SIMULTANEOUS ESTIMATION OF DRUGS PRESENT IN MICROBEADS FOR COLON-TARGETED DRUG DELIVERY SYSTEM

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ABSTRACT

Objectives: The present study was aimed to develop Eudragit S100 coated colon-targeted sustained-release formulations of alginate-pectin and alginate-hydroxypropyl methylcellulose microbeads containing norfloxacin (NF) and tinidazole (TZ) for the treatment of amebiasis which was simultaneous estimated.

Methods: Taguchi L9 orthogonal array design has been used to optimize the composition and operating conditions for the preparation of formulations. Nine batches (P1-H5) were prepared by taking three independent variables (X_1 – drug:polymer ratio, X_2 – concentration of sodium alginate, and X_3 – curing time) at three levels (1, 2, and 3). Response variables studied for batches (P1-H5) were mean particle size (μm) (Y_1), drug entrapment efficiency (% w/w) (Y_2), and drug loading (% w/w) (Y_3). NF and TZ were simultaneous estimated by ultraviolet spectrophotometric method. Drug-polymer compatibility study was carried out by differential scanning calorimetry and Fourier-transform infrared spectroscopy and indicates no physicochemical interaction.

Results: Microbeads were analyzed for morphological characteristics, mean particle size, drug entrapment efficiency, drug loading, and *in vitro* drug release. The average size of optimized alginate-pectin microbeads was found to be 881±0.05 μm with an entrapment efficiency of 78.50±0.28% (NF) and 86.50±0.32% (TZ) which was simultaneous estimated.

Conclusion: The studies concluded that formulated enteric-coated alginate-pectin microbeads after enteric coating can be used effectively for the delivery of NF and TZ to colon.

Keywords: Simultaneous estimation, Taguchi, Response variables, Independent variables, Drug entrapment efficiency, Drug loading, Curing time.

INTRODUCTION

Targeted drug delivery into the colon is highly desirable for local treatment of a variety of bowel diseases such as ulcerative colitis, Crohn's disease, amebiasis, colon cancer, and systemic delivery of proteins and peptide drugs [1].

Optimization of process parameters by Taguchi design is an effort to minimize the variation in quality as well as to attain the quality near to the intended value simultaneously [2].

Tinidazole (TZ) is the drug of choice in most forms of amebiasis. TZ provided significantly higher cure rates in the treatment of symptomatic intestinal amebiasis.

Norfloxacin (NF) is effective orally and is rapidly bactericidal. It has relatively broad spectrum of action and is effective against Gram-positive and Gram-negative organisms.

NF and TZ combination therapy is available in conventional dosage form for the treatment of amebiasis. Conventional dosage forms (tablets and capsules) are inefficient for delivering drug to colon in appropriate concentration due to absorption or degradation in the hostile upper gastrointestinal tract (GIT).

To overcome these problems, colon-targeted multiparticulate drug delivery or microbeads can be formulated which have many advantages over single-unit preparations such as uniform dispersion and uniform absorption of drug in GIT.

The objective of the present study was to develop multiparticulate delivery system for site-specific delivery of NF and TZ using natural polysaccharide (pectin and hydroxypropyl methylcellulose [HPMC]) and pH-sensitive polymer (Eudragit S100). This system was designed so as to protect the degradation of drug in the upper part of GIT using Eudragit S100 and to deliver TZ specifically to the colon, to reduce systemic dosing, and also to alleviate gastric distress [3,4].

MATERIALS AND METHODS

Materials
NF and TZ were obtained as gift sample from Macleods Pharmaceuticals Limited, Mumbai, India. Sodium alginate was purchased from Titan Biotech Ltd., Bhiwadi (Rajasthan). Calcium chloride and HPMC were purchased from SD Fine-Chemicals Ltd., Mumbai, while pectin was procured from Thomas Baker Ltd., Mumbai. All other reagents and solvents used were of analytical grade.

Experimental design and analysis
Taguchi orthogonal array L9 design was used to optimize the composition and operating conditions for the preparation of microbeads. Factors selected for the study were drug:polymer ratio, concentration of sodium alginate, and curing time taken at low, medium, and high levels as shown in Table 1.

