Response Surface Design: Optimization of a Sustained Release Formulation of Ketorolac Tromethamine

A project submitted

by

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to

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Dedicated to my parents

Certification Statement

This is to certify that the project titled “Response Surface Design: Optimization of a Sustained Release Formulation of Ketorolac Tromethamine” submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Dr. Eva Rahman Kabir, Chairperson, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the language, ideas or writings of another.

Signed,

_____

Counter signed by the supervisor,

_____

Acknowledgement

The blessing and mercy of the Almighty who is the source of our life and strength of our knowledge and wisdom, has helped me to continue my study in full diligence which I hope will reflect in my project. This research could not also have been completed without the support of many people who are gratefully acknowledged here.

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Last but not the least I would like to give special gratitude to my parents for their constant invaluable support and prayers which have enabled me to dream bigger and pursue something which can only be attainable after passing hurdles.

Abstract

The main objective of this present study is to formulate sustained release tablets of Ketorolac Tromethamine using a 3^2 full factorial design and to systemically optimize the drug release profile using a design expert software. Ketorolac Tromethamine is a potent NSAID drug that is used to treat moderate to severe pain, including pain after any surgery, acute musculoskeletal pain as well as pain associated with post uterine cramping. Ketorolac Tromethamine has a short biological half-life of 4-6 hours and frequent administration of the drug can cause GI problems. It is thus considered a suitable drug for doing formulation development to reduce the frequent administration of drug and also to reduce adverse effects of GI, thus making it more patient compliant and giving a constant therapeutic effect. In total, nine formulations of sustained release Ketorolac Tromethamine were prepared using different concentrations of the rate retarding polymer - Methocel K100M CR and Methocel K4M CR by direct compression. Each of the excipients were selected by performing compatibility study using Fourier transform infrared (FTIR) and Differential scanning calorimetry (DSC). *In vitro* dissolution study was performed for all the formulations to obtain the drug release profile of sustained release tablets of Ketorolac Tromethamine and the dissolution profile were fitted into the different kinetic models for analysis. Among all the kinetic models, most of the formulated matrix tablets followed first order drug release kinetics which indicated that the drug release follows anomalous (non-Fickian) mechanism. It was found, from the responses produced by the software, that the polymer combination used in the formulation of sustained release matrix tables at different ratios has control on the drug release. From the nine (F1-F9) formulations, F5 was finally selected as optimum formulation.

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List of acronyms

API = Active Pharmaceutical Ingredient

CR = Controlled Release

DSC = Differential Scanning Calorimetry

EM = Electromagnetic

FT-IR = Fourier Transform Infrared Radiation

FI = Factor Interaction

GI = Gastrointestinal

HPMC = Hydroxypropyl methyl cellulose

HPLC = High Performance Liquid Chromatography

IR = Infrared

KT = Ketorolac Tromethamine

MDT = Mean Dissolution Time

NSAID = Non-Steroidal Anti-Inflammatory Drug

R-squared = Multiple Correlation Coefficient

SD = Standard Deviation

1. Introduction

Oral route is the most preferred route for the administration of a drug. Conventional dosage form is often referred to as the immediate release form of the drug which after administration is rapidly absorbed into the systemic circulation (Aulton, 2007). On the other hand, sustained release dosage forms are designed to achieve a prolonged therapeutic action by the continuous release of medication over an extended period of time (Figure 1.1) after administration of a single dose (Lachman, Lieberman & Kanig, 1985). Sustained release dosage forms results in prolonged therapeutic effects but not necessarily in a uniform release of drug. It helps to avoid frequent administration of dose, helping to maintain a constant therapeutic blood level and tissue level for prolonged period of time (Kumar, Satyaprasad, Venkateswarlu, Brahma, & Gunda, 2015). Ketorolac tromethamine has a short biological half-life and this study is to be done to develop a sustained release ketorolac tromethamine tablets. It helps to lower the dosing frequency of the drug, making it more patient compliant with maximum therapeutic effect and minimum adverse effects. This sustained release dosage form, thus helps to give a constant therapeutic effect for an extended period of time (Vatsaraj, Zia & Needham, 2002).

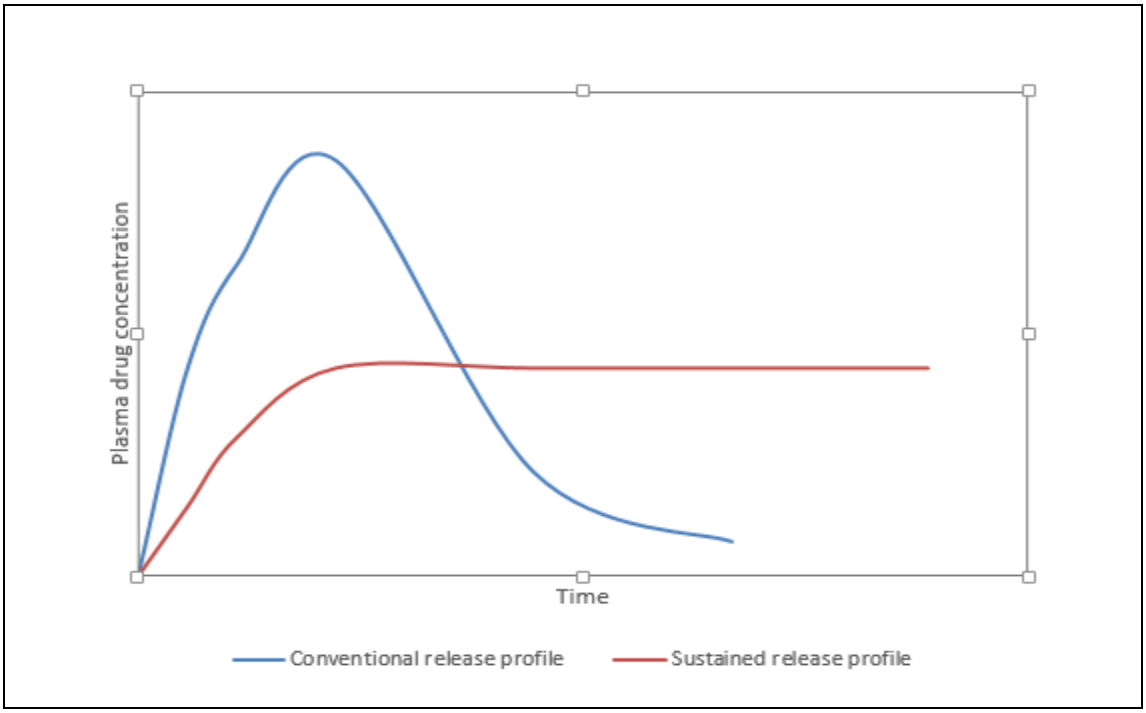

Figure 1.1: Conventional versus sustained release profile

The sustained release KT matrix tablets were prepared by direct compression technique using different grades of polymer. Though there are several methods for the formulation of sustained release KT matrix tablets, this method was chosen because it is a simple and cost effective method (Genc & Jalvand, 2008). Matrix tablets formulation can be defined as a drug or active ingredient studded in insoluble excipient in order to achieve release by a continuous release of the drug from the inert matrix core. There are different types of polymer used in this experiment. In recent years hydrophilic matrix system has been found to be widely used in the formulation of sustained release Ketorolac dosage forms (Genc & Jalvand, 2008).To perform this study HPMC (hydroxypropyl methyl cellulose) polymer of different viscosity (Table 1.1) were used in the formulation of sustained release KT tablet where they helped in prolonging drug release because of their rapid hydration, cost effectiveness, availability, good compression and gelling characteristics and very low toxicity.

Table 1.1: Composition of Methocel K 100M CR and Methocel K4M CR with their apparent viscosity

Different grade of Methocel Products	% of methoxyl in Mehocel product	% of Hydroxypropoxyl in Mehocel product	Apparent viscosity 2 % in water at 20°C (cP)
K100M	19-24	4-12	80000-120000
K4M	19-24	4-12	3000-5600

(Siepmann & Peppas, 2001)

There are different types of technique employed for compatibility study. These techniques include:

- Differential scanning calorimetry (DSC)
- Isothermal microcalorimetry
- Hot stage microscopy (HSM)
- Vibrational spectroscopy, Powder X-ray diffraction (PXRD)
- Solid state nuclear magnetic resonance spectroscopy (ss NMR)
- Scanning electron microscopy (SEM)

- Fourier Transform Infrared spectroscopy (FT-IR) and
- High performance liquid chromatography

Among these techniques Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared spectroscopy (FT-IR) are widely used for compatibility study.

1.1 Rationale of the Study

Sustained release dosage form of KT was designed to deliver a therapeutic dose of the drug that provides a slower and constant release of drug. Since immediate release tablets eliminate from the body at a faster rate and often fails to give a constant therapeutic effect resulting in need of additional dose of the drug (Shargel, Wu-Pong & Yu, 1941).

Ketorolac tromethamine, an NSAID drug with short biological half-life, is commercially available as immediate release tablet. Due to its short half- life, Ketorolac is eliminated from the body quickly and this leads to frequent administration of the drug to retain its therapeutic effect. Moreover, Ketorolac tromethamine may cause gastrointestinal irritation from increased number of doses. Thus, to enhance patient compliance in drug consumption and minimize gastrointestinal problems, the sustained release dosage form of the drug was formulated with different polymer composition using 3^2 full factorial design and optimization was done by Design Expert Software.

In the development of the sustained release tablets of KT, compatibility study of drug-excipient and excipient-excipient was done by Fourier transformer infrared (FTIR) and Differential scanning calorimetry (DSC). FTIR helps to determine the interaction of drug with each excipients in the formulation of tablets (EI-Gizawy, Zein El, Donia & Yassin, 2014). For further confirmation of compatibility, another study was done known as DSC with the same ratio as used for FT-IR (Reddy, Reddy & Devanna, 2016).

1.2 Drug Profile

Ketorolac tromethamine (5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol) is a non-steroidal anti-inflammatory drug, 800 times more potent than aspirin (Kalsriya et al., 2014). It contains substituted arylacetic acid and it can exist in three crystalline forms, all of them are equally soluble in water. KT is a highly potent analgesic that acts at the cyclooxygenase pathway of arachidonic acid metabolism for the inhibition of prostaglandin biosynthesis (Sinha, Kumar & Singh, 2009). It is a non-selective COX inhibitor. This non-narcotic drug is used for the management of moderate to severe post-operative pain (Dhumal, Bhusari, Tajne, Ghante & Jain, 2014) as well as to relieve abdominal pain, gynecologic pain, oral or urologic surgery pain (Sinha, Kumar & Singh, 2009).

Ketorolac tromethamine is used orally, intravenously, intramuscularly as well as a topical ophthalmic solution. It is commercially available as an immediate release tablet in a dosing regimen of 10 mg every 4 to 6 hours and also has a short biological half-life of 4 to 6 hours. The initial dose of KT required for patients above 65 years old and weighing less than 50 kg is 10 mg every 4-6 hours. However, the short biological half-life exhibited by KT makes the patients susceptible to its frequent administration (Vatsaraj, Zia & Needham, 2002).

2. Methodology

The proposed research study was designed to formulate an optimum sustained release matrix tablet of ketorolac tromethamine. The formulation involved the use of several excipients which was selected through extensive literature review and later confirmed based on the compatibility study with the active pharmaceutical ingredient. In this study, KT was mixed with two widely used hydrophilic polymers - Methocel K100M CR and Methocel K4M CR-to produce the sustained effect along with other excipients. The proposed formula for the formulation of matrix tablets is mentioned in Table 2.1.

Table 2.1: A proposed formula for the formulation of a drug

Ingredients	Justification
Ketorolac	API
Methocel K100M CR	Rate controlling polymer
Methocel K4M CR	Rate controlling polymer
Starch 1500	Binder
Avicel PH 101	Filler
Aerosil 200	Glidant
Talc	Lubricant

2.1 Standardization of Ketorolac Tromethamine by HPLC

10 mg of KT powder was weighed and dissolved in phosphate buffer up to the volume of 100ml to obtain a concentration of 100µg/ml. From the stock solution 80%, 90%, 100%, 110% and 120% concentration of standard solution were prepared. To prepare the five different concentrations, 2.4ml, 2.7ml, 3ml, 3.3ml and 3.6ml of the stock solution were taken in five separate 10ml volumetric flasks that resulted in concentrations from 24µg/ml to 36µg/ml respectively. Vials were prepared for the five concentrations and standardization was performed by using a validated HPLC method at a wavelength of 322nm.

2.2 Drug-Excipient Compatibility Study

Excipient selection has great importance in the formulation development of a drug. Drug-excipient compatibility study is thus considered an important portion of formulation development of a drug. Incompatibility of drug-excipients can cause undesirable interaction or can alter the product quality including chemical, physical or other properties (Zhou, 2009). Drug-excipient compatibility study was performed in the initial stage of product development so that any possible interaction can be identified before formulating the final product. FT-IR (Fourier Transform Infrared spectroscopy) and DSC (Differential Scanning Calorimetry) studies were performed for the drug-excipient compatibility study.

2.2.1 FTIR Study

Fourier Transform Infrared spectroscopy for drug-excipient compatibility study was done by passing the IR radiation through the sample where some amounts of radiation is absorbed by the sample and some are transmitted. The IR region is 4000-400 cm^{-1} (Maria et al., 2012). Functional groups are very important parameters in the analysis of the presence of any incompatibility between the drug and the excipients in FTIR test. Compounds with different structures give different IR spectrums and any interaction that occurs within the drug and the excipient causes a change in the molecular structure resulting in different IR spectrums relative to that of pure drug.

Six FT-IR test samples were prepared for drug-excipient compatibility test by mixing each of the excipient with the drug entities separately in a 1:1 ratio. The pure KT was also run through the FT-IR as reference. The IR spectrum region for every sample was studied to see whether the drug and excipients undergo any interaction or not. The following are the combinations of sample prepared for the compatibility test-

Ketorolac Tromethamine and Methocel K100M CR

Ketorolac Tromethamine and Methocel K4M CR

Ketorolac Tromethamine and Starch 1500

Ketorolac Tromethamine and Avicel PH 101

Ketorolac Tromethamine and Aerosil 200

Ketorolac Tromethamine and Talc

2.2.2 DSC Study

A Differential Scanning Calorimetry instrument was used for the thermal analysis of drug and the mixtures of drug and excipients. The samples for DSC were prepared in an appropriate ratio of 1:1 with the same ratio as prepared for FT-IR. The samples were analyzed in sealed aluminum sample pans with a nitrogen atmosphere and were subjected to a flow rate of 30ml/min and a heated at temperature range 30-300°C.

2.3 Factorial Design

The full factorial design is a very useful method that helps in the influence of certain factors involved in a process and also facilitates the determination of their relative importance. Its construction usually involves the selection of parameters and choice of responses (Gunda, Kumar, Babu & Anjaneyulu, 2015).

The formulations were designed by using 3-level, 2-factor (3^2) full factorial design. The concentration of Methocel K100M CR to be used in the matrix tablet formulations were selected as 35 %, 40%, 45% of the total weight of the tablet represented by the coded levels -1, 0 and +1 respectively (Table 2.2) and for Methocel K4M CR the amounts used were 5%, 7.5%, 10% of total tablet weight, also coded by -1, 0 and +1 respectively. Design Expert Software was used for the optimization of the formulations through the evaluation of statistical and graphical experimental design outcomes. A layout of 3-level, 2-factorial design for the nine (F1-F9) formulation is given in the Table 2.3 where A and B represents the Methocel K100M CR and Methocel K4M CR.

Table 2.2: Layout of 3-level, 2- factor full factorial design

Coded value	Actual value	
	A (%)	B (%)
-1 (low level)	35	5
0 (mid-level)	40	7.5
+1 (high level)	45	10

Table 2.3: Polymer combination of nine (F1-F9) different formulations

Formulation	Batch coded		Methocel K100M CR: Methocel K4M CR (%)	
	A	B		
F1	-1	-1	35	5
F2	-1	0	35	7.5
F3	-1	+1	35	10
F4	0	-1	40	5
F5	0	0	40	7.5
F6	0	+1	40	10
F7	+1	-1	45	5
F8	+1	0	45	7.5
F9	+1	+1	45	10

2.4 Formulation of Matrix Tablets

A proposed formula was employed for the development of nine different formulations (F1, F2, F3, F4, F5, F6, F7, F8, and F9) of the matrix tablet. Drug, polymers and all the excipients were weighed separately for the formulations (Table 2.4). Matrix tablets of KT were prepared whose total weight was 200 mg where the amount of API was 30 mg. The mixture of drug, polymer and excipients were sieved through size 60 mesh sieve prior to direct compression. The weight, hardness of the tablets of each formulation were checked before the starting of dissolution (Reddy, Reddy, & Devanna, 2016).

Table 2.4: Formulation of sustained release Ketorolac tromethamine matrix tablets

Ingredients	Amount (mg)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Ketorolac tromethamine	30	30	30	30	30	30	30	30	30
Methocel K100M CR	70	70	70	80	80	80	90	90	90
Methocel K4M CR	10	15	20	10	15	20	10	15	20
Starch 1500	43	40.5	38	38	35.5	33	33	30.5	28
Avicel PH 101	43	40.5	38	38	35.5	33	33	30.5	28
Aerosil 200	2	2	2	2	2	2	2	2	2
Talc	2	2	2	2	2	2	2	2	2
Total	200mg								

2.5 Dissolution Study

Dissolution is a process by which the drug substances are dissolved in the solvent. It helps in the determination of the rate and extent of dissolution or the release of the drug from a particular dosage form by using an aqueous media under certain specified conditions such as temperature, pH, time, rpm, etc. Dissolution test is an important technique for the formulation development of

a drug. Different types of apparatus are used to perform dissolution test based on the nature of the drug product and its formulation. The various apparatus types include rotating basket, paddle method, reciprocating cylinder, flow-through-cell, paddle-over-disk, cylinder, and reciprocating disk (Shargel, Wu-Pong & Yu, 1941).

2.5.1 *In vitro* Dissolution Study

For the *in vitro* dissolution study of KT matrix tablets, phosphate buffer of pH 6.8 was used. To prepare the dissolution medium, 6.8 gm of potassium dihydrogen orthophosphate anhydrous powder (KH_2PO_4) and 0.94gm of sodium hydroxide (NaOH) pellets were taken and dissolved into distilled water to make the volume up to 1000 ml. After that the pH of the dissolution medium was checked and regulated using orthophosphoric acid or sodium hydroxide if necessary.

For the dissolution study, 900 ml of phosphate buffer solution was poured into the vessel of dissolution test apparatus. Six tablets of KT were weighed from each of the nine formulations and immersed into the dissolution media. The temperature of the dissolution media was maintained at 37±0.5ºC and paddle was rotated at the speed of 100 rpm while dissolution was performed for a period of 8 hours. 5 ml of the samples were withdrawn after 30minutes, 1hr, 2hr, 4hr, 6hr and 8hr and immediately replaced by the same volume of dissolution media. After that the collected sample were filtered through the filter paper and their percentage release was determined using a validated RP-HPLC method at 322 nm. Initially UV-spectrometry was used to determine the release kinetics but due to release variations HPLC method was adopted.
Equation of the standard curve obtained was:

$$y = 67626x + 172921$$

where,

y = Area and

x = Concentration (µg/ml).

2.5.2 Kinetic Models for *in vitro* Dissolution Study

There are different mathematical models which can describe the dissolution release profile. The data obtained from different formulations (F1-F9) through the *in vitro* dissolution studies were inserted into different kinetic model equations to determine the mechanism of drug release and the model that best suits for the drug release (Dash, Murthy, Nath & Chowdhury, 2010).The kinetic model includes zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell kinetic models.

2.5.2.1 Zero Order Equation

Zero order refers to the kinetics where the rate of reaction is independent of the concentration of the reactant (Lachman, Lieberman & Kanig 1985). To study the drug release kinetics, data obtained from the *in vitro* dissolution studies were plotted as cumulative % of drug release versus time (Talukder, Ahmed, Haque & Chowdhury, 2010).The equation is:

$$\mathbf{C = K_0\, t}$$

where,

C=drug concentration at time ‘t’

K_0=zero rate constant (concentration/time)

T= time in hour

2.5.2.2 First Order Equation

First order refers to the kinetics where the rate of reaction is dependent on the concentration of the reactant (Lachman, Lieberman & Kanig, 1985). To study the drug release kinetics, data obtained from the *in vitro* dissolution studies were plotted as cumulative percentage of drug remaining *versus* time (Talukder, Ahmed, Haque & Chowdhury, 2010). The equation is:

$$\mathbf{\log C = \log C_0 - K\, t / 2.303}$$

where,

C= amount of drug remaining at‘t’ time

C_0 = drug concentration at t = 0

K = first order rate constant

2.5.2.3 Higuchi Kinetic Model

Higuchi model describes that cumulative percentage of drug release is proportional to the square root of time-dependent process based on Fickian diffusion (Siepmann & Peppas, 2001). To study the drug release kinetics, data obtained from the *in vitro* dissolution studies were plotted as cumulative percentage of drug release *versus* square root of time. The equation is:

$$\mathbf{Q= K_h t^{1/2}}$$

where,

Q= (100-C) i.e. the amount of drug released at time 't'

K_h= the constant reflecting the design variables of the system

Hence, drug release rate is proportional to the reciprocal of the square root of time (Siepmann & Peppas, 2001).

2.5.2.4 Korsmeyer-Peppas Model

The Korsmeyer-Peppas model is generally used to analyze the release of a drug molecule from a polymeric matrix. To study the drug release kinetics, data obtained from the *in vitro* dissolution studies were plotted as log fraction of drug release versus log time. The equation is:

$$\mathbf{\log (M_t/M_f) = \log K + n \log t}$$

where,

M_t = amount of drug release at time 't'

M_f = amount of drug release after infinite time

K = release rate constant incorporating structural and geometric characteristics of the dosage form

n = diffusional exponent indicative of the mechanism of drug release

Based on the 'n' value of a spherical shaped tablet, drug transport mechanism from the tablet is classified into four types which is shown in table 2.5 (Siepmann & Peppas, 2001).

