Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 335 (2007) 147–153
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Low molecular weight heparin loaded pH-sensitive microparticles

Yvette Meissner [a], Nathalie Ubrich [a], Fatima El Ghazouani [a], Philippe Maincent [a], Alf Lamprecht [b,*]

[a] *InsermU734-EA3452, Faculty of Pharmacy, Nancy, France*
[b] *Laboratory of Pharmaceutical Engineering, Faculty of Medicine and Pharmacy, University of Franche-Comté, Place Saint Jacques, F-25030 Besançon Cedex, France*

Received 6 September 2006; received in revised form 2 November 2006; accepted 3 November 2006
Available online 10 November 2006

Abstract

Low molecular weight heparins (LMWH) have shown efficacy in the treatment of inflammatory bowel disease after parenteral administration however risking severe hemorrhagic adverse effects. Therefore, an oral colonic targeted heparin dosage form allowing the release of LMWH directly in the inflamed tissue would be of major interest. Enoxaparin was entrapped into pH-sensitive microspheres using Eudragit P4135F that dissolves at pH > 7.2. Particle preparation was based on a double emulsion technique with either solvent extraction or evaporation. In order to increase the entrapment efficacy several preparation parameters were optimized, such as inner phase volume, polymer concentration, stabilization of the internal interface by surfactants. Solvent evaporation led to higher entrapment rates (evaporation: 70.1 ± 9.9%; extraction: 46.5 ± 6.4%). When increasing the volume of the inner aqueous heparin phase, lower encapsulation rates and larger microspheres (≈100–400 μm) were obtained. Sorbitan monostearate (1.75–28% of the total particle mass) had a stabilizing effect on the primary water/oil emulsion. Indeed, higher encapsulation rates (7%: 78.2 ± 3.5%; 14%: 76.4 ± 10.1%) and smaller particles (≈120–160 μm) were obtained whereas hexadecyltrimethylammonium bromide destabilized the primary emulsion. Interfacial tension studies at a simulated internal water/oil interface confirmed these results. As expected, in vitro drug release was found to be strongly pH-dependent; LMWH was retained in microspheres at pH < 6 (<20% release within 4 h) whereas a fast drug release was obtained at pH 7.4. The developed microspheres exhibited a particle size adapted to the needs of inflammatory bowel disease therapy, an efficient LMWH encapsulation, and a pH-controlled drug release. These microspheres represent a promising tool for the selective oral delivery of heparin to the colon, especially interesting in the treatment of inflammatory bowel disease.

Keywords: Microparticles; Low molecular weight heparin; Oral delivery; Oil/water; pH-sensitive; Double emulsion

1. Introduction

The use of pH-sensitive polymers is interesting for controlled release formulations and can protect the active drug from the intestinal fluid as well as providing selective drug delivery towards certain regions of the gastrointestinal tract (Lamprecht and Kawashima, 2006). There is a large variety of polymers commercially available, among them polyvinyl acetate phtalate and cellulose acetate phthalate or copolymers of acrylic and methacrylic acid known as Eudragit. In all cases, carboxylic acid containing monomers inside those polymers trigger the polymer dissolution depending on the environmental pH (Lamprecht and Kawashima, 2006).

For instance, the treatment of inflammatory bowel disease (IBD) relies on this strategy involving a variety of colon delivery systems (Chourasia and Jain, 2003). As described earlier, drug carrier systems with a size larger than 200 μm are subjected to diarrhea and subsequently the gastrointestinal transit time decreases followed by lack of efficacy (Hardy et al., 1985; Davis et al., 1986). Administering smaller drug delivery systems would affect the transport velocity of the carrier system in the bowel thus, reducing the risk of undelivered drug. In this context, the design of pH-sensitive microspheres (MS) for colonic drug delivery has been described in the literature (Rodriguez et al., 1998). Combining both factors by designing pH-sensitive MS led to promising results for the IBD treatment where studies in an experimental colitis indicated a significant therapeutic benefit from this carrier system (Jeong et al., 2001; Lamprecht et al., 2005).

* Corresponding author. Tel.: +33 3 81 66 55 48; fax: +33 3 81 66 52 90.
E-mail address: alf.lamprecht@univ-fcomte.fr (A. Lamprecht).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.11.014

Besides its anticoagulant properties, heparin was recently found to possess anti-inflammatory properties and to be efficient in the treatment of ulcerative colitis after subcutaneous administration (Törkvist et al., 1999; Dotan et al., 2001; Siveke and Folwaczny, 2004). Although mechanisms of action are not completely clear, its clinical application in IBD appears to be interesting. Surprisingly, oral delivery of heparins in IBD has been paid only very little attention (Cui and Jiang, 1999) which may be due to the lack of appropriate delivery systems.

