MYRRH RESIN (*Commiphora myrrha*) AS RATE CONTROLLING EXCIPIENT IN SUSTAINED RELEASE MATRIX TABLETS OF THEOPHYLLINE: EVALUATION, FORMULATION AND OPTIMIZATION STUDY

BY

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ADDIS ABABA, ETHIOPIA

DECEMBER, 2014

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A THESIS SUBMITTED TO

SCHOOL OF PHARMACY, DEPARTMENT OF PHARMACEUTICS AND SOCIAL PHARMACY

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PHARMACEUTICS

ADDIS ABABA UNIVERSITY

ADDIS ABABA, ETHIOPIA

DECEMBER, 2014

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Muluken Nigatu, entitled: *Myrrh Resin (Commiphora myrrha) As Rate Controlling Excipient In Sustained Release Matrix Tablets of Theophylline: Evaluation, Formulation and Optimization Study* and submitted in partial fulfillment of the requirements for the degree of Master of Science 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

Myrrh is an oleo-gum resin, obtained from the stem of various species of *Commiphora*, Family Burseraceae. The chief source is *Commiphora myrrha*. Myrrh is phytotoxically safe raw material in industries like pharmaceuticals and food industries. The plant based excipients have been studied for their application in different pharmaceutical dosage forms like matrix sustained release system.

Theophylline is used as bronchodilator in the management of asthma and chronic obstructive pulmonary disease. Its narrow therapeutic index (10-20 µg/ml) requires suitable formulation strategies and sustained-release oral formulations have emerged as the most useful approach.

The aim of this study was to investigate the myrrh resin from *Commiphora myrrha* as a rate controlling excipient for sustained release matrix tablets of theophylline.

The moisture content and total ash values of myrrh resin was found to be 4.67±0.577% and 0.24±0.047%, respectively. The sustained release matrix tablets of theophylline were prepared by wet granulation technique using myrrh resin as rate controlling excipient. The hardness of different formulations were found to be between 89.8±2.86 and 133.7±3.53N while all tablets have passed the friability test (<1%). Analysis of dissolution data of the formulations indicated that the best fitting is with first order kinetics, whereas the mechanism of drug release pattern follows anomalous or non-fickian diffusion.

The myrrh resin amount (A) and compression force (B) were selected as independent variables and drug release at 1 h ($rel_{1\ h}$), 12 h ($rel_{12\ h}$) and time to 50% drug release ($t_{50\%}$) were taken as the response variables. Therefore, the effects of myrrh resin amount (A) and compression force (B) were further studied and optimized for the desired outputs using central composite design statistical approach. Design-Expert 8.0.7.1 software was employed to carry out the experimental design, statistical analysis, numerical and graphical optimization.

By comparing several statistical parameters, linear model was selected for both $rel_{1\ h}$ and $rel_{12\ h}$ whereas quadratic model was found to be the best fit model for $t_{50\%.}$ The adequacy of the models was checked by analysis of variance (ANOVA). The ANOVA results revealed that both models

have significant values indicating the terms in the models have significant effect ($P < 0.05$) on the responses except the interaction effect (AB) of quadratic model which was found to be insignificant. Model simplification was carried out by eliminating this term (AB) in polynomial equations in order to improve the model.

Optimization was achieved by simultaneous optimization of $rel_{1\ h}$, $rel_{12\ h}$ and $t_{50\%}$. An optimum region of 24.981%, 91.217% and 2.861 h was obtained by the software for $rel_{1\ h}$, $rel_{12\ h}$ and $t_{50\%}$, respectively when 17.5% myrrh resin amount (MRA) and 13.9 KN compression force (CF) was used. The kinetic study showed the optimized formulation followed first order kinetics model with anomalous release mechanism.

In validation of the optimized formulation, the experimental values were found to be in close agreement with the predicted values confirming the predictability and validity of the model. This demonstrated that the optimization technique was successful in designing the theophylline sustained release matrix tablet formulation by using myrrh resin as rate controlling excipient.

The Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR) analysis confirmed the absence of incompatibility between theophylline and the myrrh resin. Therefore, the myrrh resin can be used as a potential alternative rate controlling excipient for sustained release formulation.

Keywords: Myrrh resin, theophylline, sustained release, matrix tablet, optimization, central composite design

Acknowledgements

Above all, I magnify the Almighty God with my whole heart for all indescribable things he rendered to me.

I would like to express my deepest appreciation and gratitude to my advisors Dr. Anteneh Belete and Dr. Nisha Mary Joseph for their valuable advice, comments and follow-up throughout the course of this work.

I also thank Ethiopian Pharmaceutical Manufacturing Sh. Co. (EPHARM) for donating theophylline raw material and providing me access to their laboratory facilities.

My special appreciation also goes to the staff members of the Department of Pharmaceutics and Social Pharmacy and Department of Pharmaceutical Chemistry and Pharmacognosy for their generous support during my entire work.

I extend my heartfelt thanks to Fekade Tefera, Bilal Tessema and all my classmates for their support and encouragement during this work.

Last but not least, my acknowledgement goes to Wollo University and Addis Ababa University for sponsoring my study.

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Acronyms

ANOVA	Analysis of Variance
CCD	Central Composite Design
CF	Compression Force
CV	Coefficient of Variation
DE	Dissolution Efficiency
DoE	Design of Experiments
DSC	Differential Scanning Calorimetry
FTIR	Fourier Transform Infrared
LOD	Loss on Drying
LOF	Lack of Fit
MRA	Myrrh Resin Amount
OFAT	One-Factor-At-a-Time
PRESS	Predicted Residual Sum of Square
RH	Relative Humidity
RSM	Response Surface Methodology
SD	Standard Deviation
SR	Sustained Release

1. INTRODUCTION

1.1 Oral sustained release drug delivery

A sustained release (SR) dosage form is defined as "any drug or dosage form modification that prolongs the therapeutic activity of the drug". The primary objectives of sustained drug delivery are to ensure safety and enhancement of efficacy of drug with improved patient compliance. This delivery system is increasingly being used in the treatment of acute and chronic diseases as it provides blood levels that are devoid of the peak and valley effect, which are characteristics of the conventional intermittent dosage regimen, for an extended period of time. Thus, sustained drug delivery results in optimum drug therapy with reduced frequency of dosing and side effects (Deore *et al*., 2010; Maderuelo *et al*., 2011; Pawan *et al*., 2011; Isha *et al*., 2012). SR preparations provide an immediate release of drug that promptly produces the desired therapeutic effect, followed by the gradual release of drug in amounts sufficient to maintain the therapeutic response for a specific extended period of time usually 8-12 hrs (Salger *et al.*, 2010; Pawan *et al*., 2011; Isha *et al*., 2012; Kumar *et al*., 2013). SR dosage forms are designed to complement the pharmaceutical activity of the medicament in order to achieve better selectivity and longer duration of action (Isha *et al*., 2012).

Oral SR systems continue to dominate the market despite the advancements made in other drug delivery systems in order to increase the clinical efficacy and patient compliance (Prabu *et al*., 2009; Ramakrishna *et al*., 2011). For SR systems, the oral route of drug administration has received the most attention as it is natural, uncomplicated, convenient, safer route, and due to its least sterility constraints and flexible design of dosage forms (Kannan *et al*., 2010; Kumar *et al*., 2010; Salger *et al.*, 2010; Khachane *et al.*, 2011; Isha *et al*., 2012; Kumar *et al*., 2012a).

Many strategies are available for the design and development of SR drug delivery formulations **(**Prabakaran and Vishalini, 2010; Pachuau and Mazumder, 2012). Among the different approaches for oral SR dosage forms, matrix tablets still appear as one of the most efficient and interesting formulations from both the economic and the process development point of view (Ceballos *et al*., 2005; Ferrero and Jimenez-Castellanos, 2014). This popularity of matrix

systems can be attributed to their technological simplicity, low cost, ease of fabrication and convenience (Pachuau and Mazumder, 2012).

Matrix tablets usually consist of polymer (natural, synthetic or semi-synthetic), drug and other excipients which are homogenously distributed throughout the system and prepared through either direct compression or wet granulation methods (Chansanroj and Betz, 2010; Salger *et al.*, 2010; Pachuau and Mazumder, 2012; Khan, 2013; Ferrero and Jimenez-Castellanos, 2014). Several polymers including hydrophilic and hydrophobic types have been used in the preparation of matrix based SR drug delivery systems. Drug-release retarding excipients are the key performers in matrix systems. These polymers are instrumental in giving the desired characteristics to the tablets and also influence the mechanism of drug release from the system (Pawan *et al*., 2011; Pachuau and Mazumder, 2012; Prajapati *et al.*, 2013; Grund *et al.*, 2014).

1.1.1 Natural excipients for sustained release

Numerous types of excipients are currently employed to sustain the drug release from the pharmaceutical dosage forms (Jain *et al*., 2008; Prabu *et al*., 2009; Prajapati *et al*., 2013).

Plant based excipients have been studied for their application in different pharmaceutical dosage forms like matrix controlled system, film coating agents, microspheres, nanoparticles, viscous liquid formulations like ophthalmic solutions, suspensions, implants and their applicability and efficacy has been proven (Chamarthy & Pinal, 2008; Alonso-Sande *et al*., 2009; Pawan *et al*., 2011).

The nontoxic behavior, easy availability, inexpensiveness, capability of chemical modifications, biodegradability and biocompatibility of natural excipients is making them more popular amongst formulation scientists for the development of oral SR dosage forms (Prabu *et al*., 2009; Kumar and Singh, 2010a; Shingala *et al*., 2010). Of increasing importance is the fact that plant resources are renewable and if cultivated or harvested in a sustainable manner, they can provide a constant supply of raw material (Shingala *et al*., 2010; Kumar *et al*., 2012b; Prajapati *et al*., 2013).

1.2 Myrrh

Myrrh is an oleo-gum resin, obtained from the stem of various species of *Commiphora*, family Burseraceae. The chief source is *Commiphora myrrha* (*C. myrrha*) (Hawar, 2008; Etman *et al*., 2011). Myrrh is composed of volatile oil (2–10%), ethanol soluble resin (25–40%) and water soluble gum (30–60%) (Hanus *et al*., 2005).

1.2.1 Distribution of gum and resin bearing species in Ethiopia

Ethiopia is one of the countries well endowed with various species of *Acacia*, *Boswellia* and *Commiphora* that are known to produce gum arabic, frankincense and myrrh, respectively. Available estimates prompt that the total area of oleo-gum resin bearing woodlands cover about 2.9 million ha of land in the country with over 300,000 metric tons of natural gum production potential (Tadesse *et al.*, 2007). The trees produce resin in the dry season and provide people with meaningful economic activities (Lemenih *et al*., 2003). Over 60 gum and resin bearing species are found in the country (Tadesse *et al.*, 2007). In terms of regional distribution, gum and resin producing species are found in the Afar, Amhara, Benishangul-Gumuz, Gambela, Oromia, Somali, Southern Nations and Nationalities and Tigray Regional States (Lemenih and Kassa, 2011). The most commonly known sources of gum and resin species in Ethiopia are described in Table 1.1 (Tadesse *et al.*, 2007).

1.2.2 Genus *Commiphora*

The Genus *Commiphora* (Figure 1.1) comprises over 150 species, most of which are confined to Eastern Africa, with few species also occurring in Arabia and India (Hanus *et al*., 2005). The genus is a very conspicuous and a dominant element in the dry bush lands of Northeast Africa, and a large number of species are endemic to this area. The diversity of *Commiphora* species in Ethiopia is one of the highest in the world. Fifty-two species of *Commiphora* are known to exist in Ethiopia, and 14 (25%) of the species are endemic.

The chief *Commiphora* gum of highly economic importance is myrrh, produced by *C. myrrha* (Nees) Engl., *(*synonum *C. molmol)* (Figure 1.2). This is an important commodity of commerce in Southern and South Eastern Ethiopia (Tadesse *et al.*, 2007).

Table 1.1: List of common gum and resin bearing trees and shrubs in Ethiopia by genus and species

No	**Genus *Acacia***	No	**Genus *Boswellia***	No	**Genus *Commiphora***
1	*Acacia senegal*	1	*Boswellia papyrifera*	1	*Commiphora myrrha*
2	*Acacia seyal*	2	*Boswellia microphylla*	2	*Commiphora africana*
3	*Acacia polyacantha*	3	*Boswellia neglecta*	3	*Commiphora habessinica*
4	*Acacia sieberiana*	4	*Boswellia ogadensis*	4	*Commiphora truncata*
5	*Acacia drepanolobium*	5	*Boswellia pirrotae*	5	*Commiphora boranensis*
		6	*Boswellia rivae*	6	*Commiphora guidottii*
				7	*Commiphora schimperi*
				8	*Commiphora erythraea*
				9	*Commiphora corrugata*
				10	*Commiphora cyclophylla*
				11	*Commiphora hildebrandtii*
				12	*Commiphora odia*
				13	*Commiphora kua*
				14	*Commiphora serrulata*
				15	*Commiphora monoica*

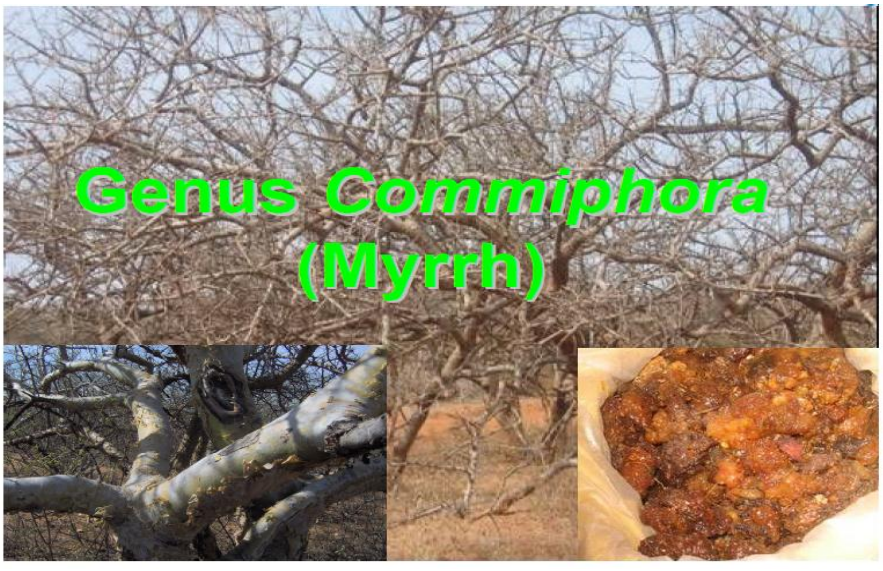


Figure 1.1: *Commiphora* tree (adopted from Tadesse, 2009)

Gum resins of *C. myrrha* (Nees) Engl. are important commercial products (fragrant oil) in Ethiopia, Kenya and Somalia. Other resin-producing *Commiphora* occur in Ethiopia, Sudan, Eritrea, Somalia, Kenya and southern Arabia (Hanus *et al*., 2005).

Figure 1.2: Myrrh from *C. myrrha* (adopted from Lemenih and Kassa, 2011)

The southeastern *Acacia–Commiphora* woodlands have the richest diversity of *Commiphora* species, with 35 (67%) of the *Commiphora* species found in the country recorded there, including 9 (64%) endemic species (Lemenih and Kassa, 2011).

C. myrrha is an indigenous tree or shrub to 4 m; bark silvery or whitish to bluish grey, peeling in small to large papery flakes, sometimes reticulately fissured on old trunks; all parts glabrous. The branches are knotted with branchlets that are pointed and perpendicular to the branches. The trifoliate leaves are small and scanty. Flowers are about 5 mm long and come out just before the rains. Fruits are about 1.2 cm long including the pronounced beak. Available in the regions: Afar, Sidamo, Bale, Hararge and out of Ethiopia in Somalia; North East Kenya and Arabia. Myrrh is also collected from other species of *Commmiphora* such as *C. africana*, *C. erythraea*, etc. but the best quality is from *C. myrrha* (Tucker AO, 1986; Tadesse *et al.*, 2007; Birkett *et al.*, 2008).

