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In Vitro Evaluation of Betamethasone-Loaded Nanoparticles

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ABSTRACT The aim of the present work was to investigate the preparation of nanoparticles as a potential drug carrier in the treatment of various inflammatory diseases. A nanoprecipitation method was used to entrap betamethasone in a poly[ε-caprolactone] matrix. Process parameters such as the initial drug load, the surfactants (polyvinyl alcohol, PVA; sodium cholate, SC), and their concentration in the aqueous phase were analyzed for their influences on particle properties. Particle size changed with increasing surfactant concentrations (PVA: 250 to 400 nm; sodium cholate: 330 to 150 nm) due to changes in interface stability and viscosity of the aqueous phase. The zeta potential was around neutrality with PVA and between −28 and −42 mV with SC. Betamethasone encapsulation rates of about 75% and 90% slightly increased with higher surfactant concentration. Drug release profiles exhibited an initial burst release with both surfactants, PVA (8–18%) or SC (25–35%) followed by a sustained release delivering 15% to 40% of the entrapped drug within 48 hours. The present nanoparticulate formulations exhibit promising properties of a colloidal drug carrier for betamethasone. Although SC seems to be advantageous due to its biocompatibility, in terms of sustained drug release pattern, the use of PVA is favorable.

KEYWORDS Nanoparticles, Inflammatory diseases, Poly[ε-caprolactone], Polyvinyl alcohol, Sodium cholate, Betamethasone

INTRODUCTION

Recent studies demonstrated the benefit from drug loaded nanoparticles (NP) as a new therapeutic tool in treating several inflammatory diseases such as arthritis and inflammatory bowel disease (Horisawa et al., 2002; Lamprecht et al., 2001). The general strategy was based on a capture of NP, into immune-related cells highly accumulated in the targeted tissue followed by a local delivery of the entrapped drug. Advantages were found in the sustained-release properties of such carriers compared to simple injections and the small size

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allowing the penetration inside the targeted tissues where usually no drug carriers can enter.

A subsequent NP design may be oriented on the desired properties, namely a low initial drug loss, sustained release, and a sufficiently small particle size, since uptake mechanisms were found to be size-dependent phenomena (Horisawa et al., 2002; Lamprecht et al., 2001. Unfortunately, the initial burst release of the drug is often also reciprocally related to the particle diameter. Therefore, an optimal combination of both may be adapted to each application separately.

Betamethasone is a derivative from hydrocortisone or prednisolone, but its anti-inflammatory potential is about five to 10-fold higher. Since corticoids are known to have severe adverse effects, they require the design of specific drug delivery systems providing a local treatment. Also, the short half-life of betamethasone (Petersen et al., 1983) demands for the use of controlled-release devices in order to reduce the number of repetitive administrations.

Based on the observation that NPs accumulate in the inflamed tissues and remain in the targeted tissue (Horisawa et al., 2002; Lamprecht et al., 2001, the use of a biodegradable polymer was advised, since, moreover, a systemic uptake cannot be completely excluded. Poly[ε-caprolactone] (PCL) was selected as the particle matrix polymer with biocompatible and biodegradable properties (Pitt et al., 1984). The particle preparation technique was based on a spontaneous emulsification solvent diffusion method described earlier (Lamprecht et al., 2001), permitting the design of nanoparticles without sophisticated equipment. Usually, surfactants are applied during the particle preparation in order to stabilize the emulsified system during the state of NP formation. Among these surfactants applied in the preparation of nanoparticles, polyvinyl alcohol (PVA) represents some kind of standard. Due to its limitations in its biocompatibility, the use of sodium cholate (SC) was tested as an alternative surfactant known for its biocompatibility and the relative simple removal from the NP surface (Quellec et al., 1998).

In this study, the two types of surfactants at various concentrations as well as the theoretical drug load were the parameters analyzed for their influence on particle properties such as NP diameter, surface potential, encapsulation rates, and drug release profile.

MATERIALS AND METHODS

Materials

PCL (MW 10,000 Da), betamethasone, and sodium cholate were purchased from Fluka (Steinheim, Germany). Polyvinyl alcohol was supplied by Sigma (Steinheim, Germany). All other chemical reagents were of analytical grade.

Nanoparticle Preparation

The preparation of NPs was based on a spontaneous emulsification solvent diffusion method (Lamprecht et al., 2001). Briefly, 250 mg of the polymer and betamethasone (4 or 10 mg) were dissolved in 6 mL of acetone followed by the addition of 4 mL of ethanol. The organic phase was then added stepwise to an aqueous phase (100 mL) containing various PVA or SC concentrations (0.1%, 0.3%, 1%, and 3%). Thereafter, the solvent mixture was removed under reduced pressure. The supernatant containing nonencapsulated drug and free surfactant was removed by repeated centrifugation steps at 42,000 g at 4°C for 20 minutes. The NPs were redispersed in either distilled water or a sucrose solution (1%) and lyophilized overnight.

