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Formulation of an anti-tuberculosis drug delivery system

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CHAPTER 3

Preliminary Design of an Enterosoluble Multiparticulate System Incorporating Isoniazid

3.1. Introduction

In this chapter, three feasible methods for the microencapsulation of INH were investigated, viz. air suspension, solvent evaporation and a novel salting-out approach for the formulation of *enterogranules, microenterospheres* and *enterospheres* respectively, as coined in this study. The methacrylic acid copolymers, methacrylic acid ethyl acrylate (MAEA) and methacrylic acid methyl methacrylate (MAMM), were instituted throughout the ensuing investigations. It is worth noting, henceforth, that the same polymer applied in a number of different ways yields exceptionally different release profiles.

The rational identification of a candidate system for further optimisation was the ultimate goal of the three ensuing experimental investigations. Coherent comparison of the dissolution data of the resultant enterogranule, microenterosphere, and enterosphere formulations thus needs to be undertaken. This may be facilitated through pairwise comparison using a model-independent dissolution approach in order to determine if any one of the preliminary formulations could be considered for optimisation.

The anatomical and physiological considerations with reference to enteric-release systems were delineated in the previous chapter. The following must be considered in developing the preliminary formulations: (1) reproduciblity of the production process, (2) particle size, and (3) entrapment and release of INH from the enterosoluble forms in acidic and phosphate-buffered dissolution media.

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3.2. Development of a Methodology for the Fabrication of Enterogranules by the Air Suspension Method

3.2.1. Materials and Methods

3.2.1.1. Materials

The poly (methacrylic acid co-methyl methacrylate) copolymers A and B with varying monomer ratios (Eudragit®L 100 and Eudragit®S 100) were received as a gift from Röhm GmbH, Darmstadt, Germany. INH (isonicotinic acid hydrazide, 99% TLC) and triethyl citrate 99% were purchased from Aldrich® (Sigma-Aldrich Inc., St. Louis, USA). Ethanol 90%$^{v}/_{v}$ and ammonia solution (NH_4OH, M_w=35.05g/mol) 25%$^{v}/_{v}$ were obtained from Rochelle Chemicals (Johannesburg, South Africa) and were of analytical grade. Sodium hydroxide (NaOH, M_w=40.00g/mol) was obtained from Saarchem (Wadeville, Gauteng, South Africa). Hydrochloric acid (HCl, 32%$^{w}/_{v}$) was purchased from Rochelle Chemicals (Johannesburg, South Africa). Potassium dihydrogen phosphate (KH_2PO_4, M_w=136.09g/mol) was obtained from Riedel-de Haen (Sigma-Aldrich Laborchemikalein GmbH, Germany). Double-deionised water (Milli-Q System, Millipore, Bedford MA, USA) was used in the preparation of all dissolution media and throughout the assay procedure.

3.2.1.2 Equipment

Standard laboratory-scale top-spray coating equipment having the configuration represented in Figure 3.1 was employed for the encapsulation process: a fluidised bed coater (Aeromatic AG, GmbH, Germany), vacuum pump (Vacutec, Johannesburg, South Africa), and a peristaltic pump (Zero-max, USA).

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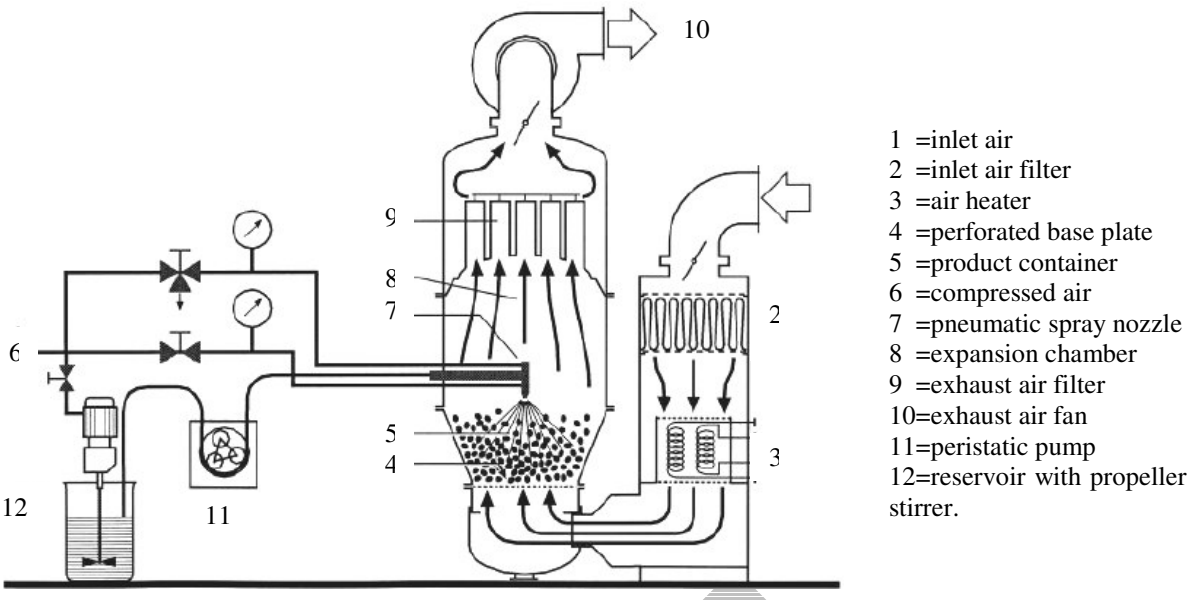

Figure 3.1: Experimental set-up for the air-suspension coating process (descriptively illustrated by Lehmann, 2001)

3.2.1.3 Identification of Processing Conditions for Successful Operation

The conditions, as elaborated henceforth, affecting the fluidised bed coating process (Table 3.1) were set during preliminary trials by identification of settings that resulted in minimal agglomeration and even enteric-coat application. The latter was determined following microscopic analysis of the coated granules employing a stereomicroscope (Olympus SZX7 stereomicroscope, Olympus, Japan) connected to a digital camera (CC 12, Olympus, Japan) and image analysis system (AnalySIS® Soft Imaging System, GmbH, Germany).

Table 3.1: Identified processing conditions

Processing Condition	**Setting**
Product charge	15g of pre-sieved matrix granules
Air flow rate/ fluidising air velocity	5m/s
Fluid application rate	5-7(x10) U/min = 5-7g/min
Drying temperature	40°C
Drying time	90min
Atomising air pressure (pressure control)	2 bar

3.2.1.3.1. Control of Airflow

Fluid bed coaters exert more mechanical stress on the cores as compared to coating pans. The proper airflow rate is important for the successful operation of the process. Although increasing the airflow rate or the temperature while maintaining the fluid application rate constant can enhance the drying kinetics, attrition of particles must be prevented. The airflow rate was thus adjusted to ensure adequate suspension of the granules with minimal granule agglomeration and attrition.

3.2.1.3.2. Fluid Application Rate or Spray Rate

The fluid application rate was adjusted for minimisation of intergranular (cohesive) and granule-fluidised bed wall (adhesive) sticking. Selecting a lower spray rate reduced any tendency to sticking or agglomeration observed during the spray process. The application rate was thus adjusted to the air handling capacity, such that the granules were always moist but never wet. In the case of excessive wetness at a low spray rate, the spraying process was interrupted for 5 minutes for intermittent drying.

3.2.1.3.3. Drying Time and Temperature

These were selected in accordance with the minimum film-forming temperatures (MFT) of the enteric-coating aqueous dispersions, having MFT of $<25^{o}C$.

3.2.1.4 Formulation of Enterogranules

For the attainment of complete enterosoluble characteristics, granules incorporating INH were prepared employing MAMM as the matrix in which the drug was embedded through institution as a polymeric binder during the wet granulation process, and as the enteric-coating material. In the GI tract, the coated granules were expected to behave as diffusion cells and release a constant

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drug quantity per unit of time.

40%$^{w}/_{v}$ solutions in 90%$^{v}/_{v}$ ethanol of the MAMM copolymer, Eudragit L® 100, were prepared by dispersing the polymer in the organic solvent with moderate agitation (700rpm) with a two-blade propeller stirrer (Heidolph®, Labotec, Gauteng, South Africa) for 1 minute. Allowing the covered solution to stand in a darkened room for 30 minutes ensured complete dissolution of the copolymer. 10.0g INH was triturated with 75mL of the MAMM-ethanol solutions (copolymer content of 30g) in a pestle and mortar. Formation of a coherent mass was facilitated by allowing a degree of volatilisation of the alcohol from the moist granulate by drying under reduced pressure at ambient conditions for 15 minutes (gravimetric weight loss: ≈5%$^{w}/_{w}$); the mass was then passed through the 1.25cm-aperture sieve of a laboratory granulator (Erweka AR400, GmbH, Germany). The granules were cooled on trays overnight at ambient conditions (21°C), then screened through stainless steel sieves of a test sieve shaker (Octagon 200, Endecotts Ltd., London, England) and the fraction >1.00cm was used as such, or subjected to microencapsulation by air suspension coating.

3.2.1.5 Enteric-Film Coating of Enterogranules

Granules were coated with either Eudragit L®100 (E L 100) and/ or Eudragit S®100 (E S 100) aqueous dispersions, prepared in accordance with Lehmann (2001) as per the formulations listed in Table 3.2. The dispersions were prepared one day in advance. The enteric-polymeric powder was dispersed at a steady rate in the stated amount of double-deionised water under moderate agitation with a two-blade propeller stirrer, ensuring that the powder was rapidly wetted without lump formation. The dispersion was stirred for 5 minutes followed by the dropwise addition of the ammonia solution to the periphery of the vortex. After adding the ammonia solution, stirring was continued for another 60 minutes, following the same procedure for triethyl citrate addition.

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Triethyl citrate was included as a plasticiser to aid polymeric coalescence and film formation. The dispersion was passed through a 0.25mm laboratory test sieve (Endecotts Ltd., London, England).

Table 3.2: Formulae for Eudragit S® 100 and Eudragit L® 100 dispersions

Eudragit S®100 dispersion	
Eudragit S 100	1.000g
1M Ammonia solution (1.7%)	0.500g
Triethyl citrate	0.508g
Water	5.532g
Content in dry polymer substance	13.3%
Degree of neutralisation	15 mole-%
Quantity employed for coating 15g granules	169.17g
Eudragit L®100 dispersion	
Eudragit L 100	1.000g
1M Ammonia solution (1.7%)	0.500g
Triethyl citrate	0.339g
Water	5.011g
Content in dry polymer substance	14.6%
Degree of neutralisation	6 mole-%
Quantity employed for coating 15g granules	154.11g

The parameters were set for fluidising the active granules in accordance with the processing conditions delineated in Table 3.1. Four preliminary formulations were investigated as set forth in Table 3.3. Three batches of each formulation were prepared. Each time, 15g of accurately weighed matrix granules were loaded into the fluidised bed. The granules were sprayed with the quantities of dispersion as listed in Table 3.2 to achieve a theoretical coat: core ratio of 1.5:1. When both E S 100 and E L 100 were employed, 84.59g E S 100 and 77.06g E L 100 were blended with mild agitation (300 rpm) for 5 minutes before spray application. The duration of the entire spraying and drying process was 90 minutes. The coated granules were evenly spread on trays and cooled overnight under ambient conditions (21°C).

