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FORMULATION AND EVALUATION OF A PECTIN BASED CONTROLLED DRUG DELIVERY SYSTEM CONTAINING METRONIDAZOLE

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ABSTRACT: A natural Pectin based formulation of Metronidazole for colon specific drug delivery is reported. The pectin was chemically modified to make it suitable for use as a pH sensitive matrix for colon targeted delivery of drugs. The formation of the modified polymer preparation was confirmed by Fourier transform infrared (FTIR) spectroscopy, Scanning electron microscopy (SEM) and thermal studies (TGA). The average particle size was found to be 43.53±12.17 μm. The drug encapsulation efficiency was found to vary between 23.44±1.83% and 45.61±0.56%. The *in vitro* drug release studies from these metronidazole-loaded formulations were carried out which showed no significant release of the drug at gastric pH. However, at colonic pH, sustained release of the drug was observed. Thus Metronidazole, which is soluble in gastric pH, can be specifically delivered at colon avoiding its release in the stomach. The *in vitro* drug release studies carried out at different time intervals, different drug content and at different pH values indicate the potentiality of the formulation as a colon specific drug delivery agent.

KEYWORDS: Heteropolysaccharide, Gastrointestinal tract, Controlled release, Coacervation, Drug loading, Encapsulation efficiency.

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1. INTRODUCTION

The use of natural polymers in pharmaceutical and biotechnology industry is increasing day by day because they are economical, readily available, non-toxic, capable of chemical modifications, potentially biodegradable and biocompatible. Pectin, a heteropolysaccharide, is an excellent carbohydrate polymer derived from plant cell wall. It is one of the major constituents of citrus by-

products. Chemically, pectin is poly α-1,4-galacturonic acid with varying degree of methylation of carboxylic acid residues. The association of pectin chains leads to the formation of gel, which is a three dimensional network [1]. This biopolymer has several unique properties that make it suitable for use as a matrix for the entrapment and/or delivery of a variety of drugs. The therapeutic efficacy and safety of drugs administered by conventional methods can be improved by a controlled drug delivery system. Controlled release refers to the use of delivery system with the objective of releasing the drug in to the patient body at a predetermined rate, or at a specific time or with specific release profiles [2]. Orally administered drugs have to transport over a long distance having different residence time in different segment of gastrointestinal tract (GIT) and experience different environmental condition like variation of pH from stomach to colon, constant mechanical pressure in the stomach, protease attack in small intestine and microflora attack in colon. Pectin has been extensively studied for colon targeted drug delivery system. Pectin passes intact through the upper gastrointestinal tract and is degraded by colonic microflora, which encouraged researchers to focus on development of pectin as a drug carrier for colon specific drug delivery [3]Though a number of polysaccharides are available for colon specific drug delivery, a limitation encountered with them is their hydrophilicity and high water solubility. This would lead to premature drug release in the tracts of the upper GIT. One approach that may alter the solubility of the polysaccharide is chemical modification like combining with other polymers or by cross-linking for improving biocompatibility [4]. Thus there is a need for modification of pectin with another biopolymer followed by cross-linking either ionically with Ca^{+2}, Ba^{+2} ions or covalently with glutaraldehyde or formaldehyde. These are again prepared using care that the system resist drug release in the tracts of the upper GIT but retain the bacterial degradability [5]. Complex coacervation is one of the simplest and oldest methods of encapsulating drugs for sustained drug delivery. Complex coacervation involves reaction between two oppositely charged polymers. Pectin is the methoxy ester of pectic acid and presence of this acidic group provides negative charge to pectin. (Fig. 1a) Gelatin is a heterogeneous mixture of water soluble proteins of high average molecular mass derived by hydrolytic action from animal collagen. Gelatin is amphoteric in nature due to the presence of carboxylic and amino guanidine group (Fig. 1b). Negatively charged polysaccharide pectin can bind with positively charged protein molecule gelatin [6].

