Optimization of a Formulation of Ketorolac Tromethamine using DoE

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 "Optimization of a Formulation of Ketorolac Tromethamine using DoE" submitted for the partial fulfillment of the requirement 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,

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Acknowledgement

The blessing of Almighty Allah, who has given us the abilities to make a difference, has helped me to continue my student life that I have tried to reflect in my project.

This research work would have not been possible without the help of many people who are mentioned below.

First of all, I would like to and I must have to mention the name of Dr. Eva Rahman Kabir (Chairperson, Department of Pharmacy, BRAC University). Without her cordial support, knowledgeable advices and wisdom, I would not have been able to complete my research project. She has constantly given me the inspiration and guidance to come up with the research and work as a project student. Her research skills and powerful conceptual ability helped me to work even after making continuous errors.

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Last but not the least, I would like to give my special heart-felt gratitude to my parents for their constant support and inspiration that helped to show the courage of doing the research

ABSTRACT

The oral sustained release drug delivery system is often considered as one of the most convenient forms of drug administration over conventional dosage forms due to its ability to maintain an effective therapeutic efficacy and contribution to rational drug therapy. As the sustained release dosage form allows slow release of the drug and maintains the constant therapeutic blood or tissue drug level for an extended period of time, it is one of the most widely used drug delivery systems. The main purpose of using the sustained release dosage form is to decrease frequency of drug intake, thus reducing adverse effects associated with the drug and also to ensure better patient compliance. The aim of the present study was to formulate an optimum formulation of the sustained release oral tablet dosage form of Ketorolac Tromethamine. Since Ketorolac Tromethamine has a short biological half-life, it can be considered to formulate as a sustained release dosage form in order to reduce the dosing regimen and subside the gastrointestinal adverse effects that is likely to occur from its frequent consumption. For the purpose of formulation design, nine formulations have been designed for 200 mg and 250 mg matrix tablets each. The different polymer ratio employed to formulate the tablet shows the impact on drug release from the dosage form. The excipients for the formulations have been selected based on the FT-IR and DSC study that justifies the compatibility of excipients with the API. The dissolution study has been done for all designed formulations in order to get the drug release profile at different time interval and these release profiles have been placed into different mathematical release kinetic models in order to get the best fitted model for release rate. Finally, the optimum formulation for the two different doses was determined using Design Expert Software which was F5 for 200 mg of Ketorolac Tromethamine matrix tablets.

Table of Contents

Chapter-3

List of Tables

List of Figures

List of Acronyms

API = Active Pharmaceutical Ingredient

SR= Sustained Release

CR= Controlled Release

FT-IR= Fourier Transform Infrared Radiation

DSC= Differential Scanning Calorimetry

FI = Factor Interaction

GI= Gastrointestinal

IR= Infrared

KT= Ketorolac Tromethamine

MDT= Mean Dissolution Time

SD= Standard Deviation

UV= Ultraviolet

NSAIDs= Non-steroidal ant inflammatory drugs

Chapter-1

Introduction

Drugs having a short biological half-life frequently are eliminated from the body, producing a plasma drug concentration that hampers effective therapeutic efficacy and may end up causing adverse effects. In contrast, the sustained release dosage form prolongs the release of drug over an extended period of time. Therefore, the sustained release drug delivery system has been considered as one of the most reliable means of administration in order to ensure the therapeutic efficacy of drug by reducing fluctuations in plasma concentration. The main purpose of formulating the sustained release of drug delivery system is to prolong the drug release for an extended period of time in order to reduce adverse effects and improve patient compliance. To fulfill this purpose, four types of polymers are available namely insoluble inert polymer, insoluble erodible polymer, hydrophilic polymer and hydrogel polymer to prepare the sustained release dosage form (Diwedi, Alexandar, & Sekhar, 2011). So, to control the rate of drug release the sustained release dosage form can be formulated with those polymers. The oral route of drug delivery system helps to improve the pharmacological properties of many drugs with the purpose of providing a safe medication to the patients. This route of drug delivery system is designed to alter the bio-distribution and pharmacokinetics of the drug in the body such that it enhances the therapeutic efficacy (Allen & Cullis, 2004). As the oral drug delivery system is often considered to increase patient compliance with the drug, this system is being treated as one of the most convenient routes of drug delivery system. Several techniques have been used to formulate the oral dosage form depending on some factors such as the disease state, symptoms of disease, duration of disease, physicochemical properties of drug, etc. (Etman et al., 2008). The conventional dosage form often leads to wide fluctuations in the plasma concentration of drugs resulting in poor efficacy of the drug in the patient's body and it may also be associated to cause some gastrointestinal toxicities. So, maintenance of the proper plasma drug concentration with lesser risk of suffering from toxicity is a tough issue in this regard (Vadivelu et al., 2015). Moreover, a conventional dosage form sometimes demands for multiple dosing therapies due to the inability of the conventional dosage form of the drug to maintain the therapeutic range of plasma drug level for an extended period of time. This routine of repetitive dosing interval is responsible for exhibiting some disadvantages such as

frequent administration especially for drugs with short biological half-life, patient non-compliance with frequent dosage regimen and sometimes, inconsistent dosing (Gennaro, 2000). In order to minimize the limitations associated with immediate release oral dosage form, the sustained release dosage form is being widely used as it allows the slow release of the drug and hence, maintenance of the therapeutic range for a prolonged period of time by acting as drug reservoirs (Allen & Cullis, 2004). The benefits of this drug delivery system are wide and these mainly include dose frequency reduction, improved patient compliance, suitable therapeutic performance with improved bioavailability and cost effectiveness. The sustained release dosage form also facilitates in reducing the adverse effects associated with potential threat of GI disorders (Etman et al., 2008). Such oral route of drug delivery system is often prepared by using particulate carries and/or polymers with the drug. Thus, the sustained release oral drug delivery system serves as a reliable dosage form of drug administration for the patients.

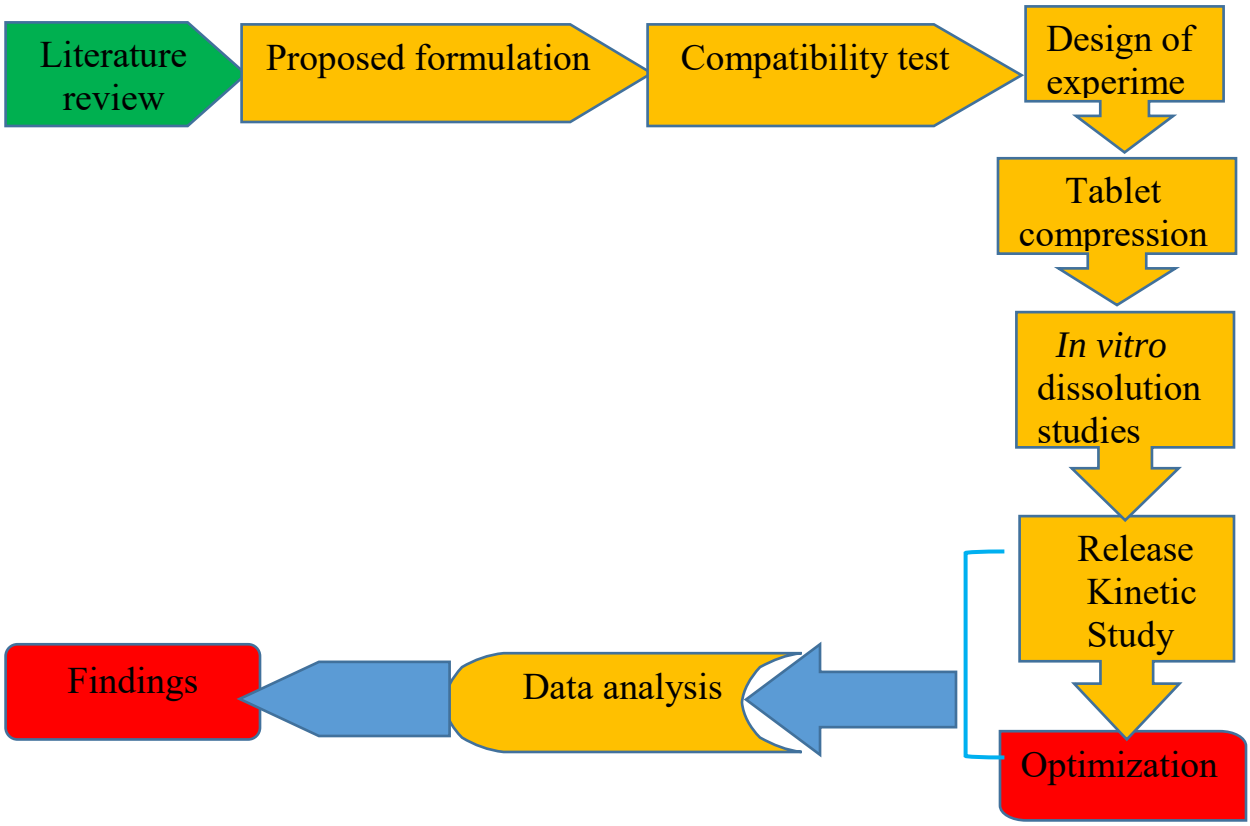

Figure 1.1: Overall design of the experiment

1.1 Rationale of the study

Ketorolac Tromethamine is a potent non-steroidal anti-inflammatory drug that has a short biological half-life often leading to its frequent administration and hence, patient incompliance when used as a conventional dosage form. Moreover, since most analgesic drugs are often likely to cause gastrointestinal side effects if consumed frequently, the increased dosing regimen of Ketorolac Tromethamine also exhibits the risk of developing gastrointestinal adverse effects. The use of this potent analgesic drug to alleviate the severe pain is wide. Consequently, the formulation of a sustained release dosage form of the drug has become quite important to ensure the safety associated with the drug accompanied by improved patient compliance by reducing dosing time intervals.

A recent attempt had been taken to design a sustained release oral tablet dosage form of Ketorolac Tromethamine where 300 mg of the matrix tablet was formulated by using polymers at different ratio and optimization of the formulations were performed. However, the formulations designed was found not to show significant sustaining effect on drug release from the tablets which is why further extensive study has been performed with two different doses i.e. 200 mg and 250 mg of the matrix tablet of Ketorolac Tromethamine. A previous study has been done with 300 mg of Ketorolac Tromethamine matrix tablet in order to find optimum formulation. Since the statistical result was insignificant, further study has been carried out with lower doses i.e. 200 mg and 250 mg of Ketorolac Tromethmine. The main purpose of lowering the dose is to ensure cost effectiveness of the study. So, the main aim of the study is to develop an optimum formulation of a sustained release dosage form of Ketorolac Tromethamine matrix tablets using different blend of polymers.

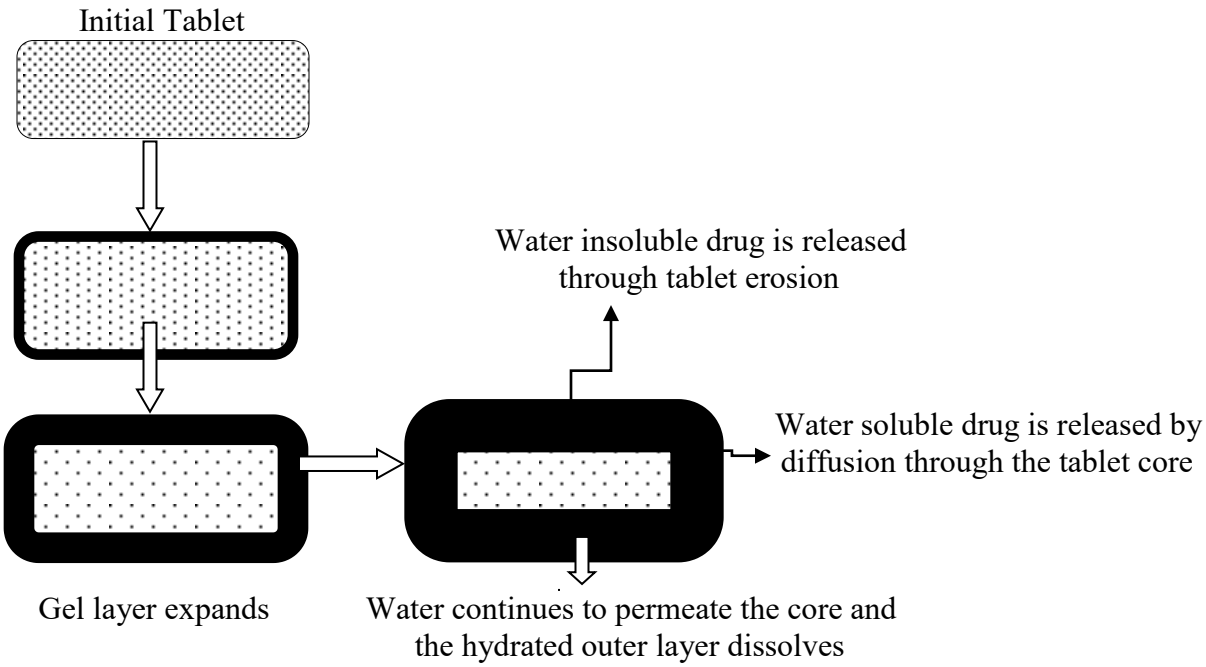

Figure 1.2: Model of drug release mechanism from a hydrophilic matrix tablet

1.2 Drug profile

Ketorolac Tromethamine

Ketorolac Tromethamine is known as a non-steroidal anti-inflammatory drug (NSAID). It has potent analgesic activity and anti-inflammatory activity as well. This drug works via blockade of the cyclooxygenase (COX) enzyme decreasing the prostaglandin synthesis responsible for producing pain (F, Schiele, & kLaus, 2005). It is the first NSAID that got approval from United States to be used as a parenteral analgesic (Buckley & Brogden, 1990). Ketorolac Tromethamine is available in different dosage forms that include oral, intramuscular, intravenous and ophthalmic dosage form. Its two enantiomeric forms, namely [-S] and [+R] with the racemic mixture, has been reported to have analgesic activity (Vadivelu et al., 2015). The main advantage of Ketorolac Tromethamine is that it is absorbed rapidly (T_{max} 1<0 hour with an effective percentage of >87%). Moreover, its single dose contains 10-30mg and plasma half-life ranges from 1.0 to 6.0 hours (Ahamed, Banik,

& Hossain, 2013). The efficacy of a single dose of Ketorolac Tromethamine has been found to be greater than other opioids such as morphine, pethidine and pentazocaine, etc to reduce severe postoperative pain in a clinical study (Buckley & Brogden, 1990).

1.4 Literature review

Extensive literature review was done before the actual project work started. Below is a list of the journals used as reference for the study:

1. Journal of Applied Pharmaceutical Science
2. American Journal of drug delivery
3. International Journal of Pharmaceutical Research and Bio-science
4. Asian Journal of Pharmaceutical Sciences
5. World Journal of Pharmaceutical Sciences
6. Asian Journal of Pharmaceutical and Clinical Research
7. Journal of Thermal Analysis and Calorimetry
8. Bangladesh Journal of Scientific and Industrial Research
9. Advanced Drug Delivery Reviews
10. International Journal of Pharmacy and Pharmaceutical Sciences
11. Dhaka University Journal of Pharmaceutical Science
12. Advanced Drug Delivery Reviews
13. Journal of Analytical and Bio-analytical Techniques
14. International Journal of Pharmaceutical Science and Research
15. Pakistan Journal of Pharmaceutical Sciences
16. Acta Poloniae Pharmaceutical-Drug Research
17. Asian Journal of Pharmaceutical Sciences

The present study identified all the gaps found in a previous study of Ketorolac Tromethamine as well as from extensive literature review.

CHAPTER 2
Methodology

Based on a proposed sustained release formulation of Ketololac Tromehamine, the research methodology of this project has been designed. In this experiment, Methocel K100M CR and Methocel K4M CR have been used with the core drug Ketololac Tromethamine which is a member of NSAID group. A compatibility study has been done for the excipients chosen for the formulation development and a verification of its compatibility has been done with the active ingredients. The proposed formula of sustained release tablet of Ketorolac Tromethamine (KT) is given in the table 2.1.

Table 2.1: Components of the sustained release formulation of Ketorolac Tromethamine

Active Pharmaceutical Ingredient	**Amount**
Ketorolac Tromethamine	30 mg
Excipients	**Justification**
Methocel K100M CR	Rate controlling polymer
Methocel K4MCR	Rate controlling polymer
Starch 1500	Binder
Avicel 200	Filler
Aerosil 200	Lubricant
Talc	Guidant

2.1 Standardization of Ketorolac Tromethamine using UV-Visible spectrophotometer

First of all, 10 mg of Ketorolac Tromethamine was weighed and dissolved in 100 ml of distilled water to obtain the concentration of 100 µg/ml. From this concentration of mixtures 8ml, 9 ml, 10 ml, 11 ml and 12 ml were withdrawn and diluted. The dilution provides the

concentration in the range between 8.4 µg/ml to 12.6 µg/ml respectively. Then, every single mixture was run into UV-Visible spectrophotometer at 322 nm to get the absorbance.

2.2 Compatibility study of drug and excipients

The interaction between the drugs and excipients suggest whether the ingredients are suitable for use in the formulation which is why, the compatibility test has been done before designing the formulation. Since the incompatibly is directly related with some disadvantages such as alteration of absorption and bioavailability of the drug, the compatibility test is an essential part of any formulation as incompatibility may affect the stability, safety and/or efficacy of the drug delivery system (Gupta & Chadha, 2015). Excipients are therefore examined for compatibility study with API and these tests mainly include FT-IR and DSC study of the components.

2.2.1 Compatibility study (FT-IR)

The Fourier Transform Infrared (FT-IR) spectroscopy is basically done in order to study the interaction of electromagnetic radiation with the chemical substances. The range that has been considered from the IR region of the electromagnetic spectrum (EM) to determine any chemical changes in the molecule passed through the IR is 4000-400 cm^{-1}. Based on the functional groups present in the substance, the nature of interaction can be determined. Different IR spectrums can be found from the compounds of different structure. If there is an interaction between the drug and the excipients, it leads to change in the molecular structure. As a result, the different IR spectrum may be obtained which indicates incompatibly. By using the separate excipients in the ratio of 1:1 to conduct the FT-IR compatibly test, test samples were prepared by mixing each drug entities individually. Then, the pure Ketorolac Tromethamine passed through FT-IR for reference. Finally, the IR spectrum was recorded within the region and observed for any interaction between the drug and the excipients. The following samples have been tested for compatibility:

1. Ketorolac Tromethamine + Methocel K100M CR
2. Ketorolac Tromethamine + Methocel K4M CR
3. Ketorolac Tromethamine + Starch 1500

4. Ketorolac Tromethamine + Avicel PH 101
5. Ketorolac Tromethamine + Aerosil 200
6. Ketorolac Tromethamine + Talc

2.2.2 Compatibility study (DSC)

The proper selection of excipients exhibits a very effective role in the successful formulation of a stable and effective dosage form. In this regard, DSC test is a successful method to identify any physicochemical interaction between the drug and excipients. If the thermograms of mixture show any pattern corresponding to the individual components, it proves the absence of incompatibility between the drug and the excipient. On the other hand, any interaction between the drug and excipients indicates the incompatibly and it can be proved by the visual presence of one or more new peaks by comparing with the thermogram obtained from pure drug (Nanjwade, Manjappa, et al. 2009).

2.3 Polymer combination of matrix tablet formulations using 3^2 full factorial design

In this study, two factors were examined each at 3 levels of low, mid and high i.e. -1, 0 and +1 polymer ratio and the experimental study was conducted for all 9 combinations of formulation both for 200 mg and 250 mg of tablets. The 3^2 randomized full factorial design was used to conduct the study. The independent variables are Methocel K100M CR (A) and Methocel K4M CR (B) whereas the percentage of drug released from the tablets of different polymer ratio acts as dependent variables (Bushra, Shoaib, Aslam, Hashmat, & Rahman, 2008).