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Table 3.3: Preliminary enterogranule formulations[a]

Formulation	E S 100 (g)	E L 100 (g)
Uncoated	0	0
E L 100-coated	0	154.11g
E L 100: E S 100-coated	84.59g	77.06g
E S 100-coated	169.17g	0

[a]*All formulations were prepared in triplicate, n=3*

3.2.1.6. Particle Size Analysis

The Feret's diameters (d_f) of the coated and uncoated enterogranules were investigated by microscopic image analysis using a stereomicroscope (Olympus SZX7, Olympus, Japan) connected to a digital camera (CC 12, Olympus, Japan) 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 (Figure 3.2). The mean diameter of each formulation was determined by measurement of the diameter of 50 randomly selected enterogranules (n=50). Results were expressed as the mean ± standard deviation (S.D.) of 50 measurements of the Feret's diameter (d_f) for each formulation.

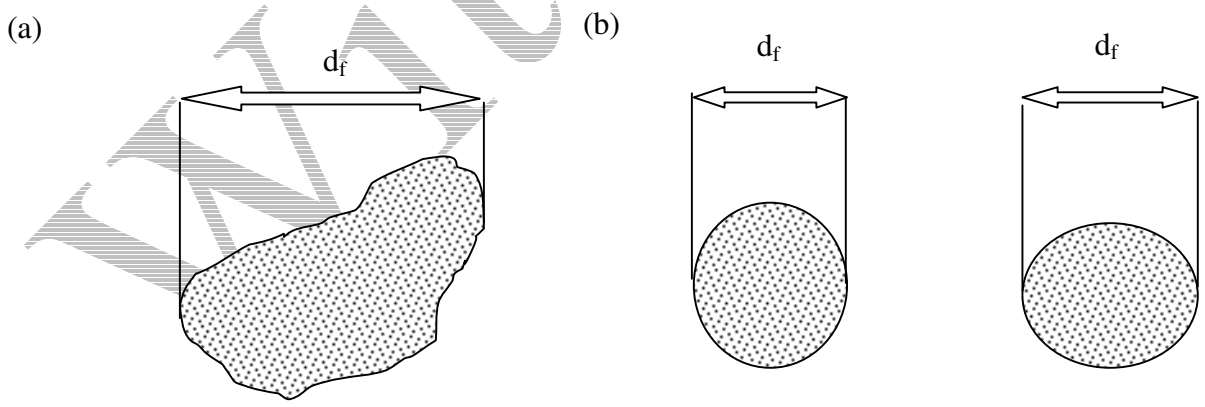

Figure 3.2: Feret's diameters (d_f): (a) Assessment of d_f of an irregular particle and (b) particle orientation for determination of shortest and longest Feret's diameters (d_f) in ovoid particles when determination of an aspect ratio is necessitated

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3.2.1.7. Construction of Calibration Curves for Spectrophotometric Determination of INH Release from the Enterosoluble Multiparticulate System

In accordance with Beer's law, the absorbance (*A*) of an absorbing species in solution is directly proportional to the pathlength (*b*) through the solution and the concentration (*c*) of the absorbing species. The concentration of a bioactive in solution can thus be determined from its measured absorbance, provided a linear relationship exists. Beer's law is applicable to dilute solutions (≤0.01M) of most substances.

Calibration curves of absorbance, measured spectrophotometrically, versus concentration were constructed for INH in 0.1M hydrochloric acid (HCl, pH 1.2) at the 265nm absorption peak and in phosphate buffered saline (PBS, pH 6.8) at 263nm, which passed through the origin. Six concentration levels (0.01-0.15 mg/mL) were prepared and three readings were made at each level.

In order to ascertain the validity and reliability of the assay method in detecting INH entrapment and release from enterosphere formualtions, precision and accuracy experiments were performed for the assay method. These studies were carried out to ensure consistency and reproducibility of the measurements obtained, as well as the accuracy of the ultraviolet (UV) data obtained.

Precision was assessed from the analysis of five sample replicates (0.03mg/mL) from an analytical sample containing 0.3mg/mL pure INH. The measured UV absorbance for each sample replicate was used in determining the precision of the method. To determine accuracy, five INH samples, each containing 0.03mg/ml INH, were assayed. These measurements were performed in both 0.1M HCl and PBS pH 6.8. The solutions employed for these determinations were separate from those used for construction of the calibration curves.

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Note that it is established at the outset that the methacrylic acid copolymer solution and/or latex and all other excipients (i.e. plasticisers, electrolytes, etc.) employed in the respective encapsulation processes did not interfere with drug analysis at the reported wavelength.

3.2.1.8. Drug Content of Enterogranules

Drug content was determined spectrophotometrically at 263nm by placing 100mg of INH-loaded enterogranules in a 200mL conical flask containing 100mL of 0.2M PBS, pH 7.0. The enterogranules were magnetically stirred for 5 hours after which time all the granular formulations were microscopically observed (Olympus SZX7, Japan) to have undergone complete erosion. This was to ensure absolute liberation and subsequent dissolution of the water-soluble INH from the enterosoluble matrix. 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. Aliquots of the filtrates were subjected in triplicate to UV spectroscopy (diode array UV spectrophotometer, Specord 40, Analytik Jena AG, Jena, Germany) at 263nm for analysis (WinASPECT® Spectroanalytical Software, Analytik Jena AG, Jena) following comparison with the standard calibration curves generated for INH in PBS media.

3.2.1.9. *In Vitro* Release Studies on Enterogranules

Characterisation of INH release from the enterogranules was assessed using a method adapted from the USP 24 general drug release standard for delayed release (enteric-coated) articles in acidic and phosphate-buffered media (USP 24, 2000). Enterogranules equivalent to 10mg INH were placed in 50mL sealed vials. For determination of the amount of INH released under acidic conditions, 20mL of 0.1M HCl was added to the vials which were then subjected to agitation at 50rpm for 2 hours in a shaker water-bath (Labex, Stuart SBS40®, Gauteng, South Africa)

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maintained at 37±0.5°C. For determination of INH release in basic media, the acid was drained from the vials whilst retaining the enterogranules and replaced with 20mL of PBS (pH 7.0). Agitation was continued for a further 6 hours. Balancing withdrawal of 1mL aliquots was performed at the appropriate time intervals and samples were then analysed by UV at 263nm following dilution for determination of the fractional INH release.

3.2.2. Results and Discussion

The regression coefficients (R^2=0.9995 and 0.9997, respectively) for the calibration curves (Figures 3.3 and 3.4) constructed for INH demonstrated linearity in acidic media (pH 1.2) and phosphate buffered media (pH 6.8) achieved over the concentration range (0.01-0.15 mg/mL).

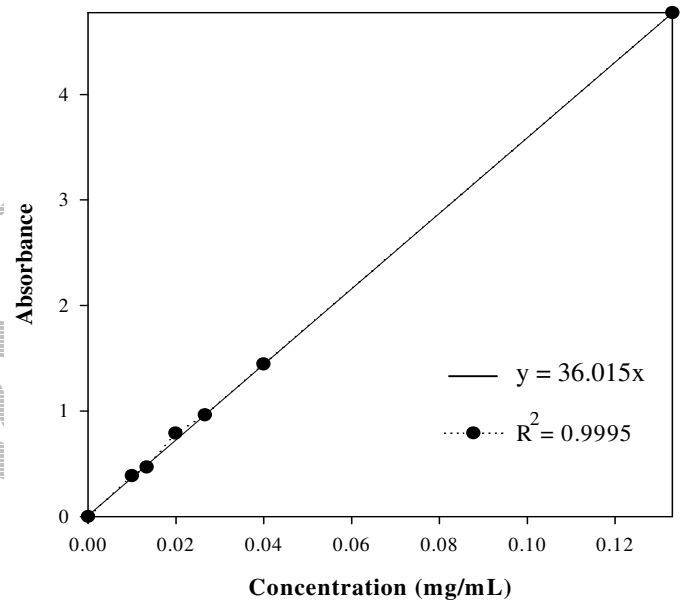

Figure 3.3: INH calibration curve at 265nm in 0.1M HCl (pH 1.2,) (S.D. within ±0.052 in all cases)

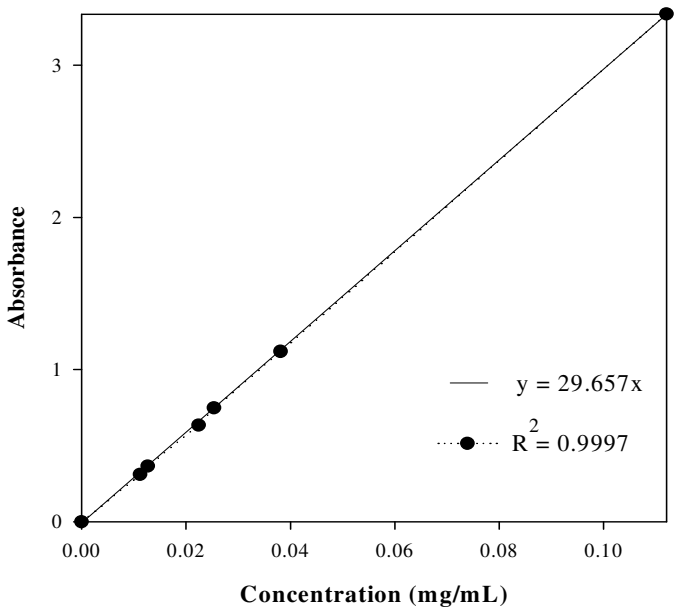

Figure 3.4: INH calibration curve at 263nm in PBS (pH 6.8), (S.D. within ±0.051 in all cases)

The accuracy of the UV analytical method in 0.1M HCl and PBS pH 6.8 was determined from the percentage recovery of INH for five samples containing 0.03mg/mL INH. This data is represented in Tables 3.4 and 3.6. The precision of the method was derived from the absorbance readings obtained from five sample replicates and is demonstrated in Tables 3.5 and 3.7.

