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A novel dissolution method for evaluation of polysaccharide based colon specific delivery systems: A suitable alternative to animal sacrifice

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ABSTRACT

The most extensively used test for predicting in-vivo release kinetics of a drug from its orally administered dosage forms is dissolution testing. For polysaccharide based, colon targeted oral delivery systems, the entire path of the gut traversed by the dosage form needs to be simulated for assessing its in-vivo dissolution pattern. This includes the dissolution testing sequentially in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and simulated colonic fluid (SCF). For SGF and SIF, simple and standardized composition is well-known. However, preparation of SCF requires addition of either the colonic contents of rodents or human faecal slurry. A method is proposed, wherein a mixture of five probiotics cultured in the presence of a prebiotic under anaerobic conditions is able to surrogate the colonic fluid. Release profiles of drug from colon targeted delivery systems in this medium were studied and compared to those generated in the conventionally used media containing rodent caecal contents and human faecal slurry. The results from the three studies were found to be quite similar. These findings suggest that the proposed medium may prove to be useful not only as a biorelevant and discriminatory method but may also help in achieving the 3Rs objective regarding the ethical use of animals.

1. Introduction

Dissolution testing is the most commonly employed in-vitro evaluation technique for predicting the in-vivo drug release kinetics from orally administered dosage forms including the modified release delivery systems (Yang, 2008; Kotla et al., 2014). There are two predominant challenges involved in designing dissolution methods for site specific delivery systems. First challenge involves creating a micro-environment that is a physiological replica of the biological milieu at the site of drug release. Second major challenge lies in simulating the conditions that the drug delivery system is likely to encounter during its transit from mouth to target site (Tomlinson, 1988). The more diverse the milieus encountered by the formulation, the more complex the dissolution method becomes. Due to the distal location of colon, the colon targeted oral delivery systems traverse the longest path, coming across the most varied milieus. Designing a suitable biorelevant medium, therefore, has proven to be a complex task. The situation is further complicated by the varied approaches that have been used for achieving colon targeting. The most commonly used strategies to formulate colon-specific drug delivery systems include timed release systems, prodrugs, pH sensitive polymer coating and colonic microflora activated delivery systems (Gulati et al., 2012; Rubinstein, 1995; Yang et al., 2002; Muraoka et al., 1998; Philip et al., 2008). Among these, the microflora activated delivery systems have been found to be quite promising. An abrupt increase in the number of microbiota and the resultant increase in enzymatic activities in the ascending colon represent an exclusive and non-continuous event independent of GI transit time and pH (Ibekwe et al., 2008; Yang et al., 2001; Prasad et al., 1998; Krishnaiah et al., 2002).

Human colon represents a dynamic and ecologically diverse environment, comprising over 400 distinct species of bacteria with a population of 10^{11}–10^{12} CFU/mL of colonic contents. These bacteria produce wide spectrum of reductive and hydrolytic enzymes, which are responsible for many biorelevant processes like carbohydrate and protein metabolism (Yang, 2008). Hence, in order to simulate the colonic milieu in vitro, the methodology should be designed in such a way that it involves the microbiota of colon. At the same time, the method should be convenient, inexpensive and reproducible.

The methods that have been reported for evaluation of colon targeted delivery systems include triggering by enzymes (Philip et al., 2008; Fetzner et al., 2004; Macfarlane et al., 1989; Maculotti et al., 2009; Maestrelli et al., 2008; Liu et al., 2012; Semde et al., 2000a,b Omar et al., 2007; Jain et al., 2007a,b; Lai

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et al., 2010), rat caecal contents (Rubinstein et al., 1993; Jain et al., 2007a,b; Watanabe et al., 1998; Takemura et al., 2000; Tiwari et al., 2011; Shukla et al., 2012, 2011; Krishnaiah et al., 2001; Shyale et al., 2005; Ravi et al., 2008; Varshosaz et al., 2010; Singhal et al., 2011; Wei et al., 2007; Seal and Mathers, 2001), human faecal slurries (Salunkhe and Kulkarni, 2008; Siew et al., 2000, 2004; McConnel et al., 2007; Karrout et al., 2009; Krenzlin et al., 2011), and multi stage compound culture system (Yang, 2008; Kotla et al., 2014; Schacht et al., 1996). Despite a large number of research reports on microbially triggered delivery systems for colon delivery, no such product is commercially available. The fact can be attributed to the lack of availability of a pragmatic dissolution method to evaluate the products during their development phase. Table 1 enumerates various dissolution methodolo gies reported so far to evaluate the drug release from microbially triggered colon-specific drug delivery systems. Their advantages and limitations are also stated in the table.

The only dissolution medium used so far, that has been found to be the closest to meet these criteria is the one that uses colon contents of rodents and thus, has been employed most frequently by the researchers. However, even this media suffers from a number of limitations like lack of reproducibility, cumbersomeness of procedure and most importantly, high cost and sacrifice of laboratory animals (Kotla et al., 2014). The dissolution method using human faecal slurries is the next best option, though the element of reproducibility is much less in this case as compared to the medium containing rat caecal content.

Probiotics are the products containing live micro-organisms that confer wide health advantages in human beings. There have been a few reports where the probiotic culture has been shown to mimic the colonic milieu in terms of the presence of polysaccharide metabolizing bacteria leading to perpetual generation of the relevant enzymes. Lactobacilli culture has been shown to release drug from soy-polysaccharide/ethyl cellulose films meant for colon targeted delivery (Karrout et al., 2009; Ursekar et al., 2012). Various strains of Lactobacillus, Bifidobacterium and Saccharomyces have been demonstrated to metabolize polysaccharides like guar gum, pectin and amylose etc. (Gainvors and Belarbi, 1995; Hartermink et al., 1999; Szwajgier and Jakubczyk, 2011).

