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pH-sensitive microparticles prepared by an oil/water emulsification method using n-butanol

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ABSTRACT

Commonly, the microencapsulation of a lipophilic drug in pH-sensitive polymeric matrix via an ordinary oil/oil emulsification allows for entrapping limited drug amounts due to its loss into the external phase. Here, we propose a microencapsulation method on the basis of an oil/water emulsification method using n-butanol. Eudragit® S100 microspheres were prepared by an oil/water emulsification solvent extraction method trapping ibuprofen as lipophilic model drug. Morphological analyses of the obtained particles showed a spherical shape and a sponge-like internal structure. In order to increase the entrapment efficacy several preparation parameters were optimized, such as theoretical drug load and surfactant concentration in the external phase. The particle size varied slightly around 170 μm, barely influenced by the modified process parameters. Drug leakage at pHs below the polymer dissolution pH was highest with microspheres prepared at low theoretical drug loading and low surfactant concentrations. In vitro drug release was found to be strongly pH-dependent; ibuprofen was retained in microspheres at pH 2.0 (<20% release within 4 h) whereas a higher leakage was observed at pH 5.5 and a nearly immediate drug release was obtained at pH 7.4. The use of n-butanol was found to be a new promising alternative for the preparation of pH-sensitive microspheres by an oil/water emulsification.

1. Introduction

Microspheres (MS) can be designed for a large variety of therapeutic applications and for a nearly unlimited number of drugs. This has led to the creation of many different microencapsulation methods specifically adapted to the requirements of each drug's properties and of each production setup (Benita, 1996). The standard preparation methods for pharmaceuticals are based on the use of preformed polymers, employed to entrap the drug, for instance by an emulsification step followed by solvent evaporation solidifying the polymer with the drug entrapped. In such cases, MS preparation is achieved by an emulsification with mechanical stirring with the prerequisite that the inner phase solvent is highly volatile to ensure fast MS formation (Bodmeier and McGinity, 1988; Lamprecht et al., 2000). In most cases, strategies based on oil/water or oil/oil emulsifications are generally related to two major decisive parameters: the solubility properties of the polymer, and of the drug to be encapsulated.

A variety of oral drug delivery approaches rely on the administration of MS, among them pH-sensitive drug release is an emerging field (Lamprecht and Kawashima, 2006). Due to their small size such particles can show additional advantages compared to standard drug delivery systems, leading to distinct differences in their therapeutic efficiency. For example, successful approaches where sparingly soluble drugs are encapsulated as a molecular dispersion inside the MS polymeric matrix in order to increase drug's oral bioavailability are described in the literature (De Jaeghere et al., 2000). This is one aspect in which the use of pH-sensitive polymers appears to be promising for oral drug delivery. On the other hand, colon delivery is relying to a high extent on pH-sensitive drug delivery systems. A colon specific drug delivery by MS formulations has been described for several anti-inflammatory drugs (Lorenzo-Lamosa et al., 1998; Rodriguez et al., 1998). In those MS preparations the pH-sensitive polymers Eudragit® S and L were applied. Usually, the preparation methods consisted in the use of an oil/oil emulsification process where Eudragit® S or L are dissolved in an acetone/2-propanol mixtures and emulsified in liquid paraffin (Lorenzo-Lamosa et al., 1998; Rodriguez et al., 1998; Jeong et al., 2001).

In emulsification methods, a low solubility of the drug in the external phase is required, otherwise low encapsulation rates may result due to drug leakage into the external phase (Nixon and Jalil, 1990). In consequence, when oil/oil emulsification is applied, the efficient entrapment of lipophilic drugs still remains a problem due

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to leakage into the external oily phase providing sufficient solubility for the drug (Lamprecht et al., 2004). Due to the lipophilicity of the external phase, these techniques were limited to hydrophilic drugs. Subsequently, other polymers have been investigated for the use in colon delivery in order to circumvent this problem. Among them, Eudragit P-4125F demonstrated different solubility properties and has been applied for microencapsulation by a simple oil/water emulsification method based on dichloromethane or ethyl acetate and water (Lamprecht et al., 2004, 2005; Meissner et al., 2007). However, these polymers are not commercialized which impedes further developments. The methacrylate polymers on the market, Eudragit® L and S are difficult to solubilize in organic solvents that are immiscible with water which hinders the preparation by an oil/water emulsification method. Eudragit® S has been used with a ternary solvent mixture allowing the application of an oil/water emulsification method (Rawat et al., 2007; Jain et al., 2005).