1.2.3 Production and Collection

The production of various gums and resins varies according to the type and place of production. Common to all, however, is the fact that production and harvesting are seasonal and performed solely during dry seasons. Ethiopia's current gum and resin production system can broadly be subdivided into 2 activities: production by tapping and collection of naturally oozing gums and resins (Lemenih and Kassa, 2011). Tapping is the artificial wounding of the stems and branches

of trees for the production of gums and resins. It involves shallow blazing (wounding) of the stems and branches by shaving off the bark using a sharp instrument, locally known as a *mingaf* or *sonke* (Figure 1.3). Under best practice, a tree is tapped for no more than 3 consecutive years, and should be rested so it can recover and regain vigour (Lemenih and Kassa, 2011).

Figure 1.3: Two of the most commonly used tapping tools (*mingaf* and *sonke*) (A) and tapping an incense tree (B) (adopted from Lemenih and Kassa, 2011)

1.2.4 Physicochemical properties

1.2.4.1 Chemical properties

Myrrh contains volatile oil, resin, and gum (Parimal *et al*., 2011). The main constituents of the volatile oil are furano-sesquiterpenes with furanodesma-1.3-diene as the main component. It also contains cadinene, elemol, eugenol, cuminaldehyde, furandiene, furanodienone, curzerenone, and lindestrene (Etman *et al*., 2011).

The resin is found to consist of a mixture of α-, β-, and γ- commiphoric acids (resin acids). It also contains two phenolic resins (α- and β-heerabomyrrhol), commiphorinic acid, heeraboresene, commiferin, and burseracin (Parimal *et al*., 2011).

The crude alcohol-insoluble fraction i.e., gum, comprises of protein (18%) and carbohydrate (64%) made up of arabinose, galactose and glucuronic acid with dominating amounts of uronic acid rich polymers (Wiendl *et al.*, 1995; Parimal *et al*., 2011).

1.2.4.2 Physical properties

Myrrh is a reddish-brown resinous material, the dried sap of a number of trees, but primarily from *C. myrrha* (Kumar and Singh, 2010a). The odour of myrrh is described as warm-balsamic, sweet, somewhat spicy aromatic, sharp and pungent when fresh (Lemenih and Kassa, 2011). They are transparent or translucent solids. They are insoluble in water, but soluble in alcohol and non-polar organic solvents like benzene or ether (Parimal *et al*., 2011).

1.2.5 Toxicological properties

Myrrh is phytotoxically safe raw material in industries like pharmaceuticals and food industries (Tadesse *et al.*, 2007). In mice, no visible signs of toxicity and no mortality were observed at oral doses of resin ≤ 3 g/kg bodyweight. Myrrh resin was also administered to mice via the drinking water at a dose of 100 mg/kg bodyweight/day during 3 months. No toxic symptoms were observed (Rao *et al*., 2001).

1.3 Optimization methods

With respect to drug formulations or pharmaceutical processes, optimization is a phenomenon of finding the best possible composition or operating conditions to result the best possible effect (Singh *et al.*, 2004; Sharma and Pancholi, 2011). It is obvious that if experiments are performed randomly the result obtained will also be random. Therefore, it is a necessity to plan the experiments in such a way that interesting information will be obtained (Lundstedt *et al.*, 1998).

The classical approach of changing one variable at a time and studying the effect of the variable on the response is a complicated technique especially in a multi-factor system (Ozdemir *et al.*, 2011). As the number of factors increases, this approach becomes highly inefficient to identify an acceptable solution. Therefore, it has been merely superseded by statistical experimental design methodology known as Design of Experiments (DoE) (Barmpalexis *et al*., 2010). DoE is a general approach that can be used effectively for optimizing such systems. The primary goal of DoE is to determine the maximum information regarding the response variables affected by input parameters by using as few observations as possible (Lundstedt *et al.*, 1998; Djoudi *et al*., 2007; Ozdemir *et al.*, 2011).

A designed experiment is a more effective way to determine the impact of two or more factors on a response than a one-factor-at-a-time (OFAT) experiment, where only one factor is changed at one time while the other factors are kept fixed because of the following reasons (Gohel and Amin, 1998; Czitrom, 1999; Ravi *et al*., 2010; Nasirizadeha *et al*., 2012):

A. It requires less resource (experiments, time, material, etc.) for the amount of information obtained.
B. The estimates of the effects of each factor are more precise. Using more observations to estimate an effect results in higher precision (reduced variability).
C. The interaction between factors can be estimated systematically. Interactions are not estimable from OFAT experiments.

Statistical experimental design methodologies are powerful, efficient and systematic tools in the design of pharmaceutical dosage forms, allowing a rationalistic study of the influence of formulation and/or processing parameters on the selected responses with short experiment time and improvement in the research and development **(**Pranav *et al*., 2013).

Among diverse DoE techniques, Response Surface Methodology (RSM) is the most frequently used optimization technique (Mandal *et al*., 2007; Ibric *et al*., 2012; Khan, 2012; Roosta *et al*., 2014) which allows using polynomial equation(s) to link monitored responses to inputs:

$$Y = f(X_1, X_2, X_3, \ldots., X_n) \pm e$$

where Y is response, f is the response function, X_i are inputs and e is the experimental error. This equation presents a mathematical description of a relationship between inputs and output (Garg *et al*., 2008; Sahu *et al*., 2009; Ibric *et al*., 2012).

RSM is a collection of mathematical and statistical techniques useful for developing, improving and optimizing processes and used to evaluate the relative significance of several affecting factors even in the presence of complex interactions (Chopra *et al*., 2007; Celebi *et al*., 2008; Sahu *et al*., 2009; Kumar and Singh, 2010b; Mujtaba *et al*., 2014). RSM is an experimental design technique by which the factors involved and their relative importance can be assessed. In addition, it is a good way to graphically illustrate the relation between different experimental variables and the responses (Lundstedt *et al.*, 1998; Meka *et al*., 2012).

RSM usually contain three steps: (1) design and conduct experiments; (2) response surface modeling through regression; (3) optimization. The main objective of RSM is to determine the optimum operational conditions for the system or to determine a region that satisfies the operating specifications **(**Celebi *et al*., 2008; Garg *et al*., 2008; Sahu *et al*., 2009). The basic idea is to change all relevant factors simultaneously over a set of planned experiments and then connect and interpret the results using mathematical models (Gabrielsson *et al*., 2002).

DoE optimization on oral controlled release matrix delivery devices started in the early 1980s. The common independent variables for all of these have been the quantities of the polymers or other ingredients, while the optimized responses invariably have been the parameters characterizing *in vitro* dissolution profile (Singh *et al*., 2005).

In the development of a SR tablet dosage form, an important issue is to design an optimized formulation with an appropriate dissolution rate in a short time period and minimum number of trials (Hamed and Sakr, 2001; Huang *et al*., 2005; Mandal *et al.*, 2007)**.** RSM is a popular and widely used technique in optimization which requires minimum experimentation and time, thus proving to be far more effective and cost-effective than the conventional methods of formulating SR dosage forms (Mandal *et al.*, 2007; Sahoo *et al*., 2011).

Central composite design (CCD), 3-level factorial design, Box-Behnken design (BBD) and D-optimal designs (D-OD) are the different types of RSM designs available for statistical optimization of the formulations (Box and Behnken, 1960; Karnachi and Khan, 1995; Sanchez-Lafuente *et al*., 2002; Iqbal *et al*., 2013).

1.3.1 Central composite design

The most common and successful experimental design used in RSM is the CCD which has equal predictability in all directions from the center (Celebi *et al*., 2008; Savic *et al*., 2014) and allowing investigation with the least number of experiments and selection of the optimal composition for achieving the presetting target (Hao *et al*., 2012).

CCD contains an imbedded (2^k) factorial design (FD) augmented with a group of star points (2k) and a "central" point. The star (axial) points allow estimation of curvature and establish new extremes for the low and high settings for all the factors (Techapun *et al*., 2002; Singh *et al.*,

2004). Hence, CCDs are second-order designs that effectively combine the advantageous features of both factorial design and the star design (Hamed and Sakr, 2001; Singh *et al.*, 2004). The total number of factor combinations in a CCD is given by $2^k + 2k + c_p$, where k is the number of independent variables, and c_p is the number of repetitions of the experiments at the center point **(**Hamed and Sakr, 2001; Celebi *et al*., 2008**).** The replicates at the center provide an independent estimate of the experimental error. The number of center points (replicates) depends on the number of variables considered in the design and is generally between 3 and 10 (Singh *et al*., 2011**).** All factors are studied in five levels: α, -α (axial points), +1, -1 (factorial points) and 0 (central point) and α-values depend on the number of variables and can be calculated by $\alpha=2^{k/4}$. Hence for two variables, α=1.41421 (Techapun *et al.,* 2002; Bezerraa *et al*., 2008; Singh *et al*., 2011; Savic *et al*., 2014).

The CCD permits the response to be modeled by a second order polynomial fit and allows estimating the constant, the linear terms, the interactions between variables and the quadratic terms which can be expressed as the following equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2$$

where X_1 and X_2 are the independent variables, β_0 is the intercept, β_1–β_{22} are the regression coefficients, and *Y* is the response function (area) (Leardi, 2009; Kamarei *et al*., 2010).

1.4 The present study

SR oral delivery systems are designed to achieve therapeutically effective concentrations of drug in the systemic circulation over an extended period of time. Over the past two decades, SR release dosage forms have made significant progress in terms of clinical efficacy and patient compliance (Kulkarni *et al*., 2011; Girish *et al*., 2012). Among the various approaches, matrix tablets composed of drug and excipient as release retarding material offer the simplest approach in designing a SR system (Kannan *et al.*, 2010; Chowdary and Reddy, 2012).

Theophylline was used as a model drug in this study. Theophylline is a methylxanthine alkaloid which is used as bronchodilator in the management of asthma and chronic obstructive pulmonary disease (Nokhodchi and Tailor, 2004; Emami *et al*., 2012). It has narrow therapeutic range (10-20 µg/ml), while toxicity generally appears at concentrations above 20 µg/ml. In addition, it

has a good thermal stability (melting between 270 and 274), and almost constant solubility (1g/120 ml) in a wide range of pH (pH 2 and 7.5), which are suitable properties for release studies (Ceballos *et al*., 2005; Roy et al., 2007; Yoon *et al*., 2009; Chaudhary *et al*., 2012).

Conventional dosage forms of theophylline should be administered 3 to 4 times a day to provide effective concentration and to avoid large fluctuations in blood concentration. This leads to poor patient compliance and enhanced risk of gastrointestinal and cardiovascular adverse effects. Therefore, its narrow therapeutic index requires suitable formulation strategies aimed at achieving and maintaining average serum levels of the drug within the therapeutic range, without significant fluctuations. SR oral formulations have emerged as the most useful preparations toward this aim (Nokhodchi and Tailor, 2004; Ceballos *et al*., 2005; Emami *et al*., 2012).

Though a wide range of excipients are reported for preparing matrix tablets, there is a continuous need to develop new, safe and effective excipients for SR matrix tablets (Nayak *et al*., 2011; Chowdary and Reddy, 2012).

Synthetic excipients have certain disadvantages such as high cost, environmental pollution during synthesis, and nonrenewable sources (Krishna *et al*., 2011; Deogade *et al*., 2012).

Today, the whole world is increasingly interested in natural medicines and excipients. In recent years, natural excipients have evoked tremendous interest due to their diverse pharmaceutical applications. These natural excipients are nontoxic, biocompatible, biodegradable, cheap, environmental friendly, and easily available and are preferred to semi synthetic and synthetic excipients (Kumar and Singh, 2010a; Deogade *et al*., 2012; Kumar *et al*., 2012b; Prajapati *et al*., 2013).

One such natural plant source is *Commiphora*. Ethiopia is well endowed with various species of *Commiphora*. As the country spends foreign currency for purchasing excipients, it is important to evaluate local alternative excipients for use in the pharmaceutical industry.

Thus, the present work attempts to evaluate the local myrrh resin extracted from *C. myrrha* as rate controlling excipient and to optimize the matrix tablets using theophylline as a model drug.

1.5 Objectives

1.5.1 General objective

- To investigate myrrh resin (*Commiphora myrrha*) as a rate controlling excipient in sustained release matrix tablets of theophylline.

1.5.2 Specific objectives

- To extract the resin fraction of myrrh;
- To characterize some physicochemical properties of myrrh resin;
- To prepare theophylline matrix tablets using myrrh resin;
- To characterize the matrix tablets;
- To study *in vitro* drug release from the matrix tablets;
- To optimize formulation and process variables of tablet preparation using central composite design; and
- To validate the optimized formulation.

2. EXPERIMENTAL

2.1 Materials

Oleo-gum resin of local myrrh (*C. myrrha*) was purchased from Natural Gum Processing and Marketing Enterprise of Ethiopia (NGPME). Anhydrous theophylline (Shandong Xinhua Pharmaceutical Co. Ltd, China) was kindly donated by Ethiopian Pharmaceutical Share Company (EPHARM). Lactose, Magnesium stearate (BDH Laboratory supplies, England), Ethyl alcohol (NALF, Ethiopia), Potassium phosphate monobasic (FARMITALIA CAROERBA, Italy), Hydrochloric acid, Sodium chloride, and Sodium hydroxide (BDH limited, Poole, England) were used as received.

2.2 Methods

2.2.1 Preparation of myrrh powder

The myrrh oleo-gum resin obtained was dried in oven (Kottermann® 2711, Germany) at 60 °C for 4 hours, powdered in a grinder and passed through sieve with a mesh size of 224 µm (Chowdary *et al.,* 2006).

2.2.2 Extraction of resin fraction of myrrh

To extract the resin from oleo-gum-resin, myrrh powder was stirred with ethanol (90%) for 2 h. The ethanol slurry was filtered through Whatman No.1 filter paper. The filtrate was concentrated to a thick paste by evaporation of ethanol at 80 °C and hydrodistilled using distillation apparatus to isolate essential oil. Then the paste was dried in oven (Kottermann® 2711, Germany) at 40 °C for a week and the yield was calculated. Finally the dry mass was powdered and passed through a mesh having pore size of 224 µm (Chowdary *et al.*, 2006; Milani *et al.*, 2007; Sharma *et al*., 2010).

2.2.3 Physicochemical characterization of myrrh resin

2.2.3.1 Swelling ratio

The swelling ratio study was carried out in distilled water, 0.1 N HCl, and phosphate buffer (pH 6.8). The study was carried out in 10 ml stoppered graduated cylinder. The initial bulk volume of 1 g dried resin was noted and then the respective medium was added in sufficient quantity to yield 10 ml uniform dispersion. The sediment volume of the swollen mass was noted after 24 h of storage at room temperature. The edge of the swollen sediments clearly differentiated from the water phase. The swelling ratio was calculated by taking the ratio of the swollen volume to the initial bulk volume (Jani and Shah, 2008). Each experiment was done in triplicate.

2.2.3.2 Loss on drying (LOD)

One gram of the sample was transferred into Petri dishes and then dried in an oven (Kottermann® 2711, Germany) at 105 °C until a constant weight was obtained. The moisture content was then determined as the ratio of weight of moisture loss to weight of sample expressed as a percentage (Emeje *et al.,* 2009). The experiment was done in triplicate.

$$\text{LOD} = \frac{weig\,ht\ of\ water\ \ in\ sample}{weig\,ht\ of\ \ sample} X\ 100 \qquad (2.1)$$

2.2.3.3 Total ash determination

Total ash value of the myrrh resin powder was determined based on the method described in the British Pharmacopoeia (BP, 2009). Two grams of powder was weighed in a pre-weighed ashing dish followed by heating in a furnace (CARBOLITE, OAF 11/1, England) at 450 ^{o}C for 8 h. The sample was then removed and kept in a desiccator and weighed. Total ash values in the samples of interest were calculated using Equation 2.2. Results are expressed as mean of three parallel determinations.

$$\%\ \text{Total ash} = \frac{m_2 - m_1}{m} X\ 100 \qquad (2.2)$$

where, m_1 is mass of the ashing dish, m_2 is mass of crucible with ash and m is mass of sample.

