# CHAPTER THREE

***THE HIGHER PERFORMANCE BOX-BEHNKEN DESIGN APPLIED TO THE SYNTHESIS (FULL MODIFICATION) AND OPTIMIZATION OF NOVEL MODIFIED POLYAMIDE 6,10 MONOLITHIC MATRIX SYSTEMS***

## 3.1. INTRODUCTION

In Chapter Two, a preliminary screening study was performed using the Plackett-Burman experimental design to establish the diverse influence of varying stoichiometry and volume ratio of the solvents on the physicochemical and physicomechanical properties of polyamide 6,10. The significant effects ($p<0.05$) at different levels of the independent variables on the measured response parameters were identified. This approach showed the enormous efficiency of statistical and mathematical designs for the execution of experiments.

The current Chapter presents an extension of this application by employing the relevant information provided by the screening process (partial modification) described in Chapter Two for the optimization of novel polyamide 6,10 monolithic matrix systems demonstrating rate-controlled drug release characteristics. This phase encompassed a full process modification which included addition of solvent phase modifiers along side with the changes in stoichiometry and volume ratios. The higher resolution Box-Behnken design was utilized for the aforementioned intent. This design is usually employed once the preliminary screening has been carried out. It is an efficient mathematical approach applied to determine the optimum level of each of the significant parameters (independent variables) that maximizes or minimizes the desired response and also the new factors that can be added (Goupy, 2005; Nutan *et al.*, 2005)**.** The Box-Behnken design is economical and therefore makes it particularly useful in reducing the number of experimental runs (Abdel- Fattah *et al.*, 2005; Kincl *et al.*, 2005; Nutan *et al.*, 2005).

Rate-controlled drug delivery technology represents one of the emerging and challenging frontier areas of research in modern medicine and pharmaceuticals (Ghosh, 2004). One

of such challenging fields of research is in the fabrication of novel monolithic matrix systems, using simple process modifications, which are flexible, allowing for easy manipulation of drug release performance in a predictable and controlled manner, thus achieving more effective therapies that eliminate the risk of both under- and over-dosing.

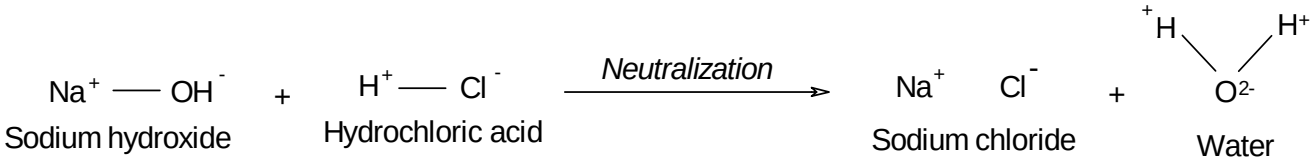
## 3.2. Full Modification of the Interfacial Polymerization Process

In an effort to enhance polyamide 6,10 matrix strength in terms of an increase in the matrix resilience due to its prominent and direct effect on matrix integrity, drug release modulation as well as degree of entanglement and disentanglement of the polymeric chain (Pillay and Danckwerts, 2002), the polar and non-polar dispersion phases (Section 1.3) of the reaction were modified using solvent phase modifiers namely; sodium hydroxide and cyclohexane to alter the pH and polarity respectively. This modification was performed simultaneously with variations in the volume ratio and stoichiometry described earlier (Chapter Two). Previously, sodium hydroxide and cyclohexane have been separately employed in the synthesis of aliphatic polyamides as solvent phase modifiers (Madan and Chareonboonsit, 1989; Gaymans and Sikkema, 1999; Phares et al., 1995) to improve on their physicochemical and physicomechanical properties. This approach explores the synergistic and antagonistic effects elicited, with regards to the integrity of the polyamide 6,10 matrices produced by using these two solvent phase modifiers (sodium hydroxide and cyclohexane) simultaneously at varying levels for the respective dispersion phases as well as using them separately.

### *3.2.1. Adjusting the pH of the polar liquid phase*

Sodium hydroxide (NaOH) which is described as a strong inorganic base otherwise described as an active proton acceptor was employed in adjusting the pH of the polar

phase. This inorganic base has been described as a neutralizing agent which improves the matrix integrity of aliphatic polyamides synthesized by interfacial polymerization by preventing disturbances in the intended reaction stoichiometry (Gaymans and Sikkema, 1999). Sodium hydroxide acts by neutralizing hydrochloric acid (a strong proton donor) to give sodium chloride which is a water-soluble inorganic salt (Equation 3.1). Hydrochloric acid (HCl) is condensation product of the interfacial polymerization reaction involved in the synthesis of polyamide 6,10 (Figure 2.1). Generation of hydrochloric acid within the reaction medium may have (despite that nylons are reported to be chemically inert) some corrosive and hydrolytic impacts on the well-structured chain configuration of the synthesized polyamide 6,10 producing relatively distorted inter-chain conformations affecting the compactness of the polyamide 6,10 matrix which may result in reduced physicomechanical strength.




***(Equation 3.1)***

In addition, generated hydrochloric acid may react with hexamethylenediamine which is a weak base (a form of neutralization reaction) as well as one of the monomers involved in the synthesis of polyamide 6,10 and this may also affect the integrity of the polyamide 6,10 matrices. Therefore, the presence of sodium hydroxide in the medium prevents the reaction between hexamethylenediamine and hydrochloric acid from taking place and makes hexamethylenediamine available for interfacial polymerization to occur.

Furthermore, inclusion of sodium hydroxide in the reaction medium removes hydrochloric acid from the system for the synthesis of polyamide 6,10 based on Equation 3.1. Elimination of hydrochloric acid from the reaction medium shifts the reaction towards the right (i.e. more production of polyamide 6,10 and hydrochloric acid; Figure 2.1) in an effect to balance the equilibrium of the process described by the *Le Chatlier's Principle* of equilibrium in chemical reactions. This may lead to an improvement in the efficiency of the polymerization process (i.e. formation of polyamide 6,10). This modification may effect the generation of polyamide structures with better-defined, more compact linear chain arrangements that could enhance matrix firmness. Furthermore, hexamethylenediamine being a moderately weak base possesses some intrinsic susceptibility to premature hydrolysis by water. Stabilization of this weak base, which is a specific contributor to the polyamide matrix integrity, becomes necessary. Therefore, the addition of sodium hydroxide, a stronger base that possesses the intrinsic potential to protect hexamethylenediamine becomes necessary. It acts by precipitating hexamethylenediamine into the reaction interface thereby preventing its untimely hydrolysis providing the system with sufficient hexamethylenediamine to achieve stoichiometric reaction levels required for an efficient polymerization process to form polyamide 6,10. The precipitation of hexamethylenediamine reduces its polarity increasing its potential to partition into the organic phase of the reaction interface supporting the process of polymerization (i.e. formation of polyamide 6,10) which occurs within organic phase of the liquid-liquid interface (Madan and Chareonboonsit, 1989).

### *3.2.2. Adjusting the polarity and density of the non-polar liquid phase*

Cyclohexane is a non-polar liquid selected to adjust the polarity and density of the non-polar phase due to its structural resemblance with that of hexane and its higher density.

The mixing of both solvents (i.e. hexane and cyclohexane) may have a prominent effect on the density and polarity of the solvent system. This may be associated with the difference in the density of cyclohexane (0.78), which is higher compared with that of hexane (0.66) as well as its polarity. A proposed explanation for this is that a mixture of these two solvents produces a denser environment for the reacting species (since polymerization takes place on the organic side of the reaction interface (Madan and Chareonboonsit, 1989). The increase in density and decrease in polarity of the organic phase reduces the rate of diffusion (due to increased viscosity) of the reacting species (sebacoyl chloride) to the interface within the reaction medium for polymerization to occur. This may enhance the efficiency of the interfacial polymerization process by improving the close packaging of the monomeric units forming polyamide 6,10 thereby improving the physicomechanical strength. Hence, an increase in the period of contact between the monomers (sebacoyl chloride and hexamethylenediamine) as a result of the increased viscosity may improve the polymeric matrix firmness and ultimately influence drug release rate from the polyamide 6,10 monolithic matrix systems.

