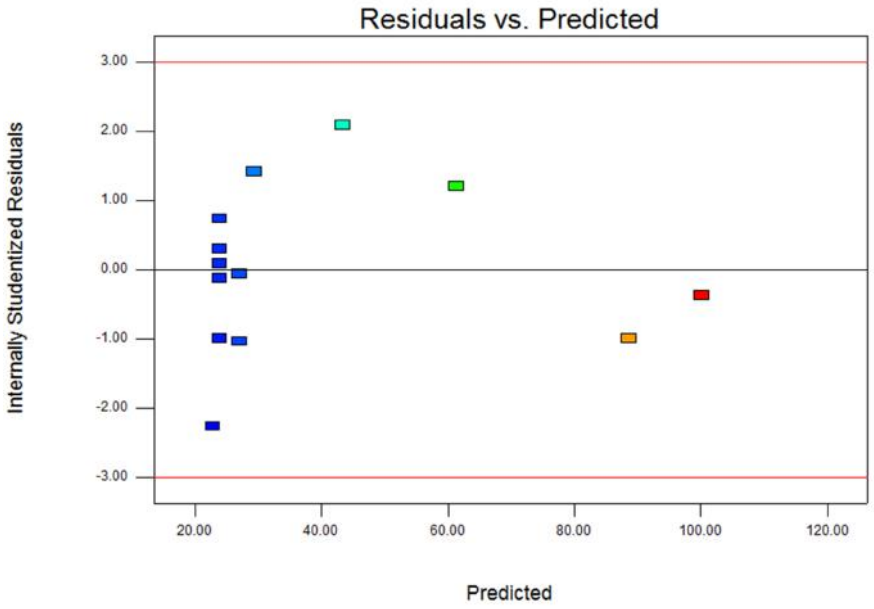

Figure 3.11: Plots of the residuals against predicted response for cumulative release at 1 hr.

From the foregoing, it is apparent that the models proposed are adequate for their respective responses and there is no reason to suspect any violation of the independence or constant variance assumption.

Since both of the response models were significant, the adjusted and predicted R^2 of both response models were in good agreements, the adequate precision were over 4 and the residuals were well behaved; it is reasonable to conclude that the selected models were fairly accurate and could be used for further analysis. The final mathematical regression models in terms of coded factors (Eq. 3.1 and Eq. 3.2) were developed using model term coefficients shown in Table 3.14. The table shows the regression coefficients (coded and actual) of model terms in the linear model of release rate and the quadratic model of cumulative release at 1 hr.

Table 3.14: Estimated model term regression coefficients for the linear model of release rate and quadratic model of cumulative release at 1 hr.

	Coded coefficients		Actual coefficients	
	Release rate	Cumulative release at 1 hr	Release rate	Cumulative release at 1 hr
Intercept	+29.98	+23.88	+32.73573	+106.48593
Main effect				
$NaHCO_3$ (X_1)	+1.54	+5.78	+0.20498	$+9.393*10^{-3}$
XG/HPMC (X_2)	-3.19	-25.03	-0.21270	-5.32993
Interaction effect				
X_1X_2	-	-7.87	-	-0.069978
Curvature effect				
$NaHCO_3^2$ (X_1^2)	-	+5.65	-	+0.10041
XG/HPMC ^{2} (X_2^2)	-	+20.41	-	+0.090724

Final polynomial equations of response variables in terms of coded coefficients of the formulation parameters were obtained as shown below:

$$\text{Release rate}\,(Y_1) = 29.98 + 1.54X_1 - 3.19X_2 \qquad \text{Eq. 3.1}$$

$$\text{Cumulative release at 1 hr}\,(Y_2) = 23.88 + 5.78X_1 - 25.03X_2 - 7.87X_1X_2 + 5.65X_1^2 + 20.41X_2^2 \qquad \text{Eq. 3.2}$$

The models can also be expressed using the actual coefficients although the common way of expression is based on the coded coefficients.

Coefficients of developed models have physical meanings on response variables. A coefficient is the amount the response changes when that term is changed by one unit, while holding the other terms constant. Both the magnitude and sign of coefficients are

important. The magnitude implies the strength whereas the sign indicates the direction of that factor variable on the corresponding response variable. A positive sign indicates a positive effect whereas a negative sign indicates a negative effect on the response [Singh *et al*., 2010]. In this study, as evidenced from (Eq. 3.1 and 3.2) and Table 3.14, both responses (release rate and cumulative release at 1 hr) are affected positively by the percentage of sodium bicarbonate and negatively by the percentage of the polymer; however, the effect of the polymer was stronger than that of sodium bicarbonate on both of the responses. The second order interaction effect negatively affects the cumulative release at 1 hr. Quadratic effects (X_1^2 and X_2^2) were found to have positive relationship with cumulative release at 1 hr. As seen from Eq. 3.1 and 3.2, the polymer percentage was more determinant factor on release rate and cumulative release at 1 hr as it had larger coefficient (-3.19 and -25.03) as compared with percentage of sodium bicarbonate (1.54 and 5.78), respectively.

3.2.6. Contour plot and surface response analysis

The 2D contour and 3D response surface plots are the graphical representations of the fitted regression models. These plots, presented on the basis of the model equations, display the interaction between the independent variables and assist in determining the optimum values of the variables within the ranges considered [Sampaio *et al*., 2006].

