UDC 541.64+678

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# Synthesis and characterization of polymeric nanoparticles based on albumin

The influence of different factors (pH of medium, nature and quantity of solvent, etc.) on physicochemical characteristics of polymeric albumin nanoparticles has been investigated in this article. Using optimal conditions which have been found out empty albumin nanoparticles with satisfactory physicochemical characteristics (particle diameter in the range of 50–100 nm, polydispersity 0.130–0.180 and ζ-potential –11,3–31,0 mV) have been obtained. Synthesized nanoparticles are promising as drug delivery systems.

*Key words:* albumin, nanoparticles, glutaraldehyde, desolvating agent, immobilization, desolvation, coagulation, polydipersity, particle size.

Prospects of using polymeric nanoparticles as drug delivery systems for the drugs which are used in the therapy of tumors and tuberculosis has been proved by the results of numerous investigations [1, 2]. Human serum albumin (HSA) is one of the most frequently used biopolymers in medicine. HSA is known to be used for treating shock, burns, hypoalbuminemia, after surgery trauma, cardiopulmonary bypass, acute respiratory distress and hemodialysis [3–5]. Albumin-conjugates are also used in treatment of arthritic diseases, for liver targeting, and others [3–5]. Albumin is known to be accumulated in malignant and inflamed tissues and nanoparticles made of HSA were found to be non-toxic and well-tolerated by human organism [3–5]. Owing to its capability to transport low molecular weight compounds it is a unique carrier for drugs. Binding drugs with albumin provided prolonged effect of such proteins and peptides as Albuferon and Levemir [5]. Functional groups (carboxylic and amine groups) which are present in the structure of albumin allow to modify the surface of the particles by attaching the molecules for targeting purposes [5–7]. For these reasons HSA is a potential drug carrier.

In [6, 7] HSA nanoparticles have been obtained by W/O emulsion polymerization, desolvation, coacervation or by protein denaturation [6, 7]. One of the most effective methods of synthesizing nanoparticles is obtaining them in emulsion. However this method has some disadvantages that limit its use, such as the removal of stabilizers and the surrounding oil phase from the system after the process [7]. Kreuter J., Langer K. et al. (Goethe University) suggested the desolvation as an alternative method for the preparation of the nanoparticles [7]. In this method HSA is dissolved in water and desolvated with ethanol and stabilized by crosslinking with glutaraldehyde.

The aim of the present work was to study the influence of various factors on physicochemical parameters of polymeric nanoparticles of HSA and selection of optimum conditions of obtaining nanoparticles with satisfactory characteristics.

## *Experimental part*

10 % solution of HSA (Blood Center), 50 % solution of glutaraldehyde and phosphate buffer 7.4 (Sigma Aldrich, Germany) were used in this work.

*Synthesis of empty human serum albumin nanoparticles.* Empty HSA nanoparticles were obtained by desolvation method. pH of 2 % albumin solution was adjusted to 8.0–8.5 using buffer solutions and then calculated amount of ethanol and glutaraldehyde was added at a constant stirring. The suspension was stirred for 24 hours. The particles of needed sizes were separated from low- and high molecular compounds by repeated centrifugation (Mini Spin plus-14500 eppendorf, Germany). Obtained particles were washed with water and dried to constant weight.

*Determination of particle size, polydispersity and surface charge.* Particle size, polydispersity and surface charge of nanoparticles were measured by photon correlation spectroscopy (PCS) at Malvern Zetasizer Nano S90 (Malvern Instruments Ltd., Malvern, UK) and Malvern Zetasizer 3000HSA (Malvern Instruments Ltd., Malvern, UK) at a temperature of 25 ºC at a scattering angle of 90º.

Pictures of empty HSA nanoparticles and nanoparticles loaded with drug were made by transmission electron microscopy (transmission electron microscope CM 12 (Philips)).

The yields of nanoparticles were determined gravimetrically. Empty aluminum cups were weighed on a balance with accuracy $1 \cdot 10^{-4}$ mg and then each cup was filled with 0.05 ml of nanoparticles' solution and was evaporated in vacuum oven at a temperature 45 ºC. Cups were again weighed after evaporation of water.

