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European Journal of Pharmaceutics and Biopharmaceutics 59 (2005) 367–371
European Journal of Pharmaceutics and Biopharmaceutics
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Note

Observations in simultaneous microencapsulation of 5-fluorouracil and leucovorin for combined pH-dependent release

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Received 24 June 2004; accepted in revised form 13 September 2004
Available online 28 October 2004

Abstract

5-Fluorouracil (5-FU) in combination with leucovorin (LV) is nowadays the standard treatment in colon cancer and would be a candidate to be delivered orally to the colon. Eudragit P-4135F or Eudragit RS100 were used separately to prepare microspheres by an oil/oil emulsification process trapping 5-FU and LV simultaneously. Scanning electron microscopy permitted a structural analysis, process parameters were analyzed and drug loading and release profiles were recorded. Particle size varied between 123 (RS100) and 146 μm (P-4135F). Generally, higher encapsulation rates were found with RS100 (5-FU, 60.3±9.7%; LV, 81.4±8.6%) compared to P-4135F (5-FU, 48.3±2.0%; LV, 55.4±2.7%). Microparticles made from Eudragit RS100 released the incorporated drug combination within 8 h not exhibiting general differences between the kinetics of both drugs. P-4135F was found to maintain the undesired 5-FU release at pH 6.8 lower than 25% within 4 h while at pH 7.4, a nearly immediate release (within 15 min) was observed. Although the release was similar at pH 7.4, at pH 6.8 LV showed a distinct initial drug loss of about 60% and a complete release within 2 h. SEM analyses revealed a substantial presence of LV crystals on the particle surface provoking a distinct burst effect of LV. These observations were concluded to be related to the high lipophilicity of P-4135F provoking a separation between P-4135F and LV during the preparation process.

Keywords: Colon delivery; Colorectal cancer; Microspheres; 5-Fluorouracil; Leucovorin

1. 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. At present, the standard regimen is an intravenous bolus injection of 5-fluorouracil (5-FU) modulated by leucovorin (LV) [1,2].

Only few approaches for an oral administration of anticancer drugs in the treatment of colon cancer 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 [3]. Alternatively, pH-dependent drug release systems have been developed for the 5-FU release in the colon [4]. Possible variations in transit time throughout the colon risking incomplete carrier disintegration and a subsequent therapy failure were thought to be reduced with this latter strategy.

However, all proposed drug delivery strategies did not consider that the optimized drug therapy for colon cancer consists of a drug combination [1,2]. The design of systems delivering an entrapped drug combination has been reported in literature earlier for other therapeutic fields [5,6]. Although an oral drug administration was aimed, these systems focused rather on a sustained than a pulsatile drug release which is proposed here.

Besides a possible local effect after absorption of the drug combination which seems to be of minor importance, a major advantage consists in the colon delivery, namely the avoidance of mucosal metabolism which has been reported to be lowest in the colon on the example of other drugs [7]. Similar findings are described for a P-glycoprotein related efflux slightly reduced in the colonic tissue. Both are reasons

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doi:10.1016/j.ejpb.2004.09.005

for a generally lower bioavailability of drugs also being responsible for a lower therapeutic effect.

Most of the commercialized systems for the local drug delivery to the lower intestine after oral administration are based on the change of pH during the gastrointestinal passage. The pH-sensitive approaches such as methacrylate/methacryl acid polymers Eudragit® S and L dissolve in aqueous media at pH 6 and 7, respectively, which may be equivalent to a drug release to the distal ileum.

Recently, an additional polymer has been described for the use in colon delivery. It has been applied for film coating purposes on pellets, tablets and the preparation of microspheres [8,9]. Similarly, the described approach of a combined and pH-sensitive drug release of 5-FU and LV is based on the use of Eudragit P-4135F which was reported to allow more effective drug retention during the gastrointestinal passage and a subsequent delivery to the colon [8,9]. Besides, diarrhea has been observed as one of the major adverse effects of the 5-FU therapy [10] which can turn oral standard formulations insufficient. A size reduction of the carrier system might be required in order to circumvent its early elimination, since size-dependent gastrointestinal retention has been reported in diarrhea with an optimum for particles smaller than around 200 μm [11]. Subsequently, the combined microencapsulation of 5-FU and LV could be an advantageous approach.

2. Materials and methods

2.1. Materials

Eudragit® RS100 and Eudragit® P-4135F were kind gifts from Roehm Pharma Polymers, Tokyo, Japan. 5-FU, LV, and polyvinyl alcohol were purchased from Sigma (Deisenhofen, Germany). All other chemicals were obtained from Nacalai Tesque Inc. (Kyoto, Japan) and were of analytical grade.