In this study, low molecular weight heparin (enoxaparin) loaded pH-sensitive MS were prepared using Eudragit P-4125F which is a copolymer of methacrylic acid, methyl acrylate and methyl methacrylate with a dissolution threshold of 7.2 (Jeong et al., 2001). This polymer has been applied for microencapsulation and subsequent colonic delivery of different drugs (Lamprecht et al., 2004, 2005). The microencapsulation of heparin based on a double emulsion method was optimized by varying several process parameters. Two different formulation techniques were tested (solvent extraction and solvent evaporation) and the influence of preparation parameters such as the amount of LMWH or the use of different surfactants were investigated. Moreover, phenomena at the internal water/oil interface were characterized by interfacial tension studies and correlated with the observed particle properties.

2. Materials and methods

2.1. Materials

Eudragit P-4135F was a kind gift from Röhm Pharma Polymers (Tokyo, Japan). For LMWH, marketed sodium enoxaparin (Lovenox® 10.000 UI anti-Xa/1 ml) was purchased from Sanofi-Aventis (Paris, France). Hexadecyltrimethylammonium bromide (CTAB), polyvinyl alcohol, sorbitan monostearate (SMS; Span® 60) and polysorbate 80 (Tween® 80) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals were of analytical grade.

2.2. Preparation of microparticles

The LMWH-loaded MS were prepared by two different methods based on a w/o/w-emulsion technique followed either by solvent evaporation or by solvent extraction using dichloromethane (DCM) or ethyl acetate (EA), respectively.

2.2.1. Solvent evaporation

Two hundred milligrams of Eudragit P-4135F were dissolved in DCM (3 ml). Then varying amounts (always given in IU per formulation batch; 10,000 IU ≈ 44 mg LMWH) of aqueous LMWH were emulsified in the polymer solution with an ultrasound probe for 15 s. This w/o-emulsion was then poured into 75 ml aqueous polyvinyl alcohol (PVA) solution (0.5%) to form a w/o/w-emulsion. This emulsion was stirred for 1 h with a three-blade propeller at 500 rpm at room temperature until the organic solvent of the internal phase was removed. MS were filtrated (Millipore®, HA, 0.45 μm), washed extensively with deionized water and dried at room temperature.

2.2.2. Solvent extraction

The solvent extraction was carried out as a two-step process: 200 mg of polymer were dissolved in 4 ml EA. Varying amounts of aqueous LMWH were added and emulsified by ultrasonication for 15 s. First, this emulsion was poured into 20 ml aqueous PVA (0.5%) and stirred for 3 min with a three-blade propeller at 500 rpm. Then, this dispersion was poured into 200 ml aqueous PVA (0.1%) and stirred for 1 h. MS were filtrated (Millipore®, HA, 0.45 μm), washed extensively with deionized water and dried at room temperature.

2.2.3. Solvent extraction and evaporation with surfactants

In order to optimize the encapsulation rates, different kinds of surfactants, namely CTAB and SMS, were added to the formulations at amounts varying from 3.5 to 112 mg per batch, expressed as percentage of total theoretical particle batch mass. Due to their solubility properties, SMS were added directly to the organic phase while the cationic surfactant CTAB was dissolved in the aqueous LMWH solution.

Fluorescent labeled MS were prepared under equivalent conditions with the same methods described above. No detailed analysis on the amount of residual organic solvents was performed although knowing that this issue might essential for the development of pharmaceutics.

2.2.4. Particle size analysis

The particle size analyses of all MS batches were carried out by laser light diffraction (Mastersizer®, Malvern Instruments, UK). The MS were dispersed in 2 ml of an aqueous solution of Tween 80 (1%).

2.2.5. Scanning electron microscopy (SEM)

The particle morphology was analyzed by SEM. The MS 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.

2.2.6. Confocal laser scanning microscopy (CLSM)

As CLSM requires a fluorescence emission from the drug in order to permit its detection LMWH was labeled with fluoresceinamine. The labeling protocol was adapted to a method described earlier (Lamprecht et al., 2006): enoxaparin was incubated with carbodiimide and fluoresceinamine overnight at room temperature and thereafter free marker and linker were removed by dialysis (Spectrapor® 7, Spectrum Ltd., USA; membrane pore size: 1000 Da) against distilled water until no fluorescence was detected in the external phase.

A Biorad MRC 1024 Laser Scanning Confocal Imaging System (Hemel Hempstead, UK) equipped with an argon krypton laser (American Laser Corp., Salt Lake City, USA) and a Zeiss Axiovert 100 microscope (Carl Zeiss, Oberkochen, Germany) was used to investigate the structure and morphology of the microcapsules. All confocal fluorescence pictures were taken with a 40× objective (oil immersion, numeric aperture 1.30).

2.2.7. Determination of encapsulation rates and in vitro drug release

The drug content was determined by nephelometry (Hoffart et al., 2003), measuring directly the amount of LMWH entrapped in the MS. Therefore 20 mg of MS were dissolved in 5 ml phosphate buffer pH 7.4 in an ultrasonic bath for 1 h and then the polymer was precipitated with the same amount of hydrochloric acid buffer pH 1.2. The precipitate was centrifuged for 10 min at 4000 rpm and supernatants were analyzed as described previously. Encapsulation rates were calculated as percentage of total recovered LMWH from the formulation divided by the initial LMWH input.

