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Local delivery of Antiparasitic drugs to the colon as a treatment for Colonic Diseases

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ABSTRACT

Amoebiasis is an infection of the large intestine caused by *Entamoeba histolytica*, and it is mainly present in the intra-intestinal lumen. The efficient treatment of amoebiasis and other colonic infections could be achieved by targeting the drug to the colon. Tinidazole is the drug of choice for intestinal amoebiasis and other colon infections and the best approach for this drug is to target the drug delivery to colon which would make the drug effective with low dose and prevent the potential hazards observed in conventional dose. The objective of the present investigation was to design a multiparticulate delivery system for site-specific delivery of Tinidazole using natural polysaccharides (pectin) and pH-sensitive polymer (Shellac) for the treatment of colonic diseases. An attempt was made to prepare and characterize Tinidazole microspheres for colon specific drug delivery in order to target the drug to the colon. Pectin microspheres were prepared using emulsion cross- linking technique. These microspheres were coated with Shellac using oil-in-oil solvent evaporation method. The method was optimized using different drug: polymer ratio (1:2, 1:3, 1:4 and 1:5) stirring rate (500, 1000, 1500, and 2000) and emulsifier concentration (1%, 1.25%, 1.5% and 2%) to produce microspheres of small size and narrow size distribution, high drug loading efficiency, and controlled drug release at the colonic pH. Microspheres prepared by using drug: polymer ratio 1:3, stirring speed 1000 rpm, and 1.25% w/v concentration of emulsifying agent were selected as an optimized formulation. Microspheres were evaluated for surface morphology, particle size and size distribution, swellability, percentage drug entrapment, in- vitro drug release in simulated gastrointestinal fluids (SGF) and stability study. The experimental results demonstrated that the prepared microspheres of Tinidazole for colon targeting may reduce the side effects of the drug caused by its absorption from the upper part of GIT when given in conventional dosage forms.

Key words: Tinidazole; Amoebiasis; Colon targeting; Shellac; Pectin microspheres.

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INTRODUCTION

Most of the conventional drug delivery systems for treating colonic disorders, infectious diseases and colon cancer are failing as the drug do not reach the site of action in appropriate concentration. Thus an effective and safe therapy of the colonic diseases using the site specific drug delivery system is a challenge to the pharmaceutical technologists. The major obstacles to delivery of drugs to the colon are the absorption and degradation pathways in the upper GIT. However a successful design of colon targeted system can overcome the obstacles. Thus colon has proven to be a potential site for local as well as systemic administration of drugs. Colon has a long retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs[1].

Amoebiasis is an infection of the large intestine caused by *Entamoeba histolytica*. The trophozoites of *E. histolytica* can invade the colonic epithelium, causing amoebic colitis[2].

Tinidazole, 1-(2-ethylsulfonylethy) l-2-methyl-5 nitroimidazole), is the drug of choice for intestinal amoebiasis and other colonic diseases such as crohn's disease, ulcerative colitis and colorectal cancer[3].

The oral bioavailability of tinidazole is about 100 percent but there are some potential hazards such as peripheral neuropathy and convulsive seizures if the drug is given by conventional dosage form which provides minimal amount for local action in the colon, and as conventional dosage form is absorbed from the stomach, side effects like nausea, metallic taste, vomiting and headache are also observed. The efficient treatment of amoebiasis and other colonic diseases could be achieved by targeting drug to the colon and the best approach is a colon targeted specific drug delivery which would make the tinidazole effective with low dose and prevent the potential hazards[4].

Hence, in the present study an attempt was made to design and characterize an oral site specific drug delivery containing tinidazole targeted to colon in pH and biodegradable manner.

MATERIALS AND METHODS

Chemicals

The drug, Tinidazole (TNZ) was purchased from Mundi Pharma, Merrut, India. Pectin and Shellac was obtained from HiMedia Laboratories Ltd, Mumbai, India. Acetone, n-Hexane, and Light liquid paraffin were purchased from Qualigens Fine Chemicals, Mumbai. Span 80 was obtained from S. D. Fine Chemicals, Mumbai. Pectinase was procured from HiMedia Laboratories Ltd, Mumbai, India. All other chemicals used were of analytical reagent grade and

were used as received.