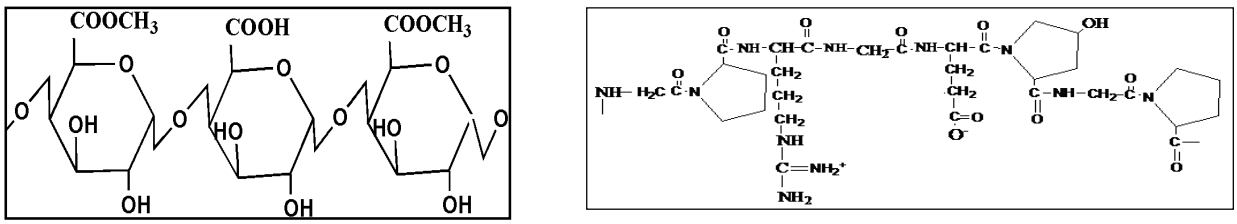

Figure: 1a) Structure of pectin, 1b) Structure of gelatin

Metronidazole is a derivative of benzimidazole having antiprotozoal, antibacterial activities. It is effective in infection caused by *Entamobea hystolitica, Giardia lambia* and Trichomomiasis [7]The objective of the present work is to prepare and evaluate polyelectrolyte complex between pectin and gelatin using glutaraldehyde as a crosslinker and metronidazole as a model drug. **2. MATERIALS AND METHODS**

Pectin was purchased from Loba Chemie Laboratory reagent, Mumbai and gelatin was purchased from Merck limited, Mumbai. Metronidazole was obtained from Tokyo Chemical Industry, Japan. All other chemicals and reagents were of analytical grade and used as obtained.

Preparation of Metronidazole –loaded pectin gelatin complex:

Powdered pectin was dissolved in double distilled water to obtain 2% (w/v) pectin solution and powdered gelatin was dissolved in double distilled water to obtain (1%, 2%, 3%)(w/v) gelatin solution. Pectin solution was added dropwise to the gelatin solution each to obtain pectin-gelatin solution until a homogenous solution was obtained. Thus the different mass ratio (1:0.5, 1:1, 1:1.5) or (2:1, 2:2, 2:3) pectin-gelatin solution was obtained. 100 mg Metronidazole was added to the blended solution and stirred for 1 hour. HCl (0.2 N) was added until pH turns 3.5 and the solution was again stirred for 30 mins. 10 ml of 1% CMC solution was added and the mixture was stirred for 1 hour. Glutaraldehyde was added to each combination and stirred for further 3 hours. 0.1M glycine solution was added as quencher and stirred for 30 mins. The entire reaction was carried out at room temperature [8].

Characterisation of Metronidazole Loaded Pectin-Gelatin Polyelectrolyte Complex

a) **FTIR SPECTROSCOPY**

FT-IR spectra of pectin, gelatin, metronidazole and metronidazole-loaded pectin-gelatin polyelectrolyte complex were obtained using FTIR spectrometer. Each sample was ground and mixed with dry potassium bromide (KBr). The spectra were recorded in absorbance mode from 4000-400 cm^{-1} (mid infrared region) at the resolution of 4 cm^{-1}.

b) **UV-VISIBLE SPECTROSCOPY**

Calibration curve of Metronidazole

Calibration curve of metronidazole was drawn spectrophotometrically at pH 2 (λ_{max} = 278 nm), 4 (λ_{max} = 320 nm), 7 (λ_{max} = 320 nm) and 9 (λ_{max} = 320 nm), which correspond to wavelengths of maximum absorbance. 10 mg of Metronidazole was accurately weighed in digital balance, dissolved in buffer solution of pH 2 and the volume was made up to 100 mL with the buffer. From this stock solution different amount of solution viz. 0.5, 1, 2, 3 and 5 mL (concentration 5-50 µg/mL) solution was withdrawn, diluted and UV-visible spectra was recorded. The experiment was conducted three times and average values of absorbance were used to draw the calibration curve. Figure 2 shows that the calibration curve was linear upto 50 µg/mL with a correlation coefficient of (R2) 0.9999.

