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PREPARATION OF POLYMERIC NANOPARTICLES OF ALBUMIN AND IMMOBILIZATION OF THEM WITH THE ANTICANCER DRUG "CYCLOPHOSPHANE"

Abstract. Nanoparticles based on biodegradable and biocompatible polymers allow to attain the targeted drug delivery to the specific organ, without affecting healthy cells of the body and prolonging the therapeutic effect of the drug substance. This study is devoted to the development of the method for the preparation of nanoparticles of human serum albumin (HSA) immobilized with the anticancer drug Cyclophosphamide. Albumin nanoparticles with the sizes of 150-170 nm and narrow particle size distribution (PDI 0.069-0.116) were obtained by coacervation. The yield of nanoparticles is 88.5%. The degree of binding of the drug to polymer nanoparticles was within the range of 54.6% to 92.4%. The drug release was prolonged. After 42 hours 68-77% of the biologically active substance was released from the polymer matrix. It is concluded that the development of HSA-based nanocarrier is promising for the Cyclophosphamide preparation.

Keywords: nanoparticles, drug carriers, human serum albumin, cyclophosphane, cyclophosphamide, anticancer drug.

Introduction

One of the serious diseases leading to death worldwide is cancer. Traditionally, cancer is treated using methods of radiation therapy, chemotherapy and surgery [1]. In order to solve the problems associated with reducing the dosage of the drug and minimize side effects, it is important to use the method of targeted drug delivery using the polymeric nanoparticles and nanocapsules. Nanotechnology-based cancer therapy became one of the promising areas of biomedicine that has been widely studied over the past few decades.[2] The use of nanoparticles in the treatment of cancer overcomes the disadvantages of conventional drug delivery systems, such as nonspecific biodistribution and targeting, insufficient solubility in water, poor oral bioavailability and low therapeutic parameters [3, 4, 5].

Among the main requirements for polymer carriers, the stability and inertness to the blood components should be outlined; also the polymer should protect the drug substance from degradation [6,7]. Human serum albumin was chosen as the polymeric carrier in this work. Albumin is a favorable macromolecular carrier and is widely used in medicine owing to its biodegradability, non-toxicity and non-immunogenicity [8,9]. The incorporation of a drug into albumin nanoparticles is an attempt to carry out the targeted delivery of drug to tumor cells to reduce side effects of the drug. Presently the antitumor drug preparations based on albumin nanoparticles have already been created, but their price is so high that it makes them inaccessible. One of the reasons for the inaccessibility of anticancer drugs created on the basis of nanoparticles is complex synthesis techniques, high energy consumption and instability of nanosystems [10]. To overcome these difficulties, in the framework of this study an attempt was made to develop a relatively simple method for producing albumin nanoparticles, to achieve a high degree of drug immobilization, and to obtain a drug with the potential for prolonged release.

Cyclophosphamide was chosen as a model preparation for immobilization. Cyclophosphamide is a cytostatic antitumor chemotherapeutic drug of an alkylating type of action; a derivative of bis-chloroethylamine and simultaneously a derivative of oxazaphosphorine or a derivative of diamidophosphate (the so-called "phosphoramide mustard"). It is recommended for the treatment of many forms of cancer, including small cell lung cancer, cancer of the ovary, cervix and body of the uterus, breast cancer and others. The drug has many side effects and limitations due to its toxicity and effects on healthy body cells, etc. Thus, ensuring the safety and effectiveness of treatment with this drug is relevant and can be solved using polymeric drug carriers.

Methods

Synthesis of polymer nanoparticles by the incorporation method

Albumin-based nanoparticles of Cyclophosphamide were synthesized using the incorporation method. To an aqueous solution of albumin (2%) was added with a certain speed (not more than 1 ml/min) a solution of the drug in ethanol with concentrations of 2; 4; 8 mg/ml (pH of the solution is 8.0-8.5). The particles formed in this process are small in size (within the range of 50-300 nm). To crosslink the particles glutaraldehyde (6.25% solution) was added to the solution, which stabilized the size and the structure of the particles. Incubation was carried out for 2 hours on a mechanical stirrer (650 rpm). The solution of unreacted serum albumin was purified by washing it three times with deionized water and centrifuging for 15 minutes at 14500 rpm. The obtained nanoparticles were separated by centrifugation (MiniSpin Plus 14500, Eppendorf, Hamburg, Germany). The yield of nanoparticles was determined by gravimetry.

