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Formulation and Evaluation Porous Microspheres of 5- Fluorouracil for Colon Targeting

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ABSTRACT: The treatment of colon cancer has been aimed by approaches of oral drug administration. 5-Fluorouracil is a candidate to be delivered orally to the colon; pH - sensitive polymers Eudragit S 100 and L 100 were used to prepare microspheres by a simple oil /water emulsification process. Process parameters were analyzed in order to optimize the drug loading and release profiles. In further attempts mixtures with Eudragit S100 and L100 were prepared to prolong drug release. Scanning electron microscopy permitted a structural analysis. The solvent extraction was preferable over solvent evaporation with a view to the encapsulation rate (extraction: 37%; evaporation: 19%) due to the hydrophilic character of the drug while release pattern were nearly unchanged. Eudragit S100, pure or in mixture, was found to retain drug release at pH 4.5 lower than 41% within 6 h. At pH 7.4, nearly immediate release (within 30 min) was observed for pure S100, while mixtures enabled to prolong the release slightly. Analysis of the morphology led to an inhomogeneous polymer distribution of S100 and L 100 throughout the particle core. However, the formulation proved its applicability in-vitro as a promising device for pH-dependent colon delivery of 5-fluorouracil. The study was aimed at develop the porous microspheres, which can control the drug release up to 6 h and hence it can prevent the acid decomposition in stomach.

KEYWORDS: Colon delivery; colorectal cancer; Microspheres; 5-Fu.

INTRODUCTION

Colon cancer is the second most cause of death after lung cancer by cancer diseases. Many different drugs or drug combinations have been tested for a successful therapy. 5-Fluorouracil (5-FU) is a commonly applied anticancer drug in the treatment of colon cancer [1]. At present, the standard regimen is an intravenous bolus injection of 5-fluorouracil (5- FU) modulated by folinic acid (leucovorin) [2, 3]. Only few approaches for an oral administration have been described in literature. Recently, enzyme dependent tablet-based systems have been proposed, which might allow an efficient treatment combined with a reduction of adverse effects [4]. However, due to variations in transit time throughout the colon, the drug release can be incomplete when the colon specific tablet matrix is not readily disintegrated and the treatment will remain insufficient. Especially, diarrhea has been observed as of one of the major adverse effects and also a toxicity limiting factor of the therapy [5–7]. This can turn oral 5-FU treatment insufficient. A size reduction of the carrier system might be an option in order to circumvent those problems, since size dependent gastrointestinal retention has been observed earlier [8]. Moreover, the so-called 'streaming' is in favor of the development of smaller drug carriers providing longer presence in the large bowel.

A colon specific drug delivery by microsphere formulations has been aimed for several anti-inflammatory drugs [9–12]. For the preparation of these microspheres the pH-sensitive polymers EudragitS and L were applied. Usually the preparation methods consisted of use of O/O emulsification process where EudragitS or L are dissolved in acetone / 2-propanol mixtures and emulsified in liquid paraffin [10–12].

These systems were developed for an oral drug delivery in the treatment of ulcerative colitis. However, the pH-dependent release properties of such a system also seem to be applicable to a potential local treatment in colon cancer. Therefore, this work aimed the development of an oral delivery system for 5-fluorouracil which allows the release of the anti cancer drug locally in the colon in dependence of luminal pH. This may reduce the systemic toxicity which is mainly the limiting factor in the treatment of colon cancer and allows a local dose increase compared to existing treatment strategies.

EXPERIMENTAL:

Materials and methods

Materials:

5-FU was kind gift from Biochem Laboratories, Mumbai. EudragitS100 and EudragitL 100 gift sample from Lupin Research Park, Pune. Polyvinyl alcohol (*M* 20 000 Da, 80% hydrolyzed), methylene chloride and other chemicals were obtained were of analytical grade.

Methods:

Formulation Design:

Formulations of 5-flurouracil microspheres containing different concentration of Drug: Eudragit were prepared using Factorial design and grouped as Batch A, B and C shown in Table 1.