The 2D and 3D plots for the interaction between percentage of sodium bicarbonate and XG/HPMC polymer percentage, and their interactive effects on the two responses are presented in Fig. 3.12 to Fig. 3.15. The contour and response surface plots shown in Fig. 3.12 and 3.13, respectively, indicate the combined effect of percentage of sodium

bicarbonate and XG/HPMC polymer on the release rate of the formulations. The series of parallel straight lines of the contour plot and the non-twisted response surface indicate that there was no interaction effect of the two parameters on the release rate. The plots show that the linear model components individually affect the release rate, with comparatively a more significant effect of XG/HPMC percentage. The same is indicated in the ANOVA results (Table 3.12), where XG/HPMC percentage showed more significant effect ($p < 0.0001$) than the percentage of sodium bicarbonate ($p = 0.004$) on the release rate.

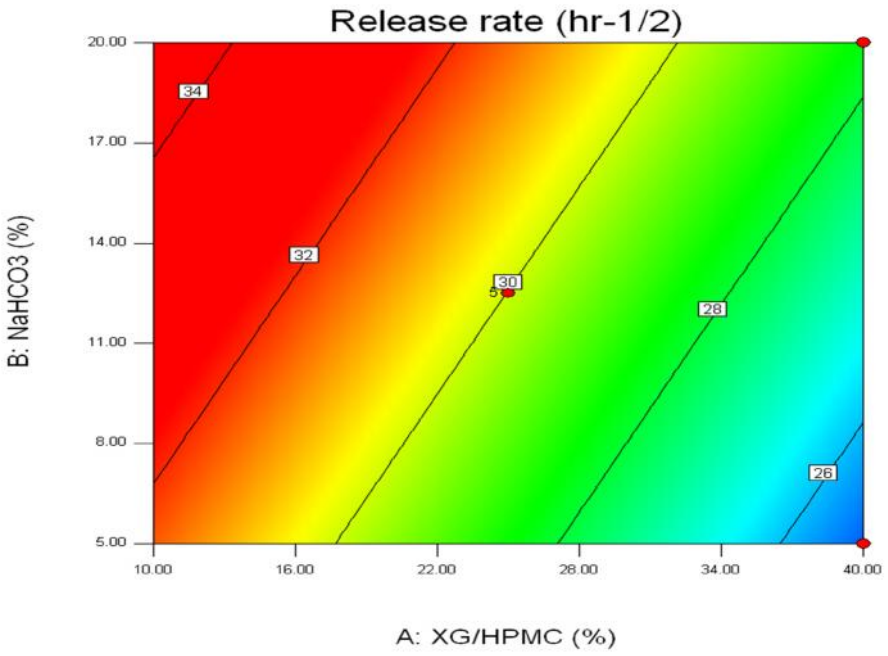

Figure 3.12: Contour plot of polymer percentage (XG/HPMC) and the percentage of sodium bicarbonate on drug release rate.

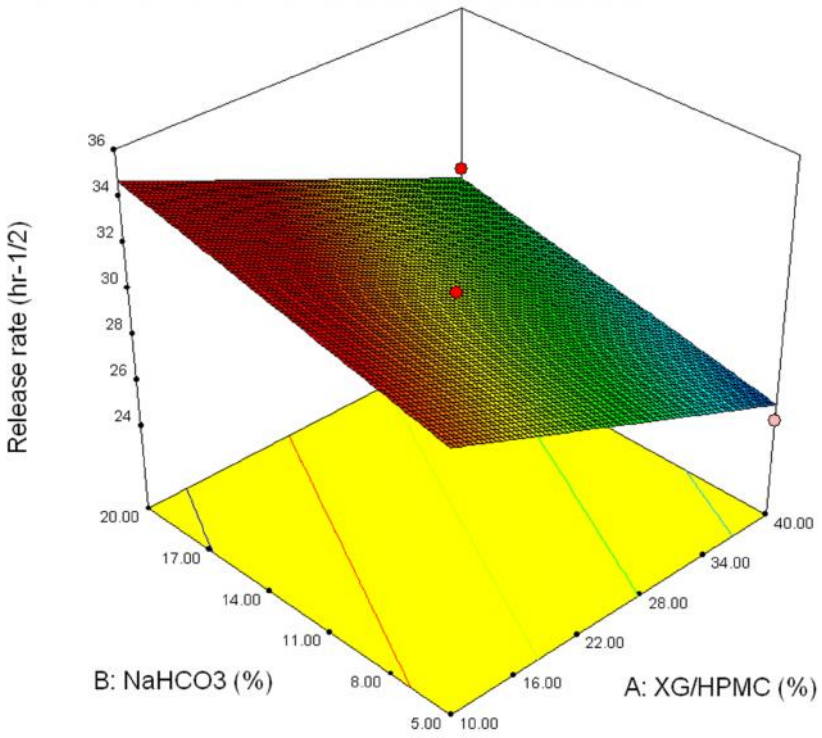

Figure 3.13: Response surface plot of polymer percentage (XG/HPMC) and the percentage of sodium bicarbonate on drug release rate.

According to Fig. 3.12 and 3.13, the most suitable conditions for slow drug release rate within the investigated ranges were found to be at the highest XG/HPMC polymer concentration (coded $X_2 = +1$) and at the lowest sodium bicarbonate concentration used (coded $X_1 = -1$).

The combined effect of sodium bicarbonate percentage and XG/HPMC percentage on cumulative release at 1 hr of the tablets is shown in Fig. 3.14 and 3.15. The plots indicate that both sodium bicarbonate percentage and XG/HPMC percentage play very important role in influencing the cumulative release at 1 hr. As the elliptical contours of Fig. 3.14, twisted response surface of Fig. 3.15, and the ANOVA results in Table 3.12 ($p < 0.0001$) indicate, the interactive effect of the two variables is highly significant. A perfect

interaction between the independent variables is characterized by formation of elliptical contours, where the maximum predicted value is identified by the surface confined in the smallest ellipse in the contour diagram [Baril *et al.*, 2010].