The in vitro drug release was carried out with three different buffer systems at pH values of 1.2 and 7.4. All buffers were prepared according to the European Pharmacopoeia. Therefore 20 mg MS were suspended in a flask containing 20 ml buffer solution and polysorbate 80 (0.1%). The suspension was incubated at 37 °C in a water bath and stirred with a magnetic stirrer at 200 rpm. Samples of 1 ml were taken at predetermined times (0.25, 0.5, 1, 2, and 4 h) and substituted with 1 ml of fresh buffer and assayed for the release of LMWH by nephelometry as described previously. In case of the phosphate buffer pH 7.4 the dissolved polymer was precipitated with 1 ml hydrochloric acid buffer pH 1.2 and then centrifuged for 10 min at 4000 rpm. All experiments were performed in triplicate.

2.2.8. Interfacial tension

Measurements of the interfacial tension (IFT) of the simulated internal water/oil interface were performed using a tensiometer (Tensiometer DSA 100, KRÜSS GmbH, Hamburg, Germany). Water or aqueous LMWH solutions were used as drop phase and the DCM phase with or without polymer as external, continuous phase. The drop volume varied from 3 to 28 μl and the external phase was 5 ml. Interface tension was recorded versus time until equilibrium was reached (no significant change within 15 min). Different parameters of the system were investigated, e.g. different concentrations of the LMWH solution and surfactants. All measurements were performed in triplicate.

2.2.9. Statistical analysis

The results were expressed as mean values ± S.D. For the pairwise comparison the Student's *t*-test was used to investigate differences statistically. In all cases, $P < 0.05$ was considered to be significant.

3. Results

The MS appeared spherical and with a rough surface, exhibiting sporadically pores irrespective the preparation method used (Fig. 1A and B). In general, the particles of the solvent extraction process had a larger diameter than those of the solvent evaporation technique. The internal structure was found to be sponge-like due to the entrapment of the internal aqueous phase which is generally in line with structural observations in MS prepared by this technique. An analysis of particle structure by CLSM proved the incorporation of FITC-LMWH inside the

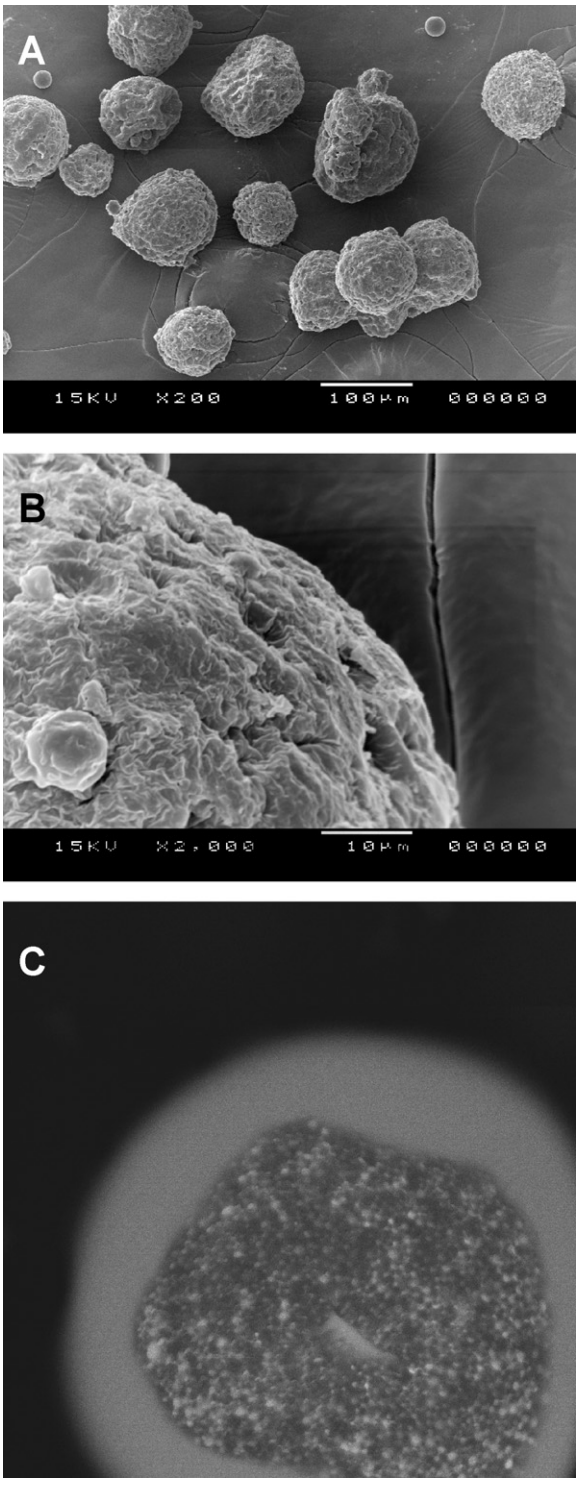

Fig. 1. SEM images of LMWH containing Eudragit P-4135F microparticles showing general appearance (A) and surface structure (B). CLSM analyses proved the incorporation of fluoresceinamine labeled LMWH using different formulation methods, here exemplarily shown for solvent evaporation with 1000 IU LMWH (C). The fluorescent aura surrounding the MS was due to marker diffusion into the aqueous dispersion medium. Image size represents 150 μm.

