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Liposomes coated with hydrophobically modified hydroxyethyl cellulose: Influence of hydrophobic chain length and degree of modification

Gro Smistad[a,*], Bo Nyström[b], Kaizheng Zhu[b], Marthe Karoline Grønvold[a], Anne Røv-Johnsen[a], Marianne Hiorth[a]

[a] Department of Pharmacy, School of Pharmacy, University of Oslo, P O Box 1068, Blindern, N-0316 Oslo, Norway
[b] Department of Chemistry, University of Oslo, P O Box 1033, Blindern, N-0315 Oslo, Norway

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ABSTRACT

Nanoparticulate systems with an uncharged hydrophilic surface may have a great potential in mucosal drug delivery. In the present study liposomes were coated with hydrophobically modified hydroxyethyl cellulose (HM-HEC) to create a sterically stabilized liposomal system with an uncharged surface. The aim was to clarify the influence of the amount of hydrophobic modification of HEC and the length of the hydrophobic moiety, on the stability of the system and on the release properties. HM-HEC with different degrees of hydrophobic modification (1 and 2 mol%) and hydrophobic groups with different chain lengths (C8, C12, C16) were included in the study, as well as fluid phase and gel phase liposomes. Both types of liposomes were successfully coated with HM-HEC containing 1 mol% of hydrophobic groups, while 2 mol% did not work for the intended pharmaceutical applications. The polymer coated gel phase liposomes were stable (size, zeta potential, leakage) for 24 weeks at 4 °C, with no differences between the C8 and C16 HM-HEC coating. For the fluid phase liposomes a size increase was observed after 24 weeks at 4 °C for all formulations; the C8 HM-HEC coated liposomes increased the most. No differences in the leakage during storage at 4 °C or in the release at 35 °C were observed between the fluid phase formulations. To conclude; HM-HEC with a shorter hydrophobic chain length resulted in a less stable product for the fluid phase liposomes, while no influence of the chain length was observed for the gel phase liposomes (1 mol% HM).

1. Introduction

Liposomes are small, nano-sized vesicles with an inner aqueous core embraced by one or more lipid membranes, usually consisting of phospholipids. They have been widely studied for drug delivery during the last decades, both for parenteral administration, *e.g.*, targeting to tumors or reduction of toxic side effects [1–3], and for topical administration to skin and mucosal membranes [4–6]. The great advantage of liposomes is their ability to encapsulate both hydrophilic and hydrophobic drugs. Some major drawbacks, however, are their short circulation time in the body after parenteral administration and the possibility of chemical degradation, aggregation, and fusion during storage [7–9]. To increase the stability, both *in vivo* and *in vitro*, liposomes have been coated with polymers [10–12]. The polymers forming the coating may be attached to the liposomal surface by electrostatic deposition, *e.g.*, chitosan [10,13,14], pectin [15,16], poly(*N*-isopropylacrylamide (PNIPAAM)-*co*-methacrylic acid) [17], and alginate [18]. Charged polymer coatings have also been introduced for improving the mucoadhesive properties of the system [19–21] and for formulation of pH sensitive [22] and temperature sensitive delivery systems [23].

The stability of colloidal systems is promoted by high surface charge of the nanoparticles [24,25]. However, charged surfaces may be problematic *in vivo* due to non-intended interactions with biological surfaces and consequently the possibility of toxic effects or precipitation due to interactions with body fluids [26,27]. In parenteral administration of liposomes an uncharged and hydrophilic, sterically protected surface is a prerequisite for long circulation time [28,29]. It has also been proposed that nanoparticles with

* Corresponding author.
E-mail addresses: gro.smistad@farmasi.uio.no (G. Smistad), b.o.g.nystrom@kjemi.uio.no (B. Nyström), kaizheng.zhu@kjemi.uio.no (K. Zhu), marthegronvold@gmail.com (M.K. Grønvold), anne.r.joh@gmail.com (A. Røv-Johnsen), marianne.hiorth@farmasi.uio.no (M. Hiorth).

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hydrophilic, uncharged surfaces may be advantageous in mucosal drug delivery, since such particles may penetrate deep into the mucosa and deliver the drug close to the cell membrane, instead of being trapped at the surface of the mucin layer [30,31]. Coating of liposomes without electrostatic interactions is possible by attaching hydrophobic groups to the polymer. During coating the polymer is anchored to the liposome surface by insertion of the hydrophobic groups into the liposome membrane [32–36]. Depending on the type, length, and amount of the hydrophobic groups, the liposome may be stabilized [37,38] or destabilized and disintegrating into mixed micelles [36,37]. Hydrophobically modified chitosan [33,39], PNIPAAM [23], and alginate [40] as some examples, have been used for liposome coating, in addition to uncharged polymers such as hydrophobically modified poly(ethylene glycol) (PEG) [41], polyvinyl alcohol (PVA) [12] and dextran [37,38].