### 3.3. Objectives

The overall aim of this section of the study is to synthesize (by the full modification strategy) polyamide 6,10 variants using the Box-Behnken design, formulate them into monolithic matrix systems, elucidate their drug release behaviour and optimize polyamide 6,10 for rate-controlled delivery of drugs that can serve diverse delivery purposes hinged on the various routes of drug administration. Furthermore, the effects of fully modifying the interfacial polymerization process (by varying the stoichiometry, volume ratios and simultaneous addition of the solvent phase modifiers) on the physicomechanical properties were assessed. Also, conductimetric analysis to assess the rate of polymeric

matrix dissolution from the molecular viewpoint based on ionic movements and matrix erosion will be investigated for all the synthesized variants. In addition, FTIR studies will be conducted to assess the integrity of the structural make-up of the formulations and confirm that no chemical interaction exists between incorporated drug and polyamide 6,10.

## 3.4. EXPERIMENTAL SECTION

### 3.4.1. Materials

Hexamethylenediamine, sebacoyl chloride, anhydrous n-hexane, anhydrous potassium bromide and deionized water are the same grade and specifications as those used in Chapter Two. Amitriptyline hydrochloride, cyclohexane and anhydrous sodium hydroxide pellets were obtained from Sigma Chemical Company (St. Louis, USA).

Amitriptyline hydrochloride was used as the model drug in this experimental phase. It is a highly water-soluble drug (100% water soluble at 25℃; Pharmaceutical Codex, 1994) and has a higher tendency of eliciting a burst release (because of its higher affinity for water molecules) that makes it more suitable for determining the controlled delivery potential of the polyamide 6,10 variants as the solubility (of both polymer and drug) influences drug release (Nutan *et al*., 2005).

### 3.4.2. Synthesis of the Polyamide 6,10 Variants in Accordance with the Box-Behnken Design Template (Full Modification)

Fourteen polyamide 6,10 variants were synthesized by the process of interfacial polymerization in accordance with the Box-Behnken design template. The different factor

levels and the design template containing combination of reactants for the synthesis of each variant are depicted in Tables 3.1 and 3.2 respectively.

**Table 3.1:** Levels of the independent variables employed in the Box-Behnken design

| Independent Variables | Levels | | | Units |
|---|---|---|---|---|
| | Low | Middle | High | |
| SC[a] | 0.25 | 1.00 | 1.75 | g |
| NaOH [b] | 0.25 | 1.00 | 1.75 | g |
| C-HXN [c] | 10.00 | 25.00 | 40.00 | mL |

[a] *Sebacoyl chloride;* [b] *Sodium hydroxide;* [c] *Cyclohexane*

The quantities of hexamethylenediamine, hexane and deionized water were kept constant throughout this experimental phase at the factor levels set for maximization of the matrix resilience from the screening design (i.e. 1.75g, 40mL and 10mL respectively; Table 2.7). Sebacoyl chloride on the other hand was varied within the previously specified limits and included the factors employed for the Box-Behnken design template. This was selected among other factors, as it was the most statistically significant (p= 0.03) of factors influencing the matrix resilience (Table 2.6). Sodium hydroxide and cyclohexane were fixed between the already-utilized levels (lower and upper) for maintenance of experimental consistency.

Each polyamide 6,10 variant was synthesized based on the same process and conditions described in Section 2.2.2. In this case, the first solution contained specific quantities of hexamethylenediamine and sodium hydroxide pellets dissolved in deionized water i.e. the polar phase, while the second solution comprised specific quantities of sebacoyl chloride evenly dispersed in a mixture of hexane and cyclohexane i.e. the non-polar phase. The percentage yield of each polyamide 6,10 variant was computed using equation 2.1.

### 3.4.3. Constructing the Experimental Design Template

A three-factor, three-level Box-Behnken design was applied to optimize the process using Minitab Statistical Software, Version 14 (Minitab Inc., State College, PA, USA). This experimental design generated fourteen experimental runs with two centre points (Table 3.2). The factor levels for the independent variables and the design template are listed in Tables 3.1 and 3.2 respectively. The selected dependent variables (or responses) were the:

(i) Matrix resilience

(ii) Mean dissolution time fixed at 8 hours

(iii) Conductivity at the first hour

For predicting the optimal formulation, a non-linear quadratic model correlating the relationship between the independent formulation variables and responses were generated. The quadratic mathematical expression is shown in Equation 3.2.

$$\text{Response} = b_0 + b_1 [SC] + b_2 [NaOH] + b_3 [C\text{-}HXN] + b_4 [SC] [NaOH] + b_5 [NaOH] [C\text{-}HXN] + b_6 [SC] [C\text{-}HXN] + b_7 [SC] [SC] + b_8 [NaOH] [NaOH] + b_9 [C\text{-}HXN] [C\text{-}HXN] \quad \textbf{(}\textbf{\textit{Equation 3.2)}}$$

where the measured (predicted) responses (i.e. matrix resilience, mean dissolution time fixed at 8 hours and conductivity after one hour) is associated with each factor level combinations, $b_0$-$b_9$ are the model coefficients and [SC], [NaOH] and [C-HXN] are independent variables.

**Table 3.2:** Box-Behnken template for the synthesis of the polyamide 6,10 variants

| Experimental Runs | Quantities of Reactants | | |
|---|---|---|---|
| | SC [a] (g) | NaOH [b] (g) | C-HXN [c] (mL) |
| 1 | 1.750 | 0.000 | 20.000 |
| 2 | 0.250 | 0.875 | 10.000 |
| 3 | 1.000 | 1.750 | 0.000 |
| 4 | 0.250 | 1.750 | 20.000 |
| 5 | 0.250 | 0.000 | 20.000 |
| 6 | 1.750 | 0.875 | 40.000 |
| 7 | 1.000 | 0.875 | 20.000 |
| 8 | 1.000 | 1.750 | 40.000 |
| 9 | 0.250 | 0.875 | 40.000 |
| 10 | 1.750 | 0.875 | 0.000 |
| 11 | 1.750 | 1.750 | 20.000 |
| 12 | 1.000 | 0.875 | 20.000 |
| 13 | 1.000 | 0.000 | 10.000 |
| 14 | 1.000 | 0.250 | 40.000 |

[a] *Sebacoyl chloride;* [b] *Sodium hydroxide;* [c] *Cyclohexane*

**Note**: Hexamethylenediamine = 1.75g, Hexane = 40mL, Deionized water = 10mL for all synthesized polyamide 6,10 variants.

### 3.4.4. Textural Profiling Analysis of the Polyamide 6,10 Variants

#### *3.4.4.1. Determination of matrix resilience*

This analysis focused on quantifying the matrix resilience for each polyamide 6,10 variant with and without the model drug (amitriptyline hydrochloride) using a calibrated Texture Analyzer (TA.*XTplus*, Stable Micro Systems, Surrey, England) fitted with a 36mm cylindrical steel probe. Matrix resilience was selected for investigation as it was the only response that was statistically significant (Chapter Two). The textural settings and procedure utilized to evaluate this physicomechanical parameter were fixed and performed as described in Section 2.2.4. A typical force-time profile employed for the

calculation of this parameter is shown in Figure 3.1. The details of the calculation are explained in Chapter Three.