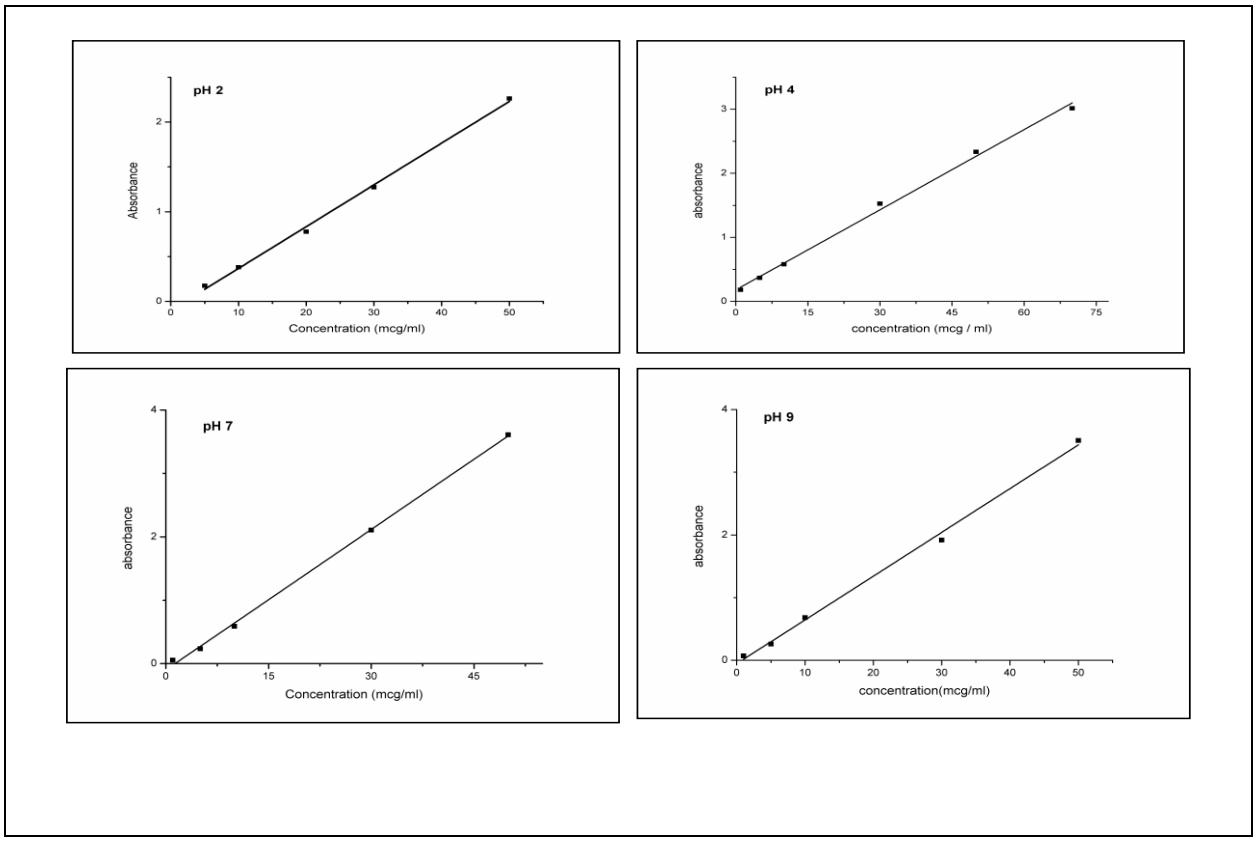

Figure 2: Calibration curve of metronidazole at a) pH 2 b) pH 4 c) pH 7 d) pH 9

Estimation of metronidazole

One hundred milligrams of the microencapsulated product was digested in 100 mL of 0.5 N HCl at room temperature. It was diluted, filtered to remove debris and the resulting solution was quantified spectrophotometrically (UV-Vis 1700) at an optimum wavelength of 277 nm, which corresponds to the absorption peak of metronidazole. The concentrations of the withdrawn sample were calculated with reference to the standard Beer's plot.

Determination of percentage of drug loading (L%)

The percentage of drug loading [9] can be estimated by using the formula:

$$L = \frac{Q_m}{W_m} \times 100$$

where,

L is the percentage loading of microcapsules,

Q_m is the quantity of drug in g present in W_m of microcapsules and

W_m is the weight of microcapsules in g.

Determination of encapsulation efficiency (EE%)

The amount of ibuprofen encapsulated [10] in the microcapsules was determined by using the following formula:

$$EE = \frac{Q_p}{Q_t} \times 100$$

where,

EE is the% of encapsulation of microcapsules,

Q_p is the quantity of drug encapsulated in microcapsules (g) and

Q_t is the quantity of drug added for encapsulation (g).

c) Scanning electron microscope (SEM)

The morphology of the microparticles was determined by Scanning Electron Microscopy on Carl Zeiss Sigma VP instrument at an accelerating voltage of 15kV. The particle size distributions were determined from the SEM images using the image J software. The average size of the particles was evaluated by measuring at least 20 particles.

d) Thermal Analysis

A thermogram was recorded using thermogravity analyser DSC 6000 TGA 4000 Perkin Elmer. A sample was tested at nitrogen atmosphere (20 mL/min flow rate) using a temperature range of 35^0C - 800^0C at a heating rate of 10^0C.

e) *IN VITRO* DRUG RELEASE STUDY

In vitro drug release from the prepared polyelectrolyte complex was performed in dissolution rate test equipment (IKON instrument, Delhi, India). The drug release study was evaluated in simulated gastric pH 2. The release study was performed in acetate buffer pH 4 followed by pH 7. Release study was done in simulated intestinal pH 9. The system was maintained at 37^0C under 50 rpm speed. 10 mL of aliquots was collected at predetermined time interval and analyzed to determine the absorbance using a UV-VIS spectrophotometer. Three replicate release studies were performed in each case and mean values were taken [11]. The amount of drug release was calculated using the respective calibration curve.

