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Studies on surface coating of phospholipid vesicles with a non-ionic polymer

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ABSTRACT

Liposomes coated with polymers may have a great potential in drug delivery. In this study, adsorption of the non-ionic hydroxyethyl cellulose (HEC) onto non-charged phospholipid vesicles was investigated. Both unmodified and hydrophobically modified (HM) HEC were included in the study. Possible interactions between the liposomes and the polymers were determined by changes in the size and the size distribution. Rheo-SALS measurements were carried out to verify the successfulness of the coating process. The stability was investigated by zeta potential measurements, UV-analysis and HPTLC. Mixing unmodified HEC (Mw 90,000 and 300,000) with the liposomes yielded no increase in the particle size. HM-HEC, however, was adsorbed onto both the fluid phase egg-PC liposomes and the gel phase DPPC liposomes. The Rheo-SALS measurements confirmed the successful coating of the liposomes. Complete coating resulted in increased chemical stability of the dispersion and in addition prevented aggregation. This study has shown that the non-ionic HM-HEC can be used to form polymer coated liposomes with neutral surface charge for enhanced stability.

1. Introduction

Phospholipid vesicles, or liposomes, are small vesicles of nano-size consisting of an aqueous core enclosed by one or more lipid bilayers. They have attracted much attention due to their structural similarity to the cell membrane and for the ability to encapsulate both hydrophobic and hydrophilic drugs [1]. Encapsulation of drugs in the liposomes has been shown to maintain the activity of the drug in environments that typically lead to degradation [2]. In addition, the liposomal drug carrier can prevent toxic side effects by the drug [3] and possess targeted drug delivery to the site of action [4,5]. One of the major drawbacks with liposomes is their tendency to aggregate and fuse. In order to protect the liposomes, the liposomes can be coated with various polymers such as chitosan [6,7], pectin [8], carbopol [9] and hydroxypropyl cellulose [10]. Decoration of the liposomal surface has shown to improve their structural stability [11] and the complex may also function as a depot for controlled release of the drug [12]. In order to obtain stable polymer coated liposomes, the surface has to be completely covered by the polymer. The stability of the coated liposomes is decreased when insufficient amounts of the polymer are utilized [7]. The liposomes then tend to form large aggregates due to bridging flocculation. However, destabilization of the coated liposomes also occurs when excess amounts of the polymer are present, due to depletion flocculation [13].

Decoration of the liposomes with a mucoadhesive biopolymer can increase the residence time at the site of action, such as the oral cavity [14]. However, biological fluids, such as saliva, can interact with charged biopolymers, thus creating larger complexes [15] which destabilizes the carrier system. In addition, polyelectrolytes can affect the activity of the drug due to electrostatic interactions between the polymer and the drug and this may affect the bioavailability of the drug [16]. These obstacles could be solved by coating the liposomes with a neutral polymer.

Hydroxyethyl cellulose (HEC) is a non-ionic and hydrophilic cellulose derivative where hydroxyethyl groups are randomly distributed along the polymer backbone [17]. HEC has been used in a wide range of pharmaceutical applications, such as a thickening agent in ophthalmic [18] and topical dosage forms [19], in mucoadhesive patches [20] and as a matrix in controlled-release solid dosage forms [21]. By grafting hydrophobic alkyl chains along the HEC backbone, the hydrophobically modified analog (HM-HEC) is obtained. The hydrophobic domains of the polymer provides self-associative features [22]. This gives the polymer unique rheological properties as a viscosity modifier suitable in pharmaceutical applications [23]. Surface adsorption of this polymer onto different surfaces, such as gold particles, has been studied. However, limited

Abbreviations: DLS, dynamic light scattering; DPPC, dipalmitoylphosphatidyl choline; Egg-PC, egg phosphatidyl choline; HEC, hydroxyethyl cellulose; HM-HEC, hydrophobically modified hydroxyethyl cellulose; HPTLC, high performance thin layer chromatography; Rheo-SALS, rheology and small angle light scattering; T_c, gel to liquid-crystalline phase transition temperature.

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literature is found on the surface coating of liposomes by HEC and HM-HEC, and to our knowledge this is the first study concerning the coating of liposomes with these polymers.

The aim of this study was to examine the surface coating of neutral liposomes with a non-ionic polymer. Commercial unmodified and hydrophobically modified HEC were chosen for the studies. The effect of the protective feature of the coating layer against aggregation and degradation was also explored. The complexes formed were characterized using dynamic light scattering to probe the size and the size distribution. The zeta potential was utilized to explore any charge alterations during storage. The combination of rheology and small angle light scattering (Rheo-SALS) was also included in order to investigate if this method could be used to verify complete coating of the neutral liposomes.

2. Materials

Egg phosphatidylcholine (egg-PC) and dipalmitoyl phosphatidyl choline (DPPC) were obtained from Lipoid GmbH (Ludwigshafen, Germany).

Two molecular weights of unmodified HEC with the commercial names Natrosol 250 L Pharm and Natrosol 250 GR Pharm were kindly donated by Azelis Denmark A/S, Aqualon Division. According to the manufacturer, the molecular weight of Natrosol 250 L Pharm was 90,000 Da (HEC-90) and Natrosol 250 GR Pharm was 300,000 Da (HEC-300), calculated from intrinsic viscosity measurements. A sample of HM-HEC (Natrosol plus 330) was offered from the same supplier. According to the manufacturer, the hydrophobic groups of HM-HEC were hexadecyl chains. The Natrosol plus 330 and Natrosol 250 GR Pharm were of comparable molecular weights and the degree of molar substitution of hydroxyethyl groups per repeating anhydroglucose unit was 2.5 (given by the manufacturer).

