Infrared spectroscopy of liposomes obtained by different variants of the injection method

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Abstract. The study by infrared spectroscopy of the structure of liposomes used in veterinary medicine as nanocontainers for the targeted delivery of medicinal substances to the cells of body tissues is a very important area of research. In the process of obtaining liposomes and liposomal preparations, processes of phospholipid modification occur. At present, the method of IR spectroscopy is used to control the deposition of substances on the surface of a liposome in order to study the delivery of drugs, etc. The comparative study of liposomes obtained by different versions of the injection method was first carried out by infrared spectroscopy. According to the results of medium-wave spectroscopy, the peaks of absorption of IR radiation in various wavelength ranges for these liposomes were revealed. This indicates the presence in liposomes of groups of atoms -CH₃, -CH₂-, primary and secondary alcohols, cis- and trans-isomers HRC = CR´H, groups –OH, groups C=O, solid fatty acids. The results of the study can be used as reference identification IR spectra for the express identification of liposomes obtained by the injection method, and the use of the Fourier IR spectroscopy method for studying the structure of liposomes with encapsulated potassium orotate can be of wide practical importance as a method of express analysis.

1. Introduction
Currently, the issues of the use of liposomes in medicine and veterinary medicine are receiving close attention. Liposomes are nanoparticles confined from the environment by a double lipid membrane, which makes them very similar to the cells of a living organism. The structural feature of these particles is their ability to include various hydrophilic and hydrophobic substances, i.e., to act as a kind of containers for the delivery of substances. Due to the very small size of liposomes, they are called nanocontainers [1]. To obtain liposomes, lipids and phospholipids of plant and animal origin are used. It is known that liposomes are selectively absorbed by tissue macrophages [2], which leads to their activation. Recent studies have shown that in addition to anti-inflammatory macrophages, activated macrophages exhibit reparative properties: they secrete VEGF (vascular endothelial growth factor), a signaling protein that stimulates angiogenesis, and the formation of granulation tissue [3].

In recent decades, infrared spectroscopy has been widely used in biology. IR analysis allows you to determine the physicochemical or biological characteristics of a sample, for example, chemical composition, granule size, density, etc. [4].
Currently, there are available databases of IR spectra of food products, technical and food additives, drugs, poly- and monomers, plasticizers, pesticides, solvents, petroleum products, toxic substances, steroids and other compounds, which are mainly inherent in plants one-component composition [5]. However, there is no reliable, complete and accessible database of IR spectra of liposomes with encapsulated potassium orotate. This method for the analysis of liposomes obtained from plant and animal raw materials is extremely poorly covered; in the available works, only the results of the analysis of some components of nanoparticles are given [6,7,8].

2. Purpose and object of research
Study of the possibility of using medium-wave infrared spectroscopy for studying liposomes obtained by different versions of the injection method.
The object of the study is liposome samples with encapsulated potassium orotate obtained by different versions of the injection method.

3. Materials and methods
3.1. Obtaining experimental liposomes
Liposomes were obtained by the injection method [9]. For this, a weighed portion of lecithin (phosphatidylcholine) was dissolved in ethyl alcohol, and α-tocopherol was added to the solution to prevent phospholipid peroxidation. The prepared solution was injected into a potassium orotate solution.
Liposome samples were obtained:
1) using a device that creates a high pressure of the solution through a hole with a small diameter;
2) using a device that creates a high pressure of the solution through a bacterial filter with many small-diameter holes;
3) using a BI-1 needleless injector;
4) using a BI-1 needleless injector by spraying over a potassium orotate solution.

3.2. Investigation of the IR spectra of liposomes obtained by different versions of the injection method
The study of the IR spectra of liposomes obtained by different versions of the injection method was carried out on a thermo fisher scientific nicolet is 10 IR Fourier spectrometer [10]. Germanium crystal attachment.

4. The discussion of the results
Figure 1 shows the results of a comparative study of the IR spectra of liposomes obtained with different versions of the injection method.