Fabrication of Tinidazole (TNZ) loaded pectin microspheres:

Pectin microspheres were prepared by emulsion cross-linking method[5, 6]. Pectin dissolved in 20 ml of distilled water and uniform solution was prepared. Dispersion of tinidazole (TNZ), prepared by dispersing tinidazole in 10 ml of dichloromethane, was added to the uniform polymeric solution with stirring. To produce an emulsion aqueous polymeric solution containing drug molecules was dispersed in 40 ml of light liquid paraffin containing Span 80 (1.25%w/v) and stirred at 1000 rpm continuously to obtain stable w/o emulsion. The solution was rapidly cooled to 15°C by placing the beaker in an ice bath. After 20 min of stirring 10 ml of 1.3% w/v $CaCl_2$ was added gradually to the system and stirred for 1hr (allows the time for cross-linking). Resultant microspheres was filtered and washed with n-hexane and then dried

Microencapsulation of Pectin microspheres:

Pectin microspheres were microencapsulated by emulsion–solvent evaporation technique[7]. The Pectin microspheres (100 mg) were suspended in 20 ml of coating solution prepared by dissolution of shellac (500 mg) in ethanol - acetone mixture and then emulsified into 40 ml of light liquid paraffin containing Span 80. The emulsification process was carried out for 2 h at 1000 rpm with mechanical stirrer. The Shellac coated microspheres were collected and rinsed with n-hexane and dried.

The method was optimized using different drug : polymer ratios (ie, 1:2, 1:3, 1:4, and 1:5), stirring rate (500, 1000, 1500, and 2000) and emulsifier concentration (1%, 1.25%, 1.5% and 2%) to produce microspheres of small size and narrow size distribution, high drug loading efficiency, and controlled drug release at the colonic pH.

Table 1: Process conditions for different batch formulations of TNZ microspheres

Formulation	Tinidazole/ Polymer ratio (gm)	Span 80 (% wt/v)	Stirring speed (rpm)	Volume of Internal phase (ml)	Cross- linking agent (% wt/v)
F1	1 : 2	1.25	1000	40	1.3
F2	1 : 3	1.25	1000	40	1.3
F3	1 : 4	1.25	1000	40	1.3
F4	1 : 5	1.25	1000	40	1.3
F5	1 : 3	1.00	1000	40	1.3
F6	1 : 3	1.50	1000	40	1.3
F7	1 : 3	2.00	1000	40	1.3
F8	1 : 3	1.25	500	40	1.3
F9	1 : 3	1.25	1500	40	1.3
F10	1 : 3	1.25	2000	40	1.3

Prepared microspheres were evaluated for following parameters:

Particle size analysis

Particle size distribution of the microspheres was determined by optical microscopy using calibrated ocular eyepiece. Product dispersed in light liquid paraffin and a smear of the dispersion was observed under compound microscope.

The size of 100 microspheres was measured in each case against a calibrated eyepiece in micrometer[8].

Determination of shape and spherecity

Morphological appearance and surface characteristics of the microspheres were studied by dispersing the microspheres in liquid paraffin and observed under microscope[8].

Scanning Electron Microscopy

The shape and surface morphology of microspheres were investigated using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (Jeol JSM-1600, Tokyo, Japan) [9].

Percentage yield

Percentage practical yield is calculated to know about percentage yield or efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of microspheres recovered from each batch in relation to the sum of starting material. The percentage yield of prepared microspheres was determined by using the formula[9].

$$\% \text{ yield} = \frac{\text{Total wt of microparticle}}{\text{Total wt of drug and polymer}} \times 100$$

Determination of drug content

Microspheres were accurately weighed (50 mg), triturated and digested in 10 ml pectinase solution (4% wt/v) and kept overnight for extraction of drug for the determination of entrapment efficiency. The digested homogenate was centrifuged and supernatant was collected. After appropriate dilution of supernatant with pH 7.4 phosphate buffer, aliquots were assayed by UV spectrophotometer at suitable wavelength. Corresponding drug concentrations in the sample were calculated from the calibration curve[10, 11].

Determination of % drug entrapment

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula:

$$\text{Drug Entrapment Efficiency (\%)} = \frac{\text{Experimental Drug Content (mg)}}{\text{Theoretical Drug Content (mg)}} \times 100$$

Theoretical drug content was determined by calculation assuming that the entire drug present in the pectin solution used gets entrapped in microspheres and no loss occurs at any stage of preparation of microspheres[10, 11].