Table 2.5: Diffusional exponent values indicating drug release mechanism

Diffusional exponent	Drug transport mechanism
0.43	Fickian diffusion
0.43<n<0.85	Anomalous/non-Fickian transport
0.85	Zero order transport
>0.85	Super case II transport

2.5.2.5 Hixson-Crowell Cube Root Law

Hixson-Crowell cube root law describes the release from systems where there is a change in surface area and diameter of the particles or tablets. This law is expressed as the cube root of the percentage of initial drug minus the cube root of the percentage of drug remaining in the matrix, as a function of time (Dash, Murthy, Nath & Chowdhury, 2010). The equation is given below:

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC}.t$$

where,

Q_0= the amount of drug in tablets

Q_t=the amount of drug remaining in time‘t’

K_{HC}= rate constant for the Hixson-Crowell cube root law

2.5.3 Successive Fractional Dissolution Time

To characterize the drug release rate in various experimental conditions, time required for 25% ($T_{25\%}$), 50% ($T_{50\%}$) and 80% ($T_{80\%}$) of drug release were calculated The Korsmeyer-Peppas release constant (K) and release exponent (n) values of Korsmeyer-Peppas model. The equations for the determination of $T_{25\%}$, $T_{50\%}$ and $T_{80\%}$ are as follows:

$$\mathbf{T_{25\%} = (0.25/K)^{1/n}}$$

$$\mathbf{T_{50\%} = (0.5/K)^{1/n}}$$

$$\mathbf{T_{80\%} = (0.8/K)^{1/n}}$$

where,

n = release exponent,

T= time

K= Korsmeyer-Peppas release constant

Mean dissolution time (MDT) is defined as the time needed for 50% of active pharmaceutical ingredients to be released from the dosage form and indicates the reduction in the release rate of the active ingredients as caused by carriers. MDT values helps to understand the drug release rate from the matrix tablets and sustaining the efficiency of the polymer. It also explains the polymer loading function as well as the nature of the polymer (Talukder, Ahmed, Haque & Chowdhury, 2010).The equation for MDT value is given below:

$$\mathbf{MDT = (n/n+1).\ K^{-1/n}}$$

2.6 Optimization Data Analysis

Optimization is a systemic and cost effective method that helps to understand the relationship between independent variables and response variables (Bushra, Shoaib, Aslam, Hashmat & Rahman, 2008). There are three methods of optimization for pharmaceutical formulations such as simplex method, Lagrangian method and search method.

The Downhill Simplex Method involves a geometric figure which has one or more points than the number of factors. The simplex is represented for two variable is triangle. This method requires only function evaluation, not derivatives. It may be the best method to use if the figure of merit is “get something working quickly” for a problem whose computational burden is small (Koshel, 2002). The Lagrangian method represents mathematic techniques to be used in the

optimization of pharmaceutical formulations. This technique require that the experimentation be completed before optimization so that the mathematical models can be generated. On the other hand search method for optimization can also be used for pharmaceutical formulations. This method can be applied for more than two independent variables (Banker & Rhodes, 2002).

Lagrangian method was chosen for optimization where experimental design was 3^2 full factorial design. In this experiment, two independent variables i.e. Methocel K100M CR (A) and Methocel K4M CR (B) and response variables for 1st hour (Y_{1hr}), 4th hour (Y_{4hr}) and 8th hour (Y_{8hr}) were selected and the the relationship between them were analyzed by Design Expert software. ANOVA was used to identify the significant effects of factors on response with the help of R^2-value, p-value and F-value. A numerical optimization technique was implemented to determine the optimum formulation (Gunjal et al., 2015).

3. Result and Discussion

3.1 Standardization of Ketorolac Tromethamine

A linear curve of Ketorolac Tromethamine was found from the plot of concentration *versus* area with a correlation coefficient (R^2) of 0.9993 as mentioned in Table 3.1 and Figure 3.1.

Table 3.1: Standardization of Ketorolac Tromethamine

Concentration (µg/ml)	Area
24.48	1833840
27.54	2030627
30.60	2244902
33.66	2436390
36.72	2665637

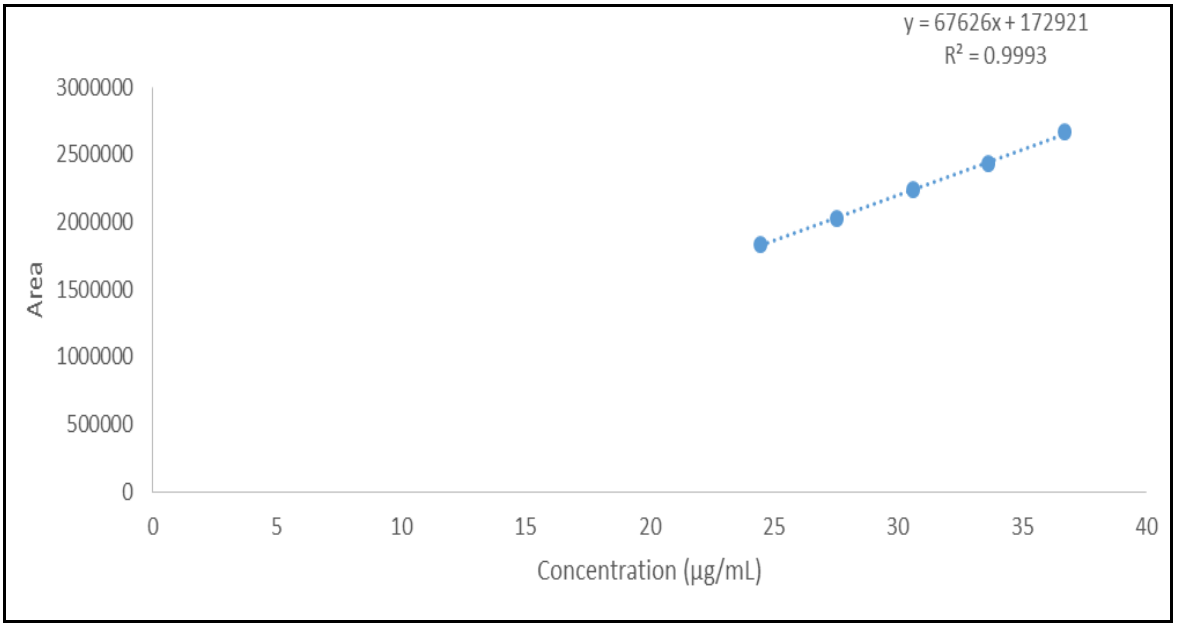

Figure 3.1: Standard curve for Ketorolac Tromethamine

3.2 Compatibility Study of Drug and Excipients

3.2.1 FTIR Characterization

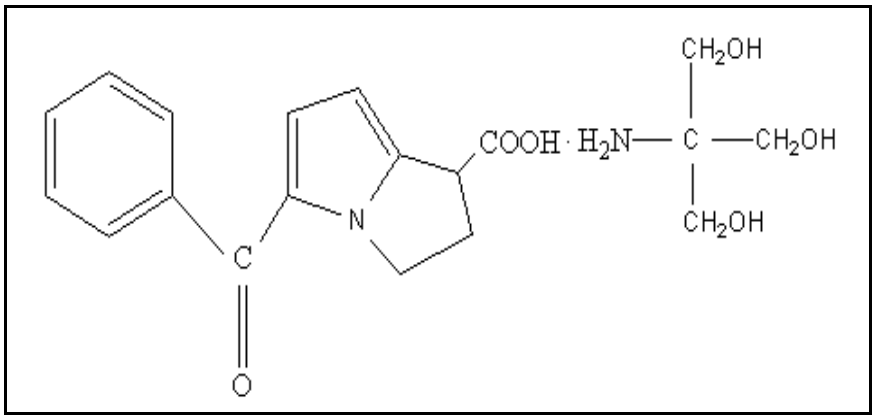

Figure 3.2: Ketorolac Tromethamine

Table 3.2: Salient functional groups of Ketorolac Tromethamine

Functional group	Frequency (cm^{-1})
Ketone (C=O)	1720-1705
Aromatic ring (C=C)	1600 and 1475
Carboxylic acid (C=O)	1725-1700
Carboxylic acid (O-H)	3400-2400
Amine (N-H)	3500-3100
Amine (C-N)	1350-1000

(Pavia, Lampman, Kriz & Vyvyan, 2008)

Ketorolac Tromethamine showed its major peak at 3349.50cm^{-1} for primary amine. It also showed other peaks including-

Alcohol (O-H) at 3079.46 cm^{-1}

Carboxylic acid (C=O) and (O-H) at 1594.22 cm^{-1} and 2898.14 cm^{-1}

Aromatic ring (C=C) at 1471.74cm^{-1}

Amine (C-N) at 1385.9cm^{-1} (Table 3.2)

FTIR studies of drug and excipients showed no interaction within the drug and excipients chosen for this experiment. Some additional peaks of Ketorolac Tromethamine were also observed but

no significant changes was seen in the original peak. The spectra of the pure drug and drug-excipients are given in Figure 3.3 to Figure 3.9.

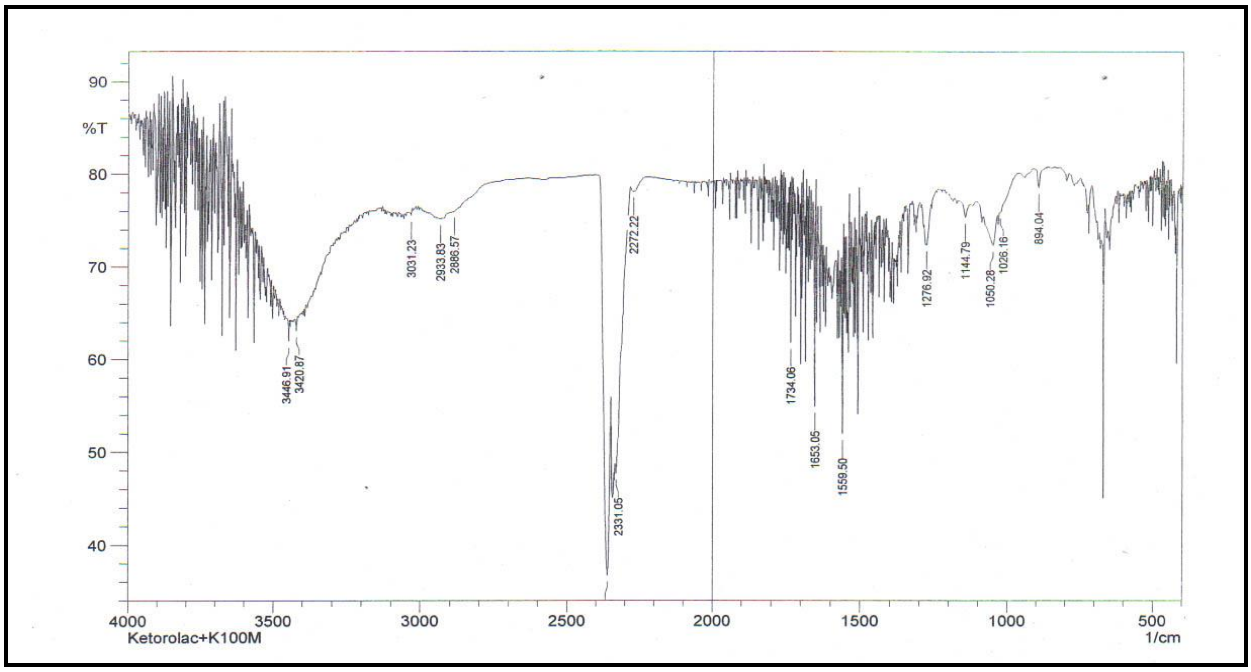

Figure 3.3: Fourier Transformed Infrared spectrum of Ketorolac Tromethamine (pure drug)

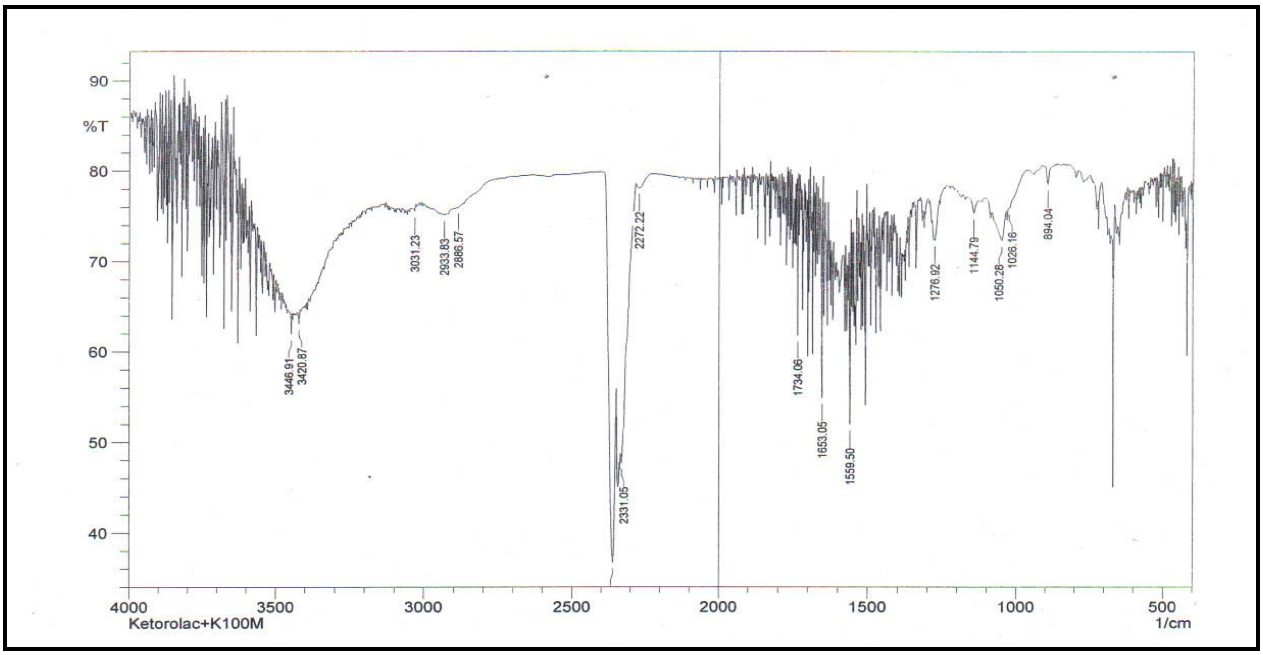

Figure 3.4: Fourier Transformed Infrared spectrum of Ketorolac Tromethamine and Methocel K100M CR

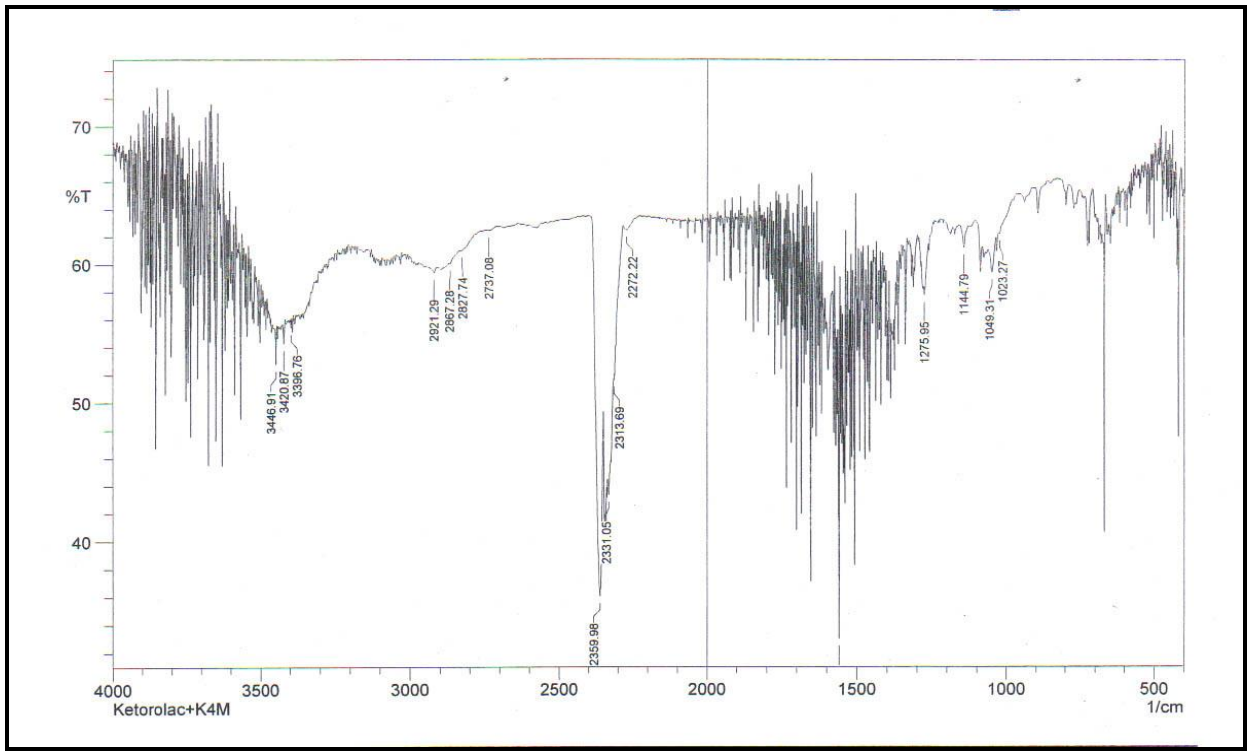

Figure 3.5: Fourier Transformed Infrared spectrum of Ketorolac Tromethamine and Methocel K4M CR

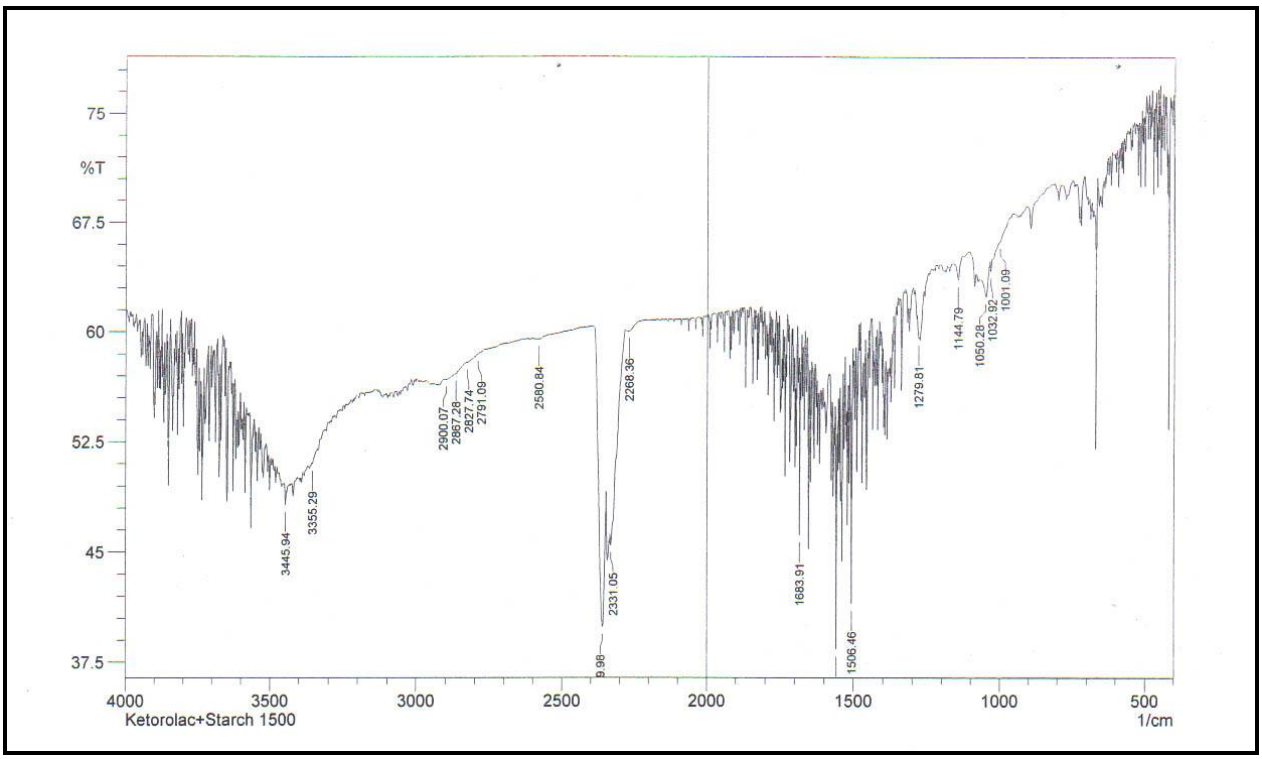

Figure 3.6: Fourier Transformed Infrared spectrum of Ketorolac Tromethamine and Starch 1500

