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Mefenamic Acid – HPMC AS HG Amorphous Solid Dispersions: Dissolution Enhancement Using Hot Melt Extrusion Technology

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ABSTRACT

Mefenamic acid, a BCS class II drug, displays high permeability and low solubility, thereby exhibiting a poor dissolution profile. Hence to improve the solubility and dissolution rate of Mefenamic acid, Hot Melt Extrusion (HME) technique was employed. The amorphous solid dispersion matrix exhibited enhanced dissolution with desired release characteristics. Hydroxypropylmethylcellulose acetate succinate (AquaSolve™ HPMC-AS HG) was used as a carrier with the poloxamer (Kolliphor P407). The drug load was varied from 20% to 40% within the blend. Drug and polymers were blended using a twin shell V-blender for 10 minutes and extruded using an 11mm twin-screw co-rotating extruder (ThermoFisher Scientific, Waltham, MA, USA). The processing conditions were selected by conducting pre-formulation studies, which included thermogravimetric analysis (TGA) to determine the thermal degradation temperature of mefenamic acid and selected polymers. Differential Scanning Calorimetry (DSC) was performed to determine the T_g of the polymers and T_m of the API. The extruded strands were collected, milled and sifted through a #30 sieve. This milled and sieved product was then subjected to DSC to characterize the state of drug in solid dispersions. The drug content of the extrudates was observed to be in acceptable range. To assess the drug release profile of the extrudates, *in vitro* dissolution testing was conducted in pH 7.4, phosphate buffer. Scanning Electron Microscopy was performed to study the morphology of the solid dispersions and Fourier Transform Infrared Spectroscopy was also performed to make sure that the drug and polymers are compatible with each other. Formulations containing Kolliphor P407 exhibited enhanced dissolution profiles compared to the

pure Mefenamic acid and solid dispersions with plain HPMC-AS HG. The DSC results illustrated the amorphous conversion of Mefenamic acid in the solid dispersions. The stability studies indicated that the optimized formulation with 20% drug load was found to be stable and in amorphous form with similar release profile after 3 months of study. Solid dispersions of Mefenamic acid with improved dissolution rate were prepared using Hot Melt Extrusion technology.

DEDICATION

This work is dedicated to my parents Archana and Shashi Bhushan Shukla, my friends Ankita, Kunal, Aditya and my family back home in India, without their tireless encouragement I would have given up long ago. Also, I would like to dedicate this work to my hometown, Indore which I missed the most.

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LIST OF ABBREVIATIONS

1. HME – Hot Melt Extrusion.
2. DSC – Differential Scanning Calorimetry.
3. TGA – Thermogravimetric Analysis.
4. FTIR – Fourier Transform Infrared.
5. MA – Mefenamic Acid.
6. HPMCAS - Hydroxypropyl Methylcellulose Acetate Succinate.
7. GIT – Gastro-intestinal Tract.
8. PVP – Polyvinyl Pyrrolidone.
9. DoE – Design of Experiments.
10. ICH – International Conference on Harmonization.
11. BCS – Biopharmaceutics Classification System.
12. SEM – Scanning Electron Microscopy.
13. SD – Solid Dispersion.
14. CMC – Critical Micelle Concentration.
15. USP – United States Pharmacopeia.
16. US FDA – United States Food and drug Administration.

CHAPTER 1: INTRODUCTION

Mefenamic acid (MA) is a Cyclooxygenase 1 and Cyclooxygenase 2 inhibitor and is classified as a Non-Steroidal Anti-inflammatory Drug [1]. It is widely used in the treatment of mild pain, specially dysmenorrhea [2]. The bioavailability of mefenamic acid is 90% but is often influenced by water intake, primary site of absorption is small intestine, t_{max} is achieved between 2-4 hours and 2-3 days are needed to reach the steady state concentration of the drug [3]. Mefenamic acid also causes certain adverse effects in the gastrointestinal tract such as bleeding and ulceration, the main reason behind these effects is believed to be its mechanism of action [4].

Mefenamic acid comes under class II of the Biopharmaceutical Classification System, which means that it has low solubility and high permeability, hence its oral bioavailability is highly dependent on its dissolution rate in the gastrointestinal tract [5,6]. If the solubility of a drug is poor, it drastically affects the in vitro dissolution and ultimately affecting in vivo absorption and bioavailability of the drug. Therefore, solubility of a drug plays a vital role in attaining its therapeutic concentration. Furthermore, the side effects caused are also enhanced by the poor solubility of the drug, attributing to the fact that dose frequency increases when the drug is poorly soluble in GIT [7].

There has been a great advancement in the field of pharmaceutical sciences when it comes to enhancing the solubility of drug substances, techniques like solid dispersions [8], inclusion complex [9], ultra-rapid freezing process [10], melt sonocrystallization [11], solvent change method [12], melt granulation technique [13], supercritical solvent are being used

extensively.

Among these approaches, Hot Melt Extrusion is a reasonably new technique which has brought various advantages over others [14]. Various perks of Hot Melt Extrusion include it being a continuous process, its solvent free and thus could also be considered one of the most economical techniques which has a very few processing steps and is devoid of drying steps. The process of extrusion involves physically mixing the drug/s with the polymers and other ingredients which are then pumped through a barrel at elevated temperature and pressure so as to get a uniform product often referred to as extrudates [15,16]. The materials which are unstable at higher temperatures may cause the process to malfunction and the product to degrade [17]. Upon addition of some specific carriers and/or plasticizers, thermal stability of a drug can be improved to a great extent, also promoting better extrusion processability while decreasing processing temperatures [18].

Solubility of many hydrophobic drugs can be improved by introducing a hydrophilic carrier into the formulation matrix, these might include PVP, HPMC or Eudragit [18]. Though the solubilization capacity of these polymers might be interrupted because of supersaturation or recrystallization [19]. This supersaturation phenomenon is exploited in this research using HPMC AS HG as a carrier. The precipitation inhibiting effect of HPMC AS has been reported in the literature [20,21].

Since HPMC AS HG solubilizes at a pH above 7, it presented a perfect environment to hinder the drug release in acidic media. The poloxamer P407 (Kolliphor) is used in this study as a solubility or dissolution enhancer which has a low melting point, surfactant properties and is orally safe to be used [22,23,24,25].

Design of Experiments (DoE) is an important tool used in the statistical analysis and plays a vital role in the application of Quality by Design approach in both industrial and laboratory based research setting. It has been observed that ever since ICH Q8 Guidelines highlighted the use of DoE approaches in the scientific and industrial domain, there has been a significant rise in the utilization of DoE. Furthermore, availability of various statistical and designing software at scientists' disposal added to the opportunities to exploit this approach at a much greater pace [26]. The software used in this study was Design Expert 11, which is one of the latest versions of the software by Stat-Ease, Inc.

The selection of design was based on the prediction based optimality criteria and the applied approach was dependent on the drug dissolution studies, taking into account the crystallinity of the formulations as observed via Differential Scanning Calorimetry. Staying as close as possible to the above mentioned responses, Mixture Design of Experiment methodology was selected [27]. Within Mixture DoE methodology, to minimize the average prediction variance over the experimental region, I optimal criterion was given a go ahead [28].

In accordance to the I optimality criterion, if P1 is considered to be the average prediction of variance of Design 1 and P2 is considered to be the average prediction of variance of Design 2, then I-efficiency of Design 1 can be stated as; P2/P1 and vice versa for Design 2. If the value of I-efficiency for Design 1 is larger than 1, it depicts that Design 1 is better than Design 2 as far as average prediction variance is concerned. When D-optimal designs were compared with I-optimal designs, it was found that in case of D-optimal designs the median variance of prediction is about 50% larger than that of I-optimal designs. Furthermore, it was observed that the I-efficiency of I-optimal designs was higher (1.00) than D-optimal designs which gave a confirmation for the effectiveness of I-optimal mixture design for this study [28].