Swellability / Degree of Swelling

The swelling ability of the microspheres on physiological media was determined by suspending them in the PBS buffer (pH 7.4). Accurately weighed amount (100 mg) of various TNZ-loaded pectin microspheres and shellac-coated pectin microspheres were placed in enzyme-free simulated intestinal fluid (pH 7.4 Phosphate buffer) in vials and allowed to swell for the required period of time. The microspheres were periodically removed and blotted with filter paper; then their change in weight (after correcting for drug loss) was measured until attainment of equilibrium[10, 11]. Degree of swelling was then calculated using the following formula:

$$\text{Degree of swelling} = (W_g - W_i) / W_g \times 100$$

Where W_i, initial weight of microspheres; and W_g, final weight of microspheres.

In vitro drug release study

Microspheres were evaluated for the *in vitro* drug release in simulated GI fluids (SGF). The drug dissolution test of microspheres was carried out using USP rotating basket method. Microspheres (100 mg) were weighed accurately and placed in the dissolution medium. The content was rotated at 50 rpm at 37°C ± 0.5°C. Perfect sink conditions prevailed during the drug dissolution study period. The simulation of GI transit condition was achieved by altering the pH of dissolution medium at different time intervals. The pH of the dissolution medium was kept 1.2 for 2 hours using 0.1 N HCl. Then KH_2PO_4 (1.7 g) and $Na_2HPO_4.2H_2O$ (2.2 g) were added to the dissolution medium, adjusting the pH to 6.8 with 1.0 M NaOH, and the release rate study was continued for an additional 5 hours. After 5 hours, the pH of the dissolution medium was adjusted to 7.4 with 0.1 N NaOH and Simulated to colonic fluid by addition of 4 % wt/v pectinase enzyme and maintained this condition up to 24 hours. The samples were withdrawn from the dissolution medium at various time intervals using a pipette fitted with a microfilter (0.45-µm). The rate of drug release was analyzed at 318 nm using UV Spectrophotometer. The receptor volume was maintained constant by replacing equivalent amount of simulated

gastrointestinal fluid. The concentration of drug in the samples was calculated based on average calibration curves (n = 3). All dissolution studies were performed in triplicate[12, 13].

Kinetic treatment of dissolution data

Data obtained from *in vitro* release studies were fitted to various kinetics equations to find out the mechanism of drug release from microspheres. The kinetics models used were zero order, first order and Higuchi models. The rate constants were also calculated for the respective models[14].

Stability Studies

The stability studies were performed as per ICH guidelines at temperature of 40º C / 75% RH (Long term stability study) for 3 months. The optimized formulation was analyzed for drug content and % drug release[15].

RESULT AND DISCUSSION

Preparation of Shellac-coated Pectin Microspheres

Pectin microspheres of TNZ were successfully prepared by emulsion cross-linking technique. The pectin microspheres were coated with Shellac by oil-in-oil solvent evaporation method, using coat: core ratio 5:1. Surface morphology and internal cross-sectional structure of the microspheres were investigated with a scanning electron microscope. The microspheres were smooth, spherical and discrete particles. SEM photographs are shown in Figure 1 & 2. The method was optimized using different stirring rate and emulsifier concentration to produce microspheres of small size and narrow size distribution, high drug loading efficiency, and controlled drug release at the colonic pH.

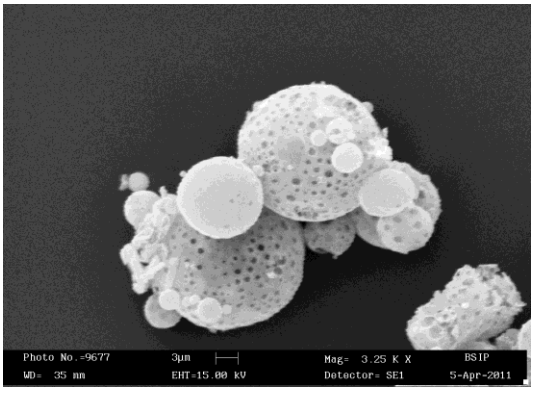


Figure 1. SEM photograph of S-F2

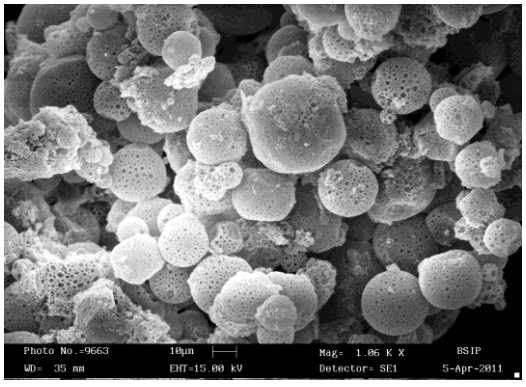


Figure 2. SEM photograph of S-F7

The mean diameter of pectin microspheres varied from 28.55 ± 0.23µm to 32.66 ± 0.07 µm with varying pectin concentration from 2% wt/vol to 5% wt/vol. The percentage drug entrapment was found in the range of 80.17 ± 0.06 µm to 72.47 ± 0.16 µm on varying pectin concentration from

