UDC 541.64+678

M.Zh. Burkeev[1], J. Kreuter[2], Ye.M. Tazhbayev[1], L.Zh. Zhaparova[1],
T.S. Zhumagalieva[1], A.T. Kazhmuratova[1], D.A. Muhanova[1]

[1]*Ye.A. Buketov Karaganda State University;*
[2]*Biocenter Niederursel, Johann Wolfgang Goethe University, Frankfurt/Main, Germany*
(E-mail: zhumagalieva79@mail.ru)

New forms of anticancer drugs «Arglabin» based on human serum albumin

In this article the possibility of creation of human serum albumin nanoparticles immobilized by anticancer drug Arglabin was considered. Physical and chemical charasterictics of nanoparticles and binding of Arglabin with polymeric nanoparticles were determined It is found that the particle size and binding of drug with polymer depend on the method of immobilization of drug.

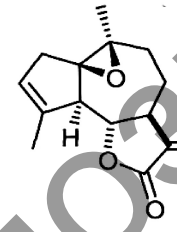
Key words: albumin, nanoparticles, immobilization, Arglabin, polydispersity, zetapotential.

1 Introduction

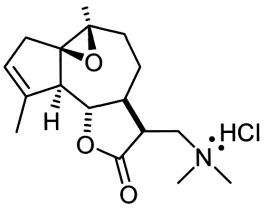
Current, the main disadvantages of drugs used in medicine are short period of validity, low selectivity to the affected organ and sufficiently high toxicity. By the results of numerous researches, it is shown that these disadvantages can be avoided by encapsulation of drugs in polymeric nanoparticles [1, 2]. Significant results were obtained by loading of pharmaceutical drugs in synthetic and natural polymers [3, 4], which are used in the treatment of diseases such as cancer, requires long-term therapy [5–7].

Using of nanosystems for drug delivery in cancer therapy allows to achieve significantly better effects than by using free drugs [3, 8–10].

One of the anti-cancer drugs is «Arglabin» obtained by Kazakhstan scientists from the plant *Artemisia glabella* growing in Central Asia [11]. The investigation of Arglabin and a number of its derivatives showed that the epoxyarglabin and hydrochloride of dimethylaminoarglabin possess the best antitumor activity. Epoxyarglabin (substance or native arglabin) hadn't found an application because of its insolubility in water. Further on the base of hydrochloride of dimethylaminoarglabin there was worked out a hydrophilic drug form preparation «Arglabin» for parenteral administration.



Arglabin



Hydrochloride of dimethylaminoarglabin

The preparation «Arglabin» is efficient in treatment of breast cancer, primary liver cancer, cancer of lungs and ovary and during several years it is used successfully in medicine practice for treating cancer diseases in the form of injection.

At the same time there is the task of prolongation of drug action and decrease its side effects.

One of the widely used polymeric carriers of drugs is human serum albumin. Because of its function to transport low-molecular substances including a variety of drug coming externally, it is unique drug carrier to target organs, which prolongs their action by binding [9, 10].

The task of present study was to consider the possibility of creation of new drug form of antitumor drug Arglabin in the form of nanoparticles on the base of HSA.

2. Materials and methods

2.1 Chemicals and reagents. Human serum albumin (HSA, fraction V, purity 96–99 %, 65.000 Da) and glutaraldehyde 8 % solution were obtained from Sigma (Steinheim, Germany). «Arglabin» (substance) and

preparation «Arglabin» were purchased from «Research-and-Production Center «Phytochemistry» (Karaganda, Kazakhstan). Solvents and all other reagents were purchased from Merck (Darmstadt, Germany).

2.2 Preparation of empty HSA nanoparticles. Empty HSA nanoparticles were prepared using a previously described desolvation method [2, 9]. By using of buffer solution, pH 2 % of albumin solution was adjusted to 8.0–8.5, then the calculated amount of ethanol and an aqueous solution of glutaraldehyde as crosslinking agent added under stirring. To complete the process, the suspension was left for 24 hours with constant stirring. The required particle on the size was separated from low- and high-molecular components by centrifugation and repeated washing with water.

2.3 Encapsulation of the drug Arglabin in nanoparticles of HSA. For immobilization of Arglabin in nanoparticles, the drug previously dissolved in an aqueous solution of albumin and desolvation was carried out. In this case two forms of the drug (lipophilic and hydrophilic forms) were used. The concentration of the drug was varied from 0.25 mg/ml to 1 mg/mL, using the following ratios of drug: albumin, 1:20, 1:10, 3:20, 1:5.

To 20 mg of serum albumin was added from 1 to 4.0 mg of drug in 4 tubes, respectively, and 1 ml water to each of them. pH of all solutions was adjusted up to 8,5 and held for 2 hours at constant stirring. In the method of incorporation water-insoluble lipophilic form of Arglabin was used. 1 ml of ethanol was added to the tubes previously. For the formation of nanoparticles 2 ml of ethanol and 8 % glutaraldehyde solution was added to the solution under constant stirring by minipump and stirred for 24 h.