Based on these facts, an effort has been made to develop a unique dissolution method using probiotic culture for evaluating polysaccharide based colon specific delivery system. Included in the wish list from the study, is the development of a convenient, inexpensive, reproducible method that can also obviate the sacrifice of experimental animals. Sulfasalazine (SFZ) has been used as the model drug while single polymer i.e. guar gum and combinations of two i.e. amylose–ethyl cellulose and pectin–ethyl cellulose have been used as the model polysaccharides for facilitating colon targeting. In order to evaluate the potential of developed dissolution medium, polysaccharide coated SFZ spheroids were formulated and their dissolution profiles were evaluated using the developed dissolution medium. The dissolution patterns were compared with those obtained using rat caecal content and human faecal slurries.

2. Materials and methods

2.1. Materials

Sulfasalazine (SFZ) was purchased from Swapnroop Drugs & Pharmaceuticals, India. Probiotic (BIOMIX-I) was gifted by Unique Biotech, Hyderabad, India. Guar gum, sodium hydroxide pellets, hydrochloric acid and isopropyl alcohol were purchased from Lobachemie, India. Microcrystalline cellulose PH 101 (MCC PH 101) was procured from Jackson laboratories, India. Talc was purchased from Qualikems Fine, India. Millipore water was used throughout the study. For in vitro dissolution studies, the following reagents were used: hydrochloric acid, potassium dihydrogen orthophosphate and sodium hydroxide (all supplied by S.D. Fine chemicals, India). Pectin was from Alliance Global, Delhi, India, ethyl cellulose was from Dhariyal Polymers Pvt Ltd, India, while, amylose was from Otto Chemie Pvt. Ltd., India.

2.2. Preparation of SFZ spheroids

Spheroids (1–1.2 mm in diameter) containing 40% sulfasalazine, 40% guar gum, 18% MCC PH 101 and 2% talc were prepared by extrusion and spheronization (Milojevic et al., 1996). The formula for a batch of 30 g of the prepared spheroids is shown in Table 2. A total of 30 g batch of physical mixture was prepared by mixing 12 g sulfasalazine, 12 g guar gum, 5.4 g MCC PH 101 and 0.6 g of talc. The entire blend was mixed homogeneously for 30 min and then passed through sieve no. 22. Wet mass was prepared by adding 30 ml of mixture of water and isopropyl alcohol (80:20 v/v) drop wise. This wet mass was subjected to extruder (RRE/EXT-05/037, R.R. Enterprises, India) and the resultant material was subjected to spheronization using spheronizer (REE/SPH-150/010, R.R. Enterprises, India) maintained at 2000 rpm for 20 min. The obtained spheroids were kept in tray dryer (CADMACH drying oven, India) at 40 °C for 1 h. The dried spheroids were divided into 3 parts, each containing 10 g spheroids. First batch (B-1) was coated with 5% w/v dispersion of guar gum till 20% w/w weight gain was obtained. Second batch (B-2) was coated using a dispersion of amylose–ethyl cellulose mixture containing 50% amylose to a total weight gain of 15%. Amylose–ethyl cellulose coating solution was prepared as reported by Siew et al. (2004). Third batch (B-3) was coated using pectin-ethyl cellulose dispersion containing 50% pectin to a total weight gain of 35%. Pectin-ethyl cellulose coating solution was prepared as per the procedure reported by Ahmed (2005). Coating was done using fluidized bed coater (Mini Glatt, Pam Glatt Pharma Technologies Pvt. Ltd., India) preheated to 60 °C prior to coating. Inlet temperature was maintained between 45 and 60 °C, spraying rate was kept 0.4–0.6 ml/min at atomising air pressure of 0.2–0.3 Bar. The coated spheroids were kept for 1–2 h at 40–60 °C.

2.3. HPLC analysis of SFZ in spheroids and dissolution fluids

The quantitative determination of SFZ was done by HPLC. A gradient HPLC (HPLC LC-20AD, Shimadzu, Japan) system with photo diode array detector SPD-M20A and RP C-18 column (Phenomenex, 250 × 4.6 mm^2 ID; particle size 5 μm) was used. The HPLC system was equipped with the software "LC solution (Shimadzu)". The mobile phase used was methanol and 10 mM ammonium acetate buffer (pH 7.0). The filtered and degassed mobile phase was pumped at a flow rate of 0.8 ml/min in the ratio of 42:52 (methanol: 10 mM ammonium acetate buffer, pH adjusted to 7.0) and analyzed at 359 nm. The retention time of SFZ was found to be 9.97 min.

2.4. Development of probiotic culture based dissolution method

2.4.1. Preparation of probiotic based culture medium

In order to evaluate the in vitro dissolution testing for polysaccharide based colon specific drug delivery, probiotic based fluid thioglycollate medium was prepared. The composition of probiotic (BIOMIX-I, Unique Biotech, Hyderabad, India) used in the present study is shown in Table 3.

Fluid Thioglycollate Medium (FTM) was prepared according to the formula specified in the FDA Bacteriological Analytical Manual (BAM) (Food and Drug Administration, 1995) that conforms to Harmonized United States Pharmacopoeia (USP)