Development of methods for obtaining and studying the properties of lipid nanoparticles for drug delivery

The relevance of the work. Nanoemulsions (NE) and solid lipid nanoparticles (SLNs) have been intensively studied in recent decades as promising means of drug delivery. The advantage of these systems is their ability to encapsulate in the lipid core and deliver poorly water-soluble lipophilic biologically active substances. At the same time, lipid nanoparticles should consist of biocompatible compounds so as not to cause side effects. In addition, they must have a sufficiently long shelf life and be resistant to temperature extremes.

The droplet size of the dispersed phase of NE and SLN does not exceed 100 nm, which contributes to the penetration of encapsulated drugs through cell barriers. The dispersion of NE and SLN depends on the composition and method of their preparation. Obtaining lipid nanoparticles is possible using high- and low-energy methods. High-energy methods include homogenization under pressure, ultrasonic action, mechanical dispersion, etc. The advantages of low-energy methods in obtaining NE and SLN compared to high-energy methods are low energy consumption and simpler hardware design. In addition, when using "soft" low-energy methods, there is no destruction of the dispersed phase of drugs encapsulated in drops. Therefore, in this work, to obtain lipid nanoparticles, the method of phase inversion with temperature change (PIT) was used.

NE and SLN are promising for use in various fields, but their application is limited due to thermodynamic instability. To stabilize NE and SLN, nonionic surfactants (surfactants) are usually used because of their lower toxicity compared to ionic ones. However, due to the low surface charge, lipid nanoparticles may be unstable to aggregation. In addition, for effective interaction of lipid nanoparticles with cells or for prolonged release of drugs from particles in the body, it may be necessary to purposefully vary the surface charge of carriers. In this case, the surface charge of lipid particles can be both negative and positive, depending on the field of application, but should not be very high in absolute value, because with an increase in surface charge, *in vivo* clearance is accelerated.

Compounds that are poorly soluble in water can be included in the composition of lipid nanoparticles, the encapsulation efficiency of which depends on the state of aggregation of the carrier. The stability of dispersions of lipid nanoparticles increases, however, the efficiency of drug incorporation into lipid nanoparticles decreases in the following order: liquid < supercooled melt < crystalline lipid. At the same time, the density of solid lipids is usually higher than the density of liquid lipids, which can prevent gravitational separation. In addition, the encapsulation of drugs in solid particles contributes to a longer preservation of the activity of these compounds. Also of interest is the question of the distribution of drugs inside the droplets of the dispersed phase of NE and SLN.

Therefore, an urgent task is to obtain NEs and SLN dispersions resistant to aggregation and sedimentation, to study the structure of lipid nanoparticles and phase transitions. This will make it possible to create on their basis nanocapsules intended for the delivery of drugs, for example, doxorubicin and thymoquinone.

The purpose and tasks of the work. Obtaining highly stable lipid nanoparticles with encapsulated drugs by the method of temperature phase inversion, study of their structure and properties.

To achieve this goal, the following tasks were set:

1. Obtain highly stable NEs with a dispersed phase consisting of paraffin oil (non-polar core) or oleic acid (polar core), with a shell that is a solid adsorption layer of Tween 60 and Span 60 or a liquid adsorption layer of Tween 80 and Span 80, by the method of temperature phase inversion.

2. Obtain highly stable SLN from stearic acid (polar core) or paraffin (nonpolar core) with a shell, which is a solid adsorption layer of Tween 60 and Span 60 or a liquid adsorption layer of Tween 80 and Span 80, by the method of temperature phase inversion.

3. To determine the influence of the core polarity and the phase state of the surfactant surface layer and the lipid core on the aggregative and sedimentation stability of lipid nanoparticles.

4. Based on the analysis of data on phase transitions, determine the structure of lipid nanoparticles.

5. Determine the cytotoxicity and penetration rate of unloaded and drug-loaded lipid nanoparticles into cancer cells.

Scientific novelty.

• The conditions for obtaining highly stable NEs and dispersions of SLN with a polar and nonpolar core and a solid shell of surfactants with a particle size of less than 100 nm have been established.

• It has been shown that the formation of a liquid-like adsorption layer upon stabilization with a mixture of Tween 80 and Span 80 NE are unstable. At the initial stage, the coarsening of drops of the dispersed phase from oleic acid (as well as paraffin oil) occurs mainly due to coalescence; at subsequent stages, both coalescence and Ostwald ripening occur.

• On the basis of thermal analysis data, an assumption was made about the structure of NE and SLN dispersed phase droplets stabilized with Tween 60 and Span 60. NE droplets consist of a liquid core of paraffin oil or oleic acid and a solid shell formed

by adsorbed surfactant molecules. SLN are covered with a solid shell of surfactants, while the core consists of a supercooled melt of stearic acid or solid paraffin.

• Based on the analysis of data on the surface activity of drugs: doxorubicin and thymoquinone and phase transitions in lipid nanoparticles, it was shown that thymoquinone is predominantly soluble in the lipid core, the solubility of doxorubicin in the core is significantly lower. At the same time, these drugs exhibit surface activity and are incorporated into the surfactant adsorption layer on the surface of lipid nanoparticles.

• It was established that unloaded NE from paraffin oil and SLN from stearic acid, stabilized with Tween 60 and Span 60, exhibit low cytotoxicity. When lipid nanoparticles are loaded with doxorubicin and thymoquine, their cytotoxicity sharply increases, which makes them promising delivery systems for these drugs.

Practical significance of the work.

• Compositions have been developed and conditions have been established for obtaining highly stable NE and dispersions of SLN.

• Possibility of encapsulation of doxorubicin and thymoquinone in lipid nanoparticles has been shown. *In vitro* studies have demonstrated the high cytotoxicity of lipid nanoparticles loaded with doxorubicin and thymoquine, which shows the promise of their use as carriers for the delivery of anticancer drugs.

• *In vivo* studies have shown that NEs from oleic acid, stabilized with a mixture of Tween 80, Span 80 and cationic surfactant CTAB or UR-16, with encapsulated indomethacin, have an effective anti-edematous effect and can be used in anti-inflammatory therapy.

Provisions to be defended.

1. Influence of core polarity, type and concentration of surfactants on the dispersion of NE and SLN obtained by the method of temperature phase inversion.

2. Structure of drops of the dispersed phase of NE and SLN depending on the polarity of the lipid core and the type of surfactant, proposed on the basis of data on phase transitions in dispersions of lipid nanoparticles.

3. Temperature ranges of stability of NE and SLN dispersions with a polar and nonpolar core, with a liquid-like and solid-like surfactant layer on the surface.

4. Cytotoxicity in vitro of NE with a dispersed phase consisting of paraffin oil and SLN from stearic acid loaded with drugs with doxorubicin and thymoquinone.

5. Effect of encapsulated indomethacin in NE from oleic acid stabilized with a mixture of Tween 80, Span 80 and cationic surfactant CTAB or UR-16 on anti-inflammatory properties *in vivo*.