Particle Size, Zeta Potential, and Scanning Electron Microscopy

The NPs were analyzed for their size distribution by photon correlation spectroscopy using a Zetasizer II® (Malvern Instruments, UK) at a fixed angle of 90°. The zeta potential was determined by the same apparatus using the zeta potential option. The NP suspensions were diluted in distilled water (1:100) prior to all analyses.

For the imaging by scanning electron microscopy, NP suspensions were dried on supports and coated with gold under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Samples were then observed with the scanning electron microscope (Cam Scan S2, Leica Cambridge Ltd., Cambridge, UK) at 20 kV.

Determination of Betamethasone Loading and In Vitro Release Profiles

The encapsulation rate of betamethasone within NPs was determined by measuring the amount of non-entrapped drug in the supernatant recovered from centrifugation by the following high performance liquid chromatography (HPLC) method: RP-18 column (LiChrospher® 100, Merck AG Darmstadt, Germany); eluent: acetonitrile:water 25:75; flow rate 1.0 mL/min. Betamethasone was detected by UV absorbance at 238 nm, samples of 20 µL were injected into the column.

Drug release experiments were performed as follows: 50 mg of lyophilized drug-loaded NPs were resuspended in 100 mL of phosphate buffer (pH 5.5) containing 0.1% of polysorbate 80 and incubated into a bath at 37°C with gentle magnetic stirring at 250 rpm under subdued light. At appropriate intervals, 0.5 mL samples were withdrawn and filtrated through a 0.1 µm PTFE Millipore filter. The filtrate was assayed for drug release and replaced by 0.5 mL of fresh buffer.

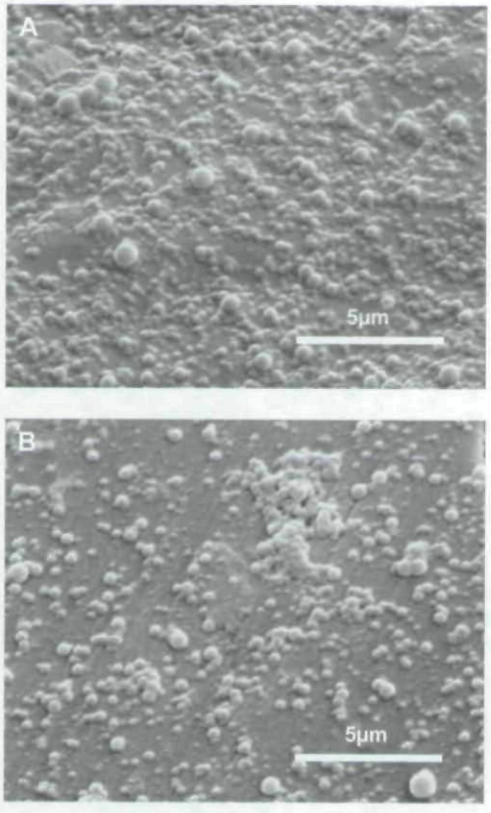

FIGURE 1 Representative Scanning Electron Microscopic Image of Betamethasone Loaded NP Prepared with 0.1% Surfactant Using Either PVA (A) or SC (B).