The required particle by size is separated by repeated centrifugation within 15 minutes at 14,500 rpm/min and was purified by washing by water for injection. The amount of free drug in a solution was analyzed by UV spectrophotometry ($\lambda = 204$ nm).

2.4 Adsorption of arglabin to HSA nanoparticles. 1.3 ml of empty HSA nanoparticles solution was taken (10 mg/ml) which were obtained by the method described above and from 50 to 800 μl of previously prepared solutions of arglabin (5.9 mg/ml) (substanive) were added. The volume of each probe was adjusted with ethanol to 2.21 ml. The mixture was stirred for 2 h (650 rpm) at room temperature. After that the particles were centrifugated and washed with water. The supernatants of the washing steps were collected and separated by size exclusion chromatography (Sephadex, Sigma Chemical Co. DE-52) and the concentration of free Arglabin in a solution was analyzed by UV-spectrophotometry ($\lambda = 204$ nm).

The yield of nanoparticles was determined by gravimetric method.

2.5 Measurement of particle size and zetapotential. The average particle size measured by photon correlation spectroscopy (PCS) using a Malvern Zetasizer 3000HSA (Malvern Instruments Ltd., Malvern, UK) at a temperature of 25 ºC at a scattering angle of 90º. The samples were diluted 1:400 with water. The zetapotential was also measured on this device. HSA nanoparticles were previously diluted 1:200 50mM phosphate-puffer pH 7.4 and the zetapotential was determined by Laser Doppler micro electrophoresis.

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

2.6 Determination of the Arglabin binding with HSA nanoparticles. The binding was determined by conductometric and UV-spectrophotometric methods on the instrument U3000 Spectrophotometer ($\lambda = 204$ nm) (Hitachi). Samples of the solutions were passed through Sephadex column (Sigma Chemical Co. DE-52) to separate the polymer from drug and washed with ethanol:water in the ratio of 1:5.7. From the obtained data a calibration chart and content of unbound drug was calculated.

Preliminary in a volumetric flask of 100 ml a drug solution with concentration of 100 mg/ml in water was prepared. Aliquots were taken from the prepared solution, was transferred into volumetric flasks of 25 ml. A series of solution with concentration of drug 5.0; 10.0; 20.0; 40.0; 60.0; 80.0; 100.0 mg/ml were prepared and electrical conductivity was fixed.

Further calibration graph constructed and the concentration of free drug was determined by it. Because the conductivity of solution is an additive value, the electrical conductivity of components of system was determined and relevant corrections were made. The degree of binding of drug was calculated from the difference between the initial and free drug.

The conductivity of the solution was measured in a thermostated cell (at 293 K), 25 ml on the instrument Conductivity meter Type OK-102 (Hungary) № 1182 and «Econics-Expert», INN / KPP 7728209000/772801001 (Moscow), provided with an electrode.

The yield of particles was found by gravimetric analysis.

10 % solution of human serum albumin (HSA) (HSA, fraction V, 96–99 %, 65,000, Sigma Aldrich (Steinheim, Germany) for the immobilization of an antitumor drug «Arglabin», and 50 % solution of

glutaraldehyde Sigma Aldrich (Steinheim, Germany) were used in the work. «Arglabin» (substance) and hydrochloride dimethylaminoarglabin was provided by the international scientific and industrial holding «Phytochemistry» (Karaganda, Kazakhstan).

With the aim of achieving an accuracy and verification of obtained results, our experimental data were studied by mathematical processing. Experimental miscount in measurements shortchanged by the method of «the least squares». It is shown by the calculations that the maximum relative miscount for the experimental data was 5 %, which was determined by specific methods of conductivity and spectrophotometry.

Typically, the physico-chemical and analytical measurements are taken a = 0,95 and b = 0,99. In this work, confidential probability is equal to 0.95.

3 Results and discussion

Nowadays three main ways of immobilization of drug to the polymer nanocarriers are known [12]. These are covalent attachment of the drug to the particle prior to preparation of particles or analogical attachment to the particle surface; adsorption of drug on the surface of preliminary prepared nanoparticles and incorporation of drug into polymer matrix during particle preparation. The first method is not widely used because the covalent attachment of drug to the polymer changes its chemical structure (entity) and may lead to changes of biological activity of drug.

In the present study it was compared two ways of loading Arglabin to the particle system: adsorption of drug on the surface of obtained HSA nanoparticles and incorporation of Arglabin into polymer matrix of nanoparticles with the use of desolvation method described above.

At the time of investigations we studied the immobilization of antitumor drug Arglabin into the matrix of serum albumin by incorporation method which involves injection of biologically active compounds directly in the reaction medium during the process of crosslinking of albumin.