There were two main purposes of this study;

i) To prepare HPMC AS HG based amorphous solid dispersions of mefenamic acid and investigate the effect of Kolliphor® on the dissolution profiles using Hot Melt Extrusion Technology.

ii) Mefenamic acid, a BCS class II drug, displays high permeability and low solubility, thereby exhibiting a poor dissolution profile. Hence Hot Melt Extrusion technique was used to improve the solubility and dissolution rate of mefenamic acid.

CHAPTER 2: MATERIALS AND METHODS

Mefenamic acid was purchased from TCI America, Portland, OR. Aquasolve HPMC AS HG was received as a gift from Ashland, Kolliphor P407 was received as a gift from BASF. Sodium hydroxide and potassium phosphate were purchased from Acros Organics. All other chemicals used in this study were of analytical grade.

Thermogravimetric Analysis:

Thermogravimetric analysis is a method that is used to ascertain the thermal degradation temperature of mefenamic acid and other components of the formulation. The equipment used in this study was a Perkin Elmer, Pyris 1, Shelton, CT, USA. TGA was performed in the heating range of 25°C - 300°C along with an accelerated rate of 10 °C/min. Nitrogen purge was set at 20 ml/min and approximately 5 mg of each sample was weighed in the platinum pan and analyzed using the Pyris 1 software. The interpretation of TGA results is based on the weight loss of the treated sample when compared to the initial weight.

Differential Scanning Calorimetry:

DSC analysis was performed to analyze the glass transition temperature and melting point of pure ingredients and eleven different formulations. The instrument used was a TA Instruments Discovery Series DSC 25, New Castle, DE, USA. Analysis was performed over the temperature range of 25°C to 300°C at 10°C and the nitrogen purge was set at 20°C/min. Samples were weighed accurately (around 5 mg) and filled in an aluminum pan, sealed with a

hermetic lid. TRIOS software was used as a means to analyze the solid state characteristics and thermal stability of the pure drug, polymers and the formulations.

Screening:

Screening studies were performed to get a better idea about setting the upper limit and lower limit while designing the experiments. These studies included going higher and lower with the extrusion temperature and testing the dissolution of the extrudates. The extrusion was performed using a 11mm twin screw co-rotating extruder (Process 11® ThermoScientific, Pittsburgh, PA, USA) at a lower temperature of up to 130°C with a 2.5 mm die insert at a screw speed of 50 rpm. The temperature was also increased up to 190°C to check the changes in the extrudates' consistency.

The dissolution studies were performed with a pH 6.8 Phosphate Buffer solution using a Hanson SR8 Plus Dissolution System (Chatsworth, CA) USP Type II (Paddle) Apparatus. The paddle speed was set to 50 rpm and the samples were withdrawn at the time intervals of 15min,30min, 45min, 60min, 90min, 120min, 150min. The results of these screening studies played a vital role in setting up the processing parameters while designing the final experiments.

Designing the Formulations:

Mixture designs consist of factors which are directly or inversely proportional to all the ingredients of a mixture. They are usually used to predict responses associated with all the formulations derived from that mixture and to predict exact ratios of all the components of that mixture.28 In this study, the I-optimal mixture design has been used which is known to minimize the average prediction variance. After selecting the optimal mixture design, the components of the formulation are incorporated. The three components of the design were A- HPMCAS HG, B- Mefenamic acid and C- Kolliphor P407. The lower limit and upper limit of each component was

set such that the mixture components of each run would add up to 100. The target profile of the optimized formulation is described in table 1:

Table 1. Target profile.

ELEMENT/ATTRIBUTE	TARGET	QTPP	CQA
Route of Administration	Oral	✓	
Dosage Form	Capsule	✓	
Assay	90-110%	✓	✓
Content Uniformity	USP <905>	✓	✓
Strength	100 mg	✓	
Stability	3 months accelerated stability	✓	
Dissolution	100% release	✓	✓
Solid State	Amorphous	✓	✓
Compatibility	Excipients	✓	✓

After the formulations were obtained, the response was added to the design. Since crystallinity of the formulations was not quantified, there was only one response which could be used to optimize the formulations. Drug release percentage was added as a response and further optimization was based on the solid state characterization of these formulations. The processing parameters used while performing extrusion are based on the results of the screening studies which were performed before the actual study.

Preparing the Formulations:

Table 2. Details of formulations

FORMULATION	% Mefenamic Acid	% Aquasolve HPMC AS HG	% Kolliphor P407
F1	20	80	0
F2	20	70	10
F3	20	60	20
F4	30	70	0
F5	30	60	10
F6	30	50	20
F7	35	50	15
F8	40	60	0
F9	40	55	5
F10	40	50	10
F11	40	40	20

After going through the pre-formulation data, processing parameters for hot melt extrusion were finalized and extrusion process was carried out. Physical mixture of the formulations mentioned in table 2 were prepared by weighing the exact amount of drug and the polymers according to the formulation design and blending them together using a turbula tumbling blender (Globe Pharma Maxiblend™, New Brunswick, NJ, USA). These blended products were then extruded using a 11mm twin screw co-rotating extruder (Process 11® ThermoScientific, Pittsburgh, PA, USA). A screw speed of 50 rpm with a standard screw configuration was employed for extrusion. A 2.5 mm die insert was used while extruding and the temperature settings have been mentioned in table 3. Feed rate was set at 4 (approximately 1.5 gm/min) and torque was carefully observed all throughout the process. The screw configuration is described in figure 1. The extrudates were then milled using a laboratory grinder and sieved through ASTM #30 mesh. The sieved powder was weighed according to the drug load and filled in hard gelatin capsules which were then used

for dissolution studies.

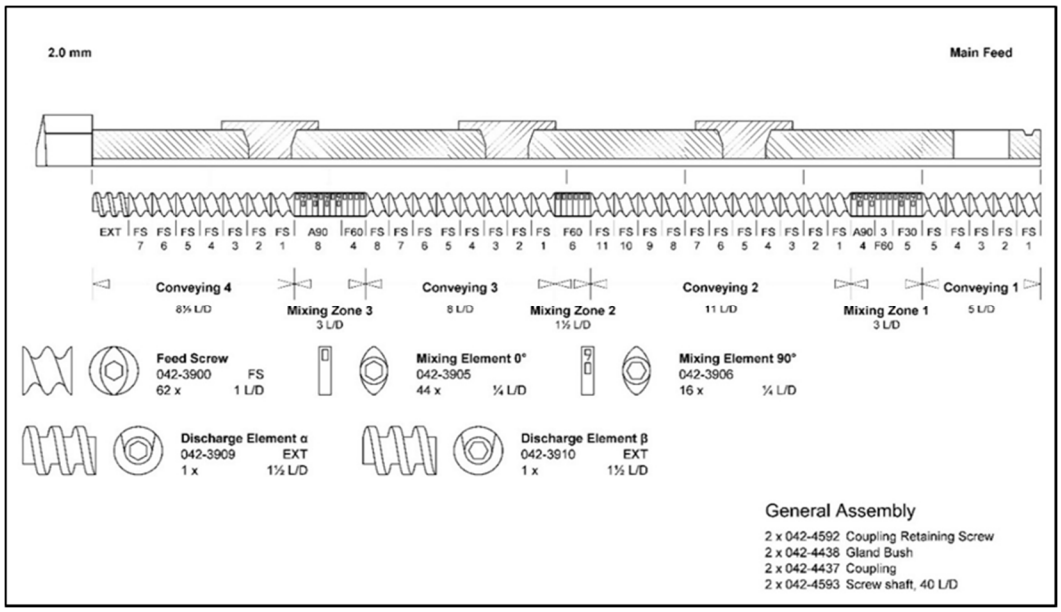


Figure1. Standard screw configuration of Twin Screw Hot Melt Extruder.

