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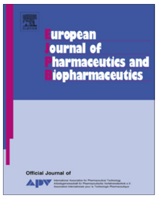

Research paper

Influence of different stabilizers on the encapsulation of desmopressin acetate into PLGA nanoparticles

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ABSTRACT

To address targeting and bioavailability issues of peptidic drugs like desmopressin, the encapsulation into nanoparticles (NP) has become standard in pharmaceutics. This study investigated the encapsulation of desmopressin into PLGA NP by the use of pharmaceutically common stabilizers as a precursor to future, optional targeting and bioavailability experiments. Polymer dry weights were measured by freeze drying and thermo gravimetric analysis (TGA). Particle sizes (ranging between 105 and 130 nm, PDI < 0.1) and zeta potentials (−35 to −45 mV) were analyzed with Dynamic Light Scattering (DLS) and Laser-Doppler-Anemometry (LDA) respectively. Highest loading efficiencies, quantified by RP-HPLC, were achieved with Pluronic F-68 as stabilizer of the inner aqueous phase (1.16 ± 0.07 μg desmopressin/mg PLGA) and were significantly higher than coating approaches and approaches without stabilizer (0.74 ± 0.01 μg/mg). Optimized nanoformulations are thus in competition with the concentration of commercial non-nanoparticulate desmopressin products. Stability of desmopressin after the process was evaluated by HPLC peak purity analysis (diode array detector) and by mass spectrometry. Desmopressin was shown to remain intact during the whole process; however, despite these very good results the encapsulation efficiency turned out to be a bottle neck and makes the system a challenge for potential applications.

1. Introduction

In recent years biologicals have become a major subject of research in pharmaceutics, as of 2013 more than 900 medicines and vaccines were in development [1]. Due to improved biotechnological production and advances in chemical synthesis, biologicals are available in quantities that are suitable for therapeutic use. However, as they often suffer from poor stability and as they are unable to efficiently pass biological barriers [2], they require a carrier system that protects them from degradation and targets them to the right compartment overcoming biological barriers [3]. Carrier systems are often particulate systems where spherical micro- or nanoparticles are used to transport drugs [4–8], but also asymmetrical particles like rods are in use [9]. Major problems when preparing nanoparticles are getting adequate amounts of particles and getting suitable uniformity of the particles to use them as well-working drug delivery vehicles. PLGA nanoparticles, however, can be easily prepared in large amounts and uniformity [10–12]. Besides the advantages of PLGA nanoparticle preparation techniques PLGA is biocompatible and biodegradable, is used in FDA and EMA approved formulations and is already used in many pharmaceutical applications [13]. As PLGA is a quite hydrophobic substance, hydrophobic drugs may be well encapsulated. The encapsulation of hydrophilic compounds is difficult, but the simplicity of preparing PLGA nanoparticles tempts to work around the hydrophilicity problem rather than addressing the problems of alternative carrier systems (uniformity, biodegradability, available amounts, etc.). One of the most common approaches as work-around for the problem is the double emulsion method [14]. Double emulsion methods have shown to be well feasible for

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Abbreviations: TGA, thermogravimetric analysis; DLS, Dynamic Light Scattering; LDA, Laser-Doppler-Anemometry; PLGA, polylactic-co-glycolic acid; NP, nanoparticles; HPLC, High Pressure Liquid Chromatography; FDA, Federal Drug Administration; EMA, European Medicines Agency; RCF, Relative Centrifugal Force; LC-MS, Liquid Chromatography coupled Mass Spectrometry; TIC, Total Ion Chromatogram; SEM, Scanning Electron Microscopy.

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microparticulate systems [15]. The architecture and chemical behavior of double emulsions on nanometer scale is debatable; however, the technique works quite well for mixing the hydrophilic compounds with hydrophobic polymers as shown for small molecules [16]. As hydrophilic therapeutic agent we chose the peptide desmopressin acetate, as it is already in use in several conventional medicaments, but without particulate delivery system. Desmopressin is the synthetic analog of Vasopressin, an antidiuretic peptide-hormone of nine amino acids length. It is used to treat Diabetes insipidus, nocturia [17] and nocturnal enuresis. Besides of its primary function as an antidiuretic, the effect of desmopressin is also tested to treat coagulopathy effects such as in case of acetylsalicylic acid [18,19], the problems of bleeding disorders [20,21] or more generally the blood loss after cardiac surgeries [22] since the late seventies [23–25].

Peptides like desmopressin tend to degrade easily compared to simple chemical compounds in a biological environment. Their encapsulation might be a possibility to meet the therapeutic window with smaller input quantities of substance as the particle could protect the drug from the environment and could promise to improve bioavailability after functionalization in future studies.

The goal of the presented study was to determine the encapsulation efficiency of desmopressin acetate in PLGA nanoparticles. Therefore, the encapsulation was conducted with several pharmaceutically relevant stabilizers in the inner aqueous phase. The effects on how encapsulation efficiencies, particle sizes, and particulate zeta potentials changed were determined. Furthermore, to prove the intact state of desmopressin after encapsulation and dissolution was a key goal of this study and was analyzed by UV spectrum analysis and mass spectrometry.