Sodium dihydrogenphosphate monohydrate and disodium hydrogenphosphate dihydrate used in the phosphate buffer, chloroform applied in the preparation of the liposomes, and copper sulfate pentahydrate and o-phosphoric acid (85%) used in the lipid degradation analysis were all obtained from Merck, Germany. All chemicals were of analytical grade.

3. Methods

3.1. Purification of HEC and HM-HEC

Commercially available HM-HEC were purified by dissolving 1.5% (w/w) in MilliQ-water and stirred overnight at room temperature (22 °C). The solutions were dialyzed against distilled water at 4 °C in a Spectra/Por® 6 dialysis membrane (Spectrum Laboratories Inc., CA, USA), with a molecular weight cut-off of 8000 Da. The water was changed twice a day the first three days, then once a day for four days. The solutions were frozen in a methanol bath (−40 °C) and freeze dried (Christ Alpha 2–4 freeze drier, Christ, Osterode am Harz, Germany). The freeze-dried products were stored in a dark place at room temperature.

3.2. Preparation of liposomes

Liposomes were made according to the film method. The lipids were dissolved in chloroform and evaporated to dryness in a rotary evaporator. The films were further dried in vacuum in the Christ Alpha 2–4 freeze drier for about 20 h to remove organic residues. The lipid films were swelled for 2 h in 5 mM phosphate buffer (pH 6.8 ± 0.1) at a temperature above the gel to liquid-crystalline phase transition temperature (T_c) and gently shaken intermittently. The liposomal dispersions were stored at 4 °C overnight. Size reduction was done at a temperature above T_c by extrusion with a Lipex extruder (Lipex Biomembranes Inc., Vancouver, Canada) using two-stacked 200 nm polycarbonate membranes (Nucleopore®, Costar Corp., Cambridge, USA.). The final lipid concentration for use in further experiments was 3 mM. The egg-PC liposomal dispersions were stored overnight at 4 °C before coating. The liposomes composed of DPPC, however, were coated immediately after extrusion due to fast aggregation.

3.3. Coating of liposomes with HEC and HM-HEC

Purified HEC and HM-HEC were dissolved in 5 mM phosphate buffer (pH 6.8 ± 0.1) and stirred overnight at room temperature. The solutions were filtered through 0.2 μm polycarbonate membranes at room temperature to minimize the risk of dust and particles. The polymer concentrations used for coating of the liposomes were in the range 0.001–0.16% (w/w).

HEC and HM-HEC coated liposomes were prepared by adding 1 ml of liposomal dispersion to 4 ml of polymer solution under constant magnetic stirring. The liposomal dispersion was added to the polymer solution in a drop wise manner (5 ml/min) using a peristaltic pump (Watson-Marlow 520S IP3, Cornwall, UK) and the stirring was continued for additional 5 min. Three parallels were prepared for each combination. The buffer concentration and the pH were kept constant in all samples, at 5 mM and pH 6.8, respectively. The coated liposomes were stored at 4 °C. At various time points samples were withdrawn and analyzed for size and size distribution, zeta potential and a combination of rheology and small angle light scattering (Rheo-SALS).

3.4. Characterization of the particles

The size, the size distribution and the zeta potential determinations were carried out using Zetasizer Nano-ZS, Model Zen3600 (Malvern Instruments, Worchester, UK). The size and the size distribution of the particles were conducted by dynamic light scattering (DLS) at 25 °C with a non-invasive back scatter detection at a scattering angle of 173°. The material setting for polystyrene latex particles was used, and the refractive index and viscosity of pure water were used as calculation parameters. The samples were diluted (1:11) with 5 mM phosphate buffer (pH 6.8) prior to size determinations and were measured in triplicate.

The zeta potential was calculated from the measurement of the electrophoretic mobility of the particles in an applied oscillating field, using laser Doppler micro-electrophoresis velocimetry at 25 °C after dilution (1:11) with 5 mM phosphate buffer (pH 6.8). The zeta potential was deduced from the mobility (U) by means of the Smoluchowski approximation, $U = \varepsilon\zeta/\eta$, where the viscosity ($\eta$) and the dielectric constant (ε) for pure water were used. Three measurements were performed for each sample.

Lipid hydrolysis was estimated from the lyso-PC content in the samples and determined by high-performance thin layer chromatography (HPTLC). Aqueous samples and standard solutions of egg-PC and lyso-PC in chloroform were applied to silica gel $60F_{254}$ HPTLC plates using a Linomat IV sample applicator (CAMAG, Muttenz, Switzerland). The plates were developed in a horizontal developing chamber (Camag) using a mixture of chloroform:methanol:MilliQ-water (32.5:12.5:2, v/v) as the mobile phase and dried [24]. The spots were visualized by immersing into a solution of 8% ortho-phosphoric acid and 10% copper sulphate pentahydrate [25]. The plates were dried at 180 °C for 20 min in an incubator, then cooled to room temperature and scanned at 510 nm using a Camag TLC scanner III. The calibration graph was made from lyso-PC standard solutions, and peak area was used for quantification. Lipid oxidation was characterized by the UV absorbance