We propose here the use of n-butanol for the preparation of pH-sensitive MS on the basis of Eudragit® S. N-butanol was observed to dissolve Eudragit® S under certain conditions suggesting its use for a microencapsulation approach. This solvent has surprisingly attracted very limited attention so far, although being of limited toxicity, falling into class 3 toxicity thus, less toxic than dichloromethane and ethyl acetate usually applied for microencapsulation (Guidance for Industry Q3C). Its miscibility with water is below 1:9 which offers a sufficiently large range to formulate an emulsion. The boiling point is cited in literature at 118 °C leading to the application of an oil/water emulsification solvent extraction method.

The objectives of this study were firstly to evaluate the use of n-butanol for the microencapsulation of drugs into a pH-sensitive polymer by an oil/water emulsification method and secondly to characterize the MS with respect to particle size and morphology, drug loading, drug leakage, and release kinetics. Ibuprofen was encapsulated as a lipophilic model drug.

2. Materials and methods

2.1. Materials

Eudragit® S 100 and Ibuprofen were kind gifts from Degussa/Roehm Pharma Polymers (Darmstadt, Germany) and Euro OTC Pharma GmbH (Kamen, Germany), respectively. Polyvinyl alcohol and n-butanol were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals were of analytical grade.

2.2. Preparation of microparticles

MS were prepared by a simple oil-in-water-emulsion technique followed by solvent extraction and optimised as follows: Two hundred milligrams of Eudragit® S 100 and varying amounts of ibuprofen (20, 60, or 100 mg) were dissolved in 8 ml of n-butanol in an ultrasonic-bath for 15 min at 60°. This polymer/drug-solution was then poured into 40 ml aqueous polyvinyl alcohol (PVA) solution to form an o/w-emulsion. This emulsion was stirred for 5 min with a three-blade propeller at 500 rpm. Afterwards 160 ml aqueous PVA (0.1% at 40 °C) were added to this first emulsion by a peristaltic pump. The heating was stopped and altogether this dispersion was stirred for 1 h. MS were filtered and washed two times with 100 ml distilled water and vacuum dried at room temperature. All formulations were performed in triplicate.

2.3. Particle size analysis and scanning electron microscopy (SEM)

Particle size analyses of all MS batches were carried out by laser light diffraction (Mastersizer® X, Malvern Instruments, UK). MS were dispersed in 2 ml of an aqueous solution of Tween 80 (0.2%). Particle morphology was analyzed by SEM. 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-5600 scanning electron microscope, Tokyo, Japan) at 24 kV.

2.4. Confocal laser scanning microscopy (CLSM)

Before preparing the first emulsion, fluorescein was added as a fluorescence marker to the external aqueous phase. A Biorad MRC 1024 Laser Scanning Confocal Imaging System (Hemel Hempstead, UK), equipped with an argon ion laser (American Laser Corp., Salt Lake City, USA) and a Zeiss Axiovert 100 microscope (Carl Zeiss, Oberkochen, Germany), was used to investigate the internal structure of the microparticles. All confocal fluorescence pictures were taken with a 40× objective (oil immersion, numeric aperture 1.30). For imaging a dispersion of microparticles in neutral oil was prepared.