*Results and Discussion*

Synthesis of empty HSA nanoparticles was done by desolvation method [5–7]. With the aim of obtaining particles of nanometric size with satisfactory characteristics a number of experiments have been done to find out optimal conditions. Initially the influence of pH of medium on particle size was studied. The results of the study are shown in Table 1.

Table 1

**Characteristics of nanoparticles depending on pH of medium**

| | pH of medium | | | | |
|---|---|---|---|---|---|
| | 2,0 | 4,0–4,5 | 7,0 | 8,0–8,5 | 11,0 |
| Portion of particles with the size below 1000 nm, % | – | – | 20 | 95–100 | – |
| Average particle diameter, *d*, nm | – | – | – | 80–100 | – |
| Polydispersity | 1,0 | 1,0 | 0,8 | 0,140–0,170 | 1,0 |

As it is seen from table 1 the particles of nanometric size were formed only at pH 8.0–8.5 which is most probably due to aldol condensation of glutaraldehyde which goes in alkaline medium at this meaning of pH.

Among the factors influencing on the formation of particles the nature of desolvating agent plays an important role. In connection with this a number of experiments directed to find out optimal solvent were conducted. Ethanol, dimethylsulfoxide (DMSO) and dimethylformamide (DMF) were used as solvents, thus there were chosen the solvents of which thermodynamic quality was better than ethanol. The rate of addition of the desolvating agent was controlled using minipump and kept constant (1 ml/min). The particles were separated from unreacted components by three-fold centrifugation and washing with water.

Physicochemical parameters of synthesized particles were measured by photon correlation spectroscopy of which the results are given in Table 2.

Table 2

**Physicochemical characteristics of empty HSA nanoparticles when using various desolvating agents**

| No | The volume of desolvating agent, ml | Average particle diameter, *d*, nm | Polydispersity | Portion of particles with the size below 1000 nm, % |
|---|---|---|---|---|
| | Ethanol | | | |
| 1 | 8 | 54,76 | 0,095 | 100 |
| | DMF | | | |
| 1 | 8 | 6210 | 0,908 | - |
| | DMSO | | | |
| 1 | 2 | 75,66 | 0,665 | 53,2 |
| 2 | 4 | 813,3 | 1,000 | 31,1 |
| 3 | 8 | 2379 | 1,000 | 53,1 |
| 4 | 10 | 590,6 | 0,800 | 53,0 |

The results of experiments when using DMF have shown the inapplicability of this solvent as desolvating agent as the particles of nanometric size were not formed (average size of obtained particles was 6210 nm with polydispersity 0.9) (Table 2).

According to the results with using DMSO when obtaining empty HSA nanoparticles varying the quantity of desolvating agent (from 2 to 10 ml) it has been found out that the portion of particles of nanometric size increases with decreasing the volume of DMSO added (Table 2). So when addition of 2 ml of DMSO the portion of formed particles of nanometric size was the highest and reached 53.2 %. However in spite of satisfactory meaning of average particle diameter the portion of particles with average size 51 nm was only 18 % (Fig. 1), which points on inapplicability of the sample for loading with drug.

| | | | Size (d.nm): | % Intensity | Width (d.nm): |
|---|---|---|---|---|---|
| Z-Average (d.nm): | 75,66 | Peak 1: | 2453 | 46,8 | 1420 |
| PdI: | 0,665 | Peak 2: | 1,325 | 35,3 | 0,6635 |
| Intercept: | 0,0625 | Peak 3: | 51,12 | 17,9 | 34,69 |
| Result quality : | Refer to quality report | | | | |