Table 3. Processing parameters for extrusion.

FORMULATION	Extrusion Temperature (°C)	RPM	Torque %
F1	160	50	40
F2	150	50	23
F3	140	50	17
F4	160	50	33
F5	160	50	20
F6	160	50	10
F7	160	50	15
F8	160	50	31
F9	160	50	27
F10	160	50	18
F11	160	50	8

Fourier Transform Infrared Spectroscopy:

FTIR spectroscopy was used as a means to study the interaction between the drug and the polymers in the extrudates. The equipment used in this study was an Agilent Technologies Cary 600 Series FTIR Spectrophotometer, Santa Clara, CA, USA. Samples of pure drug, polymers and formulations were placed on the mirror surface and the data was collected and analyzed using the Resolutions Pro software.

Scanning Electron Microscopy:

SEM study was performed to evaluate the surface morphology of the pure drug and formulations. The equipment used to perform this study was a JEOL JSM 5600 SEM (JEOL, Peabody, MA, USA) at an accelerated voltage of 5kV. Hummer Sputtering system (Anatech Ltd., Springfield, VA, USA) was used to prepare the sample in a high vacuum evaporator. Carbon pads were mounted on an aluminum base and the extrudates were powdered and placed on these adhesive pads. This step was followed by sputter coating with gold in a specific work chamber. After the gold coating, these samples were put under the microscope and the images were clicked at 50X, 200X and 500X.

Content Uniformity:

Milled extrudates equivalent to 50 mg of pure drug were weighed and transferred into 50 ml volumetric flasks. 40 ml Methanol was added to the flask and sealed with a Parafilm. This setup was sonicated for 20 minutes using a Branson 2800 Ultrasonic Bath (Cleanosonic, Richmond, VA, USA) to solubilize the drug completely in methanol. Once it is solubilized, volume was made up to the 50 ml mark using a pipette. Following this, 1 ml of drug-methanol solution was pipetted out into a 20 ml glass vial and mixed with 9 ml methanol to dilute the solution. It is further diluted to suitable dilutions and then analyzed at a wavelength of 285 nm

using a UV Visible Spectrophotometer.

Dissolution Studies:

Hanson SR8 Plus Dissolution System (Chatsworth, CA) USP Type II (Paddle) Apparatus was used to carry out the in vitro dissolution studies. The milled extrudates and the pure drug were weighed in accordance with the drug load equivalent to 100 mg mefenamic acid, sieved through sieve #30 and filled in hard gelatin capsules. Dissolution studies were performed in pH 7.4 Phosphate Buffer dissolution media for a period of 150 minutes. These capsules were placed in dissolution vessels filled with 900 ml buffer solution using sinkers. The temperature of the water bath was maintained at 37 ± 0.5 °C and paddle speed was 50 rpm. 3 mL samples were withdrawn from the dissolution vessels after 15min, 30min, 45min, 60min, 90min, 120min, 150min. The samples were withdrawn using a syringe, attached to it was a 10µ filter to keep out all the solid particles and replaced with equal volume. The samples were diluted suitably and analyzed using UV Visible Spectrophotometry.

Stability Studies:

The stability studies were conducted for three months based on the release profiles of all the formulations. The formulations with 100% drug release were selected, which were F3, F7, F9, F10. Three months accelerated stability studies were performed at RH 75% ± 5% and temperature 40°C ± 2°C. Solid state characteristics were analyzed using DSC, drug content uniformity and dissolution studies were carried out to make sure that the formulations remained stable for 3 months. All the parameters used during these studies were kept same as they were with initial samples. The Similarity Factor (f2) and Dissimilarity Factor (f1) were calculated using the formulae provided here.

$$f_1 = \{[\sum_{t=1}^{n} |R_t - T_t|]/[\sum_{t=1}^{n} R_t]\} \cdot 100$$

$$f_2 = 50 \cdot \log \{[1+(1/n)\sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \cdot 100\}$$

CHAPTER 3: RESULTS AND DISCUSSIONS

Solid dispersions of mefenamic acid (20%, 30%, 35% and 40%) with varying ratios of AquaSolve™ HPMC-AS HG and Kolliphor® P407 (0%, 10% and 20%) were successfully prepared using hot melt extrusion technology. Formulations containing Kolliphor® P407 exhibited enhanced dissolution profiles compared to the pure mefenamic acid and solid dispersions of plain HPMC-AS HG. The detailed account of the results from various studies has been discussed here.

Thermogravimetric Analysis:

Thermogravimetric analysis was performed and since it was one of the very important preformulation study, all the ingredients of the formulation were studied. TGA represents the thermal degradation temperature of mefenamic acid to be around 238°C, which denotes that the decomposition of mefenamic acid occurred at 238°C. It is same as it has been reported in the literature [29]. The thermal degradation temperature of HPMC AS HG and Kolliphor P407 was not detected when they were heated till 300°C. The processing temperature was less than the degradation temperature of mefenamic acid to make sure that it does not degrade during extrusion process. The TGA of mefenamic acid, HPMC AS HG and Kolliphor P407 is given in figure 2.

Differential Scanning Calorimetry:

DSC was performed at a temperature range of 25ºC to 300ºC. The thermogram obtained showed two endothermic peaks of mefenamic acid at 174°C and 231°C which indicates that it exists in two polymorphic forms. These two endothermic peaks correspond to conversion of Form I to Form II and then melting of Form II (231°C) respectively [30]. The solid dispersions show complete conversion to amorphous form hence improving the solubility of the formulation. However, the DSC thermograms of SDs prepared using higher concentrations of MA showed a low intensity peak. The crystalline peak of MA was either disappeared or shifted to lower temperatures in case of different SDs. This observation indicated the partial conversion of MA into amorphous form.

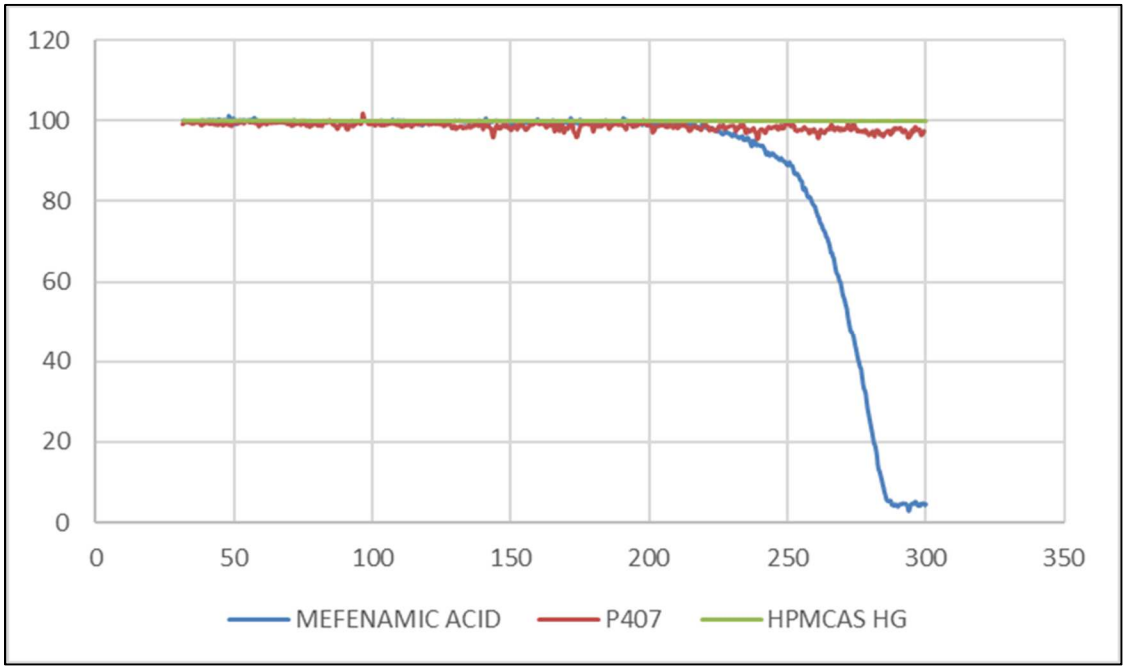


Figure 2. TGA of the drug and polymers.