Table 3.4: Accuracy determination for INH assay method in 0.1M HCl

Sample Standards	Concentration Added (mg/mL)	Concentration Found (mg/mL)	% Recovery
1	0.03	0.0291	96.9966
2	0.03	0.0304	101.3467
3	0.03	0.0299	99.5881
4	0.03	0.0292	97.1817
5	0.03	0.0295	98.3849

Table 3.5: Precision determination for INH assay method in 0.1M HCl

Sample Replicate	1	2	3	4	5
UV Absorbance	1.045	1.044	1.058	1.051	1.047

A mean of 0.0296mg/mL and a standard deviation of $5.43x10^{-4}$ were obtained for the accuracy determination in 0.1M HCl. The coefficient of variation, a measure of the relative variability for this data, was 1.834%. A mean of 1.049 and a standard deviation of $5.701x10^{-3}$ were obtained for the precision determination in 0.1M HCl. The coefficient of variation of this data was 0.543%.

Table 3.6: Accuracy determination for INH assay method in 0.2M PBS pH 6.8

Sample Standards	Concentration Added (mg/mL)	Concentration Found (mg/mL)	% Recovery
1	0.03	0.03048	101.6061
2	0.03	0.02991	99.6954
3	0.03	0.02954	98.4591
4	0.03	0.02849	94.9748
5	0.03	0.02856	95.1996

Table 3.7: Precision determination for INH assay method in 0.2M PBS pH 6.8

Sample Replicate	1	2	3	4	5
UV Absorbance	0.887	0.870	0.876	0.872	0.881

A mean of 0.0294mg/mL and a standard deviation of $8.63x10^{-4}$ were obtained for the accuracy determination assay in PBS. The coefficient of variation for this data was 2.935%. A mean of 0.877 with a standard deviation of $6.782x10^{-3}$ was obtained for the precision determination assay in PBS. The coefficient of variation of this data was 0.787%.

This indicates that INH recovery using this method of detection for determination of INH release in both 0.1M HCl and 0.2M PBS pH 6.8 is satisfactorily consistent and precise.

The morphology, mean diameter and INH release profiles in acidic media for the preliminary enterogranules are represented in Figures 3.5, 3.6 and 3.7.



Figure 3.5: Stereomicrographs (darkfield, scale bar=1cm) of enterogranules: (a) uncoated at 40X (b) E L 100-coated at 25X and (c) E S 100-coated at 40X magnification

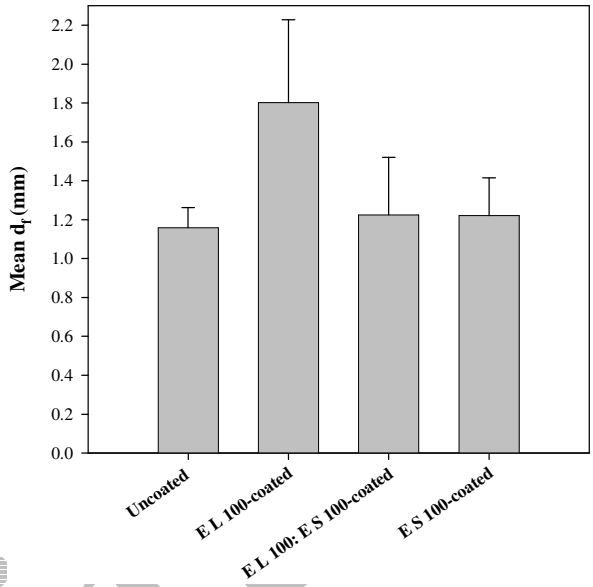

Figure 3.6: Feret's diameter (mean±S.D., n=50) of the preliminary enterogranule formulations

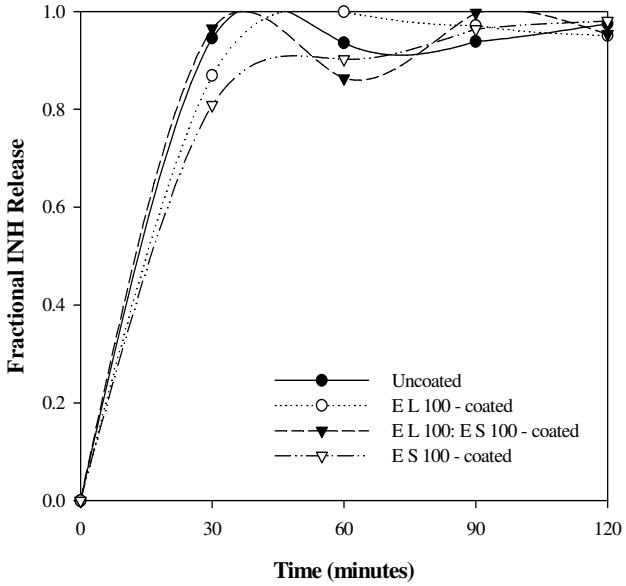

Figure 3.7: Drug release profiles of the enterogranules in acidic media (0.1M HCl, pH 1.2), (S.D. within ±0.039 in all cases)

INH release from the irregular enterogranules was uncompromisingly rapid with almost the entire entrapped amount of drug leaching out of the granule after one hour in acidic media. The following reasons were identified for the lack of enteric control of the multiparticulate system.

- The matrix granules were insufficiently compact with resultant diffusion of INH through the porous structure
- There was rapid diffusion of the water-soluble INH through the enteric-film coating
- The possibility of INH diffusion into the enteric coat during the coating process could not be ruled out even under strictly controlled coating conditions.

The shortcomings 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 than through organic-solvent-based films (Guo et al., 2002).

Bianchini et al. (1991) demonstrated the poor performance of enteric-coated dosage forms containing a water-soluble substance; these did not pass the USP 24 test unless insulation of the cores was undertaken by subcoating barriers or by employing twice the amount of coating. The lack of sufficiently effective gastroresistance has been ascribed to dissolution of a small amount of drug from the core tablet to the aqueous film during the coating process. The higher release rates from coated pellets have thus been attributed primarily to drug diffusion into the film layer during the coating process. If the active ingredients are freely water-soluble, as is the case with INH, they may dissolve in the spray mist during the coating process, resulting in active ingredients being incorporated in the film. The presence of a drug or an excipient in a film coating is not desired as it substantially alters the mechanical adhesion and permeation characteristics of the applied coating (Guo, 1993; Guo et al., 2002).

It is contemplated that fabrication of an optimal enteric-polymeric matrix system for incorporation of the water-soluble drug in a single processing step, where sufficient coalescence of the polymer is promoted, would achieve improved gastroresistance of the multiparticulate system.

3.3. Development of a Methodology for the Fabrication of Microenterospheres by the Polar Organic-in-Oil Emulsification Solvent Evaporation Method

3.3.1. Materials and Methods

3.3.1.1. Materials

The poly (methacrylic acid co-methyl methacrylate) copolymers with varying monomer ratios (E L 100 and E S 100) were received as a gift from Röhm GmbH, Darmstadt, Germany. INH (isonicotinic acid hydrazide, 99% TLC) was purchased from Aldrich® (Sigma-Aldrich Inc., St. Louis, USA). Organic solvents (acetone, ethanol) were all of analytical grade and were purchased from Rochelle Chemicals (Johannesburg, South Africa).

3.3.1.2 Formulation of Microenterospheres

A modified solvent evaporation emulsification method, employing an organic solution of the MAMM copolymers, proved feasible for the formulation of uniform, spherical multiparticulates with a narrow size distribution, capable of entrapping the water-soluble INH.

The MAMM copolymers (2.0g) were dissolved in 15.0mL acetone with 0.3mL water, or 15.0mL ethanol, or their 1:1 combination, whilst stirring with a magnetic stirrer for 30 minutes. INH (1.0g) was then dissolved in the MAMM copolymer solution by stirring for an additional 15 minutes. This solution was then emulsified in 40mL of a $1\%^{w}/_{v}$ Span 80®-liquid paraffin solution at 800rpm for 3 hours at room temperature with a Heidolph® two-blade propeller stirrer (Labotec,

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Gauteng, South Africa). Once the microenterospheres were formed, they were filtered on a Buchner funnel, washed twice with 100mL normal hexane to remove the residual oil phase, and subsequently dried under ambient laboratory conditions (21°C) overnight.

To investigate the ability of the solvent evaporation method to produce microenterospheres of adequate gastroresistance, the formulations were prepared using the proposed organic solvents. The influence of the polymer: drug ratio, stirring rate and MAMM copolymer combination (E L 100 and E S 100) were also investigated once the preferred organic solvent system was identified. All preliminary formulations were prepared and analysed in triplicate for observation of the ease of formation of a spherical non-aggregated morphology (Table 3.8).

Table 3.8: Preliminary microenterosphere formulations[a]

Formulation	Acetone (mL)	Ethanol (mL)	A:E (mL)	E S 100	E L 100	P:D	Stirring rate (rpm)	Observation
A1	15	0	0	1	1	3:1	600	Well-formed
A2	0	15	0	1	1	3:1	600	Small, Coalesced
A3	0	0	10:5	1	1	3:1	600	Small, not reproducible
A4	15	0	0	0	2	3:1	600	Well-formed
A5	15	0	0	2	0	3:1	600	Large, well-formed
A6	15	0	0	1	1	3:1	1000	Small, well-formed
A7	15	0	0	1	1	5:1	600	Large, well formed

[a]*All formulations were prepared in triplicate*

3.3.1.3. Microenterosphere Diameter Analysis

The size distribution of each of the microenterosphere formulations was determined microscopically with a stereomicroscope (Olympus SZX7, Japan) connected to a digital camera (CC 12) and image analysis system (AnalySIS® Soft Imaging System, GmbH, Germany) by measurement of the diameter of 50 randomly selected microenterospheres (n=50). Results are expressed as the mean±S.D of 50 measurements of the longest Feret's diameter (d_f) for each formulation.

3.3.1.4. Encapsulation Efficiency of Microenterospheres

Drug content was determined spectrophotometrically at 263nm by placing 100mg of INH-loaded microenterospheres in a 200mL conical flask containing 100mL of 0.2M PBS, pH 7.0. The microenterospheres were magnetically stirred for 10 hours. This period was sufficient to promote polymeric swelling and dissolution, and the resultant complete erosion of the microenterosphere, as observed microscopically (Olympus SZX7, Japan), for absolute liberation of the entrapped water-soluble 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 PBS. Aliquots of the filtrates 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. The amount of drug entrapped in the microenterospheres in each formulation was compared with the amount of drug, which was intended to be loaded in order to obtain the drug encapsulation efficiency (DEE):

$$DEE(\%) = \frac{\text{Actual quantity of drug present in enterospheres}}{\text{Theoretical quantity of drug loaded into enterospheres (actual initial loading dose)}} \times 100$$

[Equation 3.1]

3.3.1.5. *In Vitro* Release Studies on Microenterospheres

Characterisation of INH release from the microenterospheres was assessed using a method adapted from the USP 24 general drug release standard for delayed release (enteric-coated) articles in 0.1M HCl and PBS (USP 24, 2000). Microenterospheres equivalent to 10mg INH were placed in 50mL sealed vials. For determination of the amount of INH released under acidic conditions, 20mL of 0.1M HCl was added to the vials which were then subjected to agitation at

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50rpm for 2 hours in a shaker water-bath (Labex, Stuart SBS40®, Gauteng, South Africa) maintained at 37±0.5°C. For determination of INH release in basic media, the acid was drained from the vials whilst carefully retaining the microenterospheres and replaced with 20mL of a PBS (pH 7.0). Agitation was continued for a further 6 hours. Balancing withdrawal of 1mL aliquots was performed at the appropriate time intervals and samples were then analysed by UV spectroscopy at 263nm and 265nm following dilution for determination of the fractional INH release.