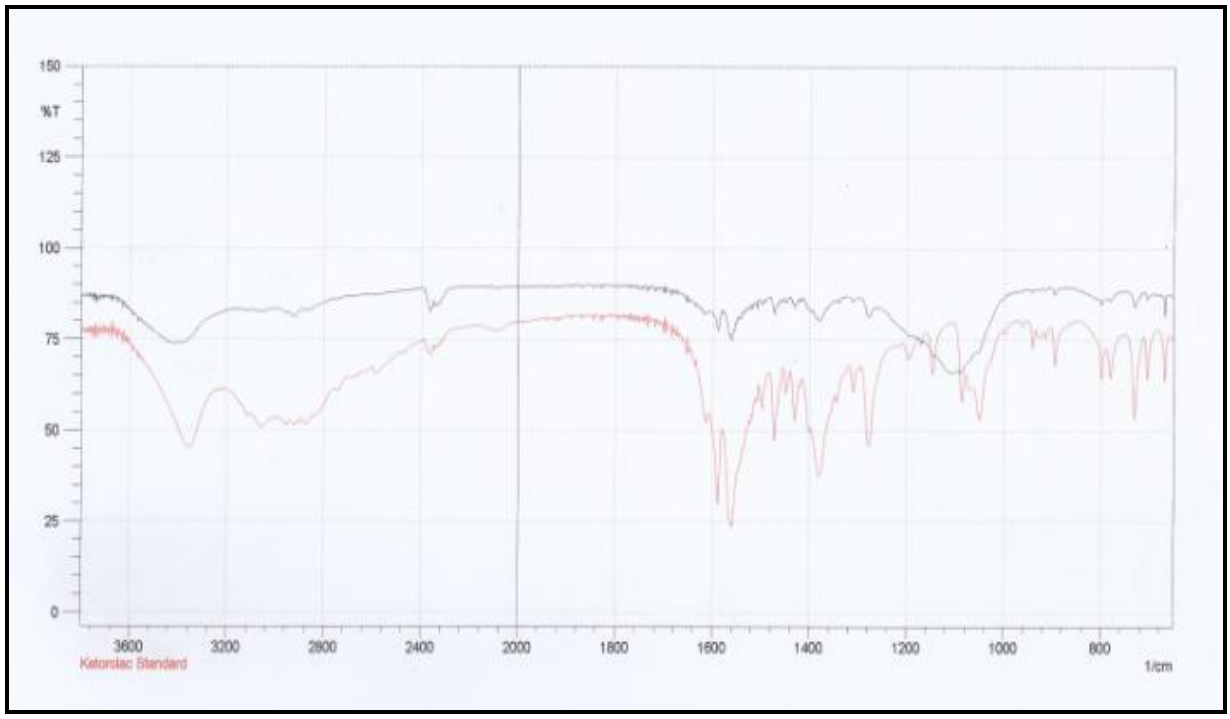

Figure 3.7: Fourier Transformed Infrared spectrum of Ketorolac Tromethamine and Aerosil 200

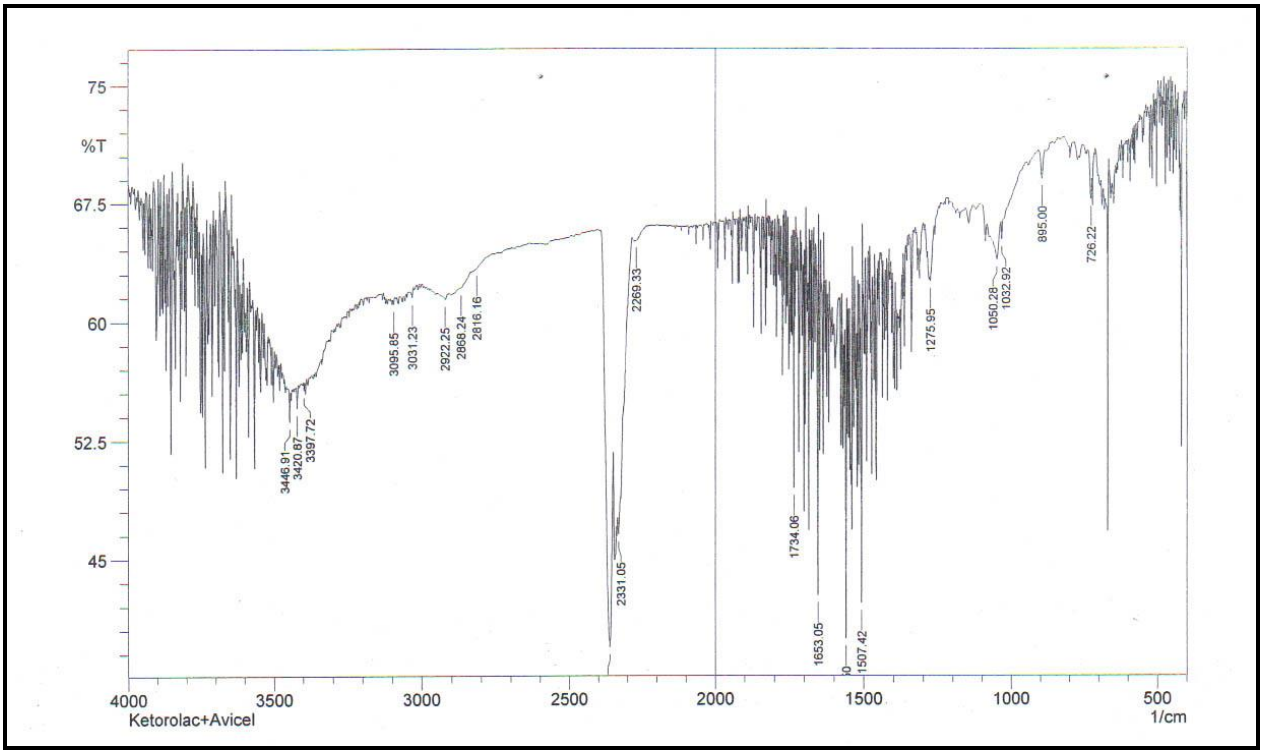

Figure 3.8: Fourier Transformed Infrared spectrum of Ketorolac Tromethamine and Avicel PH 101

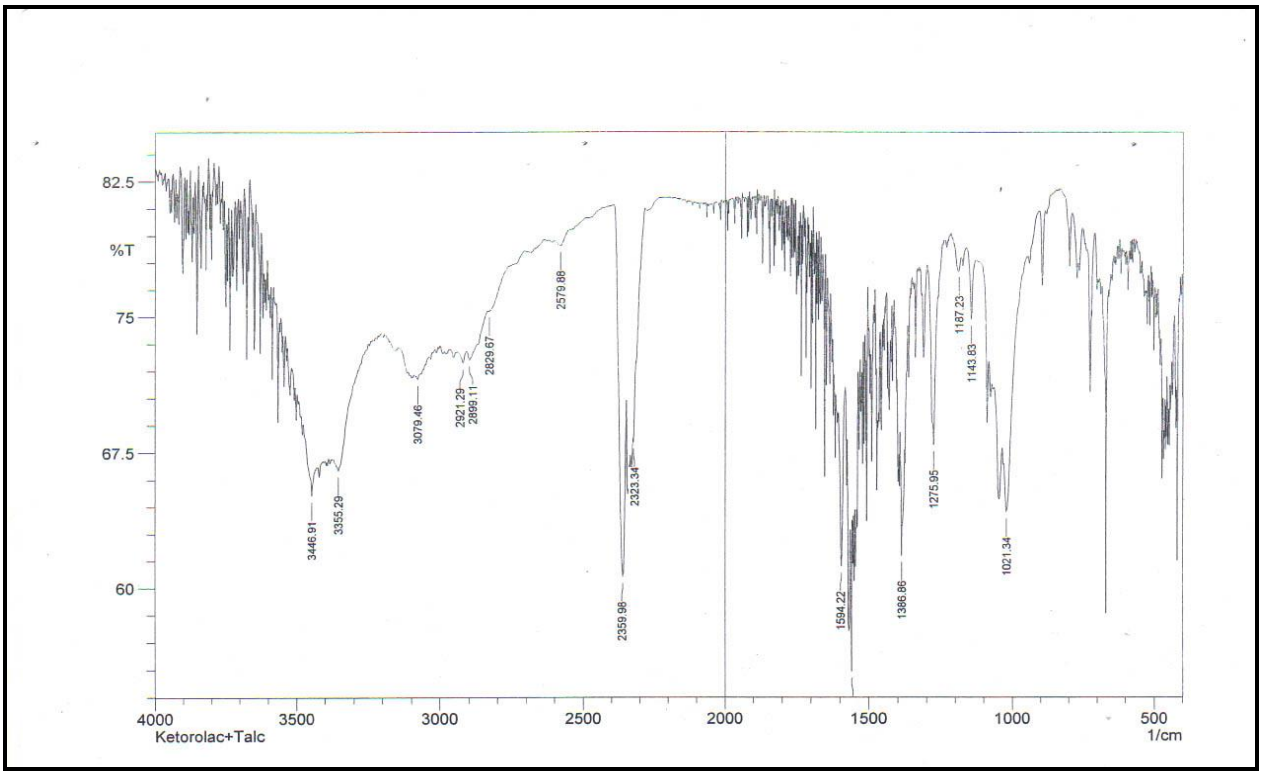

Figure 3.9: Fourier Transformed Infrared spectrum of Ketorolac Tromethamine and Talc

3.2.2 DSC Characterization

DSC is a very effective technique which helps in the identification of any pysicochemical interaction within the drug and excipients used in the formulation development of a drug (Patel, Ahir, Patel, Manani & Patel, 2015). In this method, the thermogram obtained for pure drug is compared with the thermograms obtained for the 1:1 phsical mixture of drug and excipients.

The DSC thermogram of only KT depicted a long and sharp peak at 165.98ºC and this characteristic melting point of the pure drug was found almost same in all the other thermograms with the excipients. This confirmed that there was no interaction between the drug and the excipients. However, there was a slight change in the peak shape, height and width that could be expected due to fusion of excipients that were present in the physical mixture. The thermograms for the physical mixture drug and excipients are represented in Figure 3.10 to Figure 3.16.

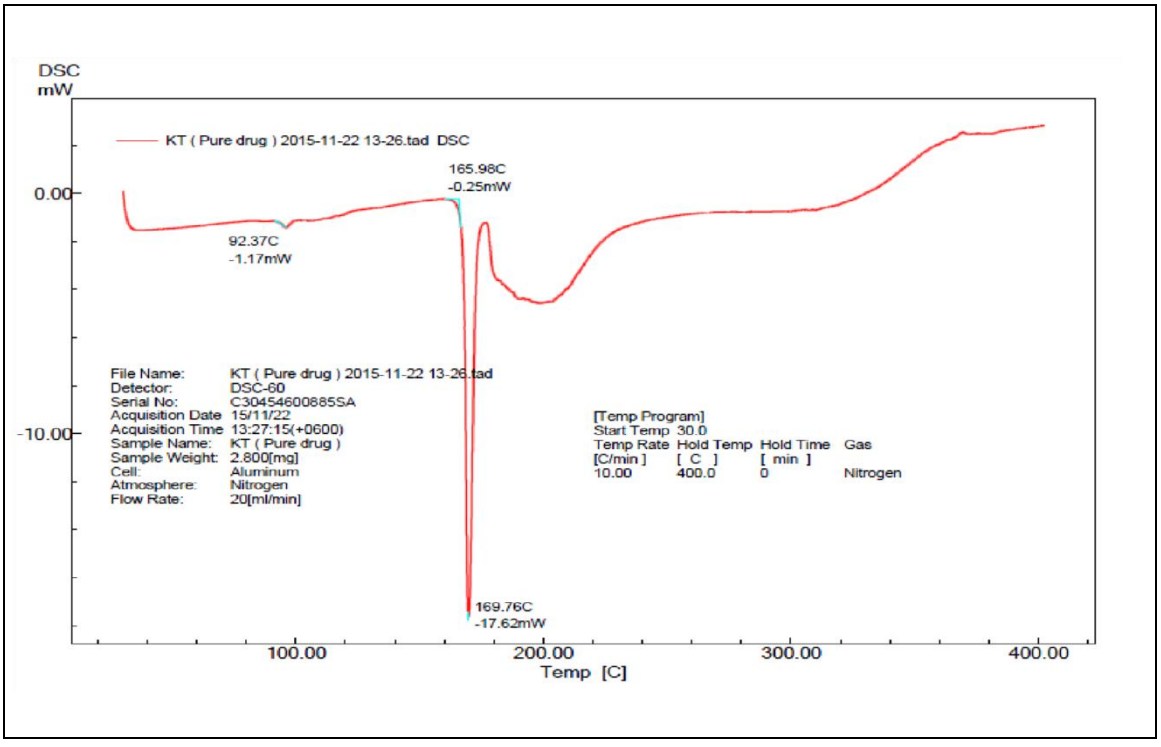

Figure 3.10: Differential Scanning Calorimetry curve for Ketorolac Tromethamine (pure drug)

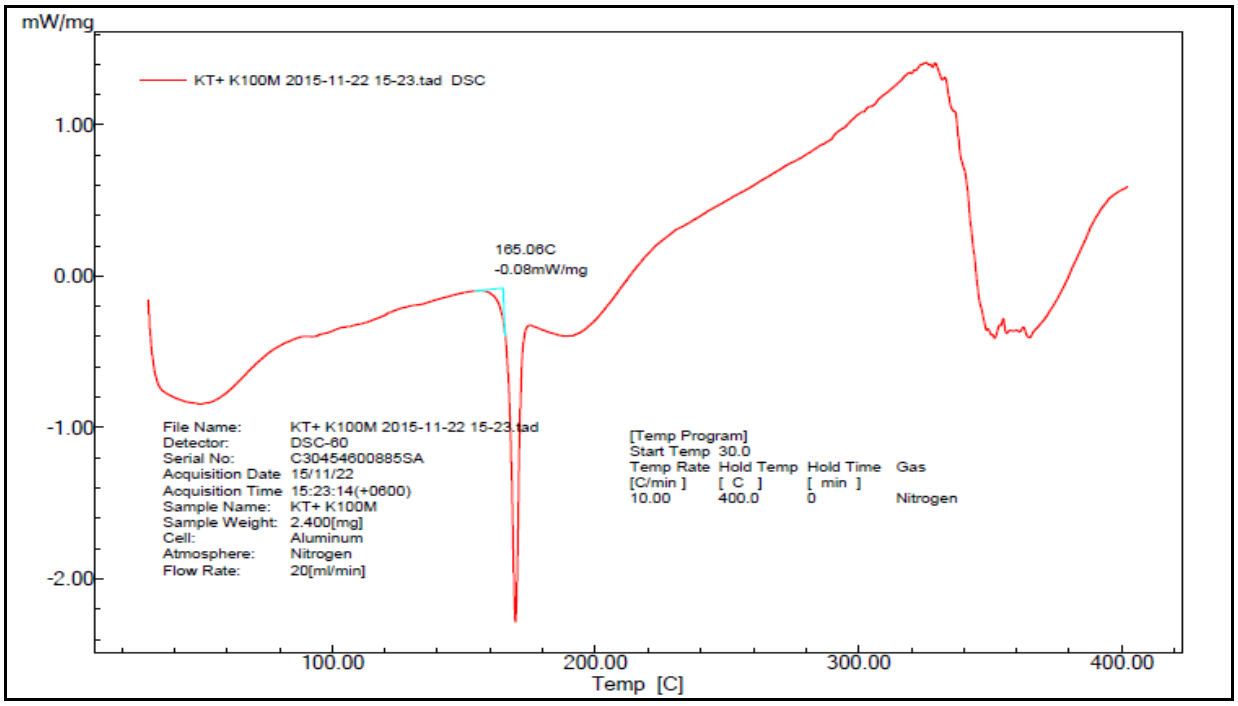

Figure 3.11: Differential Scanning Calorimetry curve for Ketorolac Tromethamine and Methocel K100M CR

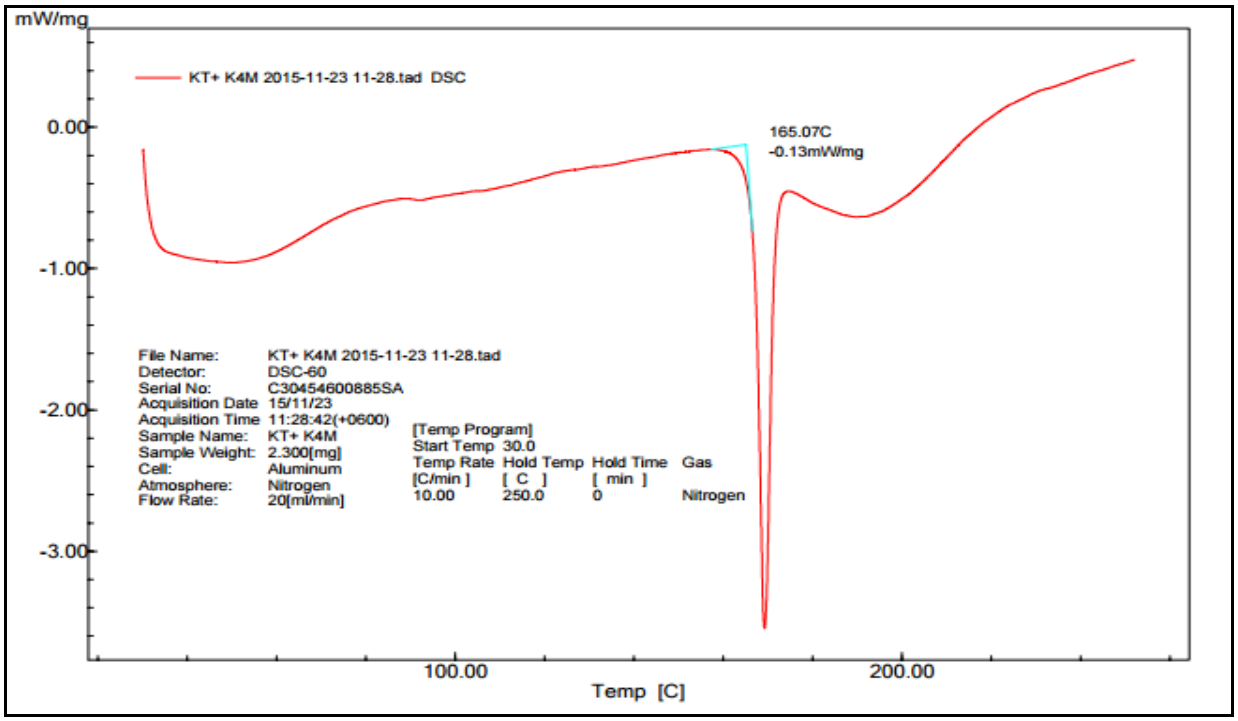

Figure 3.12: Differential Scanning Calorimetry curve for Ketorolac Tromethamine and Methocel K4M CR

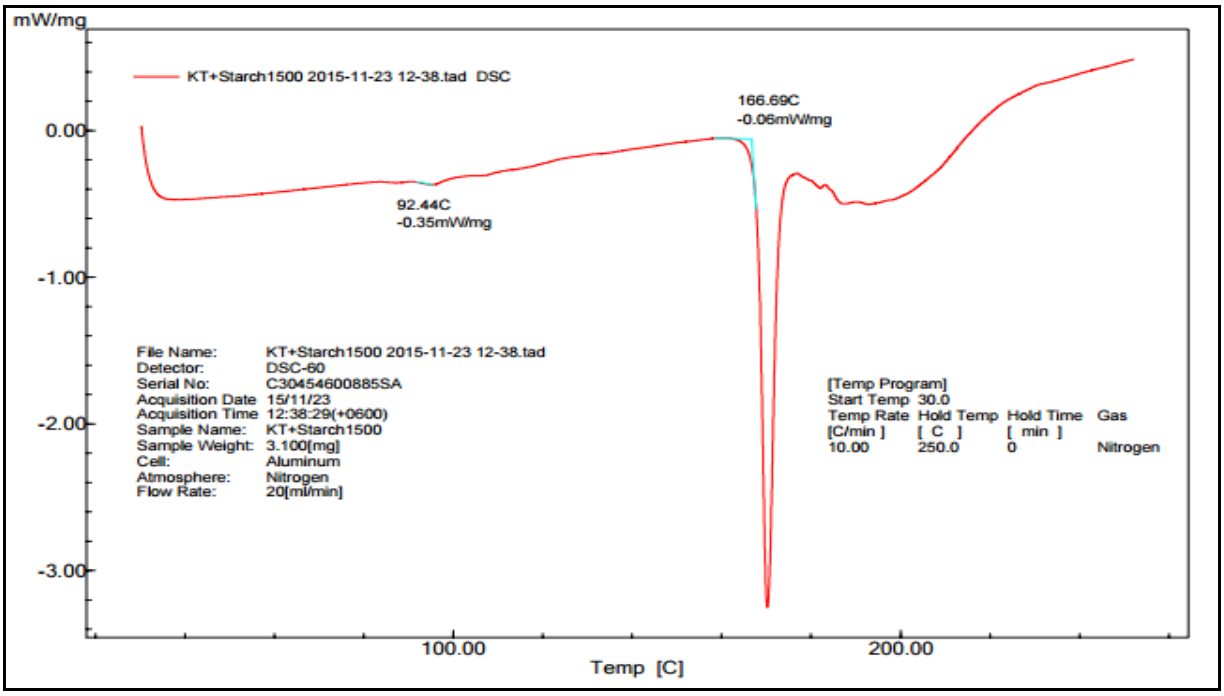

Figure 3.13: Differential Scanning Calorimetry curve for Ketorolac Tromethamine and Starch 1500

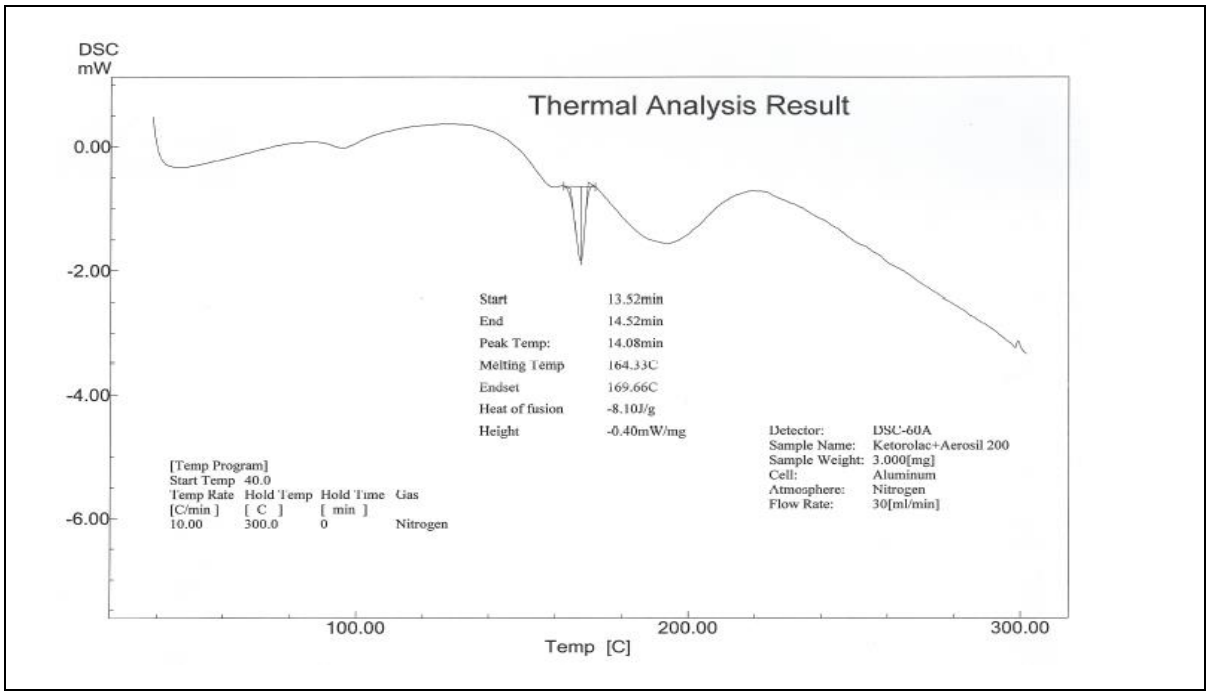

Figure 3.14: Differential Scanning Calorimetry curve for Ketorolac Tromethamine and Aerosil 200