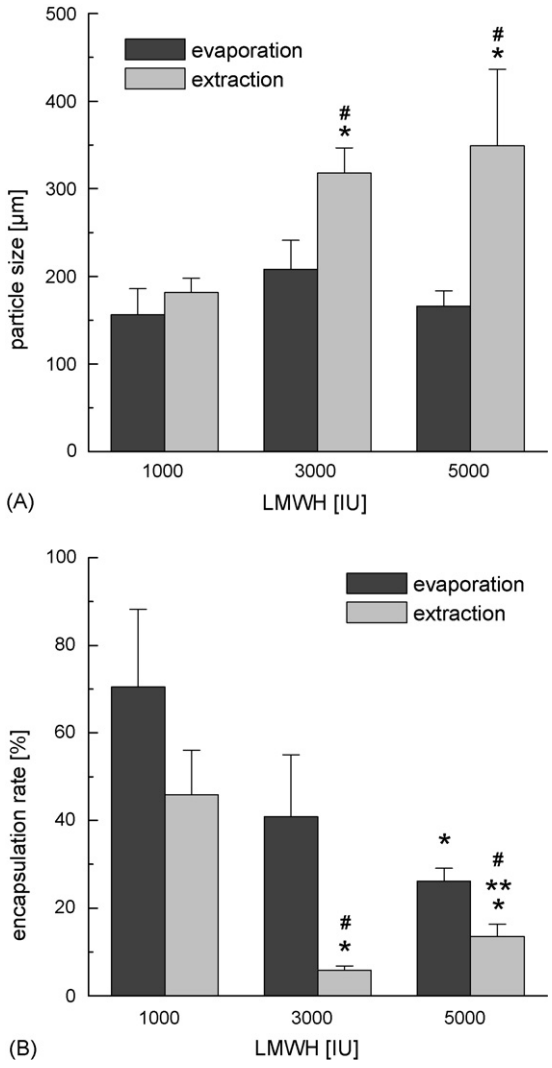

Fig. 2. Influence of the LMWH amount on the particle size (A) and on the encapsulation rates (B) after solvent evaporation and solvent extraction. Data are shown as mean ± S.D., $n = 3$. $^{*}P < 0.05$ compared to 1000 IU MS, $^{**}P < 0.05$ compared to 3000 IU MS, $^{\#}P < 0.05$ compared MS prepared by solvent evaporation.

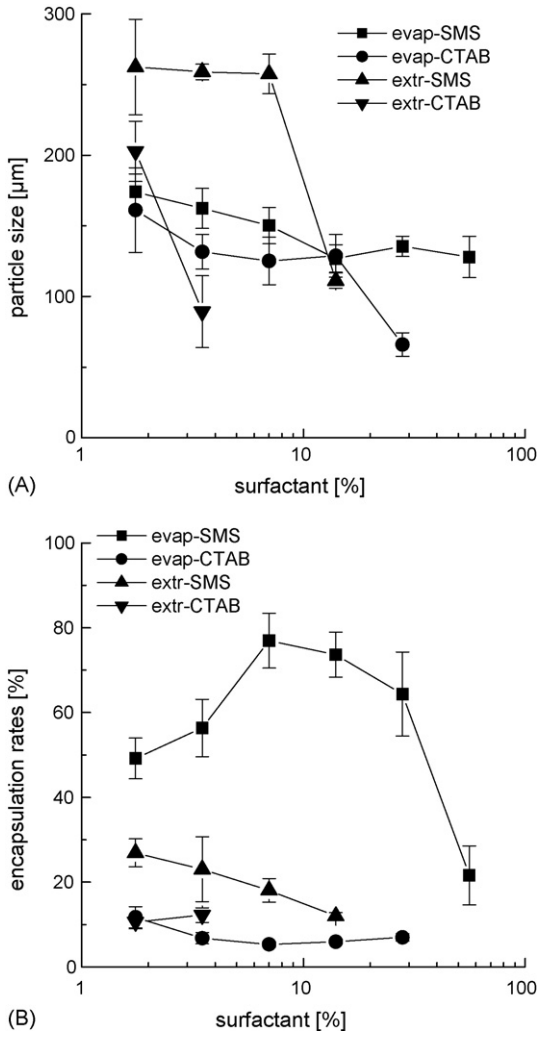

Fig. 3. Influence of SMS and CTAB amounts (given as percentage of total theoretical batch mass) on the particle size (A) and on the encapsulation rates after solvent evaporation and solvent extraction (B).

internal aqueous phase droplets (Fig. 1C). Homogeneous distribution of the inner aqueous phase droplets can be observed for solvent evaporation as well as solvent extraction method. The internal droplet size was however, essentially smaller with the solvent extraction method.

