# CHAPTER 4

# Fabrication and Statistical Optimisation of Salted-Out and Internally Cross-linked Anti-Tuberculosis Polymeric Enterospheres Employing an Experimental Design Strategy

## 4.1. Introduction

The completion of preliminary activities in Chapter 3 yielded a sufficient database to allow for the establishment of the qualitative composition of a prototype formulation and the range of components to be evaluated. For the attainment of the overall goal of efficiently determining a set of conditions that result in an optimally performing product and thereafter determine limits for critical formulation or processing variables, an appropriate statistical experimental technique is instituted (Myers and Montgomery, 1995).

Experimental design, with particular reference to Pharmaceutics, can be defined as the strategy for setting up experiments in such a manner that the information required is obtained as efficiently and precisely as possible (Lewis et al., 1999). The use of predictive models such as Response Surface Methodology (RSM) arises when the following are required:

- Optimising the process for formulation i.e. maximising or minimising one or more of the responses, keeping the remainder within a satisfactory range
- Carrying out simulations with the model equation
- Obtaining a process or product with properties (responses) within a fixed range of values
- Improved understandind of the process, thus assisting development, scale-up and transfer of formulations and processes
- Being capable of knowing at any time the optimum manufacturing conditions to obtain a product with a particular set of properties
- Plotting the responses.

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The mapping of the response is performed by describing it by means of a model equation. This equation describes the response in terms of a function of variables, which are normally quantitative and continuous. This function can then be visualised using contour plots or three-dimensional diagrams. Evidently this technique is only useful where the response is sufficiently reproducible and its dependence on the process or formulation variables can be described by a mathematical model (Lewis et al., 1999).

In this chapter, a Box-Behnken design was employed for the generation of quadratic response surfaces and construction of second order polynomial models for the prediction of the enterosphere behaviour in terms of the independent variables investigated. This facilitated a mechanistic evaluation of possible correlations between pertinent processing factors such as the concentration of $ZnSO_4$, cross-linking reaction time (CRT), concentration of triethyl citrate (TEC) and drying temperature (DT) employed in the formulation of the enterospheres on their ability to entrap the drug and target its release to the small intestine for the formulation of an optimum system. The enterosphere formulations were characterised in terms of their aspect ratio (a shape factor), molar amount of zinc ($n_{Zn)}$ incorporated within the cross-linked matrix (a stoichiometric coefficient), drug loading and drug entrapment efficiency (DEE), fractional INH release and mean dissolution time (MDT) in acidic media and textural parameters for each of the enterosphere variants (matrix resilience, deformation energy and matrix hardness). Response optimisation was then employed to identify an ideal copolymeric enterosoluble multiparticulate system with the desired drug entrapment and dissolution properties.

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### 4.1.1. Development of an Experimental Design Strategy for Enterosphere Fabrication and Optimisation

The formation and properties of the copolymeric enterospheres ionotropically cross-linked via multivalent ions for modified drug delivery depends on the concentrations and distribution of the ions incorporated within the matrix structure, which in turn is affected by the duration of exposure of the enterospheres to the salting-out and cross-linking solution. The copolymeric chains are cross-linked via cations by the formation of complexes liganded with more than one polymer group creating intramolecular and/or intermolecular cross-links (Allain and Salome, 1990). The inclusion of a plasticiser also exerts a distinctive effect on the characteristics of the enterosphere film and internal matrix due to its influence on the polymer's melt viscosity, glass-transition temperature ($T_g$), minimum film-forming temperature (MFT) and elastic modulus as a result of the plasticiser's ability to weaken copolymeric intermolecular attractions and to increase the copolymer's free volume (Nielson and Landel, 1993; McGinity, 1996).

Statistical experimental designs are strongly recommended in identifying critical formulation variables in the development of modified-release drug delivery systems (Sastry et al., 1997). In the implementation of a novel salting-out and cross-linking method for the formulation and design of ionotropically cross-linked enterospheres for delivery of a water-soluble drug to the small intestine, the use of a response surface methodology (RSM) allows for the generation of mathematical models to adequately describe or predict the drug entrapment and release behaviour of the enterospheres.

The fabrication of cross-linked enterospheres in a single processing step (precluding the use of expensive machinery and organic solvents) is an alternative approach to the standard technique for manufacturing modified-release multiparticulates, which consists of coating drug-containing

granules or beads with aqueous colloidal latex or pseudolatex copolymeric dispersions. However, as discussed and demonstrated in Chapters 3, a problem associated with enteric-coated formulations made of aqueous disperse systems or solutions is the lack of resistance against gastric fluid and the reportedly more rapid diffusion of water-soluble drug through films prepared from aqueous solutions (Guo et al., 2002). It is contemplated that fabrication of an optimum cross-linked enteric-polymer matrix system incorporating a water-soluble drug would achieve improved gastroresistance of the multiparticulate system.

As described, the salted-out and cross-linked enterosphere matrices were formed by inducing separation of the anionic polyelectrolyte, MAEA, as a polymer-rich enteric film (the 'salting-out' phenomenon) and ionotropically cross-linking the internal enterosphere matrix (Figure 4.1) following extrusion and curing of an aqueous dispersion of the polymer into a concentrated electrolyte solution. $ZnSO_4$ as the heptahydrate ($ZnSO_4.7H_2O$) was selected as the salting-out and cross-linking agent, demonstrating superior performance in comparison to other salts evaluated in Chapter 3, owing to the favourable salting-out capabilities and highly hydrated nature of the sulphate anion ($SO_4^{2-}$) in accordance with the Hofmeister series, and the superior cross-linking capabilities of the $Zn^{2+}$ for MAEA. In addition, the electrolyte demonstrates high water solubility (Eby, 2005). Sulphate ions have the ability to successfully form sufficiently strong hydrogen bonds and form a hydration shell from small rings of hydrogen-bonded water. Such a shell may consist of a symmetrical dodecahedral arrangement of 16 water molecules where each sulphate oxygen is hydrogen bonded to three water molecules; these water molecules forming small looped chains of 2 (6 occurrences per $SO_4^{2-}(H_2O)_{16}$) or 3 (12 occurrences per $SO_4^{2-}(H_2O)_{16}$) (Plumridge, 2000) as depicted in Figure 4.1.

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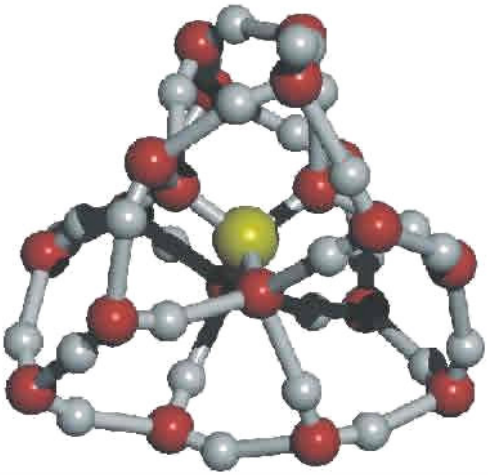

*Figure 4.1: Hydration shell of sulphate anions (Plumridge, 2000)*

MAEA is a synthetic copolymer demonstrating excellent biocompatibility, and is suitable for ionotropic cross-linking in this manner to form interconnected matrices (Figure 4.2). As anionic polyelectrolytes, they have charged carboxylic acid side groups and although they are practically insoluble in water, they are soluble in solutions of 1.0M NaOH upon neutralisation of carboxyl groups (Cohen et al., 1996). The water-dispersed polymer with charged side groups was cross-linked by reaction with a solution of $Zn^{2+}$cations.

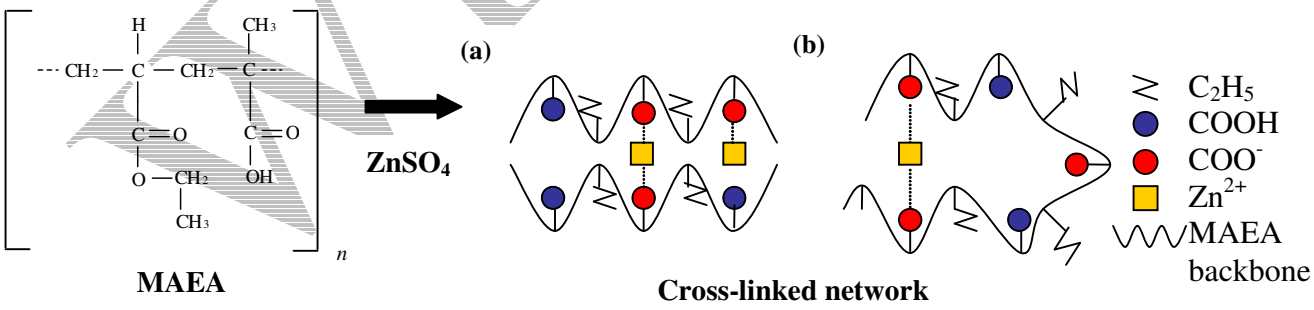


*Figure 4.2: Two-dimensional schematic of proposed non-stereo-specific interactions: (a) Inter- and (b) intra-molecular ionic interactions ('salt-bridges') between the anionic poly(methacrylic acid-co-ethylacrylate) copolymer (MAEA) and cationic agent. Such interaction (occurring in different planes) undoubtedly impacts on release behaviour of the novel form compared to the native MAEA copolymer*

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## 4.2. Materials and Methods

### 4.2.1. Materials

MAEA copolymer with a monomer molar ratio of 1:1 (Eudragit® L100-55, Methacrylic Acid Copolymer Type C) containing sodium lauryl sulphate (0.7%$^{w}/_{w}$) and polysorbate 80 (2.3%$^{w}/_{w}$) as emulsifiers was a gift from Röhm Pharma Polymers (Röhm GmbH, Darmstadt, Germany). INH (isonicotinic acid hydrazide, 99% TLC) and TEC 99% was purchased from Aldrich® (Sigma-Aldrich Inc., St. Louis, USA). NaOH ($M_w$=40.00g/mol), $ZnSO_4.7H_2O$ ($M_w$=287.54g/mol), and ethylenediaminetetraacteic acid as the sodium salt (EDTA, $C_{10}H_{16}N_2O_8$, $(HOOCCH_2)_2N\text{-}CH_2CH_2\text{-}N(CH_2COOH)_2$, $M_w$=292.24 g/mol) were obtained from Saarchem (Wadeville, Gauteng, South Africa). All other reagents were of analytical grade and were used as received.

### 4.2.2. Formulation of Enterospheres

The MAEA copolymer was redispersed, effected by addition of 1M NaOH in order to achieve neutralisation of approximately 6 mole-% of the carboxyl groups contained in the copolymer. A low degree of partial neutralisation of the copolymer was preferred to complete neutralisation: the enterospheres were notably more robust, and the level of cross-linking interaction was sufficient to induce notable release control from the enterosphere yet limit the cation content of the system. TEC, at various percentage levels, was included as a plasticiser. Dissolution of the water-soluble INH in the aqueous dispersion was achieved under agitation at 500rpm for 10 minutes with a Heidolph® propeller stirrer (Labotec, Gauteng, South Africa) to obtain a MAEA: INH ratio of 5:1. The dispersion was vortexed (Vortex Genie-2, Scientific Industries Inc., USA) before further processing to allow for homogenisation and the dissipation of any foam induced during redispersal. 10mL of the dispersion was then extruded dropwise at a rate of 2.0ml/min,

using a flat-tip needle (Terumo®, GmbH, Germany) of 0.80-mm internal diameter, into 100mL of a gently agitated $ZnSO_4.7H_2O$ solution, which induced immediate salting-out with formation of spherical enteric coating.