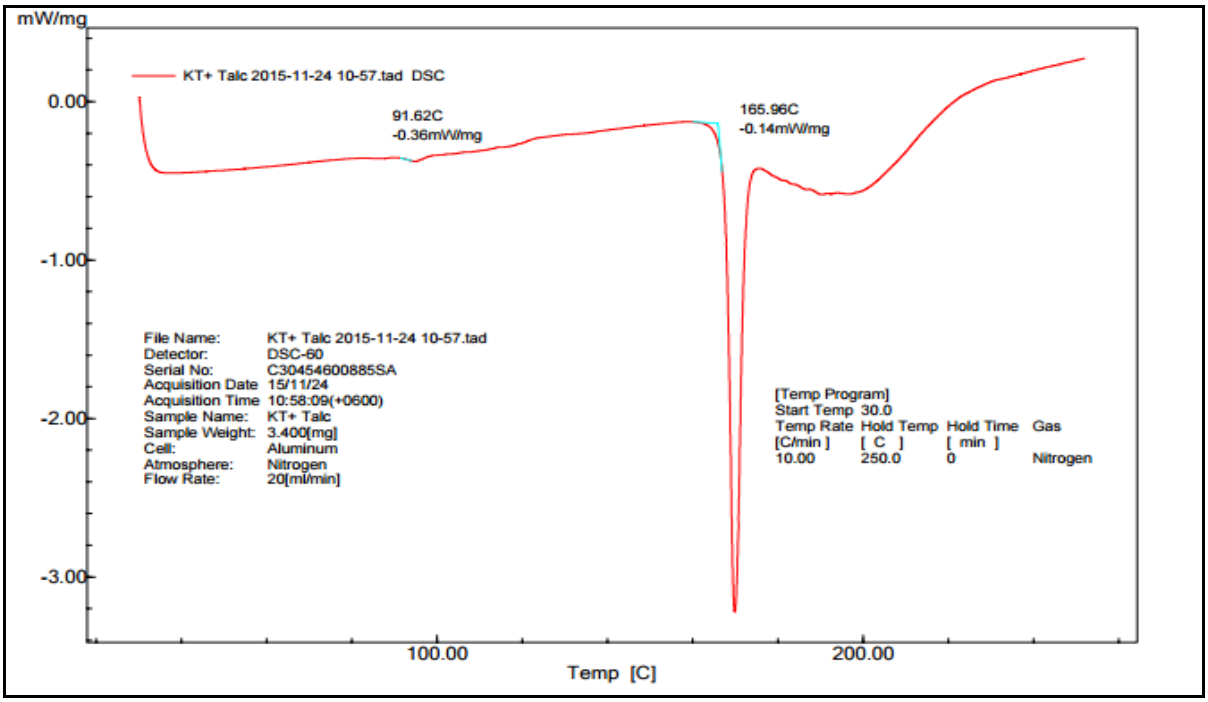

Figure 3.15: Differential Scanning Calorimetry curve for Ketorolac Tromethamine and Talc

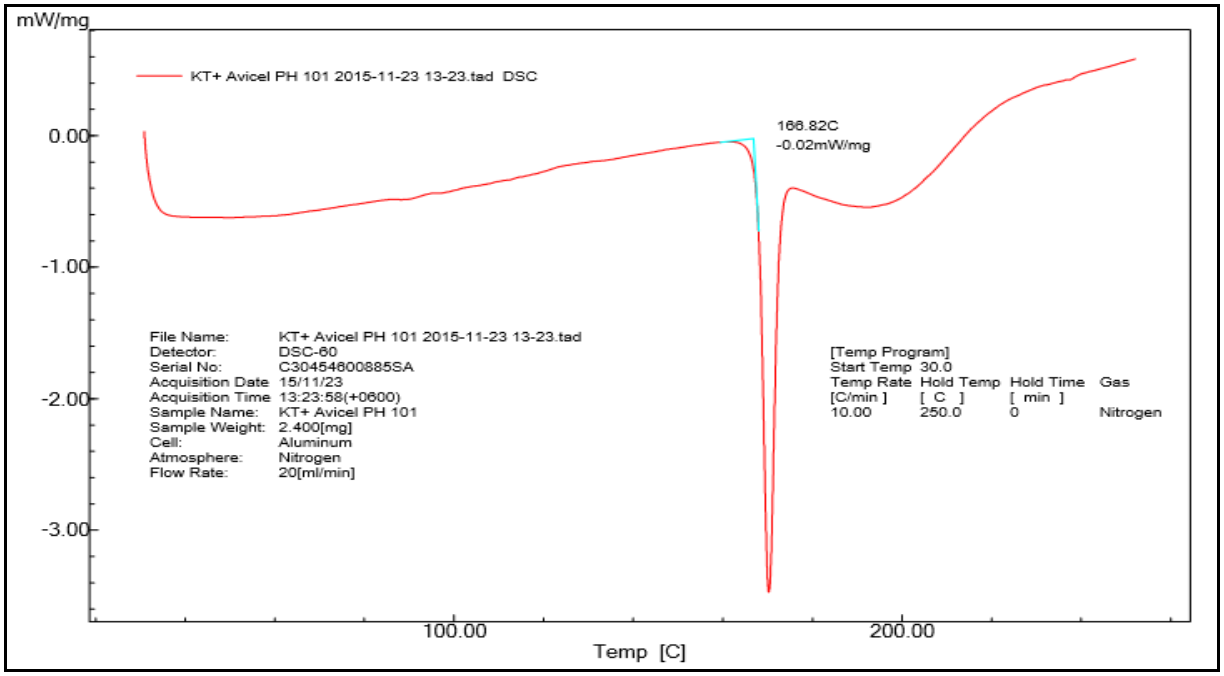

Figure 3.16: Differential Scanning Calorimetry curve for Ketorolac Tromethamine and Avicel PH 101

3.3 Release Kinetic Study

The dissolution profile obtained using a validated RP-HPLC method was employed to determine the release kinetics followed by the nine formulations. The release kinetic models that were studied for this purpose include zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell models (Table 3.3 to 3.7 and Figure 3.17 to 3.21)

3.3.1 Zero Order Plot

Table 3.3: Zero order drug release profile of nine different formulations of Ketorolac Tromethamine tablets

Time (hour)	Cumulative % of drug released								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	7.38	7.76	6.54	8.70	8.03	7.65	7.68	7.66	2.84
1	14.86	14.68	13.26	14.93	14.44	14.10	13.42	15.11	11.68
2	26.28	25.13	22.56	25.35	25.85	26.22	25.37	25.09	22.64
4	45.07	43.09	40.94	43.39	43.65	42.94	41.97	45.9	39.67
6	58.68	58.20	58.38	56.96	55.65	56.27	56.63	59.15	55.53
8	73.03	75.29	67.89	71.98	71.10	70.03	85.45	72.48	62.28

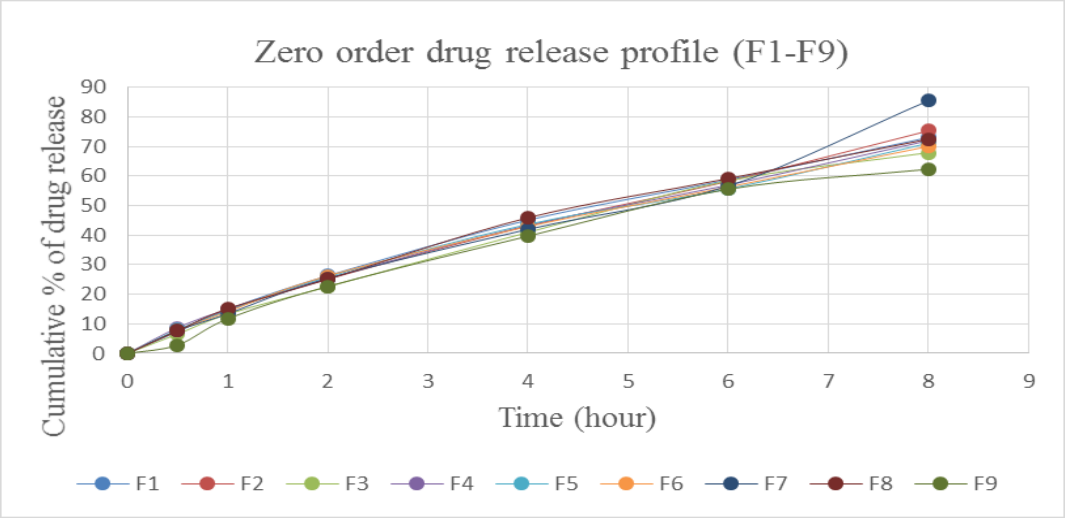

Figure 3.17: Zero order release kinetic plot of Ketorolac Tromethamine tablets

3.3.2 First Order Plot

Table 3.4: First order drug release profile of nine different formulations of Ketorolac Tromethamine tablets

Time (hour)	Log cumulative % of drug remaining								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
0.5	1.97	1.96	1.97	1.96	1.96	1.97	1.97	1.97	1.99
1	1.93	1.93	1.94	1.93	1.93	1.93	1.94	1.93	1.95
2	1.87	1.87	1.89	1.87	1.87	1.87	1.87	1.87	1.89
4	1.74	1.76	1.77	1.75	1.75	1.76	1.76	1.73	1.78
6	1.62	1.62	1.62	1.63	1.65	1.64	1.64	1.61	1.65
8	1.43	1.39	1.51	1.45	1.46	1.48	1.16	1.44	1.56

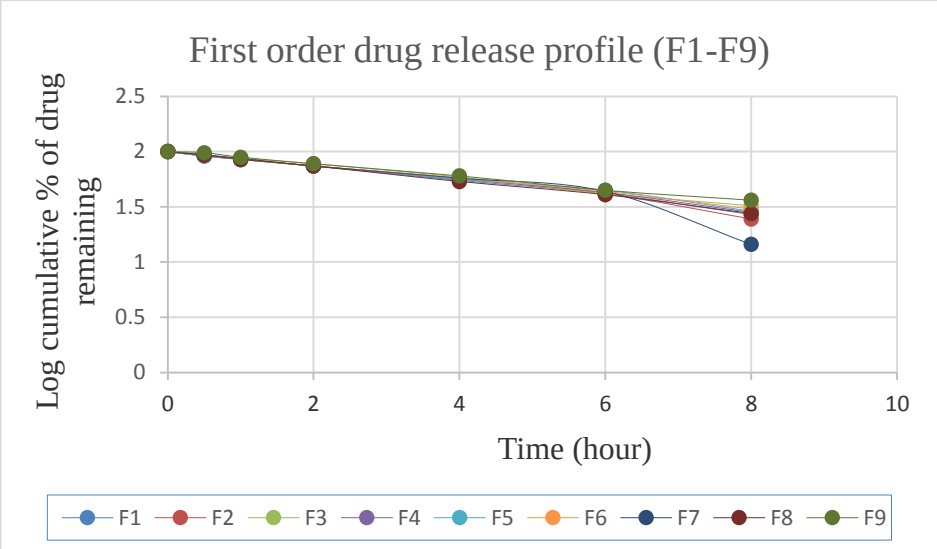

Figure 3.18: First order release kinetic plot of Ketorolac Tromethamine tablets

3.3.3 Higuchi Plot

Table 3.5: Higuchi drug release profile of nine different formulations of Ketorolac Tromethamine tablets

Square root of time (hour)	Cumulative % of drug released								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.71	7.38	7.76	6.54	8.70	8.03	7.65	7.68	7.66	2.84
1	14.86	14.68	13.26	14.93	14.44	14.10	13.42	15.11	11.68
1.41	26.28	25.13	22.56	25.35	25.85	26.22	25.37	25.09	22.64
2	45.07	43.09	40.94	43.30	43.65	42.94	41.97	45.9	39.67
2.44	58.68	58.20	58.38	56.96	55.65	56.27	56.63	59.15	55.53
2.83	73.03	75.29	67.89	71.98	71.10	70.03	85.45	72.48	62.28

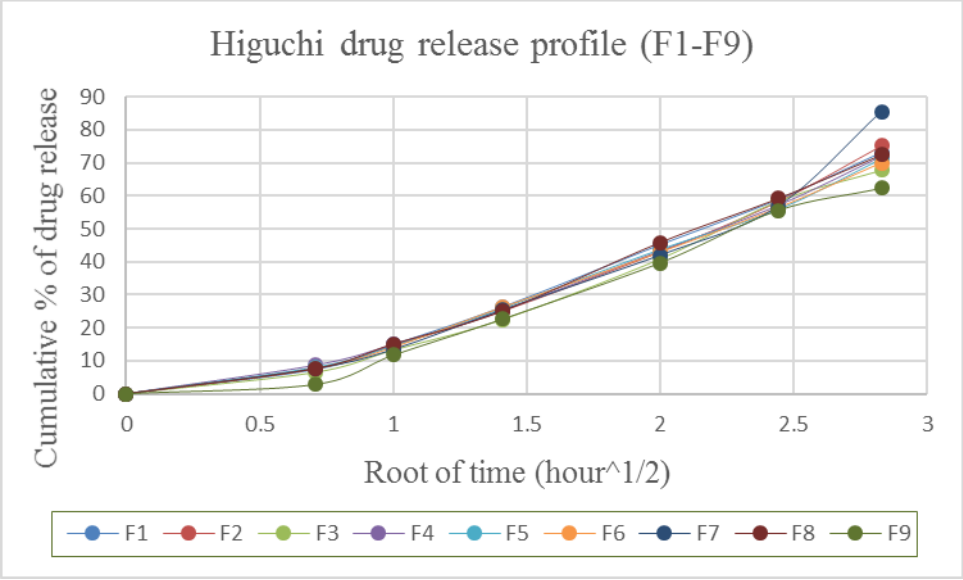

Figure 3.19: Higuchi release kinetic plot of Ketorolac Tromethamine tablets

3.3.4 Korsmeyer-Peppas Plot

Table 3.6: Korsmeyer-Peppas drug release profile of nine different formulations of Ketorolac Tromethamine tablets

Log of time (hour)	Log fraction of drug released								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
-0.3	-1.13	-1.11	-1.18	-1.06	-1.099	-1.17	-1.11	-1.12	-1.55
0	-0.83	-0.83	-0.88	-0.83	-0.84	-0.85	-0.87	-0.82	-0.93
0.3	-0.58	-0.56	-0.65	-0.56	-0.59	-0.58	-0.59	-0.60	-0.65
0.6	-0.35	-0.37	-0.39	-0.36	-0.36	-0.37	-0.38	-0.34	-0.4
0.78	-0.23	-0.24	-0.23	-0.24	-0.25	-0.25	-0.25	-0.223	-0.26
0.9	-0.14	-0.12	-0.17	-0.14	-0.15	-0.15	-0.07	-0.14	-0.20

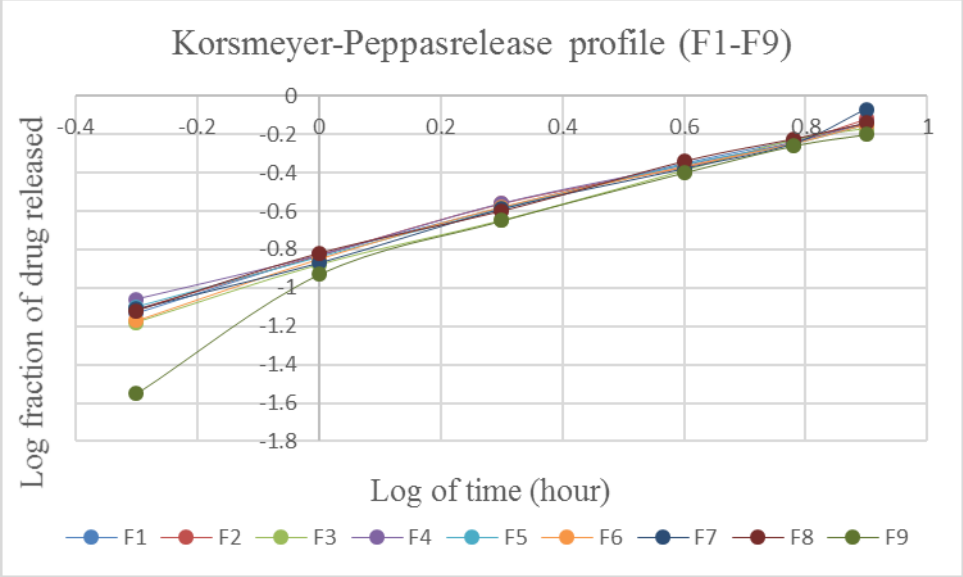

Figure 3.20: Korsmeyer-Peppas release kinetic plot of Ketorolac Tromethamine tablets

3.3.5 Hixson-Crowell Plot

Table 3.7: Hixson-Crowell drug release profile of nine different formulations of Ketorolac Tromethamine tablets

Time (hour)	Cubic root of total amount of drug (%)-Cubic root of drug remaining (%)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.12	0.12	0.1	0.14	0.13	0.12	0.12	0.12	0.04
1	0.24	0.24	0.21	0.24	0.24	0.24	0.21	0.25	0.19
2	0.45	0.43	0.37	0.43	0.44	0.45	0.43	0.43	0.38
4	0.84	0.79	0.74	0.84	0.81	0.78	0.77	0.86	0.72
6	1.13	1.17	1.17	1.14	1.10	1.12	1.13	1.19	1.10
8	1.64	1.73	1.46	1.60	1.57	1.54	2.20	1.62	1.29

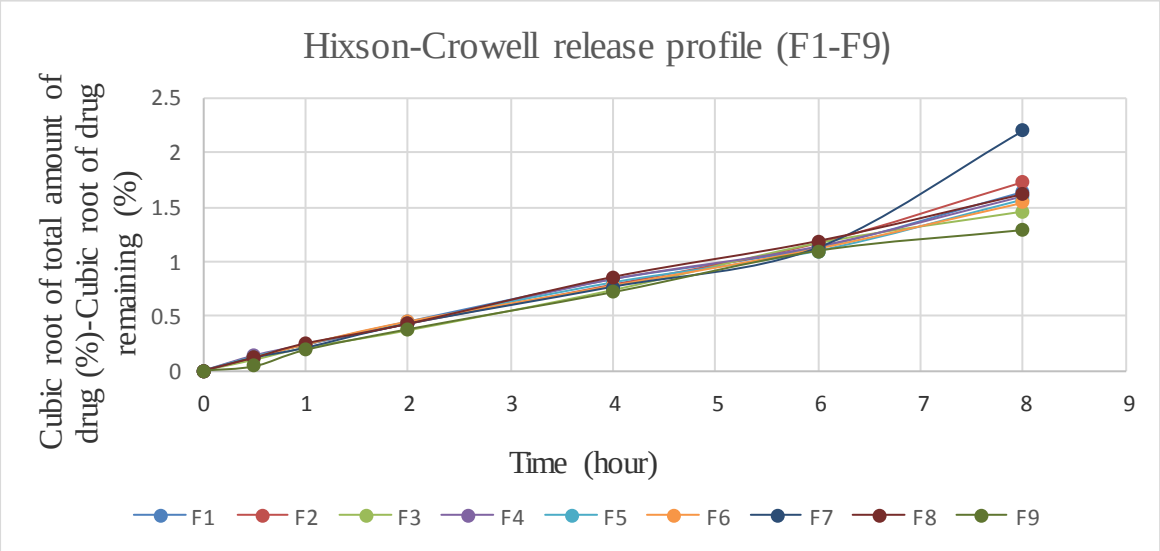

Figure 3.21: Hixson-Crowell release kinetic plot of Ketorolac Tromethamine tablets

Table 3.8: Equations from the plot of release kinetic models for the nine formulations

Formulation	Zero order	First order	Higuchi	Korsmeyer-Peppas	Hixson-Crowell
F1	y=8.9781x+4.6102 R^2 =0.9841	y=0.0689x+2.0058 R^2 =0.9941	y=26.975x-7.8528 R^2 =0.9707	y=0.8136x-0.8525 R^2 =0.9956	y=0.1971x+0.026 R^2 =0.9953
F2	y=9.1324x+3.9719 R^2 =0.993	y=0.0716x+2.01 R^2 =0.9809	y=27.214x-8.3714 R^2 =0.9615	y=0.8015x-0.8429 R^2 =0.9954	y=0.2068x+0.0047 R^2 =0.9943
F3	y=8.6052x+3.5083 R^2 =0.986	y=0.062x+2.0047 R^2 =0.9978	y=25.71x-8.2223 R^2 =0.9617	y=0.8449x-0.9044 R^2 =0.9974	y=0.1851x+0.01 R^2 =0.9979
F4	y=8.7173x+4.8283 R^2 =0.9871	y=0.0659x+2.001 R^2 =0.9941	y=26.153x-7.2162 R^2 =0.9708	y=0.7628x-0.8215 R^2 =0.9977	y=0.1935x+0.0327 R^2 =0.9972
F5	y=8.615x+4.7853 R^2 =0.984	y=0.064x+1.9993 R^2 =0.9909	y=25.899x-7.1953 R^2 =0.9717	y=0.7844x-0.8462 R^2 =0.998	y=0.1885x+0.0339 R^2 =0.996
F6	y=8.5637x+4.7271 R^2 =0.983	y=0.063x+2.0006 R^2 =0.9964	y=25.769x-7.2186 R^2 =0.9726	y=0.8293x-0.8768 R^2 =0.9923	y=0.1867x+0.0338 R^2 =0.9976
F7	y=9.9835x+2.2677 R^2 =0.9895	y=0.0919x+2.0452 R^2 =0.8966	y=29.244x-10.275 R^2 =0.9277	y=0.8357x-0.8626 R^2 =0.9947	y=0.2475x+0.0657 R^2 =0.9447
F8	y=8.9685x+4.6524 R^2 =0.983	y=0.0687x+2.0038 R^2 =0.9977	y=26.956x-7.8123 R^2 =0.9703	y=0.8099x-0.8483 R^2 =0.9964	y=0.1989x+0.0277 R^2 =0.9983
F9	y=8.162x+2.7368 R^2 =0.9728	y=0.0571x+2.0067 R^2 =0.9973	y=24.499x-8.5581 R^2 =0.9577	Y=1.0654x-1.0698 R^2 =0.9504	y=0.1692x+0.0116 R^2 =0.9898

3.4 Analysis of R^2 Values and Release Rate Constants for Nine Different Formulations

The release rate constant was calculated for the nine different foemulation from the slope of the appropriate plots and the regression coefficient (R^2) was determined (Table 3.8). In this experiment, the *in-vitro* release profile was found to best fit in the first order kinetic (R^2: 0.981 to 0.998) as it showed high linearity for most of the formulations as followed by zero order (R^2: 0.973 to 0.992) and Higuchi model (R^2: 0.928 to 0.971). The transport mechanism of the drug was also analyzed by Korsmeyer-Peppas equation which helps to describe the drug release behavior from the polymeric systems. Among all the formulations F1, F2, F3, F4, F5, F6, F7 and F8 exhibits high linearity (R^2 >= 0.992), with slope (n) values between 0.763 to 0.845 which indicates that the drug follows a non-Fickian release (diffusion and erosion) from the matrix tablet. Drug release rate constants and R^2 values for nine different formulations are given in Table 3.9.