The amount of LMWH added to the formulation had a significant influence on the particle properties. Particle size increased whereas encapsulation rates decreased with higher amounts of LMWH added to the formulation (Fig. 2). In both methods (solvent evaporation or extraction) the highest efficacy was obtained with the lowest LMWH (1000 IU) amount. Although a particle size increase was observed with both preparation methods, this effect was less explicit for MS prepared with solvent evaporation.

The use of surfactants (SMS and CTAB) in order to stabilize the internal water/oil interface aimed for the further increase of the encapsulation rates, exemplarily studied with MS containing 1000 IU LMWH. Similar to precedent results, solvent extraction led to larger MS with a significant variability in particle diameter. On the other hand, there were only minor changes in the MS size after solvent evaporation using SMS or CTAB (Fig. 3A). Using solvent evaporation, a LMWH entrapment optimum was observed for SMS at 7 and 14% (calculated on the basis of total polymer mass) while the presence of CTAB decreased LMWH encapsulation with increasing concentrations (Fig. 3B). Solvent extraction resulted in a constant decrease of entrapment efficiencies with increasing of surfactant concentrations. Moreover, no MS formulation was obtainable with CTAB at concentrations exceeding 3.5%. A study employing the mixture of both surfactants, SMS and CTAB, underlined the deteriorating influence of increasing CTAB amounts which strongly decreased the encapsulation efficacy (Fig. 4). Other preparation parameters such as the type of sorbitan ester, stirring speed, or organic phase volume did not lead to a further increase of the encapsulation rate (data not shown).

MS formulations were tested for their in vitro drug release in buffers of different pH values (Fig. 5). LMWH was retained

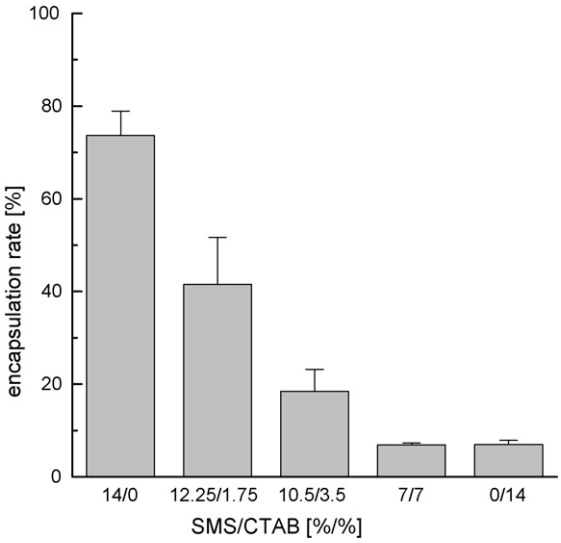

Fig. 4. Influence of SMS/CTAB mixtures in a total amount of 28 mg surfactant per formulation on the encapsulation rate after solvent evaporation. The mean particle diameter of these MS varied between 122 ± 14 and 172 ± 36 μm.

efficiently inside the MS at pH 1.2 and 6.0 for 4 h. The retained percentage of the initial drug load was at least 59% after this period while LMWH leakage at pH 1.2. A fast drug release from all formulations was observed at pH 7.4 where MS prepared by solvent evaporation slowed down slightly the release velocity. Slight increases in drug leakage at higher heparin concentrations were observed at low pHs (data not shown). Surprisingly, the presence of the surfactants had no influence on the drug release. In principle, only minor influences on the drug release behavior by the variation of the process parameters were found.

IFT was studied with a water/oil model interface in order to elucidate the effects and potential interaction between the involved components at the internal water/oil interface. The presence of the pH-sensitive polymer lowered distinctly the IFT at water/oil interface while the presence of LMWH had only a minor effect and its concentration no significant influence at the beginning of the measurement (Fig. 6A). However, the system evolved with time and when the state of equilibrium was reached significantly lower IFT values were obtained. The additional IFT lowering effects of SMS were slightly stronger than values observed with the pH-sensitive polymer alone being, moreover, concentration-dependent to a certain degree. The presence of LMWH further enhanced this effect (Fig. 6B).

When studying SMS or CTAB against distilled water, a significant decrease in the IFT was found. While the addition of LMWH into the aqueous phase resulted in a further decrease of IFT with SMS, the total destruction of the interface structure was observed with CTAB. Similar tendencies were observed with the surfactant mixture of CTAB/SMS.