The formed enterospheres were retained in the electrolyte solution for curing in a dark area for the experimentally determined protracted time intervals to promote cation diffusion with induction of cross-linking of the internal matrix. The enterospheres were then washed twice with double-deionised water (100mL) to remove any unincorporated electrolyte and then oven-dried at different temperature settings for 3 hours followed by slow cooling under ambient conditions ($21^oC$). Heating of the enterospheres at elevated temperatures below the crystalline melting point is known to result in subsequent annealing, which may cause a significant increase in the crystallinity of the MAEA copolymer, as well as relieving stresses.

### 4.2.3 Experimental Design

Optimisation of the enterospheres was conducted by constructing and analysing a four-factor, three-level ($3^4$) Box-Behnken statistical design on MINITAB®, (V14, Minitab, USA). $ZnSO_4$ (10-50%$^w/_v$), CRT (15-60 minutes), DT (25-60$^o$C) and TEC (2-10%$^w/_w$) were varied (Table 4.1) for determination of the effect of the experimental factors on $n_{Zn}$, DEE and MDT in acidic media (0.1M HCl).

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*Table 4.1: Factors and levels of independent variables generated by the $3^4$ Box-Behnken Design*

| Experimental Formulation | $ZnSO_4$ ($\%^w/_v$) | CRT (minutes) | DT ($^oC$) | Plasticiser (TEC) ($\%^w/_w$) |
|---|---|---|---|---|
| 1 | 50 | 60.0 | 42.5 | 6 |
| 2 | 30 | 15.0 | 42.5 | 10 |
| 3 | 30 | 15.0 | 25.0 | 6 |
| 4 | 30 | 37.5 | 42.5 | 6 |
| 5 | 10 | 37.5 | 42.5 | 10 |
| 6 | 30 | 60.0 | 42.5 | 10 |
| 7 | 10 | 60.0 | 42.5 | 6 |
| 8 | 50 | 15.0 | 42.5 | 6 |
| 9 | 30 | 60.0 | 60.0 | 6 |
| 10 | 30 | 60.0 | 25.0 | 6 |
| 11 | 30 | 15.0 | 42.5 | 2 |
| 12 | 50 | 37.5 | 42.5 | 10 |
| 13 | 10 | 37.5 | 25.0 | 6 |
| 14 | 30 | 37.5 | 60.0 | 10 |
| 15 | 30 | 60.0 | 42.5 | 2 |
| 16 | 30 | 37.5 | 25.0 | 10 |
| 17 | 10 | 15.0 | 42.5 | 6 |
| 18 | 30 | 15.0 | 60.0 | 6 |
| 19 | 50 | 37.5 | 60.0 | 6 |
| 20 | 30 | 37.5 | 42.5 | 6 |
| 21 | 30 | 37.5 | 60.0 | 2 |
| 22 | 50 | 37.5 | 42.5 | 2 |
| 23 | 30 | 37.5 | 25.0 | 2 |
| 24 | 10 | 37.5 | 60.0 | 6 |
| 25 | 10 | 37.5 | 42.5 | 2 |
| 26 | 50 | 37.5 | 25.0 | 6 |

### 4.2.4. Surface Morphology and Shape Analysis of Enterospheres

The Feret's diameters ($d_f$) and shape of the enterospheres were investigated by microscopic image analysis using a stereomicroscope (Olympus SZX7, Japan) connected to a digital camera (CC 12) and image analysis system (AnalySIS® Soft Imaging System, GmbH, Germany). Feret's diameter was determined from the mean distance between two parallel tangents to the projected particle perimeter. Fifty enterospheres from each of the formulations were viewed under darkfield at 16X magnification. From the longest and shortest Feret's diameters for each formulation, a shape factor (the aspect ratio) was calculated as follows:

$$\text{Aspect ratio} = \frac{d_{max}}{d_{min}} \qquad \text{[Equation 4.1]}$$

### 4.2.5. Determination of Molar Amount of Zinc Incorporated within the Cross-linked Matrix (Cross-linking Cation: MAEA Stoichiometry)

As a measure of the molar stoichiometry of the cross-linking cation and MAEA interaction, the $n_{Zn}$ was determined by complexometric/chelometric titration of $Zn^{2+}$ with EDTA (ethylenediaminetetraacteic acid, $C_{10}H_{16}N_2O_8$) demonstrating accuracy and precision in the order of ±0.1%). EDTA forms very strong 1:1 complexes with divalent and trivalent metal ions depending on solution conditions. The EDTA reacts with the $Zn^{2+}$ to form a chelate:

$$Na_2EDTA + Zn^{2+} = ZnEDTA \qquad \text{[Equation 4.2]}$$

For the analysis of the amount of $Zn^{2+}$ incorporated within the cross-linked matrix, 0.95g of accurately weighed enterospheres were hydrated in 25mL double-deionised water. Immediately prior to titration, 15mL of deionised water, 9-10mL of ammonia/ammonium chloride buffer (pH 10.0), and 3 drops of Eriochrome Black T were added. The samples were titrated with a standardised solution of 0.01M EDTA until the pink solution turned light blue.

### 4.2.6. Drug Content and Entrapment Efficiency

For determination of the drug loading of the enterospheres, 100mg of INH-loaded enterospheres were placed in a 200mL conical flask containing 100mL of 0.2M PBS, pH 6.8. The enterospheres were magnetically stirred for a minimum of 5 hours to promote and ensure erosion and disentanglement of the cross-linked structure to afford liberation and subsequent dissolution of INH. The resultant solutions were filtered through a 0.45μm membrane filter (Millipore®,

Billerica, MD, USA). The filtrates were then made up to 200mL volumes with the PBS pH 6.8. Aliquots of these solutions were subjected in triplicate to UV spectroscopy (diode array UV spectrophotometer, Specord 40, Analytik Jena AG, Jena) at 263nm for analysis (WinASPECT® Spectroanalytical Software, Analytik Jena AG, Jena) following comparison with the standard calibration curves generated for INH in PBS media. Drug entrapment was determined from the empirical relationship described in Chapter 3 (Equation 3.1).

### 4.2.7. Drug Release Studies

Characterisation of INH release from the enterospheres was assessed using a method based on the general drug release standard for delayed release (enteric-coated) articles (USP 24, 2000) employing the USP apparatus II (paddle apparatus) (USP 25, 2001). The six-station dissolution apparatus (Caleva®, Model 7ST) was modified with insertion of a ring-mesh assembly in the dissolution vessel to prevent undue sticking of the spheres to the paddle (Pillay and Fassihi, 1998). Each vessel was filled with 500mL of 0.1M HCl (pH 1.2) as the initial dissolution medium. After 2 hours, the acidic medium was drained from the vessels and replaced with 500mL PBS (pH 6.8) and samples were withdrawn for a further 3-5 hours at which time all the formulations had completely dissolved. The collected and filtered samples were diluted and the absorbance measured spectrophotometrically at 265nm and 263nm in acidic and phosphate-buffered media respectively for comparison with the standard calibration curves. All tests were performed in triplicate.

A model-independent approach was used to compare the dissolution data in acidic media (0.1M HCl, pH 1.2) of the different experimentally synthesised enterospheres. For this purpose a mean dissolution time (MDT) was calculated for each formulation, defined as the sum of different

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release fraction periods obtained for dissolution studies endured in 0.1M HCl, divided by the initial loading dose (Pillay and Fassihi, 1998):

$$MDT = \sum_{t=1}^{n} ti \frac{M_t}{M\infty} \qquad \text{[Equation 4.3]}$$

$M_t$ is the fraction of dose released in time $ti = (t_i + t_{i-1})/2$ and $M\infty$ corresponds to the loading dose and a maximum MDT refers to the fastest drug release achievable (Govender et al., 2005).

### 4.2.8. Textural Profile Analysis

Textural profiling of the enterosphere formulations was conducted for elucidation of their resilient properties, matrix deformation energy and matrix hardness. A calibrated Texture Analyser (*TA.XT.plus Texture Analyser*, Stable Microsystems®, Surrey, UK) fitted with a 50kg load cell was employed for determination of the matrix hardness and deformation energy of unhydrated spheres (using a 2mm flat-tipped steel probe) and matrix resilience of unhydrated and acid- and phosphate buffer-hydrated enterospheres (using a 36mm cylindrical steel probe). The fully integrated data acquisition, analysis and display software (Texture Exponent, Version 3.2) was employed to acquire data at 200 points/second. Studies were conducted at ambient conditions (21±0.5 $^{o}$C). Results are expressed as the mean of at least three measurements.

The matrix hardness (N/mm), calculated as the gradient of the force-displacement profile during the compression phase (Figure 4.3(a)) and deformation energy (N.m or J), calculated as the area under the force-displacement curve (AUC) (Figure 4.3(a)); was determined for the unhydrated enterosphere formulations as per the Texture Analyser settings outlined in Table 4.2.

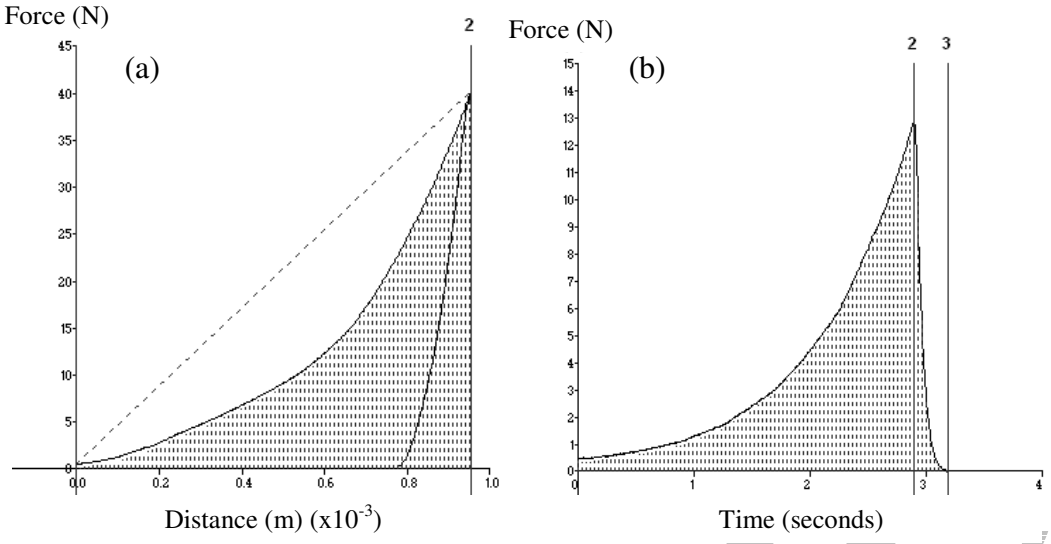


*Figure 4.3: Typical textural profiles: for the measurement of (a) deformation energy (upward gradient) and matrix hardness (AUC) and (b) resilience of enterospheress*

*Table 4.2: Textural parameters for determination of matrix hardness, deformation energy and matrix resilience*

| **Parameter** | **Matrix Hardness and Deformation Energy Settings** | **Matrix Resilience Settings** |
|---|---|---|
| Pre-test speed | 1.00mm/s | 1.00mm/s |
| Test speed | 0.50mm/s | 0.50mm/s |
| Post-test speed | 1.00mm/s | 1.00mm/s |
| Target mode | Force | 50% strain |
| Target force | 40.00N | - |
| Trigger type | Auto (force) | Auto (force) |
| Trigger force | 0.50N | 0.50N |
| Load cell | 50kg | 50kg |

In addition, resilience testing was performed on each of the formulations initially in their unhydrated state, as well as after exposure for 1 hour to 0.1M HCl and PBS (pH 6.8) at 37±0.5°C in accordance with the parameters for resilience testing (Table 4.2). Exposure to medium was accomplished by placing the enterospheres in 50mL PBS in glass reagent bottles of 100mL capacity. The resilience of the matrix was calculated as the ratio of the AUC or work done by the enterosphere on the probe after the maximum decompressive force was reached to the AUC or work done by the probe on the matrix up to the maximum compressive force (Figure 4.3(b)).