3.3.2. Results and Discussion

Microenterospheres had a spherical morphology (Figure 3.8), having a mean particle size ranging from 117.11µm to 265.31µm. The mean diameter, drug entrapment and release characteristics of the enterospheres are represented in Table 3.9.

Drug release studies revealed that the microenterospheres produced by the solvent evaporation method exhibited biphasic drug release patterns in acidic media in all cases (with an enteric-release property): an initial rapid drug release phase ('burst effect') was followed by a second, slower drug release phase (Figure 3.9). This 'burst' release cannot solely be attributed to drug diffusion through the polymer, but is probably related to the dissolution of drug aggregates located close to the microenterosphere surfaces. When exposed to the higher pH of the PBS, drug release was much more rapid, and there was prominent swelling and erosion of the microenterosphere with solubilisation of the methacrylic acid copolymer, and in all formulations, the total entrapped amount of INH was released after 3 to 6 hours.

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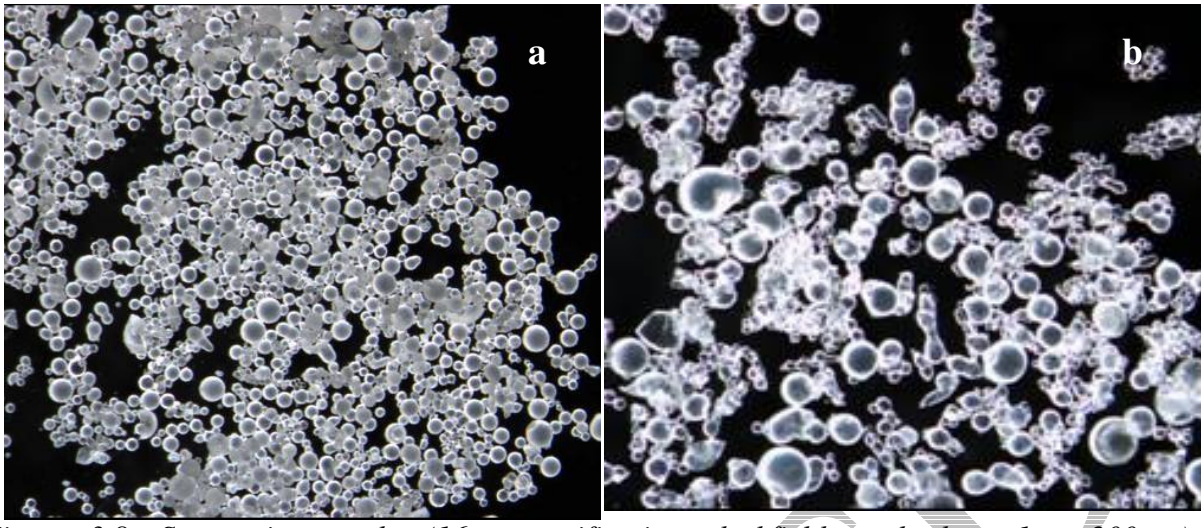

Figure 3.8: Stereomicrographs (16X magnification, darkfield, scale bar: 1cm=200µm) of representative samples [(a) and (b)] of microenterospheres

Table 3.9: Particle size, entrapment and release characteristics of preliminary microenterosphere formulations

Formulation	Enterosphere Diameter (µm)	Drug Entrapment Efficiency (%)	Fractional INH Release (t_{2h})
A1	124.49±114.60	83.25±4.60	0.522±0.015
A2	165.16±78.09	80.32±4.56	0.790±0.012
A3	119.08±66.84	86.60±2.16	0.741±0.032
A4	235.72 ±154.24	78.37±5.01	0.644±0.062
A5	265.31±185.09	87.93±4.42	0.432±0.026
A6	117.11±63.77	89.00±4.96	0.625±0.056
A7	205.10±130.60	74.62±4.83	0.389±0.010

The microenterospheres formulated by solvent evaporation demonstrated an enhanced ability to control drug release in acidic media. The most favourable results in terms of formation of reproducible microenterospheres were observed when acetone was employed as the organic solvent system (A1) due to preferential solubility of the enteric polymer in this solvent. Furthermore, microenterospheres fabricated in the acetone solvent system demonstrated less coalescence of individual spheres ascribed to the greater volatility of the solvent system.

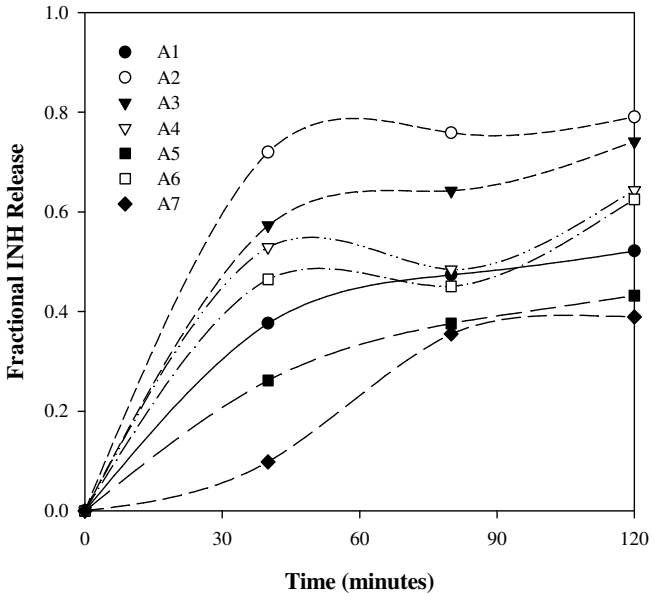

Figure 3.9: Release profiles for preliminary microenterosphere formulations in acidic media (0.1M HCl, pH 1.2), (S.D. within ±0.063 in all cases)

An increase in the polymer: drug ratio in A7 generally saw an increase in the amount of INH entrapped within the microenterospheres. An increase in the amount of polymer incorporated into the microenterospheres resulted in a decrease in the rate of INH release. The consequential increase in diffusion pathways lead to slower drug diffusion rates, in accordance with Fick's law of diffusion. This is also ascribed to the fact that the release of INH from microenterospheres increased with increasing proportion of the drug. This was due to an increased amount of drug being close to the microenterosphere surface and the likelihood of a portion of the drug being uncoated increased with higher drug loading.

Drug release from microenterospheres formulated according to the described solvent evaporation method has shown dependency on particle size and has been demonstrated by various authors (Beck et al., 1979; Barkai et al., 1990; de Brito Amorim and Ferreira, 2001; Perumal, 2001). This

was the case here, as the more rapid stirring rates employed for A6 generally formed smaller microenterospheres, which slowed the release of INH to a lesser extent, in accordance with Fick's law of diffusion. A slower stirring rate caused a resultant increase in the wall thickness, which increased drug diffusion pathways thereby protracting the diffusion process.

A5 incorporating only E S 100 was better able to prevent the release of INH under acidic conditions than E L 100 (A4). This is a consequence of the lower aqueous solubility of E S 100 due to the lower ratio of carboxyl to ester groups (approximately 1:1 in E L 100 and 1:2 in E S 100). However, its use in combination with E L 100 is preferred because use of E S 100 in isolation results in dissolution of enteric film coatings only commencing above pH 7.0 and thus usually occurs *in vivo* in the lower sections of the intestines. However, since a pH of 7.0 is frequently only just reached and not noticeably exceeded, excretion of active ingredients with the faeces should be avoided by mixing E S 100 with E L 100.

3.4. Modifications to Overcome Burst Release from Microenterospheres

Although the microenterospheres demonstrated the ability to control drug release to a certain extent, the initial burst release of INH was undesirably high owing to initial dissolution of drug aggregates located close to the microenterosphere surface and then drug diffusion through the enteric copolymer matrix. For this system, the need for double entrapment of the water-soluble INH within a reservoir or multireservoir enterosoluble system (Figure 3.10) would be warranted. Phase separation methods proved to be successful at depositing a polymeric coating upon the core material.

The process involved formation of three immiscible chemical phases: a chemical liquid manufacturing vehicle phase, a core material phase, and a coating material phase. It was therefore

essential that a polymeric phase be selected in which the core microenterosphere could not be solubilised. Utilising one of the methods of phase-separation coacervation, the coating material phase is induced to coalesce. In this case, a change in temperature and the addition of a soluble inorganic salt was instituted. Deposition of the liquid polymer coating around the core material occurs if the polymer is adsorbed at the interface formed between the core material and the liquid vehicle phase; this is a prerequisite for effective coating. In the first case, the microenterospheres were coated with an additional enteric coating affected by addition of the core phase and the aqueous polymer phase to an electrolyte solution. In the second instance, ethylcellulose, a water-insoluble polymer, was applied to the core microenterosphere following a thermal change (Bakan, 1986).

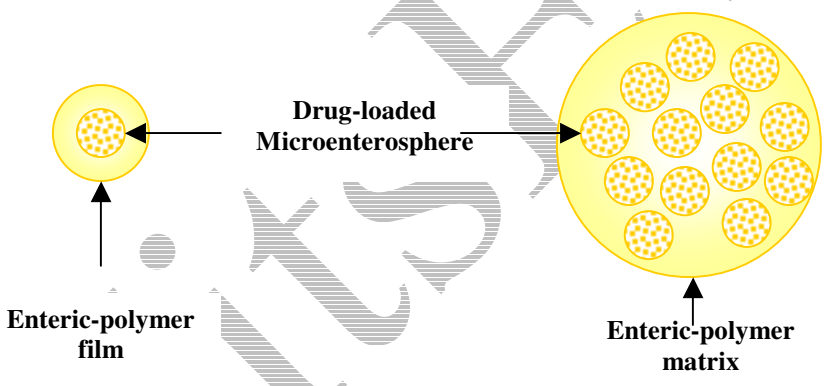

Figure 3.10: Schematic of reservoir and multireservoir enterosphere representing double entrapment of INH

3.4.1. Materials and Methods

3.4.1.1. Materials

The as-received methacrylic acid copolymer type C was a gift from Röhm GmbH, Darmstadt, Germany and contains 0.7%$^{w}/_{w}$ sodium lauryl sulphate and 2.3%$^{w}/_{w}$ Polysorbate 80 based on solid substance, added to function as emulsifiers. Sodium hydroxide (NaOH, M_w=40.00g/mol)

was purchased from Saarchem (Wadeville, Gauteng, South Africa). Ethylcellulose (Ethocel® STD 100) was purchased from Protea Industrial Chemicals (Pty) Ltd. (Wadeville, Gauteng). Cyclohexane and sodium chloride (NaCl, M_w=58.45g/mol) were purchased from Rochelle Chemicals (Johannesburg, Gauteng).