2.2. Methods

2.2.1. Preparation of microspheres

The preparation of microspheres was based on an oil/oil emulsification-solvent evaporation method. It was optimized as follows: a total polymer amount of 200 mg was dissolved in 5 ml acetone or equivalent volumes of solvent mixtures of acetone/ethanol. Fifty milligrams of 5-FU crystals (diameter [$D_{50\%}$]: 32.7 ± 3.9 μm) and 20 mg of LV crystals (diameter [$D_{50\%}$]: 17.2 ± 7.1 μm) were suspended by ultrasonication in the polymer solution. This solution was poured into 80 ml of liquid paraffin containing 1% w/w Span 80 and an oil/oil-emulsion was formed by stirring with a three-blade propeller at 600 rpm. The emulsion was stirred under vacuum until solvents were removed. Microspheres were collected by filtration and washing steps were performed with *n*-hexane followed by lyophilization.

2.2.2. Scanning electron microscopy and particle size analysis

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.

All microsphere batches were analyzed for their size distribution using a LDSA 2400A particle size analyzer (Tohnichi Computer Co. Ltd, Japan).

2.2.3. Determination of drug content and in vitro drug release

The drug loading efficiency in the microparticles was determined by high performance liquid chromatography (HPLC) based on an extraction method described elsewhere in detail [4]. The isocratic HPLC method was developed to our requirements allowing the simultaneous detection of both drugs. The system setup was as follows: RP-18 column (LiChrospher® 100); eluent:acetonitrile:acetate buffer pH 4.4 (6:94); flow rate 0.8 ml/min. Both, LV and 5-FU were detected by UV absorbance at 283 nm, samples of 50 μl were injected into the column.

The in vitro drug release was analyzed by the use of a paddle apparatus (USP XXIII). Drug-loaded microparticles (20 mg) were suspended in 500 ml phosphate buffer systems of different pH. The dissolution medium was kept under stirring at 100 rpm. All the experiments were carried out at 37 °C for 4–8 h. Aliquots of the dissolution medium (300 μl) were withdrawn at predetermined time intervals. Drug concentrations were directly analyzed by the HPLC method described before.

3. Results and discussion

The entrapment of 5-FU and LV in combination by using the newly described pH-sensitive polymer Eudragit P-4135F was intended to deliver the drug towards the distal sections of the intestine. The application of an oil/oil emulsification appeared reasonable due to the hydrophilic properties of both drugs aiming for increased encapsulation rates based on their lower solubility in the external oil phase. Moreover, a recent report on the microencapsulation of 5-FU into Eudragit P-4135F by an oil/water emulsification showed generally encapsulation rates below 40% [4] demanding for alternative preparation methods increasing the drug load.

Particle size of microspheres prepared with Eudragit P-4135F varied around 146 μm (Table 1). Generally, the encapsulation rates were higher compared to those found in

Table 1
General characteristics of microspheres

		P-4135F	RS
Diameter (μm)		146±21	123±17
Yield (%)		88.3±5.5	84.6±7.1
Encaps. rate (%)	5-FU	48.3±2.0	60.3±9.7
	LV	55.4±2.7	81.4±8.6

the above-mentioned earlier study. During drug release experiments, at pH 7.4 where complete polymer dissolution can be expected, an immediate release of both drugs was determined (Fig. 1). 5-FU showed significant drug retention inside the microparticle at pH 6.8 which was in line with the expectations since the nominal pH of polymer dissolution is around 7.2 [12]. In opposite, for LV a distinct drug leakage of around 60% was determined within the first 15 min followed by a complete release after 2 h.

During the morphological analysis of Eudragit P-4135F microparticles a distinct amount of drug crystals was observed to be attached directly to the surface of the microparticles (Fig. 2). When Eudragit P-4135F microparticles were separated from the release medium at pH 6.8 after an incubation period of 2 h LV crystals were completely dissolved leaving imprints in the particle surface behind (Fig. 3). Also the replacement of the inner organic actone phase by either acetone/2-propanol or acetone/ethanol did not exhibit distinct advantages (data not shown).

Such surprising observations were thought to be based on the polymer properties although the entrapment of a hydrophilic compound inside Eudragit P-4135F microparticles was reported recently by using an identical preparation method [8]. A rough particle surface was found which however, was related to the high theoretical drug load not impeding the pH-controlled release. Similarly, other studies entrapping hydrophilic drug containing cores inside pH-sensitive microparticles by using either

