Study of the model system for delivery and controlled release of anticancer drugs in affected areas.

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Abstract. The modern clinical oncology key task is to increase the efficiency of anticancer chemical therapy. The main direction of provided investigations are to ensure targeted delivery of drugs to cancer cells with the minimization of the harmful effect to normal cells, as well as overcoming the multiple drug resistance of cancer cells. One of the possible solutions is to use nanoporous medium filled with non-wetting liquid (drug) as a carrier. Interest to such systems is caused by two effects observed for such systems: the effect of non-wetting liquid dispersion in pores and it's anomalously slow relaxation (outflow). It has also been shown that these effects are critically dependent on temperature. The work is present results of experimental study of the kinetics of model liquid outflow from nanoporous medium at the temperature range 20-40 °C. Results obtained for systems with different granule sizes and surface modifications.

1. Introduction
The task of improving the effectiveness of chemical oncology treatment is a key in a modern clinical oncology. The main directions of carried out researches are overcoming multiple drug resistance (MDR) of cancer cells, ensuring targeted delivery of drugs to the focus onto the cancer cells, minimizing harmful side effects. The main factors of MDR are increased activity and expression of membrane transporters, some metabolic enzymes responsible for the active transport of hydrophobic chemotherapeutic substances from cancer cells and preventing apoptosis. In this regard, structures are being sought to bring the anticancer drug into the in affected areas and ensure its controlled release. Nanoparticles, such as iron oxide and chitosan, have recently attracted the attention of researchers due to their peculiarities of interaction with cancer cells [1], nanoparticles based on polyesters [2], liposomes [3], and porous media [4, 5]. Analysis of publications [6-9] has shown that the use of nanoparticles loaded with cytostatic has a number of disadvantages. The dose of nanoparticles introduced into the bloodstream is significantly limited by the relatively high intrinsic toxicity of nanoobjects, in addition, the high probability of accumulation of nanoobjects loaded with cytostatic in a liver reduces the efficiency of drug delivery to the cancer and can lead to additional harmful side effects. The important problem is the low concentration of a cytostatic loaded into a nanoparticle. Therefore, it is an important to create non-
toxic systems that allow a high concentration of the loaded anticancer drug, accumulated in affected areas and the minimum uncontrolled release of a drug in a bloodstream.

Now it is known that for many non-wetting liquids and both disordered and ordered porous media after removal of overpressure there is a non-outflow of either part of the liquid or the whole liquid was observed. The capture (non-outflow) of non-wetting liquid is established in the study of intrusion – extrusion for water, aqueous solutions of salts and organic substances, mercury, other metals, the Wood alloy and such porous media as hydrophobic silica vel and temperature (dispersion transition). In [16,17] on the example of water - porous media L23, Fluka 100 C₁₈ it was found that the volume of the captured liquid depends critically on both the initial filling level and temperature (dispersion transition). Such dependences cannot be explained within the framework of notions of intrusion – extrusion liquid from individual pores, based on Laplace ratio, phenomenological wetting angle [18]. These dependencies mean that the capture effect of a part of the non-wetting liquid can be related to the degree of filling of the collective interaction of liquid clusters in adjacent pores. Correlation effects of interaction of liquid clusters in a confinement have been previously considered in [19]. The capture of a non-wetting liquid by an irregular pore structure and the kinetics of the dispersion transition are described in [20]. In the presented approach the outflow of non-wetting liquid and its capture was described for the basic state of disordered porous medium, which is characterized by the formation of an infinite fractal percolation cluster of filled pores. The observed liquid capture is explained as a transition of a part of the liquid that was intruded into the porous medium under excess pressure into a metastable state after the subsequent decrease of the excess pressure. The energy barrier of the metastable state is defined as the difference between the "pushing out" non-wetting liquid surface energy of interaction of the liquid cluster in the pores with the skeleton of porous medium and the surface energy of the "multiparticle interaction" of the liquid cluster in the pores with the liquid clusters in adjacent pores. Description of relaxation of the metastable state of the captured non-wetting liquid based on the calculation of the function of distribution of filled pores by time of liquid outflow is also described in [21]. The latest results presented in [22, 23] experimentally confirm the idea of describing the relaxation of a non-wetting liquid dispersed in a disordered random medium [21] based on the existence of local metastable formations.

Thus, based on the previously known phenomenon of non-wetting liquid non-outflow from nanoporous medium and the discovered phenomena of transition of non-outflow liquid dispersion in nanoporous medium and anomalously slow relaxation (outflow) of liquid from nanoporous medium the granular nanoporous medium with intruded drug can be used as a carrier for controlled extraction of an anticancer drug. In this case, the combination of the characteristics of the nanoporous medium and the drug solution is determined in such a way that the drug solution dispersed in the porous medium is released from pores only when it enters the area of affected areas due to changes in environmental conditions.

In the present work model systems for controlled release of anticancer drug (Doxorubicin - "Ebeve") have been investigated. The influence of chemical modification of the surface on relaxation of non-wetting liquid was investigated. The results of relaxation of doxorubicin solution at changing external conditions (temperature, pH) are presented. It is shown that the proposed model porous media can be used to develop target drug delivery systems.

