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Research Article

FORMULATION AND EVALUATION OF METRONIDAZOLE TABLETED MICROSPHERES FOR COLON DRUG DELIVERY

SHIVANGI SINGH[1]*, NITHYA SHANTHI C[2], ARUN K MAHATO[2]

[1]Department of Pharmacy, IFTM University, Moradabad - 244 001, Uttar Pradesh, India. [2]Department of Pharmaceutics, SBSPGI, Balawala - 248 001, Dehradun, India. Email: shivangisingh.789@gmail.com

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ABSTRACT

Objective: The need of this study was to develop tableted microspheres that can be targeted to colon because metronidazole (MNZ) has good solubility at pH 1.2; hence, coating of the drug with the suitable pH dependent is done to prevent its release in the gastric region.

Methods: Colon targeted tablets of MNZ were prepared with enteric coated microspheres using pH dependent polymers such as cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, and Eudragit S 100 by solvent evaporation method. All the formulations were prepared by changing drug-polymer ratio from 1:1 to 1:5 and the interactions of the drug with polymers were studied by Fourier transform infrared and thermal analysis.

Results: Formulations F_5, F_8, and F_{14} were found to best optimized in percentage yield, drug entrapment efficiency, mean particle size and *in vitro* drug release. The result obtained were found in the desired ranges where % yield ranging from 52.56% to 98.253%, drug entrapment efficiency from 42.17% to 99.017%, and mean particle size from 36.774 to 229.961 µm. Then, tablet of optimized formulations was prepared by direct compression method and *in vitro* drug release was performed. All the parameters of tablets were found acceptable as per IP guideline. Around 4-10% drug release was in 0.1 N HCl after 2 hrs, 50% release at pH 7.4 phosphate buffer within 5 hrs, maximum retardation was found in the formulation of Eudragit S 100. Scanning electron microscopy permitted a surface topographical analysis.

Conclusion: The MNZ tableted microspheres showed their release at pH 7.4 thus this experimental work can be used to improve absorption of drug in colon for successful treatment of the disease.

Keywords: Metronidazole, Tableted microspheres, Solvent evaporation method, Direct compression method.

INTRODUCTION

Metronidazole (MNZ) is an amoebicidal drug, well known to kill the trophozoites mainly present in the colon [1]. Thus, the development of tableted microspheres is necessary by coating of the drug with the suitable pH dependent and delayed release polymer by which the drug can be targeted to colon as MNZ has a good solubility at pH 1.2 and the pH of the terminal ileum and colon is higher (pH - 6.8 and 8). Thus, coating of the drug with pH dependent polymer retards the release of drug from microsphere at low pH. Another problem associated with the MNZ is its bitter taste, which mainly lead to patient non-compliance. By entrapping the drug molecule into the polymeric layer, patient acceptance toward the drug can be increased. Microspheres are the carrier linked drug delivery system, in which particle size ranges from 1 to 1000 µm range in diameter having a core of drug and entirely outer layers of the polymer as coating material [2], constitute efficient carrier capacity by virtue of their small size [3,4]. With regards to the final dosage form and easy administration, the microspheres are usually formulated into single-unit dosage forms such as filling them into hard gelatin capsules or compressing them into tablets [5,6]. The tableted microspheres are preferred as a new approach in solid dosage form for oral drug delivery. Microspheres can be compressed into a tablet to vary the release properties of drugs [7].

METHODS

Materials

MNZ was taken as gift sample from Sunpharma New Delhi, cellulose acetate phthalate (CAP) and other chemicals used in the study were procured from CDH, Delhi. HPMC phthalate and Eudragit S 100 were taken from Yarrow Chem Product, Mumbai. Liquid paraffin was purchased from LOBA, India.

Identification of drug

The drug was identified by melting point determination (capillary tube method) on a silicone oil bath [8], Fourier transform infrared (FTIR) spectroscopy analysis on Shimadzu and differential scanning calorimeter (DSC) on precalibrated EXSTAR TG/DTA 6300.