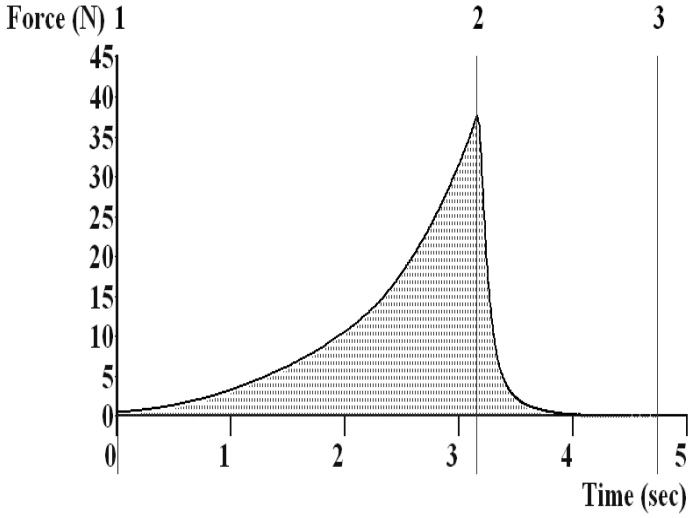



**Figure 3.1:** A Typical force-time profile employed for the determination of matrix resilience (N=10 in all cases).

### *3.4.4.2. Determination of the Brinell Hardness Number*

In addition to the matrix resilience, the Brinell hardness number (BHN) was also calculated. The BHN was computed for each compressed polyamide 6,10 monolithic matrix systems using the ball probe approach. A calibrated Texture Analyzer (TA.*XTplus*, Stable Micro Systems, Surrey, England) fixed with a ball probe indenter of diameter 0.5mm and indentation depth set at 0.25mm for all readings was employed. All other experimental settings were kept constant as specified in Table 2.3 for the determination of matrix hardness and energy of deformation. The peak force produced from indentation was assessed from the generated force-distance plots (Figure 3.2). Each analysis was performed on three replicate samples. The BHN was calculated using Equation 3.3.

$$BHN = \frac{\frac{2F}{\pi}}{D\left(D - \sqrt{D^2 - d^2}\right)}$$ ***(Equation 3.3)***

where F= force generated from indentation (N), D= diameter of ball probe indenter (0.5mm) and d= indentation depth (0.25mm).

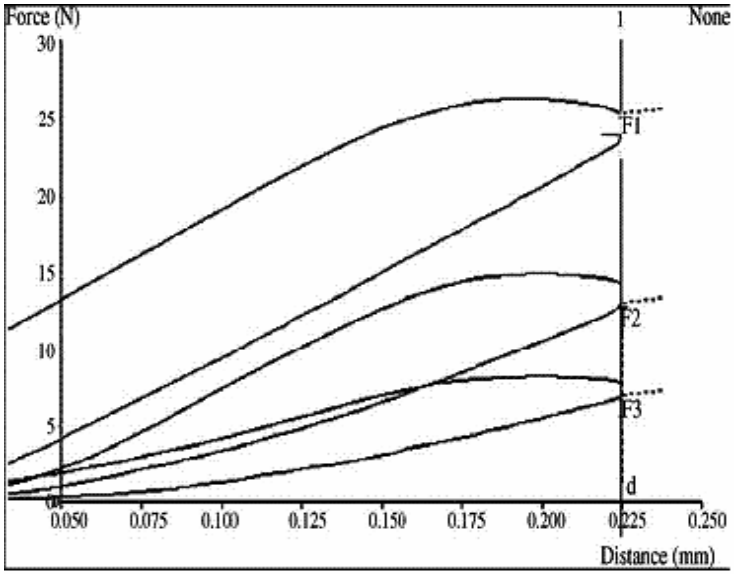



**Figure 3.2:** A typical force-displacement profile generated for the determination of the Brinell hardness number (N= 3 in all cases).

### 3.4.5. Preparation of Calibration Curves for Amitriptyline Hydrochloride in USP-Prescribed Phosphate Buffered Solution of pH 7.4

Stock solutions were prepared by separately dissolving 20mg of amitriptyline hydrochloride in 200mL of phosphate buffered saline (PBS) (pH 7.4). From the stock, a series of dilute standard solutions of the following concentrations: 0.002, 0.004, 0.006, 0.008 and 0.016 mg/mL were prepared. The absorbance of each standard solution was determined at the maximum wavelength of absorption ($\lambda_{max}$) of 240nm for amitriptyline

hydrochloride. A calibration curve (correlation coefficient; $R^2$= 0.98) was subsequently constructed (Figure 3.3).

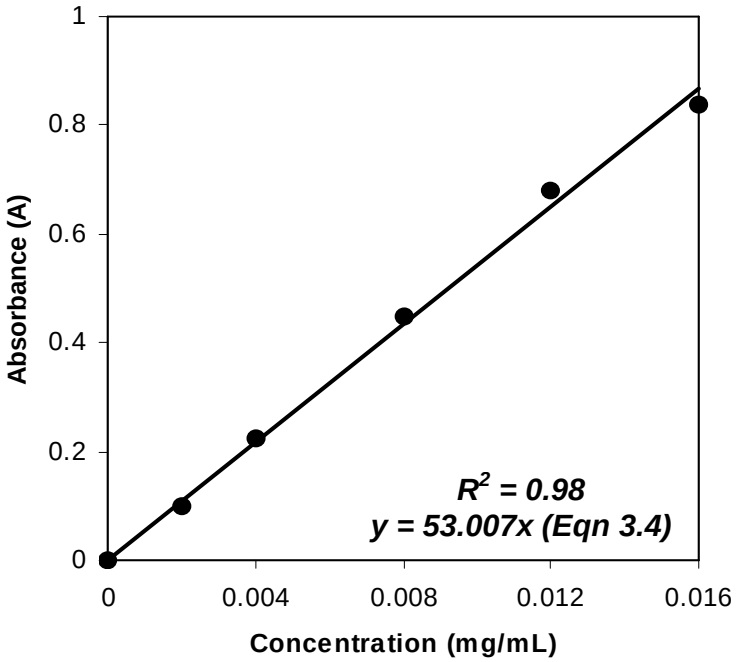



**Figure 3.3**: Calibration curve of amitriptyline hydrochloride in PBS 7.4 at 240nm (N=3 and standard deviation less than 0.35 in all cases).

### 3.4.6. Evaluation of the *In vitro* Drug Release Characteristics

#### *3.4.6.1. Formulation of the Monolithic Matrix Systems*

The fourteen polyamide 6,10 monolithic formulations were prepared in triplicate and each matrix comprised a physical mixture of 300mg of the respective ground polyamide 6,10 variant and 50mg each of amitriptyline hydrochloride. The mixture was blended for 20 minutes using a laboratory-scale blender (CG 100, Kenwood Ltd, UK) and screened through a laboratory test sieve of aperture size 1mm (Endecotts Ltd, London, UK) to ensure all particle sizes fell within 1mm and below for reproducibility**.** Final blends were compressed under a pressure of 1.0 tonne for 60 seconds (at every instance) into flat-surfaced, round compacts with each having a diameter of 13mm and a average thickness of 4mm using a Beckman hydraulic press (Beckman Instruments, Inc., Fullerton, U.S.A.).