Incorporation was carried out using of Arglabin substance and dimethylaminoarglabin hydrochloride. In both cases, concentration of drug is maintained from 0.25 mg/ml to 1 mg/ml.

The particle size, polydispersity and zetapotential of lipophilic Arglabin obtained by the incorporation method analyzed by photon correlation spectroscopy. The results are shown in Table 1.

Table 1

Characteristics of nanoparticles of HSA containing the substance Arglabin (T=25 ºC; C_{HSA}=20 mg/ml)

C_{ARGL}, mg/ml	C_{ARGL}, 10^{-5}, mol/l	drug/ polymer	*d*, nm	PDI	ζ-potential, mV	Binding, %	The yield of nanoparticles, %		Drug in NP (after adsorption), %
							Spectrophotometry	Gravimetry	
0.25	0.15	1:20	193.9 ±1.3	0.030 ±0.020	–16.3±4.0	76	88.3	82.3	3.36
0.5	0.3	1:10	256.6 ±3.0	0.074 ±0.004	–22.6±3.0	87	87.0	80.7	4.77
0.75	0.45	3:20	189.0 ±0.6	0.017 ±0.012	–26.3±2.5	73	86.5	78.8	6.57
1.0	0.6	1:5	126.2 ±1.7	0.046 ±0.018	–25.8±4.5	81	84.0	74.5	8.09

As can be seen from the data obtained particles vary in size and have a monomodal distribution. In all cases, the dispersed system is stable, as indicated by low values of ζ-potential of nanoparticles; the yield of nanoparticles obtained by two independent methods correlated.

Binding of Arglabin with nanoparticles of albumin was determined by UV-spectrophotometry at a wavelength λ = 204 nm. Before conducting the spectrophotometric analysis of particles with drug substance solutions were washed with water-ethanol and passed through a Sephadex column. Figure 1 shows the binding of nanoparticles of Arglabin from the amount of drug.

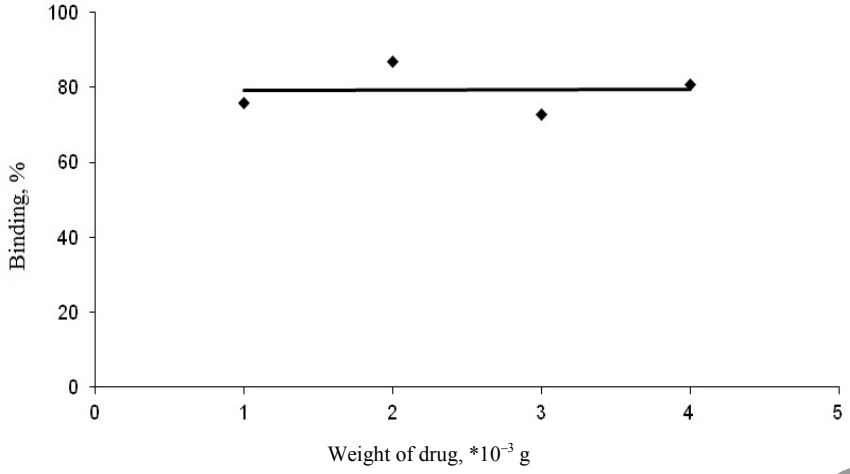


Figure 1. Dependence of binding of HSA nanoparticles from the amount of substance Arglabin

According to the presented data in the Table. 1, the effective incorporation of drug in HAS nanoparticles occured.

As it is shown in the picture, that in the result of encapsulation the lipophilic forms of Arglabin in the albumin nanoparticles the binding is always high and constitutes more than 73 % in all cases. In the proportions of drug to albumin 1:10 and 1: 5 nanoparticles immobilized by drug were obtained. The binding degree of these particles is up to 80 %, which proves the effectiveness of incorporation.

As long as the drug was included in the polymer matrix during the process of obtaining the particles, it is important to know the content of drug in the nanoparticles after incorporation. Therefore, the content of Arglabin in the obtained nanoparticles was calculated (Fig. 2).

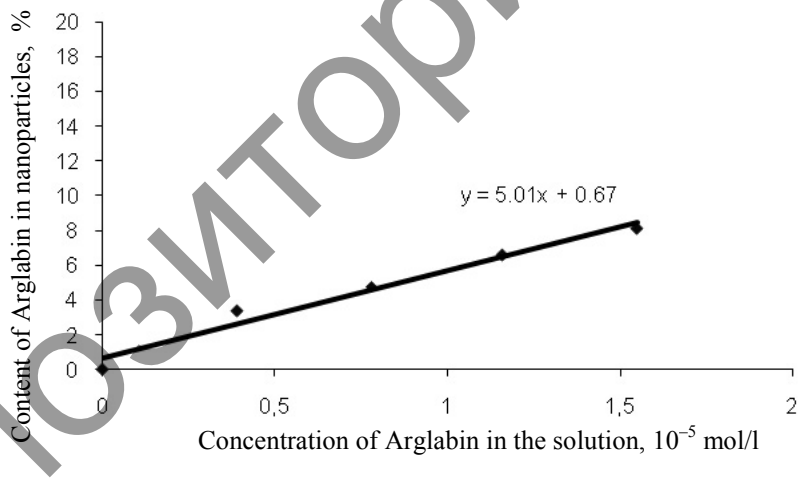


Figure 2. Content of lipophilic Arglabin in albumin nanoparticles after incorporation