2.2.3.4 Moisture sorption study

Moisture sorption pattern was studied using the method described by Odeku and Picker-Freyer, (2007). Pyrex desiccators containing distilled water, saturated solution of sodium chloride and appropriate concentration of sodium hydroxide solution were prepared to obtain different relative humidity (RH) chambers (20, 40, 60, 75.5, and 100%) and stored at room temperature. Myrrh resin powder samples were predried in an oven for 4 h at 120 ^{o}C. Two grams of myrrh resin powder was spread evenly on each Petri dish (dried and weighed) and transferred to a particular RH chamber. Samples were equilibrated for four weeks at room temperature. The weights were recorded and moisture uptake of each sample was calculated as the weight difference of the myrrh resin before and after equilibrium in a given RH chamber. Water sorption capacity of the myrrh resin was expressed as percent moisture uptake. The experiment was done in triplicate.

2.2.4 Scanning electron microscopy (SEM)

Morphology of the myrrh resin was observed under scanning electron microscope (JEOL 5400, Japan). Scanning electron photomicrograph of the gold-coated sample was taken at appropriate magnification applying voltage of 10KV.

2.2.5. Drug-excipient compatibility studies

2.2.5.1 Differential scanning calorimetry (DSC)

DSC studies were performed on theophylline alone and on physical mixture (1:1) of theophylline and myrrh resin in order to study the interaction between pure drug and myrrh resin using NETZSCH DSC 200F3 240-20-427-L. Accurately weighed samples (4-5 mg) were hermetically sealed in flat bottom aluminum pans. The scanning was carried out at a temperature ranging from 33 ^{0}C to 450 ^{0}C at a heating rate of 15 K/min under nitrogen gas flow.

2.2.5.2 Fourier transform infrared (FTIR) spectroscopy

Drug-excipient interaction was studied by FTIR spectroscopy. FTIR spectra for pure theophylline and the physical mixture (1:1) of drug and myrrh resin were acquired at room temperature using FTIR spectrophotometer (FTIR-8400S, SHIMADZU, Japan) in transmittance

mode. The samples were first ground in a mortar to reduce the average particle size. Then 5 mg of finely ground powder of each sample was 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) disk and the second window was placed on top of the first salt plate to form a thin film of the mull by compression between two plates. The sandwiched plates were placed in the spectrometer and the spectra were obtained by scanning the sample in the range of 4000 – 400 cm^{-1}.

2.2.6 Preparation and characterization of granules

2.2.6.1 Preparation of granules

Granules were prepared by conventional wet granulation method as per the formula given in Table 2.1. The required quantities of all ingredients except magnesium stearate were mixed thoroughly in a mortar and pestle by following geometric dilution technique. The granulating fluid (solvent blend of alcohol and purified water at 3:7 ratio) was added and mixed thoroughly to form a dough mass. The wet mass formed was passed through a 1.6 mm sieve to form granules and the resulting wet granules were dried in an oven (Kottermann® 2711, Germany) at 60 °C for 4 h. The dried granules were passed through 1 mm sieve to break the aggregates (Kebebe *et al*., 2010).

Table 2.1: Composition of granules for preparing theophylline matrix tablet

Ingredient	Amount(mg)
Theophylline	100
Myrrh resin	25.02-58.98*
Magnesium stearate	3
Lactose	q.s. to 300

q.s. indicates quantity sufficient

* Myrrh resin amount used in the formulations ranged between 25.02 mg (8.34%) and 58.98 mg (19.66%) as per CCD

2.2.6.2 Characterization of granules

I. Flow rate and angle of repose

Flow rate and angle of repose of the samples were determined with the funnel method. Thirty grams of granules was placed in a funnel and allowed to flow through it from a fixed height of 10 cm. Time in second for the duration of flow was recorded. Flow rate was determined and the angle of repose was calculated as per the following equations:

$$Flow\ rate = {}^{m}/_{t} \tag{2.3}$$

where m is mass in gram and t is time in sec.

$$Angle\ of\ repose\ (\theta) = \tan^{-1}\left(\frac{h}{r}\right) \tag{2.4}$$

where θ is angle of repose, h is height of granule, and r is radius of circle formed by the granule. Results were expressed as a mean of triplicate determinations.

II. Determination of density and density related properties

A. Bulk density

From each sample, 30 g of granules were carefully poured into a 250 ml graduated glass measuring cylinder. The volumes of the sample granules were then noted. The bulk densities were determined using Equation 2.5. Bulk densities recorded are average of three determinations.

$$\text{Bulk density}\left(\rho_B\right) = \frac{m}{V_B} \tag{2.5}$$

where m is mass of granule (in g) and V_B is volume of sample granule.

B. Tapped density

From each sample, 30 g of granules was carefully poured into a 250 ml graduated glass measuring cylinder and tapped 250 times using tapped densitometer (ERWEKA, SVM 20, Germany). The volumes of the sample granules were noted. Tapped density was calculated from

the weight and tapped volume of the granule by using Equation 2.6. Tapped densities recorded are averages of three determinations.

$$\text{Tapped density}\left(\rho_T\right) = \frac{m}{V_T} \tag{2.6}$$

where *m* is mass of granule and V_T is volume of the sample granule after tapping.

C. Density related properties

The Carr's index (compressibility) and the Hausner ratio were calculated from the bulk and tapped densities of the sample granules using Equations 2.7 and 2.8, respectively. Carr's index and Hausner ratio calculated are averages of three determinations.

$$Carr's\ index\ (\%) = \left(\frac{\rho_T - \rho_B}{\rho_T}\right) X100 \tag{2.7}$$

$$Hausner\ Ratio = \left(\frac{\rho_T}{\rho_B}\right) \tag{2.8}$$

where ρ_B is bulk density and ρ_T is tapped density of sample granules.

2.2.7 Preparation of matrix tablets

Theophylline tablets, 300 mg tablet weight, were compressed from granules prepared in Section 2.2.6.1. A 1% w/w magnesium stearate, used as a lubricant, was passed through a sieve with a pore size of 224 µm and blended with the dry granules for 5 min in a Turbula mixer (Willy A. Bachofen AG, Turbula 2TF, Basel, Switzerland). The granules were compressed into tablets at the desired compression force by an instrumented Korsch single punch tablet machine (KORSCH XP1-K0010288, Berlin, Germany) which was fitted with 10 mm diameter flat-faced punches. The tablets were kept for 24 h at room temperature in glass containers before their properties were evaluated.

2.2.8 Evaluation of matrix tablet properties

2.2.8.1 Thickness

Ten tablets were taken and thickness was measured using sliding caliper scale (Nippon Sokutei, Japan). Results were expressed as a mean and standard deviation.

2.2.8.2 Crushing strength

Ten tablets were taken from each batch and the crushing strengths of the tablets were determined using hardness tester (Schleuniger, 2E/205, Switzerland). Each tablet was placed between two anvils and force was applied to the anvils, and the crushing strength that just caused the tablet to break was recorded. Results were expressed as a mean and standard deviation.

2.2.8.3 Friability

The friability of the tablets was determined by placing 20 pre-weighed tablets in a friability tester (ERWEKA, TAR 20, Germany) and rotating them for 4 min at 25 rpm. Tablets were subjected to the combined effects of abrasion and shock. The loss of tablet weight was calculated as a percentage of the initial weight after dusting the tablet. Results were expressed as a mean and standard deviation.

2.2.9 Construction of calibration curve

Different concentrations of anhydrous theophylline solution were prepared to obtain 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml, 12 µg/ml, and 14 µg/ml in 0.1 N HCl and phosphate buffer of pH 6.8. Then, the absorbance of each of these concentrations was measured at λ_{max} of 271 nm by using UV-Visible Spectrophotometer (SOLAR Spectrofluorimeter, CM2203, Belarus). The absorbance readings were plotted against concentration.

2.2.10 *In vitro* drug release studies

The *in vitro* drug release studies were performed using USP type II dissolution apparatus (ERWEKA, DT600, Germany) at 50 rpm. The dissolution was done in 0.1N HCl for the first 2 h and then changed to phosphate buffer pH 6.8 (900 ml) for the next 10 h. The temperature was maintained at 37±0.5 ℃. Aliquot samples of 10 ml were withdrawn at pre scheduled intervals (0.25, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 h) and replaced with an equal volume of fresh dissolution medium which was kept at 37±0.5 ℃ to maintain sink condition. Each sample was diluted suitably and analyzed for the drug content at λ_{max} of 271 nm using a UV/Visible Spectrophotometer (SOLAR Spectrofluorimeter, CM2203, Belarus).

2.2.11 Comparison of release profiles

The dissolution efficiency (DE) of a pharmaceutical dosage form is defined as the area under the dissolution curve up to a certain time t, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. DE after 12 h ($DE_{12}^{\%}$) of release test was used to compare the results of dissolution tests of different formulations by the following equation (Costa and Lobo, 2001; Moin and Shivakumar, 2010a). One way ANOVA was applied to determine whether the existing differences were significant or not.

$$DE_{12}^{\%} = \frac{\int_0^t y dt}{y_{100}\, t} \text{x } 100 \qquad (2.9)$$

where *y* is the drug percent dissolved at time *t* and y_{100} denotes 100% dissolution

2.2.12 Analysis of kinetics and mechanism of drug release

The dissolution data were fitted into the following drug release kinetic models to evaluate the rate and mechanism of theophylline release from prepared matrix tablets:

I. Zero-order equation

$$Q = Q_0 - K_0 t \qquad (2.10)$$

where Q is the amount of drug remaining at time t, Q_o is the quantity of drug present initially in the dosage form and K_0 is the zero order release constant.

II. First-order equation

$$\ln Q = \ln Q_0 - K_1 t \qquad (2.11)$$

where Q is the amount of drug remaining at time t, Q_o is the quantity of drug present initially in the dosage form and K_1 is the first order release constant.

III. Higuchi's equation

$$M_t / M_0 = K_H\, t^{1/2} \qquad (2.12)$$

where M_t is amount of drug released at time t, M_o is amount of total drug in the tablet and K_H is the Higuchi constant.

IV. Hixson-Crowell cube root law equation

$$Q_t^{1/3} = Q_0^{1/3} - K_{HC}t \quad (2.13)$$

where Q_t is amount of drug released at time t, Q_0 is the initial amount of the drug in the tablet K_{HC} is the rate constant for Hixson-Crowell law equation.

V. Korsmeyer–Peppas model

$$M_t / M_0 = Kt^n \quad (2.14)$$

where M_t is the amount of drug released at time t, M_o is the amount of total drug in tablets, M_t/M_o is the fractional drug release at time t, K is a constant incorporating the structural and geometric characteristics of the matrix tablets and n is a diffusional exponent, indicative of the drug release mechanism.

2.2.13 Optimization of sustained release formulation by RSM

2.2.13.1 Experimental design

A CCD with five coded values (Table 2.2) was employed in optimizing an oral SR dosage form of theophylline which is the most efficient design in estimating the influence of individual variables (main effects) and their interactions, using minimum experimentation (Ravindran *et al*., 2012). The myrrh resin amount (A) and compression force (B) were selected as independent variables. The levels of the two factors were selected on the basis of the preliminary studies carried out before implementing the experimental design. Percent of drug released at 1 h ($rel_{1\ h,}Y_1$), percent of drug released at 12 h ($rel_{12\ h,}Y_2$), and time to 50% drug release ($t_{50\%}$,Y_3) were taken as the response variables. The amount of drug and magnesium stearate were kept constant while lactose was taken in sufficient quantity to maintain a constant tablet weight of 300 mg as shown in Table 2.1. All other formulation and processing variables were kept invariant throughout the study.

According to this design, the total number of treatment combinations was $2^k + 2k + n_o$, where k is the number of independent variables and n_o is the number of repetitions of experiments at the centre point which was studied in quintuplicate (Celebi *et al*., 2008; Dhiman and Singh, 2012).

For k=2, $2^2 + (2\times2) + 5 = 13$. A total of 13 experiments were carried out to find the optimum area, at which the desired responses are achieved.

Table 2.2: Experimental levels of the independent variables for optimizing theophylline sustained release formulation using myrrh resin.

Variables	**Levels**				
	-α	**-1**	**0**	**+1**	**+α**
A, Myrrh resin amount (%)	8.34315	10	14	18	19.6569
B, Compression force (KN)	7.92893	10	15	20	22.0711

α=1.41421

2.2.13.2 Validation of the experimental design

To validate the chosen experimental design, the resultant experimental values of the responses were quantitatively compared with those of predicted values and the relative error (%) was calculated by the following equation (Yas *et al*., 2013; Acharya *et al*., 2014):

$$\%\ Relative\ error = \frac{(Predicted\ \ value - Experimental\ \ value)}{Predicted\ \ value} x\ 100 \qquad (2.15)$$

2.2.14 Statistical analysis

Origin 7 Software (OriginLab Corporation, MA, and USA) was used to statistically analyze the results and 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). At 95% confidence interval, p-values of < 0.05 were considered statistically significant. All the data measured and reported are averages of a minimum of triplicate measurements and the values are expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1 Physicochemical properties of myrrh resin powder

As indicated from Table 3.1, the color of myrrh resin was reddish brown. The percentage yield of resin from *C. myrrha* was found to be 31.17% (w/w) which is in good agreement with the values reported in the literature (Wiendl *et al*., 1995; Masoud *et al*, 2000; Hanus *et al*., 2005; Su *et al.*, 2012).

Table 3.1: Physicochemical properties of myrrh resin (n = 3, mean ± SD)

S.No	Parameter	Result
1	Color	reddish-brown
2	Yield value (%)	31.17±0.404
3	Loss on drying (%)	4.67±0.577
4	Total ash (%)	0.24±0.047
5	Swelling ratio	*

* Insignificant swelling was exhibited by the resin

It was also characterized for its moisture content and total ash to indicate to certain extent its stability and purity. The moisture content was calculated as percentage loss on drying and it was found to be 4.67±0.577%. The moisture content of myrrh resin was low suggesting its suitability in formulations containing moisture sensitive drugs. It is important to investigate for the moisture content of a material because the economic importance of an excipient for industrial application lies not only on the cost and ready availability of the biomaterial but also on the optimization of production processes such as drying, packaging and storage (Martins *et al*., 2009).

The total ash content is designed to measure the total amount of residual material remaining after ignition which may include extraneous matters such as sand and soil. Thus, the ash values reflect the level of adulteration or handling of the excipient **(**Nayak *et al*., 2011; Pachuau and Mazumder, 2012). The total ash value for the myrrh resin was found to be 0.24±0.047%. The swelling characteristics of the resin was studied in different media; 0.1N HCl, phosphate buffer (pH = 6.8) and distilled water. The results showed that no swelling of the resin in these media.

Figure 3.1 shows the moisture sorption profile of myrrh resin equilibrated at various humidity levels. Moisture sorption increased generally with RH. Percent moisture sorbed ranged from 0.353±0.132% at low (20%) RH to 6.037±0.112% at high (100%) RH.

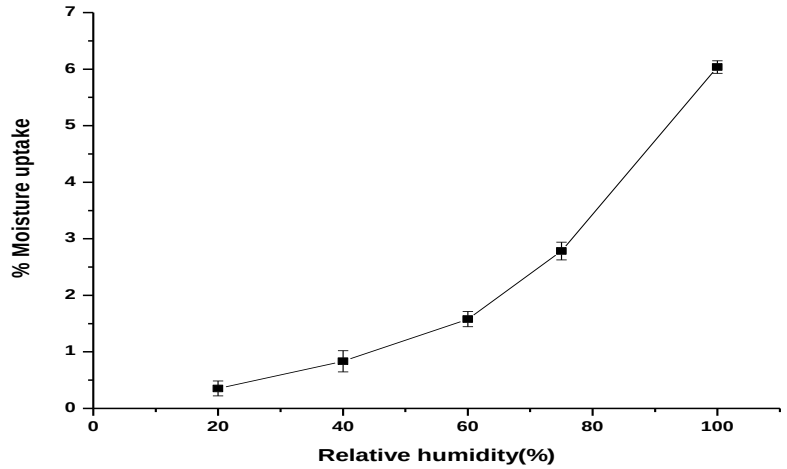


Figure 3.1: Moisture sorption pattern of myrrh resin.