Table 2.2: A randomized model of full factorial design

Coded value	A (%)	B (%)
-1 (low level)	27	5
0 (mid-level)	30	7.5
+1 (high level)	33	10

Table 2.3: Combination of polymers for F1-F9

Formulations	A	B	K100M CR	K4M CR
F1	-1	-1	27	5
F2	-1	0	27	7.5
F3	-1	+1	27	10
F4	0	-1	30	5
F5	0	0	30	7.5
F6	0	+1	30	10
F7	+1	-1	33	5
F8	+1	0	33	7.5
F9	+1	+1	33	10

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2.4 Preparation of matrix tablets:

To prepare 10 tablets both for 200 mg and 250 mg of drug, every single component from API to excipients were weighed separately in the amount as shown in Table 2.4. The formulations were coded as F1, F2, F3, F4, F5, F6, F7, F8 and F9. To ensure the homogeneous distribution, polymers were mixed thoroughly with excipients and after that, the API was mixed with it. A 40 mesh sieve was used to pass the powder mixtures of each formulation. The mass obtained from the sieve was placed into hoper of the tablet compression machine and the die and punch were placed perfectly to obtain the desired weight of the tablets for both 200 mg and 250 mg.

Table 2.4: Amount of components in the formulation of Ketorolac Tromethamine (200 mg)

Components	Amount (mg)								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Ketorolac	30	30	30	30	30	30	30	30	30
Methocel K4M CR	54	54	54	60	60	60	66	66	66
Methocel K4M CR	10	15	20	10	15	20	10	15	20
Starch 1500	49	48.5	46	48	45.5	43	45	42.5	40
Avicel PH 101	49	48.5	46	48	45.5	43	45	42.5	40
Aerosil 200	2	2	2	2	2	2	2	2	2
Talc	2	2	2	2	2	2	2	2	2
Total	200								

2.5 *In vitro* dissolution study

In order to obtain the release profile of the drug from the dosage form, *in vitro* dissolution study was performed. This *in vitro* dissolution simulates the gastrointestinal environment of the body and hence, is used to determine the percentage of drug that dissolves in a medium similar to that present in the gastrointestinal tract.

2.5.1 Process of preparing dissolution medium

To prepare phosphate buffer for dissolution, 6.8 gm potassium di-hydrogen phosphate and (KH_2PO_4) and 0.94 gm sodium hydroxide (NaOH) were dissolved in distilled water and

made up to the volume of 1000 ml. The pH of the medium was maintained at 6.8. The pH of the solution was adjusted with orthophosphoric acid or NaOH based when needed.

2.5.2 Process of preparing the sample for dissolution

In order to prepare the sample for dissolution, 900 ml of phosphate buffer dissolution medium was poured into the dissolution vessels. The temperature of medium was maintained at 37°C ± 0.5°C. Three tablets were taken from the individual 9 formulations both for 200 mg and 250 mg each, weighed and dropped into the dissolution media. The position of the tablet was in between the paddle and the bottom of the vessel. The apparatus was set at 100 rpm and left for 8 hours operation. 5ml of the samples were withdrawn after 0.5, 1, 2, 4 and 8 hours and at the same time, 5 ml of buffer was placed in the dissolution vessel. Then the samples were filtered with cotton filter and/or filter paper and absorbance was taken at 322 nm in the UV-Visible spectrophotometer. Finally, the percentage release of the drug from the tablet was obtained by using the equation of standard curve.

The formula that was used is given below:

Equation of the standard curve: $Y = 0.0657X + 0.0186$

Here, Y= Absorbance and X= Concentration (mg/ml)

So, $X = (Y-0.0186)/0.0657$

% of drug release = (X (mg/ml) × 900 ml × 100)/weight of API (mg)

2.6 Release kinetic study of the matrix tablets

The release kinetic study was performed by insertion of the percentage release values of the in vitro dissolution study into various mathematical models. These models are also useful in determining the mechanism by which drug is released from the matrix tablet.

The different mathematical models used to determine the release kinetics of the drug are as follows:

2.6.1 Equation for Zero order model

If the drug release rate is independent of its concentration, it follows the zero-order model. It represents the cumulative percentage of drug release versus time. The equation is given below:

$$C=K_0 t$$

Where, C= concentration of drug

t= time in hour.

K_0 = zero order rate constant

A graph of concentration against time may be obtained with a straight line and a slope equal to K_0 and intercept the origin of the axes.

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2.6.2 Equation for First order model:

If the release rate is dependent on concentration, it represents the first order model. It is expressed as log cumulative percentage of drug remaining versus time.

The equation is as follows:

$$\text{Log } C = \log C_0 - Kt / 2.303$$

Where, C= amount of drug remaining at time 't'

C_0 = drug concentration at t=0

K= First order rate constant

2.6.3 Equation for Higuchi square root model

This model represents the cumulative percentage of drug release versus square root of time. The Higuchi model represents the release of drugs as a square root of time-dependent process based on Fickian diffusion. The equation is given below:

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

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

K_h = the constant reflecting design variables of the system.

2.6.4 Equation for Korsmeyer-Peppas model

To analyze the release of a drug molecule, the Korsmeyer-Peppas model is basically used following the equation given below:

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

Where, M_t = amount of drug release at time 't'

M_f = amount of drug release after infinite period of time

K= release rate constant

The process of drug release from the matrix tablet is shown by the diffusional exponent that is influenced by the tablet shape. Based on the diffusional exponent (n), the transport mechanism of drug is classified into four types (Siepmann, Peppas et al 2001). These are given below:

1. Case I transport: Fickian diffusion
2. Case II transport: Drug release controlled by polymer relaxation
3. Non-Fickian or anomalous transport
4. Super case II transport

Table 2.5 Release mechanism based on diffusional exponent

Diffusional exponent(n)	Mechanism of transport
0.43	Fickian diffusion
0.43<n<0.85	Non-Fickian or anomalous transport
0.85	Case II transport
>0.85	Super case II transport

Source: Siepmann and Peppas, 2001

2.6.5 Equation for Hixson-Crowell root law model

This model is expressed by the cube root of the percentage of initial drug minus the cube root of the percentage of drug remaining in matrix. The equation is given below:

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

Where, Q_0 = initial amount of the drug in the tablets

Q_t = the amount of drug remaining at time 't'

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

2.7 Fractional dissolution time

The drug release rate followed by the tablet can be found using different release kinetic models and time required to release 25%, 50% and 80 % of the drug can be calculated by using the equations given below:

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

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

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

The mean dissolution time (MDT) is used to characterize the drug release rate from the dosage form and the retarding efficiency of the polymer which is also a function of polymer loading, polymer nature and physicochemical properties of drug (Talukder, Ahmed et al. 2010). The equation for calculating MDT is given below:

$$MDT = (n/n+1).\ K^{-1/2}$$

2.8 Process of optimization of sustained release formulation of Ketorolac Tromethamine

To obtain the optimum formulation, a 3^2 full factorial design of 9 formulations for 200 mg and 250 mg was employed. Methocel K100M CR and Methocel K4M CR were considered as the independent variable during the experiment and the percentage release of drug after respective hours was considered as the response or dependent variables. After putting the release values in Design Expert Software, the relationship between the dependent variable and independent variable can be found. The statistical model and graphs obtained from the software represents the effect of the independent variables on sustaining the release of the drug.

CHAPTER 3

Result and Discussion

3.1 Standardization of Ketorolac Tromethamine

The standardization of Ketorolac Tromethamine provides a linear calibration curve from the plot of absorbance versus concentration graph. The correlation coefficient (R^2) was found to be 0.999 as shown in the Figure 3.1.

Table 3.1: Standard curve values for Ketorolac Tromethamine

Concentration (mg/ml)	Absorbance (322 nm)
0.00806	0.574
0.00908	0.611
0.01002	0.672
0.01005	0.743
0.01204	0.810

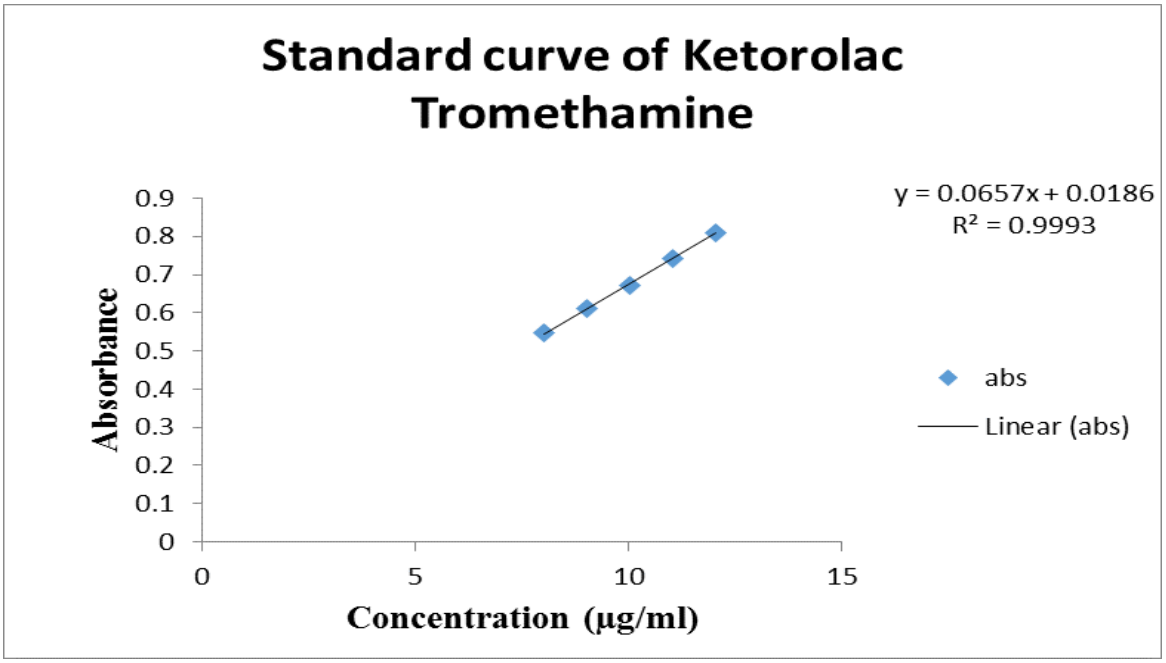


Figure 3.1: Standard curve of Ketorolac Tromethamine

3.2 Evaluation of drug-excipient compatibilty study

3.2.1 Compatibility test by FT-IR:

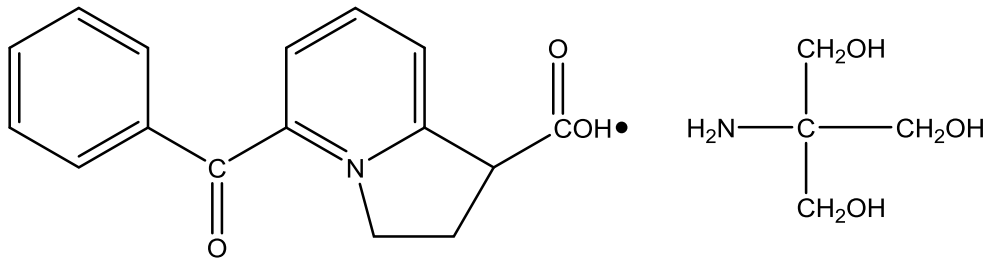


Figure 3.2: Structure of Ketorolac Tromethamine

Table 3.2: Functional groups of Ketorolac Tromethamine

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

Source: Pavia, Lampman, Kriz, & Vyvyan, 2008

The IR studies showed no interaction between the drugs and the excipients. However, some additional peaks were observed in the spectrum of IR study but it didn't show any significant change in the main peak position and indicated that no chemical interaction occurred between the drug and the excipients (Figure 3.3 to Figure 3.8).

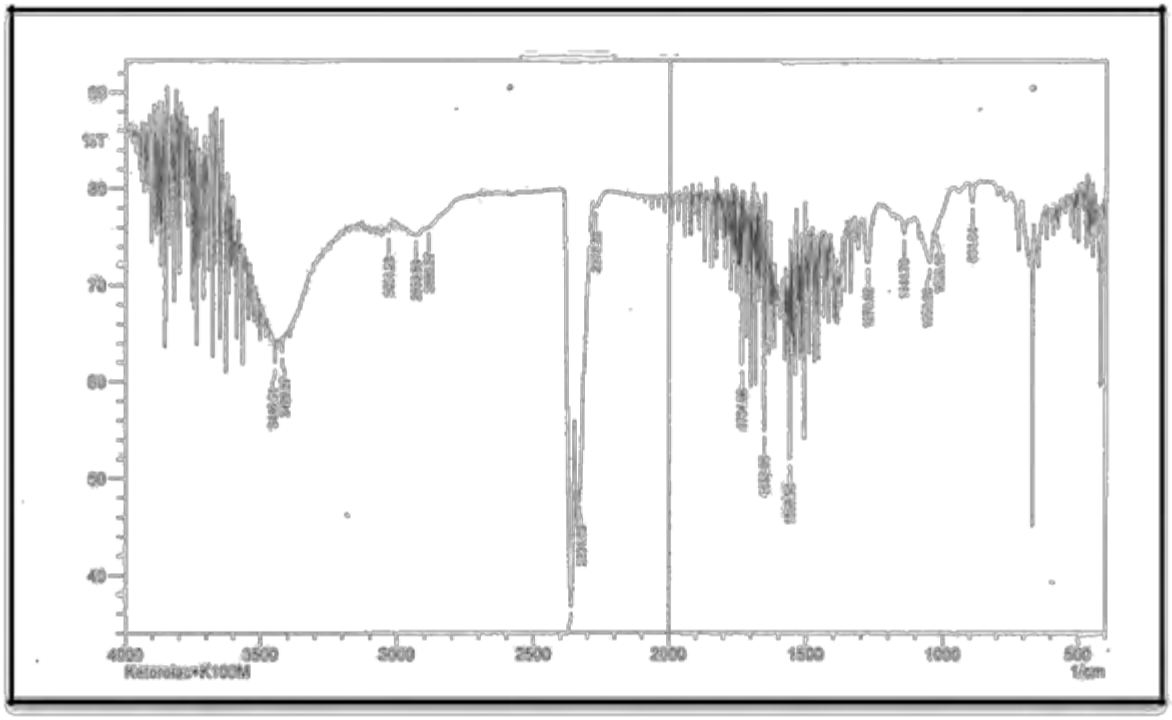


Figure 3.3: FT-IR spectrum of Ketorolac Tromethamine

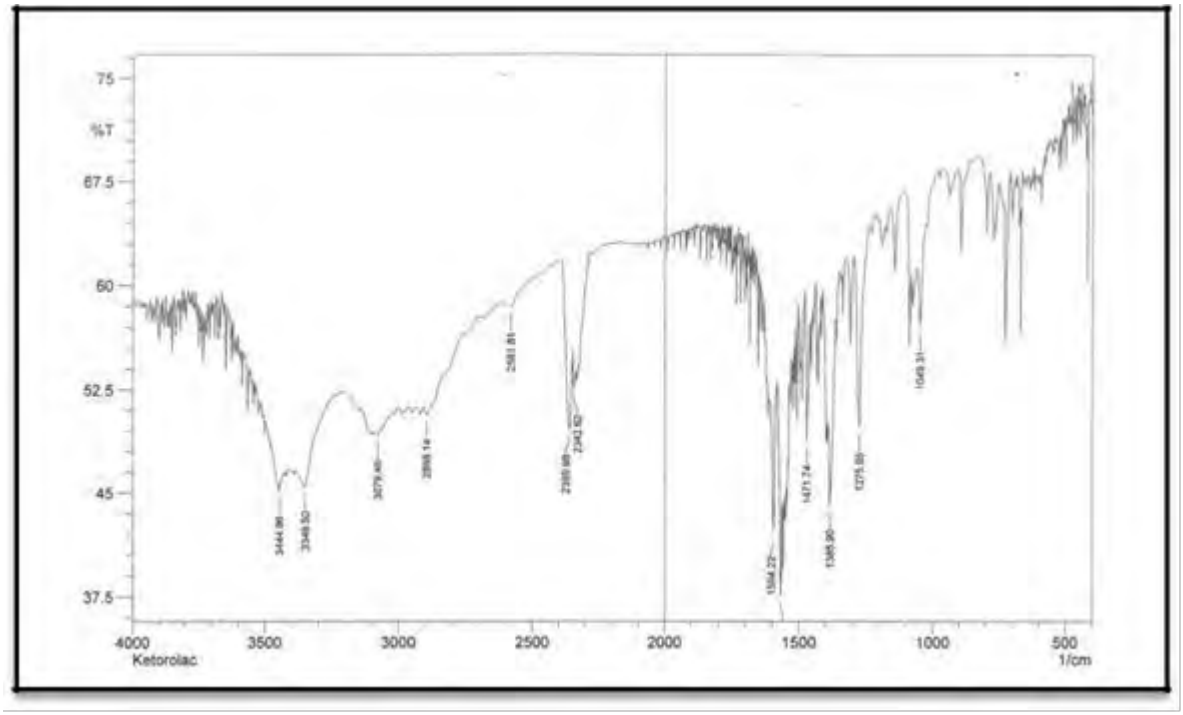


Figure 3.4: FT-IR spectrum for compatibility study of Ketorolac Tromethamine + Methocel K100M CR

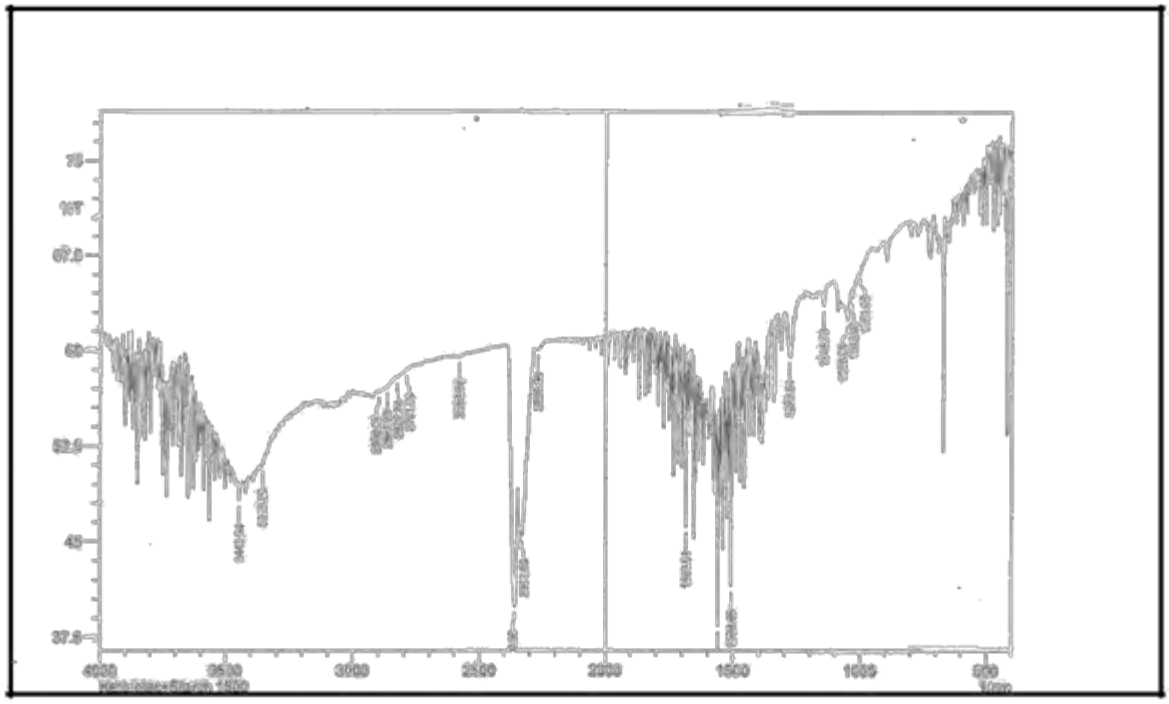


Figure 3.5: FT-IR spectrum for compatibility study of Ketorolac Tromethamine + Methocel K4M CR