4. Discussion

The selection of the microencapsulation technique depends largely on the physicochemical properties of the drug. For the entrapment of heparin and its derivates, a double emulsion

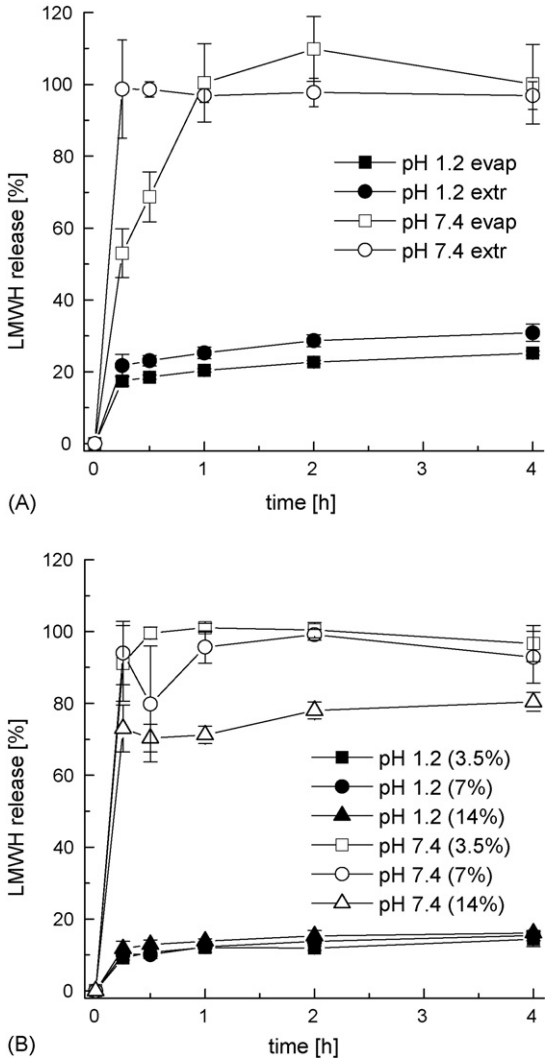

Fig. 5. In vitro drug release vs. time of LMWH loaded Eudragit P-4135F microparticles in buffer systems of pH 1.2 and pH 7.4 after solvent evaporation (evap) and solvent extraction (extr) with 1000 IU LMWH/formulation without additional surfactants (A). The influence of varying SMS amounts (given as percentage of total theoretical batch mass) on the release kinetics was determined for MS prepared by solvent evaporation (B).

(water/oil/water) followed by solvent evaporation or extraction has been reported (Jiao et al., 2002; Hoffart et al., 2003). The encapsulation of this highly hydrophilic and polyanionic compound is relatively complex. Low encapsulation rates were reported when applying standard designs of the double emulsion technique (Jiao et al., 2002). The strategy to apply counterions in order to increase the drug entrapment by attaching the drug to the particle by electrostatic interactions has been described recently (Jiao et al., 2002; Hoffart et al., 2003). SEM and CLSM revealed the formation of more or less spherical MS entrapping significant amounts of LMWH. The distinctly smaller internal water droplets observed with the solvent extraction method may be related to the partial miscibility of EA and water, lowering the volume of the internal aqueous phase during the formation of the first emulsion. As shown previously, the particle size of the formed MS is directly related to the solvent extraction rate (Ruan et al., 2002) potentially also being responsible for the difference

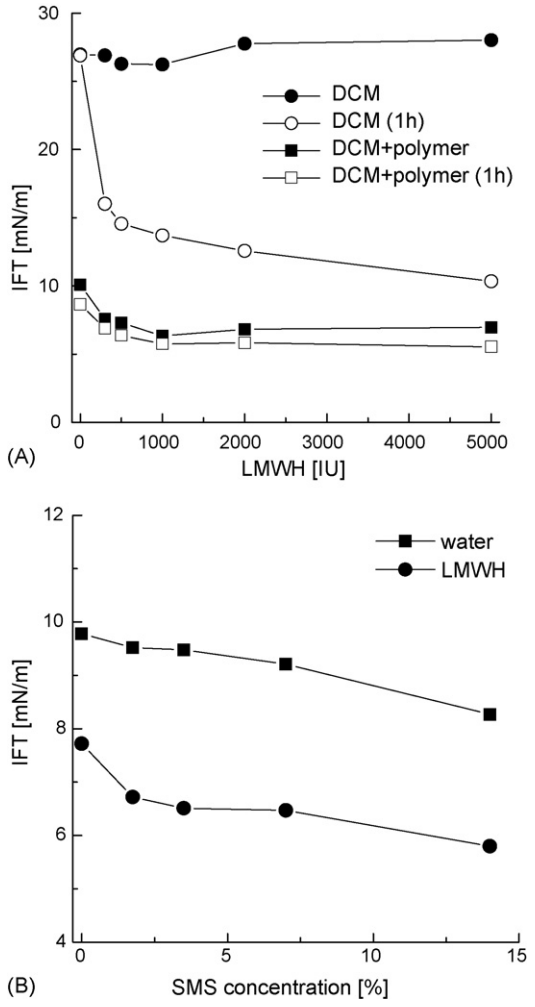

Fig. 6. IFT between aqueous LMWH and DCM or Eudragit-DCM solution (66 mg/ml) as a function of LMWH concentration (A; measurements at $t = 0$ h: full symbols, measurements at $t = 1$ h: empty symbols) and IFT between water or aqueous LMWH (500 IU/ml) and a containing SMS Eudragit-DCM solution (66 mg/ml) as a function of SMS concentration (B).