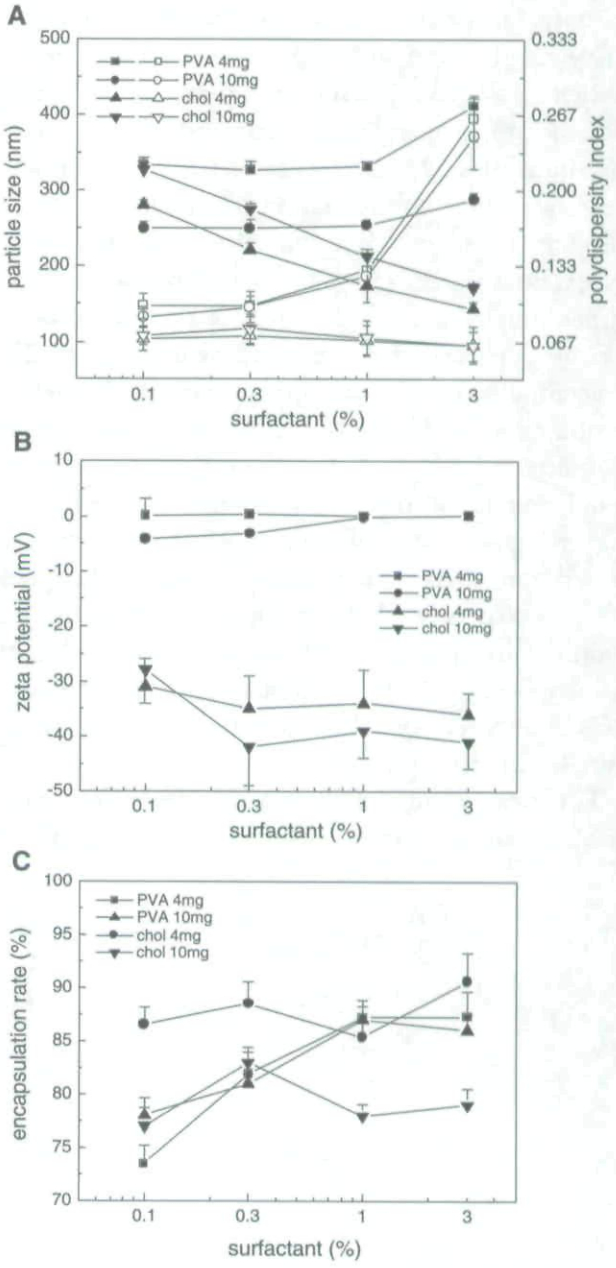

FIGURE 2 Influence of the surfactant concentration on the Mean Particle Diameter (Filled) and Polydispersity Index (Empty) (A), Zeta Potential (B), and Encapsulation Rate (C) of Betamethasone NP (n=3). Data are Shown as Mean±SD.

The amount of betamethasone in the release medium was determined by the chromatographic method described before.

RESULTS AND DISCUSSION

Nanoparticles prepared by the spontaneous emulsification solvent diffusion method exhibited a

spherical shape with a smooth surface, which was independent from the type of surfactant (Fig. 1). Particles had a submicron size prepared with either PVA or SC at all respective concentrations tested in this study (Fig. 2A). An increased SC concentration led to a particle size reduction due to stabilization of the interface with only minor changes of viscosity in the external aqueous phase as similarly described in an earlier study (Lamprecht et al., 2001). In contrast, an increasing particle size was found by using higher PVA concentrations in the aqueous phase, which might be attributed to the higher viscosity of the aqueous phase. Consequently, a less favorable mixing efficiency and larger emulsion droplets were observed, resulting in larger particles despite the increased stabilization potential at higher concentrations. Changes in the polydispersity of the particle size were observed mainly with increasing PVA concentrations, while influences by SC were minor. Similarly, these effects are based on the increased viscosity of the PVA solution.

The zeta potential of NPs with PVA varied around neutrality in all formulations. When using SC, the zeta potential exhibited generally negative values that were slightly but not significantly decreasing with increasing SC concentrations (Fig. 2B). The zeta potential values were concluded to be dependent on the varying amount of the surfactant adhering to the NP surface covering the charges by the carboxylic groups of PCL, which usually lead to distinct negative zeta potential. Attributed to the uncharged properties of the PVA overlaying the particle surface, the zeta potential exhibited a tendency towards 0 mV with higher PVA concentration. The slight SC concentration-dependent decrease of the zeta potential may be based on increasing amounts of surfactant remaining at the NP surface after the washing steps, although SC was reported to be easily removable from the NP surface (Quellec et al., 1998).

The encapsulation rates were relatively high for all batches (>70%), which is mainly due to the high lipophilicity of betamethasone (Fig. 2C). Thus, the possible general drawback of nanoprecipitation methods in general, i.e., the drug extraction by the organic solvent towards the external aqueous phase during the solvent diffusion and particle solidification step, can