From the graph, it is clear that the content of lipophilic Arglabin in formed nanoparticles increases by the growth of drug concentration in the initial solution. Thus, there is a possibility of further loading of serum albumin by lipophilic form of drug.

Our next task was the immobilization of nanoparticles by hydrophilic form of drug. Incorporation of dimethylaminoarglabin hydrochloride in the matrix of serum albumin was carried out by the same procedure as the lipophilic form of drug, but without adding ethanol. The concentration of drug was varied from 0.25 mg/ml to 1.0 mg/ml. Physico-chemical characteristics of particles also were analyzed by photon correlation spectroscopy (Table 2).

Table 2

Characteristics of nanoparticles HSA containing the dimethylamino hydrochloride arglabin (T=25 ºC; C_{HSA}=20 mg/ml)

C_{ARGL}, mg/ml	d, nm	PDI	ζ -potential, mV	Binding, %	The yield of nanoparticles, %	
					Spectrophotometry	Gravimetry
0.25	172±1	0.045±0.04	–23.1±7.0	51	59.3	63.1
0.5	200±2	0.029±0.02	–30.0±2.4	53	59.2	65.7
0.75	331±4	0.210±0.03	–39.2±2.2	67	68.5	92.7
1.0	315±1	0.138±0.01	–37.9±3.5	78	80.0	79.9

It can be seen from the Table 2, that the diameter of particles rises with increasing of drug concentration in nanoparticles of serum albumin, which is apparently caused by an increase of the content of arglabin in particles. By the value of ζ-potential we can see that polydispersity of particles is sufficiently low and the system is stable. The dependence of the degree of binding by weight of dimethylaminoarglabin is likely connected with an increase of the probability of binding with carboxyl-terminated of serum albumin. The binding degree of drug is 78 % at the maximum concentration, while the possibility of further loading of nanoparticles with Arglabin is not excluded.

Dependence of binding of HSA nanoparticles from the amount of hydrophilic form Arglabin — dimethylaminoarglabin hydrochloride is shown in Figure 3.

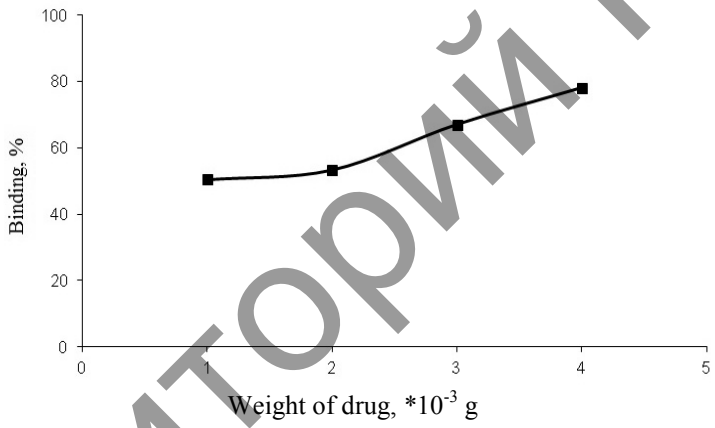


Figure 3. Dependence of binding of HSA nanoparticles from the amount of dimethylamino hydrochloride arglabin

The content of Arglabin in the obtained particles was also calculated at the end of process and the graph of dependence of drug concentration is shown in Figure 4.