### 4.2.9. Optimisation of the Formulation Ingredients

Following generation of the polynomial equations relating the dependent and independent variables, the formulation process was optimised under constrained conditions for the measured responses DEE and MDT. Simultaneous equation solving for optimisation of the formulation process was performed to obtain the levels of independent variables, which would accomplish the desired drug entrapment and enteric-release characteristics (i.e. high DEE corresponding to increased drug loading and low MDT corresponding to slowest drug release achievable in acidic media).

## 4.3. Results and Discussion

### 4.3.1. Surface Morphology of the Enterospheres

The salting-out and cross-linking approach utilised yielded spherical enterosoluble matrices in a single processing step without the use of expensive machinery and organic solvents. Typical micrographs of synthesised formulations depict the variation in the morphology as a result of formulation variables (Figure 4.4). High concentrations of plasticiser with annealing of the enterospheres at high temperatures (>42$^{o}$C) in addition to higher concentrations of the salting-out and cross-linking agent produced enterospheres with a smooth surface and translucent appearance, observed for formulations 1, 2, 6, 8, 12, 14, 18, 19, 24, and 26 due to improved phase separation and coalescence of the polymeric film. However, employing lower concentrations of the salting-out and cross-linking agent resulted in enterospheres with surface precipitates, which were not adequately incorporated within the cross-linked matrix as observed for formulations 5, 7, 13, 17, 24, and 25. Low concentrations of TEC lead to the decreased degrees of polymeric plasticisation as noted in formulations 11, 15, 21, 22, and 25.

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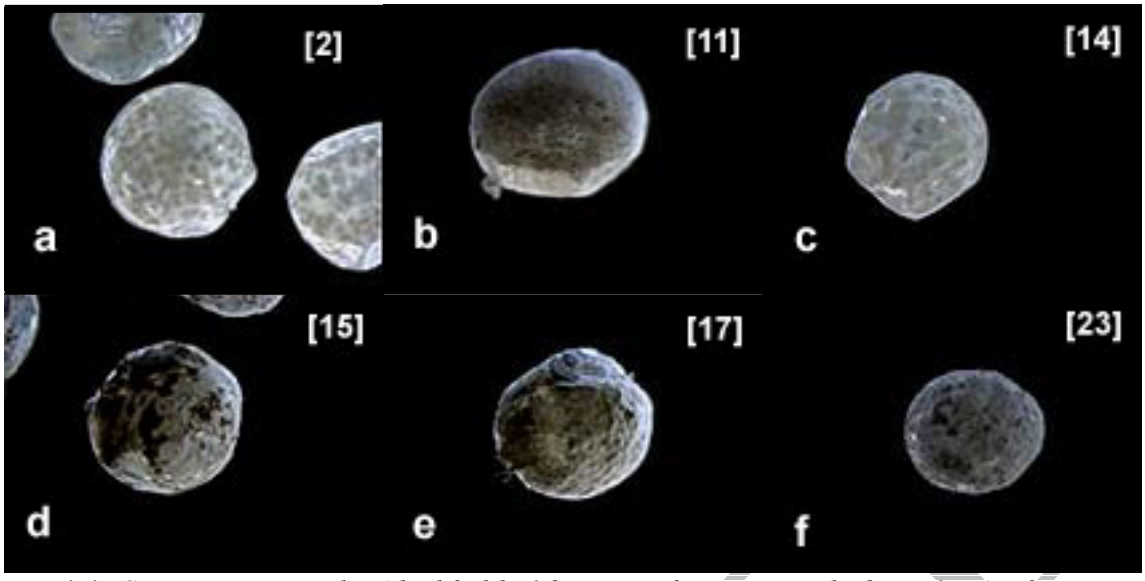


*Figure 4.4: Stereomicrographs (darkfield, 16x magnification, scale bar=1cm) of enterosphere formulations: (a) 2 (high concentration of plasticiser and salting-out agent), (b) 11 (low degree of plasticisation), (c) 14 (high degree of plasticisation), (d) 15 (low degree of plasticisation), 17 (low concentration of salting-out agent), 23 (low degree of plasticisation and no annealing).*

### 4.3.2. Measured Responses for the Experimentally Synthesised Enterospheres

The measured responses for the experimentally synthesised formulations are shown in Tables 4.3 and 4.4. The aspect ratio confirmed enterospheres of near-spherical geometry (AR≈1) with little variation in the particle diameter within each formulation (monodisperse) indicative of good flow properties, and no statistically significant variation in size between formulations ($p>0.05$). Complexometric determination of $Zn^{2+}$ revealed that 23.70 to 287.89 moles of elemental zinc was incorporated per mole of MAEA and theoretically implicated in cross-link formation. Drug content ranged from 4.74 to 13.88mg per 100mg of enterospheres. Entrapment efficiencies of 27.92% to 99.77% were obtained. The ability of the enterospheres to retard drug release in acidic media varied greatly - drug release at $t_{2h}$ ranged from 1.67% to 73.04% (Figure 4.5).

Drug release in the more alkaline PBS media was comparatively rapid for all formulations owing to hydration of the carboxylic acid groups of the methacrylic acid copolymer with the resultant dissolution of the enterosphere matrix. Enterosphere erosion in PBS was also be promoted by removal of $Zn^{2+}$ from the copolymeric matrix due to the sequestration of these cations by phosphate ions within the buffer solution to form insoluble chelates. Similar sequestration by complexing ions would be expected to occur during intestinal transit (Taha et al., 2005).

The physical and mechanical properties of polymers are extensively influenced by the chemical composition of the polymer, such as the degree of cross-linking and the type and quantity of plasticiser employed (Nielson and Landel, 1993). The textural behaviour of the enterosphere formulations (Table 4.4) accentuates the variation in their physicomechanical properties. This variation was not statistically significant ($p > 0.05$), however trends could be identified.

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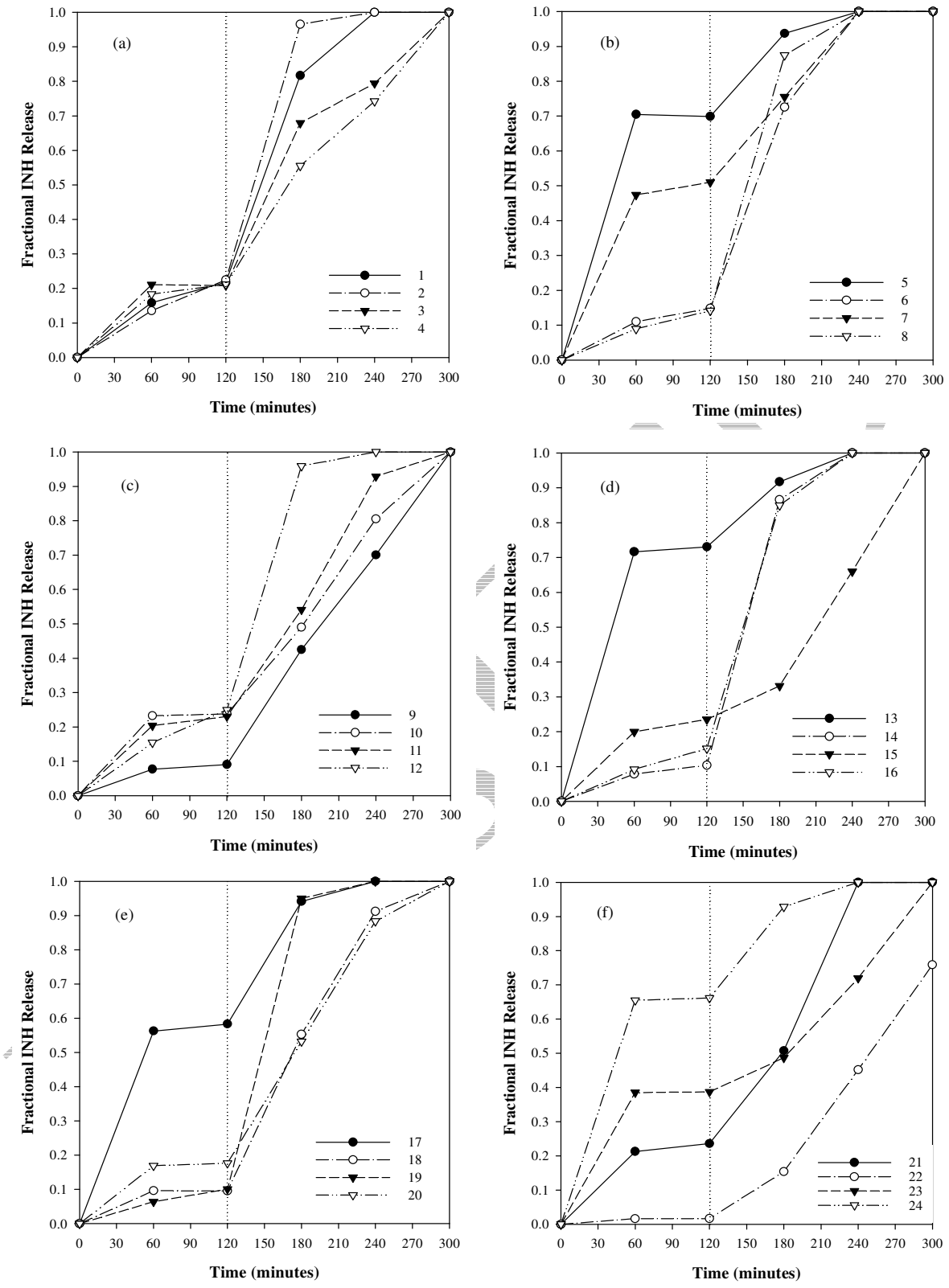


*Figure 4.5: Composite release profiles (a-f) of the enterosphere formulations in acidic (0.1M HCl, pH 1.2) and phosphate buffered media (pH 6.8) (S.D. within ±0.040 in all cases)*

*Table 4.3: Measured responses for the enterosphere formulations*[a]

| Experimental Formulation | $n_{Zn}$ (mol) | Drug Content ($\%^{w}/_{w}$) | DEE (%) | Fractional Drug Release ($t_{2h}$) | MDT (h) | Aspect Ratio |
|---|---|---|---|---|---|---|
| 1 | 89.72 | 9.51 | 92.01 | 0.218 | 0.169 | 1.22 |
| 2 | 71.53 | 9.48 | 56.79 | 0.224 | 0.202 | 1.02 |
| 3 | 136.11 | 7.02 | 50.47 | 0.208 | 0.101 | 1.16 |
| 4 | 154.17 | 7.39 | 46.19 | 0.211 | 0.134 | 1.15 |
| 5 | 145.83 | 6.96 | 40.53 | 0.699 | 0.344 | 1.03 |
| 6 | 28.33 | 13.88 | 88.37 | 0.148 | 0.113 | 1.12 |
| 7 | 115.28 | 5.31 | 30.84 | 0.510 | 0.291 | 1.10 |
| 8 | 64.86 | 9.87 | 67.41 | 0.141 | 0.123 | 1.08 |
| 9 | 140.83 | 6.83 | 45.20 | 0.091 | 0.059 | 1.11 |
| 10 | 178.61 | 6.69 | 47.21 | 0.238 | 0.125 | 1.11 |
| 11 | 203.88 | 6.68 | 42.39 | 0.230 | 0.141 | 1.21 |
| 12 | 23.611 | 12.09 | 80.57 | 0.250 | 0.221 | 1.07 |
| 13 | 181.38 | 7.97 | 52.11 | 0.730 | 0.379 | 1.17 |
| 14 | 75.55 | 7.59 | 47.59 | 0.103 | 0.076 | 1.07 |
| 15 | 205.55 | 5.68 | 35.73 | 0.235 | 0.154 | 1.14 |
| 16 | 47.91 | 9.74 | 67.99 | 0.151 | 0.135 | 0.99 |
| 17 | 130.83 | 7.16 | 39.47 | 0.583 | 0.312 | 1.01 |
| 18 | 143.88 | 6.91 | 43.02 | 0.095 | 0.047 | 1.10 |
| 19 | 72.36 | 10.27 | 62.68 | 0.101 | 0.087 | 1.13 |
| 20 | 154.17 | 7.27 | 46.61 | 0.177 | 0.096 | 1.15 |
| 21 | 229.44 | 4.74 | 27.92 | 0.236 | 0.141 | 1.10 |
| 22 | 287.78 | 7.43 | 54.39 | 0.017 | 0.009 | 1.20 |
| 23 | 203.19 | 6.02 | 39.32 | 0.387 | 0.195 | 1.11 |
| 24 | 147.50 | 7.41 | 44.54 | 0.662 | 0.337 | 1.18 |
| 25 | 155.69 | 6.52 | 39.24 | 0.708 | 0.364 | 1.24 |
| 26 | 36.39 | 11.50 | 99.77 | 0.205 | 0.171 | 1.33 |