![IR spectra of liposomes with encapsulated potassium orotate obtained by different versions of the injection method. Main components: 1 - sample 2; 2 - sample 3; 3 sample 4; 4 - sample 1.](image-url)
Liposomes obtained by different versions of the injection method (figure 1, samples 1, 2, 3, 4) contain specific components of IR radiation. The nature of the intensity of the absorption bands of liposomes obtained by different versions of the injection method can be judged by their enlarged fragments shown in figures 2-5.

In the IR spectrum of samples from 3000 to 2800 cm⁻¹ (figure 2), peaks with maxima were found:

- sample 1 - 2980.35, 2928.59, 2907.55, 2857.15 - cm⁻¹;
- sample 2 - 2928.37, 2929.86, 2906.97, 2858.19;
- sample 3 - 2980.42, 2930.11, 2905.75, 2858.22, 2840.67;
- sample 4 - 2982.12, 2958.52, 2928.92, 2908.85 and 2857.23.

This indicates that in liposomes, the \(-\text{CH}_2\)- groups, which are part of the fatty acids of lecithin, are not hidden inside the nanoparticle.

In the IR spectrum of samples 3 and 4, peaks with maxima (figure 3), 2137.65 and 2139.34 cm⁻¹.

This indicates the presence of compounds of the azide type \(-\text{N}=-\text{N}^+=\text{N}^=\) in the samples, since the \(\text{R}_4\text{N}^+\) group present in phosphatidylcholine does not have characteristic bands, probably these bands indicate its openness in these samples.

In the IR spectrum of all samples from 1800 to 1600 cm⁻¹ (figure 4) peaks with maxima were found:

- sample 1 – 1645.90; cm⁻¹;
- sample 2 – 1644.39;
- sample 3 – 1647.48;
- sample 4 – 1644.67 cm⁻¹.

This indicates the openness and presence of \(-\text{C}=\text{C}-\) double bonds in all samples, probably in the fatty acid radicals (tails) of phosphatidylcholine.
Figure 4. Fragments of the IR spectrum of samples from 1800 to 1600 cm⁻¹, shown in figure 1. Main components: (a) sample 1; (b) sample 2; (c) sample 3; (d) sample 4.

In IR samples from 800 to 1500 cm⁻¹ (figure 5), peaks with maxima were found:

- sample 1 – 1455.23, 1421.63, 1379.74, 1321.02, 1274.88, 1211.26, 1162.09, 1085.08, 1045.55, 970.96, 877.91;
- sample 2 – 1481.71, 1456.18, 1421.09, 1379.48, 1317.91, 1211.28, 1161.70, 1084.49, 1046.02, 971.28, 876.11;
- sample 3 – 1489.25, 1455.21, 1418.44, 1379.57, 1318.06, 1274.76, 1224.86, 1158.63, 1085.02, 1045.72, 971.37 920.67, 877.74, 806.46, 759.52;
- sample 4 - 1480.79, 1456.24, 1378.90, 1212.91, 1084.60, 1046.22, 971.49, 874.90.

The presence of peaks indicates the presence in liposomes of groups of atoms -CH₃, -CH₂-, primary and secondary alcohols, cis- and trans-isomers HRC=CR’H, groups –OH, C=O groups, solid fatty acids.

Figure 5. Fragments of the IR spectrum of samples from 1800 to 1600 cm⁻¹, shown in figure 1. Main components: (a) - sample 1; (b) sample 2; (c) sample 3; (d) - sample 4.

Analysis of the literature shows [6] that the method of infrared spectroscopy, in particular, is used to: control the application of chitosan (HTCC) to the liposome; studying the structure of
trimethylchitosan [7]; studying the use of albumin nanoparticles as delivery vehicles for various therapeutic agents [8].

5. Conclusion
The IR spectra of liposomes obtained by different versions of the injection method were studied. The results obtained show the presence in these liposomes of various functional groups characteristic of individual chemical compounds; this is probably due to the structure of nanoparticles (liposomes), which is consistent with the literature on the chemical structure of nanoparticles.

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