Preparation of microspheres

The enteric microspheres were prepared by the solvent evaporation method. The formulation is given in Table 1. The polymer solution was prepared by dissolving it in acetone using a magnetic stirrer. The powdered drug was then dispersed in the polymer solution. The resultant solution was then poured into a vessel of 250 ml containing of liquid paraffin while stirring at the rate of minimum 1000 rpm. Stirring was continued at room temperature until acetone evaporated completely. After evaporation of acetone, the microspheres formed were filtered and washed 4-5 times with n-hexane. Finally, the washed microspheres were dried at room temperature and collected [9].

Characterization of microspheres

Percentage yield

The percentage yield of microspheres was calculated by dividing the weight of microspheres by the total weight of the added ingredients [10].

Percentage yield = The amount of microspheres obtained (g)/The theoretical amount (g) × 100

Drug entrapment efficiency

A weighed amount of drug loaded microspheres (equivalent to 25 mg of drug) was extracted using 10 ml of ethanol [10]. The solution was suitably diluted, and the absorbance was taken at $\lambda_{max.}$ The experiment

Table 1: Formulation table for microspheres

Formulation code	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	F_{13}	F_{14}	F_{15}
Drug (mg)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
CAP	100	200	300	400	500										
HPMCP						100	200	300	400	500					
Eudragit S 100											100	200	300	400	500

CAP: Cellulose acetate phthalate, HPMCP: Hydroxypropyl methylcellulose phthalate

was done in the triplicate. The drug entrapment efficiency was calculated using the following formula:

DEE = Actual drug content/Theoretical drug content × 100

Particle size analysis

The diameter of microspheres from each formulation was determined using an optical microscope. The samples were suspended in dispersion, and individual microspheres diameter were measured using micrometers. About diameter of 500 microspheres was measured, and the mean particle diameter was calculated [10].

***In vitro* release studies**

The drug release from the microspheres was carried out using the USP Type II dissolution paddle assembly. A weighed amount of enteric microspheres equivalent to 10 mg drug were dispersed in 250 ml of 0.1 N HCl (pH 1.2) maintained at 37±0.5°C and stirred at 100 rpm. 5 ml solution was withdrawn and replaced with fresh media after every 15 minutes up to 2 hrs. Then, dissolution medium was changed by pH 7.4 phosphate buffer. 5 ml solution was collected until a constant release was found and analyzed at λ_{max} [11].

Preparation of tableted microspheres

The optimized MNZ loaded microspheres were compressed to form tablet of 250 mg using microcrystalline cellulose as diluents, crospovidone as binder while magnesium stearate as a lubricant. Each tablet contains 10 mg drug, and the tablets were coded T_1, T_2, and T_3 for each batch. The amount of microspheres equivalent to 10 mg drug and amount of excipients used to prepare 250 mg tablet is given in Table 2 [12,13].

Evaluation of tablets

Thickness

Thickness of the tablets was measured by vernier calipers. Three tablets were selected randomly from all the batches.

Hardness test

Hardness of tableted microspheres was determined using hardness tester. Three tablets were randomly picked from each batch and analyzed for hardness.

Weight variation

From each batch 20 tablets were selected at random and weight was determined. Then, the tablets were weighed individually, and each weight was compared with an average weight.

Friability test

The friability of six tablets was determined using Roche Friabilator. The initial weight of these was noted. Then, all the tablets were weighed after friabilation. Friability can be determined by the following equation:

$$\% \text{ Friability} = Wt_{initial} - Wt_{final} / Wt_{initial} \times 100$$

***In vitro* disintegration test**

The tablet disintegration was carried out by placing one tablet in each tube (6 tablets) of the basket, and the assembly was suspended in a beaker containing 0.1 N HCl (gastric pH 1.2) and operated without the disc for 120 minutes by maintaining temperature at 37±2°C. The experiment was carried out in triplicate [14].