3.2 Scanning electron microscopy (SEM)

The morphology as characterized by the particle shape and size can be used as an important feature for the identification and distinction of different polymers (Gangurde *et al*., 2013). The scanning electron microscope photograph of myrrh resin with different magnification is illustrated in Figure 3.2. The particles are of irregularly shaped with rough surfaces and it can also be seen that particles are not homogeneous in size and some of them appear as aggregates.

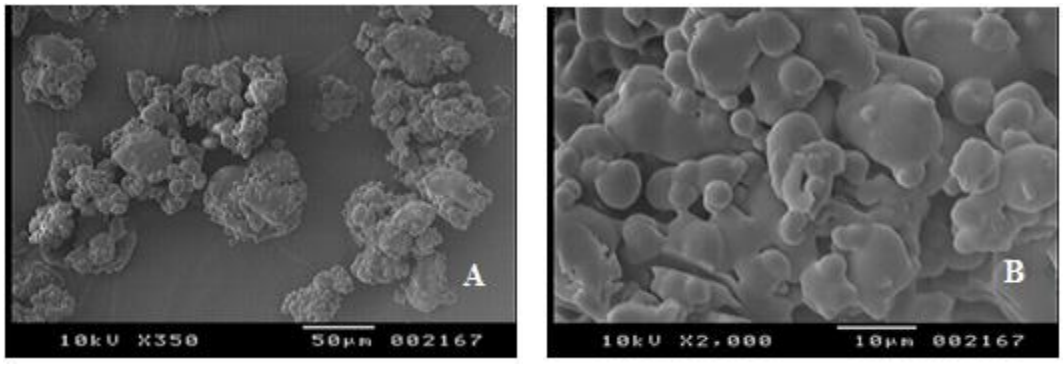


Figure 3.2: SEM photograph of myrrh resin. Magnification, **A)** x350 and **B)** x2000.

3.3. Drug-excipient compatibility studies

Assessment of possible incompatibilities between an active drug substance and different excipients forms an important part of the preformulation study during the development of solid dosage forms (Patel and Baria, 2009). DSC and FTIR were used as tools to detect incompatibility between theophylline and myrrh resin.

3.3.1 DSC studies

DSC allows rapid evaluation of possible incompatibilities between drug and excipients because it shows changes in appearance, shift of melting endotherms and exotherms, and/or variations in the corresponding enthalpies of reaction (Zaroni *et al*., 2008; Patel and Baria, 2009).

The DSC profile of pure theophylline (Figure 3.3) showed an endothermic sharp peak at a temperature of 275.4 °C indicating the fusion of theophylline. The result obtained in the current study is similar to that obtained in another study (Nokhodchi *et al*., 2010). The endothermic peak corresponding to the melting point of theophylline is reduced to 271.9 °C in the DSC thermogram of theophylline-myrrh resin mixture (1:1) (Figure 3.4), and this indicates that there is lack of significant changes in theophylline crystalline state. For further confirmation of the absence of any incompatibility, the theophylline-myrrh resin mixture was subjected to FTIR studies and its spectrum was compared with the FTIR spectra of theophylline.

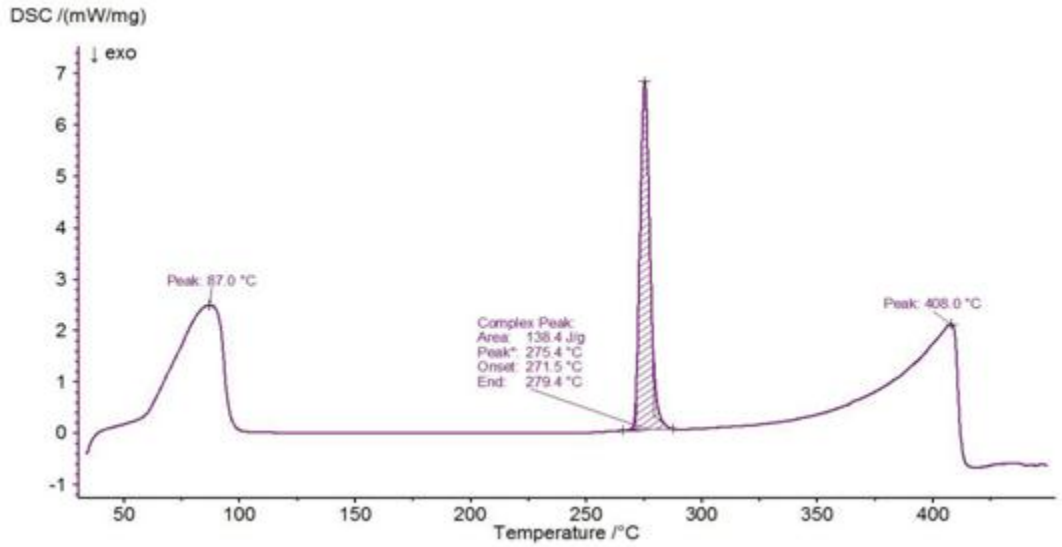


Figure 3.3: Differential scanning calorimetry thermogram of pure theophylline.

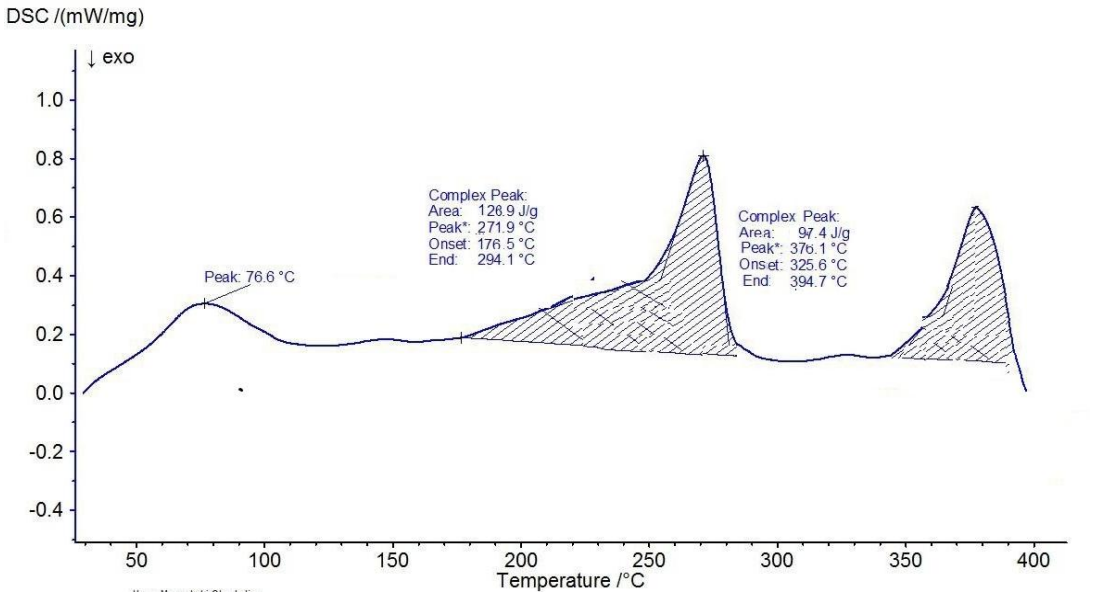


Figure 3.4: Differential scanning calorimetry thermogram of a 1:1 mixture of theophylline and myrrh resin.

3.3.2 FTIR studies

FTIR spectroscopy is a quick and simple technique for identifying any chemical changes or interaction between drug and excipient (Gangurde *et al*., 2013). Hence, it was used as supplementary technique in order to investigate the interaction between theophylline and myrrh resin and to confirm the result obtained by DSC.

The FTIR spectra of theophylline, myrrh resin and theophylline-myrrh resin physical mixture (1:1) are shown in Figure 3.5, 3.6, and 3.7, respectively. The spectrum of theophylline shows characteristic peaks at 1716 cm^{-1} and 1666 cm^{-1} attributable to the (C = O stretching vibrations). The band at 1566 cm^{-1} is attributable to the (pyrimidine ring stretching vibration), at 3118 cm^{-1} (C-H stretching vibrations), at 1186 cm^{-1} (C-N vibration). These characteristic peaks were also present in the physical mixture. The result showed that there was no significant interaction between the drug and myrrh resin and they are compatible with each other. From the results of the above studies, the compatibility of theophylline and myrrh resin can be inferred.

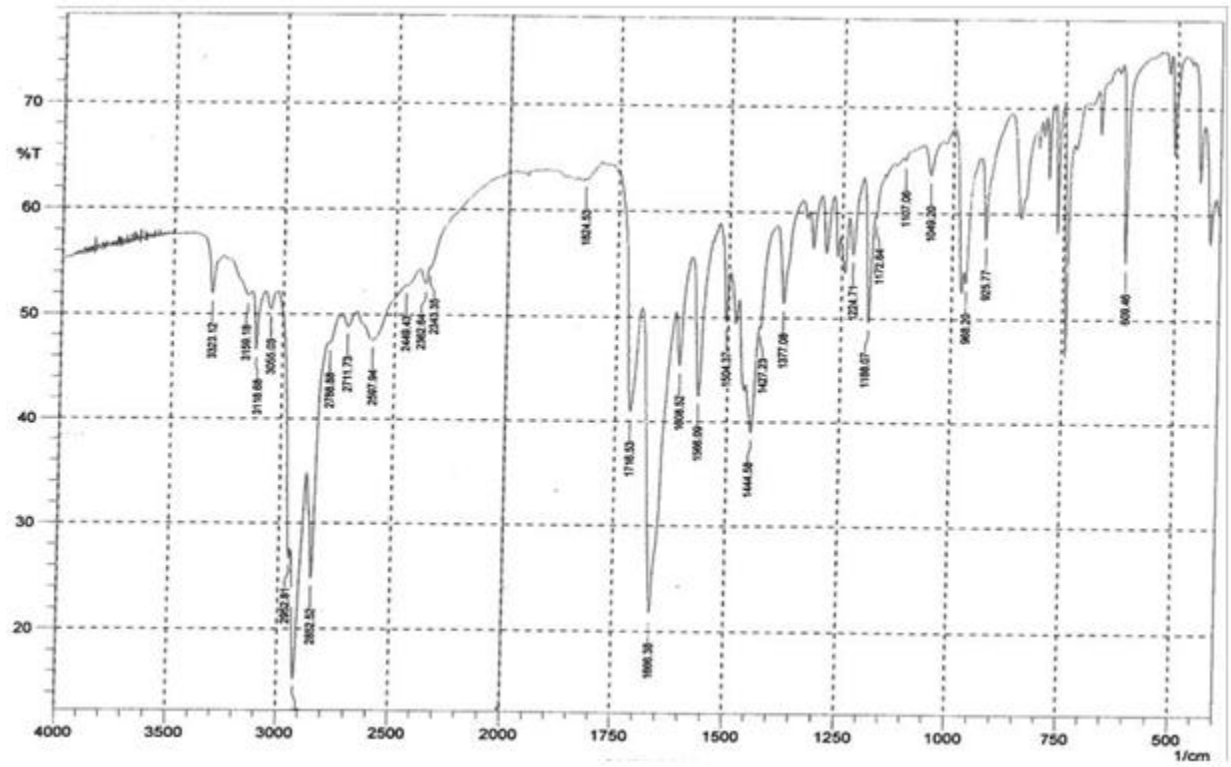


Figure 3.5: Fourier transform infrared spectrum of theophylline.

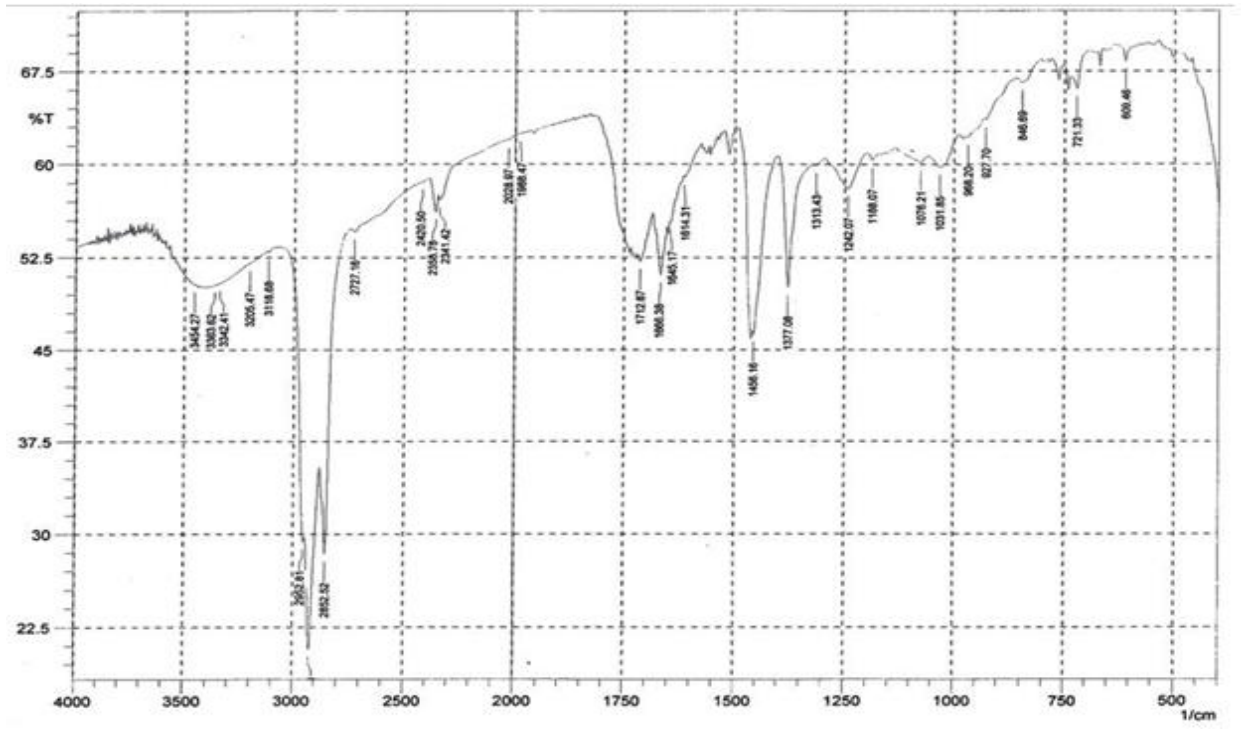


Figure 3.6: Fourier transform infrared spectrum of myrrh resin.

