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Lipid nanoparticles as drug delivery systems for biologically active compounds

The relevance of the study

The problems of increasing the bioavailability of lipophilic drugs, targeting them to affected organs and tissues, and reducing their systemic toxicity are widely discussed in the scientific literature. Lipid nanoparticles such as nanoemulsions, including liquid droplets of nanometer size, solid lipid nanoparticles (SLN), which consist of solid lipids, and nanostructured lipid carriers (NLC), which combine solid and liquid lipid components, have been proposed as carriers of active compounds to solve these problems. These nanoparticles are less than 100 nm; the small size ensures their effective penetration through the natural barriers in the human organism. Such systems are composed of biocompatible and biodegradable components; various methods, including low-energy methods, such as the phase inversion temperature (PIT) method, are used to prepare them.

Aqueous dispersions of lipid nanoparticles are thermodynamically unstable. However, they should be highly stable to aggregation and sedimentation for practical applications in the medicine, cosmetic and pharmaceutical industries. Nonionic surfactants are widespread for stabilising lipid particles due to their low toxicity. Some of them, for example, polysorbates and sorbitans, are able to provide phase inversion at certain conditions and the formation of small droplets and particles. Therefore, the influence of the composition of lipid nanoparticles and the conditions of their preparation on their size, aggregation and sedimentation stability should be investigated.

The composition of the lipid phase has a significant effect on the efficacy of encapsulation and retention of incorporated drugs or bioactive compounds. SLNs have a longer shelf life and are more efficient at delivering the encapsulated compounds than nanoemulsions. However, solid lipids in SLNs are prone to recrystallization, which can lead to uncontrolled release of incorporated compounds. The combination of solid and liquid compounds in lipid nanoparticles makes it possible to reduce the degree of crystallinity of solid components, promote the disordering of their crystal lattice and increase the efficiency of encapsulation and retention of lipophilic compounds. At the same time, the production conditions also affect the crystalline structure of lipid nanoparticles and the physicochemical properties of their dispersions.

The problem of long-term storage of lipid nanoparticles remains poorly understood. The use of thermal drying and sterilization by autoclaving (the simplest and most frequently used methods for increasing the shelf life) is not

possible due to the aggregation and destruction of lipid nanoparticles due to the melting of the components. Lyophilization is proposed for this purpose. However, there is scarce information in the literature on the storage of lyophilizes, while ageing conditions may affect the redispersion process. The irradiation of the lipid nanoparticles causes sterilization at the ambient temperature, which is of particular interest. Therefore, the effects of irradiation on the physicochemical properties of lipid nanoparticles, the effectiveness of irradiation sterilization, and its effect on the viability of microorganisms in such systems need to be investigated.

The actual problem is to study the possibility of preparing aggregation and sedimentation stable dispersions of lipid particles composed of biocompatible and biodegradable components, their subsequent lyophilization and irradiation sterilization and their effects on the physicochemical properties of systems. The studied lipid nanoparticle formulations can be promising carriers that enhance the efficacy of drugs and bioactive compounds with ophthalmologic, dermatologic, and other effects.

Aim and objectives of the study

The aim of the study was to prepare aggregation and sedimentation stable dispersions of lipid nanoparticles as drug delivery systems by the PIT method.

The following objectives had to be solved to achieve this goal:

1. to determine the conditions of the preparation of lipid nanoparticles with stearic acid, paraffin oil and paraffin wax, stabilized with nonionic surfactants: Tween 60 and Span 60;

2. to study the aggregation and sedimentation stability of lipid nanoparticles with different ratios of the above-mentioned components;

3. to study of the thermal characteristics of lipid nanoparticles with stearic acid, paraffin oil and paraffin wax;

4. to study of the effect of lyophilization on the subsequent redispersion of lipid nanoparticles and to determine the optimal conditions for their storage;

5. to study of the stability of lipid nanoparticles to irradiation and the effect of doses of different power on the sterilization efficiency of lipid nanoparticles;

6. to assess the toxicity of lipid nanoparticles and the effect of incorporation of bioactive compounds on bioavailability using astaxanthin and lutein.

Scientific novelty

The conditions for the preparation of the dispersions of lipid nanoparticles with stearic acid, paraffin oil and paraffin wax less than 100 nm by the PIT method have been determined.

Increasing the concentration of paraffin oil or wax in the composition of lipid nanoparticles with stearic acid leads to an increase in particle size and a

decrease in the degree of crystallinity of the components. On the contrary, increasing the ratio of paraffin oil in the composition of lipid nanoparticles with paraffin wax contributes to a decrease in the size of the nanoparticles.

There are no changes in the molecular structure of the components of lipid nanoparticles and no decrease in the aggregation and sedimentation stability of their dispersions after exposure to radiation doses of up to 25 kGy. The dose required for sterilization was above 15 kGy for gram-positive bacteria *Staphylococcus aureus* and above 5 kGy for gram-negative bacteria *Escherichia Coli*.

The results of Doppler ultrasonography of the vessels of the chorioallantoic membrane of chicken embryos showed that lipid nanoparticles with stearic acid, paraffin oil and paraffin wax have low toxicity to living organisms. Incorporation of astaxanthin and lutein accelerates the recovery process after modeled hemic hypoxia compared to solutions of these bioactive compounds. Moreover, the nanoemulsion with paraffin oil had the highest efficiency in the restoration of blood flow velocity.

Theoretical and practical significance

Aqueous dispersions of lipid nanoparticles with stearic acid, paraffin oil and wax stabilized with Span 60 and Tween 60 retain aggregation and sedimentation stability for over 30 days.

After lyophilization, lipid nanoparticles with stearic acid and paraffin oil can be redispersed without nanoparticle aggregates. Storage of lyophilized lipid nanoparticles with paraffin wax and with a mixture of stearic acid and paraffin wax is not recommended due to their aggregation.

Sterilization of lipid nanoparticles can be carried out under the influence of ionizing radiation with a dose of 15 kGy. This suggests that lipid nanoparticles can be used as carriers of radiopharmaceuticals and can provide prolonged maintenance of sterility of nanoemulsions, NLCs, and SLNs.

The provisions submitted for defense:

1. compositions of lipid nanoparticles with stearic acid, paraffin oil and paraffin wax stable to aggregation and sedimentation;

2. storage conditions for lyophilizates of lipid nanoparticles which maintain an average size of less than 100 nm upon subsequent redispersion;

3. conditions for sterilization of lipid nanoparticles by irradiation to preserve the molecular structure of the components, sizes, aggregation and sedimentation stability, and the dose required to sterilize from gram-positive bacteria *Staphylococcus aureus* or gram-negative *Escherichia Coli*;

4. data on increasing the bioavailability of astaxanthin and lutein incorporated into lipid nanoparticles with stearic acid, paraffin oil and paraffin wax.