2% wt/vol to 5% wt/vol. The highest drug loading efficiency was found with 2% pectin (Table 2). A higher concentration of polymer produced a more viscous dispersion, which formed larger droplets and consequently larger microspheres. In the study of effect of emulsifier concentration on formation of microspheres, the mean diameter of microspheres was found to vary from 33.31 ± 0.13µm to 27.54 ± 0.17 µm on varying emulsifier concentration (Span 85) from 1% wt/vol to 2% wt/vol for pectin microspheres. Increased surfactant concentration led to the formation of particles with a lower mean geometric diameter. Increasing Span 85 concentration from 1% to 2% wt/vol led to stabilization of the emulsion droplets avoiding their coalescence, resulting in smaller microspheres. The drug loading efficiency varied from 76.32 ± 0.16% to 79.32 ± 0.08% with varying emulsifier concentration from 1% to 2% during preparation of pectin microspheres (Table 2). The mean diameter of pectin microspheres decreased from 34.29 ± 0.14 µm to 28.43 ± 0.21 µm with increasing agitation speed of the mechanical stirrer from 500 rpm to 2000 rpm. This result was expected because high stirring rates provide the shearing force needed to separate the oil phase into smaller globules. The stirring speed of 1000 rpm was found to be optimum for pectin microspheres, as the drug loading efficiency was 77.57 ± 0.09% at this speed (Table 2). High stirring speed produced an irregular shape of microspheres but a slightly increased entrapment efficacy was found. Swellability of different microspheres was determined. No significant swelling was observed with Shellac-coated pectin microspheres as compared with pectin microspheres (Table 3), thus ensuring better resistance of Shellac-coated microspheres in the upper GI tract to swelling and preventing subsequent drug release at the nontarget site.

Table 2: Effects of process variables on mean particle size, % yield and entrapment efficiency

Process variables	Formulation code	Mean Particle Size (µm)	Percentage yield	Percentage Drug Entrapment
Effect of drug :polymer ratio	F1 (1:2)	28.55 ± 0.23	84.36 ± 0.27	80.17 ± 0.06
	F2(1:3)	29.41 ± 0.17	80.32 ± 0.21	77.57 ± 0.09
	F3(1:4)	32.27 ± 0.16	82.29 ± 0.16	73.39 ± 0.19
	F4(1:5)	32.66 ± 0.07	81.32 ± 0.15	72.47 ± 0.16
Effect of surfactant concentration	F5 (1%)	33.31 ± 0.13	79.34 ± 0.21	76.32 ± 0.16
	F2 (1.25%)	29.41 ± 0.17	80.32 ± 0.21	77.57 ± 0.09
	F6 (1.50%)	28.36 ± 0.16	80.75 ± 0.16	77.28 ± 0.10
	F7 (2%)	27.54 ± 0.17	78.25 ± 0.09	79.32 ± 0.08
Effect of stirring speed	F8 (500)	34.29 ± 0.14	86.27 ± 0.09	76.74 ± 0.25
	F2 (1000)	29.41 ± 0.17	80.32 ± 0.21	77.57 ± 0.09
	F9 (1500)	30.86 ± 0.08	82.86 ± 0.08	78.33 ± 0.16
	F10 (2000)	28.43 ± 0.21	79.33 ± 0.16	79.48 ± 0.14

Table 3: Degree of Swelling of Various Pectin Microspheres and shellac-coated Pectin Microspheres

Serial No.	Pectin Microspheres (TNZ)		Shellac-coated Pectin Microsphere(S- TNZ)	
	Formulation Code (Drug: Polymer)	Degree of Swelling	Formulation Code (Drug: Polymer)	Degree of Swelling
1	F1 (1:2)	0.85 ± 0.11	S-F1 (1:2)	0.07 ± 0.21
2	F2 (1:3)	1.26 ± 0.16	S-F2 (1:3)	0.18 ± 0.16
3	F3 (1:4)	1.28 ± 0.01	S-F3 (1:4)	0.20 ± 0.12
4	F4 (1:5)	1.29 ± 0.12	S-F4 (1:5)	0.23 ± 0.04

FTIR study

FT-IR studies were carried out for pure drug alone, along with polymers and for prepared formulations. Their FT-IR spectra were shown in Figures 3. Peaks were not affected and prominently observed in FT-IR spectra. This indicates that there is no interaction between drug and polymers and the drug was compatible with the formulation components.