Table 2: Formulation table for tableted microspheres

Ingredients	% composition		
	T_1	T_2	T_3
Microspheres	57	35.2	49
Crospovidone	4.56	2.816	3.92
Magnesium stearate	1.14	0.704	0.98
Micro crystalline cellulose	37.3	61.28	46.1

***In vitro* drug release studies**

The dissolution studies of marketed tablets and tableted microspheres were performed. Initially, dissolution was carried out in pH 1.2 for 2 hrs, and then, in pH 7.4 phosphate buffer using USP Type II Paddle method at 50 rpm until the drug completely released from the tablet under sink condition at 37±0.5°C. At specific time intervals, aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium to maintain a constant volume. After suitable dilution, the samples were analyzed λ_{max} 317.6 nm [15].

Accelerated stability testing

The accelerated stability testing of the optimized tablet was done according to ICH guidelines. Three set of formulation was packed in high-density polyethylene bottle as study would be done for 3 months. In an oven 40,°C temperature and 75% humidity was maintained, and these sets of formulations were kept. After every 1 month, one set of each formulation was removed from the oven, and the analysis of drug content and *in vitro* drug release were performed and compared with the control sets [15].

RESULTS AND DISCUSSION

The melting point of MNZ was found to be 161.33°C±0.577. The reported valve of melting point is 159-163°C [8]. From the result of melting point determination of drug, the drug was identified as MNZ. The reported peaks (cm^{-1}) of MNZ by FTIR spectroscopy of -OH, -C-CH, -N-O, -C-O, and -C-N assignments were 3230, 3105, 1538 and 1375, 1078, and 830, respectively, whereas the observed peaks (cm^{-1}) were found to be 3228.09, 3096.03, 1538.76 and 1372.41, 1074.87, and 818.59, respectively (Fig. 1). The DSC of the drug showed a sharp endothermic peak at 160°C for a pure MNZ as the melting point of drug (Fig. 2).

After comparing the FTIR spectra of given drug and physical mixture of drug-polymer (Fig. 3), it was found that there were prominent peaks of functional group in physical mixture those can be identified in the pure drug spectra. This revealed that there was no interaction between drug and polymers used to prepare the microspheres. The DSC of polymer, thermal transition occurs at 397°C (Fig. 4), which is attributed to the melting point of the Eudragit polymer. In the physical mixture of drug and polymer, the endothermic peak was observed at 160°C as the melting point of the drug. In case of the blank microspheres, an endothermic peak at 80°C was observed due to dehydration of water at the surface. The drug might have been dispersed in crystalline and amorphous form or dissolved in the polymeric matrix during formation of microspheres. After 250°C where the polymer is in the form of liquid, a mild interaction between polymer or drug or degradation may be occurred. The evaluation of thermograms revealed that there was no physical or chemical interaction found between drug and polymer. For a pure polymer, thermal transition occurred at 222°C and 397°C

attributed to the glass transition temperature (T_g) of polymer (Fig. 5). The thermogram of the physical mixture showed almost the same melting peaks at 160°C and 281°C with some lowering in enthalpy valves might be due to reduction in purity of the drug.

The microspheres were optimized with different polymers by changing the volume of external phase, stirring rate, evaporation rate, and evaporating surface area. The change in volume of external phase varied percentage yield, drug content, and particle size. It might be attributed to the fact that larger amount of external phase than 30 ml solubilized the microspheres formed. The stirring speed affects the percentage yield and particle size distribution of microspheres. Above 1000 rpm stressing speed caused breaking of microspheres. Evaporation rate also affected percentage yield and drug content. Below 2 hrs of evaporating rate resulted in incomplete emulsification of microspheres. Use of small volume beaker for dispersion of microspheres resulted in higher yield and entrapment efficiency.