*([a]Results are expressed as mean of at least 3 measurements, for Aspect Ratio n=50. S.D.s were within: $n_{Zn}$±2.50, Drug content±0.93, DEE±3.79, Drug release±0.034, MDT±0.012)*

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*Table 4.4: Measured textural properties of the experimentally synthesized variants*[a]

| Experimental Formulation | Mean Dry Resilience (%) | Mean HCl hydrated resilience (%) | Mean PBS hydrated resilience (%) | Deformation Energy (J x$10^2$) | Matrix Hardness (N/mm) |
|---|---|---|---|---|---|
| 1 | 6.80 | 6.19 | 5.42 | 1.20 | 38.10 |
| 2 | 14.69 | 5.40 | 5.03 | 1.75 | 44.11 |
| 3 | 9.43 | 17.67 | 15.52 | 0.40 | 175.62 |
| 4 | 7.91 | 9.14 | 6.25 | 0.40 | 209.86 |
| 5 | 6.29 | 6.02 | 6.33 | 0.97 | 21.16 |
| 6 | 14.46 | 5.75 | 6.95 | 2.15 | 30.83 |
| 7 | 5.97 | 6.40 | 6.10 | 0.67 | 186.88 |
| 8 | 13.24 | 5.75 | 6.51 | 0.73 | 21.73 |
| 9 | 6.40 | 9.03 | 11.33 | 0.45 | 157.47 |
| 10 | 5.25 | 13.58 | 8.84 | 0.40 | 194.14 |
| 11 | 9.19 | 9.55 | 10.30 | 0.70 | 18.91 |
| 12 | 8.95 | 9.71 | 9.64 | 1.35 | 57.48 |
| 13 | 4.38 | 7.74 | 5.95 | 0.80 | 23.36 |
| 14 | 2.36 | 6.01 | 6.73 | 0.50 | 23.89 |
| 15 | 11.41 | 12.25 | 5.10 | 1.05 | 20.90 |
| 16 | 14.19 | 6.54 | 6.67 | 1.30 | 46.11 |
| 17 | 6.94 | 3.78 | 5.12 | 0.53 | 21.47 |
| 18 | 2.93 | 11.60 | 9.12 | 1.63 | 27.02 |
| 19 | 6.88 | 5.10 | 7.04 | 0.87 | 24.22 |
| 20 | 8.77 | 9.14 | 6.25 | 0.40 | 180.48 |
| 21 | 10.27 | 5.26 | 3.75 | 0.63 | 169.27 |
| 22 | 4.62 | 14.94 | 6.44 | 0.40 | 202.14 |
| 23 | 11.96 | 17.49 | 10.72 | 0.40 | 201.31 |
| 24 | 1.05 | 15.61 | 8.85 | 1.13 | 31.33 |
| 25 | 6.77 | 11.15 | 8.10 | 0.55 | 26.63 |
| 26 | 5.79 | 6.03 | 6.47 | 1.30 | 27.73 |

*([a]Results are expressed as the mean of at least 3 measurements, S.D.s obtained were within: Resilience±0.16, Deformation Energy±4.18x$10^{-4}$, Matrix Hardness±4.37)*

Resilience is defined as the ability of a strained body to recover its size and shape after deformation caused especially by compressive stress, a concept derived from the Huber-Hencky Theory of Strength (Pillay and Fassihi, 1999). The resilience of formulations 3, 4, 9, 10, 11, 12, 13, 14, 15, 18, 20, 22, 24, 24, 26 improved by 0.36 to 14.56% with hydration suggestive of enhanced control over drug release, whereas the resilience of the other formulations was reduced (0.27 to 5.01%) following exposure to dissolution media (Figure 4.6).

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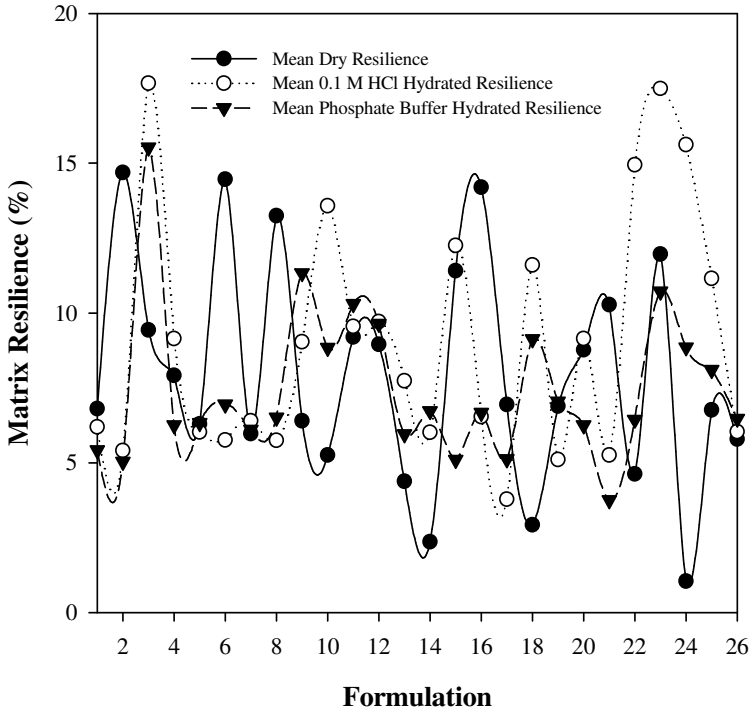


*Figure 4.6: Variable resilience of enterosphere formulations in the dry and hydrated state*

More important was the relationship between the acid-hydrated resilience and drug release in acidic media (Figure 4.7). In general, as the resilience on hydration in acidic media increased (≥6%), the extent of control over drug release increased (<30% drug release at $t_{2h}$). The increase in hydration may serve to reinforce the matrix of these variants. However, more extensive hydration resulted in the formation of a loose gel matrix in certain formulations and a lower resilience. This trend was only observed for 61.54% of the formulations and the acid-hydrated resilience of the enterospheres could not always serve as a significant predictor of drug release.

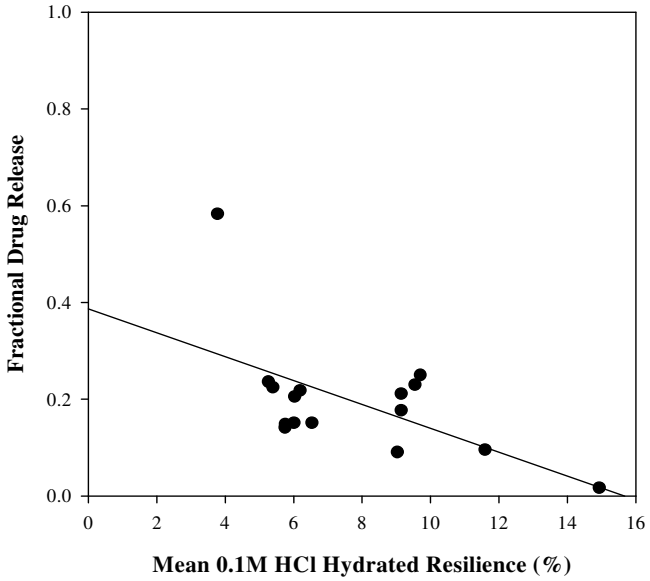


*Figure 4.7: Relationship between fractional drug release and acid-hydrated resilience*

The matrix hardness of the enterospheres was generally greater when intermediate to low concentrations of plasticiser were incorporated, due to less softening of the polymeric matrix; whereas the energy required to rupture the enterosphere matrices was greater in formulations incorporating high plasticiser concentrations, as an increased degree of plasticisation decreased brittleness and improved the flexibility and distensibility of the copolymeric chains which led to the dissipation of larger amounts of energy when exposed to shear forces (Nielson and Landel, 1993; McGinity, 1996). An increased degree of cross-linking would also be expected to improve the mechanical hardness of the enterosphere matrix (Swarbrick, 2004). Elongation or compression experiments are commonly exploited to provide an indication of the degree of cross-linking in the enterosphere formulations (Shalaby et al., 1993). In formulations that potentially exhibited a high cross-link density due to a high $n_{Zn}$, the chain segments between the cross-links were short and anchored by many points, causing a loss in flexibility and an increase in matrix rigidity. This correlational behaviour between $n_{Zn}$ (as a measure of the cross-link density) and the

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matrix hardness was demonstrated in the enterosphere formulations (Figure 4.8) by a dramatic increase in matrix hardness at high levels of $Zn^{2+}$ (>150mol).

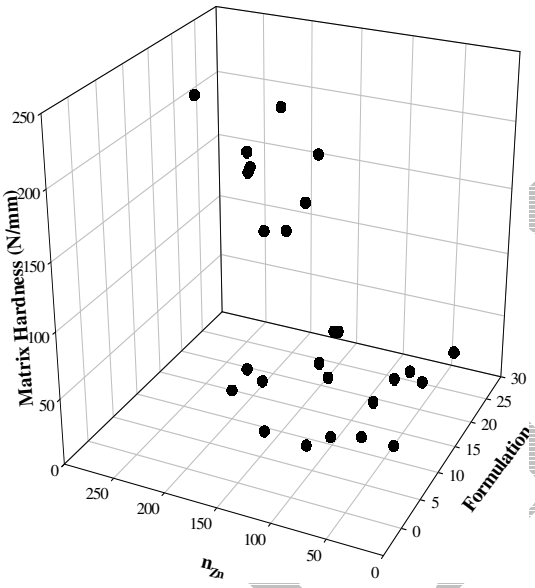


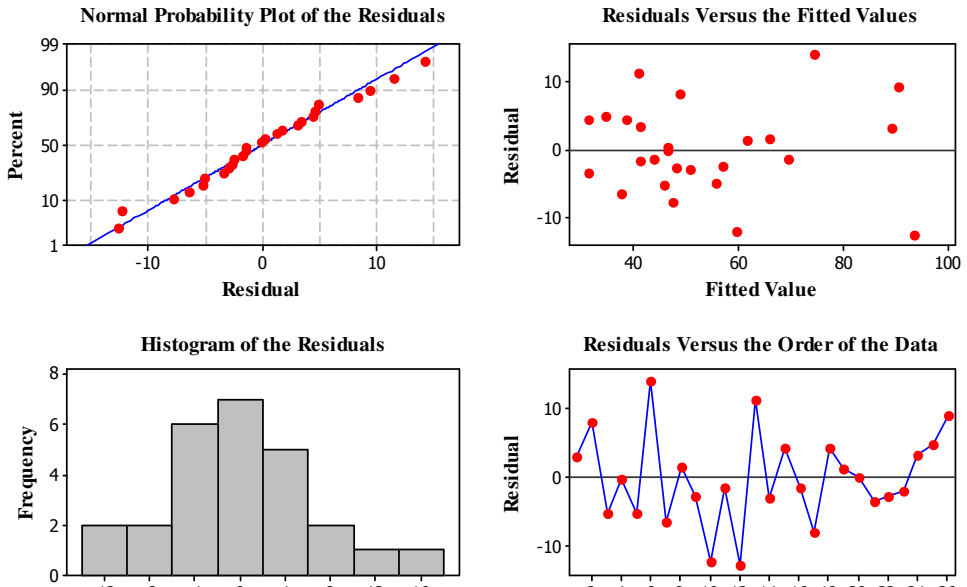

*Figure 4.8: 3-D scatter plot of matrix hardness vs. molar amount of Zn ($n_{Zn}$) vs. formulation*

### 4.3.3. Analysis of the Box-Behnken Response Surface Design

The $n_{Zn}$, DEE and MDT for the experimentally synthesised formulations were included in the statistical design for identification of a formulation with an optimal drug entrapment and dissolution profile in acidic media.