Response variables studied for prepared batches were mean particle size (μm) (Y1), drug entrapment efficiency (% w/w) (Y2), and drug loading (% w/w) (Y3) as shown in Table 2. The experimental trials were performed in random order.

Nine batches of microbeads (P1-H5) were prepared as per experimental design layout of Taguchi L9 orthogonal array design. Calculations and statistical analysis of the results were carried out by multiple

Table 1: Code for four variables at three different levels

S. No.	Variables	Levels		
		Low (1)	Medium (2)	High (3)
1.	Drug: pectin	1:1	1:2	1:3
2.	Concentration of sodium alginate	1.00%	2.00%	3.00%
3.	Curing time	30 min	45 min	60 min
4.	Drug: HPMC	1:1	1:2	1:3

HPMC: Hydroxypropyl methylcellulose

Table 2: Response variables

S. No.	Codes	Response variables
1.	Y_1	Mean particle size (μm)
2.	Y_2	Drug entrapment efficiency (% w/w)
3.	Y_3	Drug loading (% w/w)

linear regression analysis (MLRA) to determine which factors have statistically significant effect on the response parameters.

Simultaneous estimation of NF and TZ by ultraviolet (UV) spectrophotometric method

Preparation of standard stock solution

Standard stock solutions (100 μg/mL) of NF and TZ were prepared by dissolving separately 10 mg of drug each in 50 ml methanol and volume is made up with water up to 100 ml. The working standard solutions of these drugs were obtained by dilution of the respective stock solution with water.

Preparation of sample stock solutions

An accurately weighed powder sample equivalent to 10 mg of NF was transferred to a 100 ml volumetric flask and dissolved in 50 ml and sonicated for 15 min and volume made to 100 ml with double distilled HPLC grade water. It was then filtered through Whatman filter paper No. 41. The solution was suitably diluted with double distilled HPLC grade water to obtain sample solutions containing NF and TZ in the concentrations ratio of 2:3 μg/mL, respectively, as in the formulation. The final concentrations are 10 μg/mL of NF and 15 μg/mL of TZ.

Simultaneous equation method

In this method, 273 nm and 310 nm were selected as the two sampling wavelengths. NF and TZ exhibited linearity with absorbances in the range of 1–20 μg/mL and 1–40 μg/mL at their respective selected wavelengths. For simultaneous estimation of NF and TZ, mixed standards containing NF and TZ in a concentration ratio of 2:3 μg/mL each were prepared by appropriate dilution of the standard stock solutions with distilled HPLC grade water.

The absorbances of the mixed standard solutions were measured at the selected wavelengths. A set of two simultaneous equations was used for obtaining the concentration of NF and TZ as follows:

$$C_x = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \qquad (2.3.3.1)$$

$$C_y = \frac{A_2 ax_1 - A_1 ax_2}{ax_2 ay_1 - ax_1 ay_2} \qquad (2.3.3.2)$$

Where, A_1 and A_2 are absorbances of mixture at 273.0 nm and 310.0 nm, respectively, ax_1 and ax_2 are absorptivities of NF at λ_1 and λ_2, respectively, and ay_1 and ay_2 are absorptivities of TZ at λ_1 and λ_2, respectively. C_x and C_y are concentrations of NF and TZ, respectively. The concentration of NF and TZ in mixed standard and tablets formulation can be obtained by solving equation (2.3.3.1) and (2.3.3.2) [5].

Formulation development

Introduction to the preparation of microbeads

Microbeads were prepared using ionotropic gelation technique. Two different sets of microbeads were prepared using alginate-pectin and

alginate-HPMC in combination. Various formulation parameters such as the concentration of sodium alginate, pectin, and HPMC were optimized on the basis of average size, shape, entrapment efficiency, and *in vitro* drug release (Table 3).