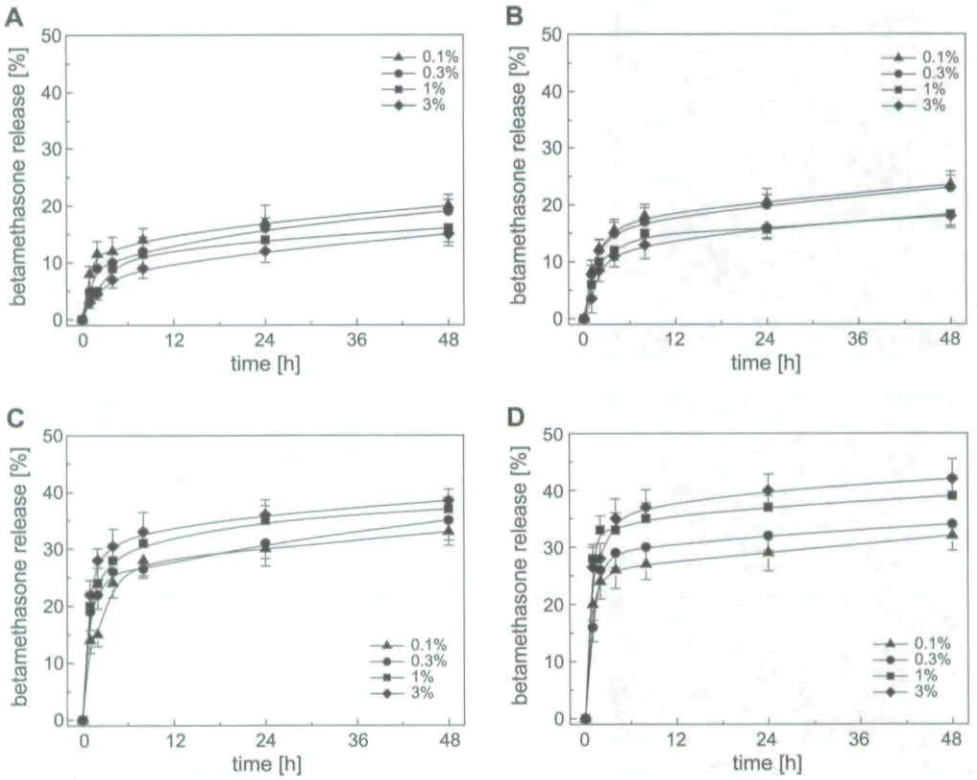

FIGURE 3 Release Profiles of Betamethasone NP in Phosphate Buffer (pH 7.4 at 37°C) During 48 hours (n=3). NP were Tested for Their Drug Release Prepared by PVA (A,B) or SC (C,D) and with a Theoretical Drug Load of 4 mg (A,C) or 10 mg (B,D). Data are Shown as Mean ± SD.

be kept at a relatively low level. An increased PVA concentration led to higher encapsulation rates based on the increased particle diameter while changes in the SC concentration did not influence the encapsulation rates distinctly. The enhanced drug solubilization by higher surfactant concentrations in the external aqueous phase was surprisingly found to be without influence.

Figure 3 illustrates the in vitro release profiles obtained by representing the percentage of betamethasone released vs. time. In general, the drug release occurred in two phases: a first initial burst release was followed by a sustained release of the drug. The drug release may be mainly controlled by drug diffusion throughout the NP matrix since the hydrolytic degradation of PCL NPs was reported to occur after 50 to 100 days (Coffin & McGinity, 1992).

Independently from the surfactant type and concentration, the burst release was increased with higher initial drug load. This is in line with conclusions of an earlier report, which describes that with higher drug loading, more drug molecules are available at the surface of microspheres, leading to higher initial release (Ravivarapu et al., 2000).

Since larger NPs exhibited a slower drug release, the surfactant concentration being responsible for the particle size variation affected, thus, the drug release indirectly. Although this factor may also have an influence on the generally higher burst release from particles prepared with SC, this hypothesis is not completely in line with observations from lower surfactant concentrations where particle diameters were similar for SC and PVA. The phenomenon of burst release was hypothesized to be an immediate dissolution of the adsorbed drug onto the particle surface (Sampath et al., 1992). Thus, the lower burst release with PVA might be related to the surfactant's hydrophilicity, which reduces a possible localization of betamathasone on the NP surface, consequently decreasing its availability for immediate dissolution.

When considering all results for the appropriate choice of the surfactant, several parameters have to be taken into account. Polyvinyl alcohol was found in earlier studies to protect NPs from their enzymatic degradation (Landry et al., 1998), which especially might be of interest for applications in inflammatory diseases where NPs are applied to tissues with high enzymatic activity. Moreover, NPs with higher drug loading and a smaller burst effect are certainly more desirable. Consequently, the use of PVA is preferred for this application. However, it has to be kept in mind that the burst effect is also due to the sink conditions in which in vitro release experiments are usually performed (50 mg of NP in 100 mL of dissolution medium). Such conditions are generally not met in vivo, suggesting that the in vivo burst effect could be much lower.

Reports on related work dealing with the entrapment of betamethasone into nanocarriers are limited in number. The encapsulation of betamethasone into lipid carriers has been described recently; however, the authors did not describe the release behavior in vitro (Sivaramakrishnan et al., 2004). Another study described the incorporation of betamethasone into polyester nanoparticles of different types of DL-lactide/glycolide copolymers with a modified emulsion solvent diffusion method in oil (Horisawa et al., 2002). Compared to this oil-in-oil emulsification method, particle purification is facilitated with this spontaneous emulsification solvent diffusion method, since the external aqueous phase of this oil-in-water system can be removed easily and also, higher drug loads are obtained.

CONCLUSIONS

The present nanoparticulate formulations can be expected to be used as colloidal drug carriers of betamethasone when prepared by a spontaneous emulsification solvent diffusion method. Moreover, the preliminary drug release tested in vitro proved that NPs had the properties of a sustained-release form. Although the surfactant sodium cholate is superior in terms of biocompatibility, a distinctly higher burst release has been found when applying this compound. Subsequently, the use of PVA for such a therapeutic approach seemed to be more appropriate.

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