Residual analysis (run order, predicted values) for the $n_{Zn}$, DEE and MDT data (Figure 4.9) generally showed random scatter i.e. no trends, indicating none of the underlying assumptions of the multiple regression analysis were grossly violated; however some fanning and an outlier was observed for $n_{Zn}$ (Figure 4.8(a) indicative of a degree of nonconstant variance. The normal probability plots of the residuals fell on a straight line indicating the data to be normally distributed with no evidence of unidentified variables.

(a)




(b)

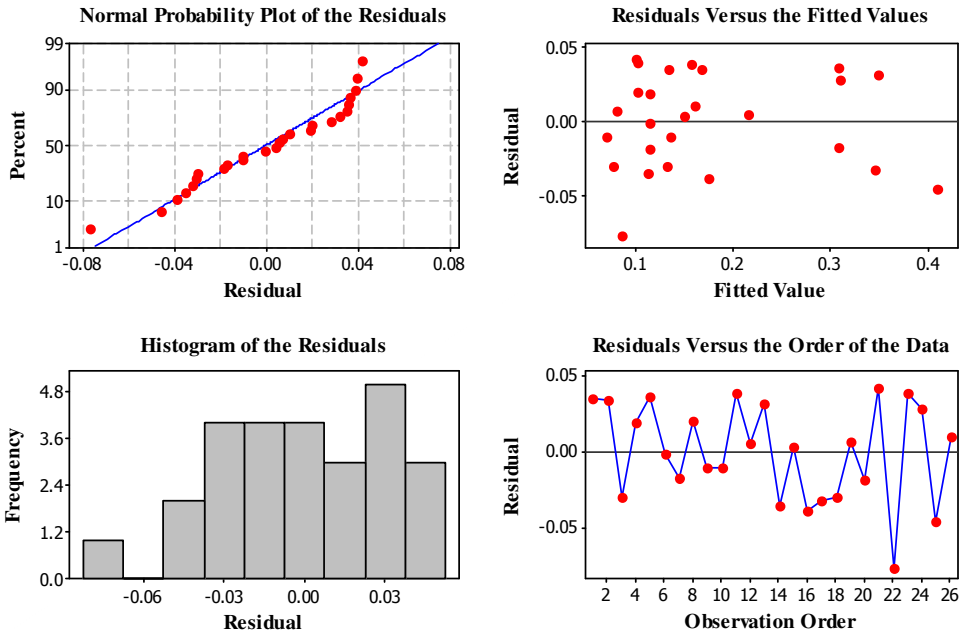



*Figure 4.9: Residual plots: (a) DEE and (b) MDT*

The residuals and standardised residuals indicated that the majority of cases were adequately fitted by the response surface model. Cook's distance was interpreted an overall measure of the combined impact of each observation on the fitted values and considers whether an observation is unusual with respect to both x- and y-values. Unusual observations generated by the model were minimal. The significance of the ratio of mean square variation due to regression and residual error was tested using ANOVA. The theoretical (predicted) values and observed (experimental) values were in close agreement as seen from Table 4.5 for $n_{Zn}$ ($R^2$=93.42%), DEE ($R^2$=93.73%) and MDT ($R^2$=95.12%) respectively, thus indicating the applicability of the regression models and usefulness of response surface plots.

The Pearson correlation coefficient (R and R-adjusted) represents the proportion of variation in the response that is explained by the model. The $R^2$ (87.3%, 87.9%, 90.5%) and $R^2$-adjusted values (71.1%, 72.4%, 78.3%) for the $n_{Zn}$, DEE and MDT models were satisfactory.

The significance of linear and higher-order interaction terms is depicted by the p-values in Table 4.6. The salting-out and cross-linking agent significantly affected $n_{Zn}$ (p=0.034) and the DEE (p=0.000), as did the plasticiser concentration employed (p=0.000 and p=0.002, respectively). High drying temperatures (≥42.5$^{o}$C) also significantly improved DEE (p=0.029). $ZnSO_4$ had a significant effect on the MDT (p=0.000). A significant interaction effect was observed between $ZnSO_4$ and TEC variables on $n_{Zn}$ (p=0.005) and on drug release in acidic media (p=0.035).

*Table 4.5: Correlation between experimental and predicted values for $n_{Zn}$, DEE, and MDT*

| Formulation | $n_{Zn}$ (mol) | | | DEE (%) | | | MDT (h) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Experimental | Predicted | Cook's Distance | Experimental | Predicted | Cook's Distance | Experimental | Predicted | Cook's Distance |
| 1 | 89.72 | 95.97 | 0.007 | 92.01 | 88.98 | 0.021 | 0.169 | 0.134 | 0.119 |
| 2 | 71.53 | 75.01 | 0.002 | 56.79 | 48.53 | 0.156 | 0.202 | 0.167 | 0.114 |
| 3 | 136.11 | 114.00 | 0.085 | 50.47 | 55.58 | 0.060 | 0.101 | 0.132 | 0.089 |
| 4 | 154.17 | 154.17 | 0.000 | 46.19 | 46.40 | 0.000 | 0.134 | 0.115 | 0.020 |
| 5 | 145.83 | 145.85 | 0.000 | 40.53 | 45.76 | 0.062 | 0.344 | 0.308 | 0.125 |
| 6 | 28.33 | 53.79 | 0.112 | 88.37 | 74.28 | 0.453 | 0.113 | 0.114 | 0.000 |
| 7 | 115.28 | 126.06 | 0.020 | 30.84 | 37.34 | 0.097 | 0.291 | 0.308 | 0.028 |
| 8 | 64.86 | 74.55 | 0.016 | 67.41 | 65.73 | 0.006 | 0.123 | 0.103 | 0.038 |
| 9 | 140.83 | 119.53 | 0.078 | 45.20 | 47.89 | 0.017 | 0.059 | 0.069 | 0.010 |
| 10 | 178.61 | 137.98 | 0.286 | 47.21 | 59.49 | 0.344 | 0.125 | 0.135 | 0.010 |
| 11 | 203.89 | 201.38 | 0.001 | 42.39 | 43.84 | 0.005 | 0.141 | 0.102 | 0.147 |
| 12 | 23.61 | 31.60 | 0.527 | 80.57 | 93.22 | 0.365 | 0.221 | 0.216 | 0.002 |
| 13 | 181.39 | 161.33 | 0.070 | 52.11 | 40.73 | 0.295 | 0.379 | 0.348 | 0.094 |
| 14 | 75.56 | 77.38 | 0.001 | 47.59 | 50.61 | 0.021 | 0.077 | 0.112 | 0.121 |
| 15 | 205.56 | 225.01 | 0.065 | 35.73 | 31.36 | 0.044 | 0.154 | 0.150 | 0.001 |
| 16 | 47.92 | 72.36 | 0.103 | 68.00 | 69.44 | 0.005 | 0.135 | 0.174 | 0.145 |
| 17 | 130.83 | 145.06 | 0.035 | 39.47 | 47.33 | 0.141 | 0.312 | 0.344 | 0.101 |
| 18 | 143.89 | 141.11 | 0.001 | 43.02 | 38.54 | 0.046 | 0.047 | 0.076 | 0.090 |
| 19 | 72.36 | 115.36 | 0.320 | 62.68 | 61.43 | 0.004 | 0.087 | 0.080 | 0.004 |
| 20 | 154.17 | 154.17 | 0.000 | 46.61 | 46.40 | 0.000 | 0.096 | 0.115 | 0.020 |
| 21 | 229.44 | 225.48 | 0.003 | 27.92 | 31.30 | 0.026 | 0.141 | 0.100 | 0.166 |
| 22 | 287.78 | 244.35 | 0.326 | 54.40 | 56.97 | 0.015 | 0.009 | 0.085 | 0.566 |
| 23 | 203.19 | 221.85 | 0.060 | 39.32 | 41.12 | 0.007 | 0.196 | 0.157 | 0.140 |
| 24 | 147.50 | 130.73 | 0.049 | 44.54 | 41.17 | 0.026 | 0.337 | 0.309 | 0.075 |
| 25 | 155.69 | 167.49 | 0.024 | 39.24 | 34.40 | 0.054 | 0.364 | 0.409 | 0.200 |
| 26 | 36.39 | 76.10 | 0.273 | 99.77 | 90.51 | 0.196 | 0.171 | 0.161 | 0.010 |

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*Table 4.6: Estimated p-values for the measured responses*

| Term | p-value | | |
|---|---|---|---|
| | $n_{Zn}$ | DEE | MDT |
| $ZnSO_4$ | 0.034 | 0.000 | 0.000 |
| CRT | 0.955 | 0.271 | 0.925 |
| DT | 0.839 | 0.029 | 0.055 |
| TEC | 0.000 | 0.002 | 0.613 |
| $ZnSO_4$*$ZnSO_4$ | 0.167 | 0.044 | 0.000 |
| CRT*CRT | 0.312 | 0.583 | 0.766 |
| DT*DT | 0.661 | 0.790 | 0.848 |
| TEC*TEC | 0.871 | 0.931 | 0.297 |
| $ZnSO_4$*CRT | 0.586 | 0.122 | 0.502 |
| $ZnSO_4$*DT | 0.353 | 0.164 | 0.668 |
| $ZnSO_4$*TEC | 0.005 | 0.235 | 0.035 |
| CRT*DT | 0.540 | 0.789 | 0.908 |
| CRT*TEC | 0.546 | 0.080 | 0.314 |
| DT*TEC | 0.985 | 0.658 | 0.965 |

The complete regression equations generated for $n_{Zn}$, DEE and MDT are indicated below:

$$n_{Zn} = 22.738+4.375[ZnSO_4]+ 4.032[CRT]+1.839[DT]+ 7.551[TEC]-0.0638[ZnSO_4*ZnSO_4]-0.036[CRT*CRT]-0.025[DT*DT]+0.179[TEC*TEC]+0.022[ZnSO_4*CRT]+0.050[ZnSO_4*DT]-0.795[ZnSO_4*TEC]-0.029[CRT*DT]-0.125[CRT*TEC] 0.005[DT*TEC]$$

[Equation 4.4]

$$DEE = 83.487-1.002[ZnSO_4]-1.588[CRT]-0.073[DT]-2.289[TEC]+0.027[ZnSO_4*ZnSO_4]+0.005[CRT*CRT]+0.004[DT*DT]+0.026[TEC*TEC]+0.018[ZnSO_4*CRT]-0.021[ZnSO_4*DT]+0.078[ZnSO_4*TEC]+0.003[CRT*DT]+0.106[CRT*TEC]-0.032[DT*TEC]$$

[Equation 4.5]

$$MDT = 0.657-0.027[ZnSO_4]+1.873E-03[CRT]+8.209E-04[DT]-0.028[TEC]+2.856E-04[ZnSO_4* ZnSO_4]-1.391E-05[CRT*CRT]-1.479E-05[DT*DT]+1.583E-03[TEC*TEC]+3.722E-05[ZnSO_4*CRT]-3.036E-05[ZnSO_4*DT]+7.256E-04 [ZnSO_4*TEC]-7.238E-06[CRT*DT]-2.831E-04[CRT*TEC]-1.536E-05[DT*TEC]$$

[Equation 4.6]

### 4.3.4. Response Surface Analysis

Main effects, interaction and response surface plots were obtained for the measured responses ($n_{Zn}$, DEE, MDT) based on the experimental model. The relationship between the independent variables and the responses can be further explained through graphical illustration of the effect of the independent variables and their interactions. The interaction effects are estimated by subtracting the mean positive response values from the mean negative response values. The estimated interaction effects of the responses studied are shown in Figure 4.10. The main effects plots (Figure 4.11) are used in conjunction with the ANOVA for the determination of the strength or relative significance of the effects across factors. The surface plots generated (Figure 4.12) represent the functional relationship between the response and the experimental factors.