2. Porous media and research methods.

As carriers were investigated nanoporous media Fluka 90 C₁₈ (#60757-50G, Sigma-Aldrich) and Fluka 100 C₈ + C₁, which was obtained by additional chemical modification of the surface of commercial sample Fluka 100 C₈ (#60755-50G, Sigma-Aldrich). The modification was carried out at the Faculty of Chemistry of Moscow State University. Porous media were investigated by the method of low-temperature sorption of nitrogen by Autosorb IQ (Quantachrome Instruments, USA) and helium picnometry by UltraPyc 1200e (Quantachrome Instruments, USA). Standard methods have determined the characteristics of nanoporous media: specific volume of pores (Vₚₒₜ), specific surface area of pores (Sₚₒₜ) (according to BET [24]), average pore size (〈r〉) and density of the material (ρ). The data are presented in Table 1. The studied porous media had differed specific surface area and specific volume.
of pores, but had the same average pore radius within error limits. It can be seen from Fig.1 that porous media have qualitatively the same pore size distribution.

As non-wetting liquids the following were used: distilled water, saline solution and doxorubicin (1.2 µmol/L) in saline solution. The surface tension, viscosity and density of liquids at 20 and 40 °C are given in Table 2. Experimental data of the non-wetting liquid dispersed in pore space relaxation were obtained on the experimental setup described in the paper [11]. All results obtained at a constant temperature. The temperature in the chamber was maintained by thermostat LOIP FT-316-40 (LOIP Ltd, Russian Federation). Nanoporous medium with mass ~ 4 g was placed in the high-pressure cell. The high-pressure cell consists of a cylindrical body, a plug with rubber seals, a cap and a rod. The free volume was filled with a non-wetting liquid (30 cm³). The assembled cell was kept at a constant temperature for about 1 - 1.5 hour. A high-pressure cell was mounted on a movable platform. By moving the rod, overpressure was created in the cell. Fig. 2 shows the dependence of the volume change on the pressure dV(P) for several sequential filling cycles Fluka 100 C₆+C₇-water system. The dependencies are given taking into account the elastic deformation of the system. The dependence dV(P) with characteristic points 1-2 corresponds to the filling of an empty pore system. The limit fill at point 2 corresponds to a fulfilling of sample by 4 g. Figure 1 shows the functions of pore size distribution, obtained according to the BJH method [24].

### Table 1. Characteristics of porous media.

| Nanoporous Medium | Vₚₑᵣ, cc/g | Sₚₑᵣ, m²/g | <r> nm | ρ, g/cc |
|-------------------|-------------|------------|--------|--------|
| Fluka 90 C₁₈     | 0.36        | 82         | 3.8    | 1.5975 |
| Fluka100 C₆+C₁   | 0.54        | 263        | 3.8    | 1.7403 |

**Figure 1.** Pores size distribution of Fluka 100 C₆+C₁ and Fluka 90 C₁₈ media.

**Figure 2.** Volume change of system Fluka 100 C₆+C₁ - water at 20 °C.

### Table 2. Characteristics of non-wetting liquids. [25].

| Liquid                  | Surface tension, σ N/m | Density, ρ g/ml | Viscosity, μ mPa*s |
|-------------------------|------------------------|-----------------|-------------------|
|                         | T=20°C                 | T=40°C          | T=20°C            | T=40°C            | T=20°C            | T=40°C            |
| Distilled water         | 0.072                  | 0.069           | 0.9982            | 0.9923            | 1.005             | 0.656             |
| Sodium chloride solution (0.9 %) | 0.073 | 0.070 | 1.0043 | 1.0006 | 1.040 | 0.675 |

The value of the total volume of pores is determined by the difference in volume (V₂-V₁). In the first fulfilling cycle of the empty porous medium, the pressure is increased and the filling up to point 2. The excess pressure is then reduced to 0. Re-filling is done after waiting time to point 2. The dependence of
dV(P) with characteristic points 3-2 was obtained. This cycle was repeated for different waiting times from 1 to $10^4$ s. As a result, series of experiments at a constant temperature for various non-wetting liquids, a set of points of fraction of the non-outflow liquid from the waiting time at different temperatures were obtained. The percentage of non-outflow liquid ($\theta$) is $\theta = \frac{V_3 - V_1}{V_2 - V_1}$. This set shows the effect of waiting time on fast and slow relaxation of non-wetting liquid dispersed in pore space. Studies were conducted for temperatures 20 and 40 °C.

3. Results and discussions
Figures 3a and 3b show the results of the study of relaxation of non-wetting liquids (water and saline) for Fluka 100 C$_8$ +C$_1$ at time intervals from 1 to $10^4$ seconds at temperatures 20 and 40 °C. The figures show that as the temperature raises, the fraction of non-outflow volume, both for water and saline solution, decreases. At the same time, the fraction of non-outflow liquid (water) decreases from 0.8 to 0.45 when the temperature increases from 20 to 40 °C at times ~ 1 s. When the waiting time increases from 1 to $10^4$ seconds at the constant temperature 40 °C the fraction of non-outflow liquid decreases from 0.45 to 0.25.