3.4.1.2. Double Entrapment in Methacrylic Acid Ethyl Acrylate Copolymer

The preferred (most gastroresistant) microenterosphere formulation (A7) was prepared as described by the solvent evaporation method. An amount of microenterospheres theoretically equivalent to 100mg INH was dispersed in 1.67mL of a 6-mole-percentage neutralised (achieved by addition of 1M NaOH) 30%$^{w}/_{w}$ MAEA copolymer aqueous dispersion equivalent to 500mg dry MAEA. The resultant dispersion was 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 magnetically agitated concentrated electrolyte solution (25%$^{w}/_{v}$ NaCl) and cured for 5 minutes in the electrolyte solution for incorporation of the microenterospheres in the salted-out enterosoluble coating for the fabrication of multireservoir enterospheres (R1). Multireservoir enterospheres were collected following decantation of the aqueous electrolyte phase and rinsed twice with 100mL double-deionised water.

3.4.1.3. Double Entrapment in Ethylcellulose

Microenterospheres were further encapsulated in ethylcellulose employing a coacervation phase separation by thermal change technique. The etherified cellulosic, containing a relatively high ethoxyl content (high degree of substitution) is insoluble in cyclohexane at room temperature but is soluble at elevated temperatures. The ethylcellulose grade selected is commonly employed for microencapsulation purposes. The ethylcellulose coating was given on the microenterospheres by employing cyclohexane as a solvent for ethylcellulose, in which neither MAMM or INH are

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soluble, and changing the temperature from 80°C with continuous stirring at 1000rpm. 500mg of ethylcellulose was dissolved in 5mL cyclohexane at 80°C (boiling point) with magnetic stirring for 30 minutes. The preferred (most gastroresistant) microenterosphere formulation (A7) was prepared as described by the solvent evaporation method. An amount of microenterospheres theoretically equivalent to 100mg INH was dispersed in the ethylcellulose solution at 80°C. The temperature of the solution was slowly lowered to 20°C at a rate of 1°C/minute. Cooling to room temperature accomplished gelation and solidification of the coating. This resulted in microenterospheres being incorporated within the ethylcellulose matrix for the fabrication of reservoir enterospheres (R2).

3.4.1.4. *In Vitro* Drug Release Studies on Reservoir Systems

R1 and R2 were prepared in triplicate for *in vitro* drug release testing. Drug release studies were conducted on the resrevoir/ multireservoir enterospheres as described previously for the microenterospheres by placing an amount of enterospheres equivalent to 10mg INH in a 50mL vessel containing 20mL of the respective release media.

3.4.2. Results and Discussion

R1 enterospheres had a mean diameter of 2535µm and R2 enterospheres had a mean diameter of 740µm (Figure 3.11). Encapsulation by coacervation-deposition of an ethylcellulose coating on the microenterospheres (R2) decreased the gastroresistance of the multiparticulate system with 70.4% of the entrapped INH being released at t_{2h}. A possible explanation for this may be the disruption of the patency of the enteric barrier created by the MAMM polymers during the thermal deposition of the ethylcellulose due to the adsorption phenomenon. Encapsulation of enterospheres in the salted-out MAEA coating enhanced the gastroresistance of the multiparticulate system, with less than 3.7% of the INH being released at t_{2h} (Figure 3.12).

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Industrially, however, the process of double encapsulation for enterosphere fabrication may not be entirely feasible as time constraints and a greater number of processing steps would limit its application.

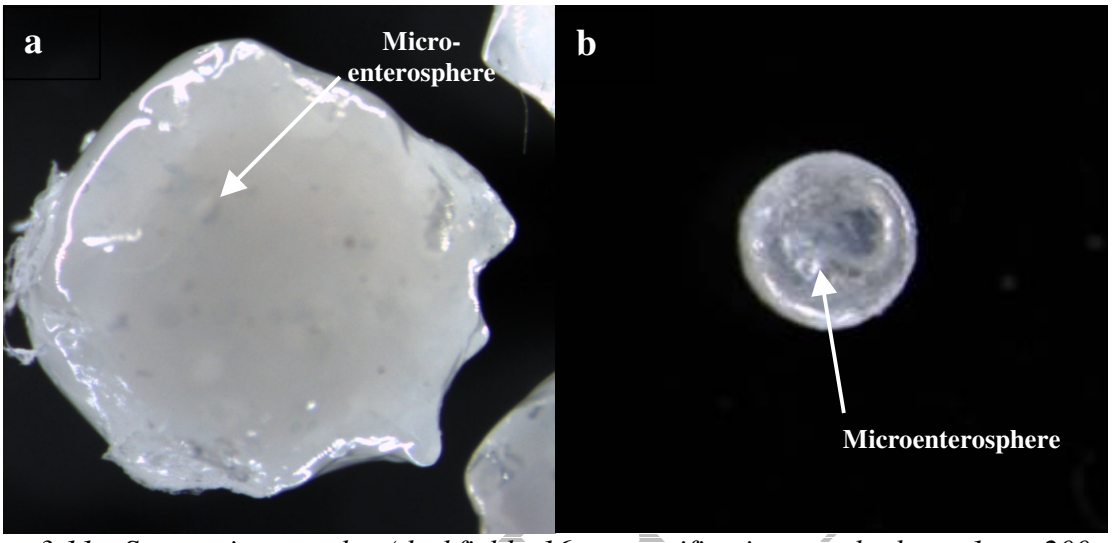

Figure 3.11: Stereomicrographs (darkfield, 16x magnification, scale bar: 1cm=200μm) of representative reservoir enterospheres: (a) R1 and (b) R2

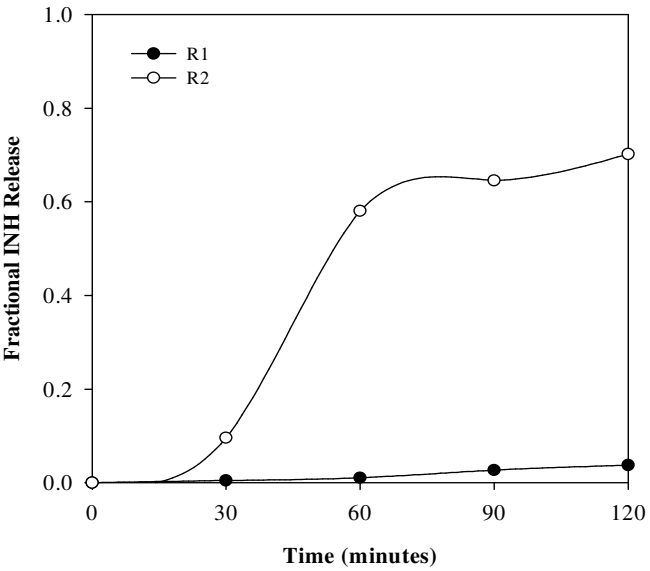

Figure 3.12: Drug release profiles of reservoir systems in acidic media (0.1M HCl, pH 1.2), (S.D. within ±0.042 in all cases)

3.5. Development of a Methodology for the Fabrication of Enterospheres by a Novel Salting-Out and Ionotropic Cross-Linking of MAEA

The principles inherent in colloid science and salting-out techniques formed the basis of this approach, which employed the more environmentally benign acrylic latex rather than an organic solution of the enteric polymer. As demonstrated previously, aqueous dispersion-coated dosage forms of water-soluble drugs have inadequate gastroresistance. It is contemplated that fabrication of a salted-out and cross-linked enteric-polymeric matrix system incorporating a water-soluble drug would achieve improved gastroresistance of the methacrylic acid copolymer coating latex.

Simple coacervation and complex coacervation were defined in Chapter 2 and result in deposition of coacervate walls from aqueous solutions of polymer by separation of a colloid-enriched phase from a dilute colloid solution (Donbrow, 1991).

This approach exploited the 'salting-out' phenomenon to induce phase-separation/ coacervation, followed by ionotropic cross-linking of the MAEA enteric copolymer. The salted-out and cross-linked enterosphere matrices were formed by inducing separation of the anionic polyelectrolyte as a polymer-rich enteric film ('salting-out') and ionotropically cross-linking the internal enterosphere matrix following extrusion and curing of an aqueous dispersion of the polymer into a concentrated electrolyte solution. The formation and properties of the copolymeric enterospheres cross-linked via polyvalent ions depends on the concentrations and distribution of the ions incorporated within the MAEA enterospheres, which is in turn also affected by the duration of exposure of the copolymer to the salting-out solution. The copolymeric chains are cross-linked via the cations by the formation of complexes liganded with more than one MAEA group creating intramolecular and/ or intermolecular cross-links (Allain and Salome, 1990). The described method precluded the use of organic solvents for dissolution of MAEA as a partially

neutralised aqueous dispersion was utilised, was highly reproducible, and the particles were formulated in a single processing step.

The MAEA is a synthetic copolymer demonstrating excellent biocompatibility, and is suitable for ionotropic cross-linking in this manner to form interconnected matrices. As an anionic polyelectrolyte, it possesses charged carboxylic acidic side groups, and the water-solubilised polymer may be cross-linked by reaction with a solution of cations. The preferred cations for cross-linking polymers with acidic side groups are divalent and trivalent ions, divalent cations being preferred due to lower toxicity; the higher the concentration of cation or the higher the valency, the greater the degree of polymer cross-linking. This phenomenon is described by the Schulze-Hardy rule, which governs the ability of an electrolyte to reduce the value of the zeta-potential of the colloidal polymer.