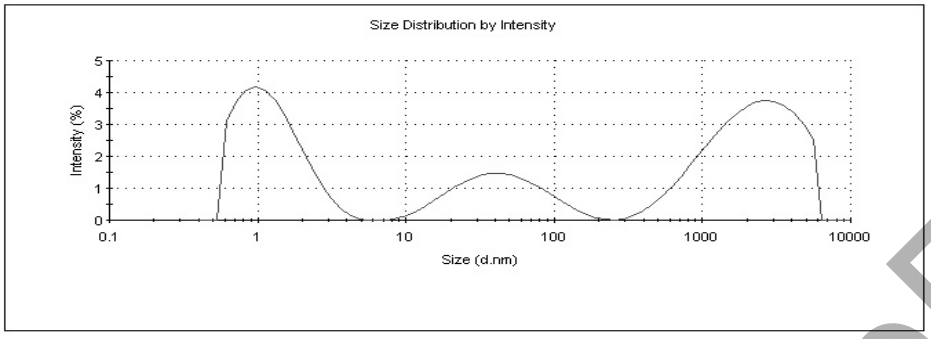



Figure 1. Particle size distribution when using DMSO (2 ml) as desolvating agent

| | | | Size (d.nm): | % Intensity | Width (d.nm): |
|---|---|---|---|---|---|
| Z-Average (d.nm): | 2379 | Peak 1: | 58,59 | 53,1 | 13,98 |
| PdI: | 1,000 | Peak 2: | 5453 | 46,9 | 263,3 |
| Intercept: | 0,168 | Peak 3: | 0,000 | 0,0 | 0,000 |
| Result quality : | Refer to quality report | | | | |

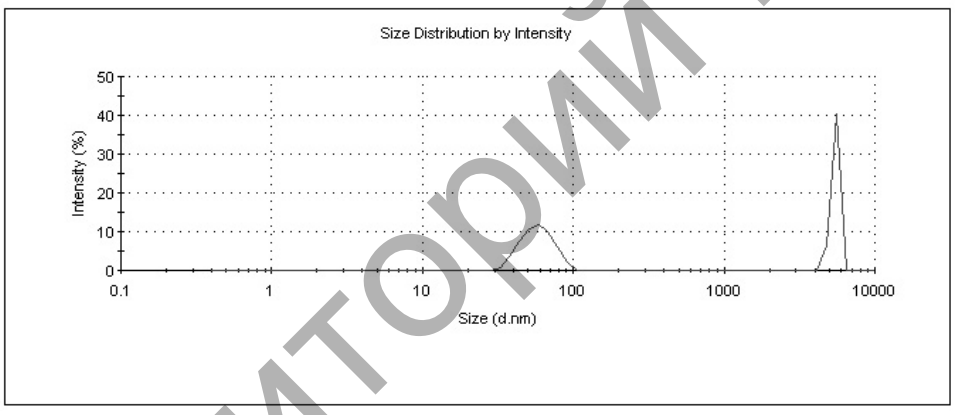



Figure 2. Particle size distribution when using DMSO (8 ml) as desolvating agent

| | | | Size (d.nm): | % Intensity | Width (d.nm): |
|---|---|---|---|---|---|
| Z-Average (d.nm): | 590,0 | Peak 1: | 570,5 | 100,0 | 80,94 |
| PdI: | 0,388 | Peak 2: | 0,000 | 0,0 | 0,000 |
| Intercept: | 0,859 | Peak 3: | 0,000 | 0,0 | 0,000 |
| Result quality : | Refer to quality report | | | | |

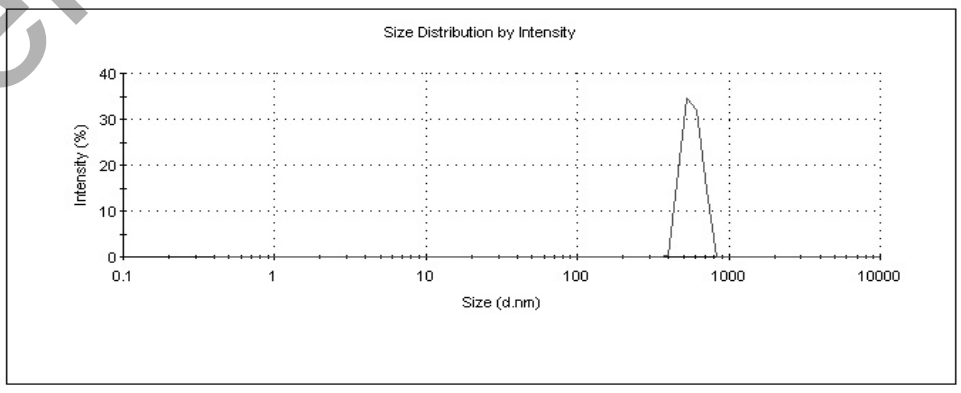



Figure 3. Particle size distribution when using DMSO (8 ml) as desolvating agent (after processing with US)

Then it was interesting to investigate the sample obtained when adding 8 ml of DMSO, as the content of particles of nanometric size (58.59 nm) in the system was more than 50 % (Fig. 2). Therefore these particles were separated from precipitation and the dispersion was processed by ultrasound (US) within 10 min. (with pauses for 20–30 sec.), then the particle size and polydisperisty were measured again. The results are shown in Figure 3.