Hydroxyethyl cellulose (HEC) is another uncharged polymer. HEC is a cellulose derivative with hydroxyethyl groups randomly distributed along the polymer chain, and has been widely used as thickening agent in pharmaceutical preparations [42], as matrix in controlled-release solid dosage forms [43] and in mucoadhesive patches [44]. In a previous paper we showed that the interaction between HEC and uncharged fluid phase liposomes was very low, or absent [45]. However, HEC can be modified by attaching hydrophobic groups to the hydroxyethyl groups giving hydrophobically modified HEC (HM-HEC). HM-HEC is commercially available and is already used as a thickening agent to provide stability in cosmetics and personal care products. Also, the *in vitro* cell toxicity of HM-HEC has been reported to be low [27]. In the previous paper we showed that by using the commercially available HM-HEC in appropriate concentrations, both fluid phase and gel phase liposomes were successfully coated [45]. In the present paper we have scrutinized these liposomal systems more closely by using HM-HEC with defined chain length of the hydrophobic groups and defined amounts of hydrophobic modification. The aim was to investigate how the amount of hydrophobic modification of the polymer and the length of the hydrophobic chain influence the coating process, the stability of the coated liposomes, as well as the lipid phase transition temperature and the release of encapsulated substance. Both gel phase and fluid phase liposomes were included in this study. Clarifying the influence of these parameters would be advantageous in designing liposomal systems consisting of uncharged liposomes coated with hydrophobically modified HEC, to obtain uncharged systems with high stability and low possibility for ionic interactions *in vivo*.

2. Materials and methods

2.1. Materials

In this work, a hydroxyethyl cellulose (HEC) sample with the commercial name Natrosol 250 GR (Lot. No. A-0382), obtained from Hercules, Aqualon Division, was utilized as a reference and as the precursor for the synthesis of the hydrophobically modified analogue (HM-HEC). The degree of molar substitution of hydroxyethyl groups per repeating anhydroglucose unit of this polymer is 2.5 (given by the manufacturer). The molecular weight (Mw = 400 000) of this sample in dilute aqueous solution was determined by intensity light scattering at 25 °C [46]. The main chemicals for the synthesis of the hydrophobically modified analogue (HM-HEC) such as glycidyl octyl ether (C8), glycidyl dodecyl ether (C12), and glycidyl hexadecyl ether (C16) were all from Aldrich and used as received without further purification. Phosphatidylcholine from soybean (SoyPC) and from egg (EggPC) (Lipoid S PC and E PC S, respectively) and dipalmitoyl phosphatidylcholine (DPPC) were obtained from Lipoid GmbH (Ludwigshafen, Germany). The chloroform used for liposome preparation, and sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate dihydrate used in the phosphate buffer were all of analytical grade from Merck (Darmstadt, Germany). The tris(hydroxymethyl)aminomethane was from VWR Chemicals (BDH, Belgium), the fluorescence marker 5(6)-carboxyfluorescein (CF), and Triton X-100 were both from Sigma (USA).

2.2. Synthesis and characterization of hydrophobically modified polymers (HM-C8-HEC, HM-C12-HEC, and HM-C16-HEC)

The hydrophobically modified hydroxyethylcellulose samples (HM-C8-HEC, HM-C12-HEC and HM-C16-HEC) were synthesized according to our previously reported procedure [47,48] and the details and characterization of the samples have been described elsewhere [46,49].

These polymers were further purified by dialyzing against Millipore water for 3 weeks and finally isolated by freeze-drying. Regenerated cellulose with a molecular weight cutoff of about 8000 was utilized as dialyzing membrane. ^{1}H NMR in DMSO-d_6 ascertained the chemical structure and purity of HM-HEC, and the degree of substitution of the glycidyl alkyl ether groups (C8, C12 and C16) were calculated from the peak ratios between the anomeric protons (4.9 ppm) and the methyl protons (0.8 ppm) of the alkyl group. We have successfully synthesized a series of samples with different length hydrophobic group (C8, C12 and C16) with different degree of hydrophobic substitution 1.0 mol% and 2.0 mol%, respectively.

2.3. Preparation of polymer coated liposomes

The liposomes were prepared by the thin-film method as described earlier [15]. In short, the lipids were dissolved in chloroform and evaporated to dryness in a rotary evaporator. The lipid film was further dried under vacuum to the next day in a Christ Alpha 2–4 freeze drier (Christ, Osterode am Harz) to remove organic solvent rests. The aqueous phase (in most cases 5 mM phosphate buffer at pH 6.8) was added to the film at a temperature above the phase transition temperature (T_c) for the lipid, the sample was slowly rotated for 15 min and then swelled for two hours, still at a temperature above the T_c for the lipid. The flask was gently shaken intermittently during the swelling. The samples were kept in the refrigerator to the next day. A Lipex extruder (Lipex Biomembranes Inc., Vancouver, Canada) with two stacked 200 nm polycarbonate membranes (Nucleopore®, Costar Corp., Cambridge, USA) was used for size reduction. The liposomes were extruded ten times through the filters at a temperature above the T_c of the lipid. Polymer solutions were made by dissolving the polymer in 5 mM phosphate buffer pH 6.8 by magnetic stirring overnight. All polymer solutions were filtered through 2 μm filters before use. The liposomes (3 mM) were coated by dropwise (~3 ml/min) adding the liposomes (one part) to the polymer solution (four parts) by means of a peristaltic pump (Watson-Marlow 520S IP3, Cornwall, UK) at room temperature during continuous magnetic stirring. The stirring continued for 5 min after all the liposomes were added. Three parallel samples were made of each combination.

When fluorescence marker was encapsulated into the liposomes, an aqueous phase containing 100 mM CF (to obtain quenching inside the liposomes [50]) in 60 mM trisbuffer pH 8.0 was used for hydration of the lipid film. The non-encapsulated CF was removed by gel filtration through PD-10 Desalting Columns (GE Healthcare Biosciences AB, Sweden) immediately prior to coating of the liposomes with polymer. The liposomes were eluted from the column with trisbuffer and diluted to 3 mM lipid concentration immediately after gel filtration with trisbuffer containing 0.35 M sodium chloride to avoid osmotic shock.