Fourier Transform Infrared Spectroscopy:

The FTIR spectra of all the samples, shown in figure 4, are consistent with the IR spectrum of the pure mefenamic acid reference spectrum. The peaks at 1646, 1255 and 889 cm−1 are representing N-H amine bending, aromatic amine stretch and aromatic C=C bending, respectively. As far as the solid dispersions of MA and Kolliphor P407 are concerned, there was no significant difference observed when all assigned peaks were compared to the pure drug. The only exception was the ether vibrations which shifted from 2910 cm^{-1} to a lower wavenumber of 2860 cm^{-1} to 2870 cm^{-1}. This might be a result of hydrogen bonding with C=C in MA.29

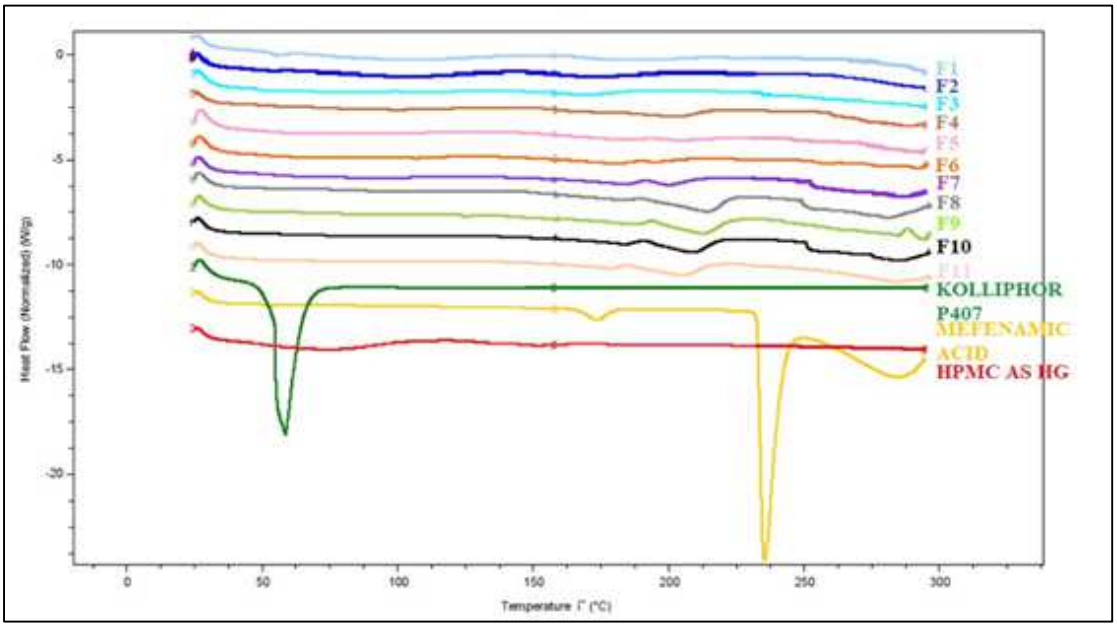


Figure 3. DSC of pure components and formulations.

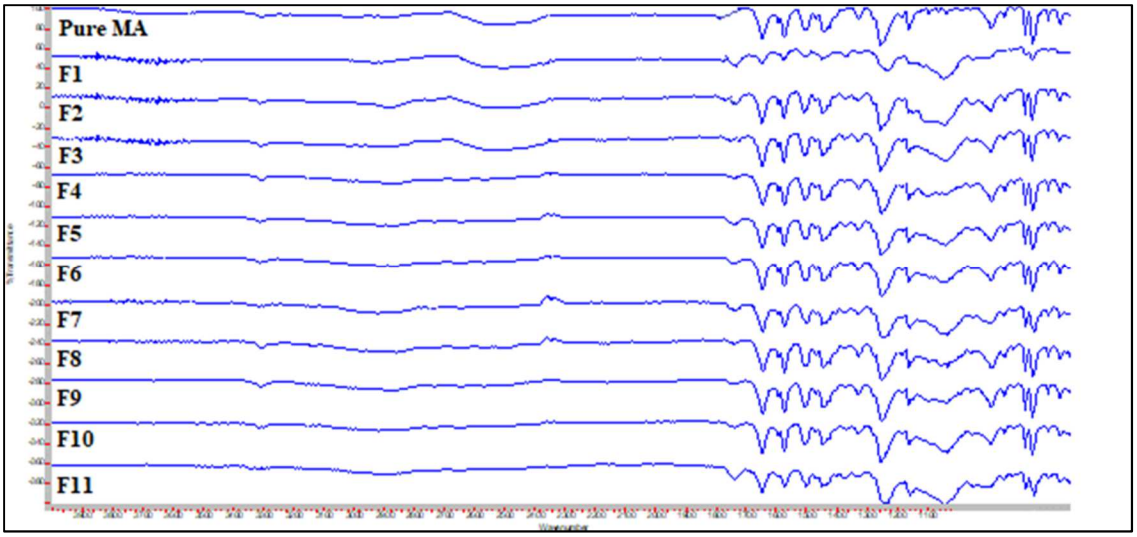
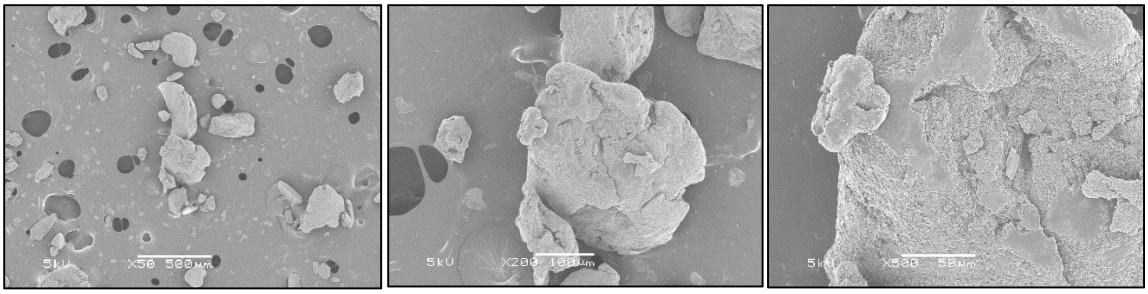


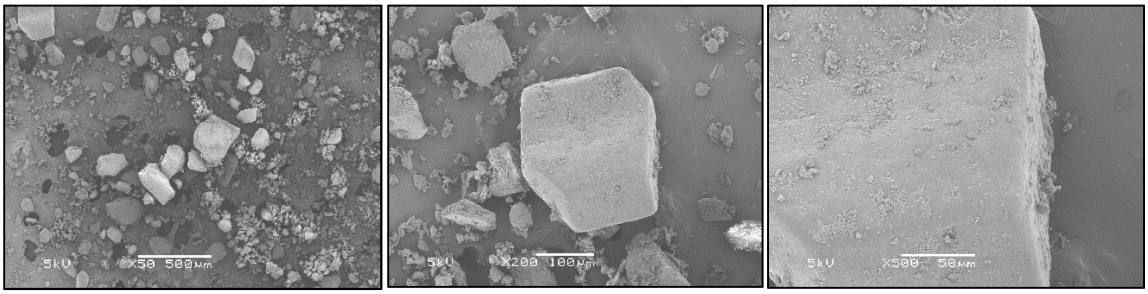
Figure 4. FTIR of the drug and formulations.