As it is seen from Figure 3 the average particle diameter was 590.0 nm and polydisperisty 0.388 which points on growing of the particles and formation of the system with distribution close to monomodal one.

From Table 2 it is also seen that the use of ethanol of 8 ml for carrying out desolvation process gives good results: the particles with satisfactory characteristics have been synthesized. So using optimal conditions found out (pH 8.0–8.5, desolvating agent — ethanol, volume of ethanol 8 ml) empty HSA nanoparticles have been obtained.

It was judged about physico-chemical characteristics on the next parameters: particle size (*d*, nm), polydispersity (*P*) and zetapotential (mV). The results of two independent particle samples are summarized in Table 3.

Table 3

**Physicochemical characteristics of HSA nanoparticles**

| Parameter | Sample A | | | Average meaning | Sample B | | | Average meaning |
|---|---|---|---|---|---|---|---|---|
| | № 1 | № 2 | № 3 | | № 1 | № 2 | № 3 | |
| Particle size, nm | 222,9 | 164,2 | 160,6 | 182,6 | 85,5 | 88,3 | 86,7 | 86,8 |
| Plydispersity | 0,154 | 0,043 | 0,033 | 0,077 | 0,191 | 0,184 | 0,176 | 0,184 |
| ζ-potential, mV | –11,3 | –31,2 | –24,2 | –22,2 | –30,4 | –28,5 | –23,8 | –27,6 |
| Particle yield, % | 40,4 | | | | 39,2 | | | |

Particle size distribution of the 3rd pattern of sample B is shown in Figure 4.

From Figure 4 it can be seen that the system 100 % consists of the particles of nanometric size of which average diameter was 86.68 nm and polydispersity was equal to 0.176.

When obtaining polymeric nanoparticles the study of aggregative and sedimentative stability of the system is of great importance. The surface charge of particles is quantitatively characterized by the value of ζ-potential and it determines the possibility and the rate of movement of disperse phase towards disperse medium. ζ-potential of colloidal system changes essentially when changing pH of medium. It is caused by the fact that proton (hydrogen-ion) and hydroxyl-ions possess high capability to be adsorbed: protons — owing to their small radius which allow them to be close to the surface of hard phase; and hydroxyl-ions are adsorbed because of their high value of dipol moment. In the acidic medium ζ-potential has positive surface charge and in a basic solution its charge will have negative meaning. As it is known the meaning of ζ-potential has to be close to –30 mV or +30 mV, as the system with the surface charge close to zero is prone to coagulation.

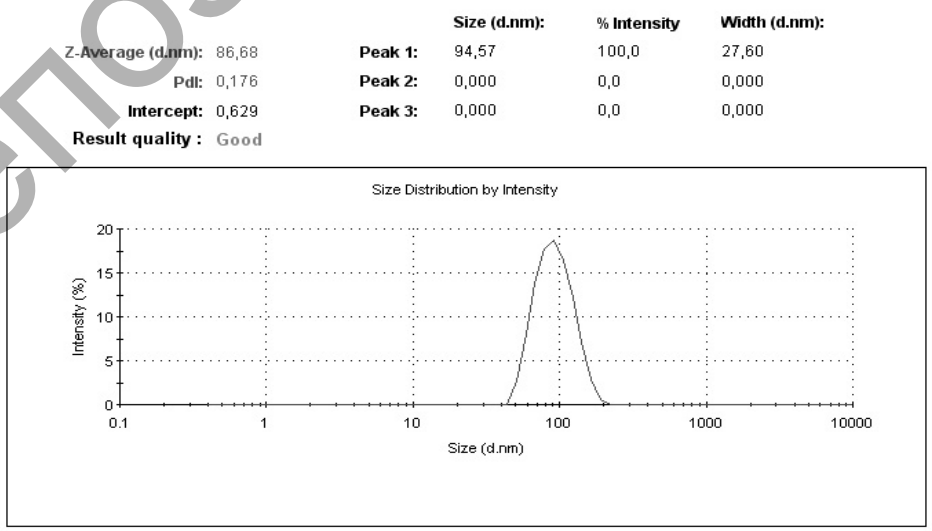



Figure 4. Particle size distribution of HSA nanoparticles when using ethanol as desolvating agent