According to Fig. 3.14 and 3.15, an increase in percentage of XG/HPMC results in a decrease in the cumulative release at 1 hr, but an increase in sodium bicarbonate percentage has the opposite effect. The effect of XG/HPMC percentage appeared to be more pronounced as compared to the percentage of sodium bicarbonate. This was confirmed from the coefficients in the mathematical model generated for cumulative release at 1 hr (Eq. 3.2) where the coefficient of XG/HPMC was larger (-25.03 vs. 5.78) than the coefficient of percentage of sodium bicarbonate.

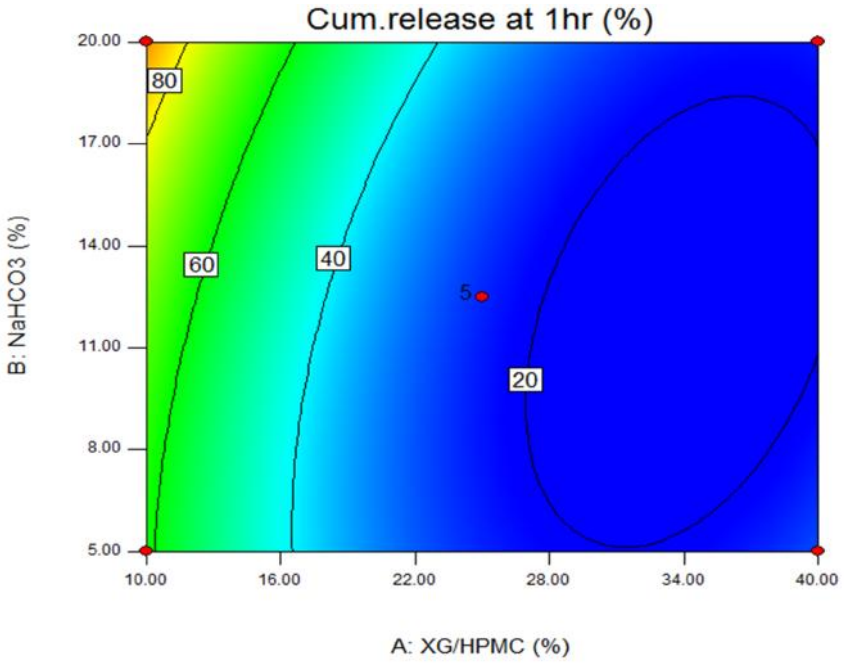

Figure 3.14: Contour plot of polymer percentage (XG/HPMC) and percentage of sodium bicarbonate on cumulative release at 1 hr.

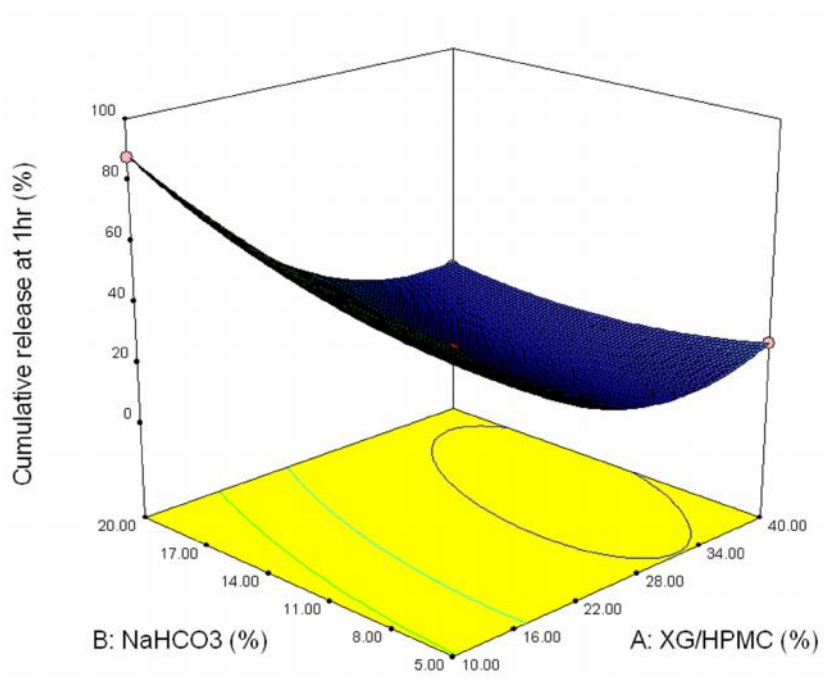

Figure 3.15: Response surface plot of polymer percentage (XG/HPMC) and percentage of sodium bicarbonate on cumulative release at 1 hr.

3.2.7. Simultaneous optimization of cumulative release at 1 hr and release rate

After generating the model polynomial equations to relate the dependant and independent variables, the formulation was optimized for the two responses simultaneously. The final optimal experimental parameters were obtained using both numerical and graphical optimization techniques of Design-Expert 8.0.7.1 software, which allows compromise among various responses and searches for a combination of factor levels that jointly optimize a set of responses by satisfying the requirements for each response in the set. Table 3.15 presents the criteria defined for factors and responses during optimization with both numerical and graphical techniques.

Table 3.15: Constraints for factors and responses used during numerical and graphical optimization.

Factor constraints				
Factor		*Low*	*High*	
$NaHCO_3$ (%)		5	20	
XG/HPMC (%)		10	40	
Response constraints				
Response	*Goal*	*Lower limit*	*Upper limit*	*Importance*
Release rate ($hr^{-1/2}$)	Target = 28	26	30	5
Cumulative release at 1hr (%)	In range	24	30	4

3.2.7.1. Numeric optimization

RSM is an effective optimization tool by which the global optimum can be obtained. The desirability function approach is one of the most widely used methods for optimization of multiple responses [Takayama *et al*., 2003]. In the use of soft-wares like Design Expert, numerical optimization is used in order to find the specific point that maximizes the global desirability function. In the numerical optimization of this study, the desired goals for responses were chosen from the menu, weight and importance to each response were assigned. To find the global (overall) desirability function, the software performs thousands of iterations and calculations and finally it comes up with the maximum desirability score and the conditions on which it was arrived. Accordingly, the predicted optimum values and the corresponding levels of parameters according to the set goals were obtained as presented in Fig. 3.16. A dot indicates the best solution found by the Design Expert solver.