Preparation of microspheres: [13]

The preparation of microspheres was based on an O/W emulsification solvent evaporation. It was optimized as follows: a total polymer was dissolved in 8 ml methylene chloride and Ethanol (3:1). Then, 5-FU crystals (diameter [*D*]: 32.763.9 mm) were suspended by ultrasonication in polymer solution. This suspension was poured into 75 ml of 1% w/w PVA and O/W type of emulsion was formed by extensive stirring with a three blade propeller at 500 rpm. When solvent evaporation was applied, the system was kept under agitation until methylene chloride was evaporated. After decantation, the microspheres were filtered (HVLP filter, Millipore, pore size 0.45 mm), washed extensively with distilled water and lyophilized overnight.

Scanning electron microscopy: [14]

The external and internal morphology of microspheres was analyzed by scanning electron microscopy (SEM). The microspheres were fixed on supports with carbon-glue, and coated with gold using a gold sputter module in a high-vacuum evaporator. Samples were then observed with the scanning electron microscope (JEOL JSM-T330A scanning microscope, Tokyo, Japan) at 10 kV.

Differential scanning Calorimetry (DSC) analysis [15]

Differential scanning calorimetric (DSC) analyses of the 5-Fu and polymer were carried out by using differential scanning calorimeter equipped with computer analyzer (Shimadzu TA–60 differential scanning calorimeter, Shimadzu Corporation, Kyoto, Japan). Samples (3-7 mg) were heated under nitrogen atmosphere on an aluminum pan at a heating rate of 10 °C/min over the temperature range of 30-300 ^{o}C.

Powder X- ray diffraction studies (PXRD) [16]

Powder X-ray diffraction (PXRD) patterns were traced employing X-ray diffractometer (Philips PW 1729, Analytical XRD, Holland) for the samples using Ni filtered CuK(α) radiation (intensity ratio(α_1/ α_2): 0.500), a voltage of 40 KV, a current of 30 mA and receiving slit of 0.2 inches. The samples were analyzed over 2θ range of 5.010-39.990^{o} with scanning step size of 0.020 ^{o} (2θ) and scan step time of one second.

Production Yield and Percentage drug entrapment [14]

Emulsion- Solvent evaporation method offers a versatile, easy and practical method for manufacturing of Microspheres. Production yield is expressed as percent yield with respect to initial amount of drug and polymer.

Table 1: Formulation Design Using Factorial Design

Drug : Polymer Ratio	Formulation Code	Polymer
1 : 1	A1	Eudragit S100
1 : 1.5	A2	
1 : 2	A3	
1 : 2.5	A4	
1 : 3	A5	
1 : 1	B1	Eudragit L100
1 : 1.5	B2	
1 : 2	B3	
1 : 2.5	B4	
1 : 3	B5	
1 : 1	AB1	Eudragit S100 : L100 (50:50)
1 : 1.5	AB2	
1 : 2	AB3	
1 : 2.5	AB4	
1 : 3	AB5	

Entrapment was determined by taking a weighed quantity of preformed microspheres (approximately 25 mg) with phosphate buffered of pH 7.4 (10 ml) was added in a vial. The solution was stirred vigorously for 24 hrs with mechanical stirrer. Supernatant was collected by centrifugation and drug content in supernatant was determined by using UV spectrophotometer at suitable wavelength (266nm). Efficiency of drug entrapment for each batch was calculated in term of percentage drug entrapment as per the following formula [14]

% Drug entrapment=

$$\frac{\text{Practical content}}{\text{Theoretical content}} \times 100$$

In vitro drug release [17, 18]

The drug release rate from microspheres was determined using USP XXIII paddle type dissolution apparatus (Lab India Disso 2000, Lab India Ltd., Mumbai). A weighed amount of microspheres equivalent to 50 mg drug was filled into a capsule (# 3) and placed in the basket. Hydrochloric acid buffer (pH 1.2), Acetate buffer (pH 4.5), Phosphate buffer pH 7.4 was used as the dissolution medium and maintained at 37 ± 2°C at a rotation speed of 100 rpm. Perfect sink conditions prevailed during the drug release studies. 5 ml sample was withdrawn at each interval, passed through a 0.45 µm membrane filter (Millipore), and analyzed by UV method to determine the concentration of drug present in the dissolution medium at 266 nm. The initial volume of the dissolution fluid was maintained by adding 5 ml of fresh dissolution fluid after each withdrawal.