Table 3.9: Drug release rate constants and R^2 values for nine different formulations

Formulation	Zero order		First order		Higuchi		Korsmeyer-Peppas		Hixson-Crowell	
	K_0	R^2	K	R^2	K_h	R^2	N	R^2	K_{HC}	R^2
F1	8.978	0.984	0.159	**0.994**	26.775	0.971	0.814	**0.996**	0.197	**0.995**
F2	9.132	0.992	0.166	0.981	27.214	0.963	0.802	**0.995**	0.207	**0.994**
F3	8.605	0.986	0.143	**0.998**	25.710	0.962	0.845	**0.997**	0.185	**0.998**
F4	8.717	0.987	0.152	**0.994**	26.153	0.971	0.763	**0.998**	0.194	**0.997**
F5	8.615	0.984	0.147	**0.991**	25.899	0.972	0.784	**0.998**	0.189	**0.996**
F6	8.564	0.983	0.145	**0.996**	25.769	0.973	0.829	**0.992**	0.187	**0.998**
F7	9.984	0.989	0.212	0.896	29.244	0.928	0.836	**0.995**	0.248	0.946
F8	8.969	0.983	0.159	**0.998**	26.956	0.970	0.810	**0.996**	0.199	**0.998**
F9	8.162	0.973	0.131	**0.997**	24.499	0.958	1.065	0.950	0.169	0.989

3.5 Analysis of Successive Fractional Dissolution Time

Successive fractional dissolution time and mean dissolution time of the nine formulations (F1 to F9) of Ketorolac Tromethamine matrix tablets were calculated (Table 3.10) and depicted in Figure 3.22. From the data obtained, it was seen that, with increase in amount of Methocel K4M CR keeping Methocel K100M CR constant, the time taken to release 25%, 50% and 80% of the drug from the matrix tablet for F1 to F3 and F4 to F6 increased, thereby, reflecting retardation of drug release rate. On the other hand, formulations F7 to F9 showed some deviation compared to formulation F1, F2, F3 and F4, F5, F6 in retarding capacity with increase in polymer loading. The mean dissolution time (MDT) for most of the formulations were also found close to each other indicating similar retarding efficiency at each polymer combinations.

Table 3.10: Successive fractional dissolution time of Sustained Release Ketorolac Tromethamine tablets for nine formulations

Formulation	$T_{25\%}$ (hour)	$T_{50\%}$(hour)	$T_{80\%(}$hour)	MDT (hour)
F1	2.06	4.78	8.50	5.02
F2	2.01	4.76	8.56	5.03
F3	2.27	5.16	8.99	5.37
F4	1.94	4.80	8.89	5.17
F5	2.06	4.98	9.07	5.29
F6	2.14	4.94	8.71	5.17
F7	2.05	4.70	8.25	4.91
F8	2.01	4.73	8.45	4.98
F9	0.94	5.28	8.21	5.21

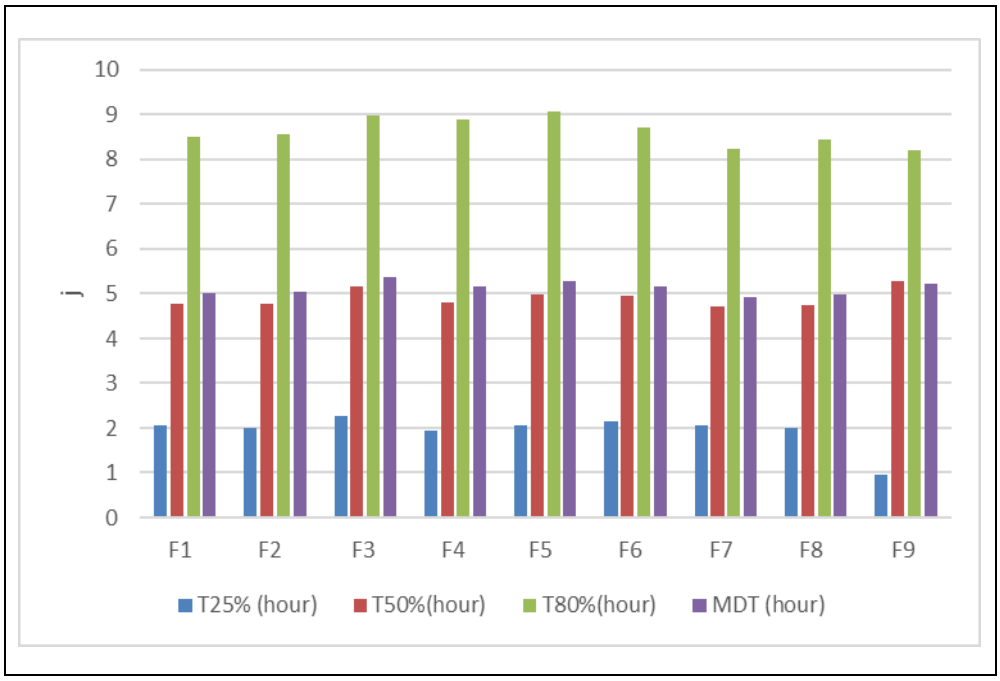

Figure 3.22: Comparison of $T_{25\%}$, $T_{50\%}$, $T_{80\%}$ and MDT values

3.6 Optimization of Sustained Release Formulation by Response Surface Methodology

3.6.1 Summary Design

Optimization is a process that facilitates the selection of the best formulation among the other possible formulations. This technique is usually implemented to save time and reduce cost in the process of drug development and manufacturing. The optimization of sustained release Ketorolac Tromethamine matrix tablets was carried out by using a three level, two factor (3^2) full factorial design where two factors were chosen as independent variable such as Methocel K100M CR (A) as factor 1 and Methocel K4M CR(B) as factor 2. For the purpose of optimization, low (-1), medium (0) and high (+1) were selected as the three coded levels. The release data of sustained release KT for each of the nine formulations were inserted into the Design Expert Software to analyze the effects of polymer on drug release at 1st, 4th and 8th hour of dissolution (Figure 3.23 to Figure 3.25).

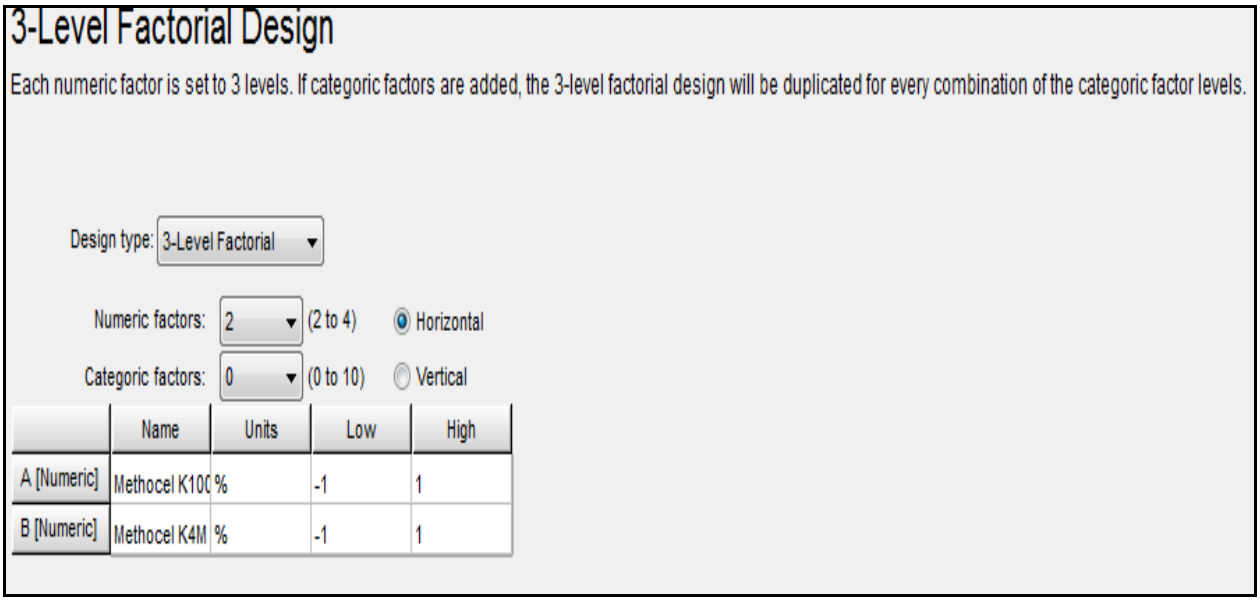

Figure 3.23: 3-level factorial design

Select	Std	Run	Factor 1 A:Methocel K10... %	Factor 2 B:Methocel K4M... %	Response 1 Response at 1s... %	Response 2 Response at 4t... %	Response 3 Response at 8t... %
	1	1	-1	-1	14.86	45.07	73.03
	2	2	0	-1	14.93	43.3	71.98
	10	3	0	0	14.44	43.65	71.1
	4	4	-1	0	14.68	43.09	75.29
	3	5	1	-1	13.42	41.97	85.45
	7	6	-1	1	13.26	40.94	67.89
	9	7	1	1	11.68	39.67	62.28
	6	8	1	0	15.11	45.9	72.48
	8	9	0	1	14.1	42.94	70.03
	5	10	0	0	14.44	43.65	71.1

Figure 3.24: The actual experimental design

Design Summary

File Version	10.0.5.0		
Study Type	Response Surface	Subtype	Randomized
Design Type	3 Level Factorial	Runs	10
Design Model	Quadratic	Blocks	No Blocks

Factor	Name	Units	Type	Subtype	Minimum	Maximum	Coded Values	Mean	Std. Dev.
A	Methocel K100	%	Numeric	Continuous	-1	1	-1.000=-1 1.000=1	0	0.816497
B	Methocel K4M	%	Numeric	Continuous	-1	1	-1.000=-1 1.000=1	0	0.816497

Response	Name	Units	Obs	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Trans	Model
R1	Response at 1	%	10	Polynomial	11.68	15.11	14.092	1.04764	1.29366	None	Linear
R2	Response at 4	%	10	Polynomial	39.67	45.9	43.018	1.82671	1.15705	None	Linear
R3	Response at 8	%	10	Polynomial	62.28	85.45	72.063	5.86752	1.37203	None	2FI

Figure 3.25: The design summary

3.6.2 Graph Column

The graph columns depicted in Figure 3.26 to 3.31 represents a plot of response at 1st, 4th and 8th hour versus each of the experimental factors, that is, Methocel K100M CR and Methocel K4M CR respectively. Each of these plots explains the individual influence of one factor on the percentage release of drug from the matrix tablets. This relationship between the response at three different times and the factors is indicated by the correlation coefficient values obtained from the interpretation of the graph column by the software after the input of the percentage release data into the Design Expert Software. The coefficient of correlation is a standardized measure of the strength of relationship between two variables which can take any value from -1 through 0 to +1. -1 indicates as one variable changes, the other changes in the opposite direction by the same amount; 0 indicates as one variable changes, the other doesn't change at all; and +1 indicates as one variable changes, the other changes in the same direction by the same amount. From the analysis of the graph columns for the independent variable of Methocel K100M CR, the correlation coefficient was found to be -0.336, -0.116 and 0.093 at 1st, 4th and 8th hour

respectively. As a result, it was concluded that the response at 1st and 4th hour decreased slightly with the increase in the amount of Methocel K100M CR whereas the release weakly increased at 8th hour. In case of graph columns with independent variable of Methocel K4M CR, the correlation coefficient was found to be -0.542, -0.506 and -0.702 at 1st, 4th and 8th hour respectively. From the interpretation of this result, it was concluded that the response at 1st, 4th and 8th hour moderately slowed down with the increase in the quantity of Methocel K4M CR.