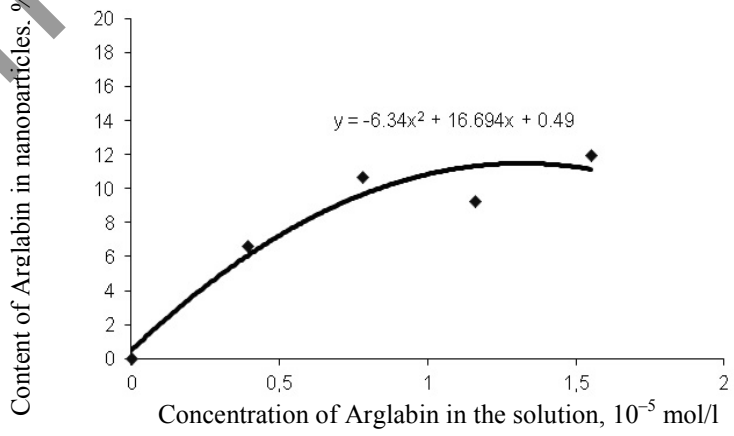


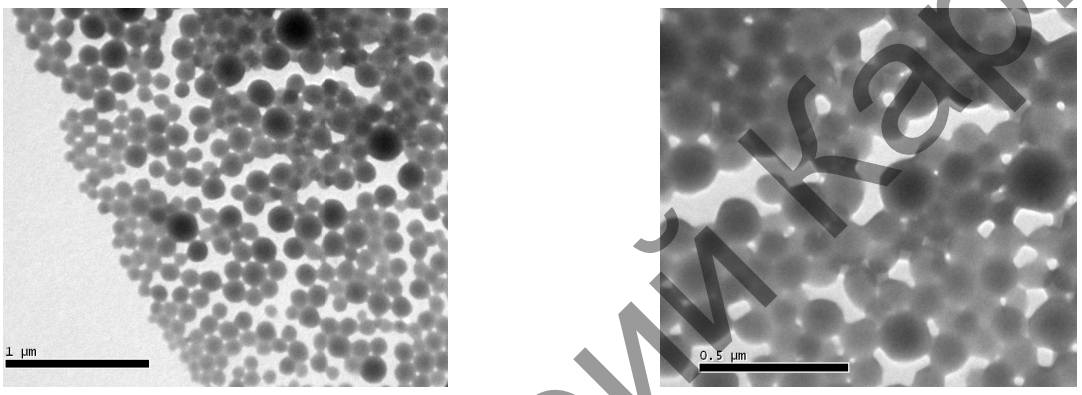
Figure 4. Content of hydrophilic Arglabin in albumin nanoparticles after incorporation

From the figure it is clear that in the content 11.97 % of dimethylaminoarglabin hydrochloride in particles we can observe the saturation in nanoparticles with drug.

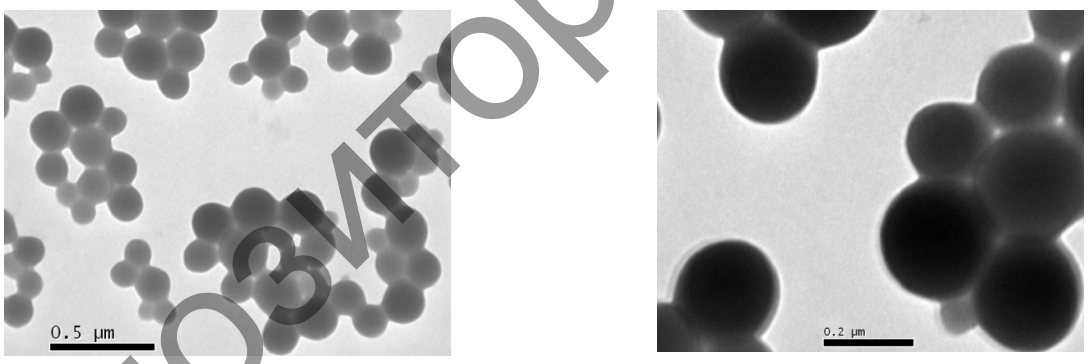
In order to confirm the obtained data by photon correlation spectroscopy, we made images of nanoparticles with drugs using transmission electron microscope (Fig. 5*a*, *b*). From the images it is seen that the size of nanoparticles with hydrochloride dimethylaminoarglabin is higher than size of nanoparticles with native form of Arglabin. The results of transmission electron microscopy correlate with obtained data by photon correlation spectroscopy.

From the above figures it is shown that obtained particles are sufficiently small and their size is 80–200 nm, which satisfies the requirements of polymer particles for drug delivery transport [13].

It is currently believed that the diameter of the nanoparticles for the treatment of cancer is should be of 10–100 nm. The lower limit was calculated by calculating of filtering ratio of walls of capillary, because the limit particle size for renal excretion is assumed to be about 10 nm in diameter. The upper limit was not determined accurately, however, it was proved that the particles with diameter of hundreds nanometers are also capable to penetrate through walls of blood vessels and assimilate in the tumor.



a) HSA nanoparticles containing arglabin substance



b) HSA nanoparticles containing dimethylaminoarglabin hydrochloride

Figure 5. HSA nanoparticles

In comparison of obtained results, earlier published material is presented in this article [13]. It is known that adsorption of drug on the preliminary prepared carrier systems bears the risk of drug loss by desorption process. To prevent the drug inactivation during storage as well as from early degradation of the complex polymer-drug after injection [12], it was studied adsorption on the nanoparticles not hydrophilic, but lipophilic or native (substantive) insoluble form of antitumor drug Arglabin. Such composition of complex allow us to hope that there are no drawbacks which were said above as there is less inclination of native Arglabin to be desorbed from the polymer matrix into water medium.

