CALORIMETRIC STUDY OF CALCIUM-CONTAINING PLGA NANOPARTICLES

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Abstract - In recent years, the frequency of use of calcium-containing drugs has increased. Placing it in nanoparticles and bringing it to the target organ with less loss is a very important step for scientists, because it allows, on the one hand, to increase the amount of calcium in the body's deficient tissue, and on the other hand, calcium placed in nanoparticles does not cause side effects.The work is about the calorimetric study of Poly(Lactic-co-glycolic Acid) (PLGA) drug-carrying nanoparticles containing calcium drugs (calcium carbonate, calcium gluconate, calcium fluoride).The thermostability of nanoparticles containing calcium salts and its structural stability at different concentrations of calcium salts were determined in the research.

Keywords - The conducted experiments and the obtained results allow us to conclude that it is possible to use PLGA nanoparticles as an effective means of transporting calcium-containing drugs in living organisms.

I. INTRODUCTION

Bone-related diseases are a huge social and economic problem today. These problems are often related to an age.It is known that elderly people often suffer from osteoporosis and osteopenia. Due to the lack of effective and rapid treatment of the above-mentioned diseases, the treatment period is very long, which affects both the patient's quality of life and the costs of the healthcare system [2,4,10,11,12]. In order to effectively administer the treatment, it became necessary for scientists to create drug carriers that would deliver the drug to the target site in the required concentration without causing side effects. As for taking large amounts of calcium supplements, it will not solve bone problems quickly and effectively. In addition, an increase in the amount of calcium in the blood will cause such problems as pancreatitis, high blood pressure, kidney stones. Severe hypercalcemia impairs neuromuscular and myocardial depolarization, leading to muscle weakness and arrhythmias. Also, the increased concentration of calcium in the blood leads to a decrease in the volume of erythrocytes, which in turn causes anemia.

Another adverse event associated with hypercalcemia is the fact that red blood cells actively promote blood coagulation and thrombus formation, which is initiated by calcium [5,8]. On the other hand, it should be noted that the low content of calcium ions (hypocalcemia) is also a great danger for the body. It should be noted that calcium levels naturally decrease with age.It is known that approximately 200 million people in the world have osteoporosis, and according to all available forecasts, this number will increase even further as the life expectancy on earth continues [7,9].This is also evidenced by the annual increase in the number of fractures (mainly in the elderly) due to osteoporosis, which reaches 8.9 million per year [1].

The essence of placing calcium ions in nanoparticles lies in the fact that, unlike the use of drugs in their pure form, the incorporation of the drug into the transport system will increase the amount of the drug that will be delivered to the destination, which in turn will increase the effectiveness of the action and, accordingly, reduce the dose of the drug.For this purpose, it is necessary to select the appropriate chemical composition and structure of the drug-carrying nanoparticles during the preparation of the complex nanoparticle.It is clear that in this case the drug-carrying nanoparticles must be made of non-toxic molecules for the living organism, because when the nanoparticles enter the cell, the enzymes of the cell must be able to break down the nanoparticles[6].In order for the advantages of nanoparticles to be fully used, the body's immune sensitivity to nanoparticles should be minimal.For this purpose, scientists have created different types of bionanotransporters, such as PLGA nanoparticles, dendrimers.It is important that these nanoparticles can interact with the membranes and have the ability to enter the ions placed in its structure through the membrane into the cytoplasm[3].

Poly (lactic-co-glycolic acid) PLGA nanoparticles are used in our research. PLGA is one of the most common biodegradable polymers[14].It is a polymer consisting of two different monomers (lactate and glycolate), which during hydrolysis in the body breaks down into its component monomers, which are endogenous to the body, therefore causing minimal toxicity[13].
We have taken it as calcium salts:
1. Calcium carbonate ($CaCO_3$), which is widely used in gene therapies [15,16];

2. Calcium gluconate ($C_{12}H_{22}CaO_{14}$), used to manage hypocalcemia, cardiac arrest, and cardiotoxicity[17]
3. Calcium fluoride (CaF_2), which is used as an anti-caries agent, and increasing the fluoride concentration in the fluid enhances the remineralization process [18].