2. Materials and methods

2.1. Materials

PLGA (Resomer RG 503 H, 50:50 ratio) was obtained from Evonik Industries Inc. (Darmstadt, Germany), Polyethylene glycol 400 (PEG), Tween 21, Pluronic F-68 and Pluronic F-127 were supplied by Sigma-Aldrich. Polyvinyl alcohol (Mowiol 4-88) was obtained from Kuraray Europe (Frankfurt a.M., Germany). The degree of deacetylation of Mowiol 4-88 is 88% indicating that 12% of the PVA monomers are actually vinyl acetate and not vinyl alcohol. Desmopressin acetate was a kind gift from Evonik Industries Inc. (Darmstadt, Germany), previously obtained from Ferring Arzneimittel GmbH (Kiel, Germany).

2.2. PLGA nanoparticle preparation and treatment for quantification

PLGA nanoparticles were prepared with a double emulsion solvent diffusion centrifugation method (see Fig. 1: 1–4): 50 mg of PLGA was dissolved in 1.5 mL ethyl acetate. To obtain the primary emulsion, 0.5 mL of an aqueous solution was added (varying amounts of stabilizer and desmopressin: either 500 μg or none for blank and coating) depending on the approach; (see Table 1). The mixture was sonicated for 1 min with a Branson Digital Sonifier (Emerson Industrial Automation, St. Louis MO, USA) and a 20 kHz probe with approx. 8 W. 2.5 mL of a 25 mg/mL PVA solution was then added for all preparations. The resulting mixture was sonicated again for 1 min with approx. 14 W to obtain the secondary emulsion. The volume of the sample was then increased with MilliQ water to 20 mL with a measuring cylinder. By increasing the water-volume PLGA particles precipitated from the emulsion. To remove the ethyl acetate the nanoparticulate dispersion was transferred into Eppendorf tubes in 1 mL quantities and centrifuged (24 kRCF, 40 min, 4 °C). The supernatant was discarded and the pellet was washed with MilliQ water and used for further analysis.

To dissolve PLGA nanoparticles for desmopressin analysis (see Fig. 1: 5–10), the pellet had to be redispersed after washing. After the washing water was removed, a small amount of water, which was wetting the walls of the Eppendorf tube, remained in the vial. The pellet redispersed automatically in this remaining water over night. After the pellet was redispersed, 400 μL of acetone (solvent for desmopressin and PLGA) was added into the vial. The vial was then vortexed and left for at least two hours to dissolve. After the dissolution the liquid was clear and 1.6 mL of ethanol absolute (solvent for desmopressin but non-solvent for PLGA) was added. The ethanol precipitated the PLGA, but not the desmopressin. The sample was centrifuged (24 kRCF, 40 min, 4 °C) to remove precipitated PLGA. The supernatant was then transferred to another Eppendorf tube. This desmopressin containing organic solvent solution was left to evaporate under a hood over night. After all organic solvent had evaporated 1 mL of MilliQ water was transferred into the Eppendorf tube to take up the desmopressin and was then used for analysis.

2.3. Coating particles

For the coating approach (CT), nanoparticles were prepared like blank particles. After the volume had been set to 20 mL, 500 μg desmopressin were added to the whole 20 mL batch. Particles and desmopressin remained at room temperature for at least 15 min before undergoing the next step (centrifugation) like all other samples.

2.4. Analysis of colloidal properties

Size and zeta potential of the particles were measured with a Malvern Zetasizer Nano ZS or ZSP (Malvern Instruments GmbH, Herrenberg, Germany). Particles were prepared as explained earlier and the pellet was redispersed in MilliQ water. The pH-value was measured and then a 1:10 dilution of the particle suspension was measured for size and zeta potential. All samples were measured with a 633 nm laser. Sizes were measured with 173° (backscatter) and zeta potentials with 13° (forwardscatter) settings.

2.5. Polymer dry weight measurements with TGA

Thermogravimetric analysis (TGA) was conducted with a Perkin Elmer TGA 4000 (Perkin Elmer, Waltham MA, USA) for determining the dry weight in 1 mL of nanoparticle dispersion. The quantity (1 mL) was treated by centrifugation as described in Section 2.2 to remove excess PVA. The pellet was then redispersed in MilliQ water and freeze dried to remove water. The freeze dried polymer was then measured by TGA to eliminate all remaining water by heat. To determine the correct temperature pure PLGA was heated with TGA and the start of its degradation was observed after 180 °C. Therefore, the value closest to 160 °C (±1 °C) was chosen to determine the polymer dry weight in a sample.

2.6. HPLC – desmopressin quantification

Desmopressin was quantified by a Dionex Summit RP-HPLC system consisting of a P680 Gradient Pump coupled with a Dionex Ultimate 3000 UV/Vis detector. Chromatographic analyses were carried out using an endcapped 125 × 4 mm, 5 μm C-18 LiChroSpher® 100 column (Merck KGaA, Germany) thermo-stated at 30 °C. Analyses were performed by isocratic elution mode using pH 7.2 KH_2PO_4 buffer:acetonitrile (80:20 v/v%) at a flow rate of