The meaning of ζ-potential of the 1st pattern of sample A (–11.3 mV) indicates on coagulative origination of the sample (Table 3). Coagulation of the particles might have taken place during cleaning nanoparticles. In spite of that fact that this sample test was excluded from further investigations, it seemed interesting for us to observe the changes of parameters of this system when undergoing to the US. With this aim pattern 1 was submerged into the ultrasonication bath. After 5 minutes of ultrasonication the diameter of particles decreased slightly and polydispersity remained at the same meaning ($d$ = 212.4 nm, $P$ = 0.153). This state of the system is observed in Fig. 5*b*), from which it is seen that distribution of particle size is still bimodal. Processing of pattern with US within 10 minutes allowed decreasing the average particle size to 200.7 nm. And the polydispersity degree also decreased ($P$ = 0,141) which is in correspondence with monomodal curve of particle distribution (Fig. 5*c*).

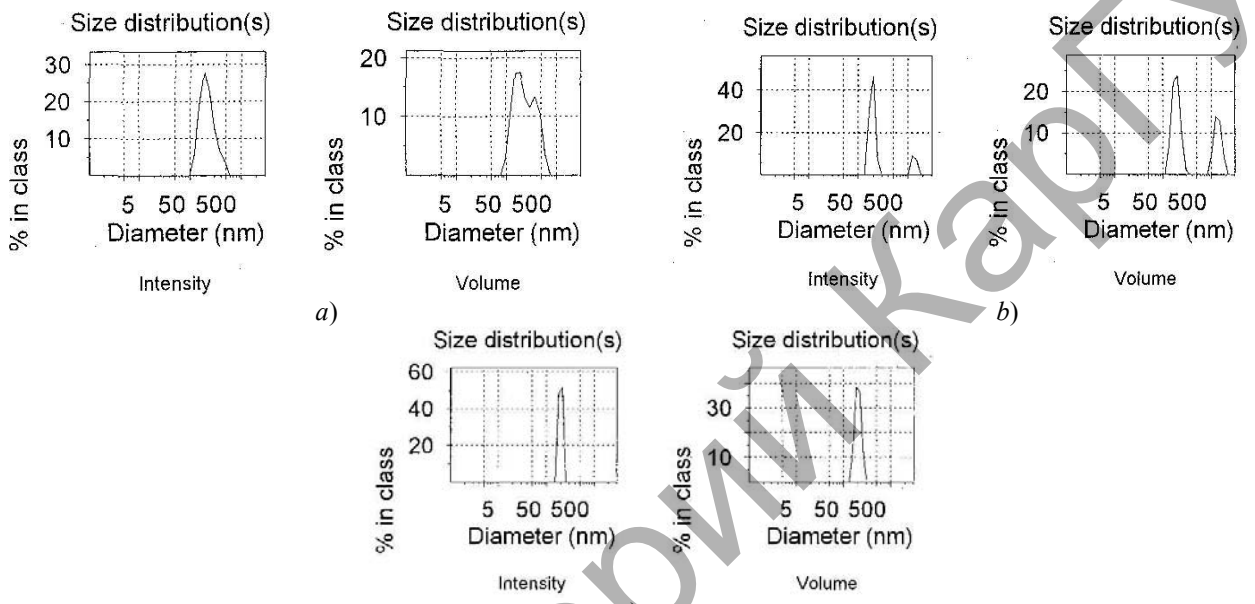
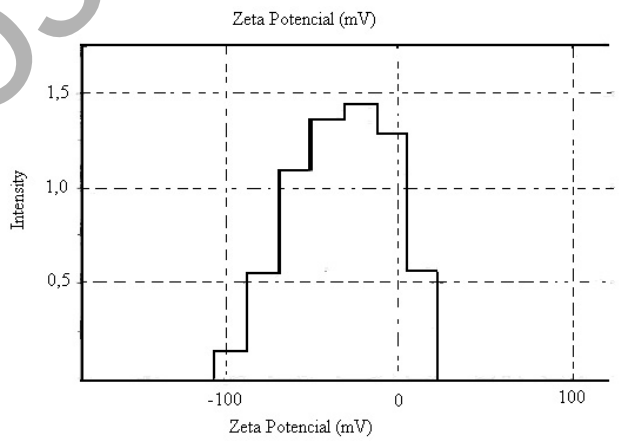