Determination of particle size and polydispersity

The average size of nanoparticles and their polydispersity were determined using photon correlation spectroscopy (FCC) at a Malvern Zetasizer Nano S90 instrument (Malvern Instruments Ltd., UK) at a temperature of 298 K and a scattering angle of 90^0. Each nanoparticle sample was properly diluted with a non-solvent immediately after preparation. The average size and polydispersity index were measured three times for each batch.

Analysis of Nanoparticles Morphology

Nanoparticles' morphology was analyzed by scanning electron microscopy (SEM) using a MIRA 3 LM TESCAN electron microscope (Czech Republic). Carbon was sprayed onto the surface of the samples using magnetron sputtering of carbon fiber to increase conductivity. The measurements were carried out in high vacuum using an SE detector at an accelerating voltage of 4–20 kV.

Investigation of the binding degree of cyclophosphamide with the polymeric nanoparticles

To determine the binding degree of drug to albumin NPs, the obtained particles were separated from the supernatant by ultracentrifugation at 14500 rpm and washed with water. The content of unbound drug was determined spectrophotometrically using a UV-1800 SHIMADZU instrument (Japan) (λ = 247.5 nm).

The Study of Cyclophosphamide Release

The kinetics of drug release from serum albumin NPs was studied using standard methods in phosphate buffered saline at a temperature of 310 K (pH = 7.4). For this, a weighed portion of nanoparticles loaded with cyclophosphamide was dispersed in 10 ml of a phosphate buffer solution. Within 2 days, 2 ml samples were taken from the dispersion at certain time intervals, which were centrifuged at a speed of 14500 rpm. The concentration of the drug in the solution was determined using UV spectroscopy at 247.5 nm [12].

Results and discussion

According to the method described above, an alcoholic drug solution was added to the albumin solution.

The concentration of the drug ranged from 2 to 8 mg/ml. To stabilize the nanoparticles they were crosslinked with glutaraldehyde. The physicochemical characteristics of the particles were determined by photon correlation spectroscopy (PCS). The results of measurements of particle size and polydispersity index are presented in Figure 1 and Table 1. As can be seen from the figure, the obtained nanoparticles have satisfactory physicochemical characteristics: the average particle size was at the range of 148 - 171.5, and the value of polydispersity index is in the interval of 0.0038-0.098. The formation of large particles is not observed in the system, and the particles are uniformly distributed.