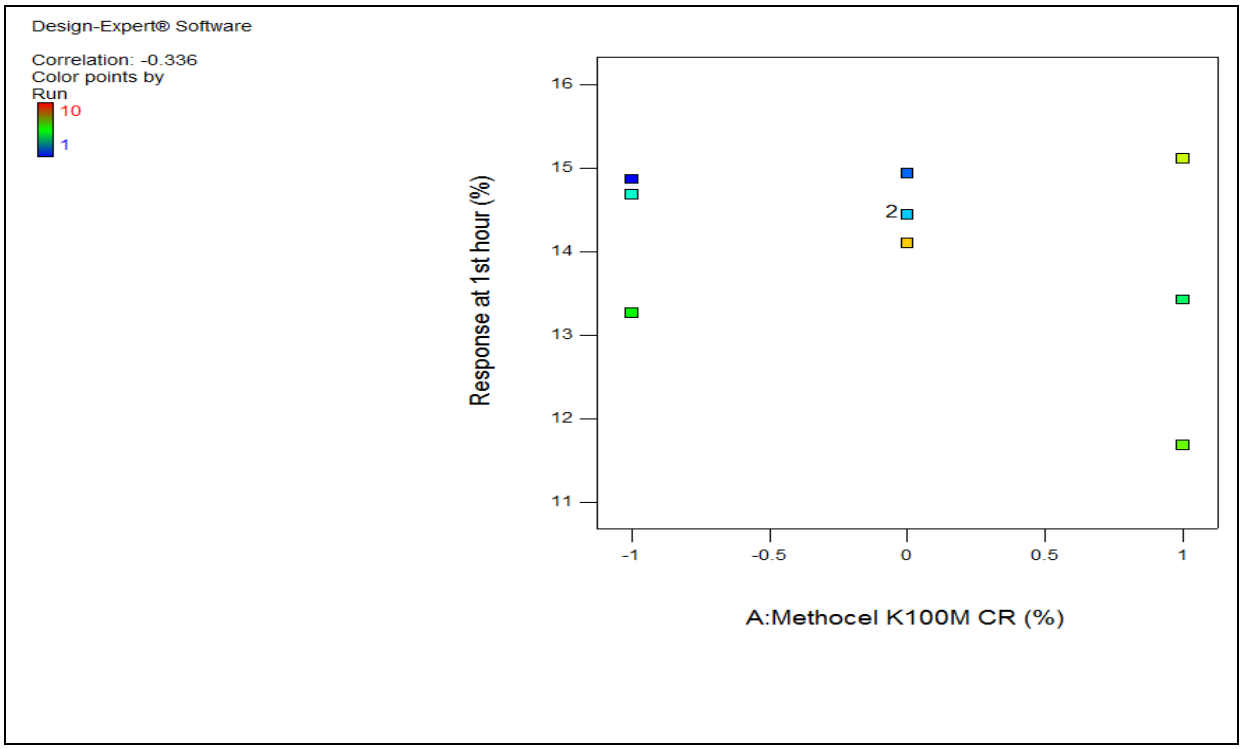

Figure 3.26: The graph column of Methocel K100M CR (A) at 1st hour

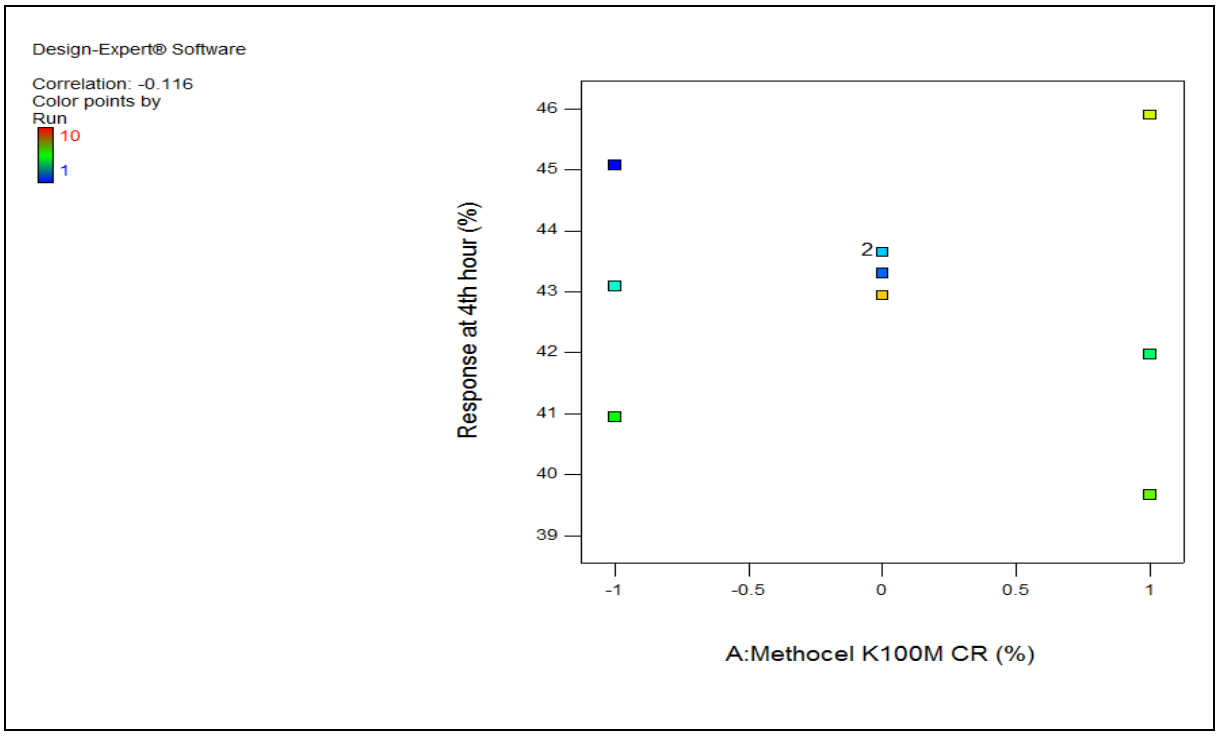

Figure 3.27: The graph column of Methocel K100M CR (A) at 4th hour

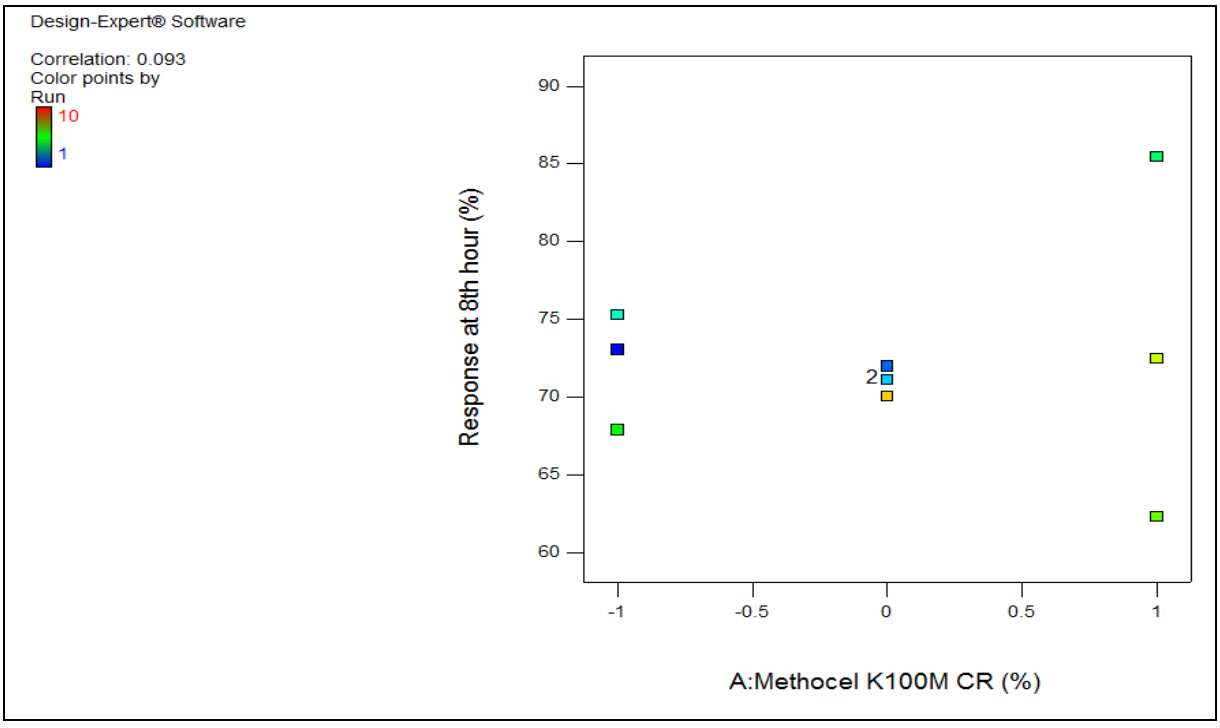

Figure 3.28: The graph column of Methocel K100M CR (A) at 8th hour

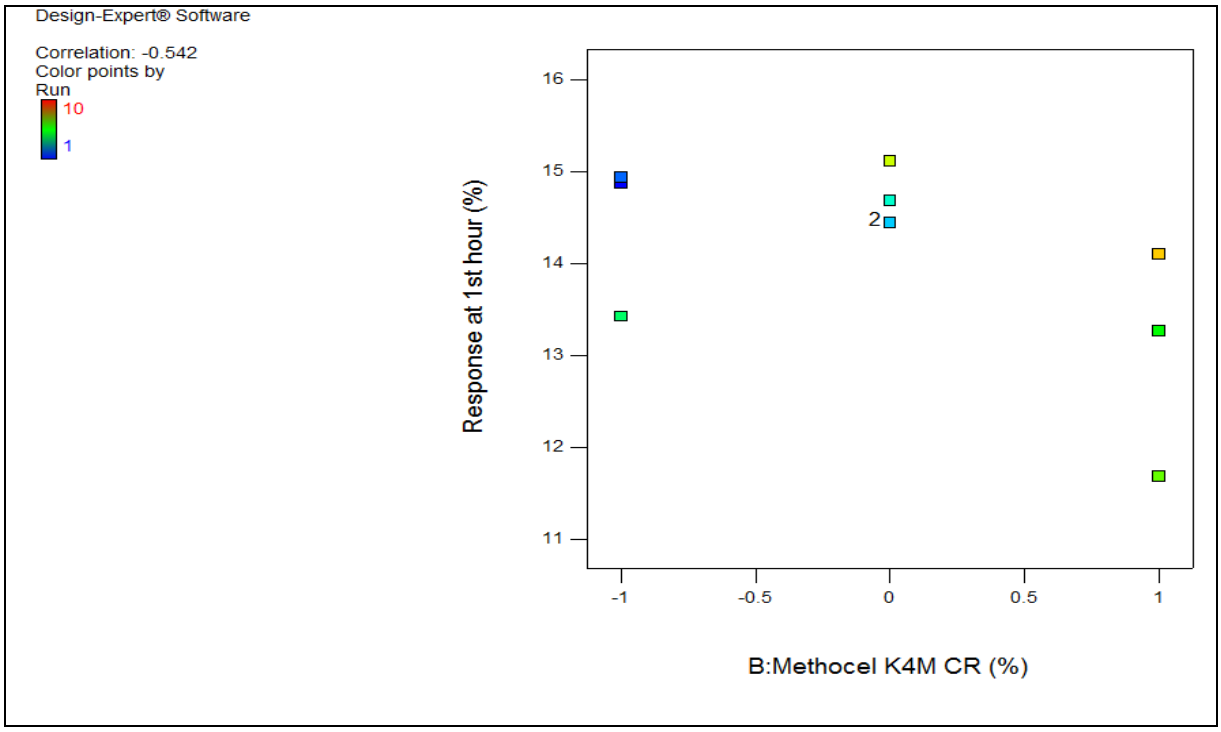

Figure 3.29: The graph column of Methocel K4M CR (B) at 1st hour

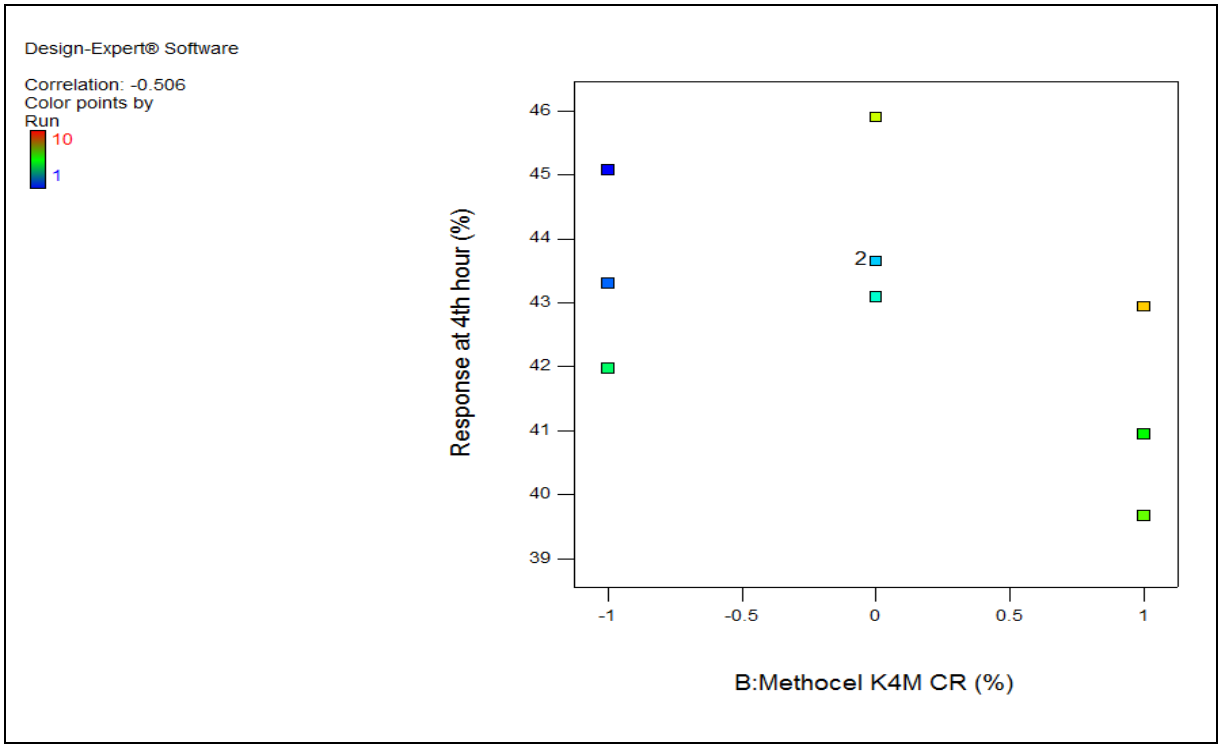

Figure 3.30: The graph column of Methocel K4M CR (B) at 4th hour

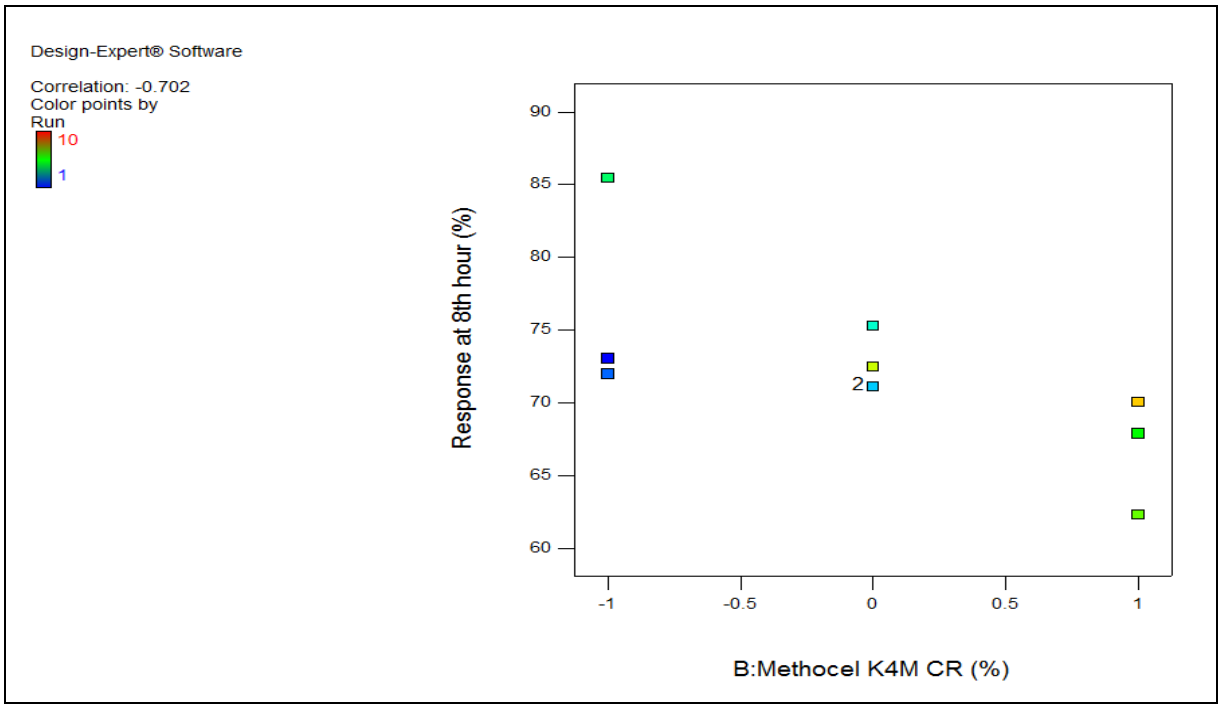

Figure 3.31: The graph column of Methocel K4M CR (B) at 8th hour

3.6.3 Analysis of 3-level, 2-factor (3^2) Full Factorial Design

Polynomial equation of 3^2 full factorial designs is represented by the following general equation:

$$Y = b_0 + b_1A + b_2B + b_3AB + b_4A^2 + b_5B^2$$

where,

Y = Dependent variable,

A= Factor 1 (independent variable),

B= Factor 2 (independent variable),

b_0= Intercept representing the arithmetic mean response of the 9 runs,

b_1 to b_5 = Estimated coefficients computed from the observed experimental response values of Y.

Different polynomial models such as linear, two factor interaction or quadratic equations were generated by the design expert software to determine the influence of the polymers on the response variables. Fit summary, ANOVA, diagnostics and model graphs were analyzed for particular sampling times.

3.6.3.1 Response at the 1st hour

At the 1st hour, for each source of terms (intercept, A, B, AB, A^2, B^2), the probability value (p-value) was examined to select the statistical model that describes the response in the best possible way. The design expert software suggested that the release at the 1st hour (Y_{1hr}) followed a linear model as the p-value was found to be 0.1610 (Figure 3.32). Lack of fit test and model summary statistics checked by the software also did not suggest significant lack of fitness for the linear model as it shows standard deviation (Figure 3.33).

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	1985.84	1	1985.84			Suggested
Linear vs Mear	4.02	2	2.01	2.40	0.1610	Suggested
2FI vs Linear	4.900E-003	1	4.900E-003	5.020E-003	0.9458	
Quadratic vs 2	2.78	2	1.39	1.81	0.2754	
Cubic vs Quad	1.49	2	0.74	0.94	0.5154	Aliased
Residual	1.58	2	0.79			
Total	1995.72	10	199.57			

Figure 3.32: Sequential model for response at 1st hour

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.92	0.4066	0.2370	-0.4665	14.49	Suggested
2FI	0.99	0.4071	0.1106	-2.5298	34.87	
Quadratic	0.88	0.6888	0.2998	-1.7451	27.12	
Cubic	0.89	0.8396	0.2783	-21.5627	222.87	Aliased

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Linear	5.86	6	0.98		
2FI	5.86	5	1.17		
Quadratic	3.07	3	1.02		
Cubic	1.58	1	1.58		
Pure Error	0.000	1	0.000		

Figure 3.33: Fit summary tests report for response at 1st hour

The response at 1st hour was also statistically interpreted by analysis of variance (ANOVA) at the 5% significance level and also confirmed the adequacy of linear model to explain the relationship between the dependent and independent variables. Values of the prob>F value less than 0.05 indicate model terms are significant. The p-value for the model was found to be 0.1610.The F value which was 2.40 also implies that the model is not significant. There is a 16.10% chance that F value this large could occur because of noise. Adequate precision measures the signal to the noise. The desirable ratio for adequate precision is greater than 4. The adequate precision was found to be 4.496 that indicates that the model generated by the software can still be used to determine the effect of the polymers on drug release as it gives adequate precision value greater than 4 (Figure 3.34).

Response 1 Response at 1st hour

ANOVA for Response Surface Linear model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	4.02	2	2.01	2.40	0.1610	not significant
A-Methocel k	*1.12*	*1*	*1.12*	*1.34*	*0.2858*	
B-Methocel k	*2.90*	*1*	*2.90*	*3.46*	*0.1052*	
Residual	5.86	7	0.84			
Lack of Fit	*5.86*	*6*	*0.98*			
Pure Error	*0.000*	*1*	*0.000*			
Cor Total	9.88	9				

Std. Dev.	0.92	R-Squared	0.4066
Mean	14.09	Adj R-Squared	0.2370
C.V. %	6.49	Pred R-Square	-0.4665
PRESS	14.49	Adeq Precisior	4.496
-2 Log Likeliho	23.04	BIC	29.95
		AICc	33.04

Figure 3.34: ANOVA for response surface linear model at 1st hour

The polynomial equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. The level of the factors are denoted as +1(high-level), 0 (mid-level) and -1 (low-level). The equation obtained from the Design Expert Software is useful for determining the relative impact of the factors by comparing the factor coefficient.

The predicted equation for response at 1st hour in terms of coded factors is:

$$Y_{1hr} = + 14.09 - 0.43A - 0.69B$$

The positive sign prior to the factor coefficient indicates accelerating effect and the negative sign indicates the retarding effect of the factors on the response variables. According to the equation, coefficient b_1 and b_2 contains negative sign which means that both A and B decreased the response. The effect of Methocel K4M CR (0.69) is greater than the Methocel K100M CR (0.43) on sustaining the release of drug at the 1st hour. The linear model generated two dimensional (2D) contour plot (Figure 3.35) and three dimensional (3D) surface response plot (Figure 3.36) for the 1st hour. The two dimensional contour plot as well as the three dimensional response surface plot shows the effect of polymer on drug release at the 1st hour. In these two plots, it can be seen that with increase in the polymer concentration, the response values are moving from red zone towards green zone and since red and green represents high and moderate percentage of drug release respectively, the retarding influence of the polymers on the release is evident. However, with the increase in concentration of Methocel K4M CR while keeping the Methocel K100M CR constant, a greater sustaining effect was produced relative to that of Methocel K100M CR.

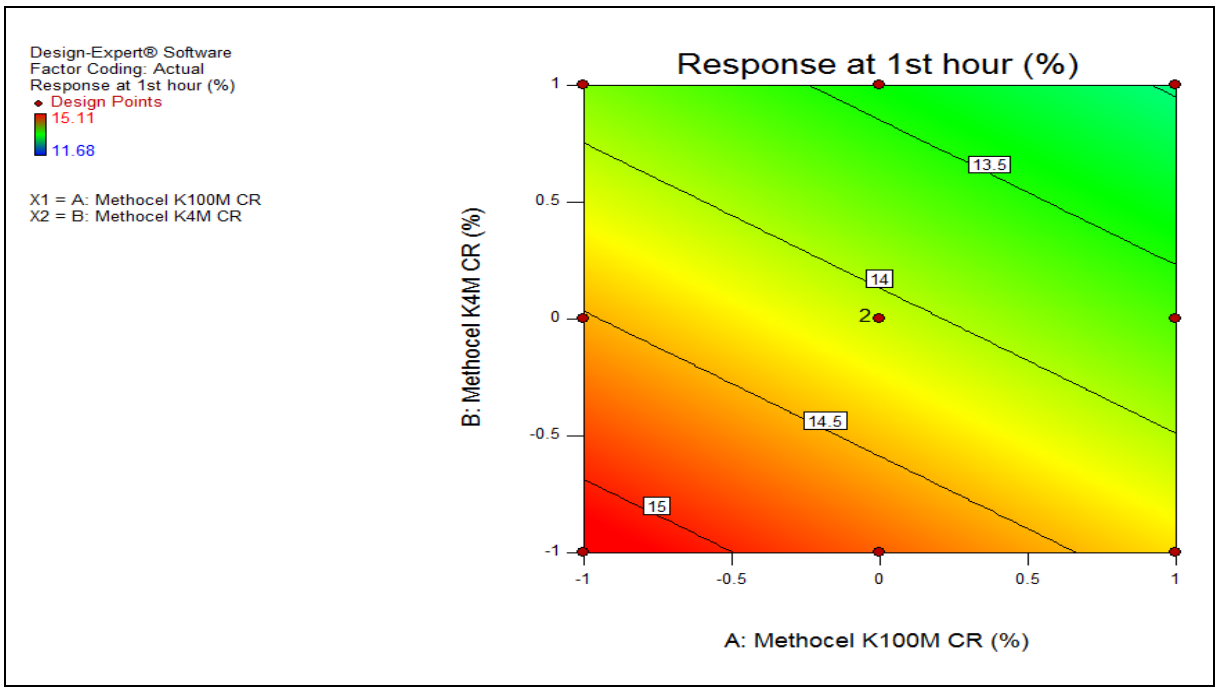

Figure 3.35: The actual 2D contour plot of drug release at 1st hour

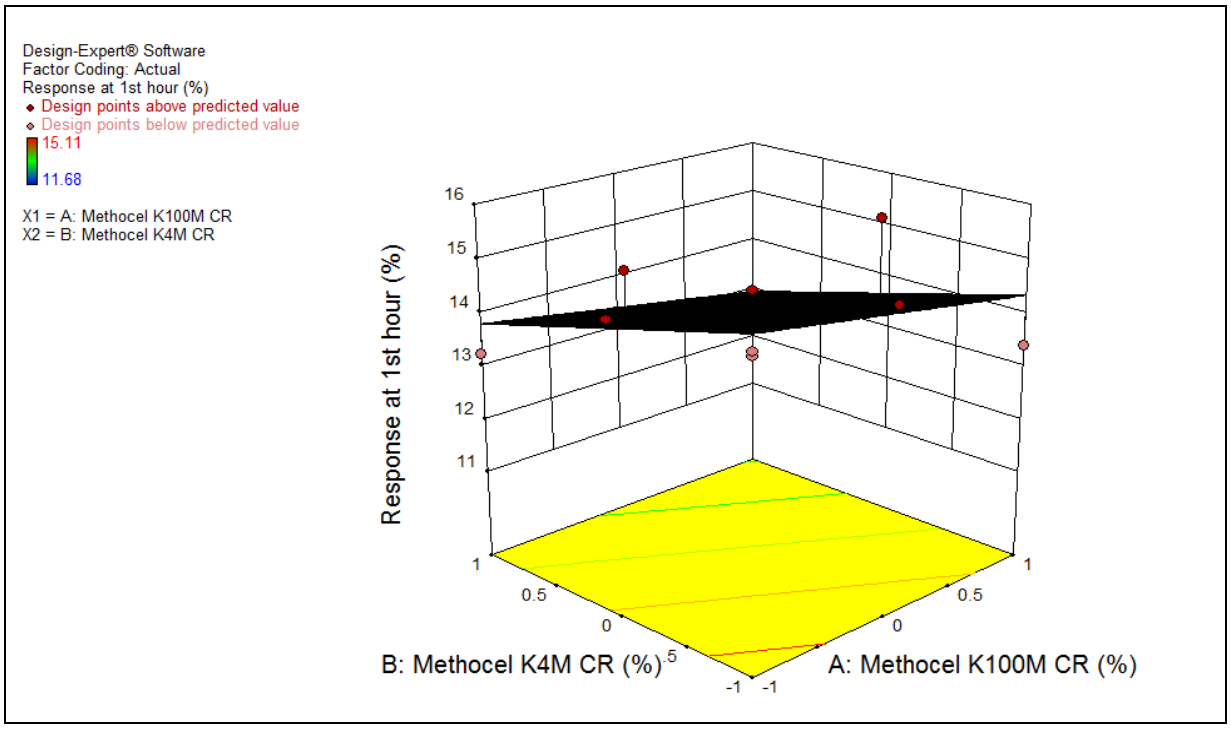

Figure 3.36: The actual 3D contour plot of drug release at 1st hour

For the 1st hour, the normal plot of residuals (Figure 3.37) showed linearity as well as close relation between each of the data points of practical and theoretical response values.

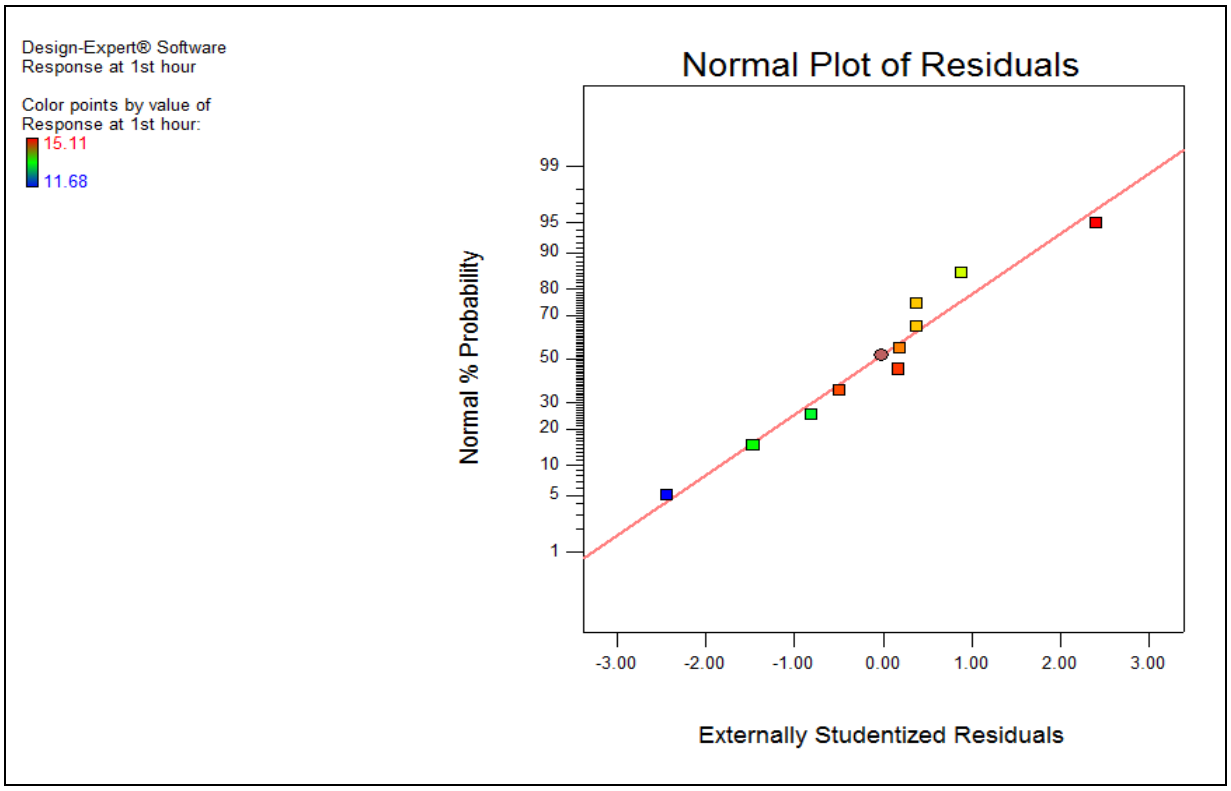

Figure 3.37: Normal plot of residuals at 1st hour

3.6.3.2 Response at the 4th hour

At the 4th hour, for each source of terms (intercept, A, B, AB, A^2, B^2), the probability value (p-value) was examined to select the statistical model that describes the response in the best possible way. The design expert software suggested that the release at 4th hour (Y_{4th}) followed a linear model as the p-value was found to be 0.3334 (Figure 3.38). Lack of fit test and model summary statistics checked by the software also did not suggest significant lack of fitness for the linear model (Figure 3.39).