Scanning Electron Microscopy:

The SEM images of the pure drug indicate that there are smaller crystalline particles accumulated on and around the bigger crystal in case of pure MA. However, when the SEM images of the amorphous solid dispersions were studied, it was found that the smaller crystalline particles were completely absent. There is an absolute mixture of the drug and the polymer at molecular level which can be clearly seen with the help of SEM images (Figure 5). This observation further confirmed the conversion of MA to an amorphous form.



F3



PURE MA

Figure 5. SEM images of the drug and selected formulation.

Content Uniformity:

The drug content uniformity of the formulations was calculated and the results were found to be under the range of accepted parameters set by the USP. The prerequisite for dose uniformity is achieved if the acceptance value of 10 units is less than or equal to L_1%. In case the acceptance value is higher, next 20 units are tested for the acceptance value, the conditions are met if the ultimate acceptance value of all 30 units is less than or equal to L_1% and no individual content of any unit is less than $(1 - L_2*0.01)$ M nor more than $(1 + L_2*0.01)$ M. The value of L_1 is 15.0 and L_2 is 25.0, unless there is some other value described in individual monograph. In other words, the preparation fails to comply with the test if more than three individual contents are outside the limit of 85% to 115% of the average content or if one or more individual contents are outside the limits of 75% to 125% of the average content [31].

Acceptance Value is calculated with the formula:

$$\mathbf{AV = |M - \bar{X}| + ks}$$

Here, M is the reference value which depends on the value of $\bar{X}$, which is the mean of individual contents. If the mean is ≥ 98.5% or ≤ 101.25%, then M = $\bar{X}$ which would mean AV = ks. If the mean is < 98.5%, then M = 98.5% and if the mean is > 101.5%, then M = 101.5%. k is the acceptability constant and s is the standard deviation.

The drug content was in the range of 90.4% to 101% for all formulations, which is pretty much under the specified values as it is described in the USP.

Dissolution Studies:

The in vitro release profile of the formulations was studied and it was observed that there was a significant improvement in the release profile of formulations which contain poloxamer. It was also clear that there was a decline in the release profiles of most of the formulations on increasing the drug load. It was also noticeable that as the amount of poloxamer was increased, drug release improved to some extent except a few cases where the drug release was enhanced on increasing the drug load.

These behaviours may be attributed to a number of sub processes, the fact that the wettability of the composition is enhanced when a hydrophilic carrier is added to the formulation is one of those justifications. The wetting of hydrophobic surfaces of the MA-HPMCAS HG solid dispersions is facilitated by the hydrophilic carrier, which in this case is poloxamer P407. As a result, the release of MA from the solid dispersion is accelerated [32]. Moreover, the hydrophilic carriers also reduce agglomeration and aggregation of the drug particles, facilitating their contact with the dissolution medium which in turn enhances the dissolution rate. The rationale behind the use of HPMCAS HG was its ability to hinder dissolution of the drug in an acidic media, which was a very significant factor in this study since the drug release was targeted

in the intestine [32]. The main reason for the targeted release of MA in the intestine is its mechanism of action, as MA is a non-selective cox inhibitor and has been known to inhibit Cyclooxygenase 1 when absorbed through stomach causing various side effects like abdominal pain, constipation, diarrhoea and heartburn. These side effects could be curbed by following the targeted absorption of MA through intestine, since its mechanism of action would be altered when absorbed through intestine. It would then inhibit Cyclooxygenase 2, which will further exhibit a conventional pain relieving effect without adding any drawbacks [33].

From figure 6 it can be observed that there is an agreement in increase of drug release from the formulation with the increase in poloxamer concentration but only up to a certain extent. This can be noticed from the difference in drug release profiles of formulations F10 and F11. Formulation F10 with 40% drug load and 10% poloxamer displays a superior drug release profile as compared to F11 with 40% drug load and 20% poloxamer. This observation can be attributed to two main reasons. First, a higher concentration of surfactant (F11) leads to formation of bigger micelles that leads to a lower surface area and hence stunned drug release as observed by Enderberg et al [34]. Second, poloxamer has a capability of enhancing the viscosity of the diffusion layer at a higher concentration. The increased viscosity of the diffusion layer due to the gel forming capability of poloxamer in F11 may be responsible for the superior performance of F10. This same pattern can be observed with formulations F5, F6, F7 because of the higher drug load and varying surfactant ratios [35]. Formulations F2 and F3 do not follow this trend because of a low drug load which is not affected by the hindering property of poloxamer 407.

Since this study involved enhancing the dissolution profile at a specific site, screening studies were performed to make sure that a minimum amount of drug would be released at a pH

below 7. When the drug dissolution studies were performed using the same formulations with a pH 6.8 Phosphate Buffer, it was observed that the drug release was barely 35% as shown in table 4. Therefore, it could be further stated that if the dissolution studies were to be performed at an acidic pH, the drug release would be retarded to a greater extent.

Table 4. Drug release at pH 6.8 phosphate buffer

Time	Pure MA	F1	F2	F3
0	0	0	0	0
15	0.21	7.09	0.28	1.34
30	0.85	14.60	3.36	8.70
45	1.77	20.26	15.29	16.87
60	1.98	23.92	24.36	21.88
90	2.91	27.48	30.90	27.86
120	3.77	30.60	34.09	33.49
150	4.04	31.78	34.36	35.96

As far as the extrusion parameters were concerned, the screening studies presented some useful insights which were handy while deciding on the final parameters. It was observed that on increasing the extrusion temperature above 160°C, the extrudates started losing their firmness and went on to get liquefied on increasing the temperature to 190°C. On the other hand, when the temperature was decreased below 140°C, the torque went beyond limit and since it could harm the extruder the temperature was not decreased below 140°C.