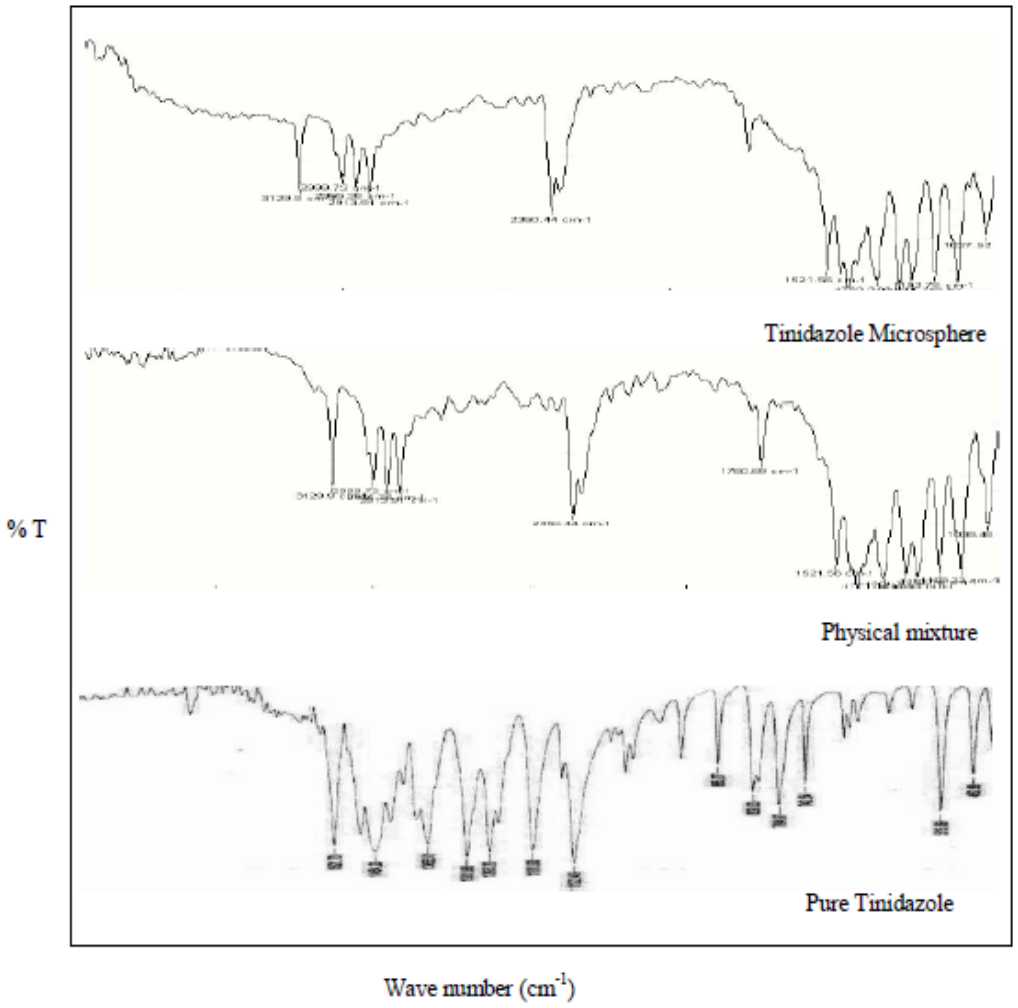


Figure 3. Comparative FT-IR spectra of pure TNZ, TNZ in combination with polymers and in optimized formulation

Differential Scanning Calorimetry (DSC) Study for TNZ

DSC studies for pure TNZ and TNZ formulation were carried out. The DSC thermograms are shown in the Figure 4 respectively for pure drug and TNZ formulation. The thermogram of Pure TNZ indicates that the melting of drug has taken place at 126.57 °C. It is matching with the literature value 127-128°°C; whereas the thermogram of TNZ formulation shows a broad peak and multiple small peaks, it is due to the excipients have undergone melting at 129.37ºC. Before the excipients completely melts, the drug might have started melting giving broad endothermic peak. Drug and polymer displayed their characteristic individual melting trends without an appreciable deviation. From this it is observed that there is no interaction between drug and polymer.