in particle size found in this study. The lower encapsulation rates obtained by solvent extraction may be due to the transfer of hydrophilic LMWH across the organic phase by its partial mix within the primary emulsion preparation and its subsequent diffusion towards the external PVA solution.

The importance of the primary water/oil emulsion stability on the encapsulation rates of MS has already been demonstrated in earlier studies (Nihant et al., 1994). The system concerned in this study was the aqueous LMWH solution emulsified in the Eudragit P-4135F containing organic phase. According to earlier observations, biological polyelectrolytes may have surface tension reducing properties (Okubo and Kobayashi, 1998). LMWH's interface stabilizing properties were concentration-dependent and evolved with time. A much higher influence on IFT was found from Eudragit P-4135F. A concentration-dependent effect on the IFT based on the higher amount of Eudragit P-4135F might be excluded since changes in polymer concentration led to no further IFT decrease. This difference may be rather due to the more expressed amphiphilic character of Eudragit P-4135F based on the lipophilic moieties in the polymer backbone and hydrophilic charges by the carboxylic groups. This observation also explains the relatively high encapsulation rates in the absence of any surfactant which is rather unusual for such a lipophilic polymer.

The addition of SMS was expected to promote a better dispersion of aqueous LMWH in the organic phase and the stability of the primary emulsion providing a higher mass transfer resistance and consequently reduce the amount of LMWH diffusing through the organic phase into the external aqueous phase. The observed optimum stabilizing the primary emulsion was apparently related to an optimal density of SMS molecules at the water/oil interface resulting in a deterioration of interface stability with a further increase of SMS concentration.

Generally, the additional effect of SMS on the IFT was relatively limited in the presence of Eudragit P-4135F. This is in line with the observation from the encapsulation studies where relatively high entrapment rates without surfactant surfactants and the additional influence by SMS was estimated secondary. A slightly stronger interface stabilizing effect was observed for CTAB compared to SMS, when measured water against polymeric DCM solution simulating a water/oil emulsion. This might be caused by interactions of the cationic surfactant CTAB and the methacrylic acid components of the polymer at the interface. The hypothesis behind using CTAB as cationic surfactant was however to form ion pairs with the polyanionic LMWH at the water/oil interface thus stabilizing the primary emulsion similar to the described entrapment strategies based on electrostatic interaction. Generally, this strategy was reported to be successful with oil/water systems (Teixeira et al., 1999). However, in our study, we found that the addition of CTAB destabilized the entire emulsion system which resulted in low encapsulation rates or a total failure of particle formation.

The in vitro drug release of all tested formulations showed a strongly pH-dependant release of LMWH. No noticeable differences could be observed in the drug release behavior of MS prepared by the two different preparation methods (solvent evaporation and solvent extraction). Similar to precedent studies, MS did not disintegrate in the buffer system at pH 1.2 and LMWH was effectively retained in the MS (Jeong et al., 2001; Lamprecht et al., 2004). The observed remaining LMWH leakage at low pHs could be related to the rough MS surface involving a certain number of pores (Rosca et al., 2004). The existence of such pores in MS made from the same polymer has been described also recently (Lamprecht et al., 2004). They were reported to result from the interconnectivity of the internal aqueous-phase droplets during the final stage of solvent evaporation when DCM displacement occurs and polymer precipitation leads to the solidification of MS. Similar to results obtained with the entrapment of other drugs comparing evaporation and extraction processes, a slightly faster drug release occurred after solvent extraction at pH 7.4 (Lamprecht et al., 2003, 2005). Already in this context a location of the drug near the particle surface could be supposed by the extraction step, increasing an early drug loss. Moreover, it was found that solvent extraction slightly increases the initial drug loss at pH 1.2 which supports the speculations about a drug accumulation near the particle surface.

5. Conclusion

Low molecular weight heparin could successfully be incorporated into pH-sensitive MS using a double emulsion technique followed by either solvent evaporation or solvent extraction. The addition of surfactants led to a benefit in terms of higher encapsulation rates in the case of SMS while in contrast, CTAB destabilized the primary emulsion. In vitro drug release has shown to be pH-dependant; LMWH could be retained in the MS at a pH < 6 and MS showed a fast release at pH 7.4. The overall optimization showed that solvent evaporation was more appropriate due to higher process yields and encapsulation rates. These MS represent a promising tool for the selective oral delivery of heparin to the colon and may to prove their efficacy as a new therapeutic strategy in IBD.