With the aim of optimization of drug loading (incorporation) into nanoparticles prepared from HSA and to establish a standard protocol for their preparation the concentration of Arglabin (native, substantive) in a sorption solution was varied between 0.25 mg/ml to 6.2 mg/ml. In this case concentrations of HSA in all solutions were the same and were equal to 5.9 mg/ml. The process of adsorption included two hour incubation of Arglabin into disperse solution of HSA nanoparticles. Drug-loaded nanoparticles were separated from

unbound Arglabin by centrifugation. The quantitative content of Arglabin was determined spectrofotometrically (λ=204 nm), preliminary separating solutions of Arglabin and HSA by size exclusion chromatography using columns Sephadex (Sigma Chemical Co. DE-52).

The results which were obtained with the help of U3000 Spectrophotometer (λ=204 nm) (Hitachi) are presented in a chart (Fig. 6).

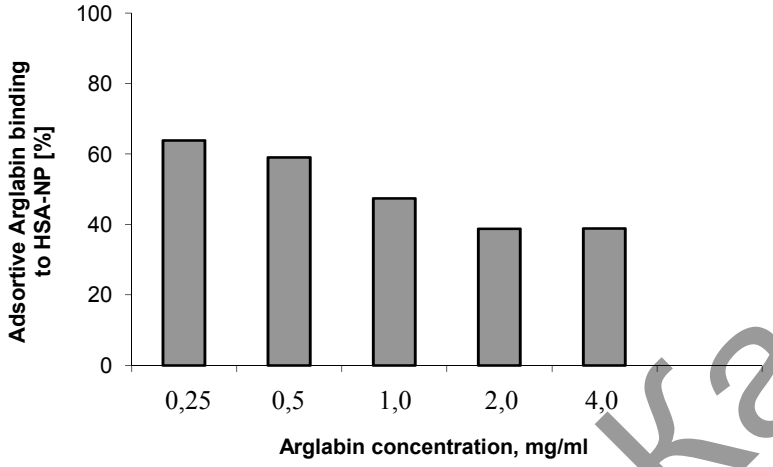


Figure 6. Adsoptive binding of Arglabin to 5.9 mg/ml of empty HSA NP

As it is shown in a chart at low concentrations of Arglabin more than 60 % of drug may be adsorbed on the surface of nanoparticles (Fig. 6). The portion of adsorbed Arglabin decreases with the increasing drug concentration in the initial solution, reaching a minimum meaning 38.8–38.9 % at a drug concentration of 2 mg/ml and 4mg/ml (Table 3).

Table 3

Characteristics of Arglabin (substantive)-loaded HSA nanoparticles by adsorption

C_{ARGL}, mg/ml	d, nm	P	Z, mV	Binding, %	Drug in NP (after adsorption), %
0.25	158.8 162.9 160.9	0.052 0.017 0.061	–14.2 –11.4 –11.0	63.9	1.6
	160.9±2.0	0.043±0.020	–12.2±2.0		
0.50	158.7 155.2 159.9	0.061 0.054 0.024	–18.7 –23.0 –16.4	59.1	2.5
	157.9±2.0	0.046±0.020	–19.4±3.3		
0.75	162.1 157.8 161.6	0.015 0.050 0.014	–20.4 –22.8 –14.6	60.0	4.3
	160.5±2.0	0.026±0.020	–19.3±4.1		
1.00	161.1 159.1 160.2	0.037 0.078 0.010	–19.0 –12.7 –10.7	47.5	4.5
	160.1±1.0	0.042±0.035	–14.1±4.2		
2.00	158.7 159.6 159.7	0.014 0.025 0.021	–14.8 –21.5 –15.2	38.8	7.2
	159.3±0.5	0.020±0.010	–17.2±3.4		
4.00	162.3 162.5 160.9	0.006 0.028 0.003	–20.0 –16.2 –15.3	38.9	13.5
	161.9±0.8	0.012±0.013	–17.2±2.4		

The obtained adsorption isotherm was analyzed using empirical equation of Freundlich which showed high correlation degree (R=0.97) and the possibility of further loading HSA nanoparticles with Arglabin. (Fig. 7).