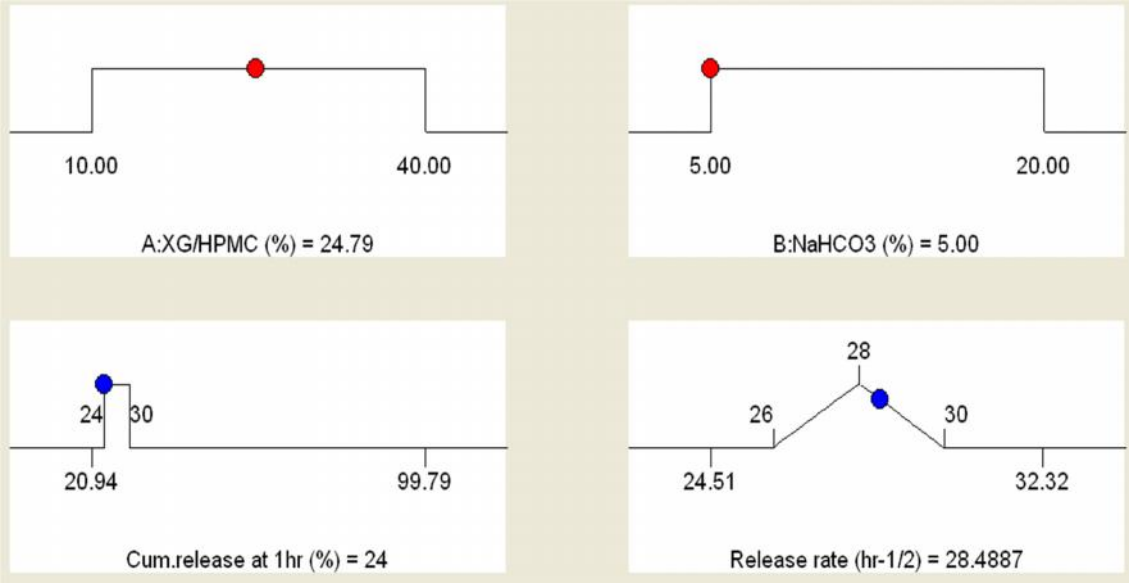

Figure 3.16: Numerical optimization results of predicted optimum values and the corresponding levels of parameters.

In multiple response optimization using desirability approach, individual desirability functions d_i indicate measures of how well the goals for each response are satisfied, whereas overall desirability function is a measure of how well the combined goals for all responses are satisfied. Desirability function ranges from 0 to 1, with value closer to one indicating a higher satisfaction of response goal(s) [Yang and El-Haik, 2008]. In this study, the values of individual desirability functions d_i of release rate and cumulative release at 1 hr were obtained from the Design-Expert solver to be 0.7556 and 1, respectively, as calculated from the optimal point obtained (Y_1 = 28.49 $hr^{-1/2}$, Y_2 = 24). This indicates that the requirement for both responses was almost achieved. The overall desirability function (D) was then obtained from the individual desirability functions to be 0.756 from the software solver calculated based on Equation 3.3.

$$D = \left[d_1^{p_1} d_2^{p_2} d_3^{p_3} \ldots d_i^{p_i} \right]^{1/\sum p_i} \qquad \text{Eq. 3.3}$$

Where i is the number of responses, d_i the individual desirability functions and p_i is the relative importance of i^{th} response as compared to the others. Importance (p_i) varies from 1 to 5, from least to most important, respectively. Fig. 3.17 shows a 3D plot of the overall desirability function D for the (X_1, X_2) plane.

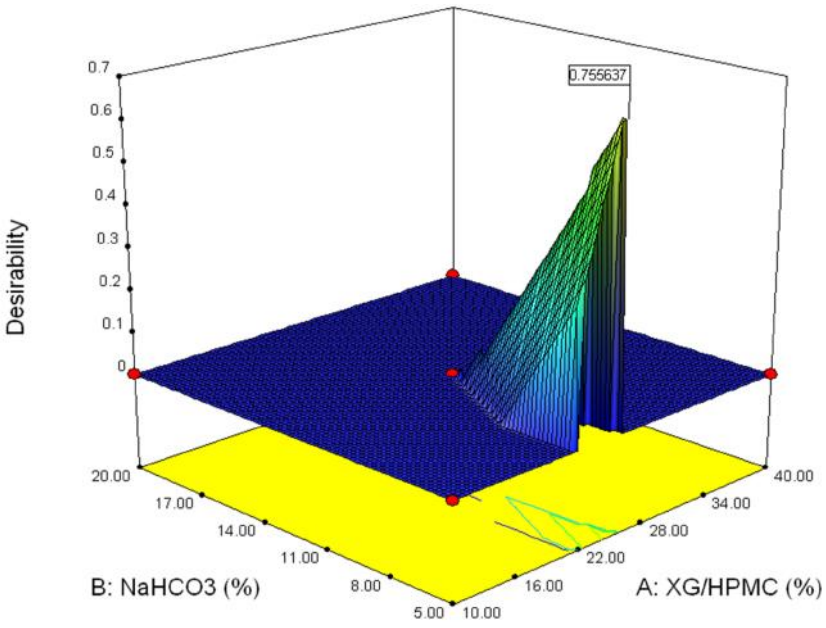

Figure 3.17: 3D plot of the overall desirability function.