Experimental part

Nanoparticles can be used as drug-carrying nanosized particles in living systems if their phase transition temperature is in the order of the physiological temperature of a living organism [1]. That is why it is important to determine the effect of temperature on the structure of PLGA nanoparticles. This can be determined using the calorimetric method.

Experiments were performed on pure and calcium-containing PLGA nanoparticles.

First, pure PLGA nanoparticles prepared in water were studied, the calorimetric curve of which is shown in Figure 1.

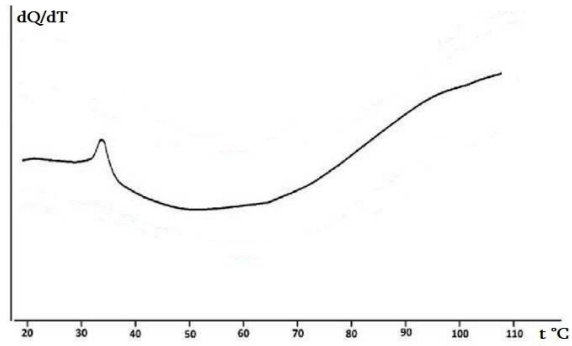


Figure 1. Dependence of heat capacity to temperature for 1 mg pure PLGA nanoparticles. Heating speed is 2^0C/min.

According to calorimetric experiments, we calculated the amount of heat absorption, which is Q=7.5 * 10^{-3} J.

After that, the experiment was performed on PLGA nanoparticles containing $CaCO_3$, at two different concentrations of $CaCO_3$ - 0.008 mg $CaCO_3$ and 0.08 mg $CaCO_3$. (see Figure 2).

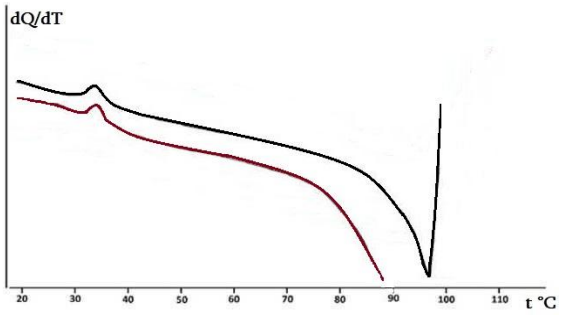


Figure 2. Dependence of heat capacity to temperature for:
a) 1 mg PLGA + 0.008 mg $CaCO_3$ (black)
b) 1 mg PLGA + 0.08 mg $CaCO_3$ (red)
The heating speed is 2^0C/min.

The amount of heat absorption for 1 mg PLGA containing 0.008 mg $CaCO_3$ is Q=1.3 * 10-3 J, and in the case of 1 mg PLGA containing 0.08 mg $CaCO_3$ is Q=0.45 * 10-3 J.

After that, two different amounts of calcium gluconate, 0.03 mg and 0.3 mg, were taken as calcium salt and complex PLGA nanoparticles containing calcium gluconate were prepared.Figure 3 shows the obtained calorimetric curves.

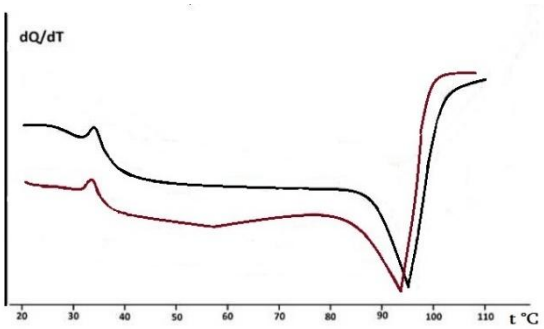


Figure 2. Dependence of heat capacity to temperature for:
a) 1 mg PLGA + 0.03mg $C_{12}H_{22}CaO_{14}$(black)
b) 1 mg PLGA + 0.3mg $C_{12}H_{22}CaO_{14}$(red)
The heating speed is 2^0C/min.

The amount of heat absorption for 1 mg PLGA containing + 0.03mg $C_{12}H_{22}CaO_{14}$ is Q=1.6 * 10-3 J, and in the case of 1 mg PLGA containing + 0.3mg $C_{12}H_{22}CaO_{14}$is Q=0.5 * 10-3 J.