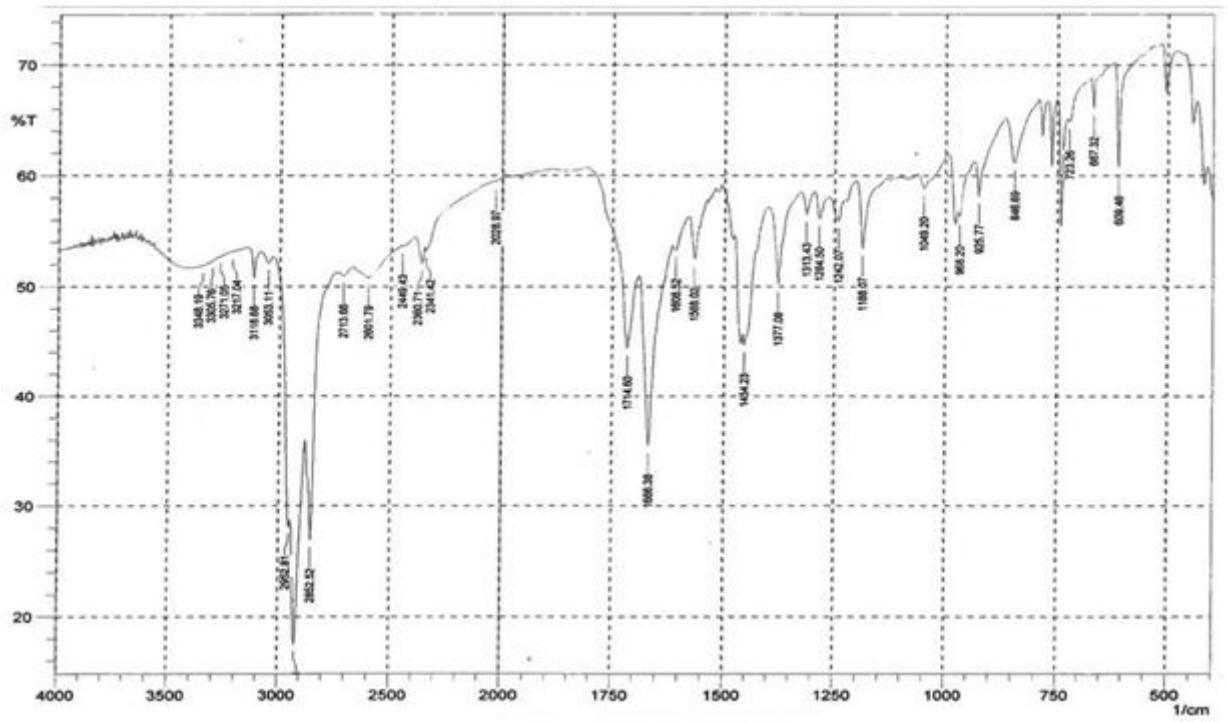


Figure 3.7: Fourier transform infrared spectrum of a 1:1 mixture of theophylline and myrrh resin.

3.4 Optimization

Different values of myrrh resin amount and compression force were used in pre-optimization investigations as a preliminary study for preparation of theophylline matrix tablets. On the basis of this preliminary study, the appropriate ranges of values were determined for the two independent variables (10 to 18% for the myrrh resin amount (MRA) and 10 to 20 KN for compression force (CF)). A CCD was used in the present study. In this design, 2 factors were evaluated, each at 5 levels, and experimental trials were performed for all 13 possible combinations. The formulation layout for the CCD batches (F1-F13) is shown in Table 3.2.

3.4.1. Characterization of granules

In the preliminary study, direct compression was tested for preparing matrix tablets but the result was not good because of the poor flowability and compressibility properties of myrrh resin powders. Hence, in the present study wet granulation technique was used for preparation of matrix tablets.

Table 3.2: The formulation for the central composite design batches of theophylline matrix tablets.

Formulation	Point type	Factors	
		MRA (%)	**CF (KN)**
F1	Factorial	18(+1)	20(+1)
F2	Factorial	18(+1)	10(-1)
F3	Factorial	10(-1)	10(-1)
F4	Factorial	10(-1)	20(+1)
F5	Axial	8.34(-α)	15(0)
F6	Axial	14(0)	22.07(+α)
F7	Axial	19.66(+α)	15(0)
F8	Axial	14(0)	7.93(-α)
F9	Central	14(0)	15(0)
F10	Central	14(0)	15(0)
F11	Central	14(0)	15(0)
F12	Central	14(0)	15(0)
F13	Central	14(0)	15(0)

Precompression parameters are the key factors in tablet production as these could affect the packing arrangements, tablet integrity, uniformity and weight variation (post compaction) (Masareddy *et al*., 2012; Ravindran *et al*., 2012). Thus, granules prepared by using myrrh resin as a matrix forming agent were evaluated for bulk density, tapped density, angle of repose, flow rate, Carr's index and Hausner ratio (Table 3.3). The bulk and tapped densities of the granules ranged from 0.42±0.01 g/ml to 0.49±0.02 g/ml and 0.45±0.01 g/ml to 0.53±0.02 g/ml, respectively. The angles of repose for all formulated granules were less than 30° indicating good flow properties. This was further supported by lower Carr's index (4–10%) and Hausner ratio (less than 1.25) values.

3.4.2. Evaluation of matrix tablets

The formulated matrix tablets were evaluated for physical properties such as tablet hardness, friability and thickness (Table 3.4). Hardness and friability are indicative of the mechanical strength of the tablets (Gangurde *et al*., 2013). The force required to break the tablet across its diameter is recorded as the hardness for the tablet (Ravindran *et al*., 2012).

The tablet thickness ranged from 2.79±0.01 to 3.02±0.02 mm. The tablet thickness, having same MRA and CF, showed low variability related to the formulation and consistency of CF. For tablets having same MRA, a decrease in tablet thickness was also observed with increasing

CF signifying an increase in tablet density. The hardness of different formulations were found to be between 89.8±2.86 to 133.7±3.53 N while all tablets passed the friability test (0.11% to 0.19%) which was less than 1%, showing enough resistance to the mechanical shock and abrasion.

Table 3.3: Flow properties of theophylline granules prepared with myrrh resin.

Formulation	Angle of repose (^{o}) ± SD	Flow rate (g/sec) ± SD	Bulk density (g/ml) ± SD	Tapped density (g/ml) ± SD	Carr's index (CI) ± SD	Hausner ratio ± SD
F1	26.56±0.64	4.79±0.09	0.49±0.02	0.53±0.02	7.55±0.25	1.08±0.01
F2	25.49±0.38	4.04±0.24	0.45±0.01	0.49±0.01	8.22±0.19	1.09±0.00
F3	29.10±0.34	4.81±0.03	0.44±0.01	0.49±0.00	9.52±1.18	1.10±0.01
F4	27.87±0.86	4.01±0.19	0.43±0.01	0.48±0.01	9.09±1.16	1.10±0.01
F5	29.28±0.70	4.33±0.07	0.43±0.01	0.45±0.02	4.42±0.15	1.05±0.01
F6	26.73±0.76	4.99±0.18	0.44±0.01	0.47±0.01	6.34±0.16	1.07±0.00
F7	29.00±0.23	4.09±0.24	0.42±0.01	0.45±0.01	6.62±0.09	1.07±0.00
F8	26.57±0.51	4.72±0.33	0.45±0.01	0.48±0.01	7.58±1.15	1.08±0.01
F9	28.93±0.69	5.03±0.03	0.46±0.01	0.49±0.01	5.47±1.14	1.06±0.02
F10	28.24±0.81	5.20±0.05	0.47±0.02	0.49±0.02	4.11±0.13	1.04±0.00
F11	26.75±0.31	4.72±0.26	0.46±0.01	0.48±0.00	6.17±0.14	1.07±0.01
F12	27.08±0.52	4.56±0.26	0.47±0.01	0.49±0.01	4.08±0.06	1.04±0.00
F13	26.57±0.51	4.88±0.24	0.45±0.01	0.48±0.01	6.29±0.15	1.07±0.00

Table 3.4: Characteristics of the formulations of theophylline matrix tablets.

Formulation	Thickness (mm)±SD	Hardness (N)±SD	Friability (%)±SD
F1	2.87±0.01	133.7±3.53	0.16±0.05
F2	3.00±0.01	92.5±2.17	0.17±0.06
F3	2.94±0.02	89.8±2.86	0.18±0.07
F4	2.79±0.01	126.6±3.53	0.11±0.02
F5	2.85±0.01	100.5±2.80	0.17±0.08
F6	2.82±0.01	120.2±3.33	0.15±0.03
F7	2.94±0.01	118.8±3.43	0.14±0.03
F8	3.02±0.02	90.0±2.31	0.19±0.06
F9	2.91±0.01	112.7±3.56	0.18±0.01
F10	2.93±0.01	110.6±2.67	0.14±0.02
F11	2.91±0.02	107.1±3.78	0.18±0.03
F12	2.90±0.02	105.4±3.66	0.14±0.04
F13	2.92±0.00	106.6±2.50	0.19±0.08

3.4.3 Construction of Calibration Curve

The absorbance readings obtained were plotted against concentration (Figure 3.8 and 3.9). The linear regression equations obtained were Y = 0.05354X - 0.00243 (R^2 = 0.9999) and Y = 0.01992 + 0.05375X (R^2 = 0.9997) in 0.1 N HCl and phosphate buffer of pH 6.8, respectively where Y is absorbance and X is concentration in µg/ml.

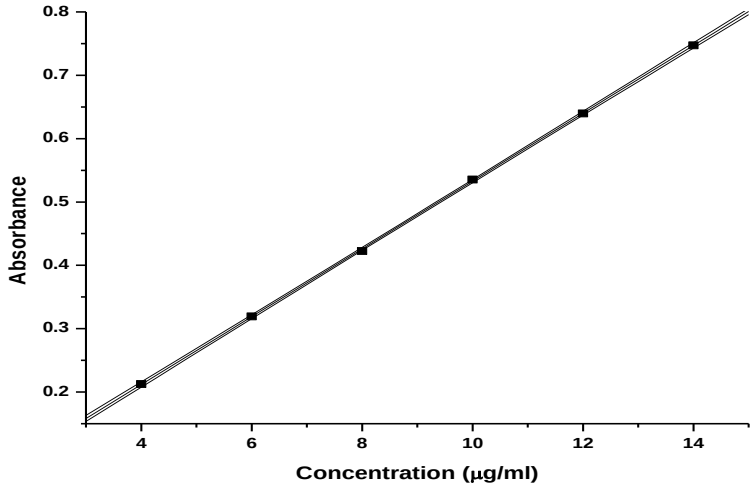


Figure 3.8: Standard calibration curve of pure theophylline at λ_{max} of 271 nm in 0.1 N HCl with upper and lower 95% confidence limits.

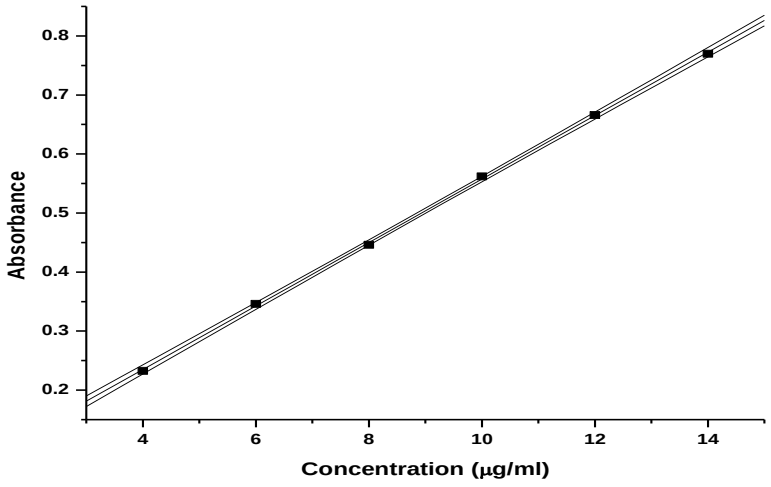


Figure 3.9: Standard calibration curve of pure theophylline at λ_{max} of 271 nm in phosphate buffer pH 6.8 with upper and lower 95% confidence limits.

3.4.4 *In vitro* drug release

The result of drug release profile from the different matrix tablet formulations is illustrated in Figure 3.10. Sustained release up to 12 h was achieved in all formulations except F5 in which about 100% of the drug release was observed within 8 h. Complete release from F5 within short time was due to less MRA (8.34%).

For the majority of formulations, the rate of drug release was found to be higher during 2 h followed by a gradual release phase for about 10 h. The initial faster release may be due to surface erosion. The release rate then decreases because the external layers of the tablet become depleted and water must penetrate deeper layers of the tablet to reach the undissolved drug. This is probably responsible for a decrease drug release in the late stage (Emeje *et al*., 2012).

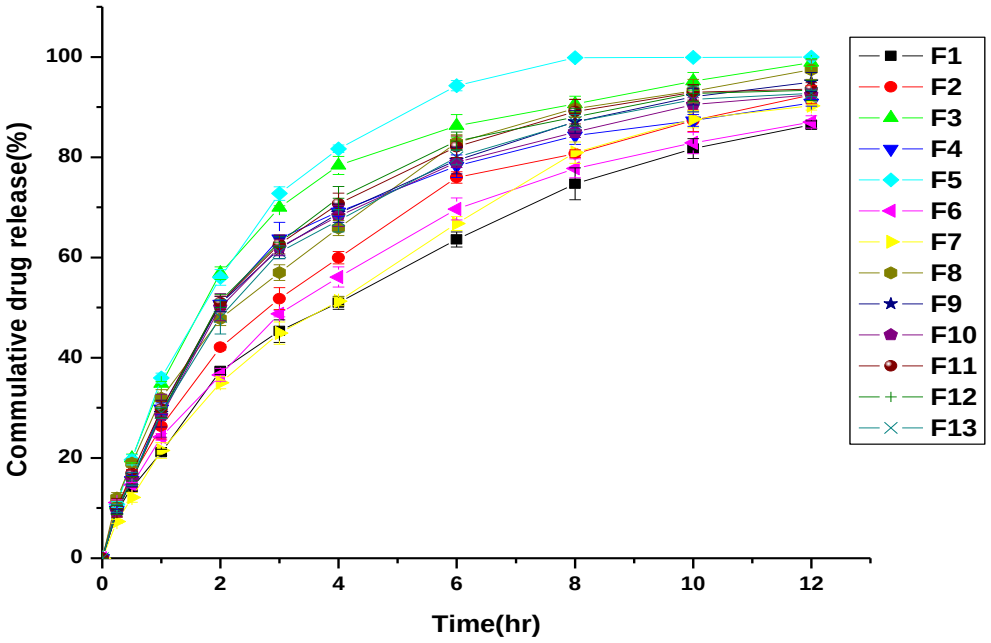


Figure 3.10: *In vitro* release profiles of thirteen theophylline matrix tablet formulations prepared as per central composite design.

An inverse relationship was also observed between concentration of myrrh resin and release rate of theophylline from the matrix tablets under similar CF. It was observed that 90.22%, 94.98% and 99.96% of theophylline was released from F7, F9 and F5 containing 19.66, 14 and 8.34 % of myrrh resin, respectively. In addition 92.48% and 98.9% drug was released from F2 (18% myrrh resin) and F3 (10% myrrh resin), respectively. This was due to hydrophobic nature of the myrrh resin which results in increase in diffusion path length and consequent retardation of drug release which is in agreement with other literature findings (Khandai *et al*., 2010; Mathure *et al*., 2011; Iqbal *et al.*, 2013).

The release profile revealed that F3, F5 and F8 showed more than 30 % drug release at 1st hour. It is reported in literature that more than 30% drug release in the first hour of dissolution indicates the chance for dose dumping (Kuksal *et al.*, 2006). This indicates formulations with lower levels of myrrh resin exhibited initial burst drug release. However, the rest of the formulations achieved sustained release with no burst or dose dumping effects.

It was observed that among the different combinations of MRA and CF used in the different formulations, F3 and F5 have shown highest drug release rate at the end of 12 h. In general, an optimal extended-release dosage form must have a minimal burst effect with most of the drug being released in a specific time period (Huang *et al*., 2005; Mujtaba *et al*., 2014).

Results of the dissolution parameters % release at 1 h, 12 h and $t_{50\%}$ which were used as responses for the formulations are listed in Table 3.5. For the matrix tablets formulated, the initial release in 1 h ranged from 21.14 – 35.94%. At the end of 12 h, the figure was between 86.45 and 99.96%. The $t_{50\%}$ varied between 1.63 and 3.76 h. These results were input into the Design-Expert 8.0.7.1 software for the optimization analysis.

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). One approach used to compare drug dissolution is dissolution efficiency (%DE). Dissolution efficiency, which is related to the actual amount of drug that is dissolved in the medium, gives an insight into the dissolution behavior of each formulation and is able to assess the individual performance of each formulation **(**Ngwuluka *et al*., 2009; Silva *et al.*, 2013).

Table 3.5: Responses of the thirteen theophylline matrix tablet formulations prepared as per central composite design.