RESULTS AND DISCUSSION

Eudragit S100 and L 100 belong to the pH-sensitive Eudragit group of polyacrylates, exhibiting a dissolution threshold pH slightly above 7.2 [19]. These properties are based on its structure, synthesized by copolymerization of methacrylic acid, methyl acrylate and methyl methacrylate. One major advantage of the polymer is the fact that it can be dissolved in methylene chloride. This makes the polymer applicable to different microsphere preparation methods replacing the complicated oil / oil emulsification process used in prior formulations [10–12]. It seems to be very advantageous to circumvent the unpleasant handling of the external oil phase including the long-term solvent evaporation under vacuum and the complex washing steps producing a distinct quantity of additional solvent waste.

Solvent evaporation and extraction:

A smoother surface was obtained for microspheres from the evaporation process, while the extraction procedure led to less regular shaped particles with a procedure led to less regular shaped particles with a generally in line with results about other polymers which was reported to be due to the rapid solvent dislocation in case of using an extraction method [16,17]. The long term contact with the external aqueous phase in the evaporation process might allow a prolonged diffusion of the hydrophilic 5-FU out of the organic phase during the preparation procedure and subsequently cause an intense drug loss. The drug release showed generally similar behavior at the different pH. 5-FU was retained efficiently inside the microspheres when tested in in-vitro buffer systems at pH 1.2 and 4.5 maintaining at least about 70 and 65% of the initial drug load after 6 h of incubation. A comparatively fast release was observed at pH 7.4, which delivered about 100% of the incorporated drug within 60 min.

When comparing evaporation and extraction process, a slightly faster drug release occurred after solvent evaporation for pH below 6.8. Since usually smoother particles have been reported to release slower [17], the closer location of the drug crystals to the particle surface might be a reason. The rather hydrophilic 5-FU diffuses towards the external aqueous phase and due to the longer contact with the external aqueous phase during the evaporation process such a location near the interface might be intensified. The drug release occurs in two step at this pH where the matrix is persistent (dissolution of the drug crystals in the particle matrix followed by the diffusion), the higher drug load with the extraction method may, therefore, also be responsible for a slower drug release. Moreover, also the slight differences in the particle diameter can have an influence on the drug release as the microspheres after solvent evaporation are smaller, exhibiting a larger surface for an enlarged drug diffusion.

pH dependent drug release:

The pH-dependent approach appears to have the lowest risk for treatment failure as compared to other delivery strategies, especially for Crohn's disease. Thus, although there is a considerable benefit in the new drug delivery approaches utilizing microparticulate approach. The colon based on pH-sensitive release is conventional and includes all possible drawbacks of this strategy.

Phosphate buffer of pH 7.4, 0.1 N HCl and Phosphate buffer of pH 4.5 were used to evaluate the decomposition of 5-FU. The results obtained from

dissolution studies of the optimised formulation are graphically represented in Fig 1, Fig 2 and Fig 3.

Scanning electron microscopy:

The results obtained from SEM studies confirmed the porous and spherical structure of microspheres (fig 4). Moreover, morphology of microspheres revealed that degree of porosity of microspheres was dependant on the composition of Eudragit present in the microspheres. High content of Eudragit led to less porosity. There was no porosity appearing in microspheres with above 200 mg of Eudragit in formulation, whereas less than 100 mg content of Eudragit resulted in loss of spherical structure and mechanical strength.

Differential scanning Calorimetry (DSC) analysis

DSC was taken by scanning formulation from 30 to 300^{o}C with heating rate of 2K/min. A sharp curve at 283.20^{o}C gives exact phase transition temperature and reveals stability of formulation at 37^{o}C.