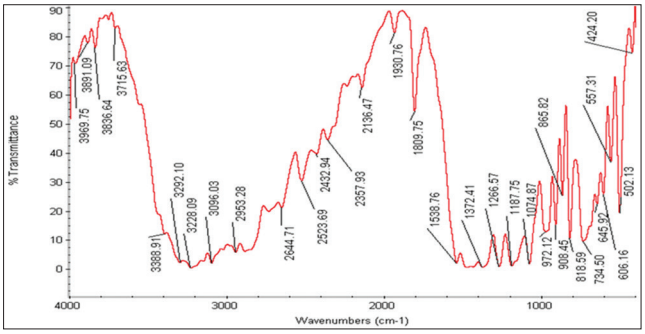

Fig. 1: Fourier transform infrared spectra of drug

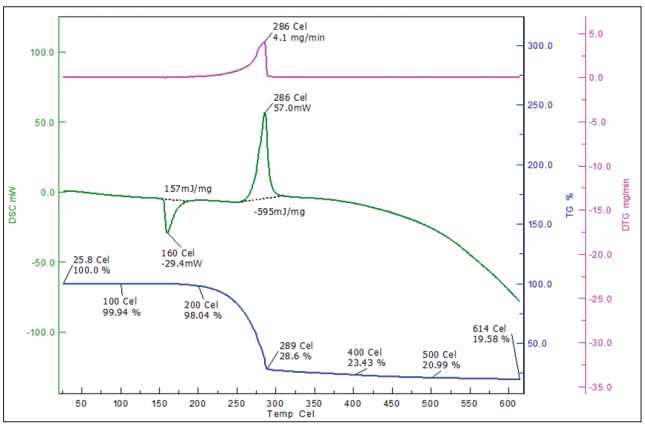

Fig. 2: Differential scanning calorimetry spectra of drug

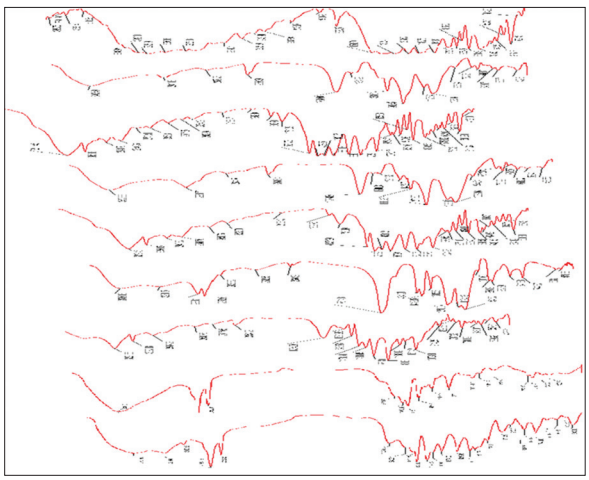

Fig. 3: Fourier transform infrared spectra of drug, cellulose acetate phthalate (CAP): Physical mixture of drug - CAP, hydroxypropyl methylcellulose phthalate (HPMCP): Physical mixture of drug - HPMCP, Eudragit S 100: Physical mixture of drug-eudragit S 100, blank formulation F_{14}, formulation F_{14}

Characterization of microspheres

Percentage yield of CAP microspheres ranges from 74 to 96, hydroxypropyl methylcellulose phthalate (HPMCP) microspheres range 52-90%, and microspheres range 63-98%. The drug entrapment efficiency of CAP microspheres varied from 42 to 94%, from 37 to 80% for HPMCP microspheres, and from 71 to 86 for Eudragit S 100 microspheres, respectively (Fig. 6).