Formulation	Responses		
	Cumulative release (%)		**$t_{50\%}$(h)(Y_3)**
	1h(Y_1)	**12h(Y_2)**	
F1	21.14	86.45	3.76
F2	26.32	92.48	2.92
F3	34.84	98.90	1.86
F4	29.85	90.86	2.40
F5	35.94	99.96	1.63
F6	24.20	87.03	3.43
F7	21.50	90.22	3.44
F8	31.93	97.47	2.30
F9	28.86	94.98	2.33
F10	28.43	92.46	2.39
F11	29.71	93.58	2.16
F12	28.95	93.34	2.15
F13	29.03	92.74	2.37

In this study, DE after 12 h of release was used to compare the results of release profiles of different formulations. The dissolution efficiency at the end of 12 h (DE_{12}) was 59 – 81% (Figure 3.11). One-way analysis of variance showed that the DE difference among the formulations was significant ($P < 0.05$). The variation among the DE results was high enough to justify the application of an optimization procedure.

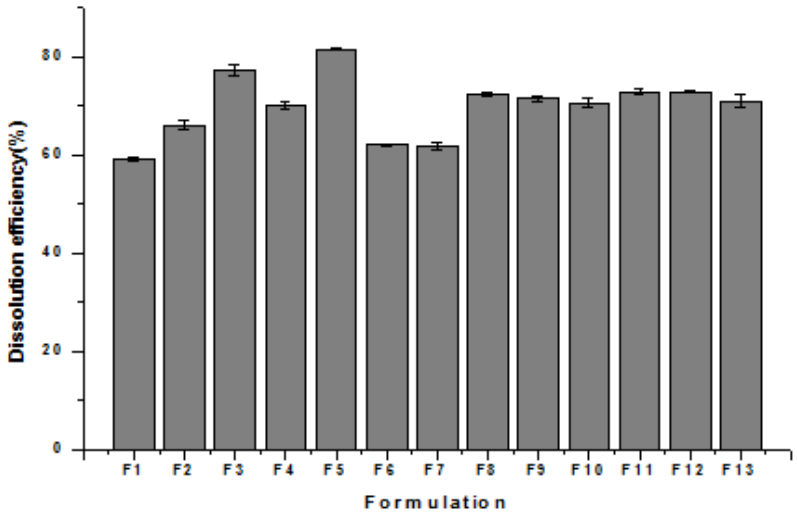


Figure 3.11: The dissolution efficiency values for the thirteen matrix tablet formulations prepared as per central composite design.

3.4.5 Drug release kinetics

The *in vitro* drug release data from the matrix tablets of different formulations were evaluated kinetically using various mathematical models: zero order, first order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas (Dey *et al*., 2014) which are useful tools for understanding and predicting drug release in matrix systems (Maderuelo *et al*., 2011). The results of the curve fitting into these mathematical models are given in Table 3.6. The correlation coefficient (r^2) was used as an indicator of the best fitting for each of the models considered (Syed *et al*., 2011; Bagdiya *et al*., 2013). The drug release data of the tablet formulations did not fit satisfactorily to zero-order, Higuchi and Hixson-Crowell models but showed good fit to the first order kinetics (r^2 = 0.9546-0.9980) describing the drug release rate to be dependent on concentration of drug and similar result was obtained by Voleti *et al*., (2013).

Table 3.6: Kinetic data from regression fitting of dissolution profiles of theophylline matrix tablets to several kinetic models.

Formulation	MODELS							
	Zero Order Kinetics		**First Order Kinetics**		**Higuchi Equation**		**Hixson-Crowell Model**	
	K_o	**r^2**	**K_1**	**r^2**	**K**	**r^2**	**K**	**r^2**
F1	6.5013	0.9329	0.1599	0.9980	26.9519	0.9948	-0.1783	0.8242
F2	6.8192	0.8961	0.2033	0.9954	28.6759	0.9832	-0.1763	0.7824
F3	6.8439	0.7826	0.3294	0.9699	29.8188	0.9218	-0.1659	0.6631
F4	6.4715	0.7975	0.1917	0.9666	28.0689	0.9309	-0.1681	0.6779
F5	7.2734	0.7675	0.7268	0.9546	31.8343	0.9122	-0.1724	0.6599
F6	6.5029	0.9089	0.1659	0.9948	27.2049	0.9869	-0.1734	0.8085
F7	7.1853	0.9367	0.1971	0.9942	29.7115	0.9937	-0.1967	0.8217
F8	7.0677	0.8763	0.2851	0.9913	29.9215	0.9745	-0.1715	0.7652
F9	6.9489	0.8337	0.2414	0.9952	29.8255	0.9530	-0.1771	0.7069
F10	6.7430	0.8225	0.2131	0.9844	29.0377	0.9463	-0.1737	0.7001
F11	6.8829	0.8184	0.2373	0.9802	29.6828	0.9443	-0.1731	0.7017
F12	6.9044	0.8056	0.2334	0.9753	29.8776	0.9362	-0.1758	0.6896
F13	6.8609	0.8355	0.2229	0.9852	29.4332	0.9541	-0.1748	0.7167

In order to investigate the mechanism of drug release, the dissolution data were also fitted to the well known exponential equation, Korsmeyer-Peppas model (Table 3.7), which is often used to describe drug release behavior from matrix systems when the mechanism is not well known or when more than one type of release phenomenon is involved (Sriamornsak *et al*., 2007; Moin and Shivakumar, 2010b).

In case of controlled or sustained release formulations, diffusion, swelling and erosion are the three most important rate controlling mechanisms. Any or all of these mechanisms may occur in a given release system (Chaudhary *et al*., 2012; Bose *et al*., 2013).

Table 3.7: Kinetic data from regression fitting of dissolution profiles of theophylline matrix tablets as evaluated by the Korsmeyer–Peppas model.

Formulation	Korsmeyer-Peppas Model		
	K	**r^2**	**n**
F1	22.1233	0.9947	0.6169
F2	25.5288	0.9975	0.6491
F3	32.5724	0.9935	0.7618
F4	28.3119	0.9959	0.7822
F5	32.8965	0.9944	0.7704
F6	24.1819	0.9957	0.6013
F7	20.1818	0.9959	0.6996
F8	29.5148	0.9936	0.6193
F9	27.7511	0.9962	0.7861
F10	27.7351	0.9974	0.7779
F11	28.8749	0.9974	0.7519
F12	28.0938	0.9958	0.7832
F13	27.8119	0.9973	0.7491

The value of diffusion exponent (n) of Korsmeyer-Peppas is used to characterize different release mechanisms as given in Table 3.8 for cylindrical tablets (Siepmann and Peppas, 2012). When determining the n exponent, data for the first 60% of drug release was used (Costa and Lobo, 2001; Papadopoulou *et al*., 2006; Quinten *et al*., 2009).

Table 3.8: Diffusion exponent and drug release mechanism for cylindrical tablets (Siepmann and Peppas; 2012).

Diffusion exponent (n)	**Drug release mechanism**
0.45	Fickian diffusion
0.45<n<0.89	Anomalous (non-Fickian) diffusion
0.89	Case-II transport
n>0.89	Super case-II transport

As depicted in Table 3.7, the value of diffusion exponent (n) ranged from 0.6013 to 0.7861 indicating an anomalous diffusion ($0.45<n<0.89$) mechanism. Anomalous diffusion of drug release mechanism signifies a coupling of both diffusion and erosion mechanisms which indicate that the drug release is controlled by more than one process during the entire period of drug release (Acharya *et al*., 2014; Mujtaba *et al*., 2014).

3.4.6 Selection of mathematical model

The relationship between the two independent variables (MRA and CF) and the three dependent variables (% drug release at 1 h, 12 h and $t_{50\%}$) were analyzed using response surface methodology. Polynomial models including linear, interaction and quadratic terms were generated for all the response variables using Design Expert software. The best fitting mathematical model was selected based on the comparisons of several statistical parameters including the coefficient of variation (CV), the multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (adjusted R^2) and the predicted residual sum of square (PRESS) provided by the Design Expert software (Huang *et al.*, 2005; Meka *et al.*, 2012; Mujtaba *et al*., 2014). Responses rel_{1h} and rel_{12h} were found to follow linear model whereas the selected model for $t_{50\%}$ is quadratic model (Table 3.9).

PRESS indicates how well the model fits the data, and for the chosen model it should be small relative to the other models under consideration (Huang *et al.*, 2005; Mujtaba *et al*., 2014). As shown in Table 3.9 the selected models have smaller PRESS values of 8.44, 38.18 and 0.16 for $rel_{1h,}$ rel_{12h} and $t_{50\%}$ respectively, compared to other models.

The fit of the model can also be evaluated through R^2-value (Singh *et al*., 2011; Sahoo and Gupta, 2012; Nazari *et al*., 2014). For the model to fit to the experimental data better, the R^2 value should be close to 1. The smaller the value of R^2, the lesser will be the fit of the model to the experimental data (Wu *et al*., 2010; Sahoo and Gupta, 2012). It can also be observed from Table 3.9 that R^2 is high (> 0.9) for all responses, which indicates a high degree of correlation between the experimental and predicted responses. In addition, the 'Predicted R^2' value is in good agreement with the 'Adjusted R^2' value, resulting in reliable models.

Table 3.9: Fit summary statistics for responses ($rel_{1\ h}$, $rel_{12\ h}$ and $t_{50\%}$) of theophylline matrix tablets.

Response	Source	R^2	Adjusted R^2	Predicted R^2	PRESS	Remark
Release at 1 h	Linear	0.9800	0.9759	0.9645	8.44	Suggested
	2FI	0.9800	0.9733	0.9420	13.78	
	Quadratic	0.9897	0.9823	0.9466	12.69	
	Cubic	0.9954	0.9889	0.9265	17.46	Aliased
Release at 12 h	Linear	0.9029	0.8835	0.8081	38.18	Suggested
	2FI	0.9080	0.8773	0.7494	49.84	
	Quadratic	0.9523	0.9183	0.7685	46.04	
	Cubic	0.9581	0.8994	-0.4732	293.06	Aliased
$t_{50\%}$	Linear	0.8428	0.8113	0.7391	1.30	
	2FI	0.8473	0.7964	0.6528	1.73	
	Quadratic	0.9870	0.9777	0.9677	0.16	Suggested
	Cubic	0.9887	0.9729	0.9517	0.24	Aliased

3.4.7 Model adequacy checking

The information about the model reliability was verified by using the analysis of variance (ANOVA) (Mutyaba *et al*., 2012). ANOVA is used to analyze the data to obtain the interaction between process of independent variables and responses (Nazari *et al*., 2014). At 5% level of significance, a model is considered significant if the p-value is less than 0.05 (Sahoo *et al*., 2011; Mujtaba *et al*., 2014). The results of ANOVA (Table 3.10) indicated all models were significant ($p < 0.05$) for all response parameters investigated.

Lack of fit (LOF) is a special diagnostic test for adequacy of a model that compares the pure error and describes the variation of data around the fitted model (Nasirizadeha *et al*., 2012; Nazari *et al*., 2014). For a model to be successfully used for prediction, the LOF should be insignificant (Sahoo *et al*., 2011; Sahoo and Gupta, 2012). The p-values of LOF (0.1492, 0.1797 and 0.8463 for Y_1, Y_2 and Y_3, respectively) were greater than 0.05, which further strengthened the reliability of the models.

Table 3.10: Summary of ANOVA results for dependent variables from central composite design

Source	Sum of squares	df	Mean square	F-value	p-value	Remark
Release at 1 h (Linear)						
Model	232.86	2	116.43	244.38	< 0.0001	Significant
MRA (A)	177.20	1	177.20	371.93	<0.0001	
CF (B)	55.66	1	55.66	116.83	<0.0001	
Lack of fit	3.91	6	0.65	3.06	0.1492	Insignificant
Release at 12 h (Linear)						
Model	179.60	2	89.80	46.48	< 0.0001	Significant
MRA (A)	75.67	1	75.67	39.17	<0.0001	
CF (B)	103.93	1	103.93	53.79	<0.0001	
Lack of fit	15.47	6	2.58	2.68	0.1797	Insignificant
Time required for 50% drug release (Quadratic)						
Model	4.93	5	0.99	106.43	< 0.0001	Significant
MRA (A)	3.10	1	3.10	334.67	<0.0001	
CF (B)	1.11	1	1.11	119.69	<0.0001	
AB	0.022	1	0.022	2.43	0.1630	
A^2	0.13	1	0.13	13.94	0.0073	
B^2	0.63	1	0.63	68.16	<0.0001	
Lack of fit	0.011	3	0.004	0.27	0.8463	Insignificant

Adequate Precision (signal to noise ratio) values higher than four for all the responses (44.29, 20.01 and 32.35 for Y_I, Y_2, and Y_3, respectively) confirmed that all proposed models can be used to navigate the design space. For all the models % CV were not greater than 10% (2.42, 1.49 and 3.78 for Y_I, Y_2, and Y_3, respectively) which indicate that models are reproducible (Mathure *et al*., 2011; Meka *et al.*, 2012; Nasirizadeha *et al.*, 2012; Pranav *et al.*, 2013).

In the response observation for rel_{1h} and rel_{12h} (Table 3.10), both linear terms (A and B) were found to be significant. For $t_{50\%}$, the linear terms (A and B) and quadratic terms (A^2 and B^2) were found to be significant while the interaction term (AB) was insignificant ($p > 0.05$). Since the interaction effect of myrrh resin and CF (AB) was not significant, model simplification was

carried out by eliminating this term in the polynomial equations (Table 3.11) in order to improve the model (Dey *et al*., 2014; Savic *et al*., 2014).

Table 3.11: Summary of ANOVA results of surface response reduced quadratic model for $t_{50\%}$

Source	Sum of squares	Df	Mean square	F-value	p-value	Remark
Model	4.91	4	1.23	112.35	< 0.0001	Significant
MRA (A)	3.10	1	3.10	283.94	<0.0001	
CF (B)	1.11	1	1.11	101.55	<0.0001	
$\mathbf{A^2}$	0.13	1	0.13	11.83	0.0088	
$\mathbf{B^2}$	0.63	1	0.63	57.83	<0.0001	
Lack of fit	0.033	4	0.008	0.62	0.6742	Insignificant

Results in Table 3.11 indicate that the reduced model was more significant ($F = 112.35$, $p < 0.0001$) than the unreduced model ($F = 106.43$, $p < 0.0001$) indicating the model predictive efficiency might be improved, although in both cases, the model was significant. The higher the F-value, the greater is the significance of the corresponding variable (term) to cause effect.

The adequacy of the models was also confirmed with residual plot tests of regression models (Mujtaba *et al*., 2014). Hence, two plots related to residuals: the normal probability plot of residuals and the plot of internally studentized residuals versus predicted values were considered as additional tests of model adequacy checking tools (Kusic *et al*., 2010).

The normal probability plot is a graphical technique for assessing whether or not a data set is approximately normally distributed. The residual is the difference between the observed and the predicted value (or the fitted value) from the regression. If the points on the plot fall fairly close to the straight line then the data are normally distributed (Hasan *et al.*, 2009; Singh *et al*., 2011; Sahoo and Gupta, 2012; Savic *et al*., 2014).

The normal probability plot of residuals and the plot of residuals versus predicted values of the responses for Y_1, Y_2 and Y_3 are shown from Figures 3.12-3.17. The results in Figure 3.12, 3.14 and 3.16 could be seen that the experimental points were reasonably aligned suggesting normal distribution. Furthermore the residuals in Figures 3.13, 3.15 and 3.17 appear to be randomly

scattered about zero and all other points were found to fall in the range of +3 to −3. These results again indicated that the model satisfies the assumptions of the ANOVA.

It can be concluded from these analyses that the proposed models are adequate for the observed set of data and there is no reason to infer any violation of the independence or constant variance assumption (Bari *et al*., 2010; Savic *et al*., 2014).