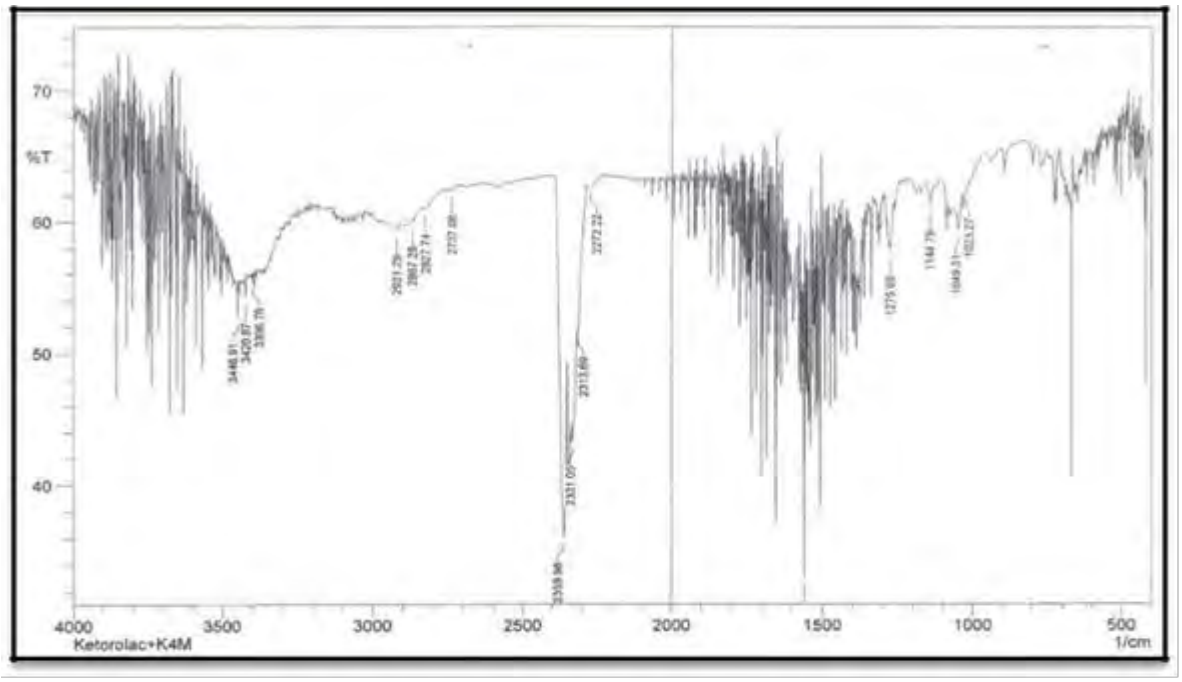


Figure 3.6: FT-IR spectrum for compatibility study of Ketorolac Tromethamine + Starch 1500

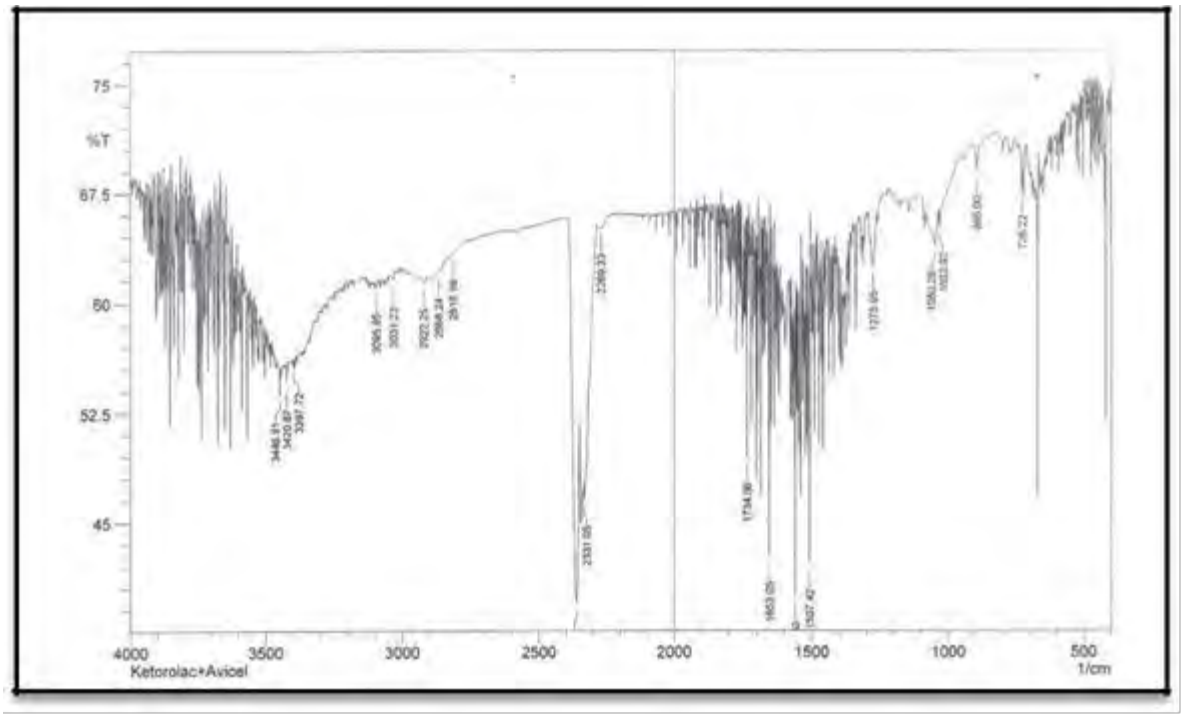


Figure 3.7: FT-IR spectrum for compatibility study of Ketorolac Tromethamine + Avicel PH 101

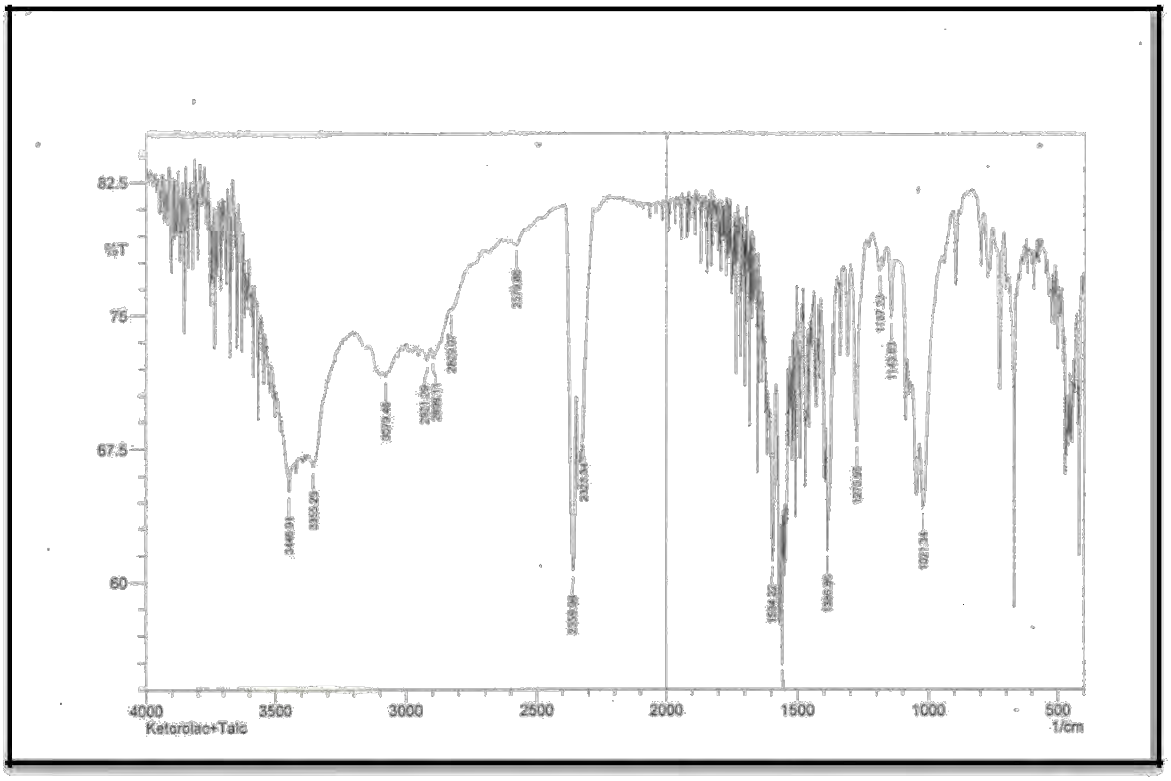


Figure 3.8: FT-IR spectrum for compatibility study of Ketorolac Tromethamine + Talc

3.2.2 Compatibility test by DSC

To formulate a successful formulation of an effective dosage form of drug, the compatibility test for ensuring the absence of incompatibility between the drugs and excipients play a significant role. There are different types of compatibility tests and DSC is one of the most efficient test among them in order to identify the interaction between the ingredients and the excipients. If the thermograms of mixtures show patterns related to the individual components, then it ensures the absence of incompatibility. The DSC thermograms of Ketorolac Tromethamine showed the long and sharp characteristic endothermic peak at 165.98^{o}C that corresponds to the melting pint of Ketorolac Tromethamine (Figure 3.9). The DSC tests also showed the endothermic peak with the pure drug and excipients that retained the characteristic peak of the drug (Figure 3.10 to 3.14). This observation from DSC test proved that no interaction occurred between the drug and the excipients. Therefore, the excipients selected were appropriate to make the sustained release formulation of Ketorolac Tromethamine.