References

Chourasia, M.K., Jain, S.K., 2003. Pharmaceutical approaches to colon targeted drug delivery systems. J. Pharm. Pharm. Sci. 6, 33–66.

Cui, H.F., Jiang, X.L., 1999. Treatment of corticosteroid-resistant ulcerative colitis with oral low molecular weight heparin. World J. Gastroenterol. 5, 448–450.

Davis, S.S., Hardy, J.G., Fara, J.W., 1986. Transit of pharmaceutical dosage forms through the small intestine. Gut 27, 886–892.

Dotan, I., Hershkoviz, R., Karmeli, F., Brazowski, E., Peled, Y., Rachmilewitz, D., Halpern, Z., 2001. Heparin and low-molecular-weight heparin (enoxaparin) significantly ameliorate experimental colitis in rats. Aliment Pharmacol. Ther. 15, 1687–1697.

Hardy, J.G., Wilson, C.G., Wood, E., 1985. Drug delivery to the proximal colon. J. Pharm. Pharmacol. 37, 874–877.

Hoffart, V., Ubrich, N., Lamprecht, A., Bachelier, K., Vigneron, C., Lecompte, T., Hoffman, M., Maincent, P., 2003. Microencapsulation of low molecular weight heparin into polymeric particles designed with biodegradable and nonbiodegradable polycationic polymers. Drug Deliv. 10, 1–7.

Jeong, Y.I., Prasad, Y.R., Ohno, T., Yoshikawa, Y., Shibata, N., Kato, S., Takeuchi, K., Takada, K., 2001. Application of Eudragit P-4135F for the delivery of ellagic acid to the rat lower small intestine. J. Pharm. Pharmacol. 53, 1079–1085.

Jiao, Y., Ubrich, N., Hoffart, V., Marchand-Arvier, M., Vigneron, C., Hoffman, M., Maincent, P., 2002. Anticoagulant activity of heparin following oral administration of heparin-loaded microparticles in rabbits. J. Pharm. Sci. 91, 760–768.

Lamprecht, A., Kawashima, Y., 2006. pH-sensitive microparticles for oral drug delivery. J. Drug Del. Sci. Technol. 16, 26–34.

Lamprecht, A., Koenig, P., Ubrich, N., Maincent, P., Neumann, D., 2006. Low molecular weight heparin nanoparticles: Mucoadhesion and behavior in Caco-2 cells. Nanotechnology 17, 3673–3680.

Lamprecht, A., Yamamoto, H., Takeuchi, H., Kawashima, Y., 2004. pH-sensitive microsphere delivery increases oral bioavailability of calcitonin. J. Contr. Rel. 98, 1–9.

Lamprecht, A., Yamamoto, H., Ubrich, N., Takeuchi, H., Maincent, P., Kawashima, Y., 2005. FK506 microparticles mitigate experimental colitis with minor renal calcineurin suppression. Pharm. Res. 22, 193–199.

Lamprecht, A., Yamamoto, H., Takeuchi, H., Kawashima, Y., 2003. Microsphere design for the colonic delivery of 5-fluorouracil. J. Contr. Rel. 90, 313–322.

Nihant, N., Schugens, C., Grandfils, C., Jerome, R., Teyssie, P., 1994. Polylactide microparticles prepared by double emulsion/evaporation technique. I. Effect of primary emulsion stability. Pharm. Res. 11, 1479–1484.

Okubo, T., Kobayashi, K., 1998. Surface tension of biological polyelectrolyte solutions. J. Colloid Interf. Sci. 205, 433–442.

Rodriguez, M., Vila-Jato, J.L., Torres, D., 1998. Design of a new multiparticulate system for potential site-specific and controlled drug delivery to the colonic region. J. Contr. Rel. 55, 67–77.

Rosca, I.D., Watari, F., Uo, M., 2004. Microparticle formation and its mechanism in single and double emulsion solvent evaporation. J. Contr. Rel. 99, 271–280.

Ruan, G., Feng, S.S., Li, Q.T., 2002. Effects of material hydrophobicity on physical properties of polymeric microspheres formed by double emulsion process. J. Contr. Rel. 84, 151–160.

Siveke, J.T., Folwaczny, C., 2004. Medical approaches and future options in chronic active ulcerative colitis. Int. J. Colorectal Dis. 19, 297–307.

Teixeira, H., Dubernet, C., Puisieux, F., Benita, S., Couvreur, P., 1999. Submicron cationic emulsions as a new delivery system for oligonucleotides. Pharm. Res. 16, 30–36.

Törkvist, L., Thorlacius, H., Sjoqvist, U., Bohman, L., Lapidus, A., Flood, L., Agren, B., Raud, J., Lofberg, R., 1999. Low molecular weight heparin as adjuvant therapy in active ulcerative colitis. Aliment Pharmacol. Ther. 13, 1323–1328.