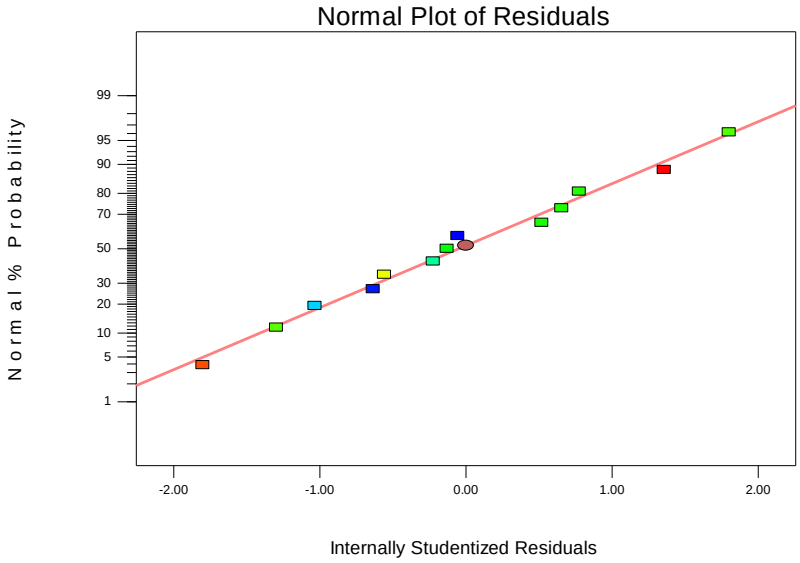


Figure 3.12: Normal probability plot of residuals for cumulative release at 1 h ($rel_{1\ h}$).

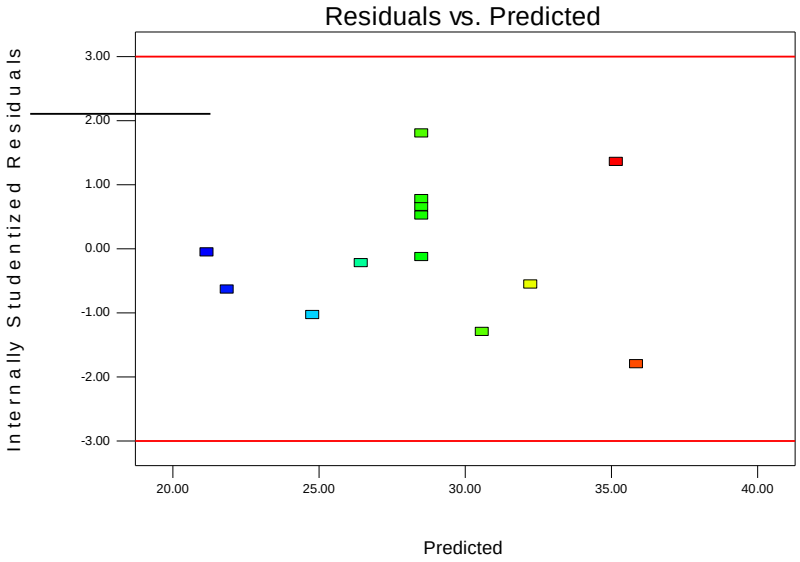


Figure 3.13: Plots of the residuals against predicted response for cumulative release at 1 h ($rel_{1\ h}$).

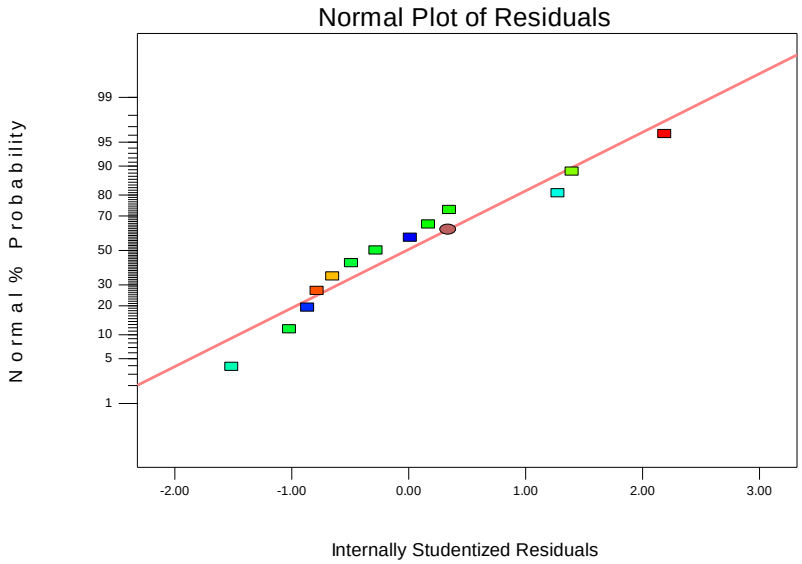


Figure 3.14: Normal probability plot of residuals for cumulative release at 12 h ($rel_{12\,h}$).

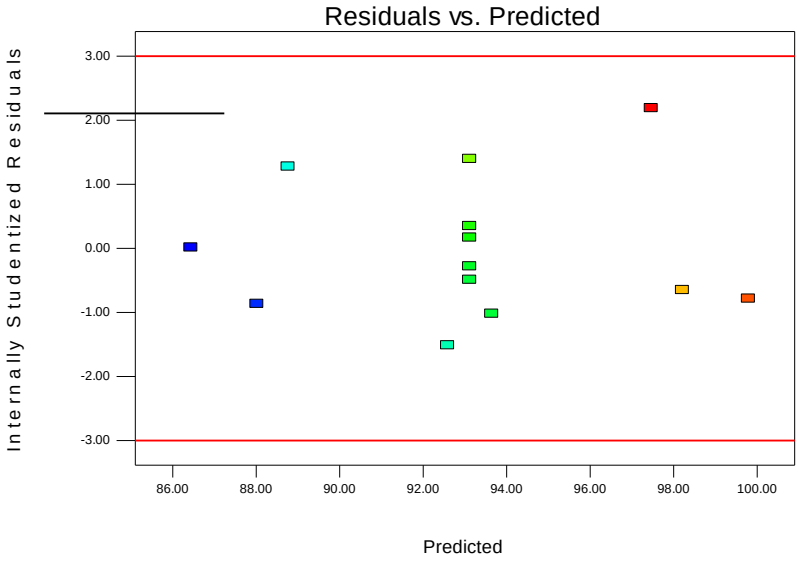


Figure 3.15: Plots of the residuals against predicted response for cumulative release at 12 h ($rel_{12\,h}$).

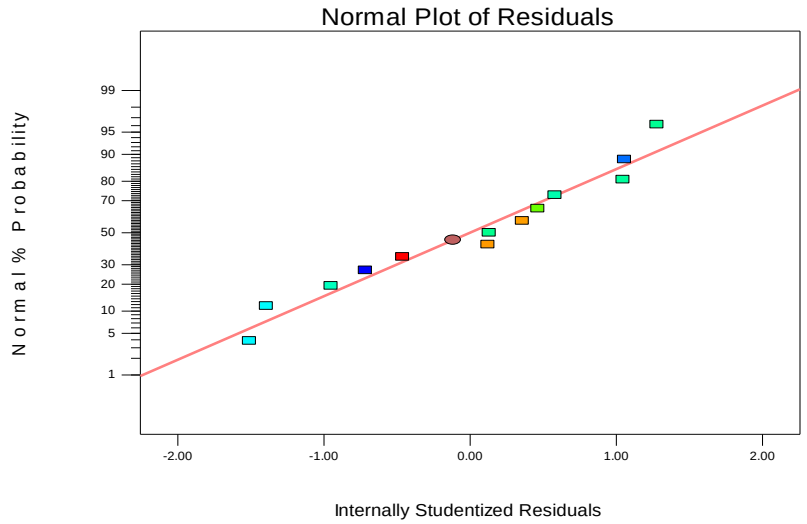


Figure 3.16: Normal probability plot of residuals for time required for 50% drug release ($t_{50\%}$).

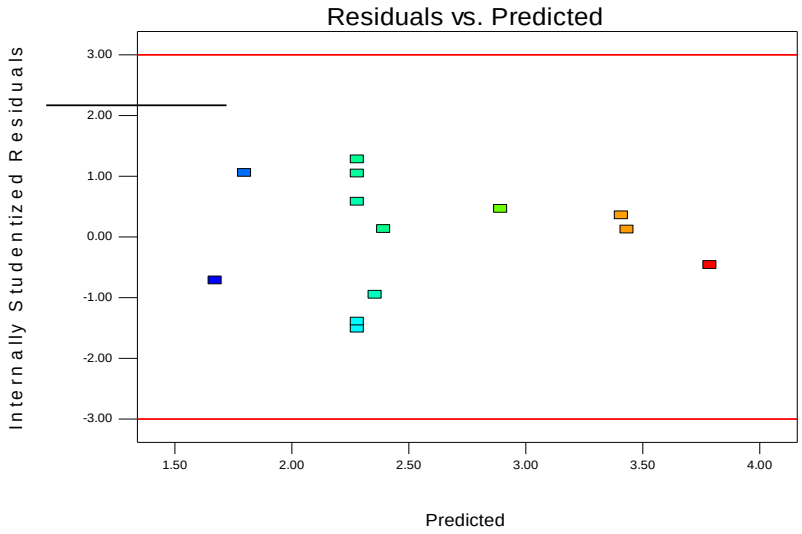


Figure 3.17: Plots of the residuals against predicted response for 50% drug release ($t_{50\%}$).

Therefore in order to determine the levels of factors which yield optimum dissolution responses, mathematical relationships (after model simplification) were generated between the dependent and independent variables. The equations of the responses are given below:

$$Y_1 = 28.52 - 4.71 * A - 2.64 * B \quad (3.1)$$

$$Y_2 = 93.11 - 3.08 * A - 3.60 * B \quad (3.2)$$

$$Y_3 = 2.28 + 0.62 * A + 0.37 * B + 0.14 * A^2 + 0.30 * B^2 \quad (3.3)$$

These equations represent the quantitative effect of independent variables (A and B) upon the responses (Y_1, Y_2 and Y_3). Coefficients with one factor represent the effect of that particular factor while coefficients with second order terms indicate the quadratic nature of the phenomena. A positive sign of coefficient indicates a synergistic effect while a negative term indicates an antagonistic effect of the factors upon the response (Akhgari *et al*., 2005; Chopra *et al.*, 2007; Hasan *et al.*, 2009; Singh *et al*., 2011; Bankar *et al*., 2012; Mutyaba *et al*., 2012; Mujtaba *et al*., 2014). Apart from the sign, the magnitude of the main effects signifies the relative influence of each factor on the response (Mandal *et al*., 2007; Singh *et al*., 2011).

It is observed from equations (3.1) and (3.2) that all the coefficients are negative. It indicates that dependent variables (Y_1 and Y_2) are significantly affected by the antagonistic effect of linear terms of MRA (A) and CF (B). The values obtained from Equation 3.1 reveals that MRA has more pronounced effect on drug release at 1 h than CF.

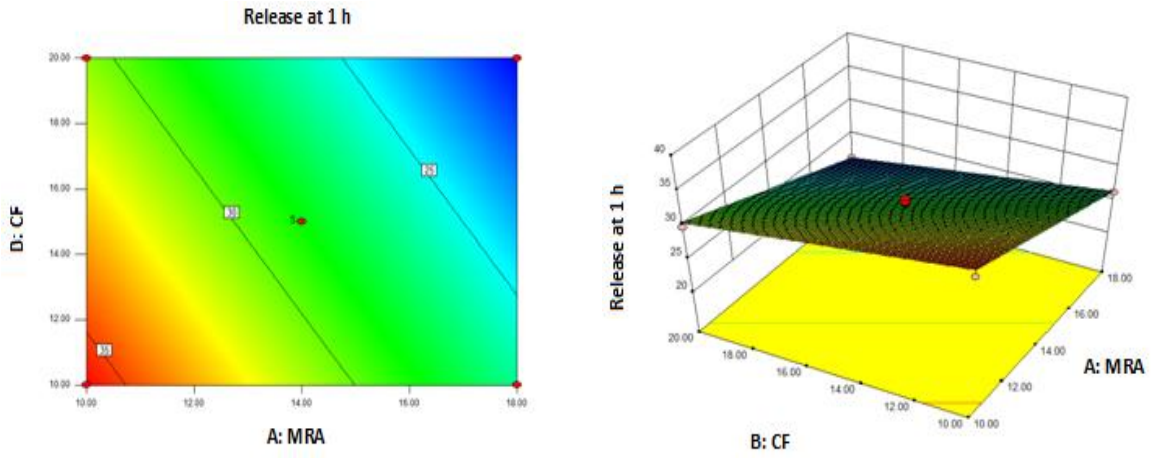
3.4.8 Contour plots and response surface analysis

Contour plot is the projection of the response surface as a two dimensional (2D) plane (Hasan *et al.*, 2009). The response surface plot is a three dimensional (3D) graphical representation of the regression equation (Lima *et al*., 2010). These plots are very useful to study the interaction effects of the factors on the responses and show the effects of two factors on the response at a time (Kshirsagar *et al.*, 2012; Mujtaba *et al*., 2014).

Contour plots (Figure 3.18a, 3.19a and 3.20a) and response surface plots (Figure 3.18b, 3.19b and 3.20b) for the obtained responses (Y_1, Y_2 and Y_3) were drawn based on the model polynomial functions to assess the change of the response surface. These plots explain the relationship between the dependent and independent variables.

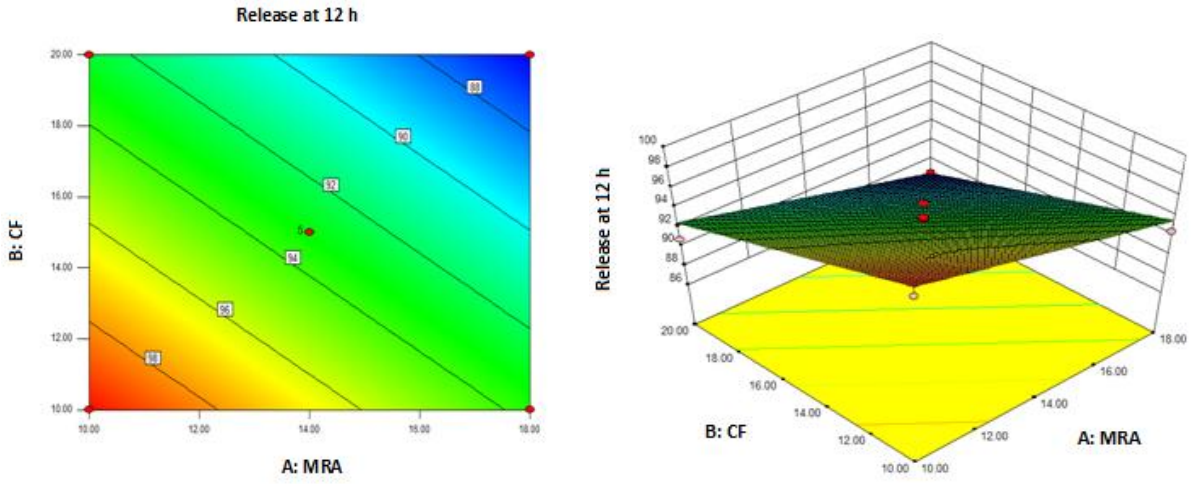
The straight lines in Figure 3.18a and 3.19a predicted linear relationship of factor A with factors B on the response Y_1 and Y_2, respectively. For responses Y_1 and Y_2, drug release decreases with increase in level of one variable while keeping other variable at a constant level. This showed that both the variables (MRA and CF) had negative effect on Y_1 and Y_2 as observed from Equations 3.1 and 3.2. However, the effect of MRA seems to be more pronounced as compared with that of CF in case of Y_1 as revealed by the response surface and the mathematical model.

Figure 3.18 and 3.19 also show that about 35.86% and 99.79% drugs are released after 1 h and 12 h respectively when both factors are at lowest level.