3.2.7.2. Graphical optimization

With the aim to definitively pointing out the optimal conditions of the release rate and cumulative release at 1 hr, a graphical optimization was conducted using the Design-Expert 8.0.7.1 software. The methodology essentially consisted of overlaying the curves of the two models obtained from the CCD according to the specific criteria imposed in Table 3.15. Fig. 3.18 shows the overlay plot in which the yellow area represents the area satisfying the imposed criteria. The point identified by the flag was chosen in the graph as

representative of the optimized area corresponding to percentage of sodium bicarbonate to be 5.00% and percentage of the polymer (XG/HPMC) to be 24.79%. Under these conditions the model predicts release rate of 28.49 $hr^{-1/2}$ and cumulative release of 24.003% in the first 1 hr..

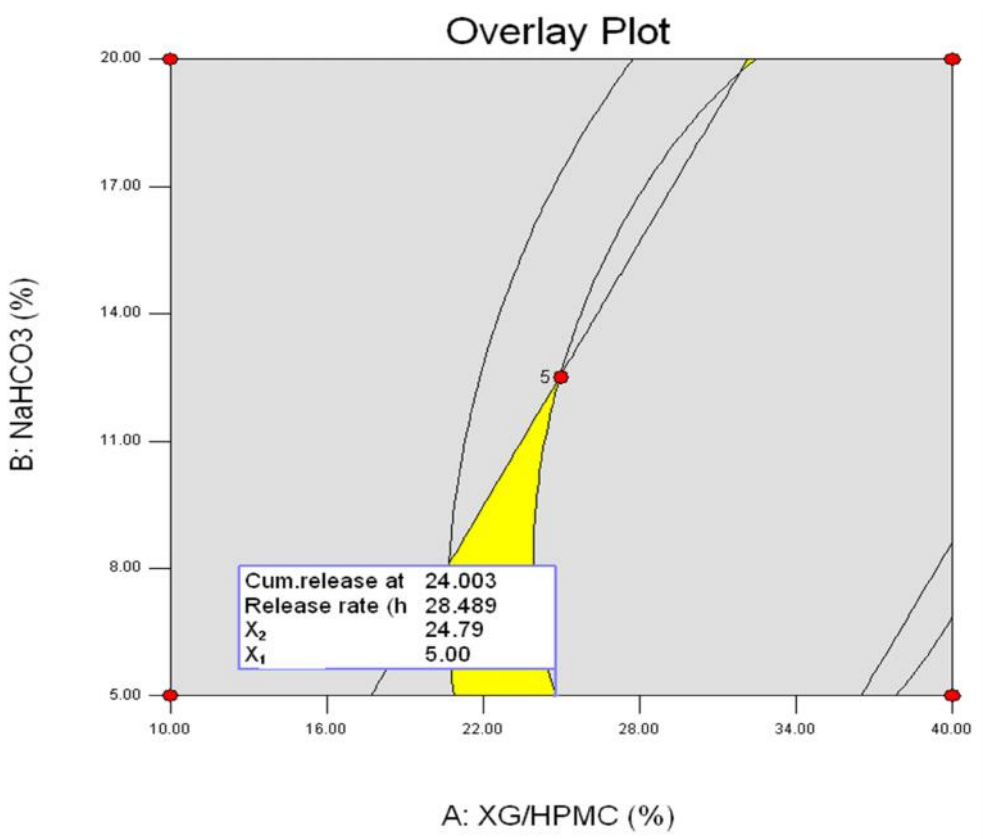

Figure 3.18: Overlaying plot of cumulative release at 1 hr and release rate as functions of percentage of sodium bicarbonate and percentage of XG/HPMC.

3.2.7.3. Confirmation test

To experimentally confirm the validity of obtained optimal point, confirmation experiments were carried out in triplicate at the optimal combinations of the factors (X_1 = 5%, X_2 = 24.79). Table 3.16 provides the predicted values, experimental results and the

percentage error values obtained at optimal levels of the factors. As seen in the table, the values of percentage errors had fallen within about 5% confirm that the experimental values of the optimized formulations agreed well with the predicted values [Mohajeri *et al*., 2010].

Table 3.16: Response values of predicted, experimental and percentage error obtained at optimal levels of the factors.

Response	Predicted value	Experimental value	% Error
Release rate (Y_1, $hr^{-1/2}$)	28.49	29.08	2.03
Cumulative release at 1 hr (Y_2, %)	24.00	25.45	5.69

3.2.8. Evaluation of the optimized floating salbutamol sulphate tablets

The optimized formulation was evaluated for its granule and tablet properties. The results are presented in Table 3.17. As shown in the table, the angle of repose, Housner ratio and Carr's index values were 28.45 ± 1.13^o, 1.09 ± 0.03 and 8.41 ± 0.026%, respectively, indicating that the granules of the optimized formulation have excellent flow property. The tablets showed low friability (0.35%), acceptable drug content value (98.96 ± 0.00) and were intact and floated for more than 12 hr with floating lag time of 2.21 sec.

Table 3.17: Granule and tablet properties of the optimized salbutamol sulphate formulation.