Figure 5. The differential intensity distribution curves of serum albumin particles according to their size

The sample B was chosen from the obtained samples for further investigations as a polymer carrier, because it has less diameter of particles (86.8 nm) and low meaning of polydispersity (0,184). It should be noted that sample B has very low negative meaning of average surface charge (–27.6 mV) (Fig. 6) which indicates on stability of colloidal system in time.




Result

| | |
|---|---|
| Zeta Potential (mV): –31.2 | Mobility (umcm/V.s): –2.447 |
| StDev(mV): 6.5 | StDev (umcm/V.s): 0.508 |
| Conductivity (mS/cm): 0.00 | F(ka): 1.50 |

Figure 6. Electrical charge distribution on the surface of HSA nanoparticles

In addition it has been found out that the reverse order of addition of desolvating agent (ethanol) and glutaraldehyde does not lead to the formation of the particles of nanometric size.

Electron microscopic pictures of prepared samples testify spherical shape of nanoparticles, narrow interval of particle size distribution and of absence of particle aggregation (Fig.7).

Pictures show that the particle size ranged between 80–120 nm.

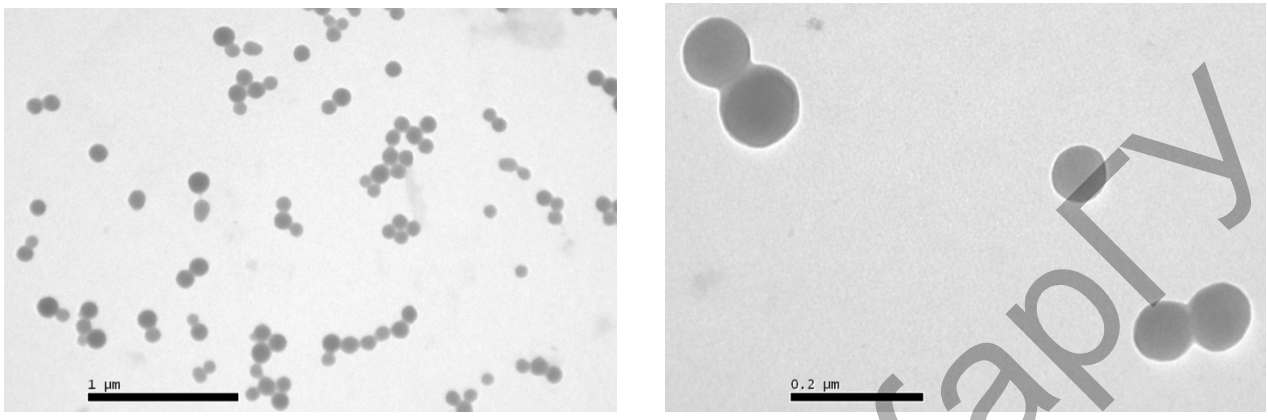



Figure 7. Electron microscopy pictures of empty HSA nanoparticles

Thus, there was obtained HSA nanoparticles with the satisfactory physico-chemical characteristics which allow using them as polymer carriers for drugs.

Obtained nanoparticles were separated from dispersion by repeated centrifugation and washing with water. Then the particles were dispersed into water using US on ULTRASONIC Cleaner LAUNCH within 10 min (20–30 sec. pause). In this case it was not possible to disperse all the particles into water, therefore the precipitate was filtered and weighed. And then dispersing degree has been calculated using obtained data which was equal to 46.74 %.