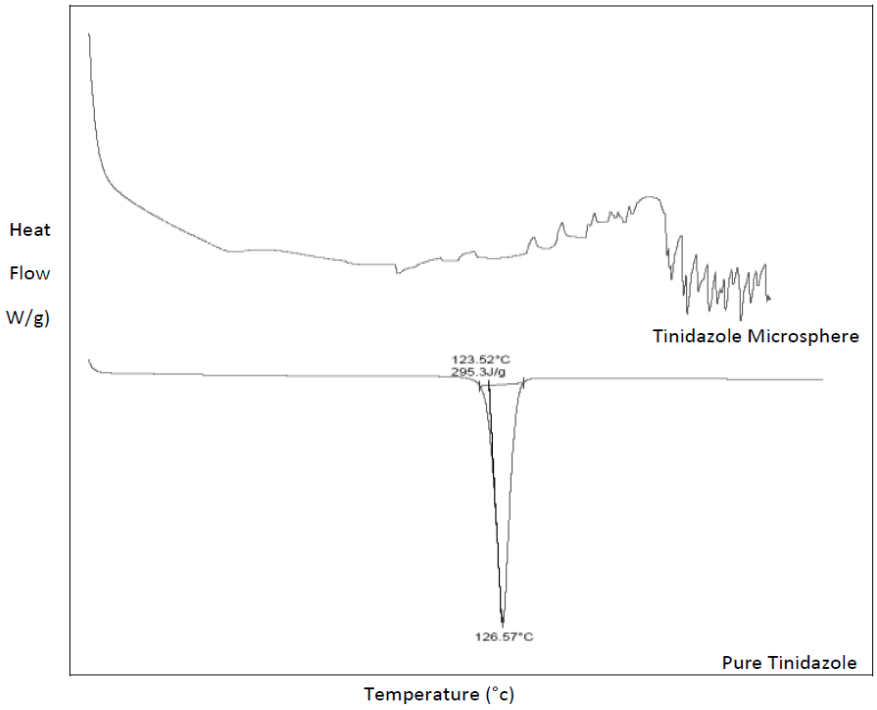


Figure 4. Comparative DSC thermograms of Pure TNZ and TNZ Formulation

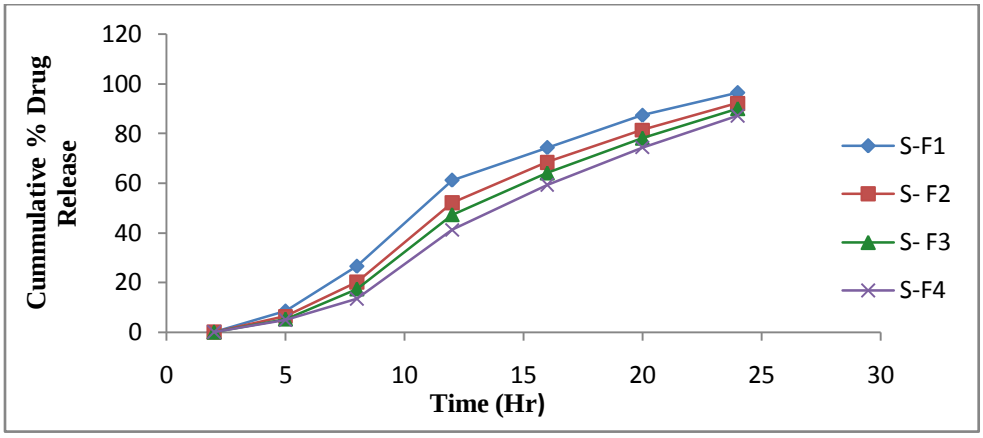
In - Vitro Drug Release Studies in Simulated Gastrointestinal Fluids

In vitro drug release study of Pectin microspheres and Shellac-coated pectin microspheres was performed in pH progression medium at 37ºC ± 0.5ºC. The plot of cumulative drug release vs. time in simulated gastrointestinal conditions is shown in Figure 5 (A, B & C).