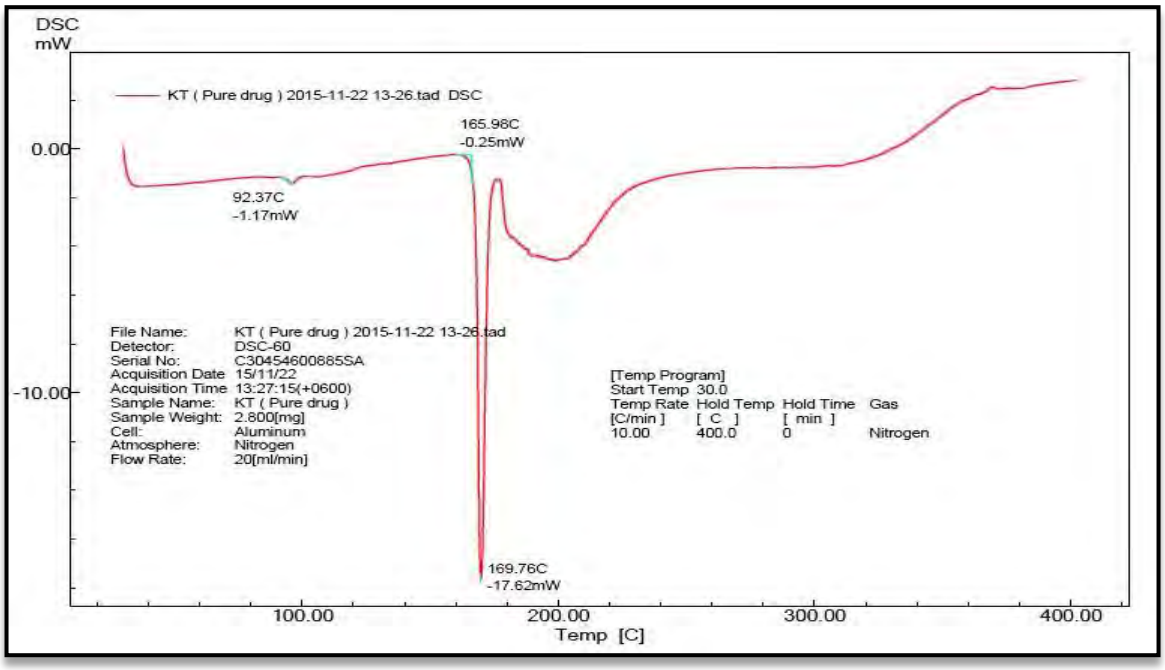


Figure 3.9: DSC thermogram of Ketorolac Tromethamine

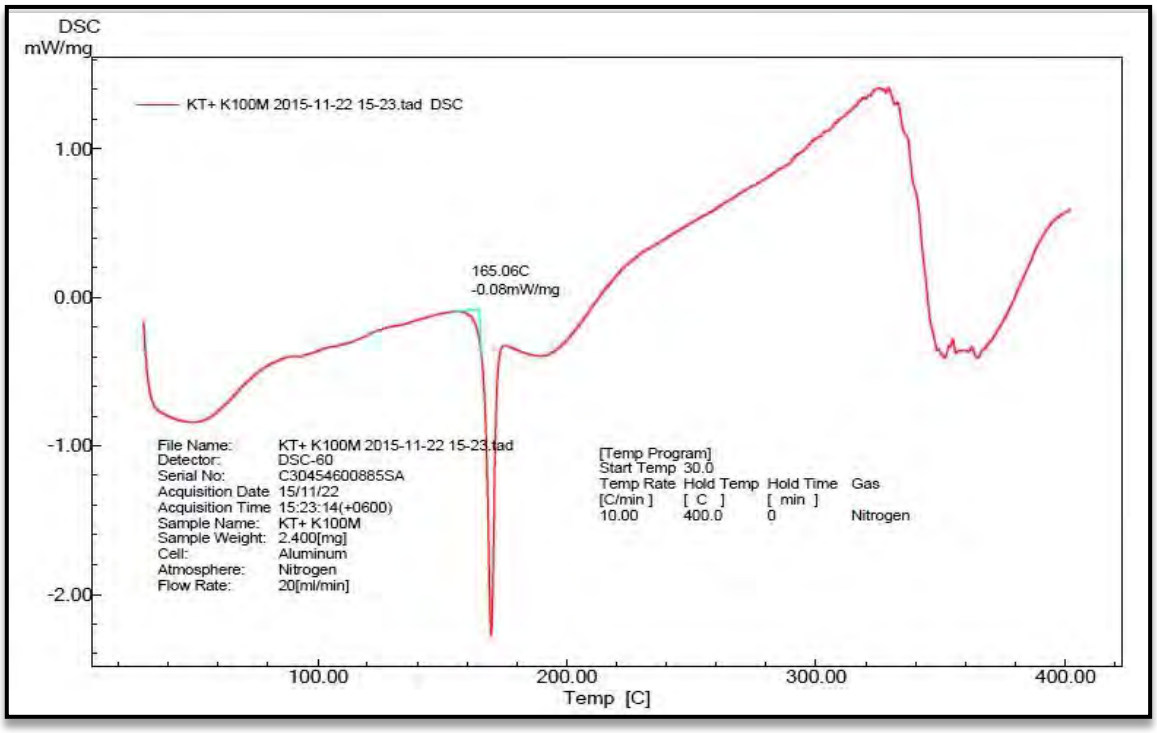


Figure 3.10: DSC thermogram of Ketorolac Tromethamine + Methocel K100M CR

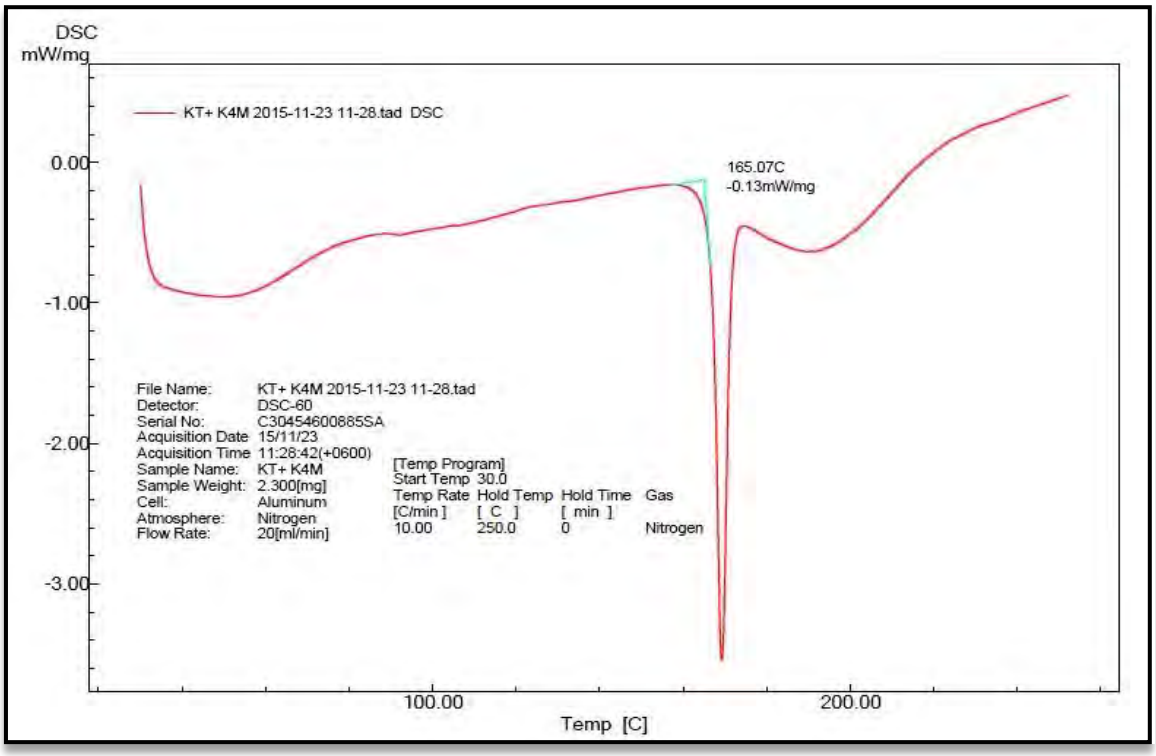


Figure 3.11: DSC thermogram of Ketorolac Tromethamine + Methocel K4M CR

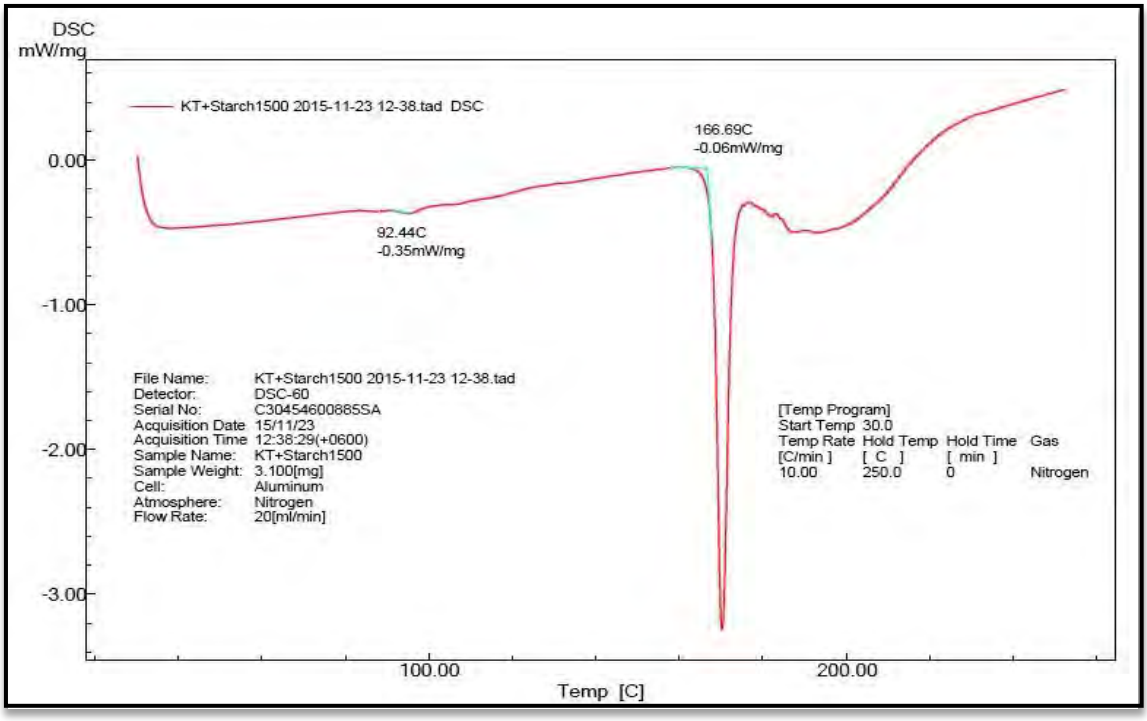


Figure 3.12: DSC thermogram of Ketorolac Tromethamine + Starch 1500

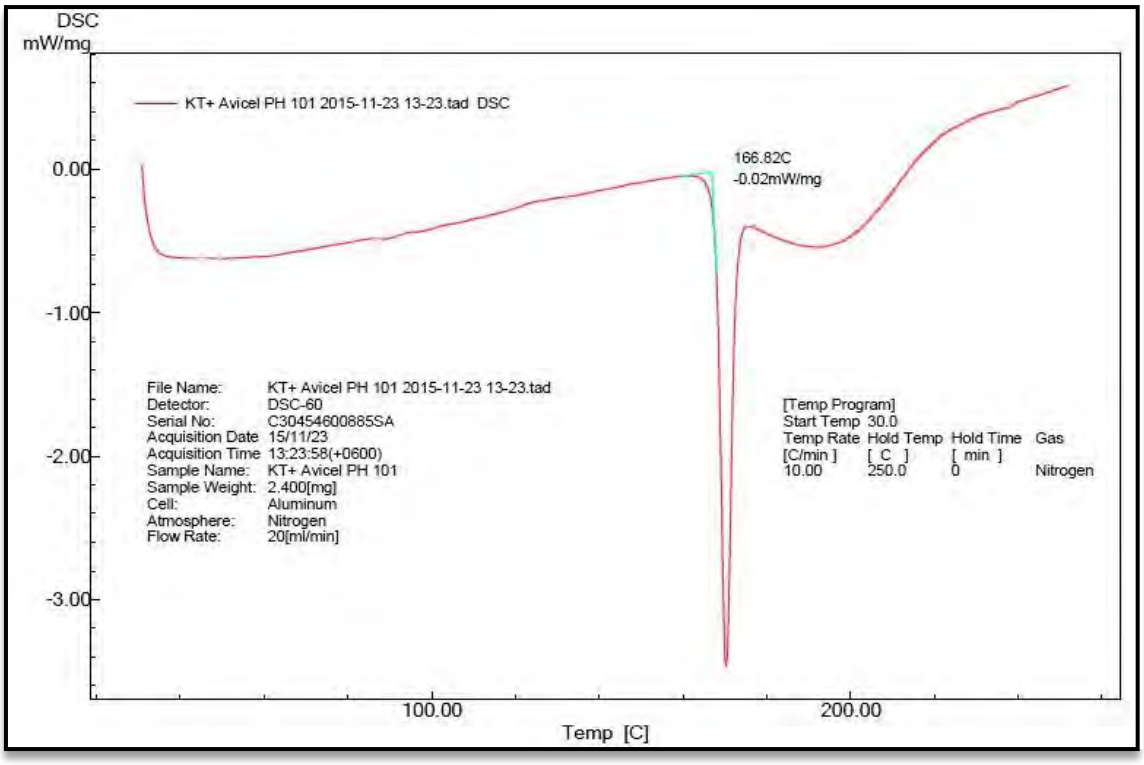


Figure 3.13: DSC thermogram of Ketorolac Tromethamine + Avicel PH 101

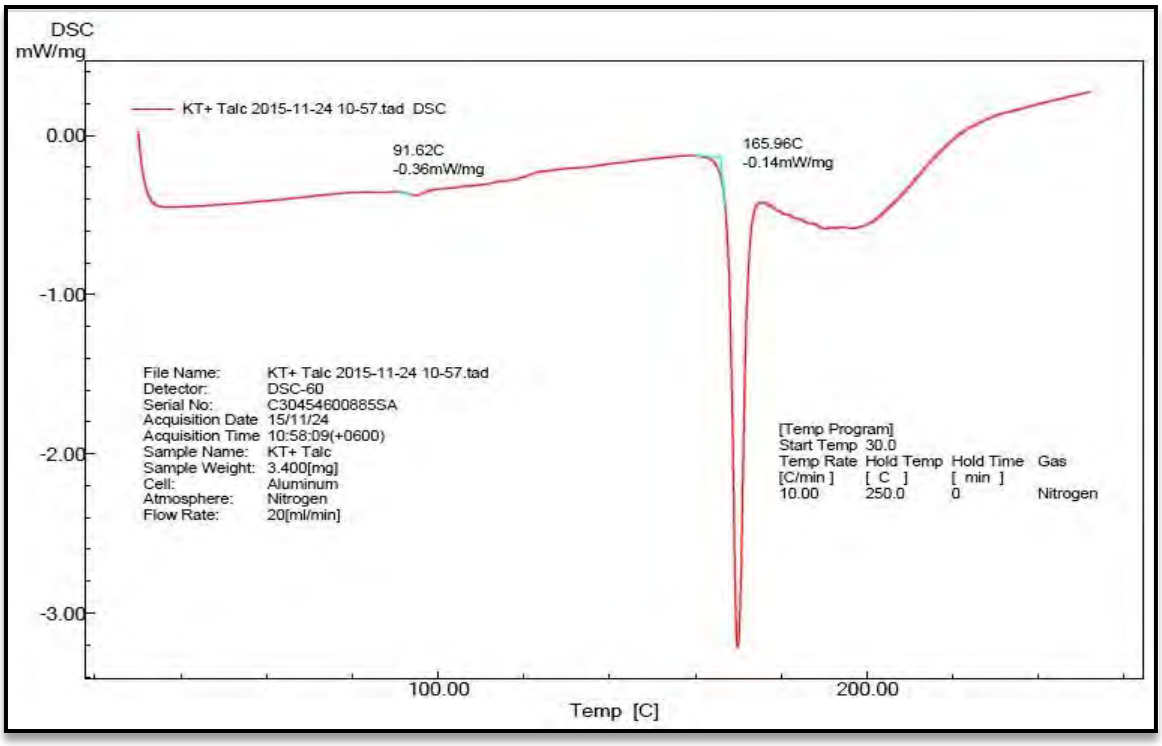


Figure 3.14: DSC thermogram of Ketorolac Tromethamine + Talc

3.3 Drug release kinetic study of Ketorolac Tromethamine matrix tablets

A polymer's ability to retard the drug release primarily depends on its viscosity and other processing factors like particle size, hardness, compressibility index, etc. Hence, Methocel K100M CR and Methocel K4M CR were used because it forms a strong viscous gel in contact with aqueous media, which may be useful in controlling delivery of drugs. The drug release data (Table 3.3 to Table 3.12) obtained from dissolution study was extrapolated by zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell equations to know the drug release kinetics followed by the nine formulations (Figure 3.15 to Figure 3.24).

Table 3.3: Zero order release profile of matrix tablets of Ketorolac Tromethamine (200 mg)

Time (Hour)	Cumulative % of drug released ± SD								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.5	14.74 ±0.41	14.35± 0.94	14.99± 0.67	18.77± 0.38	19.99 ±0.69	9.43 ±1.7	13.32 ±16.4	12.52 ±0.91	14.99 ±0.62
1	23.30 ±0.68	18.04± 0.95	24.65± 1.78	22.99± 0.85	21.64 ±3.81	13.19 ±0.53	16.54 ±1.69	17.92 ±0.48	24.65 ±1.60
2	32.96 ±0.56	30.08± 1.45	38.17± 0.93	35.70± 0.71	34.09 ±0.94	35.21 ±0.41	28.89 ±0.18	29.37 ±0.49	38.17 ±0.81
4	48.38 ±0.85	47.70± 0.04	54.98± 0.96	47.53± 0.48	47.64 ±0.71	51.55 ±0.93	39.64 ±1.40	43.68 ±3.01	54.99 ±0.58
8	60.98 ±0.91	67.85± 1.83	61.52± 0.69	69.82± 1.71	65.74 ±0.45	69.49 ±2.57	68.00 ±1.49	61.95 ±0.59	61.52 ±1.08

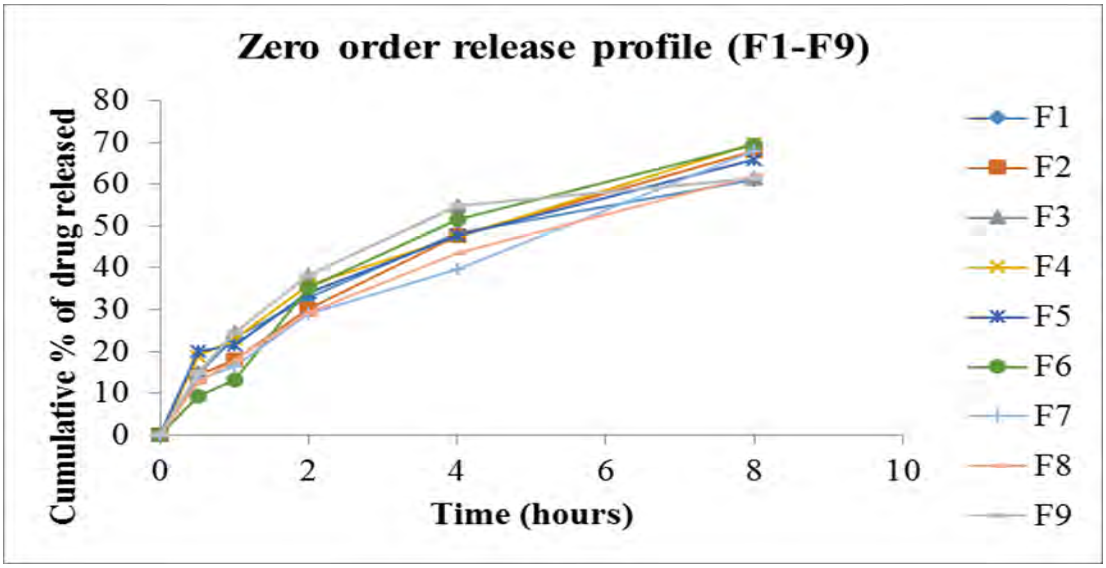


Figure 3.15: Zero order release kinetic plot of matrix tablets of Ketorolac Tromethamine (200 mg)

3.3.2 First order plot

Table 3.4: First order release profile of matrix tablets of Ketorolac Tromethamine (200 mg)

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	1.93	1.91	1.93	1.91	1.9	1.96	1.94	1.94	1.93
1	1.88	1.84	1.91	1.89	1.89	1.94	1.92	1.91	1.88
2	1.83	1.80	1.84	1.81	1.82	1.81	1.85	1.85	1.79
4	1.71	1.63	1.72	1.72	1.72	1.69	1.78	1.75	1.65
8	1.59	1.29	1.51	1.48	1.53	1.48	1.51	1.58	1.69

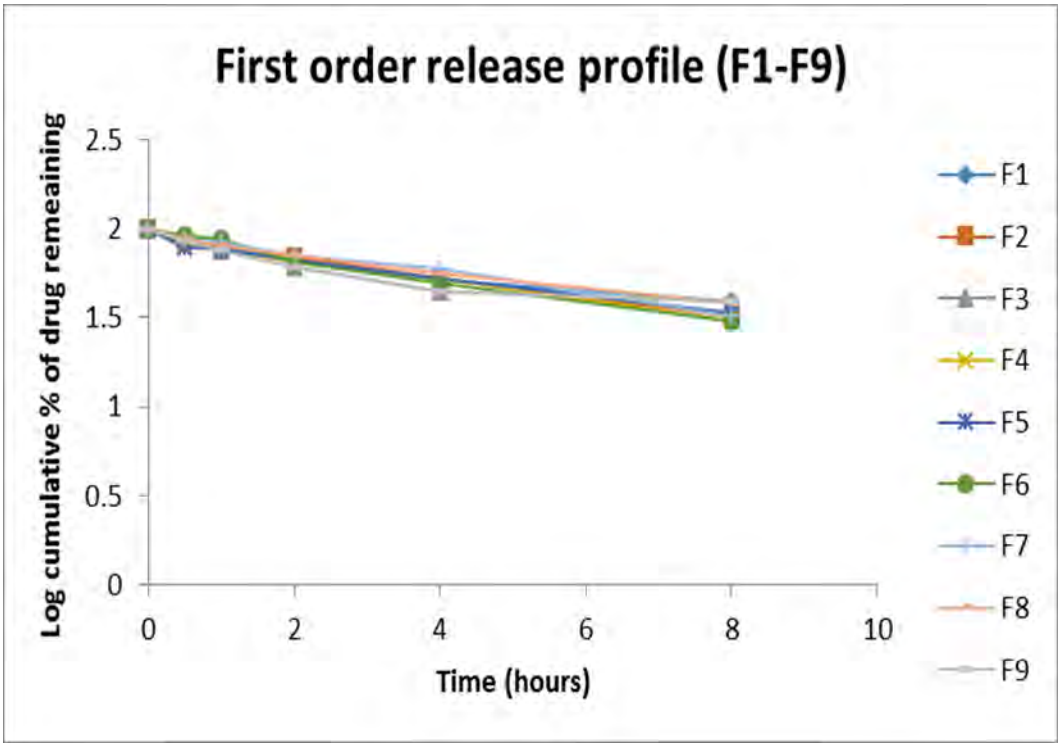


Figure 3.16: First order release kinetic plot of Ketorolac Tromethamine (200 mg)

3.3.3 Higuchi plot:

Table 3.5: Higuchi release profile of matrix tablets of Ketorolac Tromethamine (200 mg)

Square root of time (Hour)	cumulative % 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
0.71	14.74	14.35	14.99	18.77	19.98	9.43	13.32	12.52	14.99
1	23.30	18.04	24.65	22.99	21.64	13.19	16.54	17.92	24.65
1.41	32.96	30.08	38.17	35.70	34.09	35.21	28.89	29.37	38.17
2	48.38	47.70	54.98	47.52	47.64	51.55	39.64	43.68	54.98
0	60.98	67.85	61.52	69.82	65.79	69.49	68.00	61.95	61.52

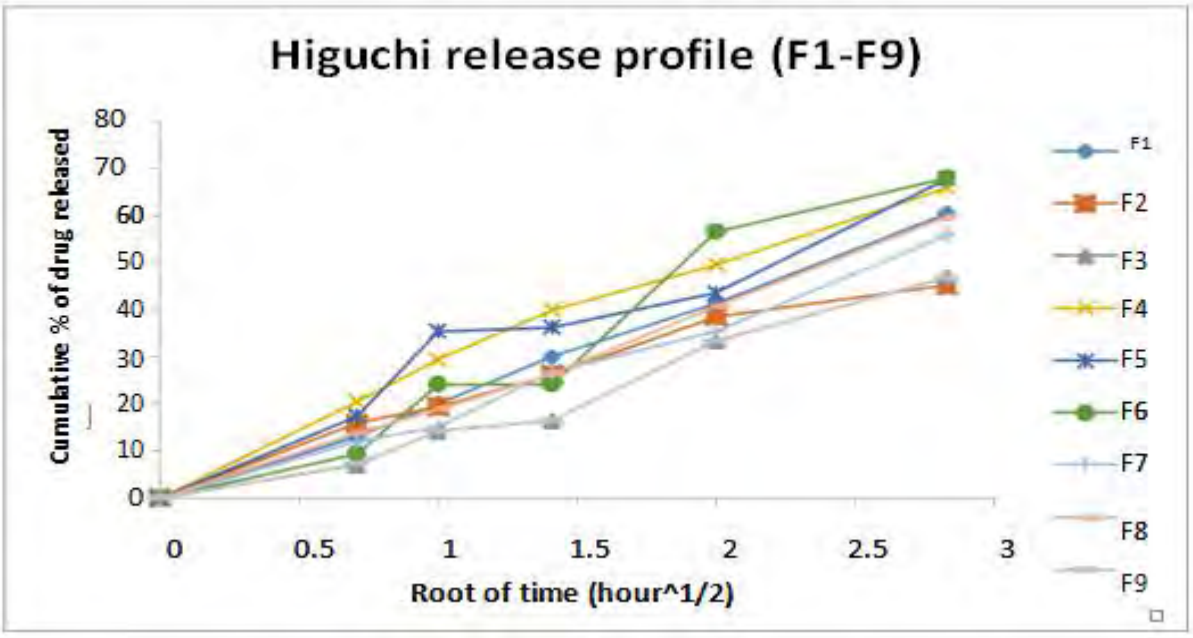


Figure 3.17: Higuchi release kinetic plot of Ketorolac Tromethamine (200 mg)

3.3.4 Korsmeyer-Peppas plot:

Table 3.6: Korsmeyer Peppas release profile of matrix tablets of Ketorolac Tromethamine (200 mg)

Log of time (Hour)	Log fraction of drug released								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
-0.30	-0.83	-0.84	-0.82	-0.73	-0.7	-1.03	-0.88	-0.9	-0.82
0	-0.63	-0.74	-0.61	-0.64	-0.66	-0.88	-0.78	-0.75	0.61
0.30	-0.48	-0.52	-0.42	-0.45	-0.47	-0.45	-0.54	-0.53	-0.42
0.60	-0.32	-0.32	-0.26	-0.32	-0.32	-0.29	-0.4	-0.36	-0.26
0.90	-0.21	-0.17	-0.21	-0.16	-0.18	-0.16	-0.17	-0.21	-0.21

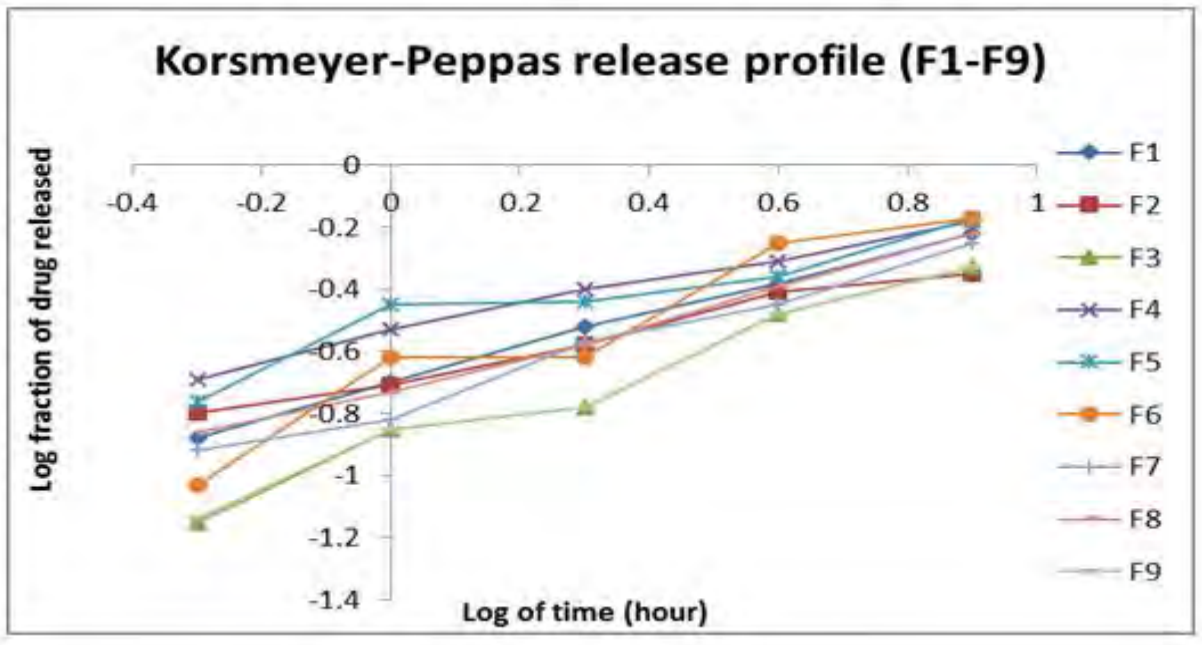


Figure 3.18: Korsmeyer-Peppas release kinetic plot of Ketorolac Tromethamine (200 mg)

3.3.5 Hixson-Crowell plot:

Table 3.7: Hixson-Crowell release profile of matrix tablets of Ketorolac Tromethamine (200 mg)

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	0.24	0.23	0.24	0.31	0.33	0.15	0.22	0.2	0.24
1	0.39	0.3	0.42	0.39	0.36	0.21	0.27	0.3	0.42
2	0.58	0.52	0.69	0.63	0.6	0.63	0.5	0.51	0.69
4	0.92	0.9	1.08	0.9	0.9	0.99	0.72	0.81	1.08
8	1.25	1.46	1.27	1.53	1.40	1.52	1.47	1.28	1.27

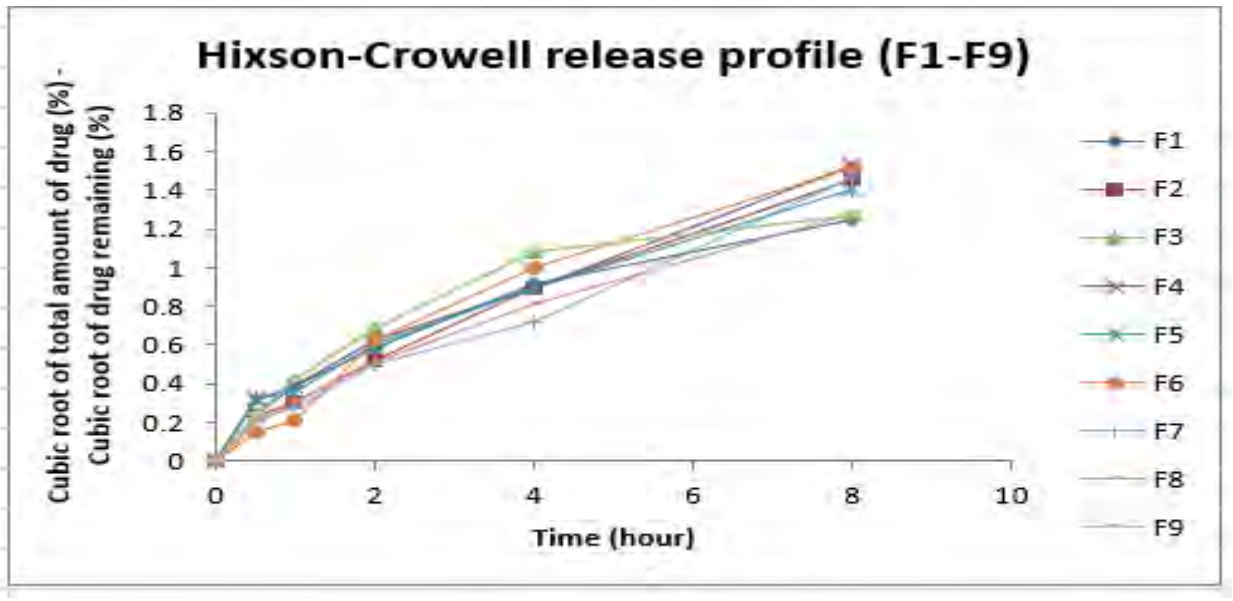


Figure 3.19: Hixson-Crowell release kinetic plot of Ketorolac Tromethamine (200 mg)

3.4 Description of drug release constant and R^2 values for different formulations

The value of drug release constant and the regression coefficients were calculated from the plot. In this experiment, the Higuchi model showed the best release kinetics profile of drug as it has values of regression coefficient for 200 mg close to 1 (R^2: 0.903 to 0.995) compared to R^2 values for zero order, first order, Korsmeyer-Peppas and Hixson-Crowell models (Table 3.8). The 'n' value obtained from the Korsmeyer-Peppas equation for all 9 formulations also showed that drug release from the tablet occurs by non-Fickian transport i.e. diffusion coupled with erosion (Table 3.8).