#### 4.3.4.1. Response Surface Analysis for Molar Amount of Zinc

Increasing the degree of plasticisation (high TEC levels) had a significant negative effect on the molar amount of $Zn^{2+}$ incorporated within the cross-linked matrix (p=0.000). The increase in polymer chain mobility afforded by the addition of a plasticiser is necessary for adequate coalescence of the polymeric film, however, high degrees of plasticisation may negate the favourable chain alignment required for incorporation of $Zn^{2+}$ within the ionic cross-link between the copolymer's carboxylic acid side chains. This is as a result of the plasticiser's ability to weaken copolymeric intermolecular attractions and to increase the copolymer's free volume. An increase in $ZnSO_4$ in the salting-out and cross-linking solution ($\geq 30\%^{w}/_{v}$) also had a significant negative effect on the amount of $Zn^{2+}$ incorporated (p=0.034). As the aqueous dispersion was extruded into the electrolyte solution, the droplet surface immediately encountered high concentrations of $Zn^{2+}$ inducing film formation. This may hinder more significant diffusional penetration of the cation into the internal matrix for participation in ionic cross-linking. The interaction between these two variables also proved to have significant opposing effects on the

molar amount of $Zn^{2+}$ implicated in cross-link formation within the internal matrix (p=0.005). $n_{Zn}$ was maximal at either high TEC levels and low $ZnSO_4$ levels and vice versa as depicted in Figure 4.10(a).

The effect of factors $ZnSO_4$ and DT at the midpoint of factors TEC and CRT on $n_{Zn}$ is shown in Figure 4.12(a). As the concentration of salting-out anions in the electrolyte solution increased, there was an observed decrease in $n_{Zn}$. This was purportedly due to the rapidly coalesced polymer film hindering a more notable penetration of the $Zn^{2+}$ into the internal matrix.

The effect of factors $ZnSO_4$ and TEC at the midpoint of factors CRT and DT on the response $n_{Zn}$ is shown in Figure 4.12(b). At low levels of $ZnSO_4$, an increase in TEC caused an increase in $n_{Zn}$, however, at high levels of $ZnSO_4$ there was a significant decrease in $n_{Zn}$ as TEC increased from 2 to 10%$^{w}/_{w}$. The optimal polymer chain alignment for ionic cross-linking with $Zn^{2+}$ thus occured at opposing levels of $ZnSO_4$ and TEC.

It must be noted for future discussion that although the cross-linking process has proved to be of significance in improving the gastroresistance of MAEA, a high degree of cross-linking does not necessarily predict greater control over drug release. This behaviour is a result of the impregnation process. The drug may better penetrate a polymeric matrix with a lower degree of cross-linking than a highly cross-linked three-dimensional matrix during the entrapment process. The drug can diffuse deeper within the enterosphere and is less likely to diffuse out on exposure to dissolution media.

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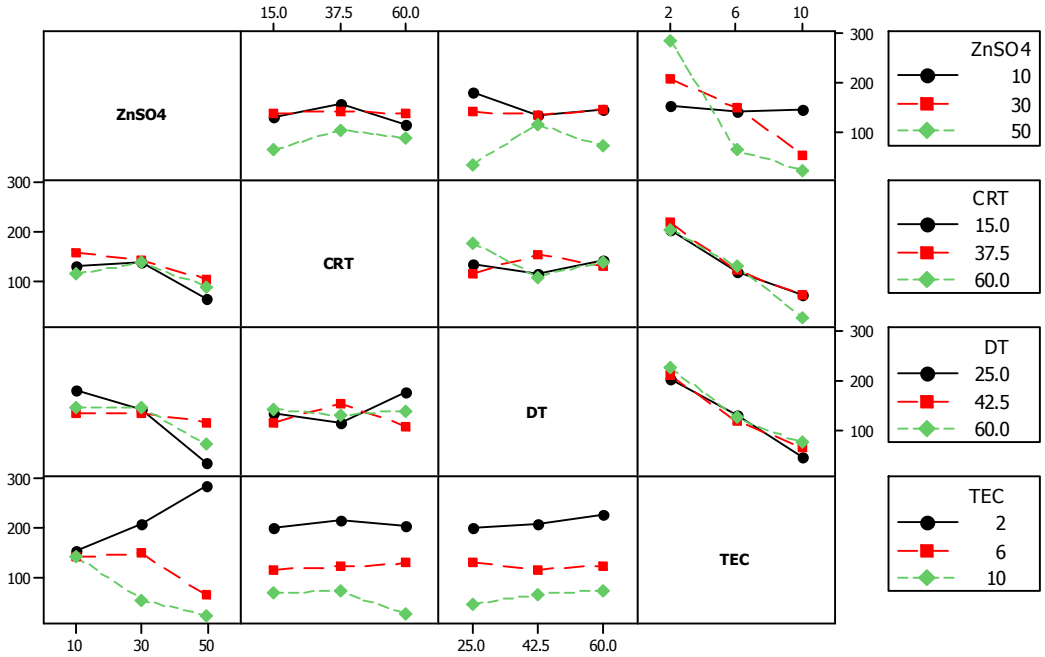
(a)

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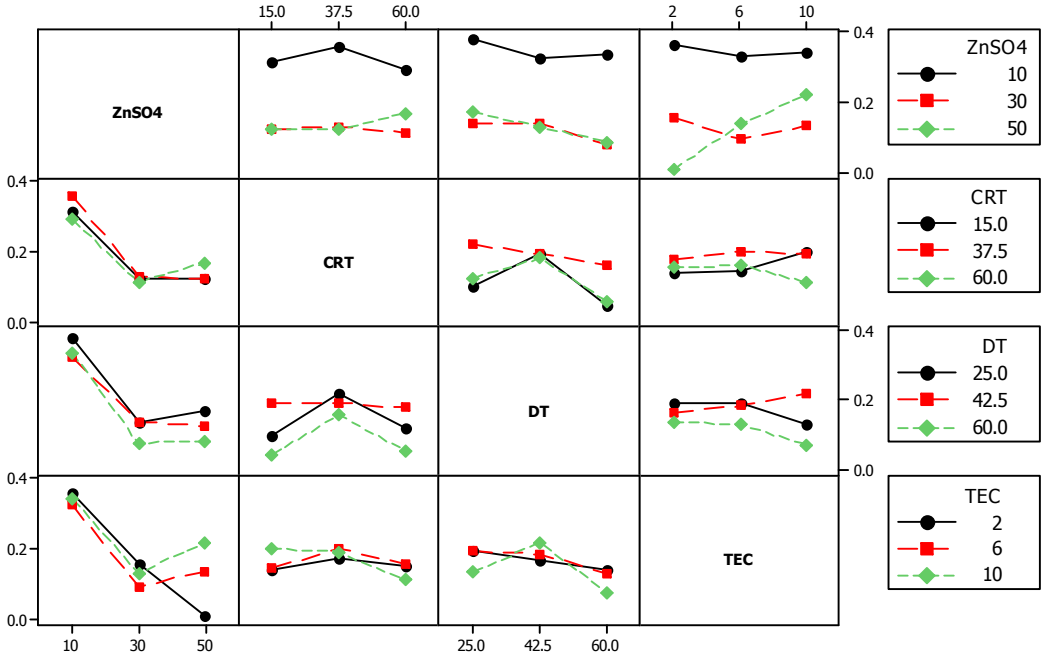