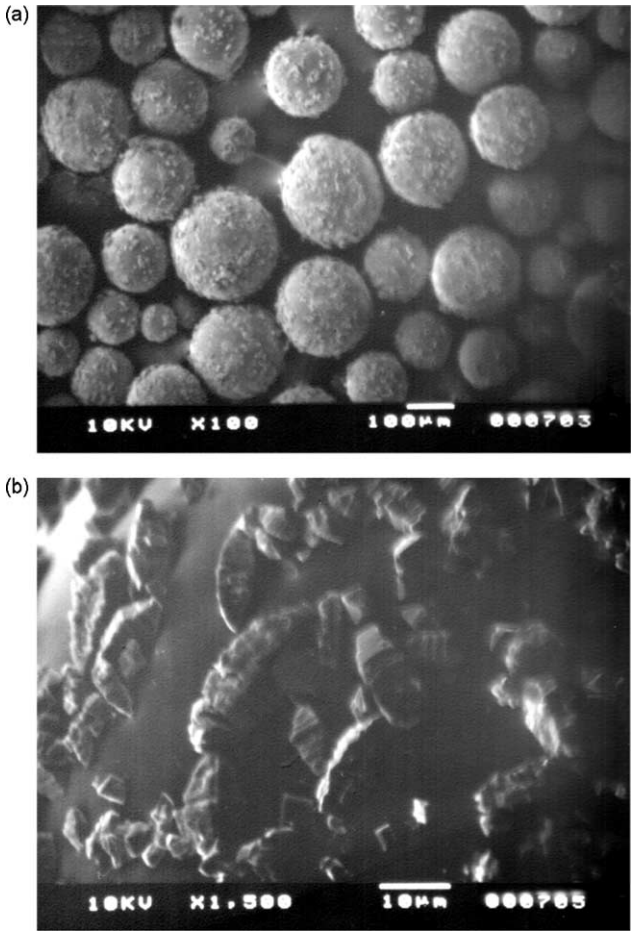

Fig. 2. SEM images of microspheres prepared with Eudragit P-4135F using an oil/oil emulsification process showing the general appearance (a) and a close-up of the particle surface (b).

Eudragit® L, Eudragit® S or their respective mixtures did not report such a separation [13,14].

Distinct lipophilic polymer properties were suggested from an earlier study where Eudragit P-4135F showed

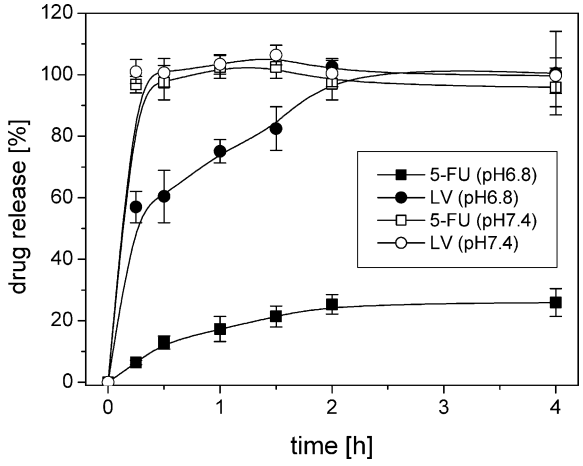

Fig. 1. Cumulated 5-FU and LV release versus time of Eudragit P-4135F microspheres at pH 6.8 or 7.4 at 37 °C ($n=3$). Data are given as mean±SD.

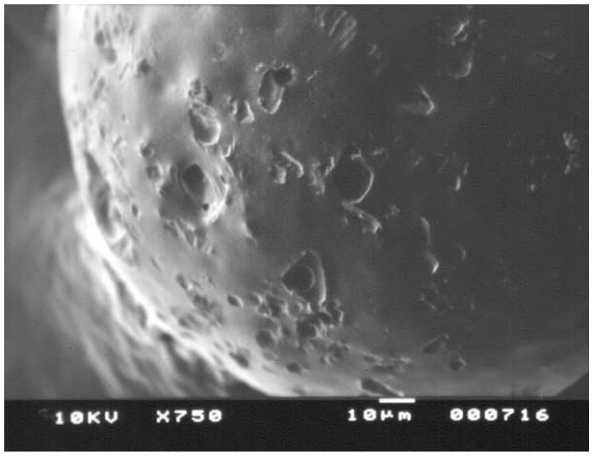

Fig. 3. SEM image of the particle surface of a microsphere prepared with Eudragit P-4135F after 2 h of drug release experiment at pH 6.8 at 37 °C.

strong interactions with a lipophilic drug [12]. Besides, a polymer separation behavior in mixtures of Eudragit P-4135F and RS100 was observed during the preparation of microparticles which was presumed to be dependent on the polymers' differences in hydrophilicity/lipophilicity [4].