Table 3.8: Release rate of drug constant and R^2 values for different formulations (200 mg)

Formulations	Zero order		First order		Higuchi		Korsmeyer-Peppas		Hixson Crowell	
	K_0	R^2	K	R^2	K_{HC}	R^2	n	R^2	K_{HC}	R^2
F1	8.191	0.901	0.178	0.981	28.092	0.929	0.690	0.948	0.111	0.978
F2	6.318	0.872	0.197	0.948	25.094	0.993	0.510	0.957	0.185	0.959
F3	7.033	0.895	0.111	0.981	28.197	0.945	0.599	0.981	0.298	0.980
F4	8.012	0.993	0.199	0.926	29.035	0.965	0.608	0.938	0.237	0.910
F5	8.330	0.738	0.147	0.971	26.036	0.993	0.518	0.980	0.115	0.991
F6	9.301	0.869	0.190	0.969	24.950	0.998	0.510	0.970	0.198	0.911
F7	8.081	0.938	0.131	0.997	25.921	0.970	0.971	0.988	0.130	0.940
F8	3.904	0.694	0.179	0.901	26.094	0.950	0.603	0.943	0.102	0.998
F9	7.116	0.717	0.196	0.920	28.688	0.981	0.611	0.988	0.193	0.930

3.5 Analysis of fractional dissolution time

The fractional dissolution time for 25%, 50% and 80% release of drug from all the 9 formulated tablets have been shown in Table 3.9. For the 9 formulations of Ketorolac Tromethamine matrix tablet, the values of MDT showed a small decrease in the drug retaining ability of the polymer for some formulations. The time taken to release 25%, 50% and 80% of the drug from the matrix tablet was found to decrease with the rise in amount of Methocel K4M CR for F1 to F3 and F5 to F9 but in case of F4, it gives the highest value than other formulations. Except F4, rest of the formulations shown the retardation of drug release hence higher MDT value as the concentration of polymer increased.

Table 3.9: Comparison of fractional dissolution time and MDT of different formulations of Ketorolac Tromethamine (200 mg)

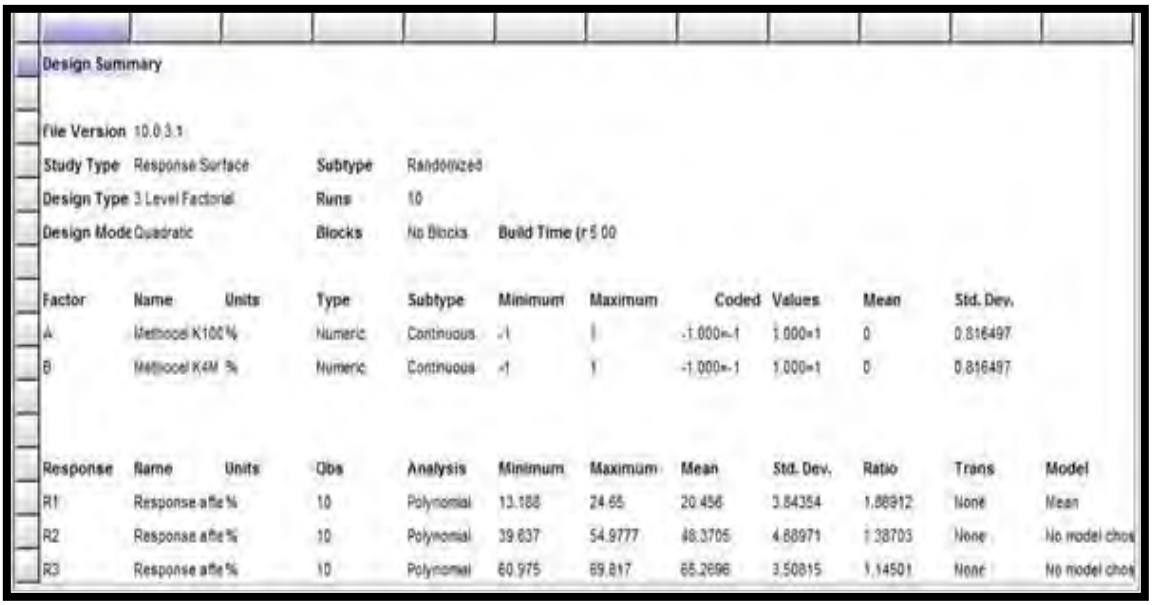
Formulations	$T_{25\%}$	$T_{50\%}$	$T_{80\%}$	MDT
F1	0.78	3.44	7.07	4.90
F2	0.99	3.32	7.06	4.10
F3	1.11	3.05	8.37	4.92
F4	1.18	3.90	10.98	5.01
F5	1.91	3.94	8.12	4.01
F6	1.23	4.39	7.90	4.72
F7	1.19	4.02	9.10	4.99
F8	1.18	3.89	8.29	4.04
F9	1.1	3.34	7.93	4.91

3.6 Analysis of the 3^2 full factorial design by using Design Expert Software

The 3^2 full factorial design has been analyzed by using Design Expert Software. Here, the two polymers have been used as independent variables and is designated as Factor 1 and Factor 2 for Methocel K100M CR (A) and Methocel K4M CR (B), respectively.

Select	Std	Run	Factor 1 A:Methocel ... %	Factor 2 B:Methocel K... %	Response 1 Response af... %	Response 2 Response af... %	Response 3 Response af... %
	3	1	1	-1	16.537	39.637	68
	5	2	0	0	21.643	47.636	65.793
	10	3	0	0	21.643	47.636	65.793
	2	4	0	-1	22.986	47.524	69.817
	4	5	-1	0	18.044	47.702	67.849
	9	6	1	1	24.65	54.977	61.516
	8	7	0	1	13.188	51.552	69.487
	7	8	-1	1	24.65	54.9777	61.516
	6	9	1	0	17.916	43.68	61.95
	1	10	-1	-1	23.303	48.383	60.975

Figure 3.20: Overall design of the experiment (200 mg)



Design Summary

File Version 10.0.3.1

Study Type	Response Surface	Subtype	Randomized
Design Type	3 Level Factorial	Runs	10
Design Mode	Quadratic	Blocks	No Blocks

Build Time (r 5.00

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 afte	%	10	Polynomial	13.188	24.65	20.456	3.84354	1.86912	None	Mean
R2	Response afte	%	10	Polynomial	39.637	54.9777	48.3705	4.88971	1.38703	None	No model cho
R3	Response afte	%	10	Polynomial	60.975	69.817	65.2696	3.50815	1.14501	None	No model cho

Figure 3.21: Design summary (200 mg)

3.6.1 Graph columns for 200 mg matrix tablets

The graph column represents the correlation between the polymers and the release rate after the respective hours. Here, for response after 1 hour, the response is negatively correlated to the concentration of Methocel K100M CR i.e. Methocel K100M CR alone did not sustain the release of drug after 1 hour. Furthermore, after 4 hours, the response is also negatively correlated with the concentration of Methocel K100M CR. So, here in this case, K100M CR did not sustain the release as well. However, in 8 hours, the response is slightly positively correlated indicating a sustaining effect on drug release.

For response after 1 hour, the value of correlation was -0.244 which proves that the response is slightly weakly correlated to the concentration of factor 1 i.e. Methocel K100M CR. This is indicated that the concentration of Methocel K100M didn't have any effect on sustaining the drug release after 1 hour.

Similarly, since the correlation value after 4 hours was -0.371, it means that the response after 4 hours is slightly negatively correlated to the concentration of Methocel K100M CR, however, poor than for response after 1 hour as negative correlation value after 4 hours is greater than after 1 hour which means at a particular level Methocel K100M has negative effect on response after 1 hour but when compared to 4 hours, the response for 1 hours was better.

For response after 8 hours, the correlation value was 0.044 which indicated that the response is slightly positively correlated to the concentration of Methocel K100M CR while keeping the Methocel K4M CR constant at all levels of Methocel K100M CR. The positive correlation after 8 hours is responsible for sustaining the release of drug and it gives better response than for 1 and 4 hours but if the values of 1 and 4 hours are compared with each other, the response after 1 hour is better than 4 hours. To sum up all responses, the response after 8 hours gives the better effect on sustaining the release of drug than 1 and 4 hours.

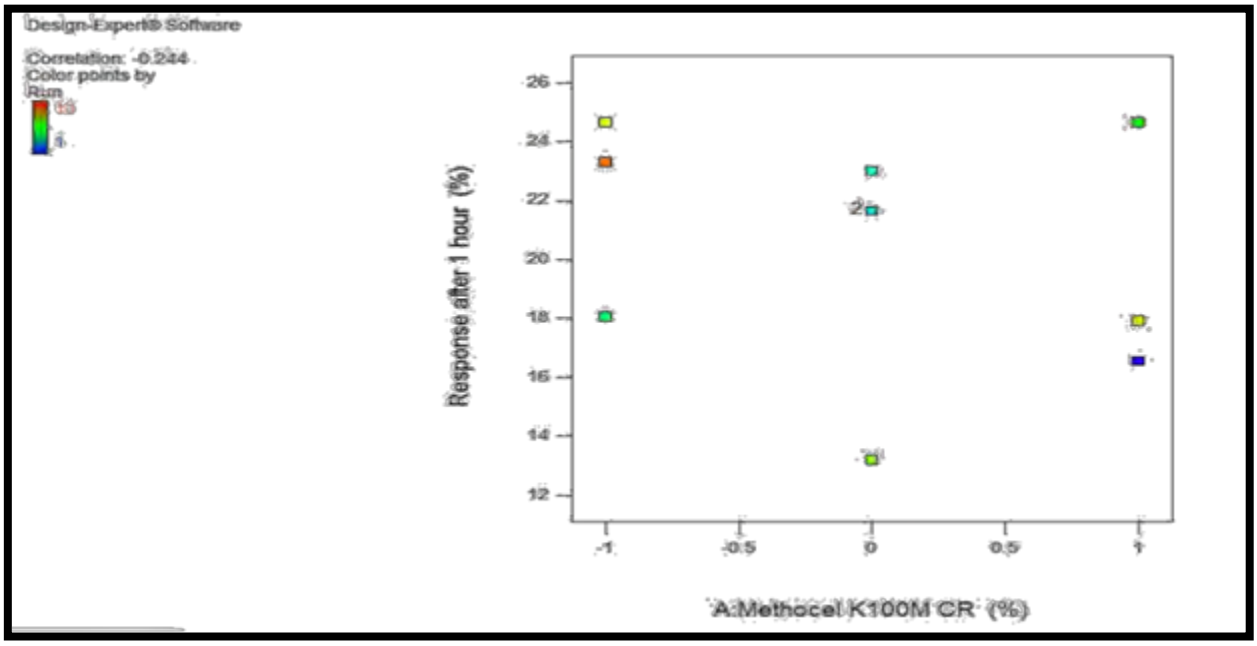


Figure 3.22: Graph column with Methocel K100M CR (A) after 1 hour (200 mg)

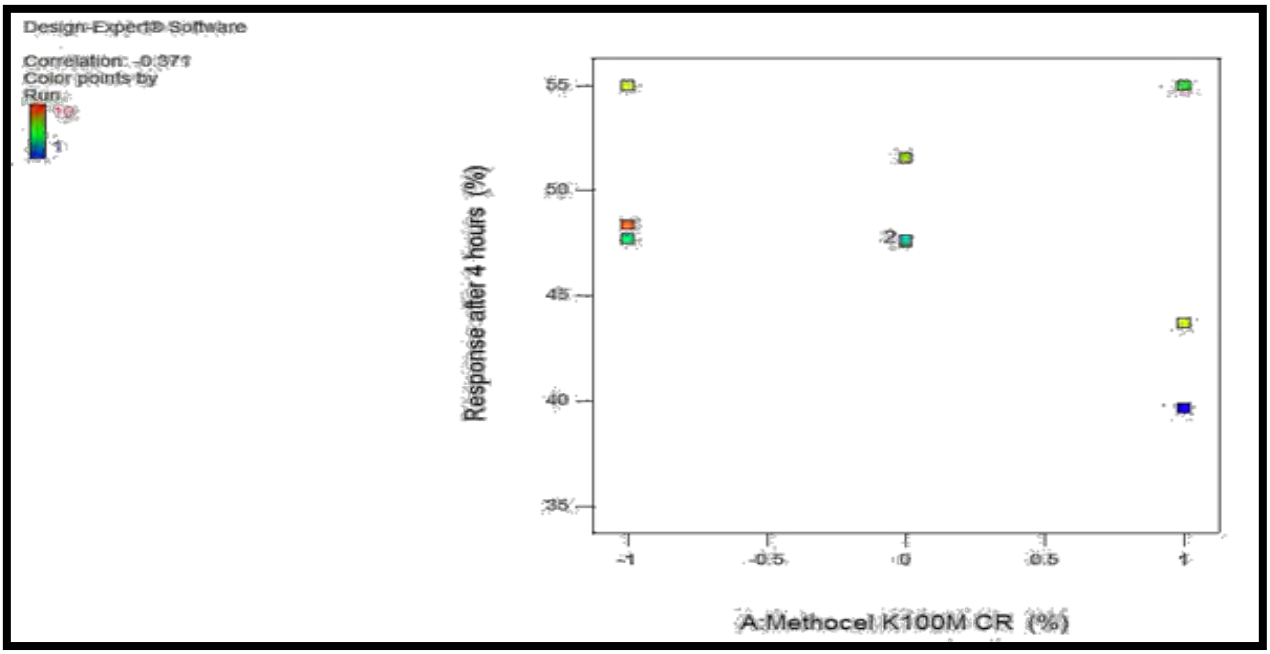


Figure 3.23: Graph column with Methocel K100M CR (A) after 4 hours (200 mg)

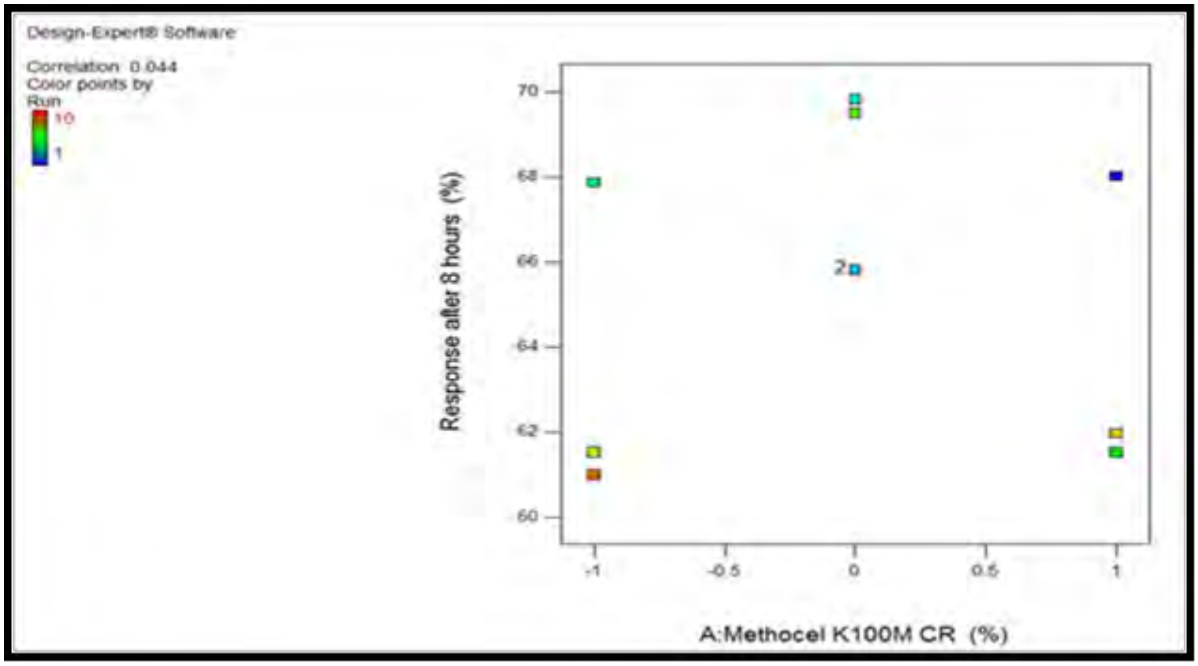


Figure 3.24: Graph column with Methocel K100M CR (A) after 8 hours (200 mg)

The three graphs depicted in Figure 3.25 to 3.27 shows the plot of response after 1, 4 and 8 hours versus Factor 2 (Methocel K4M CR). Here, the response after 1 hour is weakly negatively correlated with the concentration of polymer Methocel K4M CR with Methocel K100M CR being kept constant at all different levels of Methocel K4M CR. In contrast, the response after 4 hours is slightly positively correlated to the concentration of Methocel K4M CR indicating that the 4 hours' response has effect on sustaining the release of drug whereas it did not have any effect on sustaining the release of drug after 1 hour. Furthermore, the response after 8 hours is negatively correlated to the concentration of Factor 2. So, response after 4 hours had effect on sustaining the release of drug than that of other two responses after 1 and 8 hours. The correlation value for the effect of Methocel K4M CR on drug release after 1 hour was -0.012 indicating that the response is weakly correlated to the concentration of Methocel K4M CR. So, this polymer (Methocel K4M CR) has a very little effect on sustaining the release after 1 hour. The correlation value after 4 hours was 0.753 indicating that the response is moderately positively correlated to the concentration of Methocel K4M CR. So, it has a good effect on sustaining the release of drug after 4 hours. The correlation value for the effect of Methocel K4M CR on drug release after 8 hours was -0.243 indicating that the response is slightly weakly correlated to the concentration of Methocel K4M CR. This means that Methocel K4M alone do not sustain the release after 8 hours. So, if it is compared with the result of 4 hours, it indicates that on 1 and 8 hours, polymer B has negative effect on sustaining the release but after 4 hours the release could be slowed down at certain level of K4M CR.