Experiments were also performed on calcium fluoride, two different amounts of CaF2 0.05 mg and 0.5 mg were taken and complex PLGA nanoparticles were prepared, whose calorimetric records are shown in Figure 4.

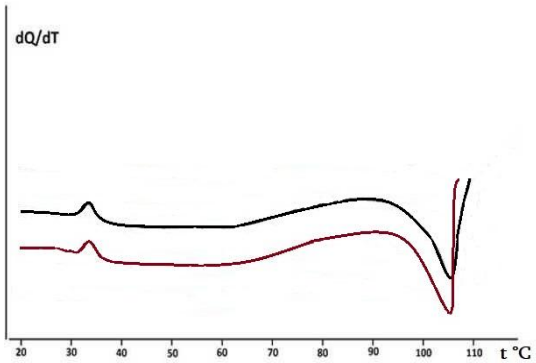


Figure 2. Dependence of heat capacity to temperature for:
a) 1 mg PLGA + 0.05 mg CaF_2 (black)
b) 1 mg PLGA + 0.5 mg CaF_2 (red)
The heating speed is 2^0C/min.

The amount of heat absorption for 1 mg PLGA containing + 0.05mg CaF_2 is Q=0.9 * 10-3 J, and in the case of 1 mg PLGA containing + 0.5mg CaF_2 is Q=0.47 * 10-3 J.

II. DISCUSSION

Knowledge of the thermostability of nanoparticles as drug-carrying nanosized particles is necessary to determine the feasibility of their use in living systems.Nanoparticles whose phase transition temperature is of the same order as the temperature of a living system can be used as drug carriers.

The phase transition temperature of the PLGA nanoparticles used by us is of the order of the temperature of a living organism, which allows us to use them as drug carriers.From our studies, it can be seen that the phase transition temperature of PLGA nanoparticles is close to 34 0C, and the amount of heat released at this time is Q=7.5 * 10-3J.From our studies, it can be seen that the phase transition temperature of PLGA nanoparticles is close to 34 ^{0}C, and the amount of heat released at this time is Q=7.5 * 10-3J.

In particular, the hydrogen atom of the hydroxyl group of one PLGA acts as a hydrogen bond donor, and the oxygen atom of the carboxyl group of the other PLGA acts as a hydrogen bond acceptor.The calorimetric curve consists of 1 cooperative peak, which allows us to conclude that the obtained nanoparticles are single-layer, unilamellar.

After incorporating calcium salts into PLGA nanoparticles, the phase transition temperature and unilamellar structure of complex nanoparticles do not change.However, there is a change in the bonds between neighboring PLGA polymers, namely, after calcium is attached to PLGA, it establishes a bond between 2 neighboring lactic acids and a neutralization reaction occurs, which causes the release of a water molecule and the establishment of ionic bonds between the carboxyl group of one lactic acid and the hydroxyl group of the other lactic acid.

In particular, hydroxyl and carboxyl groups have a negative charge and they easily interact with the positively charged calcium ion.After the calcium ion binds to the glycolic acid, a neutralization reaction takes place, the calcium ion displaces the hydrogen ions and releases water and carbon dioxide.

As a result, the amount of heat released during the phase transition in the case of complex nanoparticles is much smaller compared to pure PLGA nanoparticles.Due to this change, the thermal stability and heat decrease in proportion to the increase in calcium concentration.As the concentration of calcium salt increases, the more calcium competes with the hydrogen bonds between PLGA polymers, and therefore, with the increase in the concentration of calcium salts, the amount of heat released during melting of complex nanoparticles decreases.

III. CONCLUSION

When studying complexes containing calcium salts in PLGA drug carrier nanoparticles, we obtained the following results.The calcium salts studied in the paper (calcium carbonate, calcium gluconate, calcium fluoride) were able to incorporate into PLGA nanoparticles, as indicated by changes in the heat absorption peak compared to pure PLGA nanoparticles.The use of PLGA nanoparticles as a calcium carrier is also confirmed by its thermal stability upon addition of salts, as indicated by the fact that the phase transition temperature does not change.An increase in the concentration of calcium salts proportionally decreases the heat absorption, which indicates the ability of PLGA nanoparticles in the studied concentration range to capture the increased amount of salt.Therefore, the calorimetric experiment showed us that PLGA nanoparticles can be used as an efficient transport system for calcium salts.

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