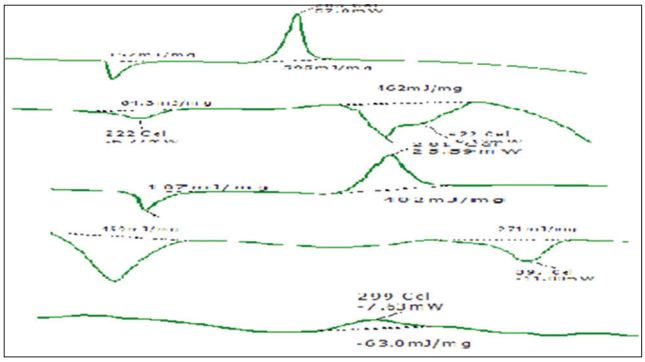

Fig. 4: Differential scanning calorimeter spectra of drug, Eudragit S 100: Physical mixture of drug-eudragit S 100, blank formulation F_{14}, formulation F_{14}

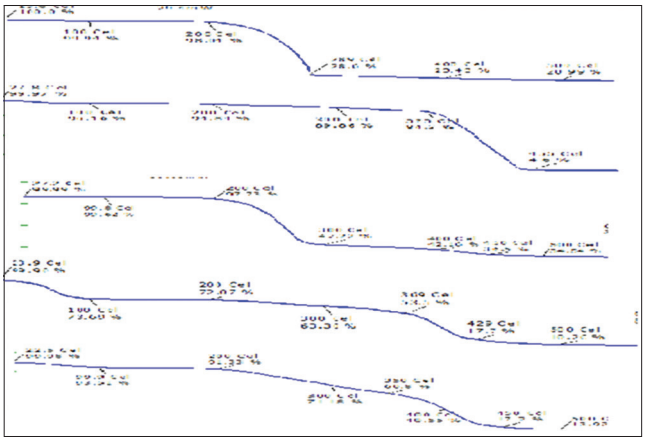

Fig. 5: T_g spectra of drug, Eudragit S 100: Physical mixture of drug-eudragit S 100, Blank formulation F_{14}, Formulation F_{14}

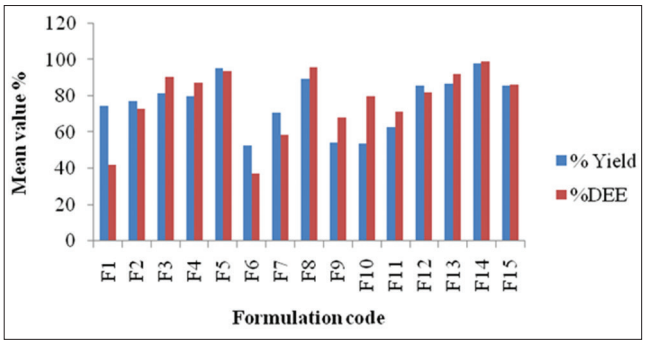

Fig. 6: % Yield and drug entrapment efficiency of different formulations of microspheres

The particle size analysis was performed on the 500 microspheres (Fig. 7). The mean particle size of microspheres ranged from 65 to 524 for CAP, 36 to 166 for HPMCP, and 63 to 256 for Eudragit S 100, respectively. It is found that by increasing the drug-polymer ratio, there is a shift toward the higher particles. Higher concentration of polymer produced a more viscous dispersion which formed larger droplets and consequently larger microspheres. The drug entrapment efficiency was found to be higher for Eudragit S 100 microspheres than the CAP and HPMCP microspheres because of its electric charge reduction [16]. The entrapment efficiency increased with increase in polymer concentration. An increase in polymer concentration resulted in the formation of larger microspheres entrapping greater amounts of the drug [17]. In case of particle size, it was found that mean size increased with increase in polymer concentration. Increasing polymer concentration produced a significant increase in the viscosity [16], thus leading to an increase of emulsion droplet size and finally a larger microsphere size [16], who suggested that the higher concentration of polymer in the sample had led to an increased frequency of collision, resulting in fusion of semi-formed particles and producing an overall increase in size of microspheres. In additional, the high viscosity of organic phase tends to restrict migration of internal oil phase to external oil phase.