### *3.4.6.2. In vitro Drug Release Studies*

*In vitro* drug release studies were performed on the fourteen polyamide 6,10 variants monolithic formulations. Each formulation was placed in a calibrated six-station dissolution testing apparatus (Caleva Dissolution Apparatus, model 7ST) using the standard USP 25 rotating paddle method at 50 rpm with 500mL PBS of pH 7.4 at 37±0.5$^{o}$C. All analyses were conducted in triplicate. The dissolution apparatus was modified by including a stainless steel ring mesh contrivance to prevent the hydrated formulation from floating (Pillay and Fassihi, 1998). For the determination of amitryptiline hydrochloride concentration, 5mL samples were manually withdrawn and filtered through a 0.45µm pore size Cameo Acetate membrane filter (Milipore Co., Bedford, Mass) at specific time intervals over a period of 24 hours. Samples were then analyzed by ultraviolet spectroscopy (Specord 40, Analytik Jena, AG) at 240nm (amtryptiline hydrochloride). An equivalent volume (to the amount withdrawn) of drug-free PBS was replaced into the dissolution medium to maintain sink conditions. A correction factor was appropriately applied in all cases where dilutions of samples were required.

The dissolution data were subjected to a model-independent analysis known as the time-point approach described by Pillay and Fassihi (1998). With this approach, the mean dissolution time set at 8 hours ($MDT_8$) for each formulation was calculated as an average of three readings. The application of the mean dissolution time provides more accurate view of the drug release behaviour and it is determined as the sum of the individual periods of time during which a specific fraction of the total dose is released (Pillay and Fassihi, 1998). This approach allows for easy and precise comparison of several dissolution data. Equation 3.5 was employed in the calculation of the mean dissolution time (MDT).

$$MDT = \sum_{i=1}^{n} t_i \frac{M_t}{M_\infty} \qquad \textit{(Equation 3.5)}$$

where $M_t$ is the fraction of dose released in time $t_i = (t_i + t_{i-1})/2$ and $M_\infty$ corresponds to the loading dose.

The approach of determining the mean dissolution time set at 8 hours was chosen based on the finding that a maximum (rapid) non-linear drug release pattern was observed for all samples up to 8 hours. Beyond 8 hours, release was relatively consistent and resembled linear, constant release patterns (Figure 3.6). It was essential to reduce the quantity of drug released during the first 8 hours to a consistent, linear, rate-controlled mode all through the period of drug release.

### 3.4.7. Evaluation of In vitro Matrix Erosion

*In vitro* polymeric matrix erosion studies were carried out on the fourteen polyamide 6,10 variants monolithic formulations. Each formulation was placed in a calibrated six-station dissolution testing apparatus (Caleva Dissolution Apparatus, model 7ST) using a standard USP 25 rotating paddle method at 50 rpm with 500mL PBS of pH 7.4 at 37±0.5$^{o}$C. The dissolution apparatus was modified by including a stainless steel ring mesh device to prevent the hydrated formulation from floating (Pillay and Fassihi, 1998). This analysis was conducted with and without amitriptyline hydrochloride in the monolithic matrix formulations to identify any possible effects of the drug on the rate of matrix loss. At predetermined time intervals, up to 24 hours, each matrix formulation was removed from the medium, blotted on filter paper (diameter 110mm and pore size 20µm) and dried to constant weight at 40± 0.5 $^{o}$C in an oven. All determinations were done in triplicate.

The mathematical expression stated in Equation 3.6 was employed to determine the percentage mass loss in % $^{w}/_{w}$.

$$\text{Mass Loss (\%)} = \frac{\text{Original Mass - Residual (dry) Mass}}{\text{Original Mass}} \times 100 \qquad \textit{(Equation 3.6)}$$

### 3.4.8. Electrolyte Conductivity Test for Evaluation of Polymeric Dissolution

*In vitro* electrolyte conductivity studies were performed on the fourteen polyamide 6,10 variants matrices in triplicate. Each formulation was placed in a calibrated six-station dissolution test apparatus (Caleva Dissolution Apparatus, model 7ST) using the standard USP 25 rotating paddle method at 50 rpm with 500mL deionized water at 37±0.5$^{o}$C. The dissolution apparatus was also modified by including a stainless steel ring mesh device (Pillay and Fassihi, 1998). Some exclusions made were that: (i) deionized water was employed instead of PBS 7.4 and (ii) each monolithic matrix device was drug-free and comprised of only 300mg polyamide 6,10 to prevent ionic interference between the electrolytes present in the buffer solution and the drug as well as the intrinsic polyamide ions.

A calibrated, conductivity tester (TDS Testr 40 with ATC, Oakton, USA) with a dual measurement range (0-199.9µs and 200-1999µs) and an automatic temperature compensation system ranging from 5-50$^{o}$C was used to detect the conductivity changes of which occurred as a result of polyamide 6,10 matrices dissolution. During a typical test, the basal conductivity reading of the blank solvent (deionized water) was recorded and the subsequent readings were subtracted. Conductivity values in microsiemens (µs) were recorded by immersing the tester into 20mL samples at various time intervals for 60

seconds and the test duration was for over 24 hours. After each determination, the conductivity tester was thoroughly rinsed and wiped dry prior to the next measurement.

### 3.4.9. Fourier Transform Infra-Red Spectrophotometric Analysis

This experiment was conducted with respect to the procedure described in Chapter Two (Section 2.2.5). FTIR was performed to ascertain the integrity of the structural backbone of the synthesized polyamide 6,10 variants and to establish the absence of any chemical interactions between the polymer and the drug.

### 3.4.10. Statistical Analysis of Data

The data generated for the physicochemical and physicomechanical parameters were statistically evaluated using response surface method and the associated one-way analysis of variance (ANOVA) (Minitab software, V14, Minitab, USA) for process optimization and evaluation of the design reliability.

## 3.5. RESULTS AND DISCUSSION

### 3.5.1. Synthesis, Physical Appearance and Percentage Yield of the Polyamide 6,10 Variants

The fourteen polyamide 6,10 variants appeared as white, crystalline and compact solids. Some of the variants appeared to have more robust surfaces when compared to those synthesized during the screening process (Chapter Two). This possibly could be associated with the full modification strategies employed. Powdered forms of these variants produced free-flowing, compressible, white particles with varying consistencies, volumes, masses and densities. The percentage yield of each variant was calculated with

respect to Equation 2.2 and the values for the fourteen samples ranged from 25% to 95% (Figure 3.4).

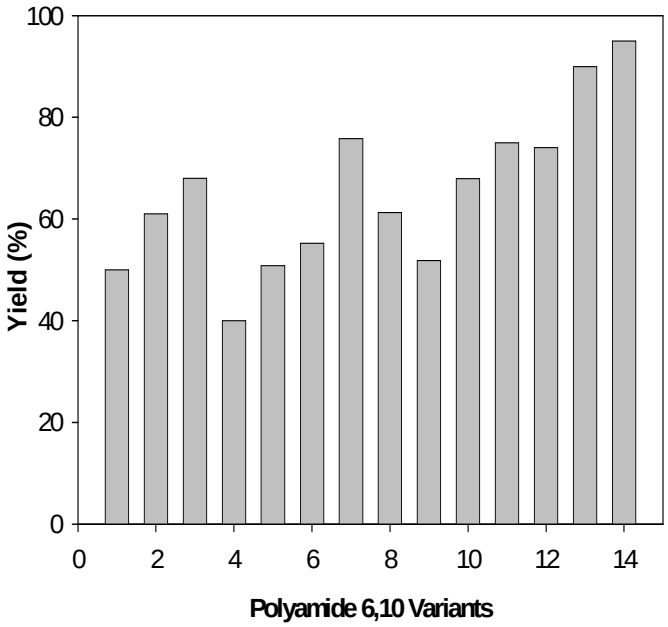



**Figure 3.4:** Changes in the yield of the fourteen modified polyamide 6,10 variants (N= 2 and standard deviation less than 5.03 in all cases).