Although methacrylic acid copolymers are practically insoluble in water, they are soluble in solutions of 1M NaOH upon neutralisation of carboxyl groups, giving clear to slightly opalescent solutions. Partial neutralisation of the aqueous polymeric dispersion of the MAEA copolymer resulted in the formation of a latex in which the polymer particles typically had a submicron particle-size distribution and behaved in the same manner as colloidal particles. The dispersed phase in the latex was thus composed of spherical polymer particles with an average diameter of 200-300nm. The dispersion medium was water containing various water-soluble compounds. The dispersions of MAEA have been demonstrated to be stabilised by a combination of electrostatic and steric mechanisms termed as electrosteric stabilisation. The electrosteric stabilisation is considered to arise in part from dissolved polymer chains with charged carboxylic groups extending out into the continuous phase. The partial neutralisation and solubilisation of the carboxyl-containing MAEA copolymer facilitated both the rapid destabilisation of MAEA in the

presence of electrolytes inducing coalescence of the colloidal particles and film formation (Nyamweya, 2001) as well as promoting interpenentration and a degree of polymeric cross-linking on protracted exposure to a solution of cations due to the presence of dissolved copolymer chains in the dispersion. As described, the efficiency of coalescence due to colloidal destabilisation is sensitive to the valency of the counterion; the concentration of counterions required for coagulation decreases drastically with increasing valency (Lieberman et al., 1988).

3.5.1. Materials and Methods

3.5.1.1. Materials

The as-received methacrylic acid copolymer type C (E L 100-55) was a gift from Röhm GmbH, Darmstadt, Germany and contains 0.7%$^{w}/_{w}$ sodium lauryl sulphate and 2.3%$^{w}/_{w}$ Polysorbate 80 based on solid substance, added to function as emulsifiers. INH (isonicotinic acid hydrazide, 99% TLC) and triethyl citrate (99% purity) were purchased from Aldrich® (Sigma-Aldrich Inc., St. Louis, USA). Electrolytes were all of analytical grade and were purchased from Rochelle Chemicals (Johannesburg, South Africa) and Saarchem (Wadeville, Gauteng, South Africa).

3.5.1.2. Formulation of Enterospheres

The novel salting-out and cross-linking method was employed for the formulation of enterospheres, instituting a partially neutralised aqueous dispersion (latex) of MAEA copolymer with a monomer molar ratio of 1:1. Among the anionic enteric polymers, MAEA is the only copolymer commercially available as an aqueous dispersion (Eudragit L 30 D-55, USP/NF methacrylic acid copolymer Type C) and as a powder for redispersion (Eudragit L 100-55, USP/NF methacrylic acid copolymer Type C) thus facilitating industrial application of this approach.

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The formula for preparation of one batch (50mL) of an INH-loaded 6-mole-% neutralised aqueous dispersion (latex) comprised 30g double-deionised water, 15g E L 100-55, 5.0g 1.0M NaOH, 5.0g triethyl citrate, and 3.0g INH.

The latex was freshly prepared each time from the powder for redispersion by slow addition of 1.0M NaOH to the latex particle agglomerates in water and dispersing in accordance with Lehmann (2001) in order to achieve neutralisation of approximately 6-mole-% of the carboxyl groups contained in the polymer for partial solubilisation of MAEA. This was undertaken with the aid of moderate agitation (700rpm) of the dispersion with a Heidolph® propeller stirrer (Labotec, Gauteng, South Africa) for a period spanning 30 minutes. Triethyl citrate ($10\%^{w}/_{w}$) was included as a plasticiser. A latex-like dispersion had formed if virtually no particles were visible in a milky liquid without any sediment formation. The pH of the dispersion thus obtained was between 5.0 and 5.2.

Dispersion of the water-soluble INH in the aqueous dispersion was achieved under agitation at 500rpm for 15 minutes with a Heidolph® propeller stirrer to obtain a MAEA:INH ratio of 5:1 (Table 3.10). The dispersion was vortexed (Vortex Genie-2, Scientific Industries Inc., USA) before further processing to allow for homogenisation and the dissipation of any foaming 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 $25\%^{w}/_{v}$ electrolyte solution, which induced various degrees of salting-out with the formation of spheres (Figure 3.13).

The formed enterospheres were cured in the electrolyte solution in a dark cupboard for an additional 30 minutes to induce a degree of cross-linking of the internal matrix. The

enterospheres were then washed twice with double-deionised water (100mL) to remove any unincorporated electrolyte and dried overnight under ambient conditions (21°C).

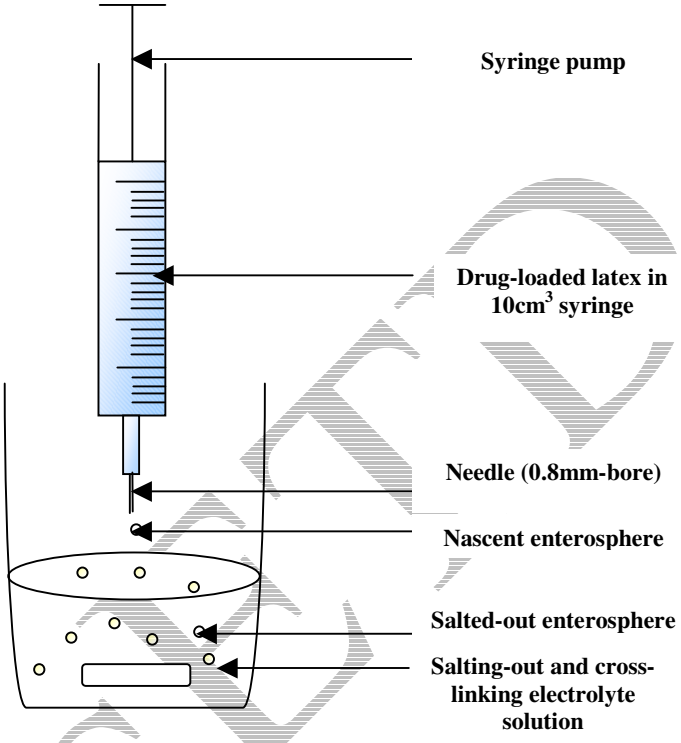

Figure 3.13: Schematic of salting-out process for enterosphere fabrication

It is proposed that salting-out and cross-linking of the MAEA copolymer in the presence of an appropriately selected electrolyte would form a dense salted-out enteric film and cross-linked interconnected enterosphere matrix that would optimally slow drug release in acidic media. Various electrolyte solutions were initially instituted for polymer separation and matrix hardening for identification of the single ideal salting-out and cross-linking agent from drug-release data. Only pharmaceutically acceptable water-soluble electrolytes were considered (Table 3.10). To evaluate the ability of anions to induce salting-out of the copolymer, corresponding sodium, zinc and magnesium chloride and sulphate electrolyte solutions were employed in the salting-out reaction. In order to elucidate the ability of monovalent, divalent and trivalent cations to

participate in ionic cross-linking, sodium, potassium, calcium, zinc, magnesium and aluminium cations (Na^+, K^+, Ca^{2+}, Zn^{2+}, Mg^{2+}, Al^{3+}) were compared. All preliminary formulations were prepared and analysed in triplicate for observation of the ease of formation of a spherical non-aggregated morphology (Table 3.11).

Table 3.10: Solubility and key hydrational properties of electrolytes tested (USP 24, 2000; BP 1998; Chaplin, 2006)

Electrolyte	Solubility in Cold Water (g/cm^3)	Ionic Volume Cation – Atomic Volume	Cation ΔH_h^o (kJ/mol)	Ionic Volume Anion – Atomic Volume	Anion ΔH_h^o, (kJ/mol)
NaCl	1 in 2.8	-6.7	-406	+23.3	-76
KCl	1 in 2.8 to 1 in 3.0	+3.5	-320	+23.3	-76
$CaCl_2$	1 in 0.7	-28.9	-1579	+23.3	-76
$ZnCl_2$	1 in 0.5	-46.0	-2047	+23.3	-76
MgCl	1 in 0.9	-32.2	-1926	+23.3	-76
$AlCl_3$	1 in 2.8	-58.7	-4680	+23.3	-76
Na_2SO_4	1 in 2.5	-6.7	-406	+25	-215
$ZnSO_4$	1 in 0.6	-46.0	-2047	+25	-215
$MgSO_4$	1 in 0.8 to 1 in 1.5	-32.2	-1926	+25	-215

(ΔH_h^o: *Absolute Enthalpy of Hydration)*

Table 3.11: Preliminary enterosphere formulations

Form	NaCl	KCl	$CaCl_2$	$ZnCl_2$	MgCl	$AlCl_3$	Na_2SO_4	$ZnSO_4$	$MgSO_4$	Observation
B1	25	0	0	0	0	0	0	0	0	Well formed
B2	0	25	0	0	0	0	0	0	0	Well formed
B3	0	0	25	0	0	0	0	0	0	Well formed
B4	0	0	0	25	0	0	0	0	0	Poorly formed
B5	0	0	0	0	25	0	0	0	0	Well formed
B6	0	0	0	0	0	25	0	0	0	V. well formed
B7	0	0	0	0	0	0	25	0	0	Poorly formed
B8	0	0	0	0	0	0	0	25	0	V. well formed
B9	0	0	0	0	0	0	0	0	25	Well formed

3.5.1.3. Enterosphere Diameter Analysis

The size distribution of each of the enterosphere formulations was determined microscopically with a stereomicroscope (Olympus SZX7, Japan) connected to a digital camera (CC-12, Olympus, Japan) and image analysis system (AnalySIS® Soft Imaging System, GmbH, Germany) by measurement of the diameter of 10 randomly selected enterospheres (n=10). Results are

expressed as the mean±S.D. of 10 measurements of the longest Feret's diameter (d_f) for each formulation.

3.5.1.4. Encapsulation Efficiency of Enterospheres

Drug content was determined spectrophotometrically at 263nm by placing 100mg of INH-loaded enterospheres in a 200mL conical flask containing 100mL of 0.2M PBS, pH 6.8. The enterospheres were magnetically stirred for 5 hours to promote and ensure erosion and disentanglement of the cross-linked structure to afford liberation and subsequent dissolution of INH. These 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. The amount of drug entrapped in the enterospheres in each formulation was compared with the amount of drug, which was intended to be loaded in order to get the encapsulation efficiency as previously described in Equation 3.1.

3.5.1.5 *In Vitro* Release Studies on Enterospheres

Characterisation of INH release from the enterospheres was assessed using a method adapted from the USP 24 general drug release standard for delayed release (enteric-coated) articles in acidic and phosphate-buffered media (USP 24, 2000). Enterospheres equivalent to 10mg INH were placed in 50mL sealed vials. For determination of the amount of INH released under acidic conditions, 20mL of 0.1M HCl was added to the vials which were then subjected to agitation at 50rpm for 2 hours in a shaker water-bath (Labex SB040, Gauteng, South Africa) maintained at 37±0.5°C. For determination of INH release in basic media, the acid was drained from the vials

whilst retaining the enterospheres and replaced with 20mL of PBS (pH 6.8). Agitation was continued for a further 6 hours. Balancing withdrawal of 1mL aliquots was performed at the appropriate time intervals and samples were then analysed by UV at 263nm following appropriate dilution for determination of the fractional INH release.