2.5. Determination of encapsulation rates and in vitro drug release

To determine the amount of encapsulated ibuprofen, 10 mg of MS were dissolved in 5 ml of isopropanol in an ultrasonic bath. Afterwards the isopropanol was evaporated by using a rotary evaporator (Bibby Sterilin Ltd., Stone Staffordshire, England) and 20 ml of a solvent mixture (acetonitrile:water:acetic acid) were added in order to solubilize the ibuprofen. The amount of ibuprofen was then determined by high performance liquid chromatography (HPLC) with a derivation from an earlier described method (Adeyeye and Price, 1997). The set-up was as follows: RP-18 column (LiChrospher® 100, Merck, Darmstadt, Germany); eluent: acetonitrile:water:acetic acid 500:477:3; flow rate 0.8 ml/min. Samples of 50 μl were injected into the column.

The in vitro drug release was carried out with three different phosphate buffer systems at pH values of 2.0, 5.5, and 7.4. All buffers were prepared according to the European Pharmacopoeia. MS were suspended in a flask containing 10 ml buffer solution. The suspension was incubated at 37 °C in a water bath and stirred with a magnetic stirrer at 200 rpm. Samples of 0.5 ml were taken at predetermined times (0.25, 0.5, 1, 2, 4 h), centrifuged at 5000 × *g* for 5 min, and substituted with 0.5 ml of fresh buffer. After centrifugation the supernatant was assayed for the release of ibuprofen by HPLC as described previously. All experiments were performed in triplicate.

2.6. Leakage experiments

Drug leakage from MS was tested at two different pHs: 3.2 and 5.5, located below and above the p*K*a of the drug (p*K*a ∼ 4.4). In order to ensure sink conditions, the buffer pH 3.2 contained additionally 0.1% polysorbate 80. MS were dispersed in 10 ml buffer solution and incubated at 37 °C for 4 h under gentle shaking. After incubation samples were centrifuged and supernatant was analyzed for ibuprofen content.

3. Results and discussion

The selection of the microencapsulation technique depends largely on the physicochemical properties of the drug. For the entrapment of lipophilic drugs in pH-sensitive polymers only a limited number of studies are reported. Especially, the limited solubility of the pH-sensitive polymers in the standard organic solvents acceptable for pharmaceutical use allowing an emulsification method is responsible for the rather little efforts that were feasible until now. Selecting n-butanol as solvent for the pH-sensitive

polymers of the polymethacrylate type appears to be interesting in order to avoid the complicated oil/oil emulsification process used in prior formulations (Lorenzo-Lamosa et al., 1998; Rodriguez et al., 1998; Jeong et al., 2001). Moreover, the reduced toxicity of the solvent is an essential aspect.

In first experiments, Eudragit® S100 was observed to be insoluble in n-butanol at room temperature (100 mg in 4 ml) while heating increased significantly the solubility. At 40 °C all polymer was soluble and reprecipitation occurred at around 35 °C. Decreasing the polymer concentration in a relevant range did not allow for temperature reduction of the polymer solution. Also the addition of other organic solvents (namely, ethanol, 2-propanol, or acetone) did not alter significantly the solubility properties. Due to the high boiling point of n-butanol solvent extraction was the preferential mean for the particle solidification step. Thus, a primary emulsion was established at 8 ml of n-butanol in 40 ml of 1% PVA. The solvent extraction step was initiated by the addition of 160 ml of 0.1% PVA. Particle quality and yields were highly increased when external phases were adjusted to the temperature of the inner organic phase.

MS appeared spherical with a rather smooth surface, exhibiting a large number of pores (Fig. 1A and B). The internal structure was found to be sponge-like (Fig. 1C). The origin of these inclusions remained unclear however, residual solvent determination directly after the preparation step led to the hypothesis that rather water is entrapped than residual n-butanol during the emulsification and extraction process since only negligible quantities of n-butanol were detected inside the MS matrix (data not shown). In recent studies similar phenomena were observed when entrapping highly hydrophilic drugs (Wang and Guo, 2008). While water influx into the particle matrix was observed during the formulation of a dichloromethane/water primary emulsion, allowing for the appearance of aqueous droplet inside the particles, it might be reasonable to consider this mechanism here, where a n-butanol/water emulsion was formulated. Although ibuprofen must not be seen as a hydrophilic drug here, the significantly higher hydrophilic properties of the particle matrix polymer could play a similar role. A control experiment where the internal n-butanol phase was "stained" with nile red proved the entrapment of external aqueous phase inside the matrix (Fig. 1D) by a total absence of fluorescent dye in the internal droplets.

