Raman spectroscopy and optical microscopy of medical infusion solutions for parenteral nutrition

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Abstract. Usage of Raman spectroscopy, optical microscopy, and retaining track membranes to find 20% SMOFlipid microparticles with a diameter of 5 to 10 μm and to prevent complications that arise during parenteral nutrition.

1. Introduction

The study was carried out on the equipment of the regional center of probe microscopy for collective use at the Ryazan State Radio Engineering University. We used an optical microscope and a spectrometric probe complex Integra-Spectra (manufactured by NT-MDT, Russia, Moscow).

Figure 1. Particle detection equipment
Spectrometric probe complex Ntegra Spectra (manufacturer NT-MDT, Zelenograd), located in the Regional Center for Probe Microscopy for Collective Use V.F. Utkin in Ryazan

A focused laser changes the vibrational state of molecules of foreign particles of the solution on the sample, and the molecules begin to emit Raman radiation. But any substance constantly emits Rayleigh radiation, with constant wavelength. Raman radiation (with dynamic wavelength) can be filtered with a dichroic filter.

Monochromatic light created by a laser is used for the initial radiation (for example, Nd: YAG – a laser based on Yttrium – Aluminum Garnets with the addition of Neodymium, with a wavelength of 532 nm – a green visible color).
The dependencies between the intensity of Raman radiation (based on the results obtained on the photosensitive matrix) and wavelength of Raman radiation is plotted on a graph. Each molecule has its own shift of Raman radiation, so the amount of molecules depends on the intensity of Raman radiation. Raman spectroscopy allows to analyze a substance in liquid, gaseous and solid states larger than 1 micron. [1]

Analysis stages:

- Determination of the chemical composition of foreign particles by Raman spectrums;
- Determination of the area of each foreign particle \( S_1, S_2, \ldots, S_i \);
- Calculation of particle concentration: \( n = N/(S \cdot d) \) [unit./ml];

Calculation of the volume fraction of particles: \( \omega = (100 (S_1+S_2+\ldots+S_i))/S \) [%]; \( N \) is the number of found foreign particles; \( s \) is the area in [cm\(^2\)] occupied by the test solution; \( d \) – layer thickness in [cm] occupied by the test solution.

Optical microscopy and Raman spectroscopy in a 20% SMOFlipid solution revealed homogeneous microparticles with sizes from 1 µm to 50 µm (average size ~ 10µm, an average concentration of 10,000 particles / ml, and a volume fraction of \( 6.4 \times 10^{-3} \)). Filters of standard medical droppers with a pore diameter of 150 microns do not retain the above described microparticles.

The circulation of microparticles through the bloodstream for 60 minutes was experimentally established when the centrifuged plasma of women aged 19 to 42 was added (10 to 1 by volume) to each of the AB0 / rhesus combinations. The average size of microparticles at 36.6°C did not change within 60 minutes, which is due to the specifics of the enzymatic activity of plasma albumin, liver and kidney enzymes and the properties of cell receptors.

The Doppler method was used to calculate the transit time of the injected solution through the subclavian vein of fullterm newborns – 0.07 s, which is incomparable with the period of lipolysis activation 60 minutes.

When microparticles enter the pulmonary and cerebral bloodstream, they cause microembolism, which leads to a pathological decrease in ventilation-perfusion relations.
2. Experiment and methods

It is proposed to filter microparticles through track membranes with a pore diameter of 3 µm, which retain microparticles with a diameter of 5 to 10 µm, which is confirmed by the homogeneous composition of the filtered microparticles and their absence in the solution during repeated filtration. The presence of microparticles and their absence on the surfaces of the track membranes used for filtration were established by optical microscopy. The homogeneity of the microparticles was established by Raman spectroscopy.

![Figure 3. Particles in test solution SMOFlipid (sizes are indicated in micrometers (µm))](image1)

![Figure 4. The microparticle of SMOFlipid in the blood plasma at room temperature 24_°C. The diameter of the image is 500 µm. Optical microscopy](image2)

![Figure 5. It is proposed to filter microparticles through track membranes with a pore diameter of 3 µm](image3)

![Figure 6. The surfaces of the track membranes](image4)

The filtration efficiency was confirmed by testing on laboratory rats – microscopy of lung sections revealed a smaller number of neutrophils in the airspace and a smaller proportion of the airspace containing hyaline membranes, in comparison with sections of the lungs of intact rats. It is caused by a reduction in capillary stasis and a decrease in the intensity of perifocal inflammation.
With parenteral nutrition of fullterm newborns with 20% SMOFlipid, the use of additional medical filters of the Octopus brand with a pore diameter of 3 µm did not reveal complications associated with impaired hemostasis, while when using filters of standard medical droppers, increased bleeding was revealed, which is manifested by point intradermal hemorrhages in the area of catheter placement, shortterm bleeding from the umbilical wound against the background of normal values of fibrinogen, antithrombin, 8 coagulation factor.

3. Results and discussion
The first paragraph after a heading is not indented (Bodytext style). Complications that arise during parenteral nutrition using standard medical dropper filters can be explained by the manifestation of hypercoagulation against the background of latent fibrinolysis. The penetration of a large number of microparticles of the infusion emulsion into the peripheral, and then into the pulmonary and cerebral
blood flow causes a deviation of hemostasis parameters and activation of the fibrinolytic link. To determine the state of hemostasis accurately, it is necessary to conduct thromboelastometry followed by analysis of the thromboelastogram. Thus, microparticles in a 20% SMOFlipid solution cause symptoms of hemorrhagic disease of newborns. The introduction of the solution must be carried out with the obligatory use of a filter with a micropore size of up to 5 microns. The infusion rate should correspond to the rate of activation of plasma enzymes and should not exceed the rate recommended in the drug’s instructions.

Acknowledgments
The work was performed on the equipment Regional center of probe microscopy collective use Ryazan State Radio Engineering University named after V. F. Utkin.

References
[1] Pardeshi N N et al 2017 Journal of pharmaceutical sciences 106(2) 511