3.5.2. Results and Discussion

The resultant matrices had a spherical morphology (Figure 3.14) and narrow size distribution (2067.5–2500.0μm) and were formulated without the use of organic solvents, harsh ingredients or time-consuming procedures. The mean diameter, drug entrapment and drug release from the preliminary enterospheres is represented in Table 3.12.

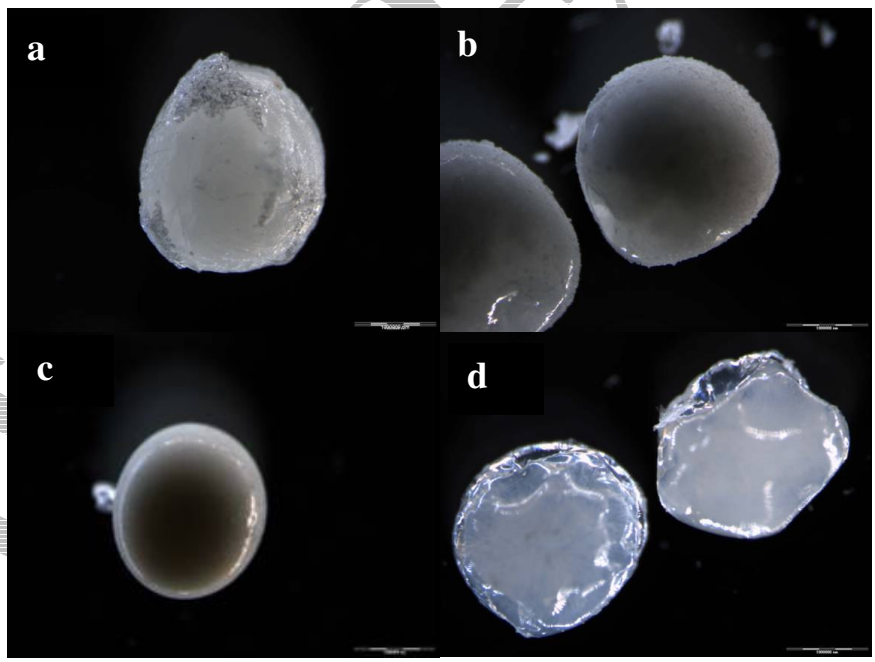

Figure 3.14: Stereomicrographs (darkfield, 16x magnification, scale bar=1cm) of representative enterospheres: salted-out employing (a) KCl (b) $CaCl_2$ (c) $AlCl_3$ and (d) $MgSO_4$

Table 3.12: Particle size, entrapment and release characteristics of preliminary enterosphere formulations

Formulation	Enterosphere Diameter (μm)	Drug Entrapment Efficiency (%)	Fractional INH Release (t_{2h})
B1	2500.00±240.42	48.56±0.53	0.381±0.019
B2	2207.50 ±141.75	48.69±7.74	0.474±0.003
B3	2515.00 ±162.17	36.33±0.76	0.756±0.065
B4	2137.50 ±241.02	18.96±1.95	0.589±0.041
B5	2352.50±230.13	45.15±3.93	0.472±0.042
B6	2067.50±149.97	26.40±0.72	0.962±0.076
B7	2220.00±266.46	70.66±0.90	0.405±0.011
B8	2170.00±199.50	69.80±3.02	0.109±0.007
B9	2272.50±145.23	53.91±0.54	0.400±0.040

In evaluating the enterospheres formulated via the salting-out approach, consideration must be given to the salting-out capabilities of the electrolytes investigated. The common mechanism underlying the systematic effects of the component ions remains obscure, but thermodynamic arguments require that the local concentration of a salting-out electrolyte be depleted in volume elements close to the polymeric macromolecule. Possibly, the depletion effect may arise from an incompatibility between different local hydrogen-bonding structures present in the water adjacent to the macromolecule and in the water of the ionic hydration shells (Al-Sagheer and Hey, 2004). The high concentrations (>1M) of neutral salts employed in this investigation result in a decrease in the solubilty of the copolymer, due to the competition between the copolymer and the ions for the water molecules, resulting in 'salting-out' of MAEA. Polymer-polymer interactions are favoured over polymer-solvent interactions at the high electrolyte concentrations due to lack of water molecules.

The ability of electrolytes to influence the conformation and stability of the copolymer depends on the concentration and ionic strength of the electrolyte. In addition, the salting-out (stabilising) action of different electrolytes increases with their hydration energy and steric hindrance. Originally described in connection with the effect of salts on protein solubility, the Hofmeister

series ranks ions in order of their effectiveness in promoting phase separation. According to the Hofmeister series, ions may be ordered as follows: $SO_4^{2-} \approx HPO_4^{2-} < F^- < CH_3COO^- < Cl^- < Br^- < NO_3^- < I^- < ClO_4^- < SCN^-$; $NH_4^+ < K^+ < Na^+ < L^+ < I^+ < Mg^+ < Ca^{2+}$, etc. Ions to the left ($SO_4^{2-} < F^- < CH_3COO^-$, NH_4^+, K^+) promote salting-out, aggregation and stabilisation of the native conformation. These ions that reduce solubility are referred to as 'structure-makers' or 'cosmotropes'. Ions to the right (I^-, ClO_4^-, SCN^-) promote unfolding, dissociation and salting-in (Damodaran, 1996). These ions that increase solubility are referred to as 'structure-breakers' or 'chaotropes'. Chaotropes are weakly hydrated and exhibit a smaller change in viscosity with concentration, having more negative hydration coefficients than cosmotropes that are strongly hydrated and have positive hydration coefficients (Chaplin, 2006).

Two general mechanisms may be proposed to account for exclusion of electrolytes from exposed surfaces. 'Crowding' is based on a steric exclusion, dependent only on solute size and shape, but not chemical nature. The spatial distribution of solute or salt concentration from the copolymer surface is determined by the nature of the forces underlying exclusion. Alternatively, the interaction of the enteric copolymer macromolecule and solute with water could be more favourable than direct solute-surface interactions also resulting in a 'preferential hydration'. In this case, the chemical nature of the solute is additionally important. The large literature on exclusion of small solutes (M_w< ~500g/mol) from proteins and nucleic acids indicates that exclusion does not only depend not only on the size but also the chemical nature of the solute (Chik et al., 2005).

With reference to Table 3.11, these effects may further be elaborated in terms of the ionic volumes of the respective anions and cations. Small ions are strongly hydrated, with small or negative entropies of hydration, creating local order and higher local density. Large singly

charged ions (e.g. Cl^-) are able to sit comfortably within dodecahedral water clathrate shells and produce the lowest apparent density for the water-based solution. Less large ions (e.g. K^+) cause the partial collapse of such clathrate structures through puckering. The puckered collapse of the water clathrate structures surrounding the smallest ions (e.g. Na^+), is tightly formed as these ions hold strongly to the first shell of their hydrating water molecules; hence there is less localised water molecule mobility and strong hydration (Dougherty, 2001). Generally, the water surrounding anions tends to retain favourable water-water hydrogen bonding whereas that surrounding small cations does not (Chaplin, 2006). Ultimately, the ionic volume of the oppositely charged electrolyte ions determines the electrolytes' solubilities.

The SO_4^{2-} anion was most effective at salting-out the enteric copolymer due to its greater propensity to induce a salting-out action, being appropriately positioned in the Hofmeister series as a structure-maker. For the sulphate salts, drug entrapment was satisfactory, ranging from 53.91 to 70.66% for INH, and fractional drug release at t_{2h} was comparatively low ranging from 0.109 to 0.405. Chloride salts were less effective in promoting salting out of the methacrylic acid copolymer than the corresponding sulphate salts. Drug entrapment ranged from 18.96 to 48.69% and fractional drug release ranged from 0.381 to 0.756 for the corresponding salts. Na as the chloride and sulphate salt demonstrated similar capabilities. Zn as the chloride salt was less effective in promoting the formation of enterospheres that effectively entrapped and controlled the release of INH in acidic media. A similar trend was observed for Mg as the chloride salt (Figure 3.15).

The contribution of the anion towards salting-out is greater than that of the cation of a particular electrolyte. The effectiveness of anions in salting-out macromolecules is thus generally highest for small, multivalent ions such as hydrogen phosphate (HPO_4^-) and sulphate (SO_4^{2-}) (Hatti-Kaul,

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2000). When Napper (1983) investigated the salting-out of polyvinyl acetate dispersions stabilised by polyoxyethylene, it was reported that the order of effectiveness for anions followed the Hofmeister series, but this was not the case for cations.

The salting-out effects of the cations employed in this investigation cannot be interpreted solely in terms of the competition for water between the polymers and the electrolyte because highly hydrated ions are not necessarily the best flocculants (Schick, 1987). Cations at the high order of the series (e.g. Ca^{2+}) have been purported to weaken intramolecular hydrophobic interaction and enhance the unfolding tendency of the polymer, however, certain cations may also promote varying degrees of ionic cross-linking due to the propensity of the anionic carboxylic acid groups of MAEA to undergo cross-linking when exposed to a suitable solution of cations. The copolymeric chains may cross-linked via the cations by the formation of complexes liganded with more than one polymer group creating intramolecular and/ or intermolecular cross-links. The methacrylic acid copolymer chains act as polydentate ligands in the complexation of di- and trivalent cations (Allain and Salome, 1990). Mg^{2+} and Zn^{2+} cations have been demonstrated to complex with available oxygens in the polymer (Hey et al., 2005). This accounts for the observed increase in gastroresistance of enterospheres formulated employing Mg^{2+} and Zn^{2+} cations. The cross-linking effect of these cations was only notably promoted, however, when coupled with the structure-making SO_4^{2-} anion. According to Hey et al. (2005) monovalent ions such as Na^+ and K^+ employed here are non-complexing and induce salting-out in accordance with the standard Hofmeister series for cations. Thus neutral salts of monovalent cations would induce a salting-out effect additive of the capabilities of the cation and anion. Although well-formed, the trivalent Al^{3+} did not prove to be effective in forming an enterosphere matrix, which favourably entrapped and/or controlled the release of INH possibly due to unfavourable steric interactions, which resulted in poor alignment or orientation of the copolymer chains.

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An overall qualitative explanation of phase separation of the aqueous copolymeric system in the presence of an electrolyte system relates the observed behaviour to the degree to which substitution of water-cation hydration associations occur by MAEA-carboxylic acid oxygen-cation interactions. Electrolytes with small multivalent anions of high-charge density are constrained from such interactions with the copolymer chain, leading to the presence of salt-depleted zones and consequent phase separation.

A key empirical feature both of Hofmeister effects and of neutral electrolyte exclusion from surfaces that distinguishes these interactions from direct binding, is the approximate linear dependence of free energy perturbations on electrolyte concentration. This linearity implies that preferential hydration coefficients are independent of electrolyte concentration (Chik et al., 2005).