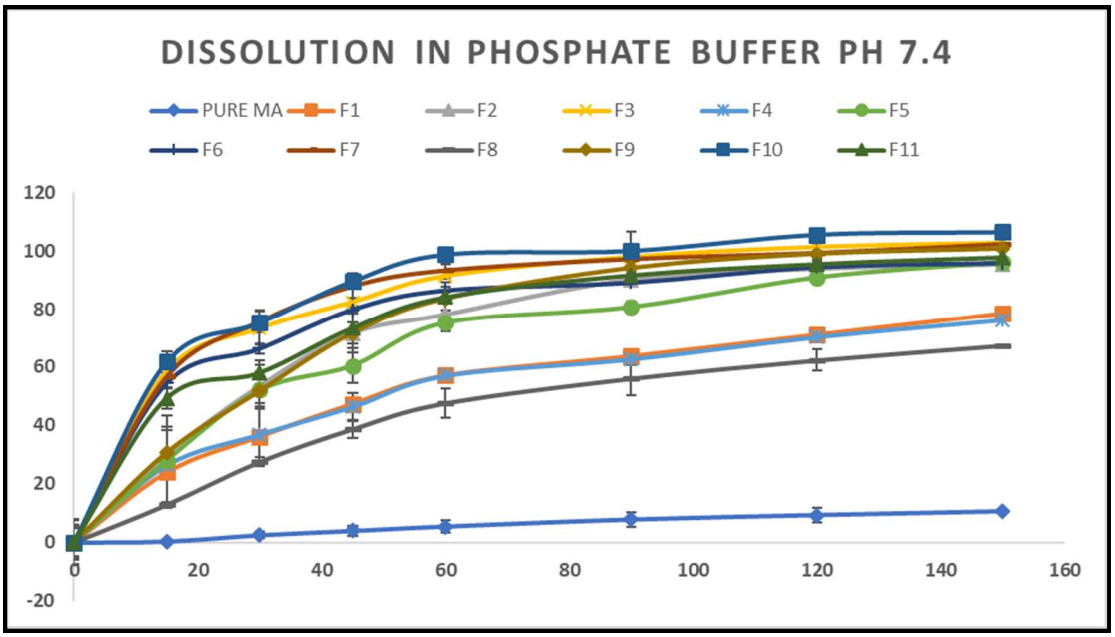


Figure 6. Release profiles of MA and all formulations

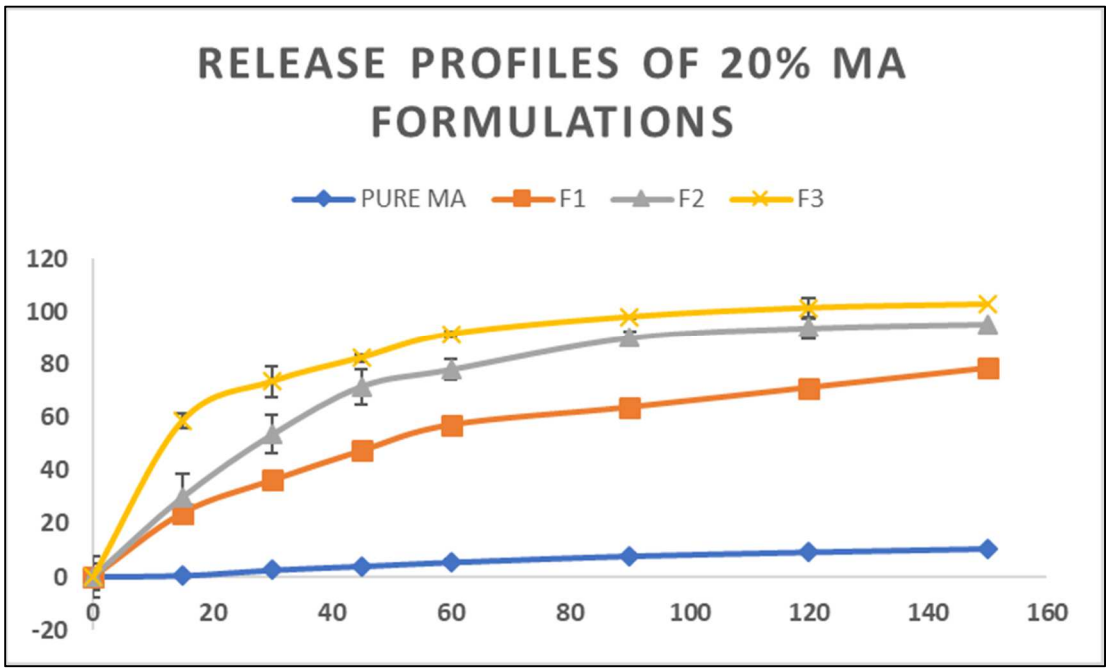


Figure 7. Release profiles of 20% MA formulations

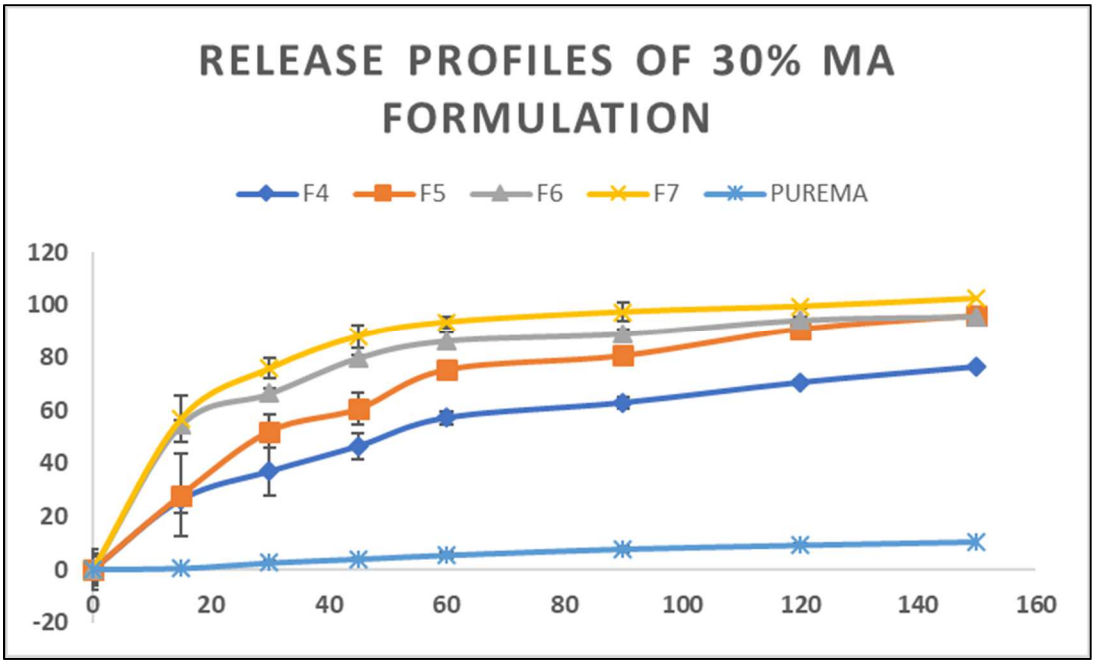


Figure 8. Release profiles of 30% MA formulations

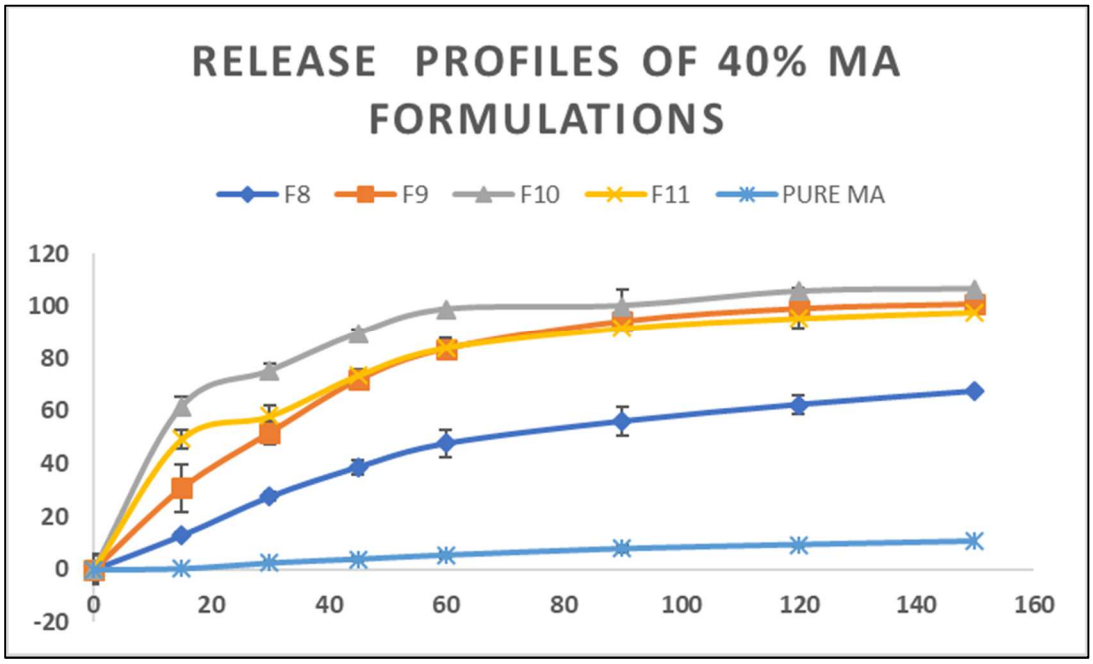


Figure 9. Release profiles of 40% MA formulations

Statistical Analysis:

Table 5. Analysis of variance.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1381.91	5	276.38	6.74	0.0189	significant
□¹□Linear Mixture	883.19	2	441.60	10.78	0.0103	
AB	37.34	1	37.34	0.9113	0.3766	
AC	491.12	1	491.12	11.99	0.0134	
BC	132.60	1	132.60	3.24	0.1221	
Residual	245.87	6	40.98			
Lack of Fit	245.87	5	49.17			
Pure Error	0.0000	1	0.0000			
Cor Total	1627.78	11				

The Model F-value of 6.74 implies the model is significant. There could only be a 1.89% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicated that the model terms are significant. In this case A, B, AC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction might improve this model.