Parameters	Experimental values
Granule properties	
Bulk density (g/cm^3)	0.32 ± 0.01
Tapped density (g/cm^3)	0.35 ± 0.00
Angle of repose (^{o})	28.45 ± 1.13
Carr's Index (%)	8.41 ± 0.03
Hausner ratio	1.09 ± 0.03
Flow rate (g/sec)	2.02 ± 0.12
Tablet properties	
Hardness (N)	65.3 3± 1.53
Thickness (mm)	3.65 ± 0.05
Friability (%)	0.35
Assay (%)	98.96 ± 0.00
Matrix integrity	Intact
Floating lag time (sec)	2.21 ± 0.1
Floating duration (hr)	> 12

The release profile and release kinetics of the optimized formulation were evaluated using three different batches as presented in Fig. 3.19 and Table 3.18. ANOVA of the release profiles based on DE values of the three batches, 65.06 ± 0.8, 65.71 ± 1.14 and 64.73 ± 0.66%, revealed that there was no statistically significant difference ($p = 0.1871$) in the release profiles of the formulations. The release profile curves presented in the figure also support the ANOVA results of DE that the release patterns are similar among the batches, leading to the conclusion that the optimal formulation obtained yields reproducible results. The results also confirmed that formulation of sustained release floating matrix tablet of salbutamol sulphate that releases the drug for 12 hr in a sustained manner was achieved.

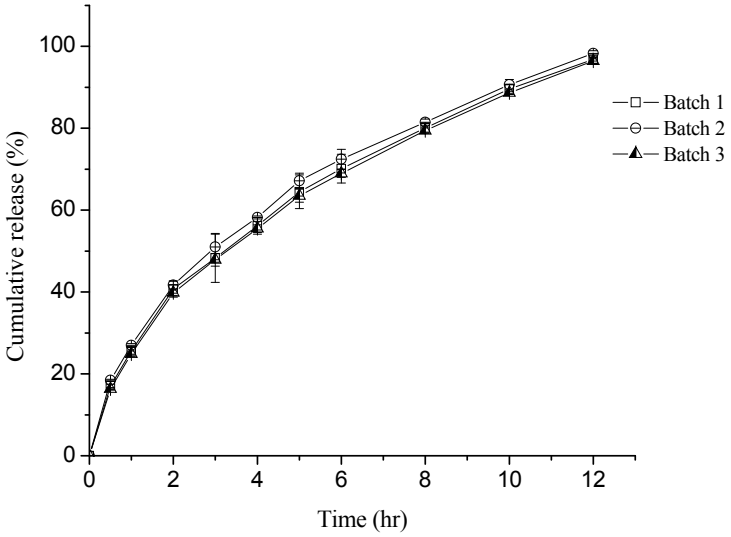

Figure 3.19: Release profile of optimized sustained release floating tablet of salbutamol sulphate.

Table 3.18 shows the release kinetics and release mechanism study results for the optimized formulation. Release kinetics study revealed that Higuchi square root kinetic model was the best fit model with $R^2 \geq 0.996$. The drug release mechanism from the optimized formulation was also evaluated using the Korsmeyer-Peppas model at 60% release and the results showed that n value ranges from 0.562 to 0.588 indicating drug release from the optimized formulation follows non-Fickian diffusion release mechanism.

Table 3.18: Release kinetics and release mechanism study results for the optimized formulation.

Formulation code↓	Zero order		First order		Higuchi matrix		Hixson-Crowell		Koresmeyer-Peppas		
	R^2	K	R^2	K	R^2	K	R^2	K	R^2	n	K
Batch 1	0.946	6.67	0.793	-0.13	0.997	29.08	0.854	-0.16	0.996	0.576	0.258
Batch 2	0.939	6,65	0.790	-0.12	0.996	29.09	0.849	-0.15	0.997	0.562	0.273
Batch 3	0.948	6.67	0.791	-0.13	0.998	29.09	0.854	-0.16	0.996	0.588	0.250

3.2.9. Drug-excipient interaction study

Drug-excipients interaction was studied using Fourier transformed infrared (FT-IR) spectroscopy. Fig. 3.20, 3.21 and 3.22 depict the IR spectra of pure salbutamol sulphate, optimized formulation and their overlap. As shown in the figures the characteristic peaks of salbutamol sulphate were observed: C-O stretching vibrations of primary alcohol at 1112 cm^{-1}, C-O vibrations of phenol at 1205 cm^{-1}, C-H bending vibrations of tertiary carbon at 1338 cm^{-1} and C-H stretching vibrations at 2952 cm^{-1} [Prasanthi *et al*., 2011].

These characteristic peaks also appear in the spectrum of the optimized formulation at the same wave numbers indicating that there was no interaction between the drug and formulation excipients.