Loading efficiency (%)

Accurately weighed 100 mg of microbeads was extracted in 100 ml phosphate buffer, pH 7.4 for 24 h. Extracted solution was filtered and a sample of 5 ml was withdrawn from this solution. Sample was diluted to 50 ml with distilled water and assayed spectrophotometric ally (Systronics AU2701) at 273 nm and 310 nm to determine the content of drug in microbeads. All the determinations were performed in triplicate. Drug loading (%) of the microbeads was calculated using the equation:

Drug loading (%)=Drug content×100 weight of drug+polymer

Statistical analysis

Response variables were analyzed using Microsoft Excel spreadsheet, 2007. MLRA was used to develop equations for mean particle size (Y_1), percentage drug entrapment efficiency (Y_2), and percentage drug loading (Y_3).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$$

Where, Y is estimated response of response variable, β_0 is arithmetic mean response of nine batches, β_1 is estimated coefficient for factor X_1, β_2 is estimated coefficient for factor X_2, and β_3 is estimated coefficient for factor X_3.

Differential scanning calorimetry (DSC)

DSC measurements were done on DSC Q10V 9.0 build 275 model water limited instrument by heating the samples from 25°C to 250°C at the rate of 20°C/min in a nitrogen atmosphere (flow rate, 10 mL/min).

Fourier-transform infrared (FTIR) spectroscopy

FTIR spectra were taken on FTIR – Shimadzu instrument to investigate the possible chemical interactions between drug and polymer. 1 mg of sample was triturated with 300 mg of KBr in a mortar. A little amount of triturated sample was compressed at 10 kg/cm² into a pellet maker. Pellet was kept on sample holder and scanned from 4000 cm⁻¹ to 400 cm⁻¹.

In vitro drug release study from core microbeads

The *in vitro* drug release study of core alginate-pectin microbeads and alginate-HPMC microbeads was carried out using USP Dissolution Apparatus II (Veego DA, 6DR Japan) at 37 ± 0.5°C at 100 rpm using 900 ml of simulated intestinal fluid of pH 6.8 as dissolution medium for 8 h. A muslin cloth was tied over the basket to prevent the slippage of beads from the basket. A fixed volume of 5 ml sample solution was withdrawn at predetermined intervals, which was then filtered through Whatman filter paper, diluted suitably, and analyzed spectrophotometrically using UV–visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu) at 273 nm for NF and 310 nm for TZ. Equal volume of fresh dissolution medium was added immediately after withdrawal of the test sample, the percentage drug release was calculated from the simultaneous equation (2.3.3.1 and 2.3.3.2) [6].

Table 3: Composition of microbeads

Formulation code	Sodium alginate (%)	Pectin (%)	HPMC (%)	Calcium chloride (%)	Cross-linking agent (ml)	Curing time (min)
P1	1.00	0.50	-	2	1	45
P2	1.00	1.00	-	2	1	45
P3	1.00	1.50	-	2	1	45
H1	1.00	-	0.75	2	1	45
H2	1.00	-	0.25	2	1	45
H3	1.00	-	0.50	2	1	45
P4	2.00	0.50	-	2	1	45
P5	2.00	1.00	-	2	1	45
P6	2.00	1.50	-	2	1	45
H4	2.00	-	0.25	2	1	45
H5	2.00	-	0.50	2	1	45
H6	2.00	-	0.75	2	1	45
P7	3.00	0.50	-	2	1	45
P8	3.00	1.00	-	2	1	45
P9	3.00	1.50	-	2	1	45
H7	3.00	-	0.25	2	1	45
H8	3.00	-	0.50	2	1	45
H9	3.00	-	0.75	2	1	45