Powder X- ray diffraction studies (PXRD)

X-ray diffraction pattern for pure drug indicated crystalline form of 5-FU. Sharp peak between 28.385^{o}2θ to 28.525^{o}2θ was characteristic of 5-FU. In drug loaded microsphere formulation, the sharp peaks of pure 5-FU was not observed. It indicates that there may be partial or complete transitions of crystalline state to amorphous state.

Production Yield and Percentage drug entrapment

Entrapment efficacy of drug loaded microspheres was found to be in the range of 24.67 - 41.23 %. Entrapment efficacy was dependent on the composition of drug as well as Eudragit. Low content of Eudragit and high drug composition in formulation led to reduce the entrapment of drug in microsphere formulations. Drug to Eudragit ratio of 1:2 yielded maximum entrapment efficacy of 41.23 %. Whereas, minimum entrapment efficacy of 24.67 % was observed with 1:3 ratio of drug to Eudragit. However, 5-Fu being high solubility drug with low dose required high concentration of polymer and/or excipients in dosage form for better formulation development.

Drug content of all the batches were found to be in the range of 24.67 % - 41.23 % so the formulations showed a high drug content in final formulation as compared to limited drug loading for any other sophisticated formulations like nano particles, emulsifications, liposomes, micelles etc.

Drug release studies

In-vitro dissolution studies were performed in three different dissolution media. Hydrochloric acid (0.1 N), Acetate buffer (pH 4.5) Phosphate buffer (pH 7.4) were used for in-vitro drug dissolution study. Phosphate buffer of pH 7.4 were used as the colonic pH is around 6.8 to 7.4 and hydrochloric acid buffer of pH 1.2 was used to study whether the decomposition of 5-Fu takes place in acidic pH or not. Microspheres containing 30 mg equivalent of 5-Fu were subjected to this study to maintain the sink condition throughout the study. Dissolution studies were carried out over the period of 3h for hydrochloric acid buffer and for 6 hr for acetate buffer and phosphate buffer, which mimic the gastric emptying time. All the prepared microspheres showed better controlled release profile. Drug to Eudragit ratio of 1:2 of Batch A shown 101% release within 6 h in phosphate buffer (pH 7.4)

The *in-vitro* drug release studies showed that about 25 % to 30 % drug released in acidic pH (pH 1.2), about 40 % to 45 % drug released at pH 4.5 and 88 %to 101 % drug release at pH 7.4 (Colonic pH).

CONCLUSION

A colon specific microparticulate drug delivery system can be prepared by a simple and fast o/w emulsification method. A relatively high drug load even for the hydrophilic drug 5-FU can be achieved. A pH-dependent release can be provided in a range of pH 6.8, where the main drug load is retained, and pH 7.4, where a fast dissolution of the carrier occurs. When polymer combinations were applied for the microsphere preparation (Eudragit S100 and Eudragit L 100) at different mixing ratios, only minor influences on particle size and drug load are observed. These simple polymer mixtures can prolong the release only for a relatively short period due to the exceptional structural arrangements of the carrier system. However, this new formulation is a good candidate for an application in oral delivery for colon cancer. It has to be proven *in-vivo* whether these relatively small property changes have an impact on the colon delivery.

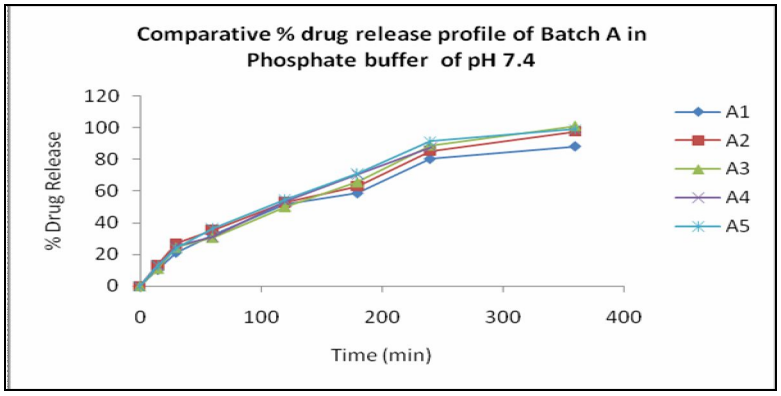

Fig. 1: Comparative % drug release profile of Batch A in Phosphate buffer, pH 7.4