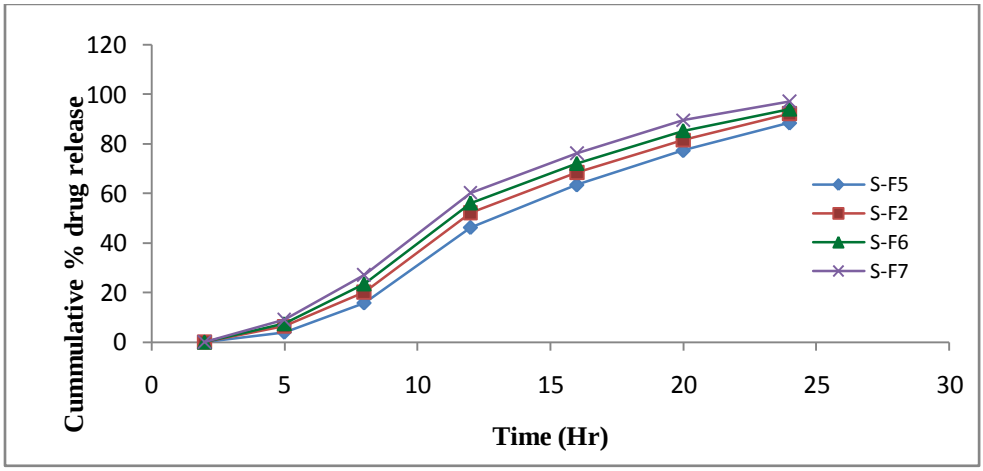
The cumulative percentage drug release from Shellac-coated pectin microspheres showed the desired rate, as there was no measurable drug release observed up to 2 hours in SGF (pH 1.2), while in SIF (pH 6.8), the drug release was quite insignificant (>1%) up to 5 hours. But in colonic fluid (SCF) maximum drug release was observed due to dissolution of the shellac coat at

pH 7.4 and the pectin microspheres were degraded on exposure to the colonic fluid and results in higher percentage of drug release. Significant release from Shellac- coated pectin microspheres in SCF followed the order S-F1 > S-F2 > S-F3> S-F3. The release of drug from microspheres decreased as the polymer concentration increased, suggesting that drug release could be controlled by varying the polymer concentration (Figure 5A). This could be attributed to an increase in the density of the polymer matrix and the diffusional path length that the drug has to traverse. Variables such as surfactant concentration and stirring speed also influence the drug release pattern. Dissolution profiles (Figure 5B) indicated that the release rate from the microspheres increased significantly as the concentration of surfactant increased. This might be attributed to the fact that average size of microspheres increased as the concentration of surfactant increased thereby free drug on microsphere surface is available for dissolution. Release curves (Figure 5 C) indicated that drug release was increased significantly with increasing in stirring speed. This can be attributed to the fact that the drug migration will be high for low stirrer speed and more amount of drug will remain in the microspheres surface but when stirring speed was increased drug migration will be less due to collision of emulsion droplets.

A)



B)



C)

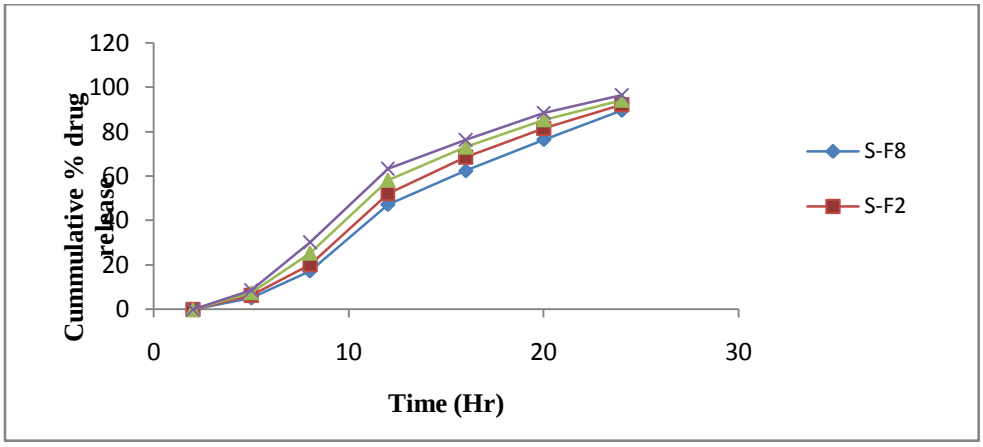


Figure 5. Percentage cumulative in-vitro tinidazole release from different Shellac-coated pectin microspheres in simulated gastrointestinal fluids of different pH. (A) Effect of Drug/ Polymer ratio on drug release (B) Effect of surfactant concentration on drug release (C) Effect of Stirring speed on drug release. Values are average of 3 readings ± standard deviation.