The surface morphology and structure of microspheres were investigated using SEM. The surface of CAP microspheres was smooth, spherical, and exhibited pores on its surface (Fig. 8). Such pores were due to the interconnectivity of internal phase droplets during the final stage of solvent evaporation [16, 18]. Whereas the surface of HPMCP and Eudragit S 100 microspheres were rough and exhibited large pores and cracks within crystalline drug on the surface of the microspheres (Figs. 9 and 10).

The polymer solidified at the same time as the hardcore was formed, resulting in the creation of coarse microspheres as reported [19]. The pores might form passages to help the drug release from inner pores of microspheres (Fig. 11). The crystals of MNZ adsorbed on the surface of microspheres contribute to a burst release and help to achieve effective concentration quickly after oral administration [20].

In vitro release profile of microspheres showed that increase in polymer concentration; decreased rate of drug release from microspheres.

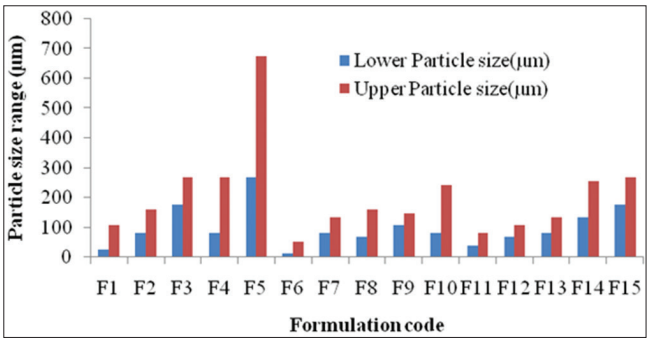

Fig. 7: Particle size range of different formulation of microspheres

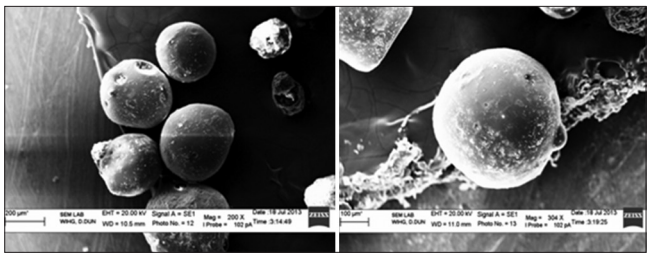

Fig. 8: Scanning electron micrograph of cellulose acetate phthalate microspheres

Around, 12% of drug was found to be released in 0.1 N HCl (pH 1.2) in CAP microspheres. Once the media changed to buffer of higher pH, 40% of drug was released in initial hours. At pH 7.4 more than 50% of drug released within 3 hrs and more than 90% of drug was released within 4.5 hrs (Fig. 9). The HPMCP microspheres showed about 7-9% of drug release within 2 hrs at 0.1 N HCl, which is not significant. More than 40% of released in pH 7.4 phosphate buffer within 3 hrs and 70-90% of drug released within 4 hrs (Fig. 10). Since the acrylic polymer used is not soluble in acidic pH and starts to dissolve above pH 7, no significant amount of drug was released in 0.1 N HCl after 2 hrs, around 4% of drug was released in 0.1 N HCl after 2 hrs. 50% of drug released within 5 hrs at pH 7.4 phosphate buffer (Fig. 12).

Evaluation of tablets

The optimized microspheres formulation among above were compressed into the tablet form, and they were evaluated for various parameters such as thickness, hardness, weight variation, friability, *in vitro* disintegration test, and *in vitro* dissolution testing. The various evaluation parameters of tableted microspheres are given in Table 3. The scanning electron microscopy of microspheres of tableted microspheres (T_3) containing individual microspheres scattered. There was no visible damage to microspheres. The microspheres inside the tablet maintained their shape with no significant changes in their surface properties (Fig. 13).