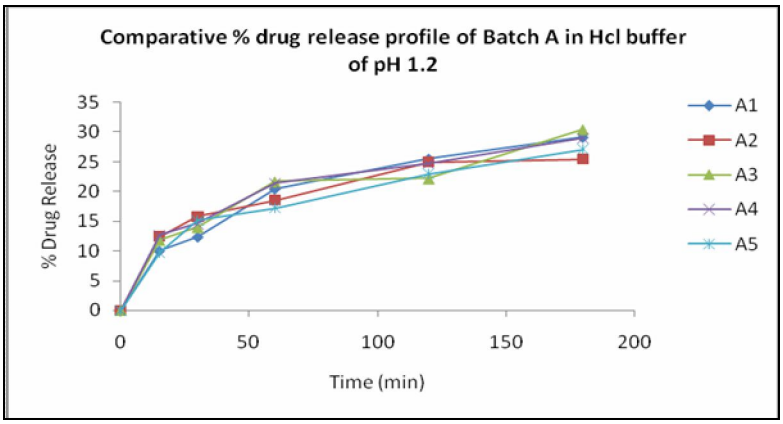

Fig. 2: Comparative % drug release profile of Batch A in Phosphate buffer, pH 7.4

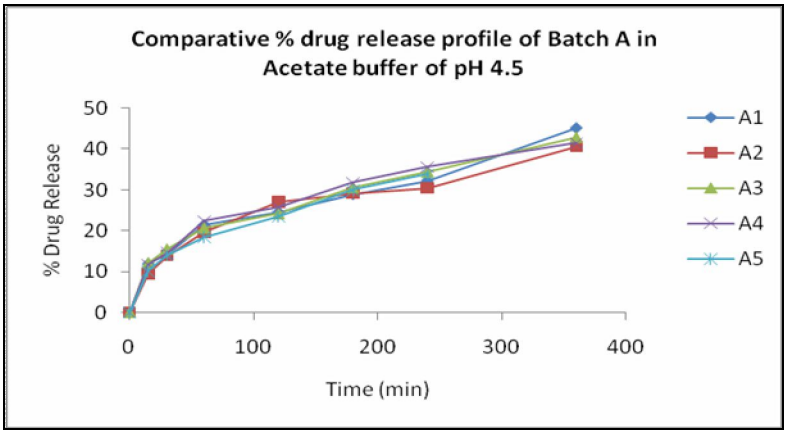

Fig. 3: Comparative % drug release profile of Batch A in Phosphate buffer, pH 7.4

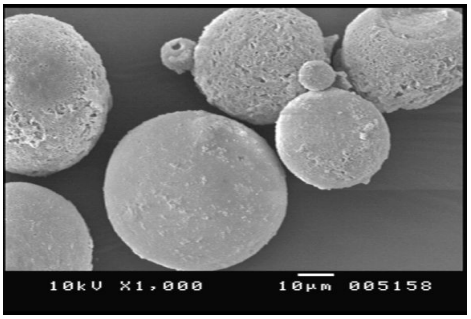

Fig 4: SEM images of empty Microsphere

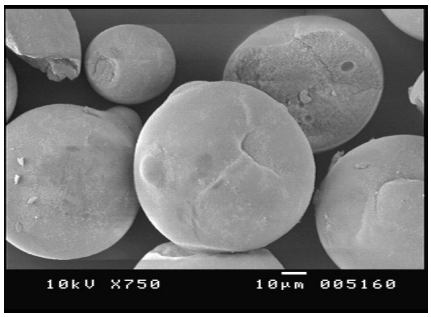

Fig 5: SEM images of Microspheres of Batch A

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