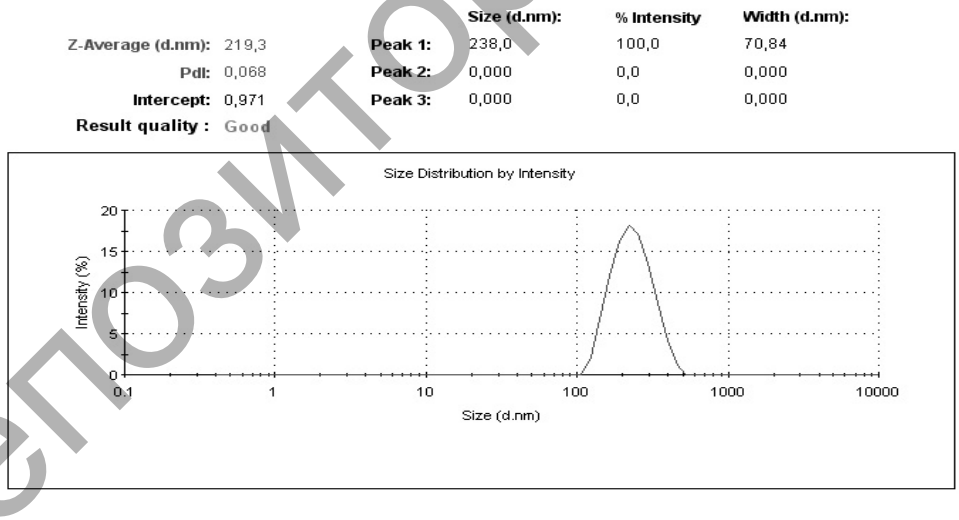



Figure 8. Particle size distribution of empty HSA nanoparticles dispersed into water

Then the particle size of nanoparticles dispersed in water has been measured. Average particle diameter was 152.6 nm and polydispersity meaning was 0.244. And the portion of the particles with the size below 1 µm was 96.8 %. With the aim of increasing dispersing degree the processing with US was repeated and second-time dispersing degree has been determined (Table 4).

As it can be seen from table 4 after 2$^{nd}$ time dispersing of empty nanoparticles into water almost 90 % dispersing was achieved. Particle size of nanoparticles dispersed into water 2$^{nd}$ time was 219.3 nm and polydispersity 0.068 (portion of nanoparticles was 100 %) (Fig. 8).

Table 4

**Characteristics of empty HSA nanoparticles**

| | Characteristics of nanoparticles | | | |
|---|---|---|---|---|
| | Average particle diameter, *d*, нм | Polydispersity | The yield of nanoparticles, % | Dispersing degree, % |
| Initially synthesized nanoparticles | 80,8 | 0,237 | 96,8 | – |
| Nanoparticles after 1st time dispersing | 152,6 | 0,244 | – | 46,7 |
| Nanoparticles after 2nd time dispersing | 219,3 | 0,068 | – | 88,4 |

So in this article optimal conditions of synthesizing polymeric HSA nanoparticles by desolvation method have been worked out and empty HSA nanoparticles with satisfactory physicochemical characteristics for further loading them with drugs have been obtained.

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## Альбумин негізіндегі полимерлі нанобөлшектердің синтезі және сипаттамасы


Мақалада альбуминнің полимерлі нанобөлшектерінің физика-химиялық сипаттамаларына әр түрлі факторлардың (pH ортаның, еріткіштің табиғаты мен мөлшерінің және т.б.) әсері зерттелген. Анықталған нанобөлшектердің қолайлы жағдайларын қолданып, қанағаттанарлық физика-химиялық сипаттамаларға ие (орташа өлшемі 50–100 нм, полидисперстілігі 0,130–0,180, ζ-потенциал –11,3–31,0 мВ) сарысулы альбуминнің бос нанобөлшектері алынды. Ол бөлшектер дәрілік заттарды тасымалдағыштар ретінде болашағы зор.



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## Синтез и характеристика полимерных наночастиц на основе альбумина


В статье изучено влияние различных факторов (pH среды, природа и количество растворителя и др.) на физико-химические характеристики полимерных наночастиц альбумина. Используя найденные оптимальные условия синтеза наночастиц, получены пустые наночастицы сывороточного альбумина с удовлетворительными физико-химическими характеристиками (со средним размером в пределах 50–100 нм, полидисперсностью 0,130–0,180, ζ-потенциалом –11,3–31,0 мВ). Синтезированные частицы перспективны в качестве носителей лекарственных веществ.

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