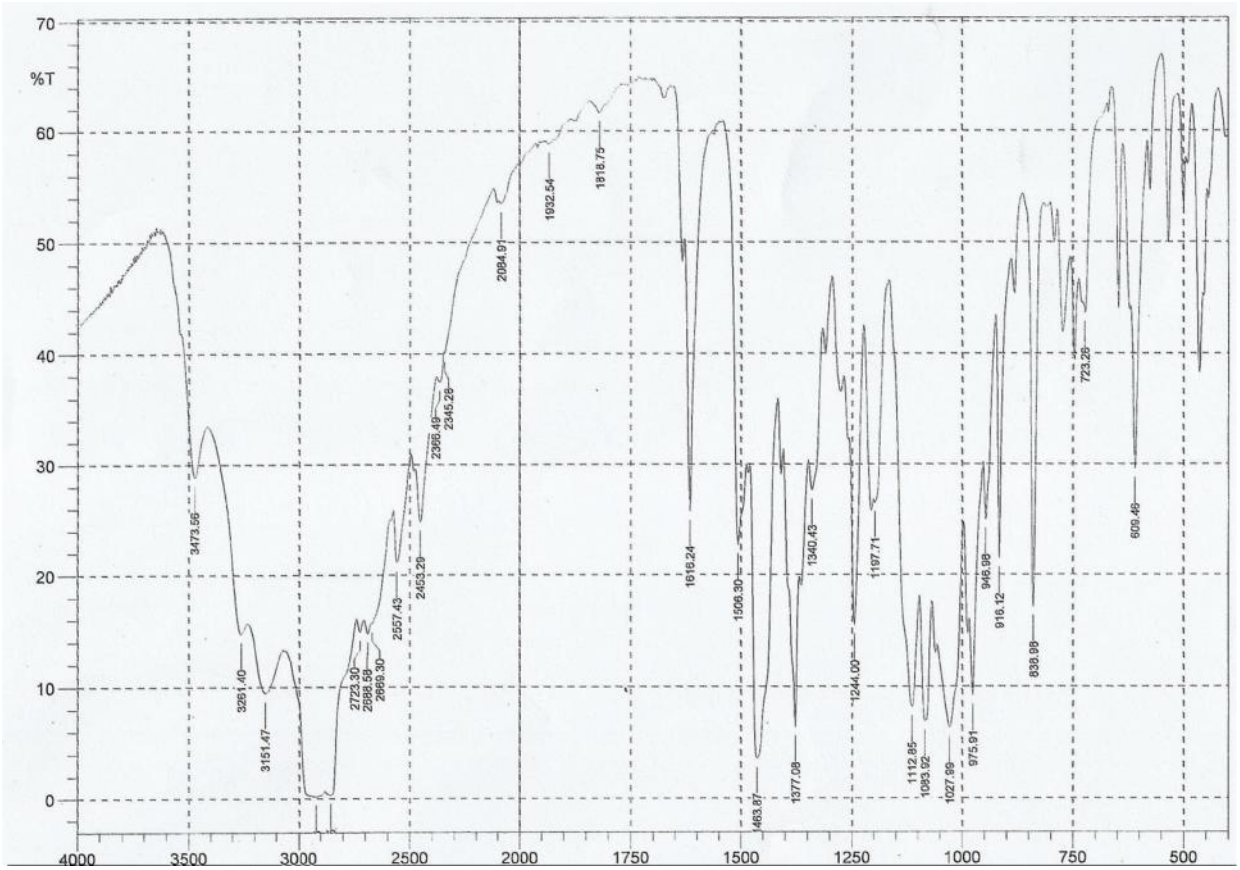

Figure 3.20: FTIR spectrum of pure salbutamol sulphate.

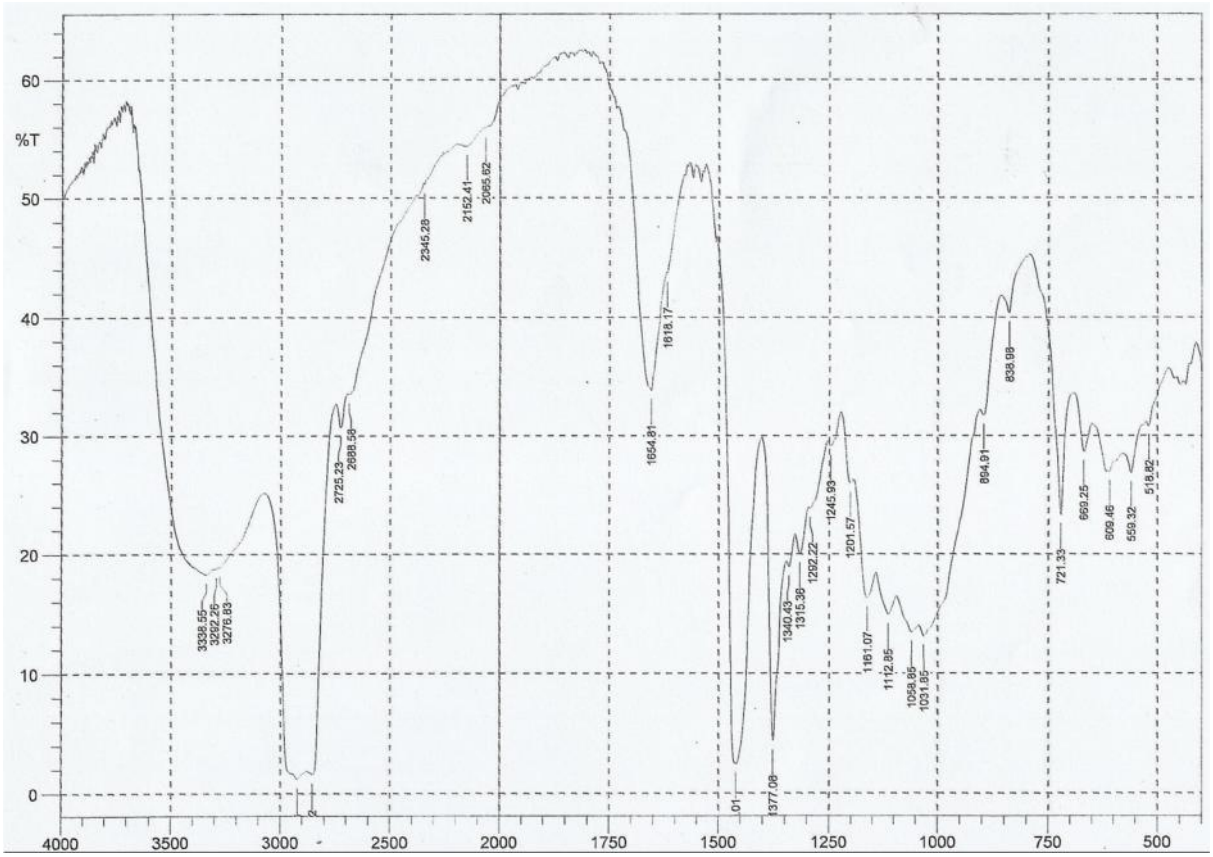

Figure 3.21: FTIR spectrum of optimized floating tablet of salbutamol sulphate.