*Figure 4.10: Interaction plots: (a) $n_{Zn}$, and (b) MDT*

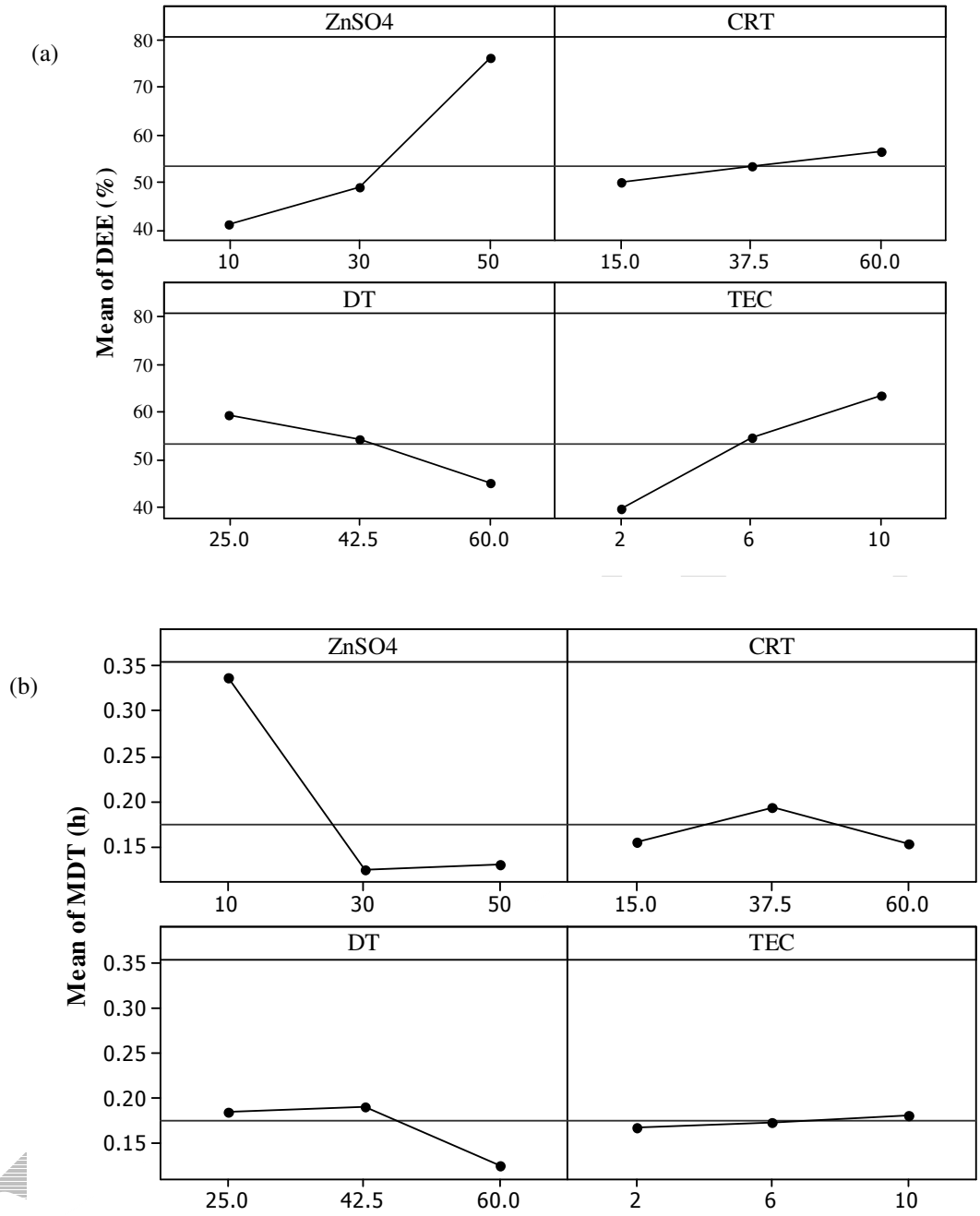


*Figure 4.11: Main effects plots: (a) DEE and (b) MDT*

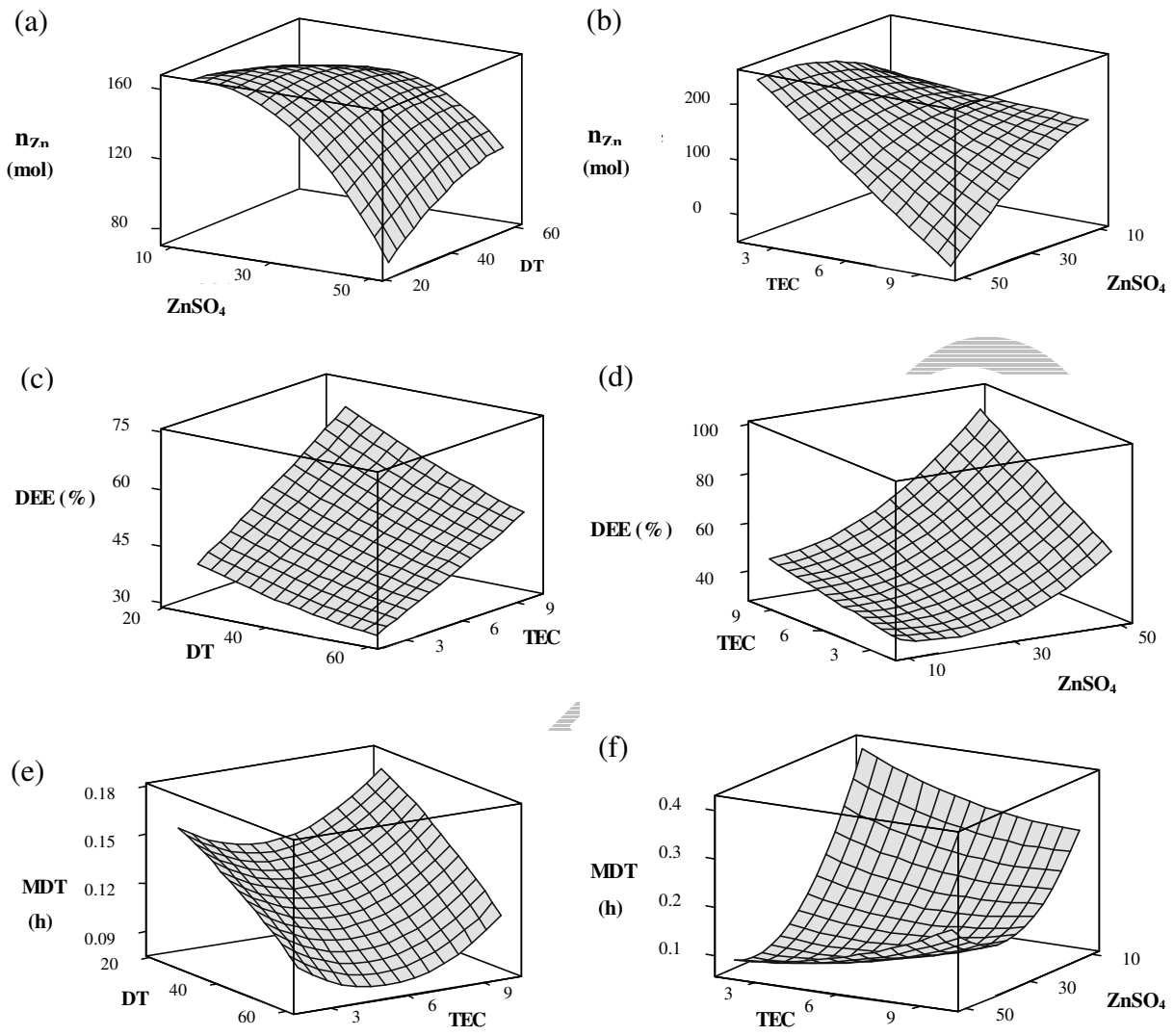


*Figure 4.12: Response surface plots: $n_{Zn}$ [(a) and (b)], DEE [(c) and (d)], and MDT [(e) and (f)]*

### 4.3.4.2. Response Surface Analysis for Drug Entrapment Efficiency

Inspection of the main effects plots generated (Figures 4.11(a)) illustrates the significance of the effect of the concentration of the salting-out and cross-linking agent and the plasticiser on the effective amount of drug entrapped within the enterosphere matrices. Factors promoting cross-link formation and polymer coalescence facilitate the formation of a dense matrix, which retards drug release to a greater extent (Guo, 1993; Saettone et al., 1995; Sriamornsak, 1999). The increased availability of the $Zn^{2+}$ in the salting-out and cross-linking solution at higher concentrations promoted gel shrinkage and the formation of intra- and intermolecular ionic cross-

links within and between the copolymer chains, producing a dense, interconnected enteric film in which drug entrapment is more likely and which retained its integrity in acidic dissolution media, slowing the release of INH through the reduced interstices of the enterosphere. The drug entrapment was increased with an increase in TEC. Because matrix formation is considered to be dependent on the concentration of incorporated plasticiser, the coalescence of the polymer particles is likely to be enhanced by increasing TEC, with improved entrapment of INH. High DT values however, had a noted negative effect on the DEE ($p=0.029$). Annealing at temperatures $\geq 42.5^{o}C$ used in this study resulted in an increased drug leaching to the enteric film surface with resultant surface loss.

The effect of factors DT and TEC at the midpoint of factors $ZnSO_4$ and CRT on response DEE is shown in Figure 4.12(c). At low levels of factor TEC, the DEE was low and increasing factor DT further lowered the DEE. At high levels of TEC, the DEE improved; however, an increase in the DT factor still resulted in a reduction in the DEE due to leaching of the drug out of the highly plasticised, pliable structure when exposed to temperatures $\geq 42.5^{o}C$. The DEE was maximal at a low DT level and at high TEC levels.

The effect of factors $ZnSO_4$ and TEC at the midpoint of factors CRT and DT on the response DEE is shown in Figure 4.12(d). At low levels of factor TEC, an increase in $ZnSO_4$ caused an increase in the DEE. High levels of TEC resulted in a significant improvement in the DEE as factor $ZnSO_4$ increased from 10 to $50\%^{w}/_{v}$. The increase in polymeric chain mobility afforded by increased degrees of plasticisation could aid in rapidly orientating the polymeric chains for formation of a coalesced matrix, which facilitated INH entrapment within its network structure.

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#### 4.3.4.3. Response Surface Analysis for Mean Dissolution Time

A significant interaction effect was observed between $ZnSO_4$ and TEC variables (p=0.035), and drug release in acidic media was minimal when a high concentration of $ZnSO_4$, in combination with a low concentration of TEC was employed in the formulation of enterospheres. Increasing the concentration of $ZnSO_4$ from 10 to 30%$^{w}/_{v}$ had a significant negative effect on the MDT (p=0.000).

An increase in the availability of $Zn^{2+}$ in the salting-out and cross-linking solution promoted the formation of a cross-linked spherical enteric film of improved gastroresistance. Cross-linking of the internal matrix was also promoted, except at very high concentrations of the salting-out and cross-linking solution (>30%$^{w}/_{v}$). The formation of cross-links between copolymer chains ultimately formed a dense, interconnected polymeric film and internal matrix which retained its integrity in acidic dissolution media, slowing the diffusion of INH through the reduced interstices of the enterosphere structure (as depicted in Figure 4.13(a), (b), and (c)). The phenomenon of enhanced salting-out and cross-linking of the enteric copolymer in the presence of a higher concentration of salting-out anion and cross-linking cation is described by the Schulze-Hardy rule, which governs the ability of an electrolyte to reduce the value of the zeta-potential of the polymer.

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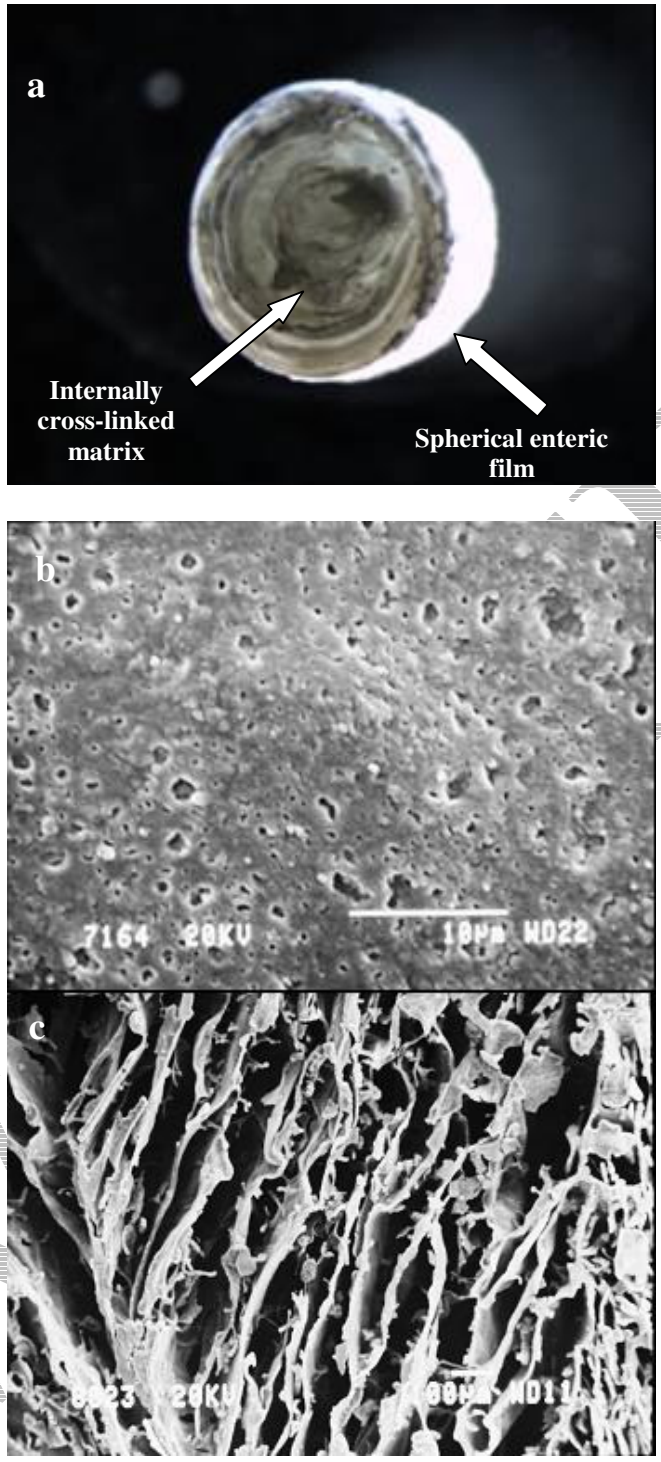



*Figure 4.13: Stereomicrographs and corresponding scanning electron micrographs of enterosphere formulation 22: (a) cross-section of enterosphere at 16X, (b) patent spherical enteric film at 3000X, (c) cross-linked internal matrix at 100X magnification*

An increase in the drying temperature from 42.5 to 60°C caused an overall reduction in the amount of drug released in acidic media. Sufficient annealing of the enterosphere (at temperatures ≥42.5°C) softened the copolymer causing it to fill the interstices and resulting in the observed morphological changes. The reduced porosity and enhanced coalescence of enterospheres dried at temperatures ≥42.5°C resulted in prolongation of the total release time and a decrease in the MDT. The drying temperature employed may thus be related to the $T_g$ and MFT of the copolymer-plasticiser systems constituting the enterospheres. Coalescence of particles within the enterosphere matrix was improved when the drying temperature was set above the MFT of the copolymer-plasticiser system. The MDT was minimal at high levels of both $ZnSO_4$ and DT.