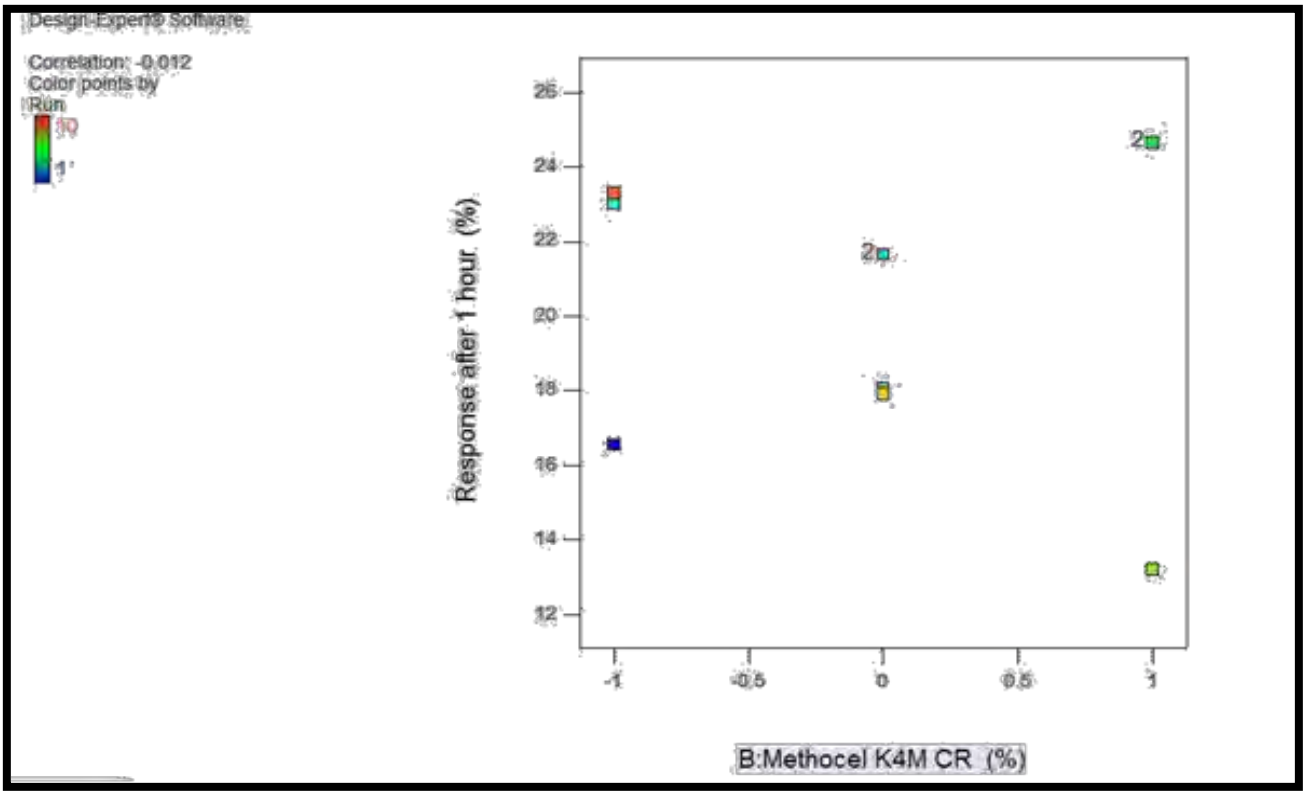


Figure 3.25: Graph column with Methocel K4M CR (B) after 1 hour (200 mg)

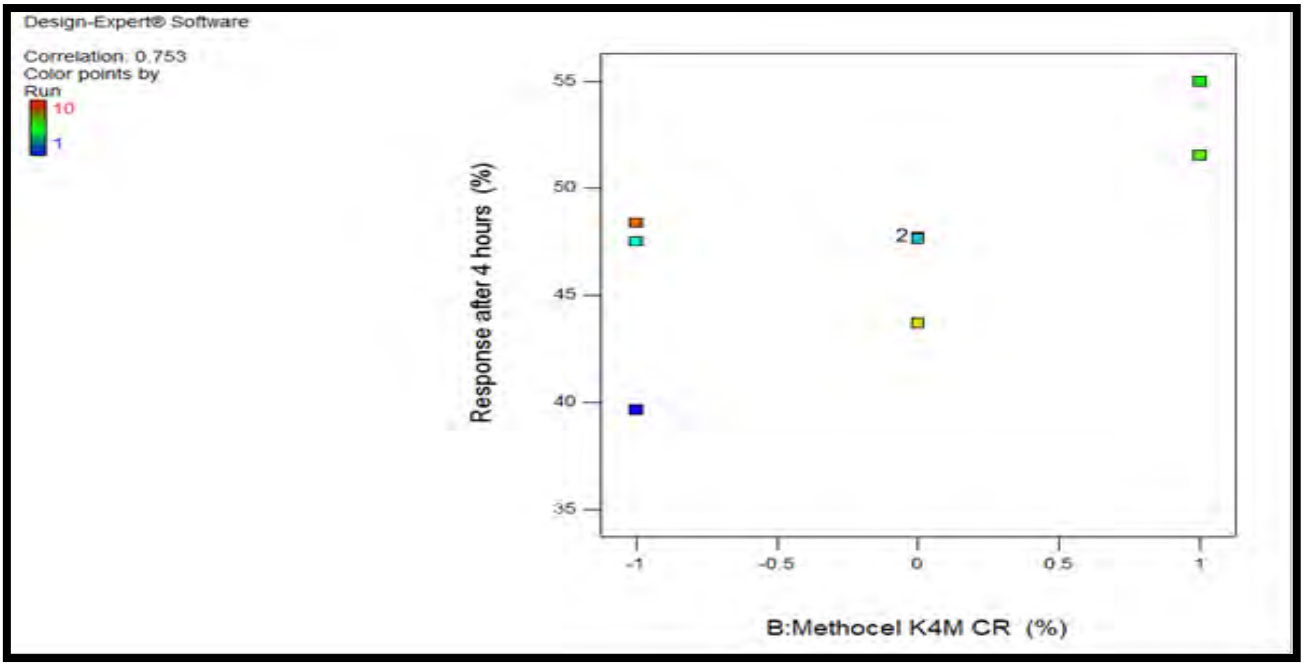


Figure 3.26: Graph column with Methocel K4M CR (B) after 4 hours (200 mg)

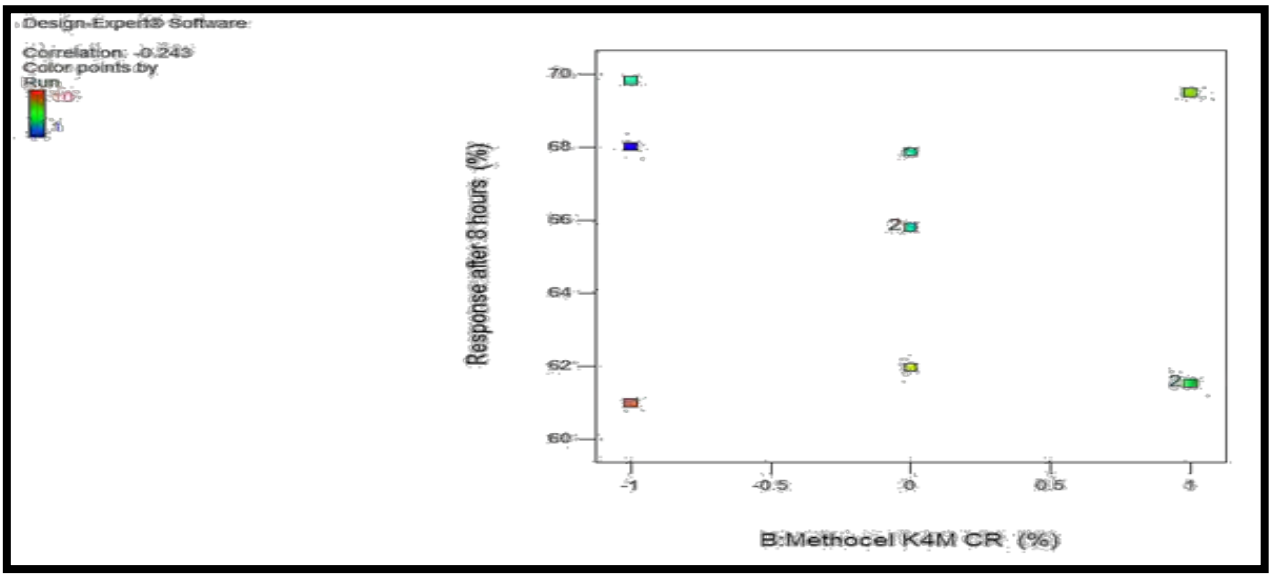


Figure 3.27: Graph column with Methocel K4M CR (B) after 8 hours (200 mg)

3.6.2 Statistical model, Mathematical model, Response surface and Normal plot analysis (200 mg)

A. Statistical model analysis for response after 1 hour

After 1 hour, for each source of terms (intercept, A, B, AB, A^2, B^2), the probability value (p-value) was examined to choose the statistical model that best evaluates the response. So far, the Design Expert Software predicted that the release pattern after 1 hour (Y_{1hr}) followed a 'Mean *versus* Total' model where the mean represents the intercept (b_0) of the statistical equation $Y= b_0 + b_1A + b_2B + b_3AB + b_4A^2 + b_5B^2$. However, no p-value was mentioned for the suggested model (Figure 3.28).

Moreover, the 'Lack of Fit Tests' performed by the Software on the basis of the response values at different levels of polymers used suggested neither any F-value (Fitness value) nor p-value for the model, thereby, indicating that the model for response after 1 hour lacks fitness but fitness lacks to what extent could not be figured out since the result from software only represents the significance at 5% level (Figure 3.29).

The response after 1 hour was also statistically analyzed using analysis of variance (ANOVA) at 5% significance level. From the ANOVA, the ability of the model to provide adequate information on the dependence of drug release on polymer was determined. The p-

value for this model was not mentioned by the software indicating that the model is not significant at 5% level and the chance of error is more than 5% (Figure 3.30).

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	4184.48	1	4184.48			Suggested
Linear vs Mear	7.94	2	3.97	0.22	0.8061	
2FI vs Linear	11.44	1	11.44	0.60	0.4664	
Quadratic vs 2	4.37	2	2.19	0.080	0.9245	
Cubic vs Quad	73.89	2	36.94	2.09	0.3234	Aliased
Residual	35.31	2	17.66			
Total	4317.43	10	431.74			

Figure 3.28: Sequential model for response after 1 hour (200 mg)

Lack of Fit Tests

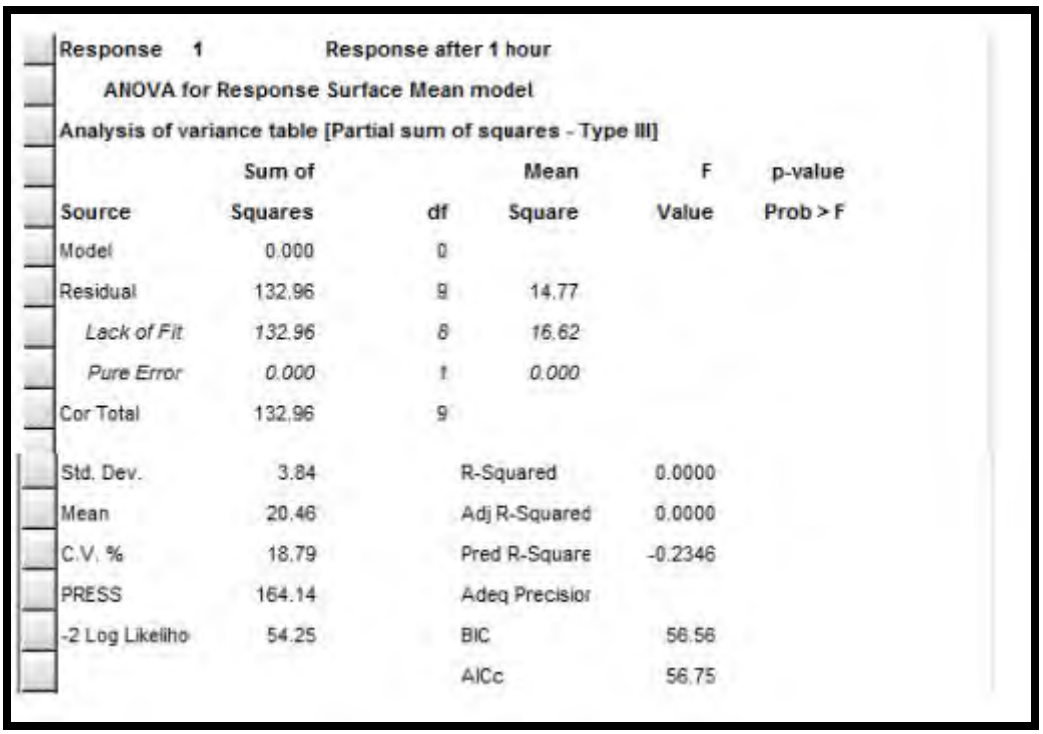
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Linear	125.02	6	20.84		
2FI	113.57	5	22.71		
Quadratic	109.20	3	36.40		
Cubic	35.31	1	35.31		
Pure Error	0.000	1	0.000		

"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	4.23	0.0597	-0.2089	-1.2019	292.75	
2FI	4.35	0.1458	-0.2813	-2.8945	517.79	
Quadratic	5.22	0.1787	-0.8479	-6.9358	1055.10	
Cubic	4.20	0.7344	-0.1952	-36.3662	4968.04	Aliased

Figure 3.29: Summary of the Lack of fit test (200 mg)



Response 1 Response after 1 hour

ANOVA for Response Surface Mean 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	0.000	0			
Residual	132.96	9	14.77		
Lack of Fit	*132.96*	*8*	*16.62*		
Pure Error	*0.000*	*1*	*0.000*		
Cor Total	132.96	9			

Std. Dev.	3.84	R-Squared	0.0000
Mean	20.46	Adj R-Squared	0.0000
C.V. %	18.79	Pred R-Square	-0.2346
PRESS	164.14	Adeq Precisior	
-2 Log Likeliho	54.25	BIC	56.56
		AICc	56.75

Figure 3.30: ANOVA response after 1 hour (200 mg)

B. Mathematical model analysis for response after 1 hour

The predicted equation for Y_{1hr} in terms of coded factors is mentioned below:

$$\mathbf{Y_{1hr} = 20.456}$$

The above polynomial equation indicates that the response after 1 hour is independent of the polymer concentration. Since there are no coefficients of A and B, it means no effect was determined from the change in polymer concentration on drug release.

C. Response surface analysis for response after 1 hour

The two dimensional (2D) contour plot and the three dimensional (3D) surface plot illustrated by the Software for response after 1 hour also supported the statistical model which showed that the release was independent of the polymer loading. In the 2D contour and 3D surface plot, the whole region is green that is neither A nor B nor even AB interaction is affecting (increasing or sustaining) drug release (Figure 3.31 A and 3.31 B).

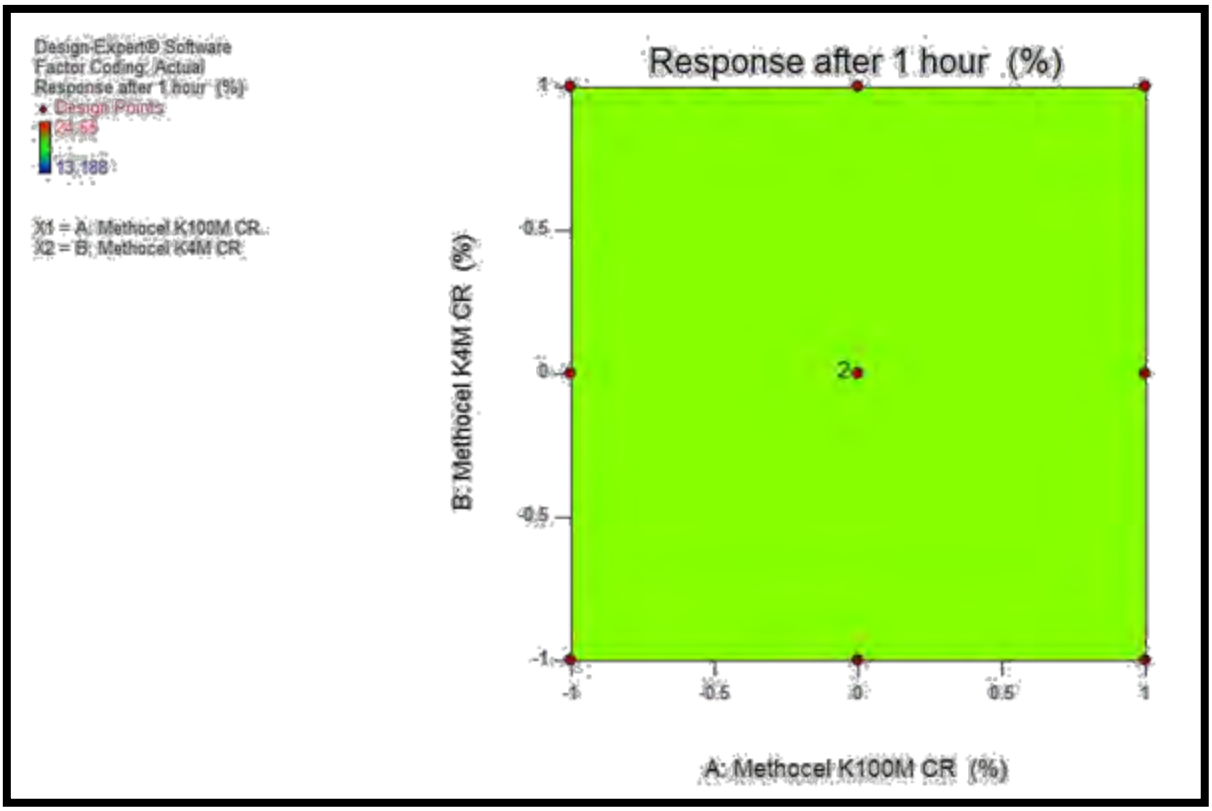


Figure 3.31 A: 2D response plot after 1 hour (200 mg)

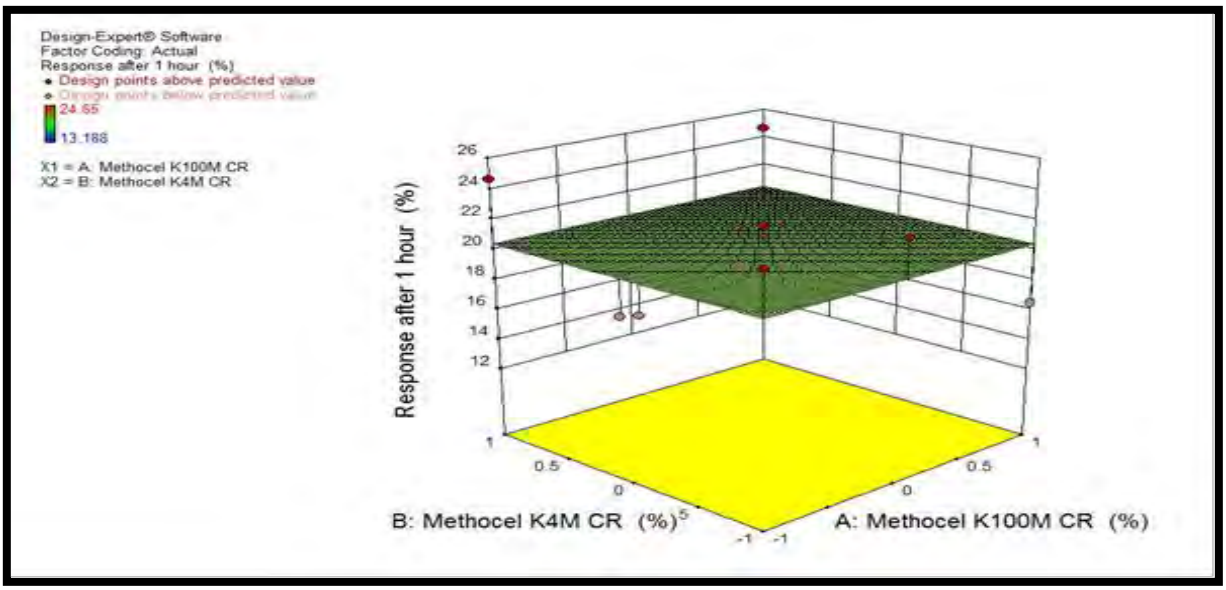


Figure 3.31 B: 3D response plot after 1 hour (200 mg)

D. Normal plot analysis for response after 1 hour

The diagnostic-normal plot of residuals determines the linearity of the data points and for the first hour, the practical values for all the formulations were found in close proximity to the best fit line drawn, thereby, showing linearity in response values at different polymer level used (Figure: 3.32)

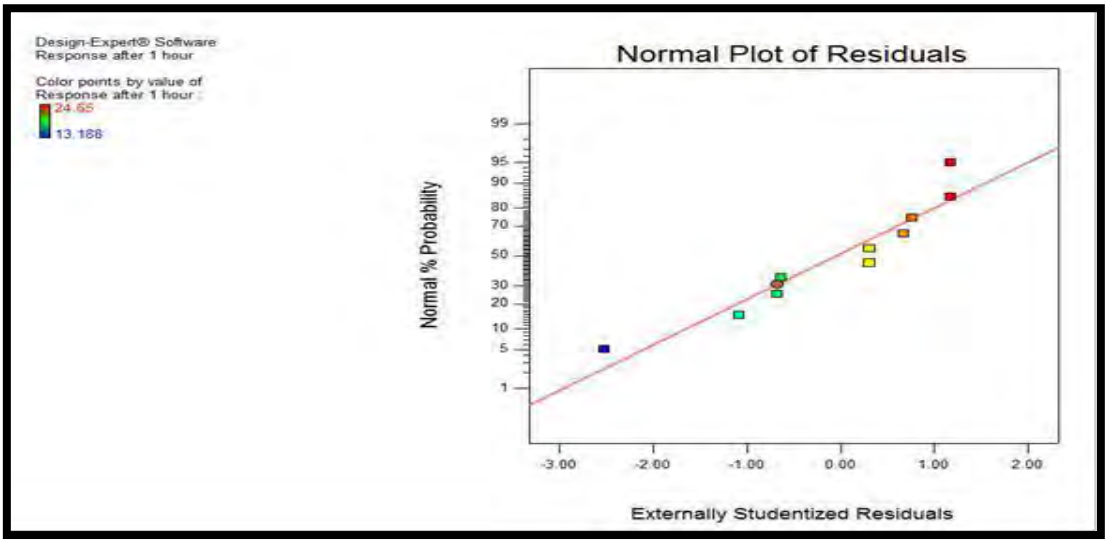


Figure 3.32: Normal plot residual after 1 hour (200 mg)

A. Statistical model analysis for response after 4 hours

After 1 hour, for each source of terms (intercept, A, B, AB, A^2, B^2), the probability value (p-value) was examined to choose the statistical model that best evaluates the response. So far, the Design Expert Software predicted that the release pattern after 4 hours followed a linear model with a p-value of 0.0140 (Figure 3.33).