3. RESULTS AND DISCUSSION

In this work two biopolymers were taken into account viz. pectin and gelatin. Gelatin is positively charged below its isoeletric point and is expected to form polyelectrolyte complex with negatively charged polysaccharide such as pectin at low pH. The complex coacervation of pectin-gelatin takes place at pH around 3-4 [12]. The purpose of the modification was to reduce the polarity of pectin by reducing the number of free hydroxyl group.

FT-IR Spectral analysis: Pectin has free carboxyl group whereas gelatin has the presence of amino group. During complex cocervation the carboxyl group interacts with amino group to form a complex

that contains amide group. FT-IR (Fig. 3) spectrum of gelatin displays characteristic group vibration at 3415 cm^{-1}which is characteristic of free amino group. FT-IR spectrum of pectin showed stretching frequency for carboxylic acid group at 1600 cm^{-1}. The peaks of free amino group present in gelatin disappeared in pectin–gelatin complex coacervate and a characteristic peak for amide in the region of 1500–1600 cm^{-1} appeared in the complex coacervate and confirms formation of complex (amide) due to reaction between amino group of gelatin and carboxylic group of pectin [8]. FT-IR spectrum of drug loaded microcapsules showed characteristic peaks of the corresponding drug and revealed intact nature of the drug in the microcapsules.

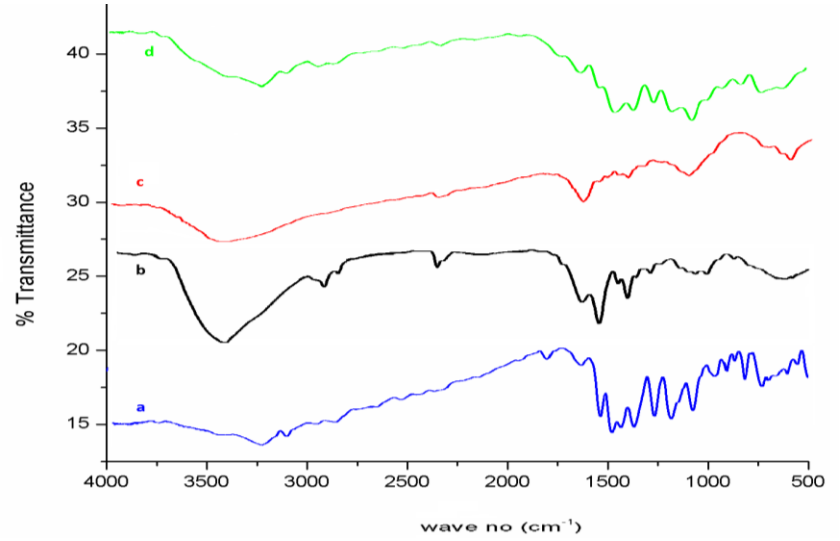

Figure 3: FT-IR spectra of a) Metronidazole b) Pectin c) Gelatin d) formulation

UV-Visible Spectral Analysis: The absorbance of the samples was determined at wavelength according to the dissolution medium pH 2 (λ_{max} = 278 nm), 4 (λ_{max} = 320 nm), 7 (λ_{max} = 320 nm) and 9 (λ_{max} = 320 nm) using UV–Visible spectrophotometer (UV-1700, Shimadzu) against respective buffer solution. A formulation chart of pectin-gelatin polyelectrolyte complex microparticles containing metronidazole is prepared and estimated using UV-visible spectra and is provided in Table 1. Metronidazole being a water-soluble drug is poorly encapsulated. The microparticle yield was good indicating that the formulation technique was reliable. There was no evidence of correlation between the amount of glutaraldehyde added and the yield of microparticles. However, higher quantity of crosslinking agent may produce slow release of drug and result in ineffective therapy. Entrapment efficiency was expressed as the percentage of drug entrapped in these prepared pectin-gelatin polyelectrolyte complex compared to the initial amount of the drug included in the formulation of all the samples, PG-1a (1:1 ratio of pectin and gelatin with 1ml of cross linker added) showed maximum drug encapsulation efficiency and hence this composition was chosen for drug release study.