The tableted microspheres showed release of about 10% in CAP microspheres, 5% in HPMCP tableted microspheres, and about 2% in case of Eudragit S 100 within 2 hrs in 0.1 N HCl. The following are the rank order of the drug release T_1 (CAP) > T_2 (HPMCP) > T_3 (Eudragit S 100) (Fig. 12). Eudragit S 100 tableted microspheres showed slower release than CAP and HPMCP in pH 6.8 maximum of about 7%, whereas CAP showed release of 61% and HPMCP around 53%. This might be due to the difference in solubility of polymer and interaction of the drug with polymer. The *in vitro* drug release of MNZ loaded tableted microspheres was affected strongly by the pH of media.

One of the most important properties of a delayed release system is its resistance against the gastric condition. It is required that no more than 10% drug degradation would occur after 2 hrs in 0.1 N HCl [21]. When these tableted microspheres were kept in 0.1 N HCl for 2 hrs, the structural integrity of microspheres was almost maintained. However, when these microspheres were kept in pH 7.4 phosphate buffer for 3 hrs, the microspheres progressively developed pores and tortuous

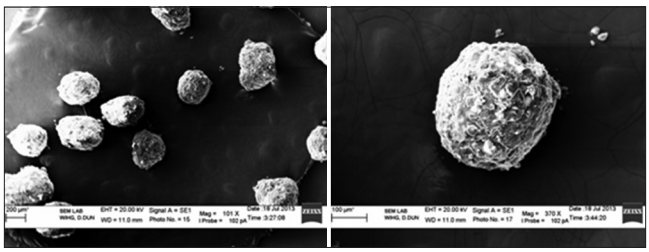

Fig. 9: Scanning electron micrograph of hydroxypropyl methylcellulose phthalate microspheres

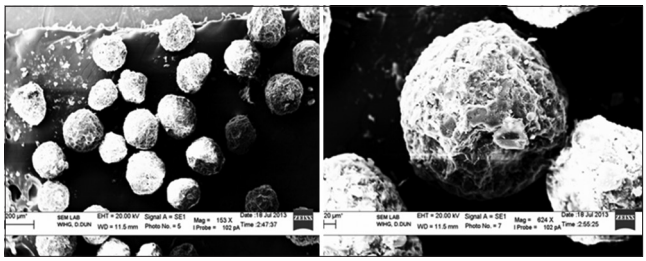

Fig. 10: Scanning electron micrograph of Eudragit S 100 microspheres

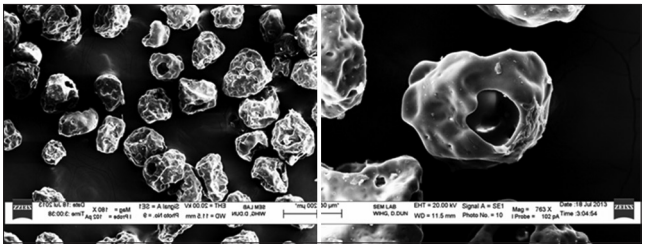

Fig. 11: Microspheres after drug release

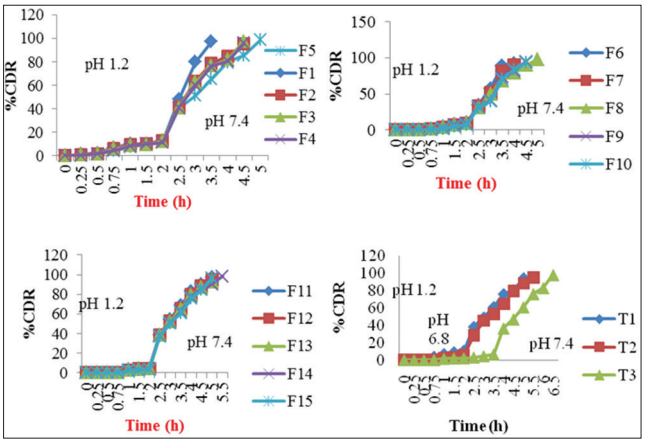

Fig. 12: *In vitro* release studies of different formulation of microspheres and tableted microspheres