### 3.5.2. Evaluation of the Physicomechanical Parameters

#### *3.5.2.1. Analysis of Matrix Resilience*

A general increase in matrix resilience when compared to the polyamide 6,10 variants obtained from the screening design (Chapter Two) was observed (Table 3.3). These results demonstrated the capability of the polar and/or non-polar phase modifications to synergistically or individually enhance polymeric matrix firmness (Table 3.2). Furthermore, the ability of the higher resolution Box-Behnken design to generate effective stoichiometric combinations of monomers solvent volume ratios as well as the quantities of the solvent the phase modifiers that could enhance the strength of the synthesized polyamide matrices which subsequently improves matrix resilience was envisaged (Table 3.2).

**Table 3.3:** Matrix resilience values of the polyamide 6,10 variants synthesized using the Box- Behnken design template

| Experimental Runs | Matrix Resilience (%) |
|---|---|
| 1 | 40.70 |
| 2 | 41.85 |
| 3 | 45.85 |
| 4 | 42.96 |
| 5 | 56.99 |
| 6 | 46.97 |
| 7 | 45.60 |
| 8 | 42.97 |
| 9 | 43.92 |
| 10 | 46.34 |
| 11 | 46.09 |
| 12 | 46.02 |
| 13 | 54.67 |
| 14 | 63.48 |

Furthermore, the numerical values obtained from the assessment of matrix resilience for the formulations with and without amitryptiline hydrochloride were observed to be closely related. This is illustrated in Figure 3.5. This established the fact that the addition of drug to the polymer did not interfere with the structural integrity of the polymeric matrix confirming the absence of any form of chemical interaction between the drug and polymer that is desired as these formulations are intended to be physical mixtures for direct compression to produce the monolithic matrix systems.

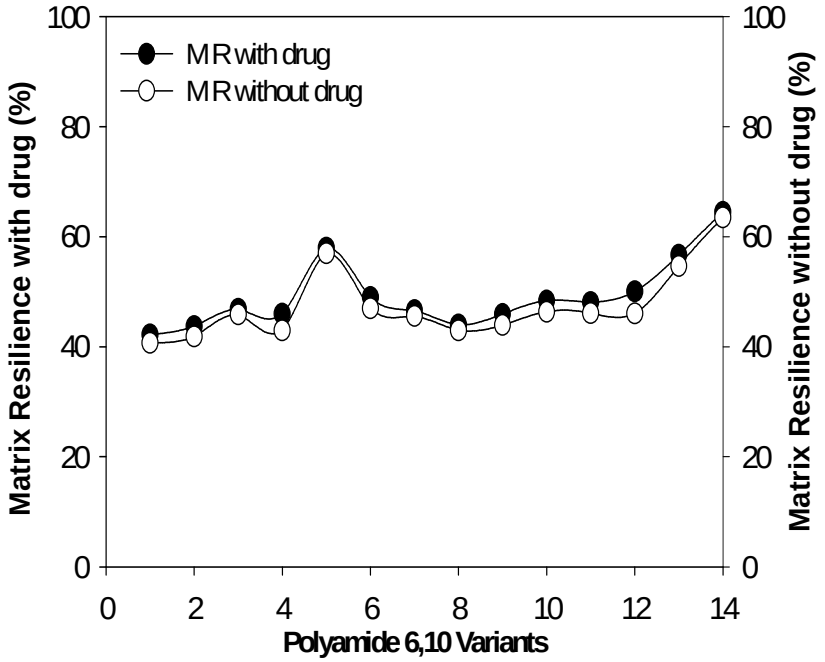



**Figure 3.5**: Relationship between matrix resilience for polyamide 6,10 matrix formulations with and without drug (N= 10 and standard deviation less than 5.47 in all cases).

### *3.5.2.2. Determination of the Brinell Hardness Number*

The Brinell Hardness Number is used to evaluate the resistance of solid materials to deformation, which is a measure of the hardness of the material. In this case, the compressed polyamide 6,10 monolithic matrix systems are likened to solid materials. The determination of this parameter demonstrates further the highly varied stress-strain transitions (measured as physicomechanical strength in terms of matrix resilience) observed for the polyamide 6,10 variants. It was observed that the overall profile generated for both Brinell Hardness Number and matrix resilience were comparable (Figure 3.6). This indicated that there was a directly proportional relationship between matrix resilience and the Brinell Hardness Number.

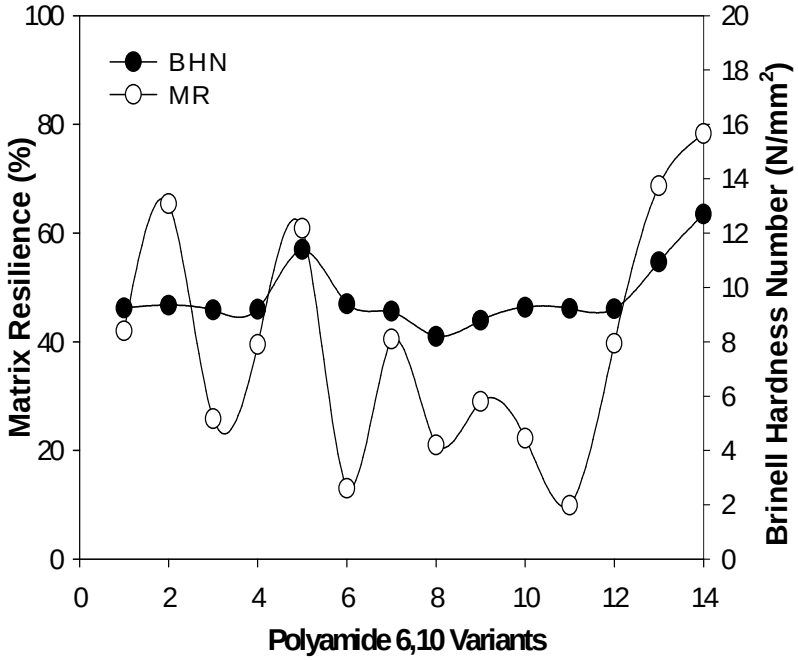



**Figure 3.6**: Correlation between the Brinell Hardness Number and matrix resilience.

A hypothetical statement with respect to data generated that the measure of resistant force computed for each variant using the Brinell Hardness Number is a function of their ability to compress to various hardnesses despite the fact that they were subjected to the same formulation conditions (i.e. compressibility efficiency) can be made. Therefore, the disparities in the derived values of Brinell Hardness Number values can be associated with the changes in the stoichiometry of the reaction, volume ratios as solvent phase modifiers employed. These may alter the polyamide 6,10 polymeric chain structure by influencing the inter-molecular hydrogen bond configuration, which affects the consistencies of the powdered forms as well as their compressibility and packing efficiency (during compression) by the influence on the particle-particle electrostatic interactions and these may produce differences in the hardness of each compressed matrix.

### 3.5.3. *In vitro* Drug Release Characteristics

Diverse release patterns were observed for the fourteen formulations which may be associated with the various degrees of polymerization attained for each polyamide 6,10 variant based on the modification strategy applied during synthesis. Figure 3.7 illustrates the dissolution profiles of the fourteen formulations.

The capability of a relatively higher matrix resilience (41.9%-63.5%) to improve matrix strength and firmness consequently minimizes the rate of matrix disentanglement and dissolution thus prolonging and controlling the quantity of drug released was evident for from the synthesized polyamide 6,10 variants, e.g. Formulations 2, 3 and 5-14 (Table 3.3 and Figure 3.7). Exceptions to this trend were Formulations 1 and 4 (Figure 3.7) which displayed relatively high matrix resilience values of 40.7% and 42.9% (Table 3.3) but rapid drug release patterns which may be attributed to some chemical transformations beyond the scope of this study.

The mean dissolution times fixed at 8 hours ($MDT_8$) for each dissolution profile using Equation 3.3 described earlier were calculated. The $MDT_8$ was employed as the release profiles displayed a relatively dynamic, non-linear, rapid release patterns up to 8 hours after which drug release approached constant, steady-state release patterns (Figure 3.7). A numerical representation of the $MDT_8$ for all fourteen formulations is presented in Table 3.4. It was observed in this study that a low $MDT_8$ was associated with slow releasing formulations while the converse was the case with a high $MDT_8$ (Figure 3.7 and Table 3.4).