The rough surface resulting from a high density of intraparticular cavities may have an impact on drug encapsulation and also on drug leakage and thus in vitro drug release. The formation of a very thin polymer layer formed on the particle surface, potentially also including small pores may facilitate the drug diffusion towards the external phase. Besides, pores in MS made from similar polymers and their influence on drug leakage has been described repeatedly (Rosca et al., 2004; 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 dichloromethane displacement occurs and polymer precipitation leads to the solidification of MS. This is in line with findings that entrapped cavities inside the particle matrix rather contain water than organic solvent. The subsequently hypothesized structure comes close to observations done with particles made from double emulsion techniques.

Process yields varied between 76% and 89% in all preparations. Particle size remained more or less unchanged over the whole range of ibuprofen and PVA amounts (Fig. 2A). However, the amount of ibuprofen added to the formulation had a significant influence on the encapsulation rates (Fig. 2B). With increasing drug amount the encapsulation rate increased while the presence of higher concentrations of PVA led to lower encapsulation rates.

While for hydrophilic drugs a short contact time between unsolidified organic and aqueous phase is crucial in order to obtain high drug loadings for lipophilic drugs however, other factors play dominant roles. Although nearly immediate particle solidification during the solvent extraction can be achieved, lower encapsulation rates were observed. Similar to ethyl acetate, n-butanol diffuses into the external aqueous phase during the solvent diffusion due to its miscibility with the external aqueous phase. Indeed, the drug has a good solubility in the organic phase, which in parallel may enhance its transport towards the aqueous phase during solvent diffusion. Thus, the organic solvent 'extracted' the drug out of the particle matrix during the polymer precipitation. This phenomenon has been already reported from MS preparation using diverse solvent extraction methods (Lamprecht et al., 2004).

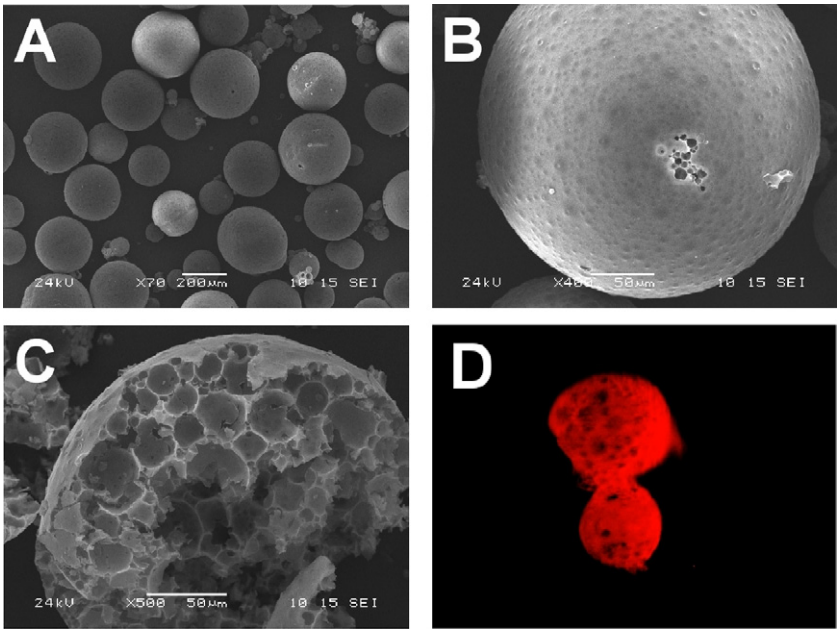

Fig. 1. SEM images of ibuprofen containing Eudragit® S100 MS showing general appearance (A), surface structure (B), and internal morphology (C). The spherical shape as well as the rough surface can be easily seen. Internal structure analysis by CLSM confirmed the partial entrapment of the external aqueous phase which appears here as the unstained droplets inside the particle matrix (D).