Table 6. Fit statistics

Std. Dev.	**6.40**	**R^2**	**0.8490**
Mean	92.96	Adjusted R^2	0.7231
C.V. %	6.89	Predicted R^2	-0.0164
		Adeq Precision	6.6442

A negative Predicted R^2 implies that the overall mean may be a better predictor of the response than the current model. In some cases, a higher order model may also predict better.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Here the ratio of 6.644 indicates an adequate signal. This model can be used to navigate the design space.

Table 7. Coefficient in terms of coded factors

Component	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
A-HPMC AS-HG	76.28	1	5.67	62.42	90.15	2.71
B-Mefenamic acid	106.59	1	33.94	23.53	189.64	39.10
C-Poloxamer P407	9.26	1	36.18	-79.27	97.79	36.94
AB	-60.91	1	63.80	-217.03	95.21	19.11
AC	223.57	1	64.58	65.55	381.59	16.23
BC	171.81	1	95.51	-61.90	405.51	27.88

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

When the Predicted versus Residual graph was analysed, it was observed that all the formulations are in linearity and the variability was uniform all along the axis. There was no curvature or outliers and hence the assumptions of this model are believed to be true. The variance was constant with the mean all throughout the graph and the constant variance assumption was not violated.

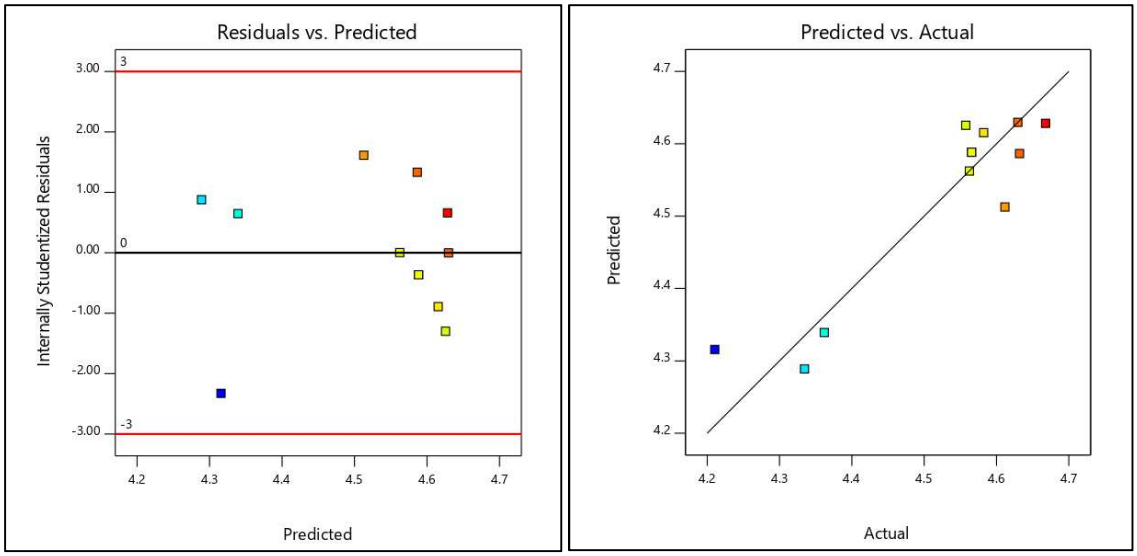


Figure 10. Graphs of actual, predicted and residual data.

The regression graphs given in figure 10 describe the relationship between the independent variables and the dependent variables in the formulation. As it can be very easily noticed from the Actual versus Predicted graph, there is a positive relationship between the independent and the dependent variables in the formulation. The response used in this prediction was % drug release and with the help of regression analysis, it could be further confirmed that the formulations were significantly influenced by poloxamer 407. The formulations with 0% poloxamer 407 can be seen at the lower end of the graph while the formulations with some amount of poloxamer 407 are at the top end of the graph.

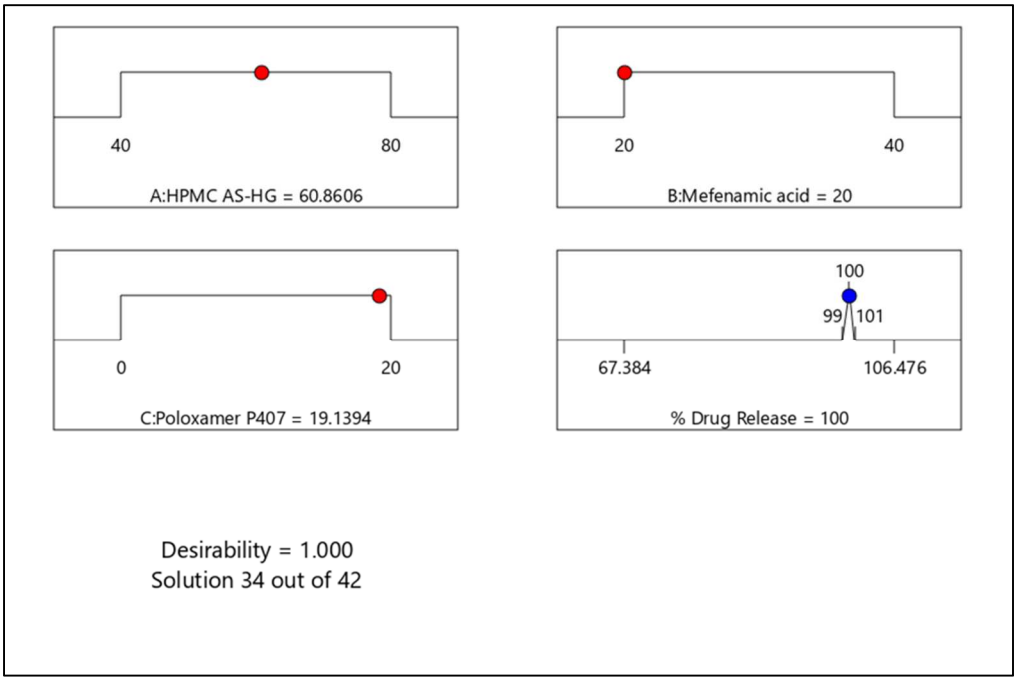


Figure 11. Numerical Optimization

Figure 11 demonstrated that there could be 42 possible formulations within the limits of this design that could give a 100% drug release and the optimized formulation can be selected from these 42 formulations. Formulation #34 could be selected as an optimized formulation owing to fact that it closely resembles formulation F3. Furthermore, formulation F3 had completely converted into amorphous form, was compatible to the ingredients and had acceptable drug content uniformity.

Stability studies:

The thermogram obtained after DSC (Figure 12) showed that there is no recrystallization peak in case of formulation F3, while there appears to be some recrystallization in formulations F7, F9 and F10. The drug content uniformity was checked and it was found to be within the acceptable range in accordance to the USP. The optimized formulation was also evaluated for its drug release profile by performing dissolution studies, and the release profile can be seen in Figure 13. The Similarity Factor (f2) and dissimilarity Factor (f1) were calculated using the

formulae and they were found to be well within the range of a stable formulation [36].

Table 8. Drug content after 3 months accelerated stability study

F3	F7	F9	F10
93.1888	98.452	106.6047	102.8896

Table 9. Table of similarity and dissimilarity factors after accelerated stability studies.