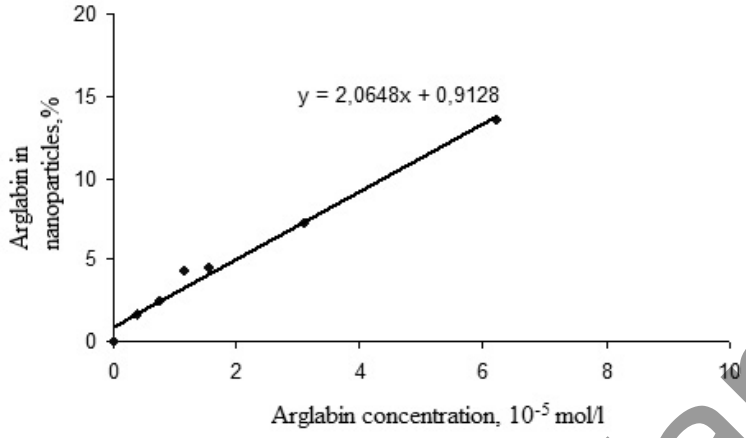


Figure 7. Adsorption of Arglabin on nanoparticles of serum albumin

So with the increasing of Arglabin concentration in the solution together with decreasing binding degree as it is said above the portion of drug on nanoparticles' surface increased and in a limit case it reaches 13.5 % from HSA nanoparticles' mass. The equation of adsorption (x/m = 3.06) shows that the portion of Arglabin in polymer-drug complex may be increased essentially.

As it was expected, because of electroneutrality of native Arglabin absolute meaning of zetapotential remained to be high enough (between –11.0 and –22.8 mV) which provides mobility of nanoparticles and prevents them from coagulation, and as a consequence the particle size changed slightly (160.8±2).

From the Table 3 and Figure 7 of polydispersity it is seen that the size distribution of particles is in relatively narrow meaning interval and the system is sufficiently stable.

Arglabin-loaded nanoparticles is seen on pictures obtained with the use of CM 12 transmission electron microscope (Philips) and they are shown in the Figure 8.

It is seen in a pictures that there might a formation of associates but separated particles are small enough. Thus, it was shown a possibility of obtaining a new drug form of antitumor drug «Arglabin» by loading HSA nanoparticles with native Arglabin.

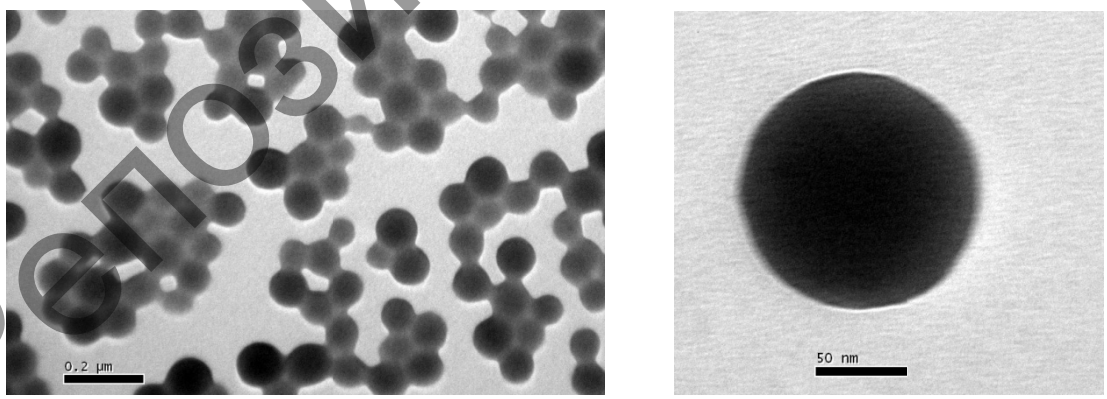


Figure 8. Electron-microscopy pictures of nanoparticles with Arglabin adsorbed on a particle surface

So, comparing the results of research on the production of nanoparticles by two methods one can conclude that the particle size, the degree of binding depend on the method of immobilization drugs. Thus, larger particles can be obtained by immobilization by incorporation than when immobilized by absorption method. And the maximum degree of binding is achieved by first one.

References

1 *Платэ Н.А., Васильев А.Е.* // Высокомол. соед. — 1982. — Т. А.24, № 4. — С. 675–695.

2 *Kreuter J.* Nanoparticles: Colloidal Drug Delivery Systems. — New-York: Marcel Dekker, 1994.

3 *Шварц И.Ш., Титов А.П., Васильев А.Е.* Биодеградирующие наночастицы и нанокапсулы — носители лекарственных веществ с регулируемыми свойствами // Актуальные проблемы создания лекарственных форм с заданными биофармацевтическими свойствами: Тезисы докл. Всесоюзн. науч.-техн. конф., 24–26 окт. 1989 г. — Харьков, 1989. — С. 141.

4 *Сметанкина О.Н., Приходько Т.В., Турко Я.А.* // Биотехнология. Теория и практика. — 2002. — № 3. — С. 89–97.

5 *Couvreur P., Dubernet C., Paisieux F.* // Eur. J. Pharm. and Biopharm. — 1995. — Vol. 41, No. 1. — P. 2–13.

6 *Скидан И., Гельперина С., Северин С. и др.* // Антибиотики и химиотерапия. — 2003. — Т. 48, № 1. — С. 23–26.