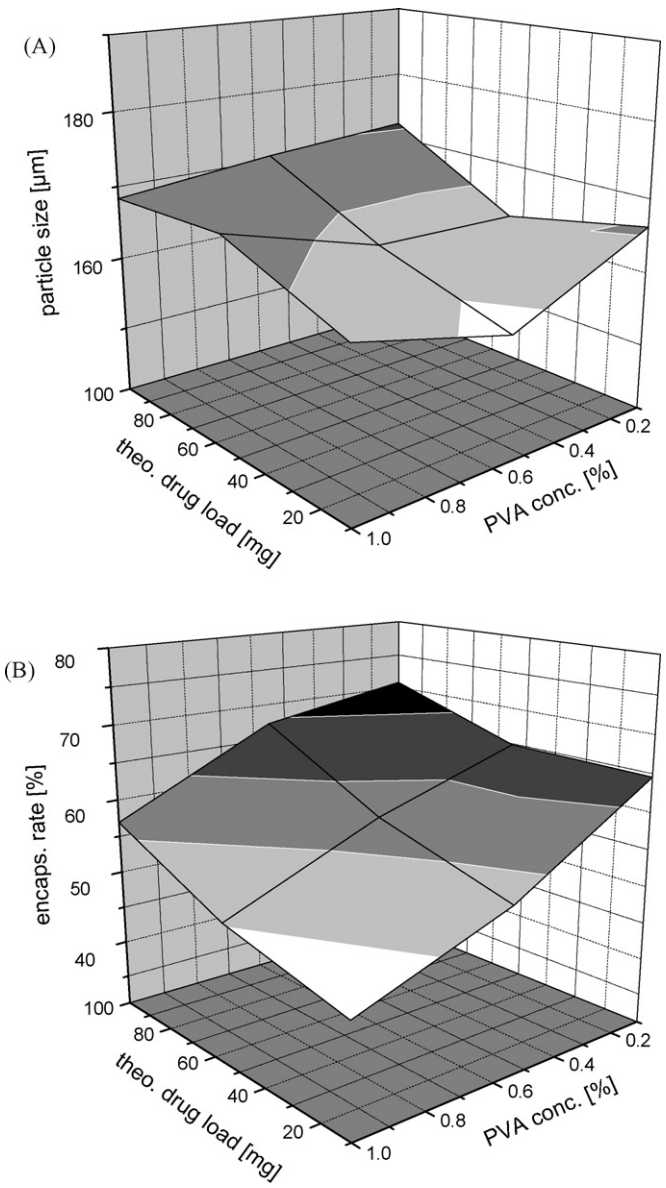

Fig. 2. Influence of the PVA concentration and the theoretical drug load on the particle size (A) and on the encapsulation rates (B). Data are shown as mean ± S.D., $n = 3$.