Moreover, the 'Lack of Fit Tests' performed by the software on the basis of the response values at different levels of polymers used suggested neither any F-value (Fitness value) nor p-value for the model, thereby, indicating that the model for response after 4 hour lacks fitness but fitness lacks to what extent could not be figured out since the result from software only represents the significance at 5% level (Figure 3.34).

The response after 4 hours was also statistically analyzed using analysis of variance (ANOVA) at 5% significance level. From the ANOVA, the ability of the model to provide adequate information on the dependence of drug release on polymer was determined. The suggested p-value from the ANOVA was found to be 0.0140 indicating that the model is significant at 5% level. So, the statistical response after 4 hours indicates that the model is significant at 5% level for 200 mg of tablets and that the release of drug from the tablet is significantly dependent on the polymers used at different levels (Figure 3.35).

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	23397.02	1	23397.02			
Linear vs Mear	139.52	2	69.76	8.36	0.0140	Suggested
2FI vs Linear	19.12	1	19.12	2.92	0.1384	
Quadratic vs 2	21.15	2	10.57	2.33	0.2134	
Cubic vs Quad	16.09	2	8.05	7.80	0.1137	Aliased
Residual	2.06	2	1.03			
Total	23594.96	10	2359.50			

Figure 3.33: Sequential model for response after 4 hours (200 mg)

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Linear	58.42	6	9.74		
2FI	39.30	5	7.86		
Quadratic	18.16	3	6.05		
Cubic	2.06	1	2.06		
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	2.89	0.7048	0.6205	0.2674	145.02	Suggested
2FI	2.56	0.8014	0.7022	0.2075	156.87	
Quadratic	2.13	0.9083	0.7936	0.0295	192.11	
Cubic	1.02	0.9896	0.9531	-0.4673	290.43	Aliased

Figure 3.34: Summary of Lack of fit test after 4 hours (200 mg)

Response 2 Response after 4 hours

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	139.52	2	69.76	9.36	0.0140	significant
A-Method1	27.17	1	27.17	3.28	0.1142	
B-Method2	112.34	1	112.34	13.46	0.0080	
Residual	58.42	7	8.35			
Lack of Fit	58.42	6	9.74			
Pure Error	0.000	1	0.000			
Cor Total	197.94	9				

Std. Dev.	2.89	R-Squared	0.7048
Mean	48.37	Adj R-Squared	0.6295
C.V. %	5.97	Pred R-Squared	0.2674
PRESS	145.02	Adeq Precision	8.119
-2 Log Like	46.03	BIC	52.94
		AICc	56.03

Figure 3.35: ANOVA response after 4 hours (200 mg)

B. Mathematical model analysis for response after 4 hours

The equation for response after 4 hours has been found as follows:

$$Y_{4hr} = 48.3705 - 2.13\ A + 4.33B$$

This equation is linear determined by the presence of A and B in the equation. According to the above equation, coefficient of A i.e. b_1 bears a negative sign which means that A decreased the response by 2.13 times. So, increasing the amount of the Methocel K100M CR polymer in the formulations increased the time taken by the drug to leave the formulation and retarded release of drug into the medium by 2.13 times. Coefficient of B i.e. b_2 bears positive sign indicating that Methocel K4M CR increased the response by 4.33 times. So, polymer A has greater impact on sustaining the drug release after 4 hours.

C. Response surface analysis for Response after 4 hours

From the bar present beside the 2D contour plot it can be seen that the lowest percentage release was 39.637% and the highest percentage release was 54.9777% after 4 hours. The blue zone represents the lowest response values and the red zone represents the highest response values. It is evident from the 2D and 3D plot that with decrease in the level of Methocel K4M CR and with increase in the level of Methocel K100M CR, the response values are moving towards the blue zone (Figure 3.36 A and 3.36 B). This indicates the using the polymer content in such pattern slows the release of the drug from the tablet in a linear manner. Therefore, the plots are also supported by the linear equation obtained for response after 4 hours.

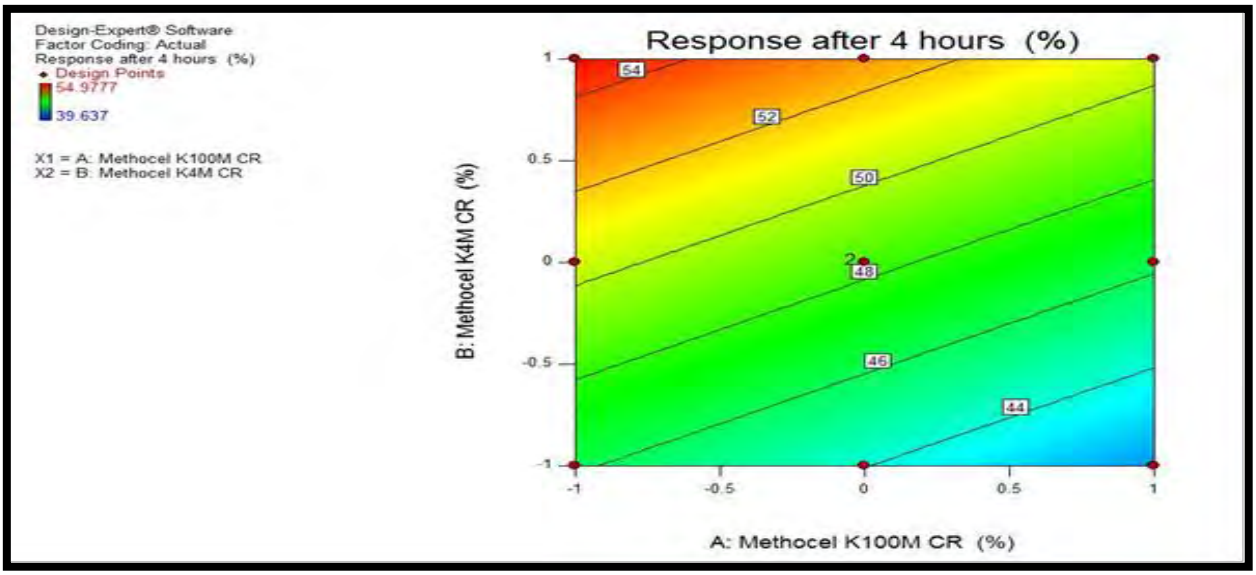


Figure 3.36 A: 2D response plot after 4 hours (200 mg)

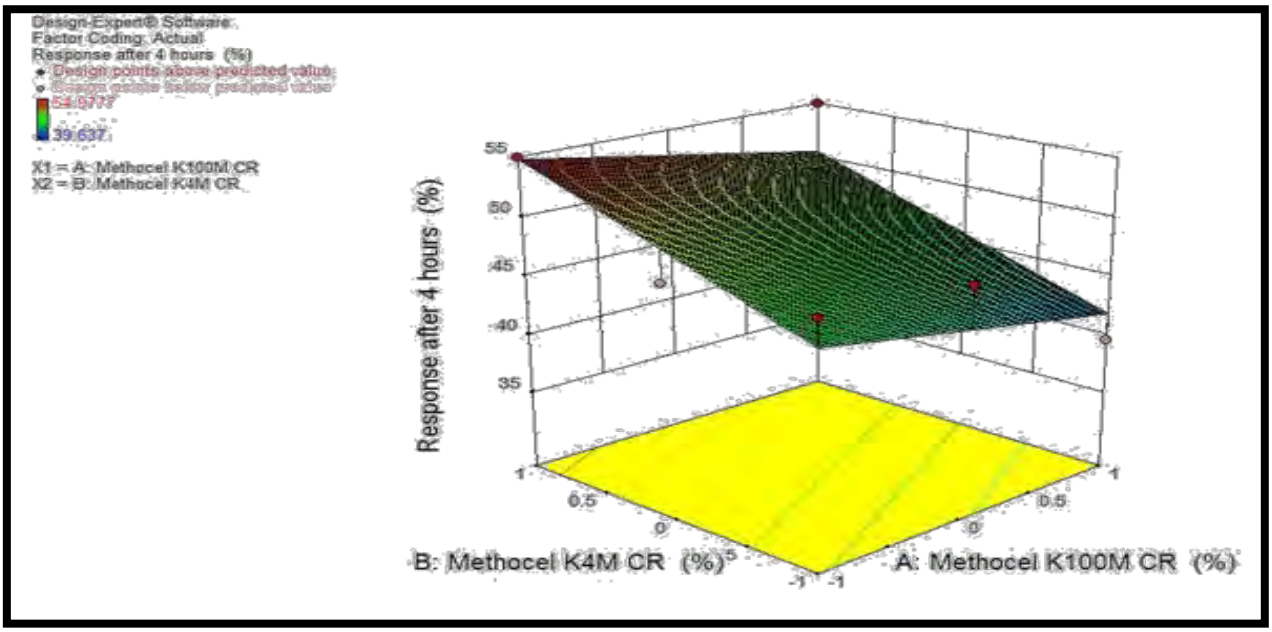


Figure 3.36 B: 3D response plot after 4 hours (200 mg)

D. Normal plot analysis for response after 4 hours

The diagnostic--normal plot of residuals determines the linearity of the data points and for 4 hours, the practical response values for all the formulations were found in close proximity with the best fit line drawn, thereby, showing linearity in response values at different polymer levels used (Figure 3.37).

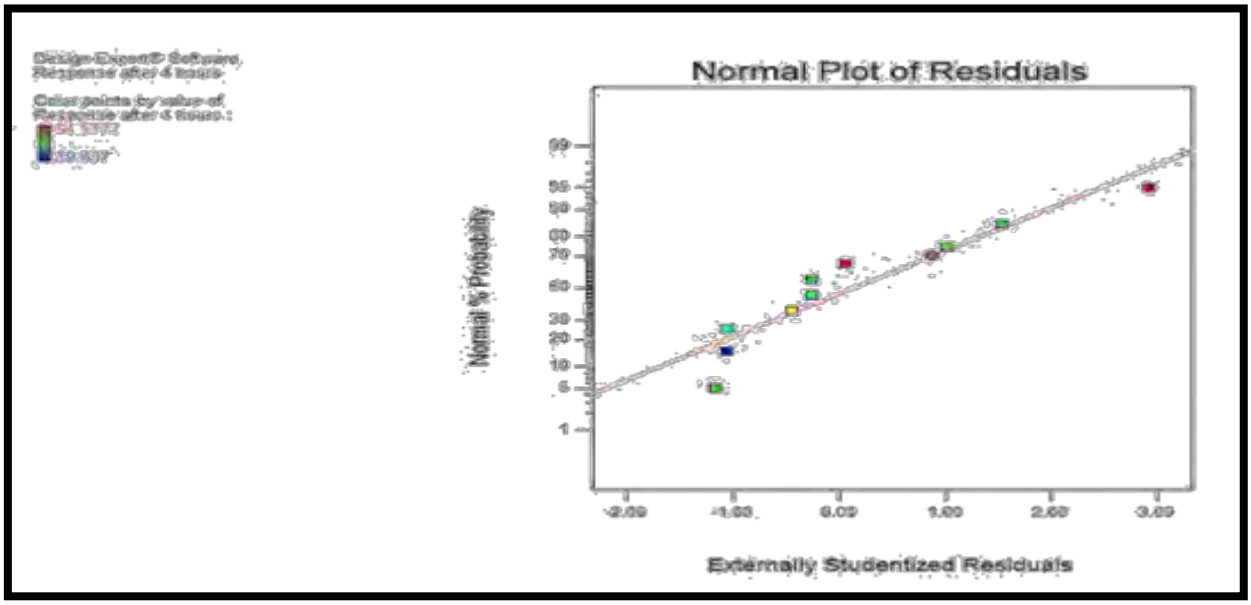


Figure 3.37 Normal plot residuals after 4 hours (200 mg)

A. Statistical model analysis for response after 8 hours

After 8 hours, for each source of terms (intercept, A, B, AB, A^2, B^2), the probability value (p-value) was examined to choose the statistical model that best evaluates the response. So far, the Design Expert Software predicted that the release pattern followed a 'Mean *versus* Total' model where the mean represents the intercept (b_0) of the statistical equation $Y= b_0 + b_1A + b_2B + b_3AB + b_4A^2 + b_5B^2$. Moreover, there was no p-value was mentioned for the suggested model (Figure 3.38).

Moreover, the 'Lack of Fit Tests' performed by the software on the basis of the response values at different levels of polymers used suggested neither any F-value (Fitness value) nor p-value for the model, thereby, indicating that the model for response after 4 hour lacks fitness but fitness

lacks to what extent could not be figured out since the result from software only represents the significance at 5% level (Figure 3.39).

The response after 8 hours was statistically analyzed using analysis of variance (ANOVA) at 5% significance level. From the ANOVA, the ability of the model to provide adequate information on the dependence of drug release on polymer was determined. The p-value for this model was not mentioned by the Software indicating that the model is not significant at 5% level and the chance of error is more than 5% (Figure 3.40).

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	42601.21	1	42601.21			Suggested
Linear vs Mear	6.77	2	3.38	0.23	0.8019	
2FI vs Linear	12.34	1	12.34	0.81	0.4034	
Quadratic vs 2	40.87	2	20.43	1.61	0.3070	
Cubic vs Quad	31.85	2	15.93	1.68	0.3729	Aliased
Residual	18.94	2	9.47			
Total	42711.97	10	4271.20			

Figure 3.38: Sequential model for response after 8 hours (200 mg)

Lack of Fit Tests

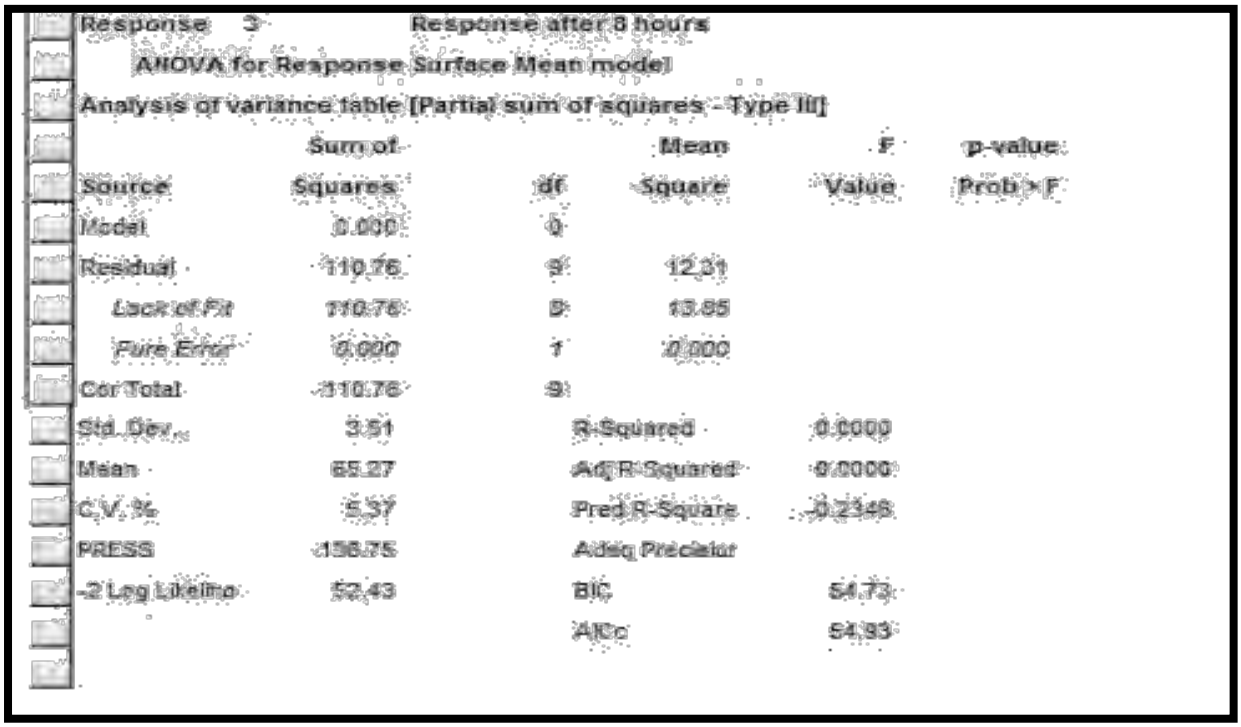
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Linear	103.99	6	17.33		
2FI	91.66	5	18.33		
Quadratic	50.79	3	16.93		
Cubic	18.94	1	18.94		
Pure Error	0.000	1	0.000		

"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	3.85	0.0611	-0.2071	-1.2358	247.65	
2FI	3.91	0.1725	-0.2412	-2.8201	423.13	
Quadratic	3.56	0.5415	-0.0317	-3.3311	479.73	
Cubic	3.08	0.8290	0.2306	-23.0539	2664.31	Aliased

Figure 3.39: Summary of Lack of fit test after 8 hours (200 mg)



Response 3 Response after 8 hours

ANOVA for Response Surface Mean model

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

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	0.000	0			
Residual	110.76	9	12.31		
Lack of Fit	110.76	8	13.85		
Pure Error	0.000	1	0.000		
Cor Total	110.76	9			

Std. Dev.	3.51	R-Squared	0.0000
Mean	65.27	Adj R-Squared	0.0000
C.V. %	5.37	Pred R-Squared	-0.2345
PRESS	136.75	Adeq Precision	
-2 Log Likelihood	52.43	BIC	54.73
		AICc	54.93

Figure 3.40: ANOVA response after 8 hours (200 mg)

B. Mathematical model analysis for response after 8 hours

The predicted equation for Y_{8hr} in terms of coded factors is mentioned below:

$$\mathbf{Y_{8hr} = 65.2696}$$

For the response after 8 hours (Y_{8hr}), only b_0 with a value of 65.2696 was obtained indicating that the polymer has no control on the release of drug after 8 hours. The polynomial equation represents the response after 8 hours is independent of the polymer concentration. Since there are no coefficients of A and B, it means no effect of changing polymer concentration on drug release.

C. Response surface analysis for response after 8 hours

The two dimensional (2D) contour plot and the three dimensional (3D) surface plot illustrated by the Software for response after 8 hours also supported the statistical model which showed that the release was independent of the polymer loading. In the 2D contour and 3D surface plot, the whole region is green that is neither A nor B nor even AB interaction is affecting (increasing or sustaining) drug release (Figure 3.41 A and Figure 3.41 B).