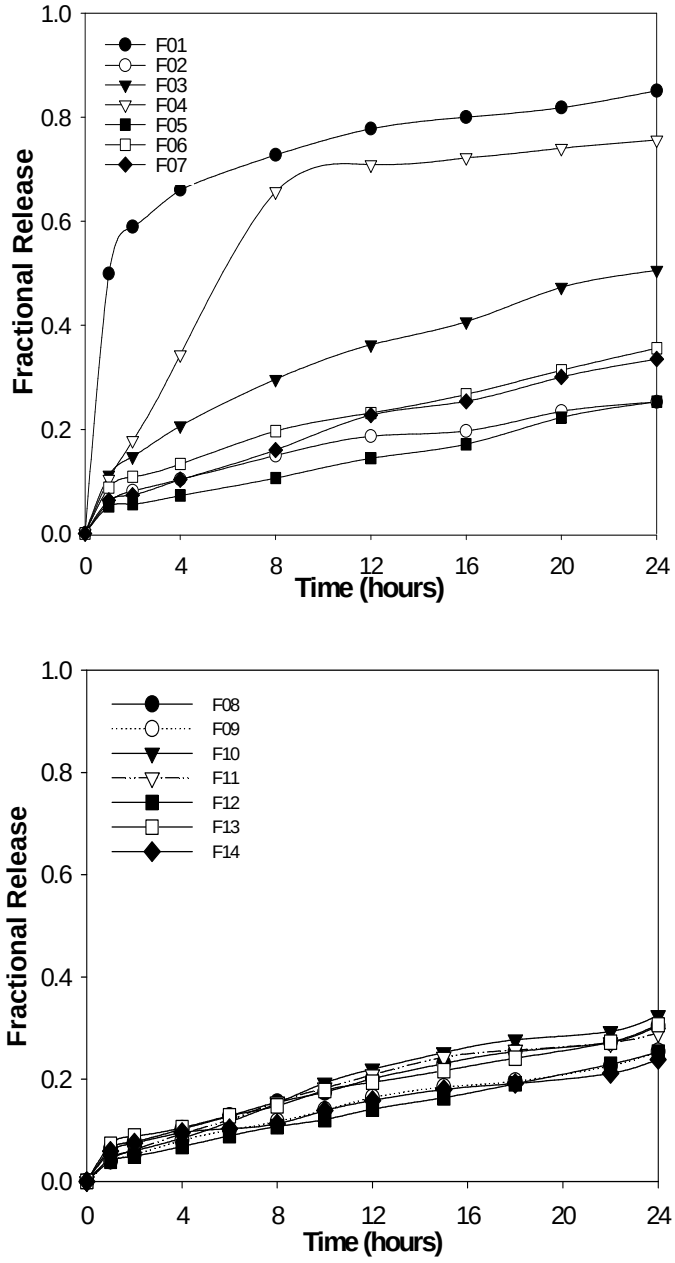



**Figure 3.7**: Drug release profiles for the fourteen formulations in PBS 7.4 prior to optimization (N=3 and standard deviation less than 0.18 in all cases).

**Table 3.4:** Mean dissolution times ($MDT_8$) for the fourteen formulations in accordance with the Box-Behnken design template

| Experimental Runs | Mean Dissolution Time |
|---|---|
| 1 | 11.701 |
| 2 | 2.452 |
| 3 | 4.270 |
| 4 | 8.500 |
| 5 | 2.511 |
| 6 | 2.383 |
| 7 | 2.242 |
| 8 | 2.183 |
| 9 | 2.664 |
| 10 | 2.220 |
| 11 | 2.189 |
| 12 | 2.596 |
| 13 | 2.672 |
| 14 | 2.100 |

### 3.5.4. Analysis of Matrix Gravimetric Changes

Many of the release profiles presented a non-proportional relationship between the cumulative quantity of drug released in PBS over 24 hours and the residual mass of matrix. Formulation 14 for instance released 23.84% of its drug-load at 24 hours while 48.59% of the matrix remained intact. Based on these observations, an assumption that drug release from the polyamide 6,10 variant matrices was prompted by an initial rapid matrix loss accompanied by a process of molecular diffusion to the inner core of the matrix followed by a gradual process of diffusion of drug molecules accompanied with a slower and more consistent pattern of polymeric matrix dissolution and drug liberation can be made. Thus, the initial rapid release (up to 8 hours) of drug molecules occurred due to initial matrix disentanglement and not diffusion of drug molecules. The percentages of matrix remaining at 24 hours for the fourteen formulations are represented in Figure 3.8.

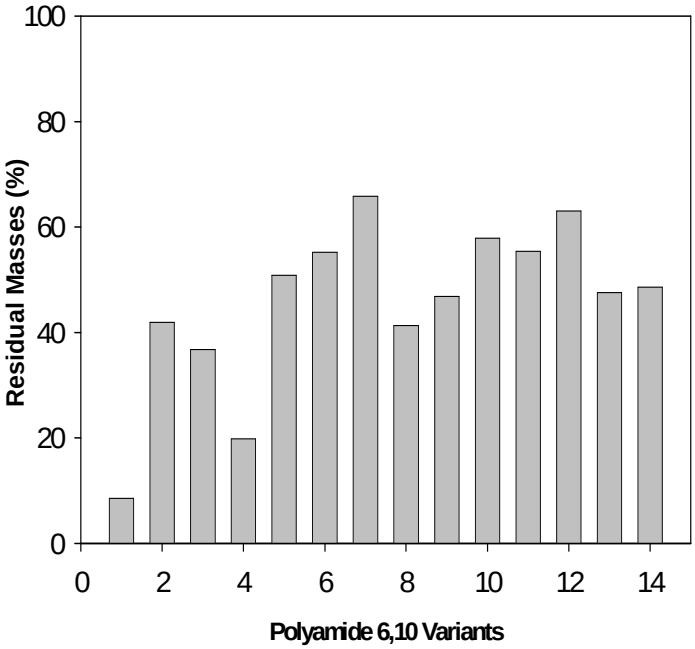



**Figure 3.8**: Percentage residual masses of eroded matrices after 24 hours (N= 3 and standard deviation less than 4.69 in all cases).

### 3.5.5. Electrolyte Conductivity Assessment

This study was performed to evaluate matrix erosion from the micromolecular perspective by visualizing it as actual matrix dissolution involving the movement of polymeric ions due to hydration. A proposed mechanism of dissolution of polyamide 6,10 to generate its ions is described in Figure 3.9. The profiles generated from this study can be described as bi-phasic. The first phase (at the first hour) demonstrating an initial sharp increase in conductivity followed by an apparently steady phase (beyond the first hour). This is indicative of the fact that there is an initial rapid process of matrix dissolution generating conducting electrolytes followed by a more steady-state. This may be responsible for the initial burst and irregular, diverse release profiles observed for the first eight hours (Figure 3.7). Plots of the change of conductivity values with time for the fourteen formulations are presented in Figure 3.10.