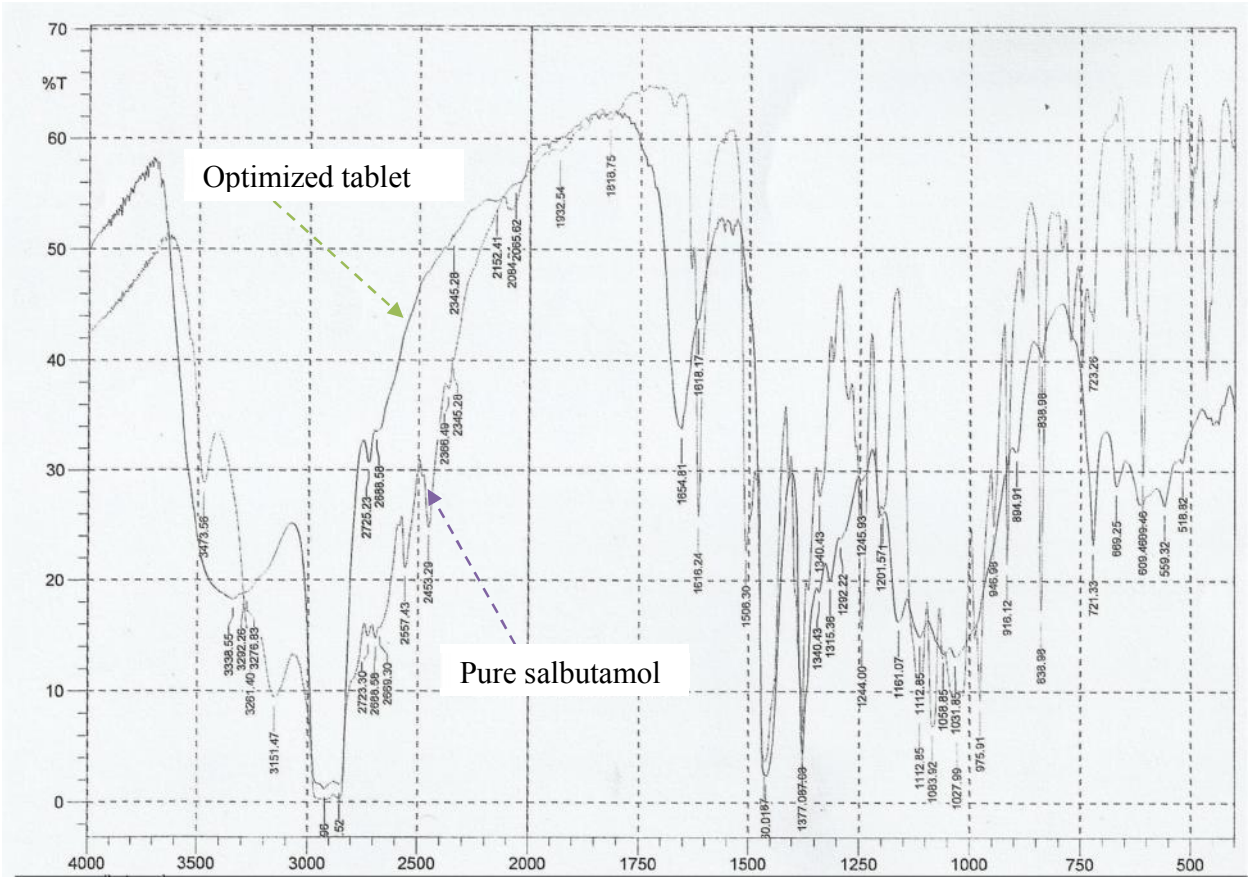

Figure 3.22: Overlap of FTIR spectra of pure salbutamol and the optimized tablet.

4. CONCLUSION

Sustained release floating matrix tablet of salbutamol sulphate was prepared by wet granulation technique using HPMC and xanthan gum, as release retarding agent and $NaHCO_3$ as floating aid.

Preliminary studies revealed that HPMC has low flow property than XG and showed burst release pattern at low concentration. Formulation variables like polymer type, polymer ratio, polymer concentration, and $NaHCO_3$ concentration showed significant difference in release rate, cumulative release at 1 hr, and floating lag time, but mot on floating duration. Formulations at low concentration of HPMC didn't keep their matrix integrity.

In general speaking, among formulations developed in the preliminary study, those formulations with XG and combination of HPMC and XG showed good release pattern at low and high concentration levels. Of these formulations, the one with 1:3 (XG/HPMC) ratio was selected as one factor for optimization due to that it contains relatively high amount of HPMC, which has low hydration power than XG, that can release enough amount of drug in the first 1 hr which can be used as bolus dose for rapid relief of asthma. So, this polymer combination and $NaHCO_3$ at low and high level of concentration were used as independent variables and cumulative release at 1 hr and release rate were used as dependent variables for optimization. Since all formulations floated below 10 seconds, floating lag time was not considered during optimization. Optimization was done using central composite design statistical approach by design expert 8.0.7.1 software. The optimized formulation containing 24.79% of XG/HPMC

(1:3) and 5% of $NaHCO_3$, as predicted from the software, was experimentally evaluated and showed good agreement with the predicted response values and resulted floating tablets of standard quality. In conclusion, this study has come up with an optimum formulation for the preparation of floating tablet of salbutamol sulphate that could remain buoyant in the gastric content and release the drug over a period of 12 hr in a sustained manner. From *in vitro* perspective, this optimized formulation may improve the overall bioactivity of oral salbutamol sulphate and patient compliance.

5. SUGGESTIONS FOR FURTHER WORK

The results of this study suggest further investigations for the optimized formulation on:

- Evaluation of accelerated and long term stability studies, and
- Investigations on *in vivo* performance using animal models and human volunteers.

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