European Journal of Pharmaceutical Sciences 86 (2016) 50–57

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Enhancement of site specific delivery of diloxanide furoate as an antiamoebic drug

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ARTICLE INFO

Article history:
Received 30 August 2015
Received in revised form 3 March 2016
Accepted 3 March 2016
Available online 4 March 2016

Keywords:
Colon
Pectin
Zn acetate
Diloxanide furoate
X-ray imaging

ABSTRACT

The basic aim of the present research work is to deliver the diloxanide furoate (DF) at specific area using pectin microspheres. The microspheres were prepared by spray drying method and cross-linked by zinc acetate. Different concentrations of polymer (pectin 0.5–3%) and cross-linking agent (0–3% w/v in a mixture of ethanol:water) are taken to optimize the entrapment efficiency, swelling behavior, size and first 6 h in-vitro release in simulated gastric fluids. Optimized formulation was characterized in the terms of in-vitro release, in-vivo drug disposition in various organs and in the blood of Sprague–Dawley albino rats and in-vivo gastrointestinal tract transit behavior using X-ray imaging method on albino rabbits. Findings suggested that microspheres containing a concentration of polymer (2% w/v) have average size of 100–500 μm, entrapment efficiency 85.82 ± 0.5 with swelling index 18.77 ± 5.21. In-vitro results and in-vivo gastric transit behavior (using X-ray imaging) have shown no release in first 3–6 h that proved the colon specific delivery of DF. The results also suggested that the above approach have not only site specific delivery, but it improves the conversion of active drug by increasing the enzyme mediated hydrolytic degradation of DF due to the presence of polysaccharide polymer:water gel complex.

1. Introduction

Amoebiasis is a universal disease, prominently affecting regions of Asia, Africa and South America, where it is endemic. The disease affects thousands of people worldwide and is the third leading cause of death due to parasitic disease. Different classes of drugs, such as, tissue amoebicide (Metronidazole, Tinidazole, Chloroquine, and Emetine) and luminal (Diloxanide furoate, Iodoquinol, Paromomycin) are needed to treat the disease, but many of them have serious side effects (Tepe et al., 2011; Cimanga et al., 2006; Sinha et al., 1995).

Diloxanide furoate2, 2-dichloro-4-hydro-N-methylacetanilide-2-furoate (DF) is a luminal amoebicide that destroys the trophozoites of *E. histolytica*. DF converts as active molecule Diloxanide by hydrolytic degradation in the colon mediated by colonic microflora (Perhson and Bengtsson, 1983; McAuley et al., 1992). Recently, greater emphasis has been placed on site specific delivery to ensure the safety, efficacy and effectiveness. Colon targeted drug delivery has benefits of local and systemic treatment of many chronic diseases like irritable bowel syndrome, colitis, Crohn's disease, colon cancer and infections. (Yassin et al., 2010). Colon provides a less hostile environment for drug due to low diversity and intensity of digestive enzyme activities and near neutral pH and transit time of 4 to 78 h that increases the time of drug availability in action and absorption (Singh et al., 2015; Pinto, 2010). Different systems are being developed for the purpose of site-specific drug delivery of the colon like pH dependent, time dependent, pressure dependent, enzyme dependent systems (Yang et al., 2002). One or combinations of the above approaches are utilized to achieve colon targeted drug delivery system (Macleod et al., 1999). But due to some drawbacks related to, poor site specificity of the pH dependent system, large variations in pH of the Gastrointestinal tract (GIT), variation in gastric emptying time of different age, sex result in failure to deliver the drug at site (Krishnaiah et al., 2002). The best alternative approach to colon specific drug delivery is the use of carriers containing carbohydrates (pectin, chondroitin sulfate, amylase, inulin HP and guar gum) that are degraded exclusively by colonic bacteria since large intestine contains bacterial count 10^{11} cfu/ml compared to 10^{4}cfu/ml in small intestine (Krishnaiah et al., 2003).

Pectin is a polysaccharide, found in the cell walls of plants, is a predominately linear polymer of mainly α-(1–4) — linked D-galacturonic acid residues interrupted by 1, 2-linked L-rhamnase residues. It is totally degraded by colonic bacteria but is not digested in the upper GIT. Pectin has a few hundred to about 1000 building blocks per molecule.

The aim of the present research was to develop the microspheres of DF using pectin as a polymer with no release in upper GIT and targeted

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http://dx.doi.org/10.1016/j.ejps.2016.03.001
0928-0987/

release in colon mediated by colonic enzymes for achievement of high and effective drug concentration on lower GIT. Pectin is a polysaccharide hydrophilic polymer and works as a nutrient for colonic enzymes, but it swells with aqueous environments and is not able to shield its drug load effectively during its passage through the stomach and small intestine (Beneke et al., 2009; Sinha and Kumria, 2002). This can however be adjusted by cross-linking of its hydrophilic groups as a polyionic complex with zinc acetate to produce Zinc-pectinate complex. The targeted release was achieved by enhancement of interaction of colonic enzymes, especially hydrolytic enzymes for fast and easy conversion to active drug Diloxanide from DF.