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	18505.48	1	18505.48			Suggested
Linear vs Mear	8.09	2	4.04	1.29	0.3334	Suggested
2FI vs Linear	0.84	1	0.84	0.24	0.6430	
Quadratic vs 2	7.66	2	3.83	1.14	0.4056	
Cubic vs Quad	11.03	2	5.52	4.58	0.1791	Aliased
Residual	2.41	2	1.20			
Total	18535.51	10	1853.55			

Figure 3.38: Sequential model for response at 4th hour

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Linear	21.94	6	3.66		
2FI	21.10	5	4.22		
Quadratic	13.44	3	4.48		
Cubic	2.41	1	2.41		
Pure Error	0.000	1	0.000		

"Lack of Fit Tests": Want the selected model to have insignificant lack-of-fit.

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	1.77	0.2694	0.0606	-0.7349	52.10	Suggested
2FI	1.88	0.2972	-0.0541	-2.6043	108.24	
Quadratic	1.83	0.5524	-0.0070	-3.6072	138.36	
Cubic	1.10	0.9198	0.6393	-10.2776	338.68	Aliased

Figure 3.39: Fit summary tests report for response at 4th hour

The response at 4th hour was also statistically interpreted by analysis of variance (ANOVA) at the 5% significance level and also confirmed the adequacy of linear model to explain the relationship between the dependent and independent variables. Values of the prob>F value less than 0.05 indicate model terms are significant. The p-value for the model was found to be 0.3334.The F value which was 1.29 also implies that the model is not significant. There is a 33.34% chance that F value this large could occur because of noise. Adequate precision measures the signal to the noise. The desirable ratio for adequate precision is greater than 4. The adequate precision was found to be 2.87 that indicates inadequate signal to use the model to evaluate the response (Figure 3.40).

Response 2 Response at 4th hour

ANOVA for Response Surface Linear model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	8.09	2	4.04	1.29	0.3334	not significant
A-Methocel k	*0.41*	*1*	*0.41*	*0.13*	*0.7297*	
B-Methocel k	*7.68*	*1*	*7.68*	*2.45*	*0.1614*	
Residual	21.94	7	3.13			
Lack of Fit	*21.94*	*6*	*3.66*			
Pure Error	*0.000*	*1*	*0.000*			
Cor Total	30.03	9				

Std. Dev.	1.77	R-Squared	0.2694
Mean	43.02	Adj R-Squared	0.0606
C.V. %	4.12	Pred R-Square	-0.7349
PRESS	52.10	Adeq Precisior	2.870
-2 Log Likeliho	36.24	BIC	43.14
		AICc	46.24

Figure 3.40: ANOVA for response surface linear model at 4th hour

The predicted equation for response at 4th hour in terms of coded factors is:

$$Y_{4hr} = + 43.02 - 0.26A - 1.13B$$

According to the equation, coefficient b_1 and b_2 contains negative sign which means that both A and B retarded the response. But the effect of Methocel K4M CR (1.13) is greater than the Methocel K100M CR (0.26) on sustaining the release of drug at 4th hour.

The linear model generated two dimensional (2D) contour plot (Figure 3.41) and three dimensional (3D) surface response plot (Figure 3.42) for the 4th hour. The two dimensional contour plot as well as the three dimensional response surface plot shows the effect of polymer on drug release at the 4th hour. In this two plots, it was visualized that with increase in the polymer concentration, the response values are moving from yellow zone towards green zone and the retarding influence of the polymers on the release is evident. However, with the increase in concentration of Methocel K4M CR while keeping the Methocel K100M CR constant, a greater sustaining effect was produced relative to that of Methocel K100M CR.

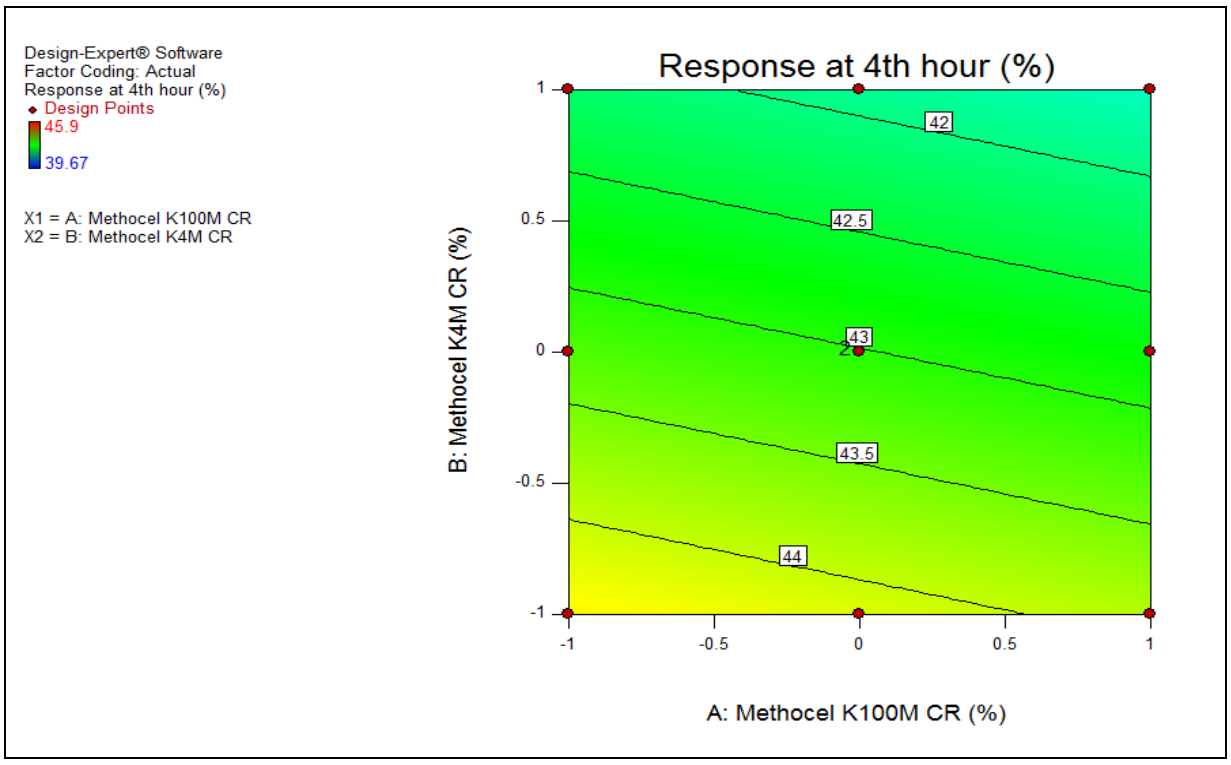

Figure 3.41: The actual 2D contour plot of drug release at 4th hour

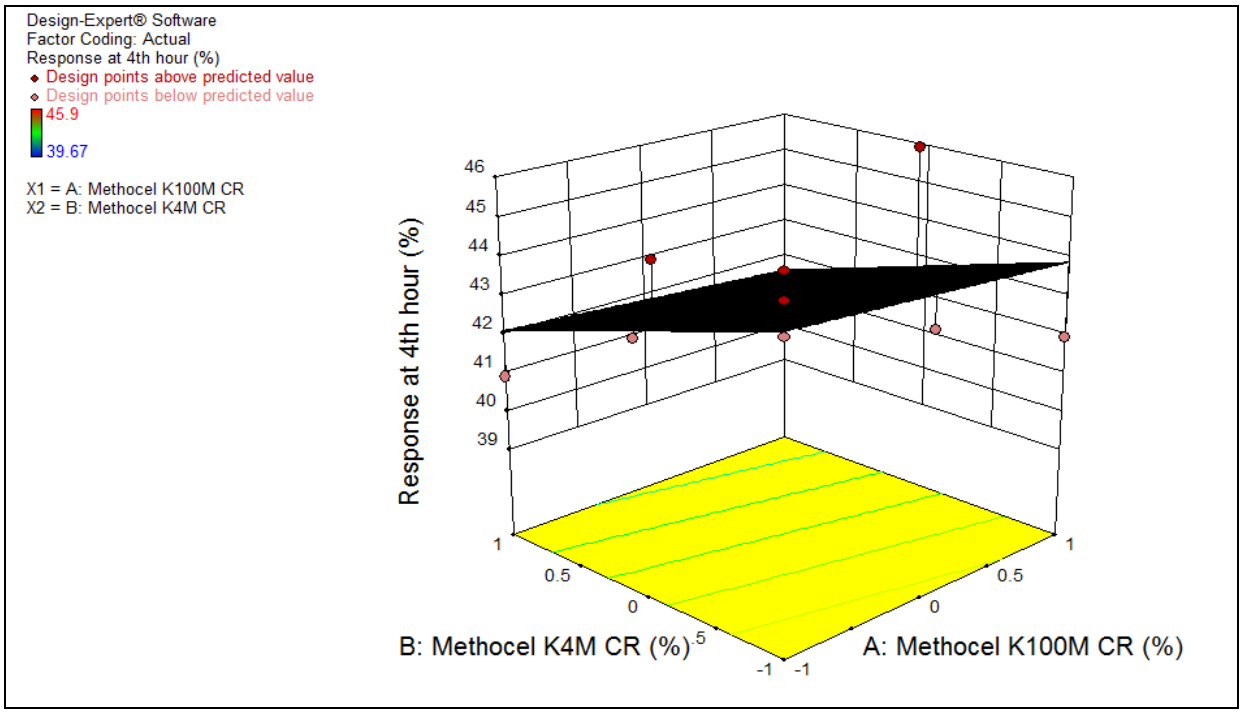

Figure 3.42: The actual 3D contour plot of drug release at 4th hour

For the 4th hour, the normal plot of residuals (Figure 3.43) showed linearity as well as close relation between each of the data points of practical and theoretical response values.

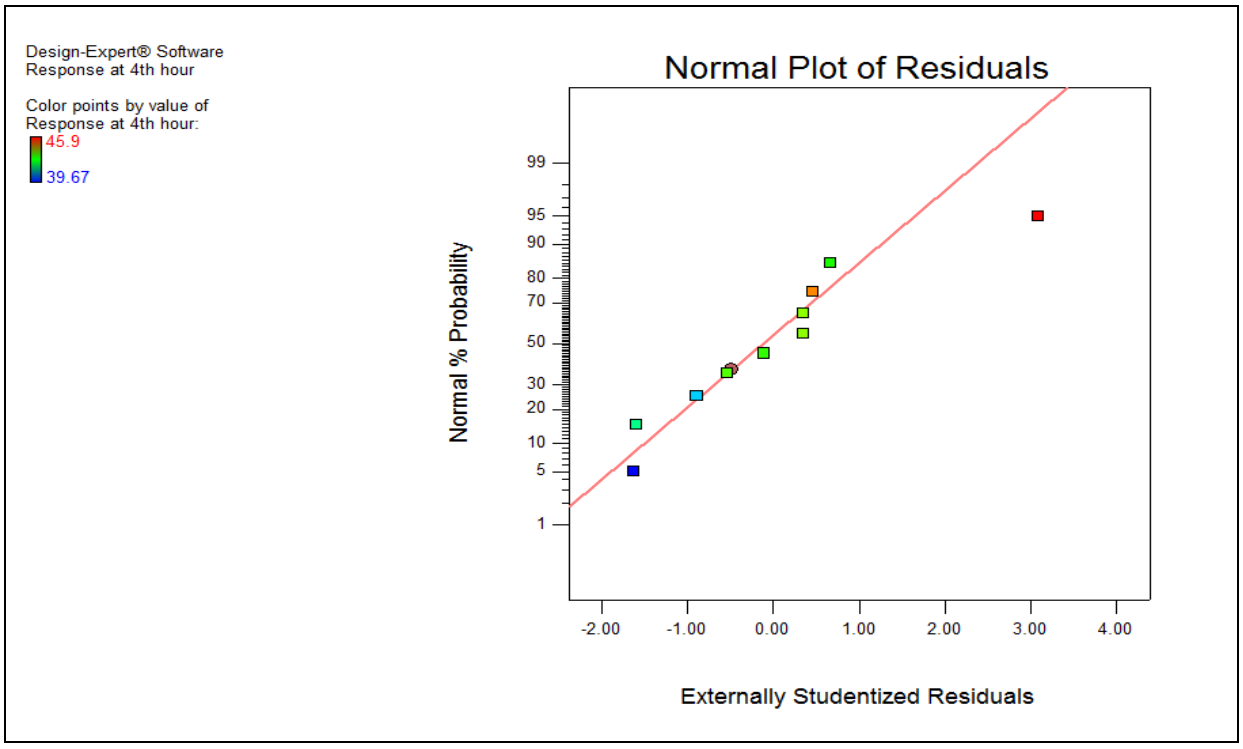

Figure 3.43: Normal plot of residuals at 4th hour

3.6.3.3 Response at the 8th hour

After 8 hours, for each source of terms (intercept, A, B, AB, A^2, B^2), the probability value (p-value) was examined to select the statistical model that describes the response in the best possible way. The design expert software suggested that the release at 8th hour (Y_{8hr}) followed a 2FI model as the p-value for this model was found to be 0.0418 (Figure 3.44). Lack of fit test and model summary statistics checked by the software also recommended no lack of fitness for the 2FI model (Figure 3.45).

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	51930.76	1	51930.76			
Linear vs Mear	155.28	2	77.64	3.52	0.0877	
2FI vs Linear	81.27	1	81.27	6.65	0.0418	Suggested
Quadratic vs 2	9.26	2	4.63	0.29	0.7633	
Cubic vs Quad	62.53	2	31.26	41.31	0.0236	Aliased
Residual	1.51	2	0.76			
Total	52240.61	10	5224.06			

Figure 3.44: Sequential model for response at 8th hour

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Linear	154.57	6	25.76		
2FI	73.30	5	14.66		
Quadratic	64.04	3	21.35		
Cubic	1.51	1	1.51		
Pure Error	0.000	1	0.000		

"Lack of Fit Tests": Want the selected model to have insignificant lack-of-fit.

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	4.70	0.5011	0.3586	-0.3375	414.44	
2FI	3.50	0.7634	0.6451	0.0171	304.56	Suggested
Quadratic	4.00	0.7933	0.5349	-1.2684	702.87	
Cubic	0.87	0.9951	0.9780	0.3127	212.95	Aliased

Figure 3.45: Fit summary tests report for response at 8th hour

The response at 8th hour was also statistically interpreted by analysis of variance (ANOVA) at the 5% significance level. Values of the prob>F value less than 0.05 indicate model terms are significant. The p-value for the model was found to be 0.0263.The F value which was 6.45 also implies that the model is significant. There is a 2.63% chance that F value this large could occur due to noise. Adequate precision measures the signal to the noise. A ratio greater than 4 is desirable. The adequate precision was found to be 8.641 that indicates that the model generated by the software can still be used to determine the effect of the polymers on drug release as it gives adequate precision value greater than 4 (Figure 3.46).

Response 3 Response at 8th hour

ANOVA for Response Surface 2FI model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	236.55	3	78.85	6.45	0.0263	significant
A-Methocel k	*2.67*	*1*	*2.67*	*0.22*	*0.6568*	
B-Methocel k	*152.61*	*1*	*152.61*	*12.49*	*0.0123*	
AB	*81.27*	*1*	*81.27*	*6.65*	*0.0418*	
Residual	73.30	6	12.22			
Lack of Fit	*73.30*	*5*	*14.66*			
Pure Error	*0.000*	*1*	*0.000*			
Cor Total	309.85	9				

Std. Dev.	3.50	R-Squared	0.7634
Mean	72.06	Adj R-Squared	0.6451
C.V. %	4.85	Pred R-Square	0.0171
PRESS	304.56	Adeq Precisior	8.641
-2 Log Likeliho	48.30	BIC	57.51
		AICc	64.30

Figure 3.46: ANOVA for reponse surface 2FI model at 8th hour

The predicted equation for response at 8th hour in terms of coded factors is:

$$Y_{8hr} = + 72.06 + 0.67A - 5.04B - 4.51AB$$

According to the equation, coefficient b_1 contains positive sign and b_2 and b_3 contains negative sign which means that both B and AB decreased the response but A does not have adequate retarding effect on the response alone.

The 2FI model generated two dimensional (2D) contour plot (Figure 3.47) and three dimensional (3D) surface response plot (Figure 3.48) for the 8th hour. The two dimensional contour plot as well as the three dimensional response surface plot shows the effect of polymer on drug release at 8th hour. In these two plots, it was visualized that with the increasing concentration of Methocel K4M CR keeping the Methocel K100M CR, the sustaining effect was produced as the response values are moving from green zone towards blue zone. Thus, the retarding influence of the polymers on the release is evident. However, with the increase in concentration of Methocel K4M CR and Methocel K100M CR simultaneously, a sustaining effect was also produced but the effect was slightly lower relative to the condition when only the concentration of Methocel K4M CR was increased.

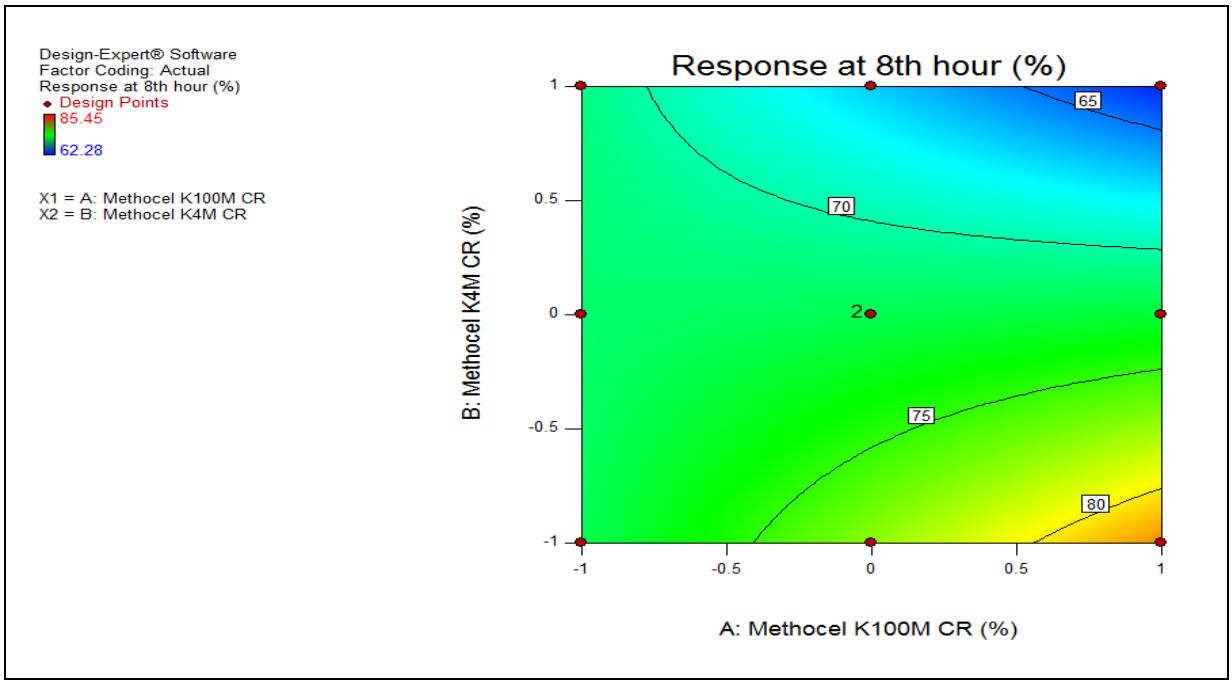

Figure 3.47: The actual 2D contour plot of drug release at 8th hour

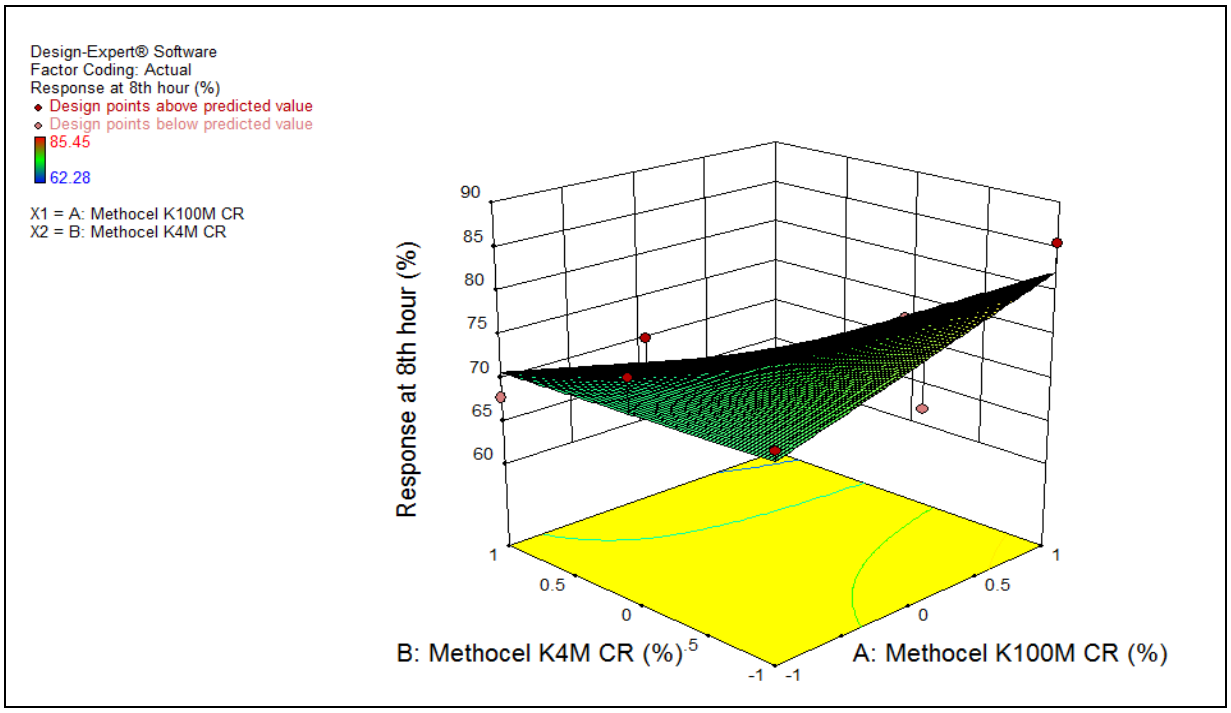

Figure 3.48: The actual 3D contour plot of drug release at 8th hour

For the 8th hour, the normal plot of residuals (Figure 3.49) showed linearity as well as close relation between each of the data points of practical and theoretical response values.