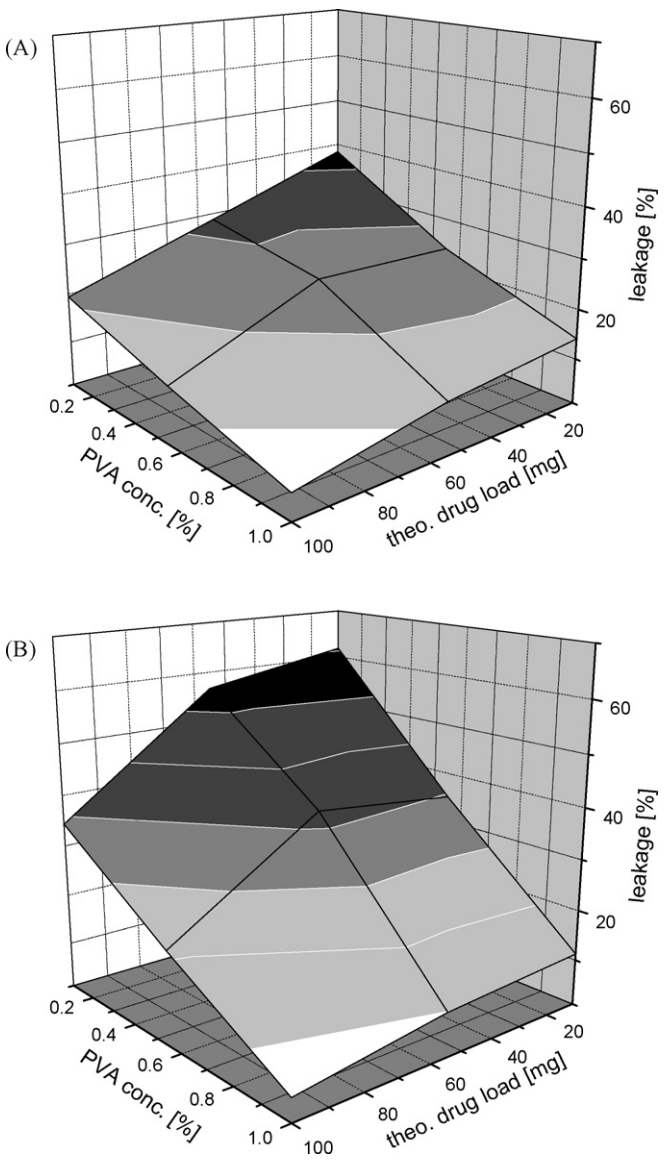

Fig. 3. Influence of the PVA concentration and the theoretical drug load on the on the drug leakage at pH 3.2 (A) or at pH 5.5 (B).

The mechanism is furthermore enhanced by the increased solubilization of ibuprofen in the external aqueous phase by the presence of PVA. This is line with the obtained data since the higher PVA concentration lowered the encapsulation rate while the total surface area (particle diameter) remained constant.

Besides, there are other observations to be taken into account having a lowering effect on the encapsulation rate. Lipophilic interactions between polymer and drug may significantly differ from studies with polyesters. Furthermore, the internal sponge-like structure leads to an unintentional large inner surface of the particles which facilitates drug leakage from inside the matrix after swelling.

Drug leakage from MS when incubated in different buffer systems was generally lower at pH 3.2 than at 5.5 (Fig. 3). Again, a clear tendency can be observed towards lowest leakage percentage from formulations with high drug load.

Particle morphology revealed that drug leakage occurred by diffusion out of the particle matrix, since MS appear completely intact after 4 h (Fig. 4A). Potentially drug loss occurs by the drug diffusion across the very thin undissolved polymer layer building the particle surface, since no major open pores were observed on the MS surface (Fig. 4B). Drug leakage experiments also underlined that drug solubility is indeed decisive on leakage. Observations with ibuprofen at pHs where its high solubility is caused by its anionic form, showed that the leakage from particles is enhanced while structural analyses did not reveal differences of the particle surface. In literature, the opposite was reported which is the accelerated drug release with increased ibuprofen load in non-water soluble polymers (Tamilvanan and Sa, 2000; Kawashima et al., 1989). Although Eudragit® S100 can be considered as a non-water soluble matrix at pH < 6 (and is therefore comparable to polymers used in these studies), theoretical drug loads were distinctly higher in these reports completely changing the particle structure. Drug loadings led partially to a collapse of the particle matrix structure exposing drug crystals directly to the particle surface which was not the case in our study.

MS formulations were tested for their in vitro drug release in buffers of different pH values (Fig. 5). Ibuprofen was retained efficiently inside the MS at pH 2 for 4 h. The retained percentage of the initial drug load was at least 79% after this period while