Plasticisers are added to film-forming polymers to modify physical properties of the polymers and to improve their film-forming characteristics as well as their permeability, hence controlling the drug release (Guo, 1993; Saettone, 1995). Though the plasticisers included in the copolymeric dispersion served to decrease the MFT and $T_g$, they also increased the free volume in the enterosphere matrix, which in turn facilitated the release of drug from the enterosphere (Sinko and Amidon, 1989; Sastry et al., 1998). A significant interaction effect was observed between $ZnSO_4$ and TEC variables (p=0.035), and a low MDT was observed when a high concentration of $ZnSO_4$, in combination with a low concentration of TEC, was employed in the formulation of the enterospheres.

The effect of factors $ZnSO_4$ and TEC and their interaction at the midpoint of factors DT and CRT on response MDT is shown in Figure 4.12(e). At low levels of TEC, an increase in $ZnSO_4$ from 10 to 50%$^{w}/_{v}$ resulted in a significant decrease in MDT. Although high levels of plasticiser

improved copolymer coalescence, the free volume of the enterosphere was increased, with a resultant increase in drug release as discussed above.

The effect of factors DT and TEC at the midpoint of factors $ZnSO_4$ and CRT on response MDT is shown in Figure 4.12(f). At high and low levels of TEC, an increase in DT significantly reduced MDT. Increasing the TEC concentration employed in the formulation of the enterospheres did not significantly slow the rate at which drug diffused out of the enterosphere matrix, even when elevated drying temperatures ($\geq 42.5^{o}C$) were employed.

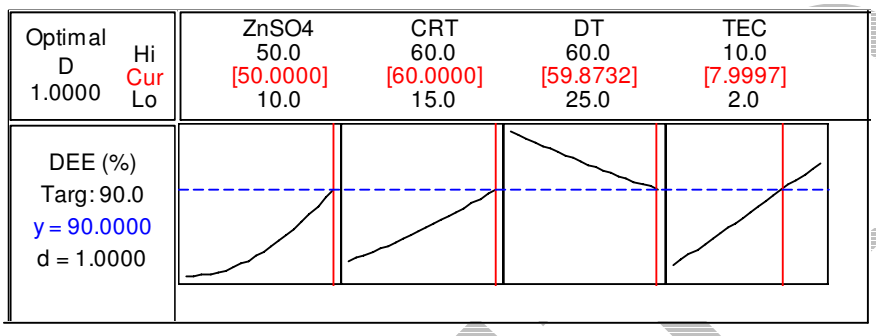
### 4.3.5. Response Optimisation

Response optimisation procedure (MINITAB®, V14, Minitab, USA) was used to obtain the optimised levels of $ZnSO_4$, CRT, DT and TEC. Three optimal formulations were developed following constrained optimisation of DEE, constrained optimisation of MDT and simultaneous constrained optimisation of DEE and MDT (F1, F2, and F3). An MDT value representing sufficiently controlled release in acidic media such that ≤3% and ≤6% of the entrapped drug would be released after the first and second hour respectively was targeted (MDT≤0.07). This was in order to ensure drug release in accordance with the USP 24 specifications for drug release from enteric-release articles (<5% at $t_{1h}$ and <10% at $t_{2h}$). The optimised levels of the independent variables that would achieve the desired dissolution and entrapment properties and their predicted responses were then determined. The optimised levels of the independent variables, the goal for the response, the predicted response, y, at the current factor settings, as well as the individual and composite desirability scores are shown in Figure 4.14. Based on the statistical desirability function, it was found that the composite desirabilities for each of the formulations was > 0.9. The constrained settings utilised are outlined in Table 4.7.

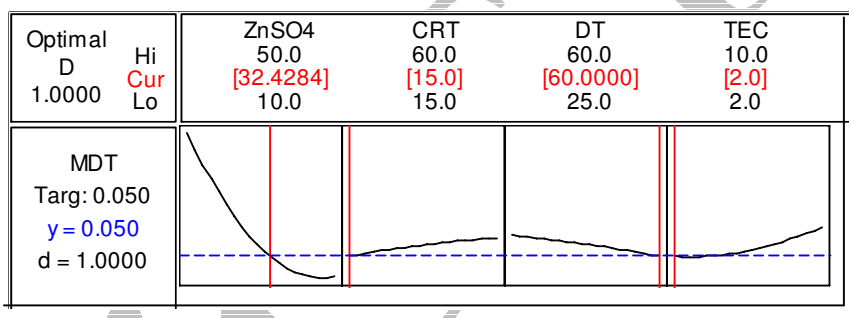
*Table 4.7: Constrained settings for response optimisation*

| Parameters | Constraint |
|---|---|
| $ZnSO_4$ ($\%^w/_v$) | 10-50 |
| CRT (minutes) | 15-60 |
| DT ($^oC$) | 25-60 |
| TEC ($\%^w/_w$) | 2-10 |
| DEE (%) | 80-90 |
| MDT (h) | 0.05-0.07 |

(a)




(b)




(c)

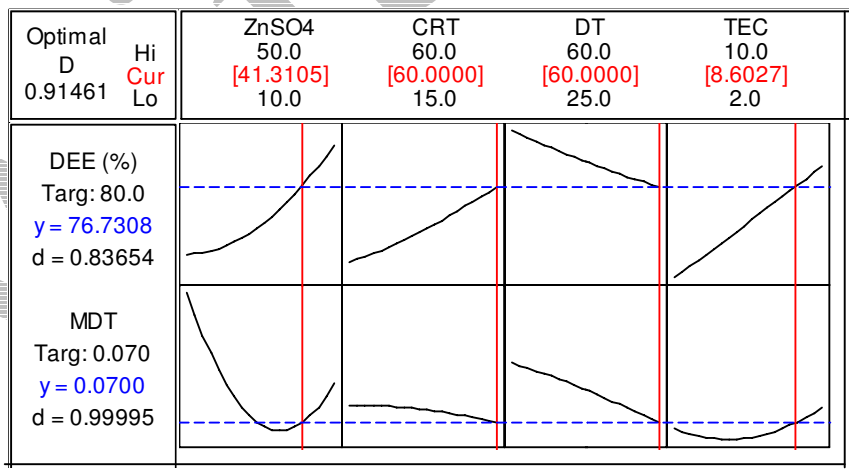



*Figure 4.14: Optimisation plots delineating factor settings and desirability values (d) for optimal formulations: (a) F1, (b) F2, and (c) F3*

The ideal formulations were prepared according to the optimal predicted settings. The experimentally derived values for the DEE and MDT of the optimal formulations were in close

agreement with the predicted values (Table 4.8), demonstrating the reliability of the optimisation procedure in predicting the dissolution behaviour of the novel salted-out and cross-linked enterosoluble systems and ascertaining the significance of the effect of $ZnSO_4$, DT and TEC on INH entrapment and release from the enterospheres.

*Table 4.8: Experimental and predicted response values for the optimised formulations*

| Measured Response | Formulation | Predicted | Experimental | Desirability |
|---|---|---|---|---|
| **DEE (%)** | F1 | 90.000 | 91.066 | 1.000 |
| | F2 | [a] | 47.414 | [a] |
| | F3 | 76.731 | 72.515 | 0.837 |
| **MDT (h)** | F1 | [b] | 0.107 | [b] |
| | F2 | 0.050 | 0.066 | 1.000 |
| | F3 | 0.070 | 0.069 | 1.000 |

[a] *Formulation optimised for DEE only*
[b] *Formulation optimised for MDT only*

Simultaneous optimisation of DEE and MDT resulted in the fabrication of an optimum gastroresistant system (F3) with adequate drug entrapment. The dissolution profile of the optimum enterosphere formulation is depicted in Figure 4.15. Drug release was sufficiently retarded in accordance with the USP 24 specifications for enteric-release articles and this formulation was employed in future investigations (USP 24, 2000). However, it needs to be asertained whether significant segregation of RIF and INH is attained (i.e. adequate RIF release in acidic media simulating the gastric environment) upon co-administration, with INH delivered as enterospheres.

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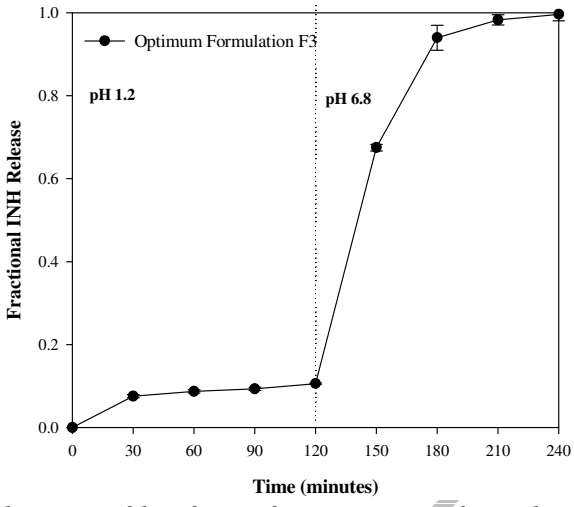


*Figure 4.15: Composite release profile of INH from optimum formulation (F3)*

Inclusion of a cross-linking cation in the enterosoluble delivery system must not be without toxicity considerations. Significantly, it must be noted that the elemental zinc content of a quantity of the optimum enterosphere formulation equivalent to a once daily dose of INH (300mg) was 34.69±0.075mg, which is at least 5 times lower than the levels which precipitate low copper status, altered iron function and reduced immune function (150-450mg/day) and at least 50 times lower than the elemental level precipitating zinc toxicity in an adult, evidenced by nausea, vomiting and fever (Hooper et al., 1980; Institute of Medicine, 2001).

## 4.4. Concluding Remarks

In this chapter, the Box-Behnken Response Surface Design found application in the development and optimisation of a novel approach for the fabrication of ionotropically salted-out and cross-linked enterospheres for delivery of INH to the small intestine. The design generated a range of spherical formulations, which varied in their resilient nature, matrix hardness, deformation energy, and drug entrapment and release characteristics. The use of RSM proved to be a compelling option for the identification of critical and significant formulation variables and

processing variables such as $ZnSO_4$, TEC and DT. The salting-out and cross-linking agent and plasticiser significantly affected $n_{Zn}$ and the DEE. The temperature at which the enterospheres were annealed also significantly affected the DEE. $ZnSO_4$ and the interaction between $ZnSO_4$ and TEC had a significant effect on the MDT.

Regression analysis demonstrated the agreement between the predicted and observed responses obtained, indicating the applicability of the models generated by the Box-Behnken design. Additional experiments performed at the optimal variable settings confirmed the validity and reliability of the proposed models in predicting the drug entrapment and dissolution behavior of the salted-out enteric-release systems.

Simultaneous optimisation of the formulation and processing variables resulted in the fabrication of an optimal formulation having a DEE of 72.51% and a MDT of 0.069 in acidic media, which was capable of adequately controlling INH release in acidic media and releasing >90% of the drug after 2 hours at intestinal pH. The fabrication of an optimal cross-linked enterosphere system in a single processing step is thus a satisfactory alternative to the standard technique for manufacturing enteric-release multiparticulates.

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