2. Material and method

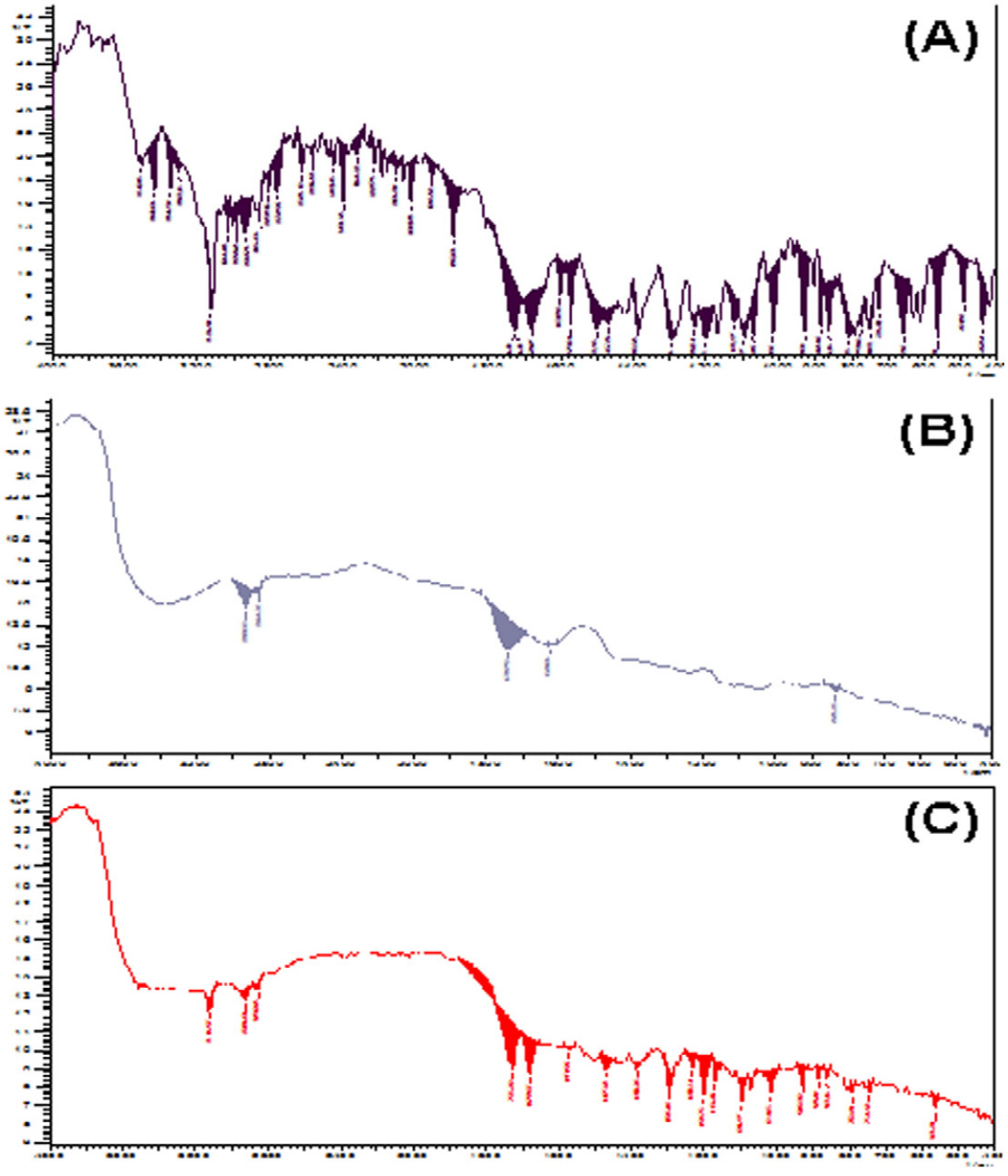
2.1. Material

Pectin from apples (M_r 30,000–100,000; degree of esterification 70–75%),(CDH India), Diloxanide furoate (purity > 99%) was obtained as a gift sample of Unichem lab. (Mumbai, India). Other chemicals and solvents were purchased from CDH Mumbai India. Sprague–Dawley albino rats (male and female average weight 210–265 g) were procured by Animal House Institute of Pharmaceutical Sciences, G. G. Central University, Bilaspur, C.G. India. The animals were allowed water and laboratory *Chowad libitum*. The animals were maintained in a 12 h light–dark circle. All animal procedures were performed in accordance with protocols approved by institutional animal ethical committee.

2.2. Method

2.2.1. Preparation of microspheres of DF

Microspheres were prepared by spray-drying method (Lee et al., 2004; Bigucci et al., 2003). Briefly 20 g of pectin was dissolved in 1000 ml of de-ionized water and then 1 g of the drug was mixed in pectin solution for overnight stirring. The prepared solution was a spray dried to get microspheres through the nozzle (0.2 mm diameter) of the spray-dryer (Labultima Laboratory Spray Dryer, LU-228, India) having a pressure on 200 kPa to 300 kPa. The inlet temperature of spray dryer was 110 °C fixed to get an air outlet between 45 and 50 °C. The



Graph 1. FTIR spectra of DF (a), pectin (b) and DF and pectin mixture (c).