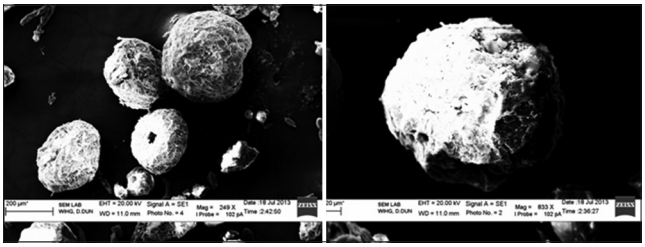

Fig. 13: Scanning electron micrograph of tableted microspheres

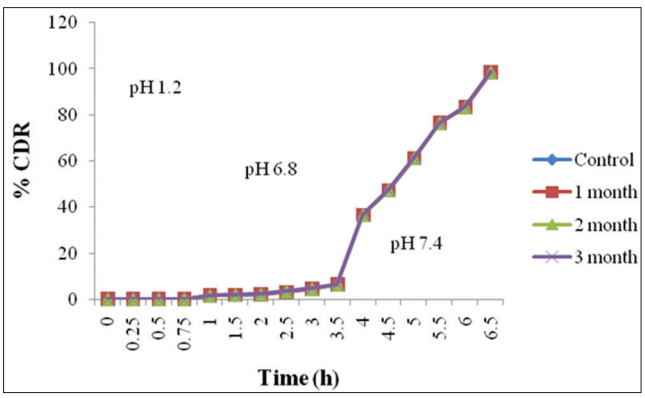

Fig. 14: *In vitro* release studies of T_3 tableted microspheres after accelerated stability

pathways which may be the reason for the uniform release of drug. The rate of drug release from the microspheres is also dependent on the polymer concentration of the prepared system, which indicates that the release rate decreases with increasing amount of polymer. This can be explained by a decreased amount of drug present close to the surface and also by the fact the amount of uncoated drug decreases with higher polymer concentration [16]. Further, smaller microspheres are formed at a lower polymer concentration and have a larger surface area exposed to dissolution medium giving rise to faster drug release.

Table 3: Evaluation parameters of tableted microspheres

Evaluation parameters	Tableted microsphere		
	T_1	T_2	T_3
Thickness (mm)	3.85±0.035	4.34±0.03	4.02±0.015
Hardness (kg/cm^2)	5.69±0.02	5.60±0.015	5.74±0.03
Weight variation (mg)	252.33±1.15	248.33±0.57	252.66±0.57
Friability (%)	0.8	0.78	0.8
Disintegration time (min)	56±1	57.66±2.30	58.33±0.57
Drug content (%)	93.893±0.01	95.345±0.02	98.817±0.02

Accelerated stability of T_3 formulation was performed at 40°C±75% RH for 3 months. The percentage drug content and *in vitro* drug release studies were performed after every month for 3 months. The result of percentage drug for control was 98%, whereas after 3 months was 97.56% (Fig. 14). The result was interoperated after similarity factor. The similarity factor was found 99.76%, which is in the range 50-100%. Thus, the formulation was considered to be stable.

CONCLUSION

The MNZ tableted microspheres showed their release at pH 7.4 thus this experimental work can be used to improve absorption of drug in colon for successful treatment of the disease. As MNZ has good solubility at gastric pH and coating of drug with pH dependent polymer retards, its release in pH 1.2. Among all the formulations tablet of F_{14} formulation (T_3) gave the good release. Its 3% part released in 0.1 N HCl in 2 hrs, about 7% in pH 6.8 phosphate buffers, and rest of drug was released in pH 7.4 phosphate buffer. There was 50% release in 4.5 hrs and 98% release in 6.5 hrs.

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