These observations can be explained by the chemical composition of Eudragit P-4135F. It contains besides the monomers of methacrylic acid and methyl methacrylate the components of the standard pH-sensitive polymers Eudragit® L and S additionally large amounts of methyl acrylate (around 65%) turning it more lipophilic. Also the hydrophilicity of LV (around 8-fold higher solubility in water compared with 5-FU) is contributing significantly to the repulsion between LV and polymer while 5-FU can be entrapped more successful. Consequently, LV insoluble in both, internal and external phase and, moreover, highly hydrophilic is transported during the evaporation process towards the oil/oil interface and subsequently located at the particle surface when the matrix polymer is precipitating with the solvent removal. The presence of the drug on the particle surface after the phase separation is persistent due to the fact that LV is, moreover, insoluble in the washing medium *n*-hexane and only shear forces may have removed some of the LV crystals from the particle surface mechanically. Consequently, the non-encapsulated LV crystals are still located at the particle surface in the final product leading to the unacceptable drug release behavior.

In opposite, the use of an oil/water emulsification method is no alternative option due to the differences in hydrophilicity/lipophilicity between drug and polymer being unfavorable for satisfying drug entrapment.

In order to prove the influences by the polymer, reference microparticles were prepared with a similar preparation setup. Using the matrix polymer Eudragit® RS100, which is a swellable, more hydrophilic but not pH-sensitive polymer, permitted to observe the influences on the general appearance of the microparticles, in dependency of the physico-chemical properties of the polymer. This, however, would surely not fit in a comparable therapeutic strategy due to completely different release properties.

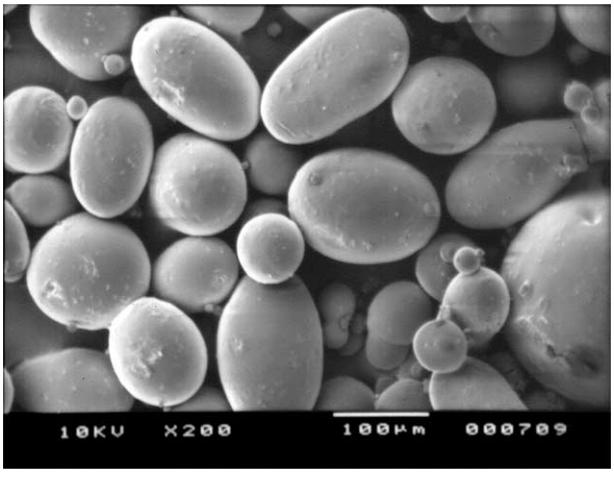

Fig. 4. SEM image of microspheres prepared with Eudragit® RS100 using an oil/oil emulsification process showing their general appearance.

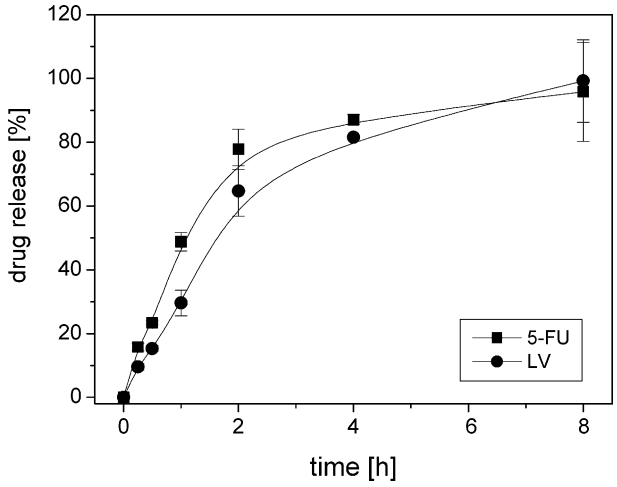

Fig. 5. Cumulated 5-FU and LV release versus time of Eudragit® RS100 microspheres at pH 7.4 at 37 °C ($n=3$). Data are given as mean ± SD.

Eudragit® RS100 microparticles appeared more or less spherical with a smooth surface subsequently fully entrapping the drug crystals (Fig. 4). The mean particle diameter was similar for both polymers which might be based on the exactly equivalent preparation setup and higher encapsulation rates were determined with Eudragit® RS100 due to the apparently higher repulsion between Eudragit P-4135F and both hydrophilic drugs (Table 1). Drug release kinetics at pH 7.4 led to a continuous combined drug release of both drugs from Eudragit® RS100 microparticles being completed after around 8 h. Such release properties are based on the drug diffusion out of the swollen non-degradable particle polymer matrix (Fig. 5).

4. Conclusions

A combined pH-sensitive drug release of 5-FU and LV for their delivery was aimed. However, due to repulsion between polymer and LV, no successful drug entrapment and subsequent release was achieved. These observations were concluded to be related to the high lipophilicity of P-4135F where the increase of drug's hydrophilicity enhanced the subsequent drug/polymer separation. Thus, Eudragit P-4135F combined with an oil-in-oil emulsification method was observed to be very limited in use for the entrapment of hydrophilic compounds into pH-sensitive microspheres.

Acknowledgements

We would like to acknowledge the financial support by the Japanese Society of Promotion of Science with

the JSPS/Alexander-von-Humboldt-Foundation Postdoctoral Fellowship no. P-02701.

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