Release Kinetics

Data obtained from in-vitro release studies was utilized for release kinetics. The values of in-vitro release were attempted to fit into various mathematical models i.e. zero order, first order, higuchi matrix, Korsmeyer-peppas and hixson crowell. These values were compared with each other for model fitting equation. Based on highest regression value (r), formulations gave good fit to the Zero order and Korsmeyer- Peppas model. Since the diffusion exponent (n) value was 0.5<n<1, the drug release follows Anomalous (non- Fickian) diffusion.

Table 4: Percentage drug content of optimized formulation subjected for stability testing

Before stability study	After stability study
77.57 ± 0.09	75.42 ± 0.23 %

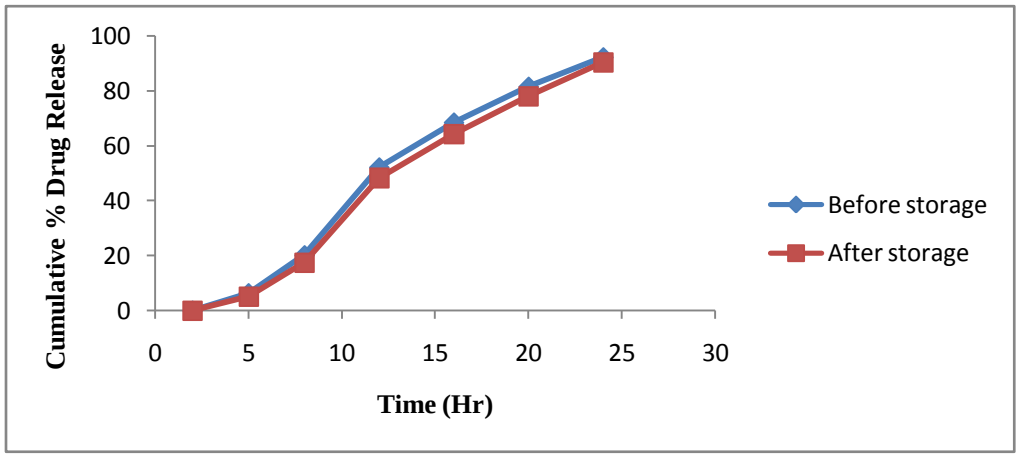


Figure 6 Percentage drug release of optimized formulation subjected for stability testing

Stability study

In view of the potential utility of optimized formulation for targeting of Tinidazole to colon, stability studies were carried out at 40°C / 75% RH for 3 months to assess their long term stability. There is no appreciable change in drug content (Table 4) and dissolution profile of optimized formulation after storage at 40°C / 75% RH for 3 months as shown in Figure 6.

CONCLUSION

The present study has been a satisfactory attempt to formulate microparticulate system for colon targeted delivery of Tinidazole using natural polysaccharides (Pectin) and pH sensitive polymer (Shellac). From the reproducible results of the executed experiments, it can be concluded that:

- The IR spectra revealed that there was no interaction between drug and polymer, thus indicating the compatibility of the drug with the polymers used.
- Pectin and Shellac are suitable, biocompatible polymers for the preparation of microspheres. The polymer Shellac can be used to delay the drug release until the formulation reaches the colon and thereafter the drug is released in colon.
- Drug-polymer ratio significantly influences the particle size as well as drug release pattern of microspheres.
- Particle size analysis revealed that the microspheres were in the acceptable range, and all the formulations showed good surface morphology.
- Good entrapment efficiencies and percentage yield were obtained with the polymers.
- The swelling ability of the microspheres in physiological media was determined. Thus ensuring better resistance of Shellac coated microspheres in the upper GIT to swelling and preventing subsequent drug release at the non target site.
- In vitro release studies showed that as the amount of the polymer increases, the extent of drug release decreases. The cumulative percentage drug release from Shellac based microspheres showed the desired rate, as there was no measurable drug release observed up to 2 hours in SGF (pH 1.2) and no drug release occurred below the pH of polymer solubility while at pH 7.4, the significant drug release was observed.
- Data obtained from *in vitro* release studies were fitted to various kinetic models. On the basis of regression values (r), it was concluded that formulations gave good fit to the Zero order and Korsmeyer- Peppas model.
- Stability studies indicated that the formulation is stable at 40°C / 75% RH and is the suitable temperature for storage.

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