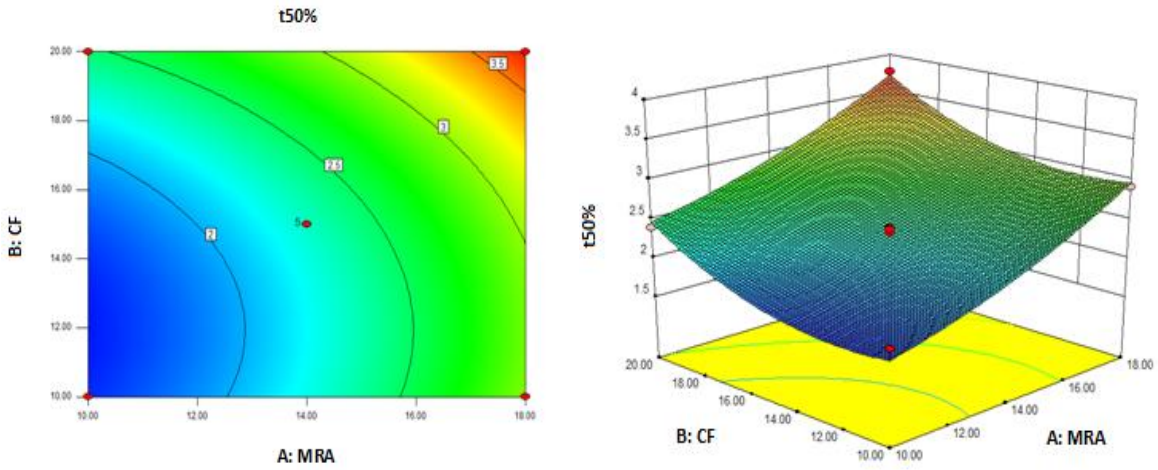
a **b**

Figure 3.18: a) Contour plot and b) Response surface plot showing the effect of MRA (A) and CF (B) on $rel_{1\,h}$ (Y_1).



a **b**

Figure 3.19: a) Contour plot and b) Response surface plot showing the effect of MRA (A) and CF (B) on $rel_{12\,h}$ (Y_2).



a **b**

Figure 3.20: a) Contour plot and b) Response surface plot showing the effect of MRA (A) and CF (B) on $t_{50\%}$ (Y_3).

However, factors A and B have non-linear relationship on the response Y_3 (Figure 3.20a and 3.20b). Response surface plots show the relationship between these factors even more clearly. The contribution of the second order term from Equation 3.3 was interpreted as the presence of curvature and represents the nature of the response surface system (maximum, minimum or saddle system). Thus, the positive sign for quadratic terms (Equation 3.3) demonstrated the concave form of the curve, as observed in Figure 3.20b (Cunha *et al.*, 2009).

From these figures (Figure 3.20a and 3.20b) it is found that about 1.72 h is required for the tablet to release 50% of the total drug amount (Y_3) when both the MRA (A) and CF (B) are at lowest level. This value is about 3.71 h when both factors are at highest level. Analysis of Figure 3.20b also shows that at constant level of MRA, $t_{50\%}$ decreased with increasing CF to a minimum after which it increased.

3.4.9 Optimization of $rel_{1\ h}$, $rel_{12\ h}$ and $t_{50\%}$

After generating the model polynomial equations to relate the dependent and independent variables, the process was optimized for all three responses. To optimize all the responses with different targets, a multi criteria decision approach like a numerical optimization technique by the desirability approach and graphical optimization technique by the overlay plot were used (Meka *et al.*, 2012). Optimum formulation was obtained based on the constraints set on

dependent variables (Table 3.12). These constrains are common for all the formulations. Hence the optimum values of the variables were obtained by graphical and numerical analyses using the Design-Expert 8.0.7.1 software.

Table 3.12: Constraints for responses used during numerical and graphical optimization.

Response	*Goal*	*Lower limit*	*Upper limit*	*Importance*
$rel_{1\ h}$	In range	24	28	5
$rel_{12\ h}$	In range	90	100	4
$t_{50\%}$	In range	2.75	4	3

3.4.9.1 Numerical optimization

A numerical optimization technique using the desirability approach was employed to develop a new formulation with the desired responses (Arora *et al*., 2011). 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). Figure 3.21 shows the predicted optimum values and the corresponding levels of parameters according to the set goals. A dot indicates the best solution found by the Design-Expert solver. In this study, the overall desirability of 1 was obtained as shown in the Figure 3.22.

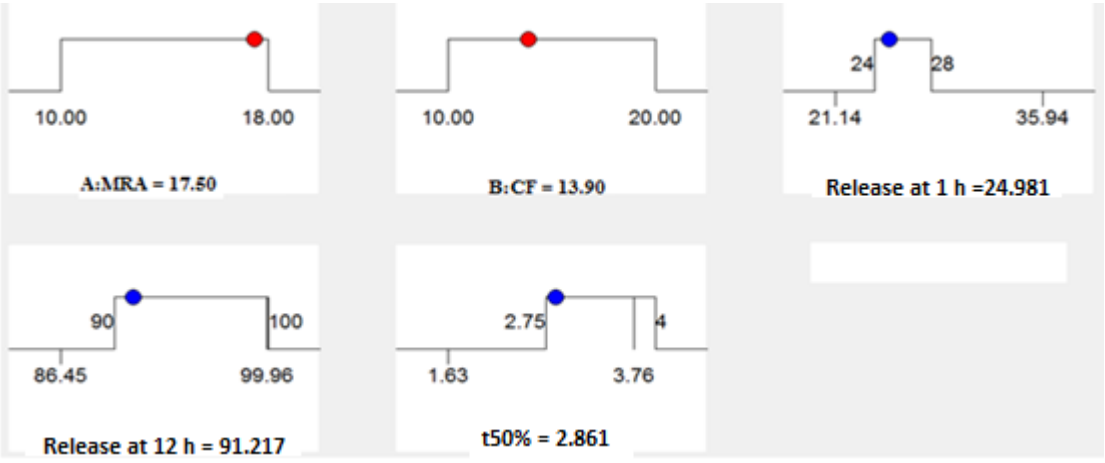


Figure 3.21: Numerical optimization results of predicted optimum values and the corresponding levels of parameters.

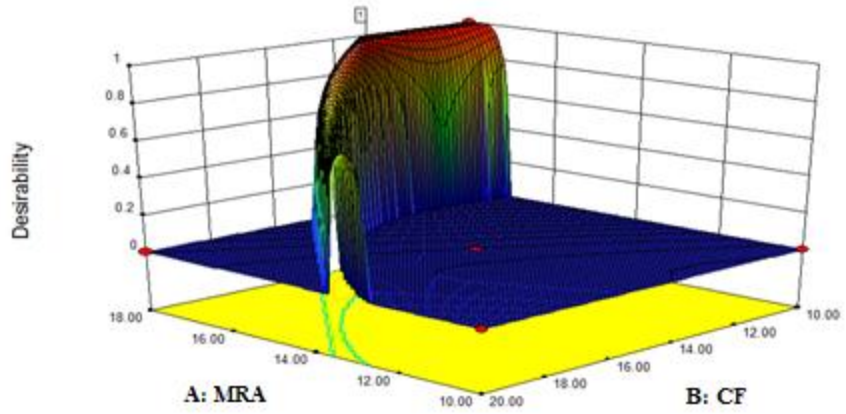


Figure 3.22: 3D plot of the overall desirability function.

3.4.9.2 Graphical optimization

The region of optimized formulations was also ratified using overlay plot (Singh *et al*., 2010). Based on the criteria indicated in Table 3.12, the overlay plot (Figure 3.23) was drawn in which the yellow area represents the area satisfying the imposed criteria. The point identified by the flag was one of the solutions given by the software (same solution shown in Figure 3.21). This flag predicts Y_1, Y_2 and Y_3 to be 24.981%, 91.217% and 2.861 h, respectively under the given condition for independent variables (17.5% for MRA and 13.9 KN for CF).

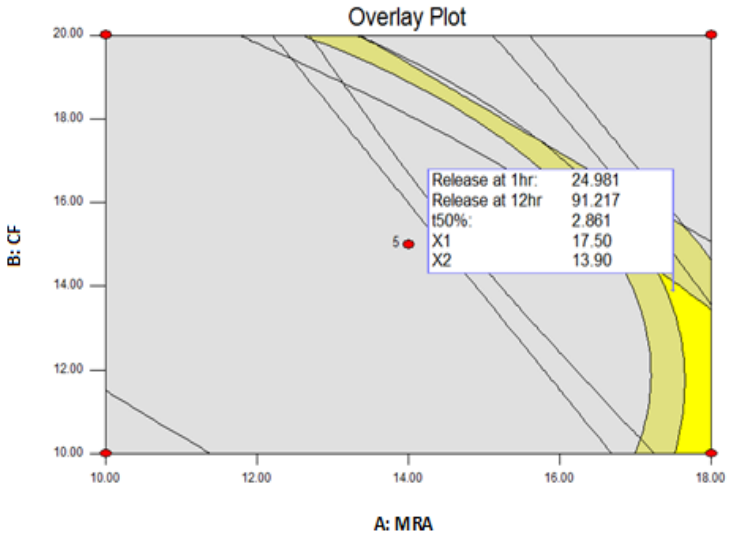


Figure 3.23: Overlay plot of the three responses as functions of MRA and CF (X_1= MRA, X_2= CF).

3.4.10 Evaluation and validation of the optimized theophylline matrix tablet formulation

3.4.10.1 Granule and tablet characteristics

The optimized formulation (MRA of 17.5% and CF of 13.9 KN) was evaluated for its granule and tablet characteristics (Table 3.13). As depicted in the table, the granule bulk and tapped densities were found to be 0.48±0.02 and 0.52±0.02, respectively. The angle of repose (25.61±0.37), Carr's index (7.03±0.91) and Hausner ratio (1.07±0.01) values showed the good flowability of the granules of the optimized formulation. Moreover the tablets showed hardness value of 99.30±1.65 and low friability (0.17±0.02) value which are acceptable.

Table 3.13: Characteristics of the optimized formulation of theophylline matrix tablets.

Parameters	Experimental values (mean±SD)
Granule characteristics	
Angle of repose (^{0})	25.61±0.37
Flow rate (g/sec)	5.11±0.23
Bulk density (g/cm^3)	0.48±0.02
Tapped density (g/cm^3)	0.52±0.02
Carr's index	7.03±0.91
Hausner ratio	1.07±0.01
Tablet characteristics	
Thickness (mm)	2.92±0.02
Hardness (N)	99.30±1.65
Friability (%)	0.17±0.02

3.4.10.2 *In vitro* drug release and release kinetics of optimized formulation

In vitro dissolution study was carried out on the prepared optimized formulation using three different batches (Figure 3.24). The result indicated that 24.11% and 92.45% of drug release was obtained at the end of 1 h and 12 h, respectively.

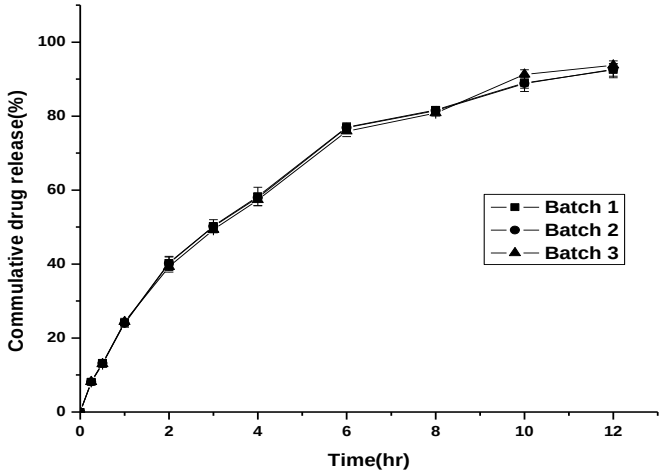


Figure 3.24: *In vitro* release profile of the optimized theophylline matrix tablet formulation.

The drug release of optimized formulation was best fitted to the first order kinetics model (Table 3.14). The value of the diffusion exponent (n) was found to be 0.7257 (Table 3.15) indicating that the mechanisms of drug release was diffusion coupled with erosion.

Table 3.14: Kinetic data from regression fitting of dissolution profile of optimized theophylline matrix tablets to several kinetic models.

Optimized formulation composition	MODELS							
	Zero Order Kinetics		**First Order Kinetics**		**Higuchi Equation**		**Hixson-Crowell Model**	
	K_o	**r^2**	**K_1**	**r^2**	**K**	**r^2**	**K**	**r^2**
MRA=17.50% CF=13.90 KN	7.1475	0.8974	0.2128	0.99627	30.0225	0.9825	-0.0280	0.9637

Table 3.15: Kinetic data from regression fitting of dissolution profile of optimized theophylline matrix tablets as evaluated by the Korsmeyer–Peppas model.

Optimized formulation composition	Korsmeyer-Peppas Model		
	K	**r^2**	**n**
MRA=17.50% CF=13.90 KN	22.7562	0.9955	0.7257

3.4.10.3 Validation of the optimized formulation

Three checkpoint formulations (that are selected to be at factor levels different from those used in the CCD) were prepared and evaluated for dependent responses. Table 3.16 shows the composition of optimum checkpoint formulations with their experimental and predicted values of all the response variables, and the percentage error in prognosis.

The relative errors (RE) (%) between the predicted and experimental values for each response were calculated and the values found to be within 5% (ranged between -4.496 and 4.814%). Thus, the experimental values were found to be in close agreement with the predicted values confirming the predictability and validity of the model. This demonstrated that the optimization technique was successful in designing the theophylline sustained release matrix tablet formulation.

Table 3.16: Checkpoint formulations comparing experimental and predicted values of the optimized formulations.

No	Optimized formulation composition	Response variable	Experimental value	Predicted value	%RE
1	A=17.50%	Y_1	24.107	24.981	3.499
	B=13.90 KN	Y_2	92.446	91.217	-1.347
		Y_3	2.975	2.861	-3.998
2	A=17.58%	Y_1	23.983	24.941	3.841
	B=13.8 KN	Y_2	92.629	91.229	-1.535
		Y_3	2.989	2.874	-4.001
3	A=17.64%	Y_1	24.378	25.611	4.814
	B=12.39 KN	Y_2	93.698	92.197	-1.628
		Y_3	2.975	2.847	-4.496

4. CONCLUSION

In this study, myrrh resin extracted from *C. myrrha* was successfully used as rate controlling excipient in the preparation of theophylline sustained release matrix tablet.

Physicochemical, pre-compression and tablet properties of formulations prepared by wet granulation as per CCD were within the specifications. Moreover, the DSC and FTIR studies indicated the absence of significant interaction between theophylline and the myrrh resin.

The MRA and CF were found to be the determinant factors for formulation of theophylline sustained release matrix tablets. Accordingly, the RSM based on CCD was successfully applied to optimize the combined effect of MRA and CF with respect to cumulative drug release at 1 h, 12 h and $t_{50\%}$.

The findings of this study demonstrate that both variables (MRA and CF) had negative effect on cumulative drug release at 1 h and 12 h. *In vitro* release kinetic study indicated that the drug release predominately follows first order kinetics and the mechanism was found to be diffusion coupled with erosion.

The optimal conditions were obtained at 17.5% of MRA and 13.9 KN of CF. Under these conditions, the cumulative drug release at 1 h, 12 h and $t_{50\%}$ were 24.981%, 91.217%, and 2.861 h, respectively. The kinetic study showed the optimized formulation followed first order kinetics model with anomalous release mechanism. It was found that the mathematical models generated were statistically significant and valid for predicting values of response parameters at selected levels of formulation variables.

Therefore, the results of the present study suggest that optimum formulation with theophylline release over a period of 12 h in a sustained manner with absence of burst release can be prepared by using myrrh resin as rate controlling excipient and myrrh resin can be used as an alternative pharmaceutical excipient in the formulation and manufacture of sustained release matrix tablets.

5. SUGGESTIONS FOR FURTHER WORK

The results of this study suggest further investigation in the following directions:

- Stability study of the matrix tablet made with myrrh resin;
- *In vivo* study of the release profile from the matrix tablet;
- Evaluation of myrrh resin as film forming material

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