Formulation code	Pectin (mg)	Gelatin (mg)	Glutaraldehyde (mL)	Drug (mg)	Yield[a] (P%)	Drug loading[b] (L%)	Encapsulation efficiency[c] (EE%)
PG-1a	500	500	1	100	71.35±1.06	3.61±0.14	45.61±0.56
PG-1b	500	500	2	100	63.13±0.64	3.27±0.57	39.44±0.18
PG-2a	500	625	1	100	67.9±1.46	4.64±0.72	37.89±1.33
PG-2b	500	625	2	100	68.22±0.63	3.15±0.70	33.47±0.51
PG-3a	500	375	1	100	57.99±0.52	3.56±0.16	28.62±0.65
PG-3b	500	375	2	100	59.86±0.73	3.72±0.11	23.44±1.83

Table 1 Formulation chart of pectin –gelatin complex

Thermal Analysis (TGA): The samples present a characteristic thermal degradation. In pure pectin (Fig. 4a) the first step occurring 35^0 C to 130^0 C corresponds to water loss and can be evaluated as 10% of the initial mass. The second step between 200^0 to 400^0 C, leading to 60% of weight loss, corresponds to pyrolytic decomposition of pectin, which may include primary and secondary decarboxylation of acid side group and carbon in the ring. The third step between 500^0 to 700^0 C corresponds to oxidation region [13]. In case of the modified pectin (Fig. 4b), an improvement in thermal stability can be seen from the TGA curve. The first weight loss is due to evaporation of water. The second weight loss pattern reveals a greater thermal stability as compared to pure pectin and corresponds to 45% of the weight loss. The weight loss in this region is less sharp as compared to the pure pectin. Thermal degradation of the complex in this region is lower than the thermogram of pure pectin. This suggests an interaction between pectin and gelatin, which can be assumed to be due to loss of organization.

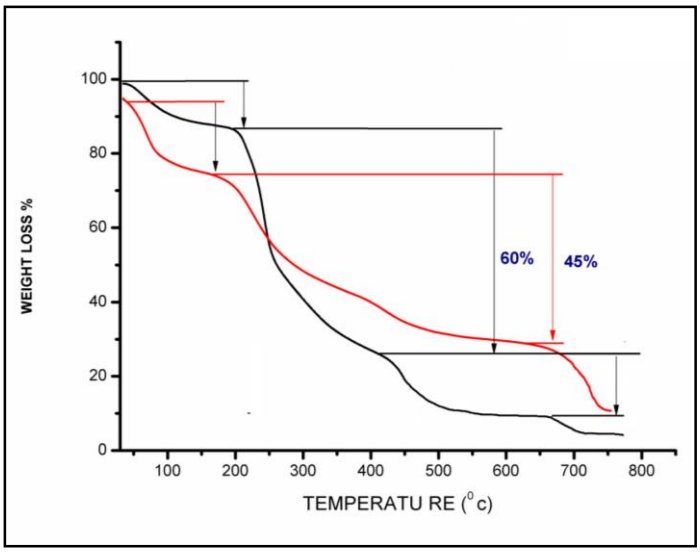

Figure 4 : TGA thermogram of (a) Pectin (b) Modified Pectin

Scanning Electron Microscope (SEM): The morphology of the complex was analysed by SEM (Fig. 5), which revealed its uniformity, homogeneity and interconnected porous nature. Such a structure allows easy incorporation of bioactive materials within it.