![Figure 3. Relaxation of liquids for systems a) Fluka 100 C$_8$ +C$_1$ – water, b) Fluka 100 C$_8$ +C$_1$– saline solution](image)

![Figure 4. Relaxation of liquids for systems a) Fluka 90 C$_{18}$ – water, b) Fluka 90 C$_{18}$ – saline solution](image)

In Figure 4a and 4b show results of liquids relaxation for the Fluka 90 C$_{18}$ - water system and the Fluka 90 C$_{18}$ - saline solution. According to the data presented in Fig. 4a and 4b, for each system there is a decrease of non-outflow liquids fraction when the temperature increases from 20 to 40 °C. However,
the fraction of outflow liquid is ~ 0.1 in the case of a water system. And when the waiting time increases to $10^4$ seconds at a constant temperature 40 °C, the fraction of non-outflow liquid changes from 0.7 to 0.6.

Thus, in the Fluka 100 C₈+C₁ system - water - the fraction of the outflow liquid (0.4) is much higher when temperature increasing and at short observation times (~ 1 s) than for the Fluka 90 C₁₈ - water system (0.1). When observing relaxation at a constant temperature of 40 °C and waiting time of $10^4$ seconds, the fraction of non-outflow liquid is also higher for the Fluka 100 C₈+C₁ – water system.

According to the results, the Fluka 100 C₈+C₁ was selected as a model system for drug delivery. Fig.5 shows results of the study of doxorubicin solution relaxation at 40 °C. The figure shows that the relaxation of doxorubicin solution within the error limits coincides with the results for saline solution (see Fig. 3b).

Also for the model system, studies were carried out on the outflow of the non-wetting liquid into buffer solutions with different pH. The pH control was performed on a Hanna EDGE pH-meter (1% accuracy). The relaxation of the doxorubicin solution (1.2 µmol/L) at 40 °C in buffer solutions with pH 5 and pH 7.4 at a relaxation time of $10^3$ s was about 0.38. Thus, the pH of buffer solutions within the measurement error limits does not affect the outflow of the doxorubicin solution from the pore space.

In addition, the stability of cytotoxic properties of Doxorubicin – “Evbe” in the solution under conditions of overpressure was studied at Pirogov RNIMU. For this purpose the solution of doxorubicin (1.2 µmol/L) in the saline solution was placed into the cell without a porous medium, and then excess pressures of 100, 200 and 300 bar were achieved in the chamber. The selected concentration of the preparation corresponded to the IS50 value determined experimentally for the given cancer cell culture.

For each pressure, after decreasing the overpressure, samples were studied on the culture of HeLa cancer cell culture vitality by the MTT cell method. The results are shown in Figure 6.

![Figure 5](image-url)

**Figure 5.** Relaxation of doxorubicin solution dispersed in Fluka 100 C₈+C₁ pore space at 40 °C.

![Figure 6](image-url)

**Figure 6.** Vitality of HeLa cell culture as a function of an excess pressure.

Figure 6 shows the preservation within the limits of the measurement error limits of cytotoxic properties in comparison with the control solution, which was not exposed to high pressure. It should be noted that the studies of HeLa cell culture vitality decline depending on the time of incubation with doxorubicin exposed to high pressure showed that the maximum number of cells dies in the interval from 8 to 12 hours, which corresponds to the data of untreated preparation and indicates the preservation of the basic pharmacological mechanisms of its action and molecular structure.

4. Conclusion

The paper presents the study of model systems for targeted drug delivery. The relaxation effect of non-wetting liquid for nanoporous media with qualitatively similar pore size distribution and surface chemistry has been investigated: Fluka 100 C₈+C₁ and Fluka 90 C₁₈. For each system, there was a decrease in the fraction of outflow liquid with increasing waiting time, at a constant temperature and a
decrease in the fraction of outflow liquid with increasing temperature for a fixed observation time. According to the results presented, the better outflow under changing external conditions (temperature) was observed for the Fluka 100 Cₘ+C₁ sample.

The relaxation of doxorubicin solution with porous Fluka 100 Cₘ+C₁ medium at 40°C was also studied. The relaxation of doxorubicin solution within of error limits was found to coincide with the results obtained on this sample for the saline solution. In addition, it has been shown that the pH of the external environment in the pH range of 5 - 7.4 within the measurement error limits does not affect the fraction of the non-outflow liquid from the Fluka 100 Cₘ+C₁. The method of MTT-test on the culture of HeLa cancer cells shows the preservation of the main pharmacological mechanisms of action of doxorubicin and its molecular structure in case of exposure to excess pressure up to 300 atm. Thus, the presented results show that it is possible to use such systems as nanoporous medium - a non-wetting liquid as model systems for the targeted delivery of anticancer drugs to affected area.

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