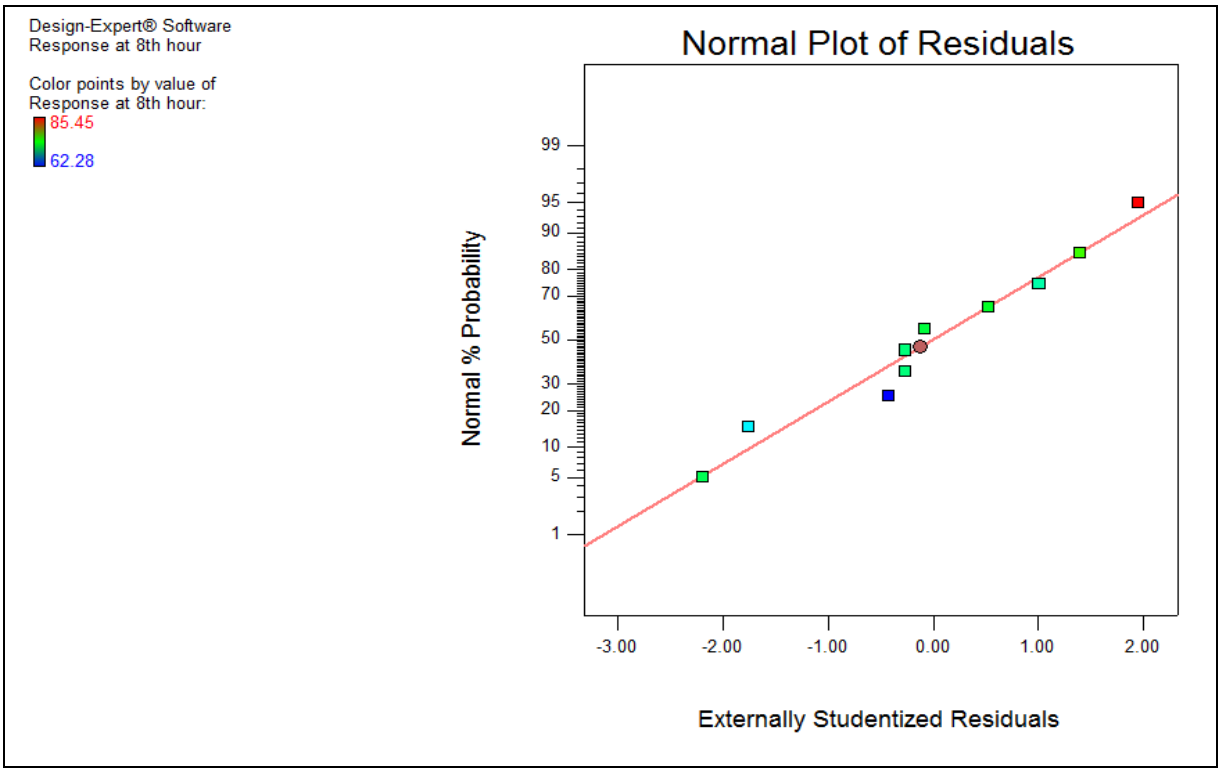

Figure 3.49: Normal plot of residuals at 8th hour

Response	Intercept	A	B	AB	
Response at ...	14.092	-0.431667	-0.695		
p=		0.2858	0.1052		
Response at ...	43.018	-0.26	-1.13167		
p=		0.7297	0.1614		
Response at ...	72.063	0.666667	-5.04333	-4.5075	
p=		0.6568	0.0123	0.0418	
Legend		p <.01	.01<= p <.05	.05<= p <.10	p >=.10

Figure 3.50: Summary of the responses at 1st, 4th and 8th hours

3.4 Optimization of the Formulation

A numerical optimization technique was implemented to determine the optimum formulation. The desirable ranges of these responses were confined to 11.68% < Y_{1hr} < 15.11%, 39.67% < Y_{4hr} < 45.90% and 62.28% < Y_{8hr}< 85.45% (Figure 3.51). The optimum formulation was chosen to be F5 among the nine different formulations as the observed responses were found to be in close association with the predicted values for the optimized formulation (Table 3.11). An overlay plot was also generated by the software that showed a yellow region for the optimum formulation at 0, 0 coded level (Figure 3.52)

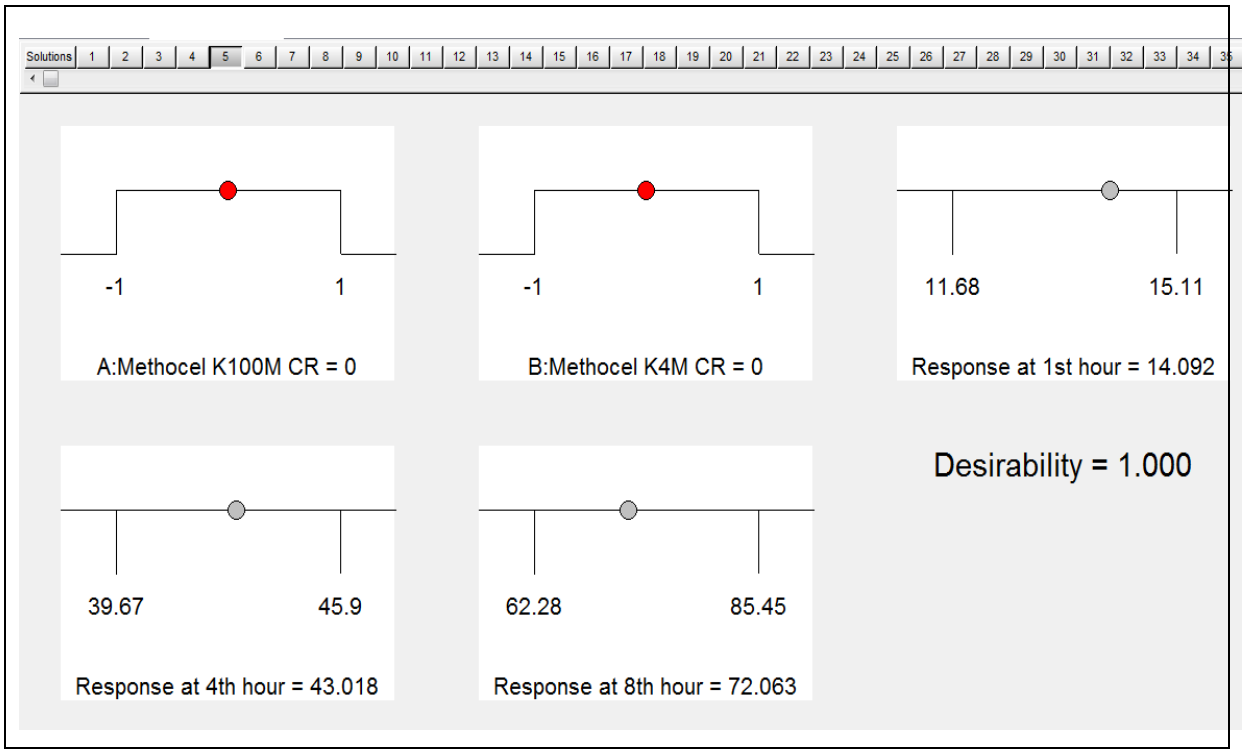

Figure 3.51: The predicted drug release profile for the optimum formulation

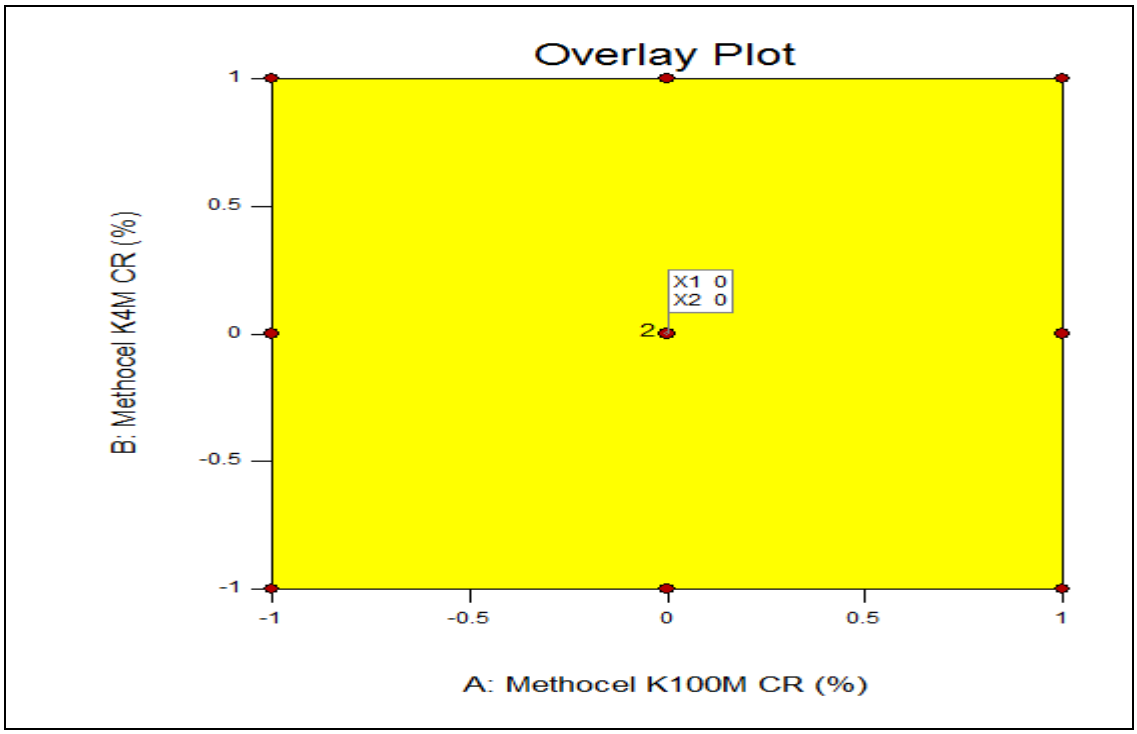

Figure 3.52: The overlay plot of the optimum formulation

Table 3.11: Optimum formulation with their experimental and predicted values

Methocel K100M CR: Methocel K4M CR (%)	Response variables	Experimental value (%)	Predicted value (%)	Prediction error (%)
F5= 40:7.5	Drug release at 1st hour	14.44	14.09	2.48
	Drug release at 4th hour	43.65	43.02	1.46
	Drug release at 8th hour	71.10	72.06	1.33

4. Conclusion

The present study involved the formulation of a sustained release oral tablet dosage form of Ketorolac Tromethamine where an optimum formulation was successfully determined. The design of this experiment was done by formulating nine different formulations of the matrix tablet using different ratio of hydrophilic polymers for each. All the excipients involved in the formulation showed compatibility with Ketorolac tromethamine. *In vitro* dissolution study of the tablets were performed for eight hours which generated drug release profile for each of the nine formulations. The data obtained from the dissolution study were fitted into several mathematical models to identify the release kinetics and mechanism of drug transport that is followed by the matrix tablets of Ketorolac Tromethamine. Finally, optimization of the formulation was done by using Design Expert software.

Recommendation

Further work such as stability study and quality control testing can be performed with the optimum formulation of Ketorolac Tromethamine that was determined through the research work.

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Appendix

Appendix 1

Ketorolac tromethamine

Ketorolac tromethamine is a non-steroidal anti-inflammatory drug. The properties of Ketorolac Tromethamine is-

Chemical name: 5-Benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid compound with 2-amino-2-(hydroxymethyl)-1, 3-propanediol

Molecular formula: C_{19} H_{24} N_2 O_6

Molecular weight: 376.40 g/mol

Melting point: 162°C

Appearance: White or off-white crystalline powder

Solubility: Freely soluble in water and methanol, slightly soluble in ethanol and partially insoluble in acetone, methylene, Chloride, toluene.

pKa: 3.46

pH: The pH of a 1%(w/v) solution in distilled water is 5.7-6.7 (Vadivelu et al., 2015).

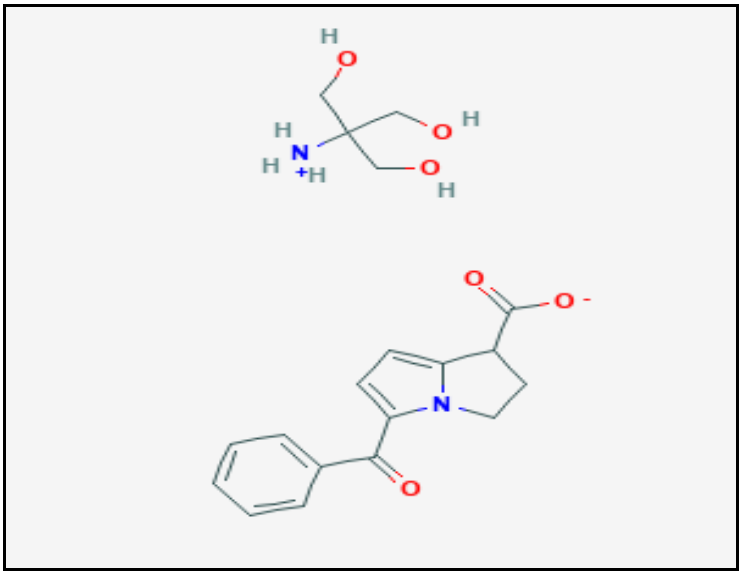

Figure: Structure of Ketorolac Tromethamine

Appendix 2

High performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) is an analytical technique which is used to separate, identify and quantify each component in a mixture. It pumps the sample mixture or analyte in solvent (mobile phase) at a high pressure through the column with chromatographic packaging material (stationary phase). It is very useful in the analysis of pharmaceutical products ("Hiq For High Performance Liquid Chromatography (HPLC)")

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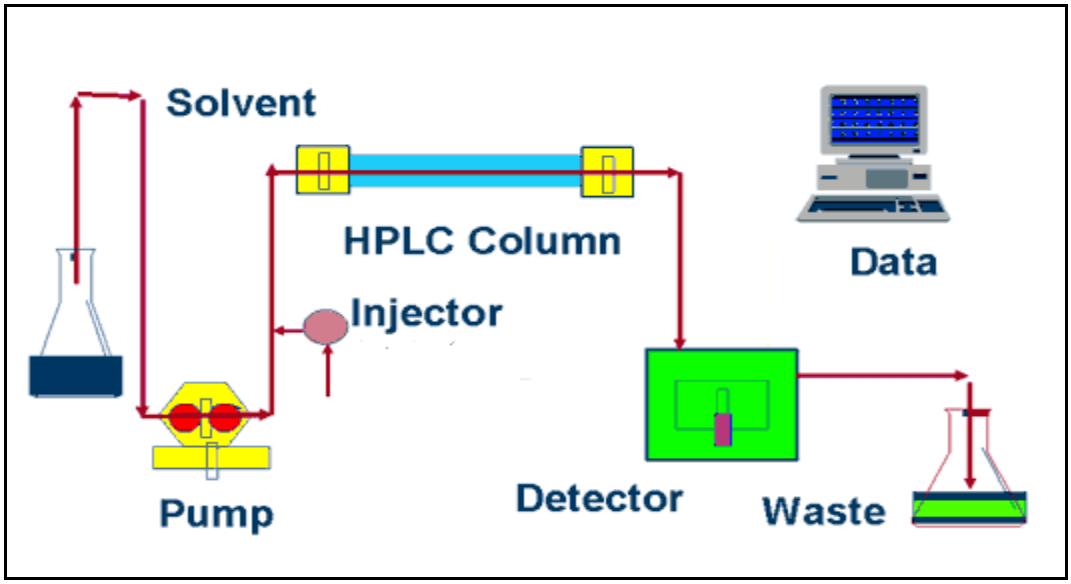

Figure: HPLC system

Appendix 3

Fourier transform infrared (FTIR)

Fourier transform infrared, more commonly known as FT-IR, is the preferred method for infrared spectroscopy. FT-IR or Fourier Transform Infrared spectroscopy is the study of the interaction of electromagnetic radiation from the IR region of the EM spectrum (4000-400) cm^{-1} with a molecule through which IR radiation is passed. When IR radiation passed through a sample (solid, liquid or gas), certain frequencies of the radiation are absorbed by the atoms of the substance leading to molecular vibration (Pavia, Lampman, Kriz, & Vyvyan, 2008)

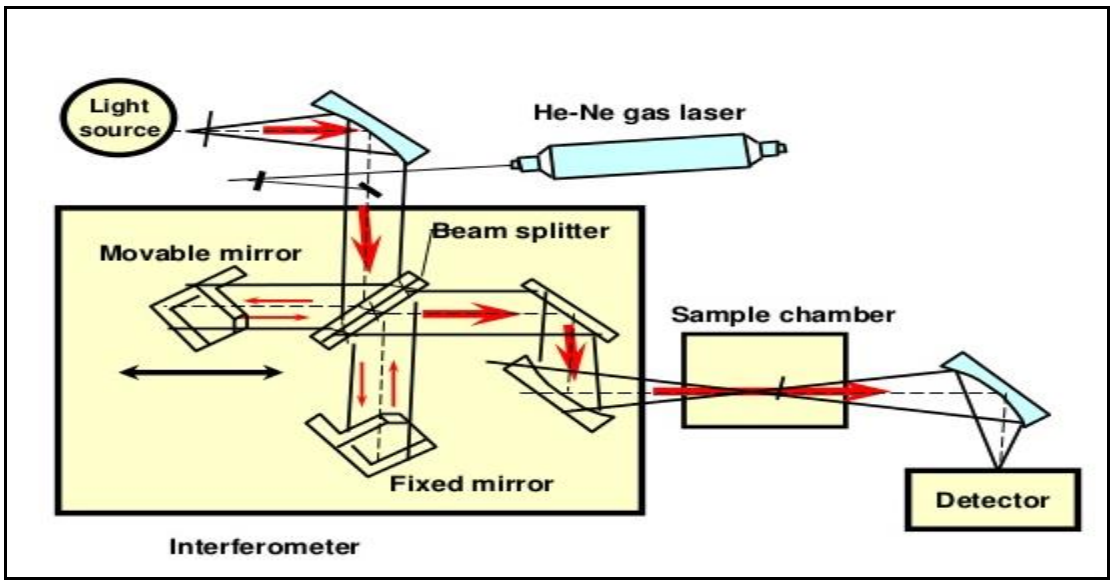

Figure: FTIR instrument

Appendix 4

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) is a thermal analysis technique used for the analysis of drug and the mixtures of drug and excipients.DSC is also used in polymers, food, paper printing, manufacturing, agriculture, semiconductors, electronics etc. The biggest and important advantage of DSC is the ease and speed with which it can be used to see transitions in materials (Satpute & Sayed, 2015).

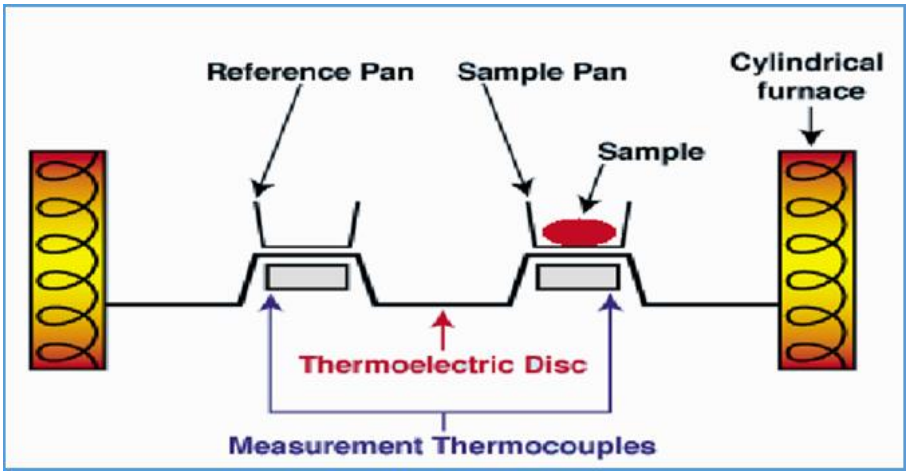

Figure: DSC instrument