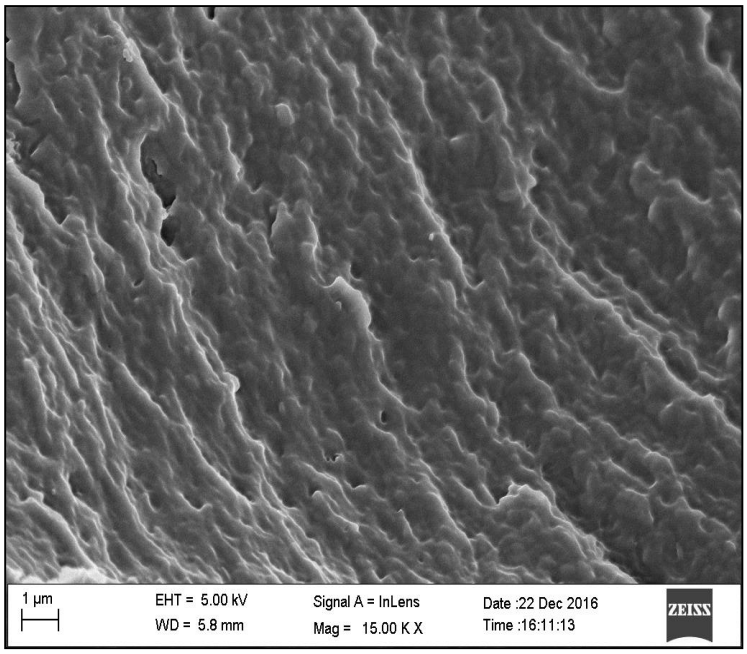

Figure 5: SEM image of modified Pectin

The interconnected porous matrix of the sample signifies an appropriate crosslinking of negatively charged pectin and positively charged gelatin which is expected to interact through electrostatic interaction forming a three dimensional network. Addition of glutarldehyde as a crosslinker might have produced heterogeneity to the structure. The particle size distribution of the prepared complex was measured using an image J software. The average size of the particle was calculated by measuring size of 20 particles and found to be 43.53 ± 12.17 µm.

Drug Release studies:

Drug release was influenced by the pH of the medium showing a significant difference in both acidic and the alkaline medium (Fig. 6). The drug release was less in acidic medium and it may be due to less solubility of drug in acidic pH thereby decreasing its corrosive effect in gastric environment. The drug release % increases considerably on shifting to alkaline pH. High release may be due to high solubility of both the drug and the polymer in alkaline medium hence it could be used for colon specific drug delivery.

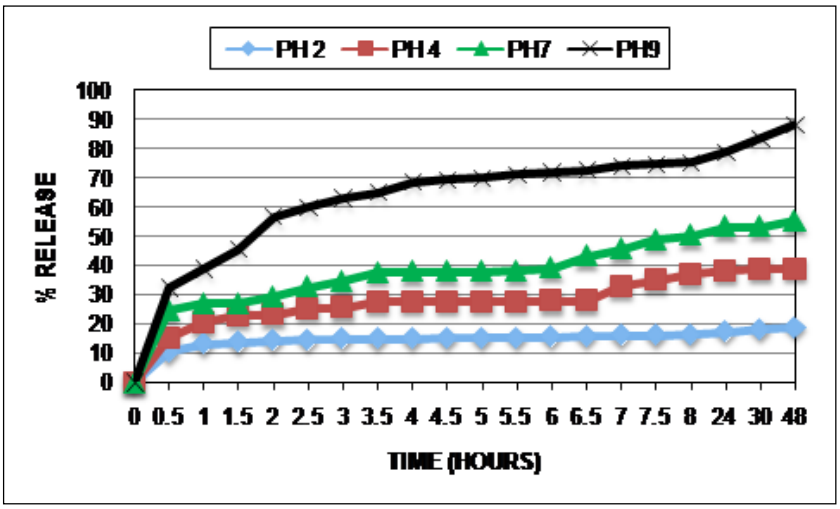

Figure 6: Release pattern of Metronidazole at different pH

4. CONCLUSIONS:

The use of natural polymers for pharmaceutical applications is attractive. Pectin based system is ideal for controlled drug delivery since it is not digested in the gastrointestinal tract until it reaches colon where it is susceptible to microbial degradation thereby releasing the transported material. However, pectin alone is unable to control premature release of drug in the upper gastrointestinal tract because of its swelling and solubility in aqueous medium so pectin was allowed for complex cocervation with gelatin. Compatibility of the drug in polymer matrix was analysed with FTIR and TGA studies. The FTIR spectra obtained from the powdered tablet showed the presence of the all above characteristic bands of the drug almost at the same wave numbers. The drug release was less in acidic medium while it showed sustained release in alkaline medium. The prepared formulation could be successfully used in controlled drug delivery of metronidazole in the colon to cure various colonic diseases like amoebiasis, giradiasis, trichomonasis and anaerobic infections. Controlled drug release would minimise side effects by preventing the release of drug in the upper part of GIT. Hence this drug delivery system can be an option for controlled drug delivery. However, future works involve to further decrease the release of drug in gastric and small intestinal fluid and to provide complete release of drug selectively in colon.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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