7 *Гельперина С.Э., Смирнова З.С., Халанский А.С. и др.* // Рос. биотерапевт. журн. — 2004. — Т. 3. — С. 56–64.

8 *Speiser P.P.* // Clin. Pharmacol. — 1994. — Vol. 13. — P. 337–345.

9 *Langer K., Balthasar S., Vogel V. et al.* // Int. J. Pharm. — 2003. — Vol. 257. — P. 169–180.

10 *Maeda H., Wu J., Sawa T. et al.* // J. Controlled Rel. — 2000. — Vol. 65. — P. 271–284.

11 *Адекенов С.М.* // Рос. биотерапевт. журн. — 2002. — Т. 1, № 2. — С. 5–7.

12 *Dreis S., Rothweiler F., Michaelis M., Cinatl Jr., Kreuter J., Langer K.* Preparation, characterization and maintenance of drug efficacy of doxorubicin-loaded human serum albumin (HSA) nanoparticles // International journal of Pharmaceutics. — 2007. — Vol. 341. — P. 207–214.

13 *Zhaparova L., Tazhbayev Ye., Burkeev M., Adekenov S., Ulbrich K., Kreuter J.* Preparation and investigation of antitumor drug Arglabin loaded human serum albumin nanoparticles // Trends in Cancer Research. Ltd. India. — 2008. — Vol. 4. — P. 43–47.

М.Ж. Буркеев, Й. Кройтер, Е.М. Тажбаев, Л.Ж. Жапарова, Т.С. Жумагалиева, А.Т. Кажмуратова, Д.А. Муханова

Сарысулы альбумин негізінде қатерлі ісікке қарсы «Арглабин» препаратының жаңа үлгілері

Мақалада қатерлі ісікке қарсы «Арглабин» препаратымен иммобилденген альбумин нанобөлшектерін алу мүмкіндігі қарастырылған. Нанобөлшектердің физика-химиялық сипаттамалары және «Арглабиннің» полимерлі нанобөлшектермен байланысу дәрежесі анықталды. Бөлшектердің өлшемі және байланысу дәрежесі дәрілік затты иммобилдеу әдісінен тәуелді екені дәлелденді.

М.Ж. Буркеев, Й. Кройтер, Е.М. Тажбаев, Л.Ж. Жапарова, Т.С. Жумагалиева, А.Т. Кажмуратова, Д.А. Муханова

Новые формы противоопухолевого препарата «Арглабин» на основе сывороточного альбумина

В статье рассмотрена возможность получения наночастиц альбумина, иммобилизованных противоопухолевым препаратом «Арглабин». Определены физико-химические характеристики наночастиц и степень связывания «Арглабина» с полимерными наночастицами. Установлено, что размер частиц и степень связывания зависят от способа иммобилизации лекарственного вещества.

References

1 Platee N.A., Vasiliev A.Ye. *Macromolecular compounds*, 1982, A.24, 4, p. 675–695.

2 Kreuter J. *Nanoparticles: Colloidal Drug Delivery Systems*, New-York: Marcel Dekker, 1994.

3 Shvarts I.Sh., Titov A.P., Vasiliev A.Ye. *Actual problems of creating formulations with desired biopharmaceutical properties*: Abstracts. Proc. scientific and engineering. conf., Kharkiv, Oct. 24–26, 1989, p. 141.

4 Smetankina O.N., Prikhod'ko T.V., Turko Ya.A. *Biotechnology. Theory and practice*, 2002, 3, p. 89–97.

5 Couvreur P., Dubernet C., Paisieux F. *Eur. J. Pharm. and Biopharm.*, 1995, 41, 1, p. 2–13.

6 Skidan I., Gelperina S., Severin S. et al. *Antibiotics and Chemotherapy*, 2003, 48, 1, p. 23–26.

7 Gelperina S.E., Smirnova Z.S., Khalanskiy A.S. et al. *Russian biotherapeutics J.*, 2004, 3, p. 56–64.

8 Speiser P.P. *Clin. Pharmacol*., 1994, 13, p. 337–345.

9 Langer K., Balthasar S., Vogel V. et al. *Int. J. Pharm*., 2003, 257, p. 169–180.

10 Maeda H., Wu J., Sawa T. et al. *J. Controlled Rel*., 2000, 65, p. 271–284.

11 Adekenov S.M. *Russian biotherapeutics J.*, 2002, 1, 2, p. 5–7.

12 Dreis S., Rothweiler F., Michaelis M., Cinatl Jr., Kreuter J., Langer K. *International Journal of Pharmaceutics*, 2007, 341, p. 207–214.

13 Zhaparova L., Tazhbayev Ye., Burkeev M., Adekenov S., Ulbrich K., Kreuter J. *Trends in Cancer Research. Ltd. India*, 2008, 4, p. 43–47.