HPMC: Hydroxypropyl methylcellulose
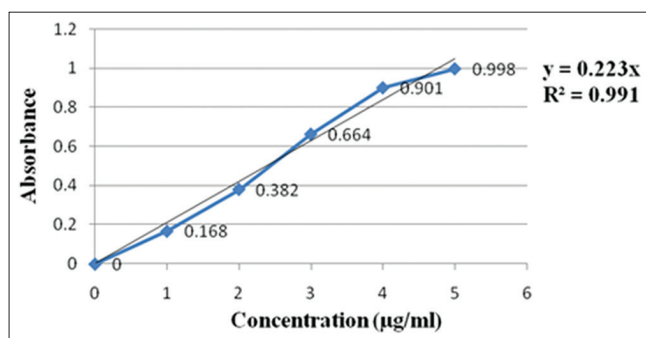

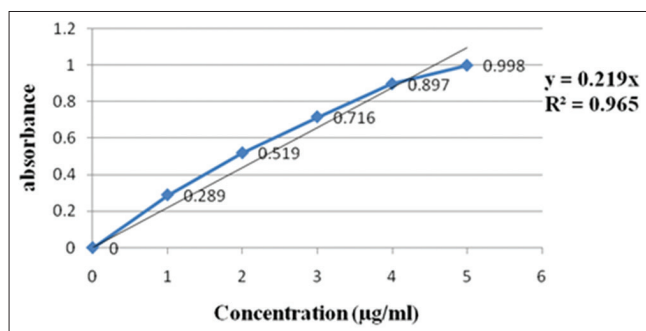Fig. 1: Regressed curve of norfloxacin in methanol at 273 nm

Fig. 2: Regressed curve of tinidazole in methanol at 310 nm

In vitro drug release study from coated microbeads

The drug release study of coated alginate-pectin microbeads was carried out using USP Dissolution Apparatus II (Veego DA, 6DR Japan) at 37 ± 0.5°C at 100 rpm using 900 ml of simulated gastric fluid of pH 1.2 as dissolution medium for initial 2 h, followed by phosphate buffer (pH 7.4) for next 3 h. The beads were then transferred in PBS pH 6.8 and the release was noted up to 8 h. A fixed volume (5 ml) of sample solution was withdrawn and analyzed spectrophotometrically for quantitative estimation of drugs released as described in earlier section 2.4.7 [7,8].

RESULTS AND DISCUSSION

Preparation of standard curve of NF and TZ

Identification of drug was carried out using UV spectroscopy. The calibration curve of NF and TZ was prepared in methanol. The data were regressed to obtain straight line. The regression coefficient of NF and TZ was found to be 0.991 and 0.965, respectively, indicating a good linearity, Figs. 1 and 2. The calibration curve obeyed Beer's Lambert law in the concentration range of 0–6 μg/ml.

Simultaneous estimation of NF and TZ by UV spectrophotometric methods

An accurately weighed microbeads sample equivalent to 10 mg of NF was transferred to a 100 ml volumetric flask and dissolved in 50 ml and sonicated for 15 min and volume made to 100 ml with double distilled HPLC grade water. It was then filtered through Whatman filter paper No. 41. The solution was suitably diluted with double distilled HPLC grade water to obtain sample solutions containing NF and TZ in the concentrations ratio of 2:3 μg/mL, respectively, as in the formulation. The final concentrations are 10 μg/mL of NF and 15 μg/mL of TZ.

Absorbance value of Norfloxacin and Tinidazole is given in Tables 4 and 5.

Observation of Norfloxacin and Tinidazole in Simultaneous estimation is given in Tables 6-8.

The concentration of NF and TZ was found to be 9.67 μg/ml and 14.40 μg/ml which were very close to the actual concentration of drugs present in microbeads formulation.

Drug excipient interaction

Drug excipient interaction was carried out by DSC. In this, DSC thermogram of NF, TZ, sodium alginate, and pectin polymer recorded using a DSC.

The thermogram of mixture of drugs (NF and TZ) and excipients shown that there was no interaction between drugs and polymer used in Fig. 3.

SUMMARY AND CONCLUSION

The aim of the present study was to develop Eudragit S100 coated colon-targeted sustained-release formulations of alginate-pectin microbeads and alginate-HPMC microbeads containing NF and TZ for the treatment of amebiasis which was simultaneous estimated.

