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Polyethylene glycol as an alternative polymer solvent for nanoparticle preparation

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ABSTRACT

Solvent toxicity is one of the major drawbacks in the preparation of polymeric nanoparticles today. Here, polyethylene glycols (PEGs) are proposed as non-toxic solvents for the preparation of polymeric nanoparticles. Based on a preparation process similar to the solvent displacement technique, several process parameters were examined for their effects on the properties of the prepared nanoparticles by this method to achieve the optimum preparation conditions. The investigated parameters included polymer type and concentration, volume and temperature of the dispersing phase, methods of dispersing the solvent phase into the non-solvent phase, duration and speed of stirring and washing by dialysis. Ammonio methacrylate copolymer (Eudragit RL), poly-lactide-co-glycolide (PLGA), and PEG-PLGA were found to be successful polymer candidates for the preparation of nanoparticles by this method. Nanoparticles with diameters ranging from 80 to 400 nm can be obtained. The encapsulation efficiencies of bovine serum albumin, and lysozyme as model proteins were ranging from $7.3 \pm 2.2\%$ to $69.3 \pm 1.8\%$ depending on the strength of polymer–protein interaction. Biological assays confirmed a full lysozyme activity after the preparation process. PEG proved to be a suitable non-toxic solvent for the preparation of polymeric protein-loaded nanoparticles, maintaining the integrity of protein.

1. Introduction

Submicron particles have a wide range of applications in many different areas. Among the great variety of previously developed nanoparticles, polymeric nanoparticles are of great importance (Liu et al., 2008). For the past decade, research has accelerated towards the delivery of protein and peptide drugs. Owing to the fragile nature of those biological macromolecules, it is important to ensure that their conformation and biological efficacy are not altered during the encapsulation procedure, which usually involves potentially damaging exposure to organic solvents and/or ultrasound (Krishnamurthy et al., 2000). The strong interest in halogenated organic solvent-free methods is also driven by the potential for a reduction of toxicological risk and an improved preservation of drug integrity and activity, especially for proteins and peptide drugs. Therefore there is an increasing demand for more non-toxic solvents and less harsh conditions for the preparation of nanoparticles without affecting the biological activity of such macromolecules.

However, processes for nanoparticle preparation still use organic solvents to dissolve the water-insoluble polymers due to the lack of alternatives. Several methods have been extensively described for the preparation of polymeric nanoparticles, including emulsion/solvent evaporation, solvent displacement, emulsion and miniemulsion polymerization (Pinto Reis et al., 2006; Vauthier and Bouchemal, 2009). In all those methods, the use of volatile solvents, which are harmful to human health and the environment, can be rarely avoided. Solvents classified into classes II and III in the USP and the Pharm. Eur., respectively, are usually used for the preparation of polymeric nanoparticles (Allhenn and Lamprecht, 2011). Moreover, most of the preparation techniques require further treatment to assure that the residual solvent concentrations in the prepared nanoparticles are lower than the permitted toxic levels. Furthermore, the analytical controls on those given specifications require extensive and expensive methods (Bitz and Doelker, 1996). Hence, it is of great importance to find ways to avoid using these solvents in the preparation process.

Here, we propose PEGs as non-toxic solvents for the preparation of polymeric nanoparticles. PEG is an uncharged, hydrophilic, linear polymer whish is available in a number of molecular weights. It is non-immunogenic and has a very low order of toxicity. It is also approved by the FDA for use in drugs (parentrals, topicals, suppositories, nasal sprays), foods, and cosmetics (Fuertges

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and Abuchowski, 1990). PEG has been used experimentally in biodegradable polymeric matrices used in controlled-release systems (Mohl and Winter, 2004). Furthermore, in concentrations up to approximately 30% (v/v), PEG 300 and PEG 400 have been used as the vehicle for parenteral dosage forms (Wallick, 2009). PEG is water-miscible, and hence the solvent displacement technique was selected as the most appropriate method for preparing nanoparticles using PEG as a polymer solvent. Solvent displacement (or nanoprecipitation) technique was first developed by Fessi et al. (1989). It requires two solvents that are miscible. Both the polymer and the drug must dissolve in the first one (the solvent), but not in the second system (the non-solvent). Nanoprecipitation occurs by rapid desolvation of the polymer when the polymer solution is added to the non-solvent provided that aggregation phenomena are limited (Bilati et al., 2005). This technique presents numerous advantages, in that it is simple, rapid, economic, and yields small particle sizes with narrow polydispersity indices (des Rieux et al., 2006; Govender et al., 1999; Legrand et al., 2007). Therefore, a modified solvent displacement (MSD) method, in which the non-toxic, 'protein-friendly' PEG is used as a polymer solvent replacing the frequently employed solvents, would be of great interest.

