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Liposomes as carrier for echinochrome

Sea urchin pigment echinochrome (6-ethyl-2,3,5,7,8-pentahydroxy-1,4-naphthoquinone) (Ech) is a natural bioactive compound with many health-promoting benefits. However, its poor water solubility and bioavailability has limited biomedical application. The inclusion of Ech in complexes with kappa-carrageenan (CRG) decreased its oxidative degradation and improved its solubility. In the present study, we used CRG as matrixes for Ech and encapsulated Ech/CRG in into liposomes. Ech did not oxidize and retain stability after the encapsulation in liposomes. According of obtained dates the entrapment efficiency of the Ech in the liposome was 46 %. The lyophilization process did not violate the native form of Ech. The size distribution and ζ-potential of the developed liposomes with and without Ech were determined.

Key words: liposomes, carrageenan, echinochrome

Natural substances possess unique characteristics and abundantly available in nature. The most abundant sea urchin pigment echinochrome A (6-ethyl-2,3,5,7,8-pentahydroxy-1,4-naphthoquinone) (Ech) exhibits a wide range of pharmacological activities [2,6]. Ech is soluble in organic solvents, but its solubility in water is poor that limits its application. Early we have shown that inclusion of Ech in complexes with CRGs decreased it oxidative degradation and improved it solubility [8]. The liposomal form of Ech can be the best formulation to improve the bioavailability of Ech in cells. Liposomes are vesicles consisting of an aqueous core enclosed by one or several lipid bilayers and can encapsulate a wide range of drugs, both hydrophilic and hydrophobic, either in the aqueous core or in the lipid membrane [4].The function of the liposomes strongly depends on their properties, especially their size and charge. Liposomes can be coated by charged polymer with mucoadhesive properties [7]. The coating the liposomes with a mucoadhesive polymer as carrageenan can improve the stability of the liposomes [4, 7].

The present study is primarily focused on the preparation and characterizations of convention liposomes and liposomes containing Ech/CRG composition.

CRG was isolated from a sterile form of red algae (*Chondrus armatus*) with an average molecular weight of 560 kDa determined by viscosimetry. The substance Echinochrome (registration number in the Russian Federation is P N002362/01 [STATE REGISTER OF DRUGS (as of December 5, 2016) Part 2]) was obtained in G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Vladivostok, as powder.

Convention and CRG/Ech containing liposomes were produced using standard thin film hydration and sonication method. An extruder with membranes 0.1 μm and 0.4 μmwas used to obtained liposomes homogenic in sizes. Liposomes produced with larger pore membranes (liposomes-0.4) yielded a polydispersity suspension with a mean hydrodynamic diameter 430.3±29.8 nm. Unilamellar liposomal suspensions with a low polydispersity was prepared with membrane having a pore size of = 0.1 μm (liposomes-0.1). These liposomes were homogenic particles and had monomodal distribution with a mean diameter 125.6±2.5 nm

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The stability of liposomes during 24 hours in solution was studied by dynamic light scattering (DLS). Liposomes-0.1 were more stable than liposomes-0.4. They preserve their stability after 24 h storing in water as also in saline solution. Aggregation and increase in polydispersity index (from 0.108 to 0.315) were observed for liposomes-0.4 after 24 h storing in water. Raising hydrodynamic diameter from 430.3±29.8 nm to 616.3±45.7 nm was also registered.

It is known that liposomes are advantageous in encapsulating different drugs. Hydrophilic drugs are entrapped in the aqueous layer, while hydrophobic drugs a stuck in the lipid bilayers [1]. CRG was used as a soluble matrix. The liposomes loaded water solutions of CRG/Ech were preparated using standard thin lipids film method followed by sonication. The concentration of Ech included in liposomes was determined after its disruption with treatment of butanol/water mixture. The entrapment efficiency of Ech in butanol layer was determined spectrophotometrically at 468 nm. It was 46 % that may be due its hydrophobic nature and stuck in the lipid bilayers, as was shown for hydrophobic drug [6]. Water-soluble drugs have lower encapsulation in the liposomes compared to their lipophilic counterparts [3]. Indeed, according to our data, only 8% of carrageenan was included in the liposomes.

To evaluate of the stability of Ech in the liposomes the absorption at 468 nm were determined. Two characteristic absorption bands of the native Ech at 339 nm and 468 nm were observed in the spectrum of Ech included in liposomes. Itis known that oxidation form Ech has absorption band at 390 nm [5]. We did not registrated such a band in obtained spectrum of Ech from 300 to 650 nm. So the Ech did not transformated after lyophilization of liposomes and their storage.

Loading the liposomes with negatively charged polymers CRG resulted in reversal of the ζ-potential to negative values, which together with increase in size propose the formation of liposomes coating with polymer. ζ-potential of liposomes CRG/EchA-0.1 and CRG/EchA-0.4 were -24.4 and -15.6 mV respectively. A higher value of negative charge for liposomes-0.1 in comparison with liposomes-0.4, is probably due to a greater amount of CRG that covers of smaller liposomes.

The present study reported on the properties of liposomes with and without CRG/Ech encapsulation. The possibility of using liposomes as a suitable carrier system for different applications depends on their properties. Particle size and ζ-potential are important properties that determine stability of liposomes. Value of the ζ-potential is also useful tool in controlling the aggregation, fusion and precipitation of liposomes.

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