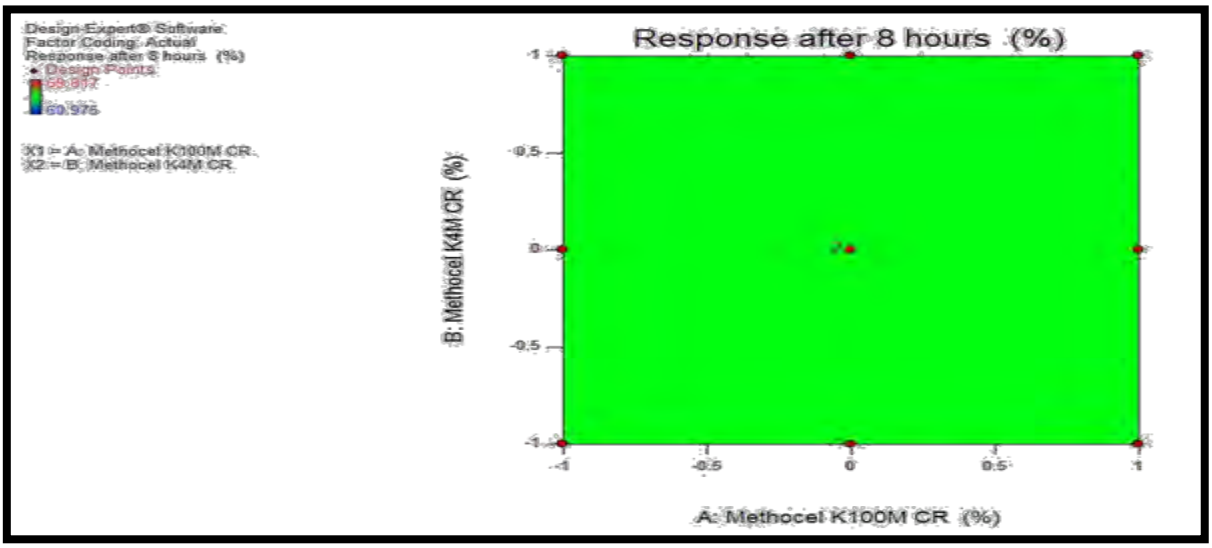


Figure 3.41 A: 2D response plot after 8 hours (200 mg)

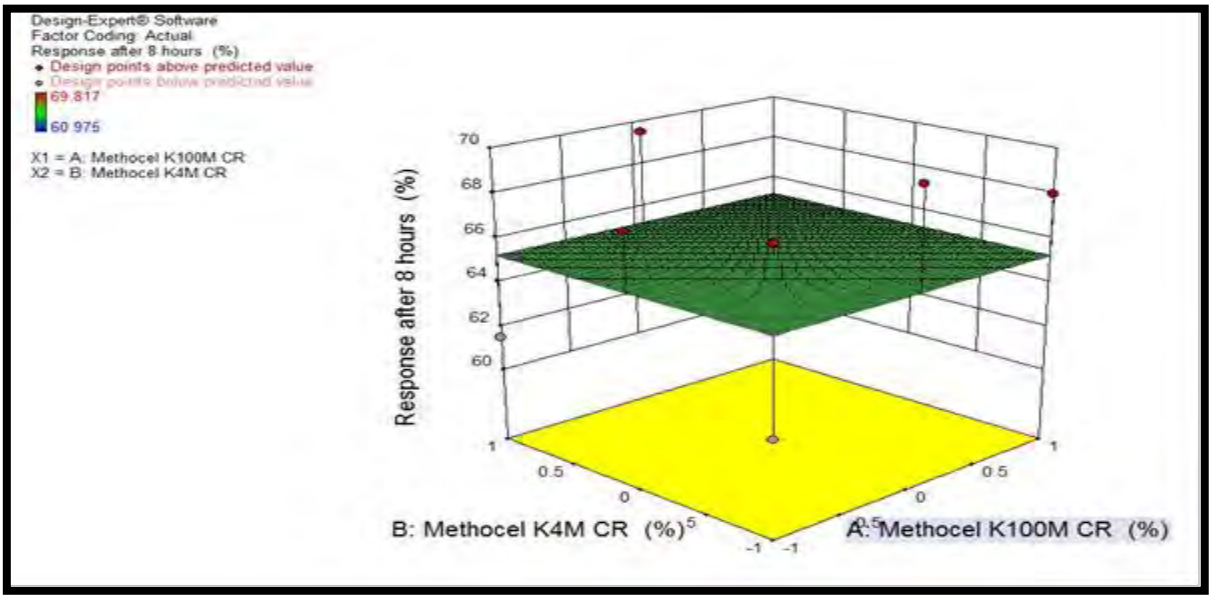


Figure 3.41A: 3D response plot after 8 hours (200 mg)

D. Normal plot analysis for response after 8 hours

The diagnostic--normal plot of residuals determines the linearity of the data points and for 8 hours, the practical response values for all the formulations were found in close proximity with

the best fit line drawn, thereby, showing linearity in response values at different polymer levels used (Figure 3.42).

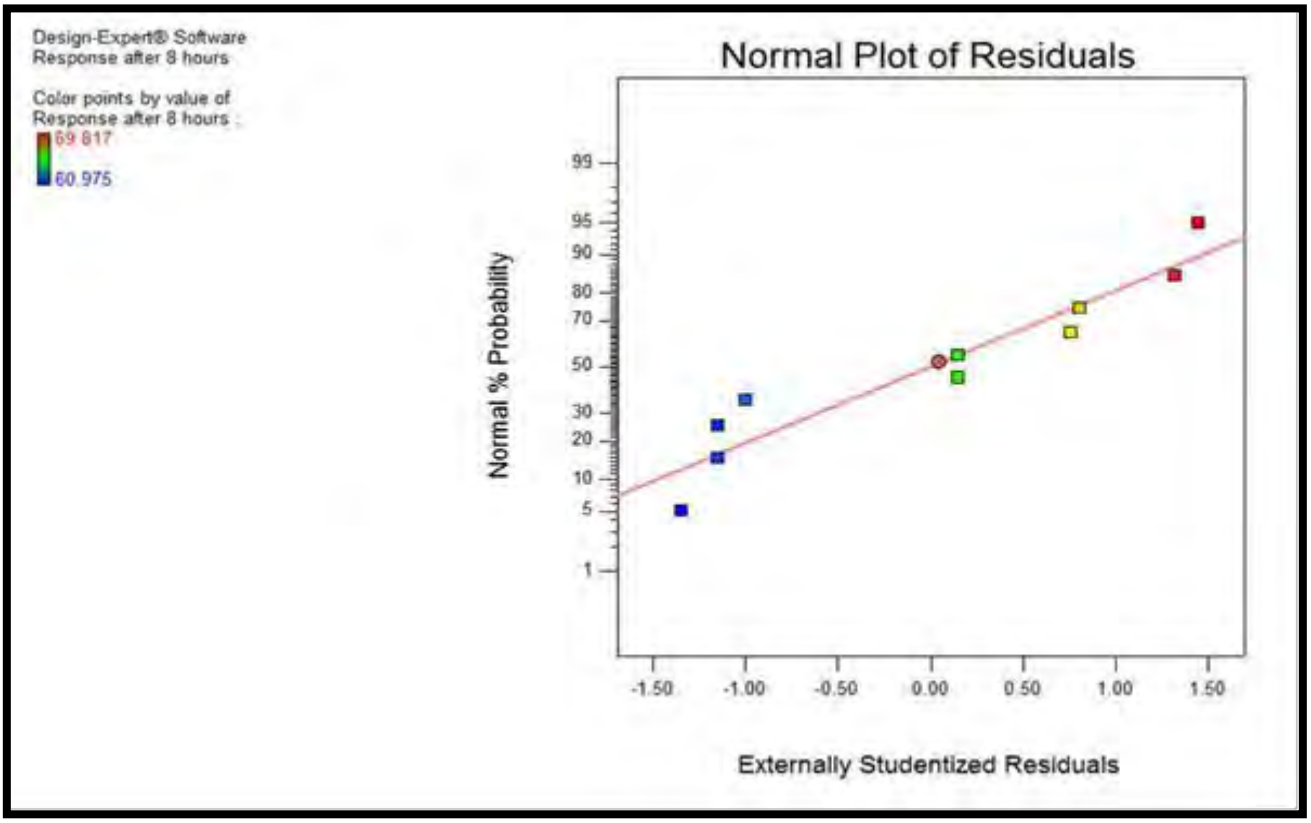


Figure 3.42 Normal plot residuals after 8 hours (200 mg)

3.6.3 Summary of the responses after 1, 4 and 8 hours

The Figure 3.43 represents the response after 1, 4 and 8 hours and effects of both polymers after respective hours. For 1 hour, both polymers have no effect on sustaining the release as it only represents the intercept and that the response after 1 hour is not statistically significant. In case of 4 hours' response, polymer A decreases the release of drug indicating that A has effect on sustaining the release after 4 hours but polymer B has no effect on sustaining the release of drug after 4 hours. The linear model suggested by the software for response after 4 hours was also found to be statistically significant at 5% level. Moreover, the response after 8 hours also indicates that both polymers have had no effect on the release of drug as it only represents the value of intercept like the response after 1 hour.

Response	Intercept	A	B		
Response aft...	20.456				
p=					
Response aft...	48.3705	-2.12812	4.32712		
p=		0.1142	0.0080		
Response aft...	65.2696				
p=					
Legend		p <.01	.01<= p <.05	.05<= p <.10	p >=.10

Figure 3.43 Summary of the response after 1, 4 and 8 hours (200 mg)

3.7 Optimization of the formulation:

After the determination of the release pattern followed by the formulations for 200mg tablets, the optimum formulation was identified through the implementation of numerical desirable ranges of the responses for 1 hour (Y_{1hr}), 4 hours (Y_{4hr}) and 8 hours (Y_{8hr}). The desirable ranges for these responses were restricted to $13.19\% < Y_{1hr} < 24.64\%$, $39.64\% <$

Y_{4hr} < 54.98% and 61.52% < Y_{8hr} < 69.49%. Based on the close proximity of the predicted response values with the experimental response values and overlay plot graphical analysis, the optimum formulation for 200mg tablet was obtained.

The optimum formulation was chosen to be F5 for 200 mg matrix tablet of Ketorolac Tromethamine based on the desirability criterion and graphical analysis of the formulation (Figure 3.44 and Figure 3.45). From the prediction error values in Table 3.12 for F5, it could be seen that the experimental responses were in close relation with the predicted values for the optimum formulation and for F5 the polymer combination at coded level 0, 0 was also found to be within the optimum region i.e. the yellow region of the overlay plot (Figure 3.45).

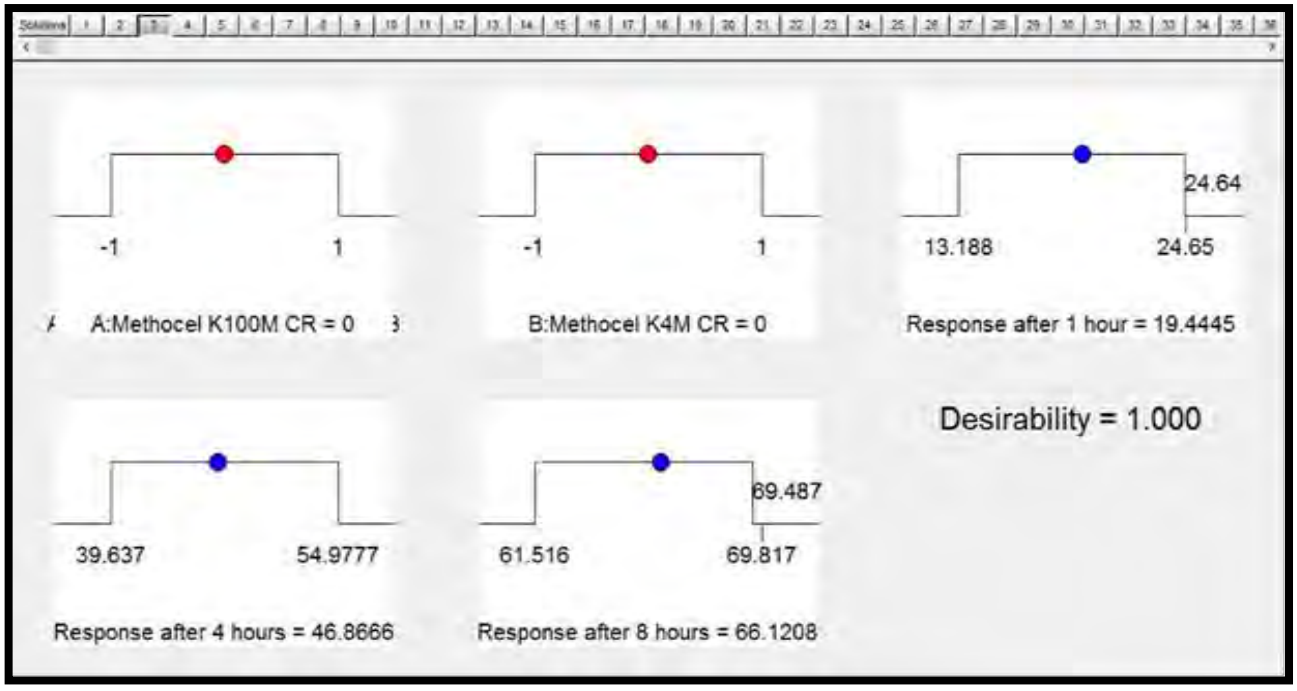


Figure 3.44: Predicted drug release profile of optimum formulation (200 mg)

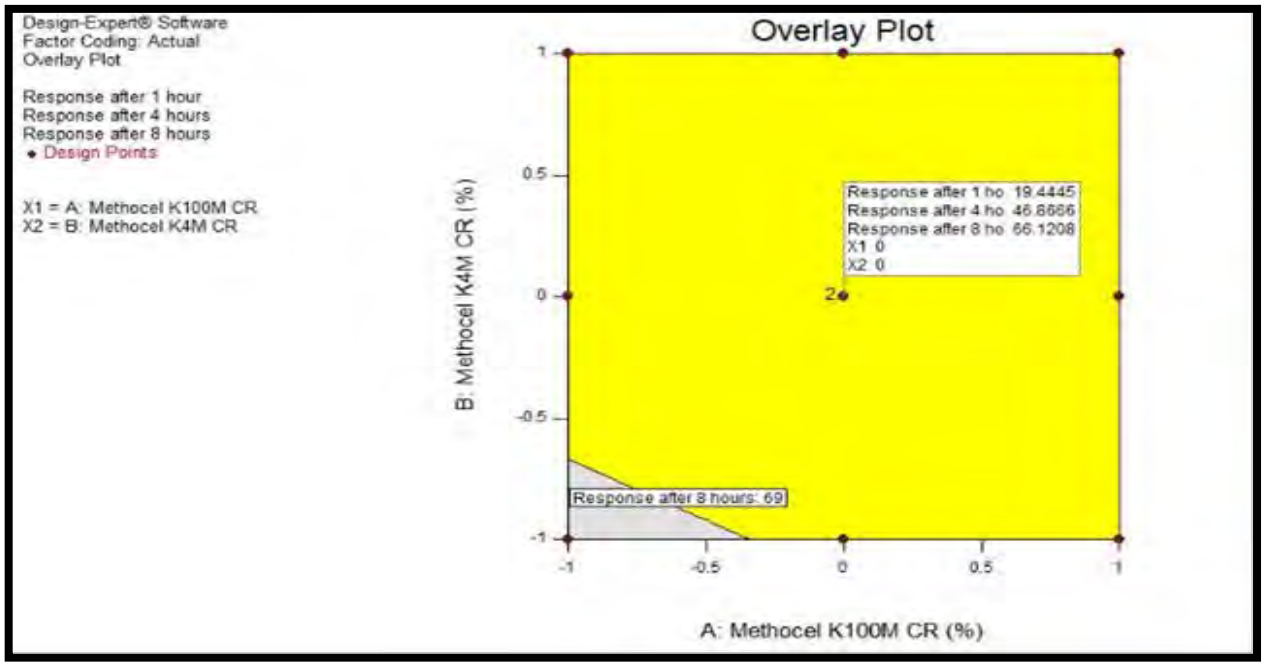


Figure 3.45: Overlay plot of the optimum formulation (200 mg)

Table 3.10: Predicted and experimental value of response for the optimum formulation

Composition Methocel K100M CR: Methocel K4M CR	Response variables	Experimental Value (%)	Predicted Value (%)	Prediction Error (%)
F5=30:7.5	Drug release after 1 hour	21.64	19.45	11.26
	Drug release after 4 hours	47.64	46.87	1.64
	Drug release after 8 hours	65.79	66.12	0.50

Conclusion

In this present study, the sustained release oral tablet dosage form of Ketorolac Tromethamine was formulated for two different doses i.e. 200 mg and 250 mg. For the purpose of formulation design, the compatibility between each of the selected excipients and the API were justified, which eventually led to the preparation of the tablets. In vitro dissolution study was performed to determine the release kinetics and the optimum formulation of the sustained release tablets for both the doses. However, statistically significant result in sustaining the drug release could not be obtained for 250 mg tablets from the Design Expert Software analysis which is why the 200 mg tablet formulations were chosen over 250 mg to proceed with the determination of the optimum formulation. From the statistical analysis of the models presented by the software for 200 mg tablets, a once-daily oral sustained release tablet formulation was identified on the basis of the close agreement between the experimental and the predicted responses, as well as the presence of the polymer combination for the chosen optimum formulation in the optimum region of the overlay plot.

Recommendation

An extensive research study could be performed with 250 mg dose of the matrix tablet of Ketorolac Tromethamine by using different polymer combinations.

Appendix

Appendix-1

Ketorolac Tromethamine:

Tromethamine salt of Ketorolac generates Ketoroalc Tromethamine. This salt is responsible for enhancing the solubility of Ketorolac that help the drug to absorb rapidly. Its chemical name is ±5-Benzoyl-2-3-dihydro-1-H-Pyrrolizine-1-carboxylic acid, 2-amino-2-(hydroxyl)-1, 3-propanediol. The molecular weight of Ketorolac is 376.40g/mol. Its pH in distilled water is 5.7-6.7 and pK_a 3.46 . The appearance of Ketorolac Tromethamine is white or white crystalline powder.

Appendix-2

Design Expert Software

Design Expert is a piece of software designed to facilitate the design of formulations and determine the interactions of multiple factors. This tool usually exhibits test matrices to screen up to 50 factors. It also offers the use of a power calculator that helps to establish the number of test runs needed. ANOVA is provided to establish statistical significance. Based on the validated predictive models, a numerical optimizer helps the user determine the ideal values for each of the factors in the experiment. The software determines the main effects of each factor as well as the interactions between factors by varying the values of all factors in parallel. A response surface model (RSM) can be used to map out a design space using a relatively small number of experiments. RSM provides an estimate for the value of responses for every possible combination of the factors by varying the values of all factors in parallel, making it possible to comprehend a multi-dimensional surface with non-linear shapes. The optimization feature can be used to calculate the optimum operating parameters for a process.

Appendix-3

FT-IR

Fourier Transform Infrared FT-IR represents the study of interaction of electromagnetic radiation. This radiation maintains the IR region of the EM spectrum (4000-400 cm^{-1}).IR radiation is passed through this region. The interaction of the spectrum is completely depend

on functional groups present in compound. The molecular vibration starts when certain frequencies of the radiation are absorbed by the molecule. The resulting IR spectrum represents two region namely functional group region (4000-1500 cm^{-1} and finger print region (<1500 cm^{-1}). Finger print region only represents the peak and value for the compound. No two compounds have same finger print region. In contrast, functional group region shows peaks for different functional groups of that compound.

Appendix-4

DSC

Differential Scanning Calorimetry or DSC is a thermo-analytical method in which in which the difference in the amount of heat is required to increase the temperature of a sample. A sample is heated or cooled and the changes of heat is required to increase the temperature of that particular sample. The changes of heat is recorded as changes of heat flow that is required for the sample. DSC has wide application in industries, pharmaceuticals, polymers, food, paper, printing, manufacturing and electronics.

Appendix-5

Dissolution Study

Dissolution study is necessary to examine the release of drug from pharmaceutical dosage form. The main purpose of dissolution study is to evaluate the *in-vitro* release kinetics .The dissolution study carries out by placing tablets into a prepared buffer dissolution medium and the release rate has been identified after several hours interval. To find out the level of API presents in the diluted solution, HPLC or UV-spectrophotometer have been used. When a method has been developed in terms of mathematical release kinetics model, further method validation is required to ensure the efficacy and effectiveness of formulations.

References

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