Formulations	f1	f2
F3	5.99	62.86
F7	9.05	56.66
F9	5.91	62.28
F10	5.55	64.89

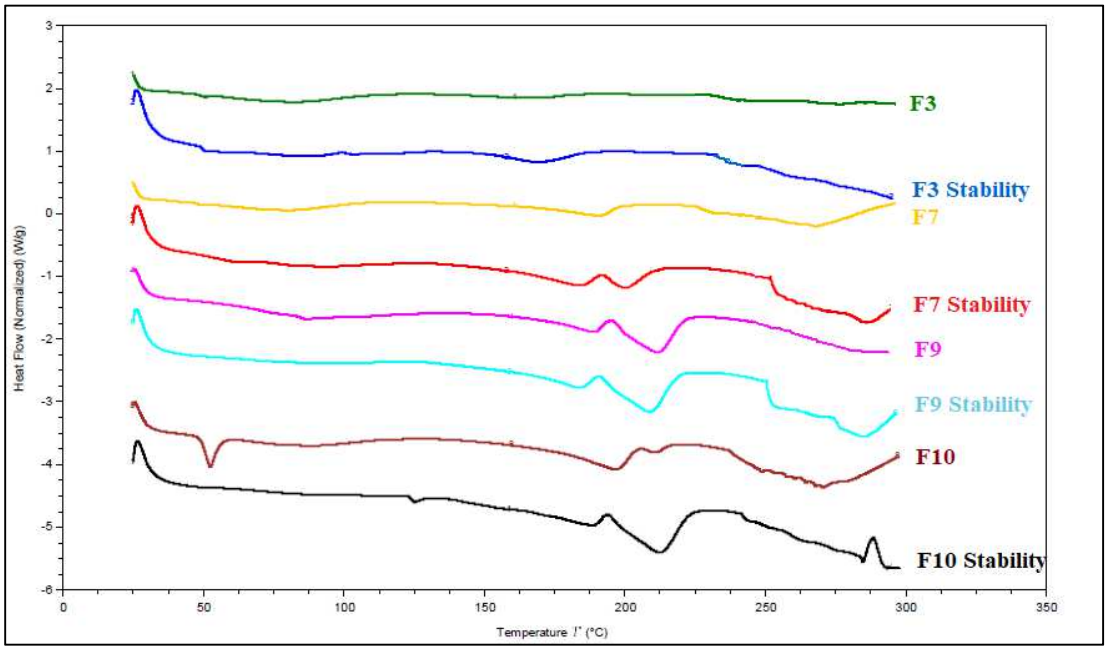


Figure 12. DSC thermograms of the stability samples

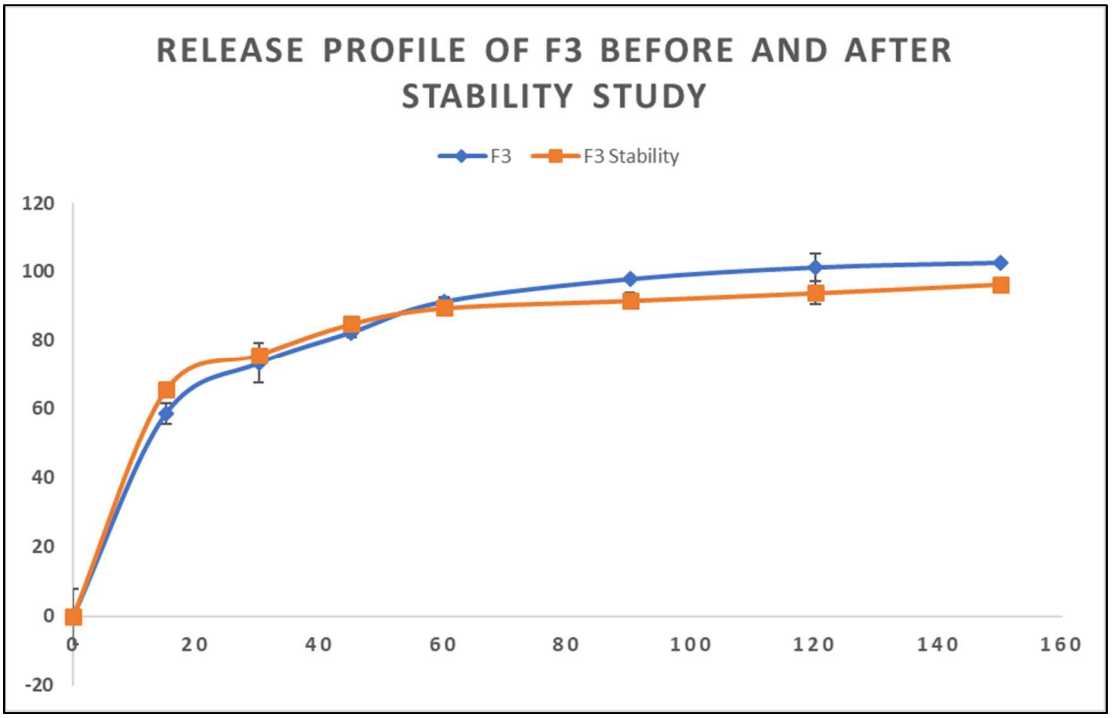


Figure 13. Release profiles after accelerated stability studies.

As far as the optimized formulation was concerned, there was only one formulation which could possibly be considered as an optimized formulation. It could be clearly observed from the release profiles, solid state characterization and stability studies that formulation F3 is the one which had a 100% drug release, had completely converted into amorphous form and remained stable after 3 months of accelerated stability studies.

CHAPTER 4: CONCLUSION

The dissolution of mefenamic acid in HPMCAS HG solid dispersions was observed to be increased. Furthermore, addition of Kolliphor® P407 enhanced the dissolution rate compared to pure mefenamic acid but on increasing the drug load above 20%, the poloxamer started forming bigger micelles inhibiting the drug release after reaching Critical Micelle Concentration (CMC). Mefenamic acid was converted into an amorphous form, which facilitated the increased rate of dissolution. FTIR data reveal that the drug and polymers studied were compatible. Stability studies showed that formulation F3 was stable after 3 months of accelerated stability study at RH 75% ± 5% and temperature 40°C ± 2°C. Therefore, it was confirmed that F3 could be considered the optimized formulation. Solid dispersions of mefenamic acid with improved dissolution rate were prepared using hot melt extrusion technology.

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CHAPTER 5: BIBLIOGRAPHY

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VITA

Ashay Shukla is an adaptable, inquisitive and energetic professional with deep experience in business development, management, pharmaceutical research and market analysis. Hailing from Indore, a fast growing city in central India, he completed his bachelors in 2012 from Rajiv Gandhi Proudyogiki Vishwavidyalaya majoring in pharmaceutical sciences. He has been involved in various entrepreneurial initiatives which include a pharmacy, a distributorship agency and an automation based startup. In the year 20017, he joined Master of Science program at the University of Mississippi with an emphasis on Pharmaceutics and Drug Delivery and has worked under the guidance of Dr. Michael A. Repka. His major projects include Mefenamic acid – HPMC AS amorphous solid dispersions using Hot Melt Extrusion Technology: Impact of surfactant on drug release characteristics, enhancing solubility and stability of ketoprofen by developing Ketoprofen-Nicotinamide co-crystals using Hot Melt Extrusion and development of a novel semisolid dosage forms for the effective treatment of Recurrent Aphthous Ulcers. Ashay also worked as a Pharmaceutical Market Analyst with the Office of Technology Commercialization at the university. He was presented with a Summer Research Fellowship Award in the year 2018 by the Graduate School, University of Mississippi. He is a member of the American Association of Pharmaceutical Scientists and Rho Chi Honor Society.