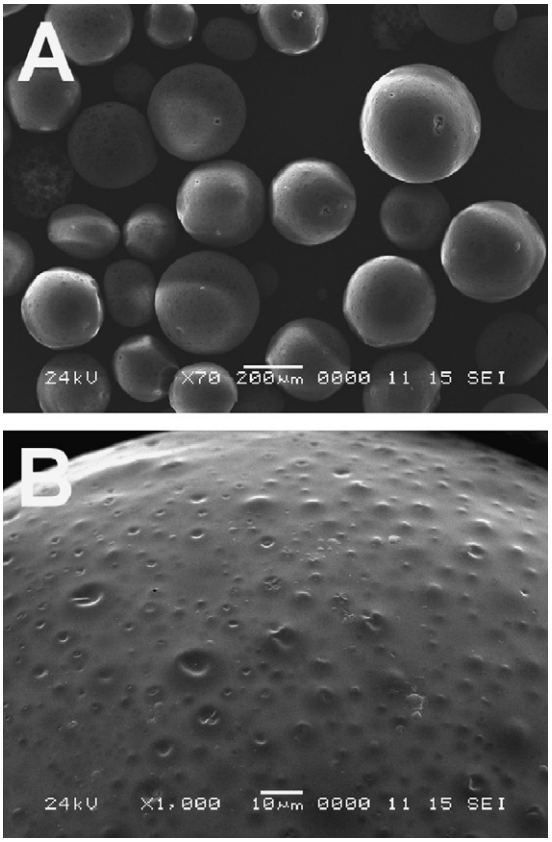

Fig. 4. SEM images of ibuprofen containing Eudragit® S100 MS showing general appearance (A) and surface structure (B) after the leakage experiments, distinctly showing the particle integrity after 4 h of incubation.

ibuprofen leakage at pH 5 was significantly higher varying between 35% and 50% of the initial drug load. A slightly slower drug release was observed for lower theoretical drug loadings, mainly visible at pH 2. A fast drug release from all formulations was observed at pH 7.4 where a complete dissolution was observed after 15 min.

Generally, the in vitro drug release of all tested formulations showed a strongly pH-dependant release of ibuprofen. Similar to precedent studies, MS did not disintegrate in buffer systems at low pHs and the drug was effectively retained within the particle matrix (Jeong et al., 2001; Lamprecht et al., 2004). The observed ibuprofen leakage at low pHs is potentially again based on the slow diffusion of the drug across the polymer film separating the very porous interior of the particle matrix from the release medium. Similar to results obtained with the entrapment of other drugs, at pH 7.4 a nearly immediate release occurred with all formulations based on the dissolution of the pH-sensitive polymer, since total absence of particles is observed after 15 min. Overall, only minor influences on the drug release behavior by the variation of the process parameters in the given ranges were found. Although particle size was relatively constant throughout the different batches, an influence of the particle size on drug release and also leakage cannot be excluded.

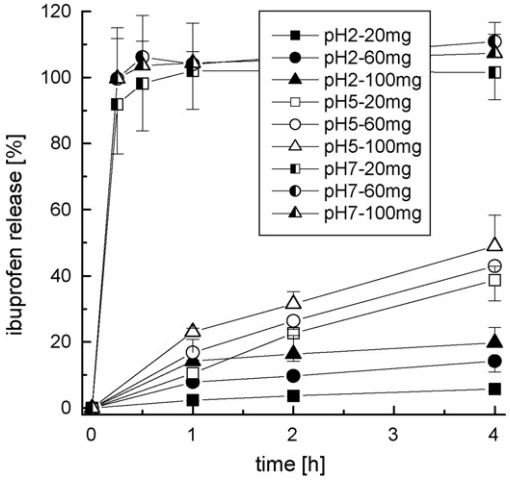

Fig. 5. In vitro drug release vs. time of ibuprofen loaded Eudragit® S100 MS in buffer systems of pH 2.0, 5.5, and pH 7.4.

4. Conclusions

N-butanol allows the preparation of pH-sensitive MS on the basis of an oil/water emulsion technique followed by solvent extraction. This is especially interesting for the entrapment of lipophilic drugs in such MS and avoids complex solvent mixtures with relative high toxicity risk. In vitro drug release has shown to be pH-dependant; ibuprofen could be retained in the MS at a pH below the p*K*a of the drug and a fast release was observed at pH 7.4. The overall summarized preparation parameters showed that a high theoretical drug loading can significantly increase the encapsulation rates as well as reduce the leakage phenomenon. MS prepared by this new method can fulfill the requirements of pH-sensitive carriers for oral drug delivery.

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