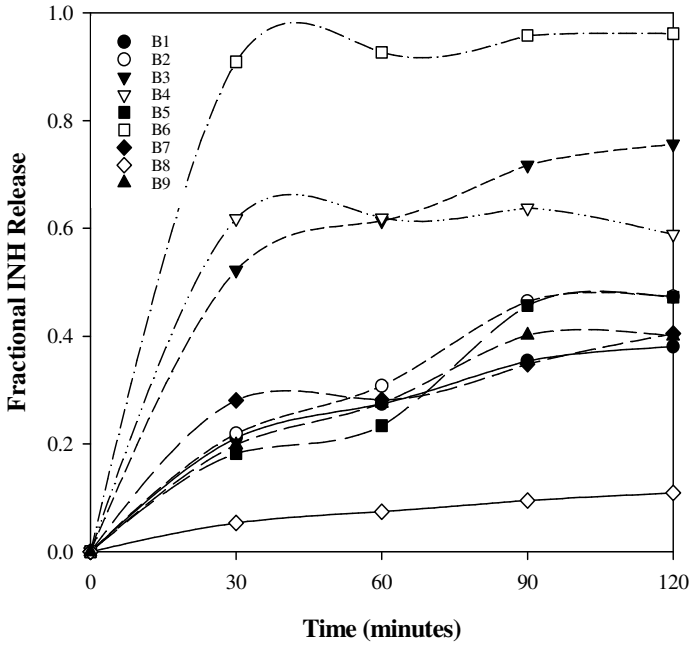

Figure 3.15: Drug release profiles of preliminary enterosphere formulations in acidic media (0.1M HCl, pH 1.2), (S.D. within ±0.076 in all cases)

$ZnSO_4$ had the most notable effect on INH release due to the ability of the SO_4^{2-} anion to induce the most favourable salting-out of a patent/unmitigated enteric polymeric film. Zn^{2+} in the salting-out solution and cross-linking solution demonstrated superior performance in relation to other cations for promoting gel shrinkage and the formation of intra- and intermolecular ionic cross-links within and between the polymer chains, producing a dense, interconnected enteric film and matrix in which drug entrapment was more likely and which retained its integrity in acidic dissolution media, slowing the release of INH through the reduced interstices of the enterosphere (Sriamornsak, 1999).

3.6. Treatment of Dissolution Data for Selection of a Candidate Enterosoluble Formulation

Dissolution data of the enterogranules, microenterospheres, enterospheres and a reference multireservoir enterosphere system, R1, were subjected to pairwise comparison using a model-independent dissolution approach in order to determine if any one of the preliminary formulations could be considered for optimisation, or if further modifications to a fabrication method were necessary. The resultant values generated by the model-independent approach do not depend on the selection of a specific parameter for fitting the data, but are dependent on the sampling times.

3.6.1. Methodology

R1 was employed as the reference formulation for pairwise comparison with the dissolution data of the enterosoluble formulations at time points 1 hour (t_{1h}) and 2 hours (t_{2h}).

In the pairwise approach, determination of a difference factor and a similarity factor (outlined in the SUPAC and IVIVC guidelines) using the mean percentage released values was performed using the following equations. The similarity factor (f_2) is a logarithmic reciprocal square root

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transformation of the sum of squared error and is a measurement of the similarity in the percent dissolution between the two curves:

$$f_2 = 50\log\left\{[1+\frac{1}{n}\sum_{i=1}^{n} w_t(R_t - T_t)^2]^{-0.5} \times 100\right\} \quad \text{[Equation 3.2]}$$

n is the number of pull points, w_t is the optional weight factor, R_t is the reference assay at time point t and T_t is the test assay at time point t. An f_2 value between 50 and 100 suggests that the dissolution profiles are similar. An $f_2$value of 100 suggests that the test and reference values are identical and as the value becomes smaller, the dissimilarity between release-profiles increases.

Moore and Flanner (1996) have also described a difference factor (f_1) as follows:

$$f_1 = \left\{\frac{\sum_{t=1}^{n}|R_t - T_t|}{\sum_{t=1}^{n} R_t}\right\} \times 100 \quad \text{[Equation 3.3]}$$

f_1 describes the relative error between the two curves, n is the number of time points, R_t is the dissolution value of the reference (pre-change) batch at time t, and T_t is the dissolution value of the test (post-change) batch at time t. The percent error is zero when the test and reference profiles are identical and increases proportionally with the dissimilarity between the two profiles. Generally, dissolution curves are considered equivalent when difference values are less than 15 and similarity values are greater than 50 (CDER, Center for Drug Evaluation and Research, 1997).

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3.6.2. Results and Discussion

The similarity and difference factors (f_2 and f_1) for the preliminary formulations appear in Table 3.13. The reference system, R1, instituting double encapsulation of the INH in a multireservoir enterosphere, enhanced the gastroresistance of the multiparticulate system, with less than 3.7% of the INH being released at t_{2h} (Figure 3.16). Industrially, however, the process for their fabrication may not be entirely feasible as time constraints and a greater number of processing steps would limit its application. Model-independent approach was thus instituted for identification of a candidate enterosphere formulation with a similar favourable release profile for further optimisation.

Enterosphere formulation B8, prepared by the salting-out approach and employing $ZnSO_4$ as the salting-out and cross-linking electrolyte, had an overall f_2 value >50 suggestive of a similar dissolution profile in acidic media for this formulation and the reference (Figure 3.16) and f_1 values at each time point close to zero suggestive of only a small relative error between the dissolution behaviour after 1 and 2 hours in acidic media of B8 and the reference. B8 proved to be the best formulation for controlling drug release in acidic media and was selected as the candidate formulation for further investigation and optimisation.

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Table 3.13: Similarity and difference factors of the preliminary enterosoluble formulations

Formulation	t_{1h}		t_{2h}	
	f_2	f_1	f_2	F_1
R1	100.00	0.00	100.00	0.00
Uncoated	9.22	88.32	8.93	25.03
E L 100-coated	7.77	94.38	9.49	24.39
E L 100: E S 100-coated	10.98	81.43	9.42	24.47
E S 100-coated	10.00	85.16	8.79	25.19
A1	26.64	39.57	18.39	12.93
A2	14.40	69.57	13.68	20.11
A3	18.72	56.99	15.14	18.80
A4	22.74	47.35	23.27	16.19
A5	33.04	29.46	27.71	10.54
A6	25.00	42.68	19.06	15.70
A7	40.73	20.65	30.19	9.40
B1	36.41	25.21	30.72	9.17
B2	33.84	28.39	25.53	11.65
B3	18.48	57.64	14.69	19.20
B4	18.28	58.17	20.44	14.73
B5	40.03	21.33	25.59	11.62
B6	9.42	87.49	9.24	24.68
B7	35.83	25.90	29.27	9.81
B8	66.67	6.12	64.37	1.91
B9	36.33	25.31	29.51	9.69

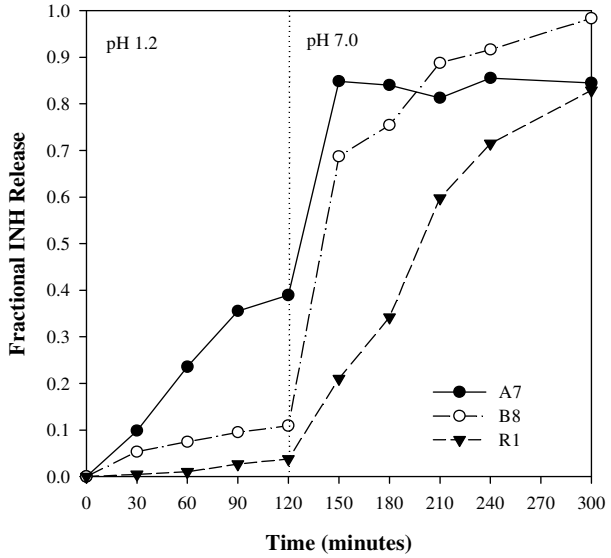

Figure 3.16: Composite drug release profiles for A7, B8 and R1 representative of the degree of similarity between R1 and the candidate formulation, B8

3.7. Concluding Remarks

Three microencapsulation methods for the formulation of enterosoluble multiparticulate formulations incorporating the water-soluble anti-TB drug, INH, were investigated for identification of a candidate formulation for further development and optimisation. The enterosoluble formulations entrapped and controlled the release of INH in acidic media to varying degrees. The enterogranules provided unsatisfactory bioactive release control in acidic media.

The initial burst release of INH was fairly high for microenterospheres formulated by the solvent evaporation method owing to initial dissolution of drug aggregates located close to the microparticle surface and then drug diffusion through the enteric matrix. For this system, the need for double entrapment of the water-soluble INH within a reservoir/multireservoir system would be warranted. Double encapsulation within a MAEA matrix employing a salting-out approach has demonstrated the ability to improve the gastroresistance of the system and succeeded in the achievement of a drug release profile in acidic media within the USP 24 specifications for drug release from enteric-coated dosage forms (<5% drug release after 1 hour and <10% drug release after 2 hours in acidic media) (USP 24, 2000). The large number of processing steps implicated in this method may escalate the cost of manufacturing the system, but its favourable release profile validated its implementation as a model system for fitting of drug release data.

The enterospheres formulated by the salting-out and cross-linking approach demonstrated varying degrees of gastroresistance, which showed dependence on the salting-out and cross-linking electrolyte employed. Use of the appropriate electrolyte succeeded in the fabrication of an

enteric-release system of adequate gastroresistance (10.9% INH release at t_{2h}). Institution of a model-independent approach aided in identifying a candidate formulation for optimisation.

The advantages of the selected candidate device over traditional MAMM- and MAEA-coated dosage forms can be anticipated, such as (i) the replacement of the coating process by a simpler less time consuming treatment without solvent vapours, (ii) cross-linking cations impart the enterosphere with a network structure and physical integrity, which is of utmost significance in drug delivery and dosage form design and (iii) there is a more gradual delivery of the dose to the designed site which, for many drugs and therapies, is more suitable than the dose dumping, typical of the traditional dosage forms. On the other hand, potential disadvantages of the present enterosphere matrices should be recognised, such as (i) the necessity of a drug load not exceeding the percolation threshold, and (ii) an incomplete inhibition of release in the protected GI zones (i.e. the stomach), due to some drug diffusion in the hydrated matrix (Carelli et al. 2000), however, this can be minimised by careful optimisation of the enterosphere to ensure adequate polymeric coalescence and cross-linking.

Furthermore, scale-up can be performed by the appropriate equipment currently available (i.e. extrusion apparatus) or by employing a spray-drying approach with various atomisation, drying and separation techniques.

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