The design of the colon-targeted microbeads combines two approaches: pH-sensitive delivery and biodegradation in the colonic environment. For microbeads preparation, drug was first entrapped within the alginate-pectinate beads using ionotropic gelation technique and then these microbeads were coated within Eudragit S-100 polymer by solvent evaporation method. Polymer was selected to target the colon as it starts dissolving at pH 1.2 in the stomach,
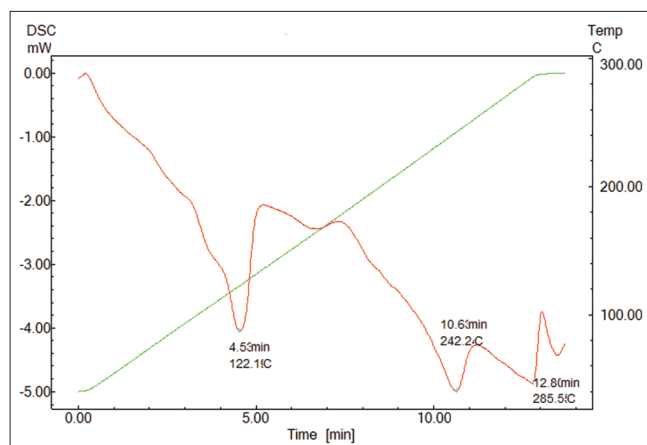
Fig. 3: Differential scanning colorimetric report of sodium alginate, pectin, norfloxacin, and tinidazole mixture

Table 4: Absorbance value of Norfloxacin

Concentration (μg/ml)	Absorbance (nm)
0	0
1	0.168±0.06
2	0.382±0.05
3	0.664±0.07
4	0.901±0.06
5	0.998±0.06

Table 5: Absorbance value of Tinidazole

Concentration (μg/ml)	Absorbance (nm)
0	0
1	0.289±0.03
2	0.519±0.04
3	0.716±0.03
4	0.897±0.05
5	0.998±0.05

Table 6: NF in simultaneous estimation

NF	
ax_1 273 nm	0.0913±0.03
ax_2 11 nm	0.0254±0.05

NF: Norfloxacin

Table 7: TZ in simultaneous estimation

TZ	
ay_1 273 nm	0.017±0.07
ay_2 311 nm	0.060±0.03

TZ: Tinidazole

Table 8: NF and TZ in simultaneous estimation

NF+TZ (2:3 μg/ml)	
(A_1 273 nm)	1.188±0.02
(A_2 311 nm)	1.100±0.03

NF: Norfloxacin, TZ: Tinidazole

but the microbeads coated with Eudragit E-100 prevented the drug release and prolonging its release while transit through the intestine, thereby, maintaining its effective plasma concentration over longer duration. Eudragit E-100 is insoluble in acidic medium but dissolves above pH 7.0 [9].

The calculated regression coefficients for zero-order, first-order, and Higuchi models were 0.777, 0.906, and 0.951. The *in vitro* drug release of alginate-pectin microbeads was best explained by Higuchi models as the plot showed the highest linearity, followed by the first order. The Korsmeyer–Peppas release exponent n for the optimized formulation (P5) was 1.416 indicating that the release was governed by Non-Fickian Super Case II Transport.

Microbeads stored at 40±0.5°C showed K value as 3.42×10^{-4} and $t_{10\%}$ value as 304 days, while those stored at room temperature (25±0.5°C) showed K value as 2.66×10^{-4} and $t_{10\%}$ value as 390 days. This indicates that microbead formulations are stable.

The concentration of NF and TZ was found to be 9.67 μg/ml and 14.40 μg/ml which were very close to the actual concentration of drugs present in microbeads formulation.

CONCLUSION

Microbeads were developed using Taguchi L9 orthogonal array design and drugs were simultaneous estimated. The concentration of NF and TZ was found to be very close to the actual concentration of drugs present in microbeads formulation. Hence, the studies concluded that formulated enteric-coated alginate-pectin microbeads after enteric coating can be used effectively for the delivery of NF and TZ to colon.

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AUTHORS' CONTRIBUTIONS

Dr. Meenakshi Bharkatiya constructed an idea for manuscript and planned methodology to reach the conclusion. Mr. Abhishek Kumar was taken responsibility in execution of the experiments, data management, reporting, logical interpretation, and presentation of the results. Mr. Abhishek Kumar organized the manuscript and Dr. Meenakshi Bharkatiya supervised the manuscript preparation and reviewed the manuscript.

CONFLICTS OF INTEREST

The author confirms that there are no conflicts of interest, financial or otherwise.

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