The first aim was to study the feasibility of using PEG as a non-toxic solvent for the preparation of polymeric nanoparticles, using the MSD method. In this aspect, different formulation parameters were studied for the most prominent polymers in nanoparticle preparation to optimize the formulation process. Secondly, a preliminary study was carried out on adapting this method to prepare polymeric protein-loaded nanoparticles. Lysozyme and bovine serum albumin (BSA) were employed in this study as model basic and acidic protein molecules, respectively, because of their detailed characterization profiles.

2. Experimental

2.1. Materials

Polyethylene glycols (Macrogol 300 and 400; PEG) were from Caesar & Loretz GmbH (Hilden, Germany). Poly (DL-lactide-co-glycolide) (Resomer RG 502 H; PLGA), and PEGylated PLGA (Resomer RGP d 50155; PEG-PLGA) were obtained from Boehringer Ingelheim, Germany. Poly(meth)acrylates: Eudragit L 100, S 100, RL PO (EDRL), and RS PO (EDRS) were kind samples from Evonik Röhm GmbH (Darmstadt, Germany). Ethyl cellulose (Ethocel standard 4 premium and 10 premium) were kind gifts from Colorcon, UK. Lysozyme, from hen egg white, was from Roche Diagnostics (Mannheim, Germany). Bovine serum albumin (albumin fraction V, purity ≥ 98%, BSA) was from Carl Roth (karlsruhe, Germany). Polyvinyl alcohol (PVAL) 98–99% hydrolyzed, and chitosan (medium molecular weight) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals were of analytical grade or equivalent purity.

2.2. Screening of polymers solubility in PEGs

A wide range of polymers was screened for the possibility of preparing nanoparticles by the solvent displacement method, using PEG 300 or PEG 400 as polymer solvents. Polymers investigated in this screening are listed in Table 1. The screening was carried out using 100 mg of each polymer, except PEG-PLGA (40 mg), due to its limited solubility in PEG. The dispersing phase (the non-solvent) was constituted either from distilled water, or PVAL solution (1%, w/v).

2.3. Nanoparticle design

The MSD method is graphically presented in Fig. 1. For the preparation of the particles, the amount specified of the polymer was dissolved in 3 ml of PEG to form the diffusing phase. This phase was then added dropwise by means of a burette to the dispersing phase under magnetic stirring. The effects of various processing parameters on the feasibility of nanoparticle formation and mean particle size were studied. The investigated processing parameters include: volume and temperature of the dispersing phase, methods of dispersing the PEG into the non-solvent medium, duration and speed of stirring, polymer concentration, and washing by dialysis. Unless otherwise mentioned, all the experiments were conducted by varying one of the parameters while keeping all the other processing parameters at a set of standard conditions. For EDRL and PEG-PLGA, the standard conditions were: 15 ml distilled water as a dispersing phase (the non-solvent) kept at 37 ± 1 °C, stirring speed 900 rpm. For PLGA, the standard conditions were: 15 ml PVAL solution (1%, w/v) as a dispersing phase kept at 37 ± 1 °C, stirring speed 600 rpm.

For determination of the optimum duration of stirring, the dispersing phase was kept under stirring for 6 h with continuous investigation of the particle size.

To investigate the feasibility of using ultrasonic cell disruptor for nanoparticle formation using PEG as a solvent, the diffusing phase was added to the dispersing phase as a massive bulk by means of a syringe in the presence of ultrasonic cell disruptor (Banoelin sonopuls, Berlin, Germany) for 2 min.

The freshly formed nanoparticles were then washed using dialysis membranes (regenerated cellulose, MWCO 50 KDa) in order to gradually remove the PEG and to replace it with water. The size, PI and zeta potential of the prepared particles were measured before and after washing, and the % shrinkage was calculated:

$$\%\,\text{shrinkage} = (\text{mean diameter before washing} - \text{mean diameter after washing})/\text{mean diameter before washing} \times 100\% \quad (1)$$

Protein-loaded nanoparticles were prepared by dissolving 5 mg of protein (either BSA or lysozyme) in 100 μl of distilled water which is then dispersed into 3 ml of the polymer-containing PEG by vortex mixing, forming the diffusing phase. This phase was then added to the dispersing phase (the non-solvent) dropwise by means of a burette under magnetic stirring.

2.4. Nanoparticle characterization

2.4.1. Determination of the particle size and zeta potential

The prepared particles were analyzed for their particle size and size distribution in terms of the average volume diameters and polydispersity index (PI) by photon correlation spectroscopy using particle size analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA) at fixed angle of 90° at 25 °C. The nanoparticle suspension was diluted with distilled water before particle size analysis. Samples were diluted with a solution containing sodium chloride to adjust the conductivity to 50 μS/cm for zeta potential measurements. All samples were analyzed in triplicates at 25 °C and the error was calculated as standard deviation (S.D.).

2.4.2. Scanning electron microscope (SEM) analysis

Samples were prepared by finely spreading a diluted drop of the nanoparticle suspensions after washing over slaps and allowing them to dry in a desiccator. The samples were then coated with a fine gold layer using a gold sputter module and observed by field-emission SEM (FE-SEM; LEO SUPRA 55, Carl Zeiss, Reutlingen, Germany).