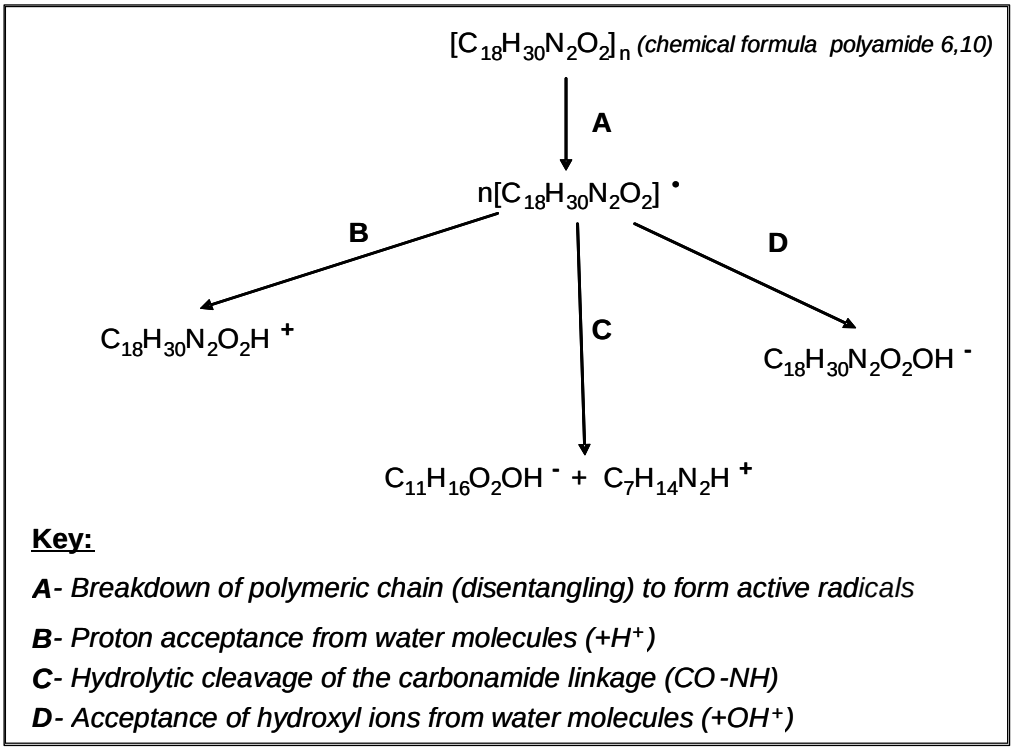



**Figure 3.9**: Proposed mechanism of ionic transfer and formation of conducting electrolytes of polyamide 6,10 .

Conductivity values at the first hour ($COND_1$) were employed in the optimization process because of its direct influence on the overall matrix erosion process, which also affects the drug release patterns. Minimizing the initial sharp rise in the conductivity values could reduce and control the amount of drug liberated. Furthermore, the intrinsic potential of the polyamide variants to generate polar, ionic particles is demonstrated. This capability will favour the process of bio-erosion as well as metabolism and excretion via the human renal system.

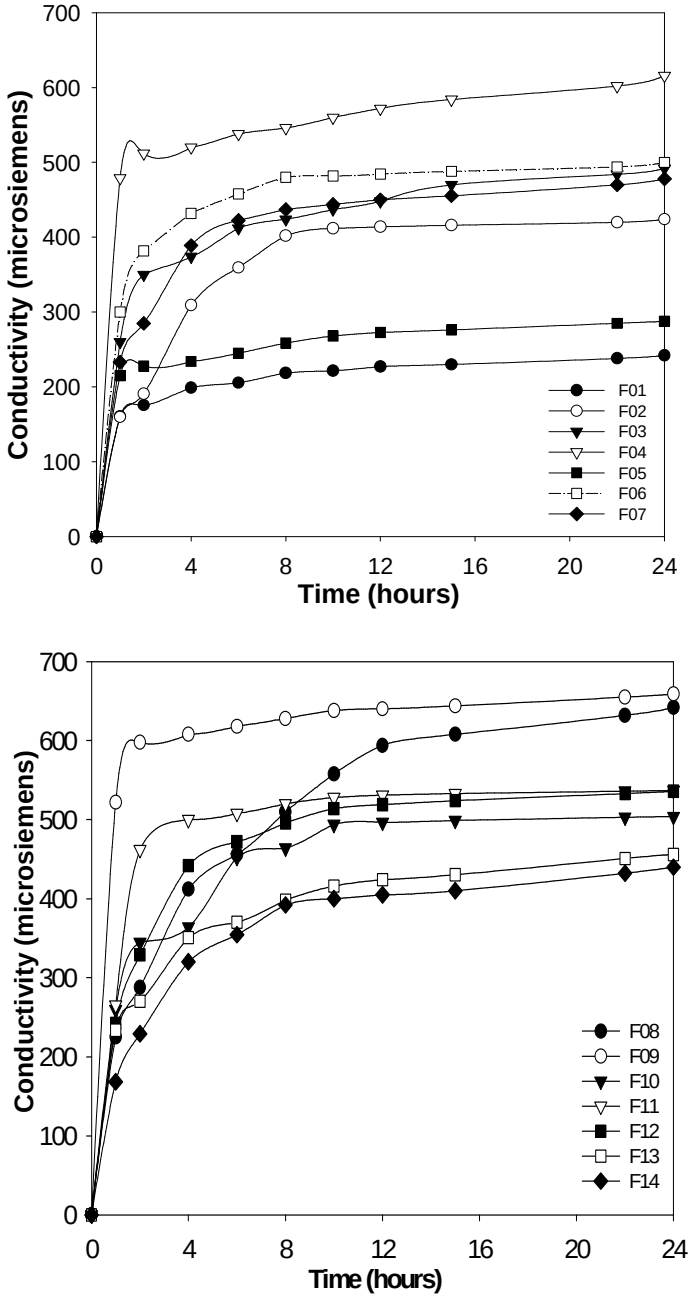



**Figure 3.10**: Change in conductivity values for the fourteen polyamide 6,10 formulations (N= 3 and standard deviation less than 10.2 in all cases).

### 3.5.6. Structural Analysis by Fourier Transform Infrared Spectroscopy

Spectrophotomeric analyses were conducted on the fourteen polyamide 6,10 variants as well as the optimized formulations; pure drug and a mixture of both comprising the monolithic system.

Pure drug (amitriptyline hydrochloride) displayed characteristic absorption bands of aromatic C=C (1,600.26cm$^{-1}$), aromatic C-H (3092.25cm$^{-1}$) stretch vibrations; peaks at 3440.32cm$^{-1}$ and 597.27cm$^{-1}$ indicative of a secondary amine moiety and the C-N-C scissors vibrations respectively.

Specific transmitting bands of amide (N-H, 3306.44 - 3401cm$^{-1}$), methylene segments (C-H stretch, 2850 - 2900cm$^{-1}$), $CH_2$ wag (1442 - 1466.15cm$^{-1}$) and rock (700 - 750cm$^{-1}$) movements, carbonyl groups (C=O, 1690 - 1740cm$^{-1}$), C-N-C scissors vibrations (480 - 510cm$^{-1}$) of the fourteen polyamide 6,10 variants as well as the optimized formulation were also recorded.

The above-stated vibrational frequencies correlate with the established chemical structures of amitriptyline hydrochloride and polyamide 6,10 respectively. In addition, the vibrational frequency values obtained for the present analysis as regards to the polyamide 6,10 variants are similar to the numerical value ranges generated in Chapter Two (Section 2.3.5 and Table 2.8) for the variants synthesized using the screening design. This reveals that despite the variations in the physicochemical and physicomechanical properties exhibited by the variants, a consistency in their basic chemical backbone structure is evident. In other words, the modification strategy employed brings about physical and not chemical changes.

Infrared analysis conducted on the monolithic matrix formulations produced from a mixture of amitriptyline hydrochloride and the respective polyamide 6,10 variants revealed that none of the formulations displayed measurable shifts in the values of the vibrational frequencies of characteristic functional moieties described above for both the drug and

polymer. A conclusion that there were no chemical interactions, confirming that they are physical mixtures, between the drug and polymeric carrier (i.e. polyamide 6,10) for each formulation can be made.

Furthermore, the ranges of vibrational frequencies for the specific bonds observed for the polyamide 6,10 variants synthesized using the Box-Behnken design were closely related to those prepared using the Plackett-Burman design (Table 2.8). An inference that the modification strategies had no influence on the intactness of the polyamide 6,10 structural backbone.

### 3.5.7. Constrained Optimization

The primary aim of this optimization process was to develop diversified monolithic matrix systems with polyamide 6,10 as the polymeric carrier displaying slow, intermediate and controlled release rates. This is based on the finding that modification of the interfacial polymerization process of synthesizing polyamide 6,10 employed in this study influenced their drug release characteristics (Figure 3.6).

After generating the quadratic polynomial regressions relating the independent to the dependent variables (Equation 3.2), experimental results were fitted within set constraints for predicting the optimal formulation. The simultaneous optimization was performed using Response Surface Optimizer (Minitab V14, USA). With this technique, constraints were set to obtain levels of independent variables that will simultaneously maximize or minimize matrix resilience and the $MDT_8$ with respect to the desired release profiles. With regards to the purpose of this optimization process which is to achieve slow, intermediate and controlled release (pseudo zero-order) systems, matrix resilience and the mean

dissolution time were targeted at different levels (Table 3.5) giving the desirability function (i.e. a value that measures the accuracy of the statistical process) equal to one. This shows the accuracy and efficiency of the statistical optimizer.

**Table 3.5**: Numerical targets set for the significant response parameters to generate the desired drug release performances

| Set Targets for the Response Parameters | | Predicted Drug |
|---|---|---|
| **Matrix Resilience (%)** | **$MDT_8$** | **Release Patterns** |
| 65.00 | 1.10 | Slow |
| 45.85 | 4.27 | Intermediate |
| 43.19 | 7.02 | Controlled |

As earlier stated, the selected response parameters for this experimental section were three namely: the matrix resilience, $MDT_8$ and conductivity at the first hour. The matrix resilience and $MDT_8$ were chosen for the simultaneous optimization process because of the correlation measures that are employed to estimate the fitness of the statistical model for accurate prediction. The model-dependent terms employed in this study include the: (i) *p-values* set at 95% confidence level ($p<0.05$) and (ii) the correlation coefficient, $R^2$ (set at values greater than 0.70). Table 3.6 lists the levels of significance for the various responses.

**Table 3.6:** Levels of significance for the response parameters

| Response Parameters | *p-values* | $R^2$ | Lack of fit |
|---|---|---|---|
| MR [a] | 0.031 | 0.907 | 0.324 |
| $MDT_8$ [b] | 0.013 | 0.948 | 0.264 |
| $COND_1$ [c] | 0.678 | 0.705 | 0.038 |

[a] *Matrix resilience,* [b] *Mean dissolution time,* [c] *Conductivity at one hour*

$R^2$ values closest to the numerical value of one were recorded for $MDT_8$ and matrix resilience. This signified that the statistical analysis of the data produced a high degree of correlation between the experimental and predicted values. Furthermore, the *p-values* for both responses (excluding conductivity values at the first hour) were of statistical significance ($p<0.05$). The *p-values* for the lack-of-fit were greater than 0.05 (with the exception of conductivity values at one hour), suggesting that the model was precise and stable. On this basis, these two parameters were selected for the optimization process with the exclusion of the conductivity value at one hour (Table 3.6). The set constraints (Table 3.5), the optimal factor levels that achieved the desired numerical values of the significant response parameters are represented in Table 3.7.

**Table 3.7:** Experimental and fitted response values performed at optimal factor levels

| Desired Drug Release Patterns | Selected Factor Levels | | | Response | | | |
|---|---|---|---|---|---|---|---|
| | | | | MR [a] | | MDT [b] | |
| | SC (g) [c] | NaOH (mL) [d] | C-HXN (mL) [e] | Fit [f] | Exp [g] | Fit [f] | Exp [g] |
| Slow | 0.63 | 0.10 | 40.00 | 64.00 | 65.20 | 1.25 | 1.17 |
| Intermediate | 1.49 | 0.10 | 2.92 | 44.00 | 45.33 | 4.50 | 5.00 |
| Controlled | 0.20 | 0.40 | 10.00 | 42.00 | 41.55 | 6.92 | 7.00 |

[a] *Matrix resilience,* [b] *Mean dissolution time,* [c] *Sebacoyl chloride,* [d] *Sodium hydroxide,* [e] *Cyclohexane,* [f] *Fitted values,* [g] *Experimenatal values*

**Note**: Hexamethylenediamine, Hexane and Deionized water were kept constant as established by the screening design (i.e. 1.75g, 40mL and 10mL respectively) for the three polyamide 6,10 matrix formulations presented in Table 3.7.

A close relationship between the experimental and predicted values was observed ($R^2>0.90$). This outcome demonstrates the stability and validity of the optimization procedure. The new monolithic matrix formulations were prepared using the three

optimized polyamide variants synthesized from the model-predicted optimal factor levels. Representative dissolution profiles of the optimized formulations are shown in Figure 3.11 below.

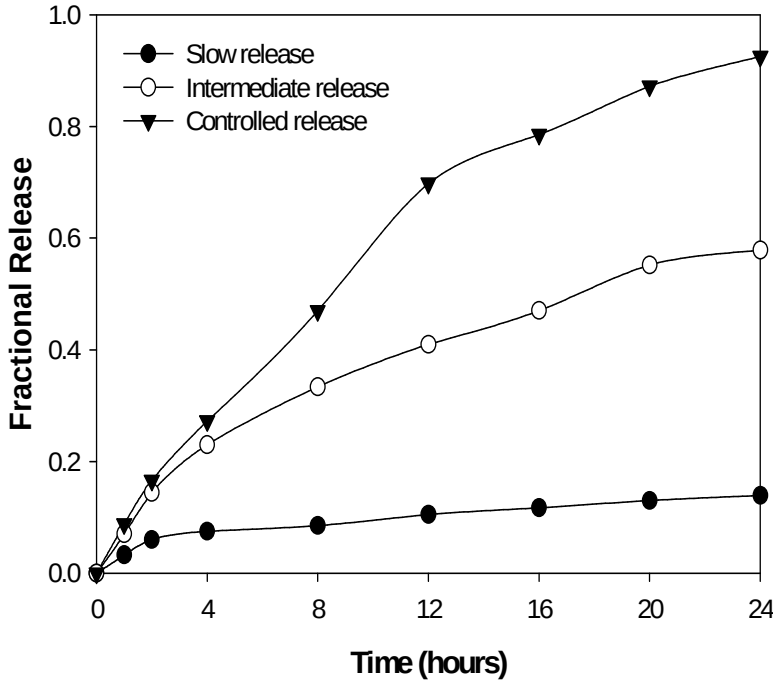



**Figure 3.11**: Dissolution profiles for the optimized monolithic matrix formulations (N= 3 and standard deviation less than 0.36 in all cases).

## 3.6. CONCLUDING STATEMENTS

This work has displayed the capability of the modification strategy employed to improve the matrix integrity of polyamide 6,10 as regards to its strength and its application in rate-controlled drug delivery. Overall, the main purpose of this Chapter, which was to obtain diversified, optimized monolithic matrix formulations exhibiting minimized burst effects and achieving prolonged release patterns was accomplished by using polyamide 6,10 as the polymeric carrier. Furthermore, the matrix systems developed in this study have effectively released amitriptyline hydrochloride, a highly water soluble drug in a pseudo zero-order manner without significant burst effect. The influence of formulation variables

on the drug release performance from the optimized polyamide 6,10 monolithic matrix systems developed in this Chapter, mathematical models to establish the drug release kinetic mechanisms as well as their physicochemical and physicomechanical properties will be investigated in the subsequent Chapters.