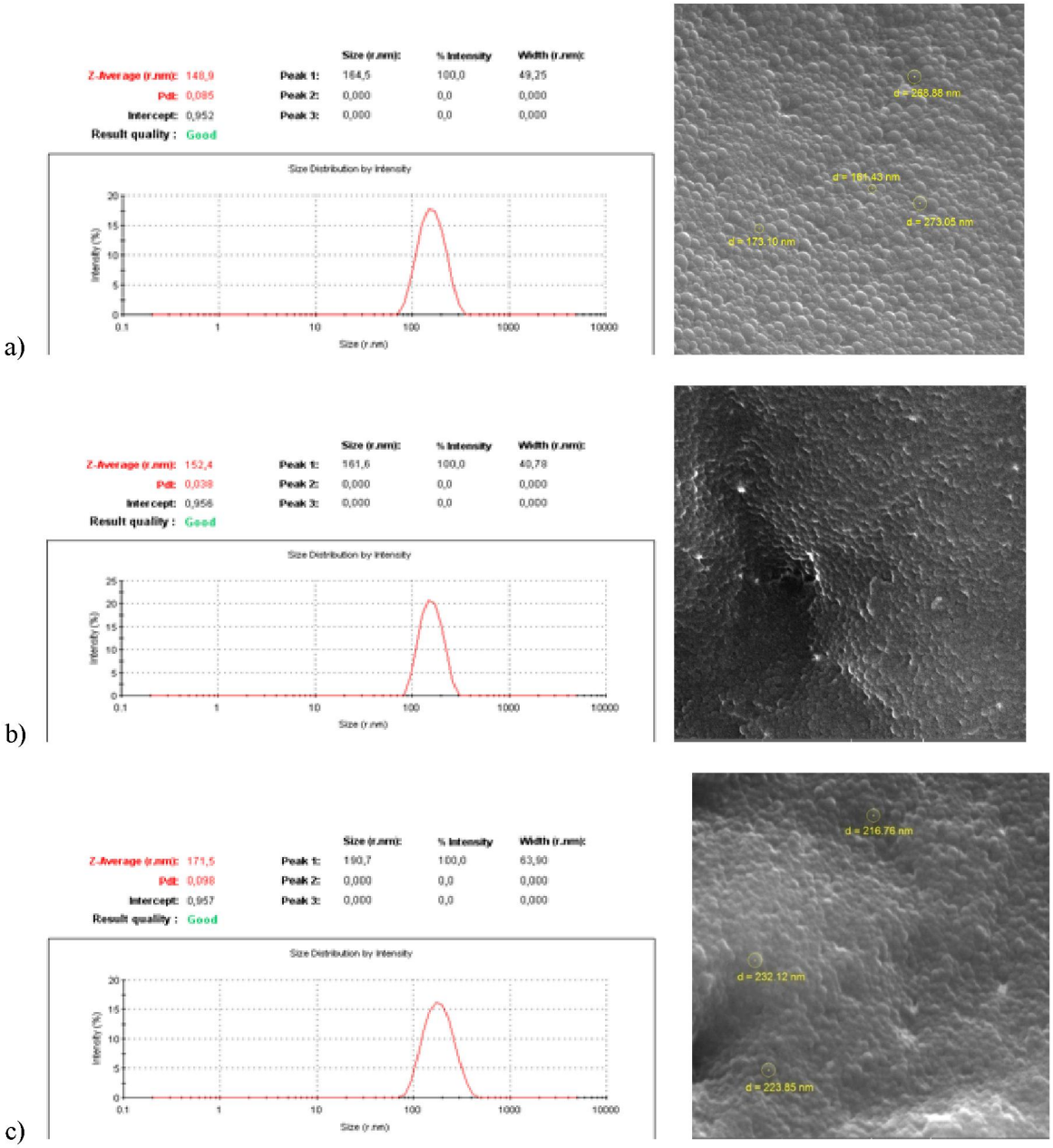

Figure 1 - PCS data and electron microscopic photographs of serum albumin nanoparticles immobilized with cyclophosphamide at concentrations of a) 2 mg / ml; b) 4 mg / ml; c) 8 mg / ml

To confirm the data obtained by the PCS the electron microscopic images of polymeric nanoparticles loaded with cyclophosphamide were made using a scanning electron microscope (Figure 1 a, b, c).

From electron microscopic images (Figure 1), it can be seen that the polymeric nanoparticles have spherical shape and they are uniformly distributed in size and shape: the average particle size of nanoparticles of all concentrations varies from 100 - 300 nm. It was found that with increasing the concentration of the drug the size of the polymeric nanoparticles increases, but their meanings meet the requirements to the nanoparticles for invasive administration. These parameters indicate that the obtained nanoparticles can be used as transporting systems.

The binding degree is an important quantity that characterizes the quantification of the immobilization of a drug substance in a polymer matrix. Therefore, the next step in our study was to determine the binding degree of the cyclophosphamide with albumin nanoparticles. It was found that the binding degree

of cyclophosphamide in the samples reached 92.4% depending on the concentration of the drug (table 1). In this case, the increase in the concentration of the drug in the solution leads to the increase in its content in the polymeric nanoparticles.

Table 1 - Physicochemical characteristics of albumin nanoparticles immobilized with cyclophosphamide

C_{ds}, mg/ml	d, nm	PDI	The yield of the particles with the size up to 1000 nm,%	The binding degree,%	The degree of release,%	The yield of nanoparticles,%
2	140.9	0.154	100	54.6	68.2	70.5
	148.9	0.085	100			
	143.8	0.116	100			
4	152.4	0.038	98.5	79.4	71.5	75.3
	149.1	0.085	100			
	151.2	0.086	99.5			
8	172.6	0.134	100	92.4	77.1	88.5
	171.5	0.098	100			
	168.8	0.116	100			

At the next stage, the release of cyclophosphamide from albumin nanoparticles was studied. The release kinetics was studied under conditions close to physiological (in phosphate-buffered saline at a temperature of 310 K, pH = 7.4) for 2 days. The results of this study are presented in Figure 2.

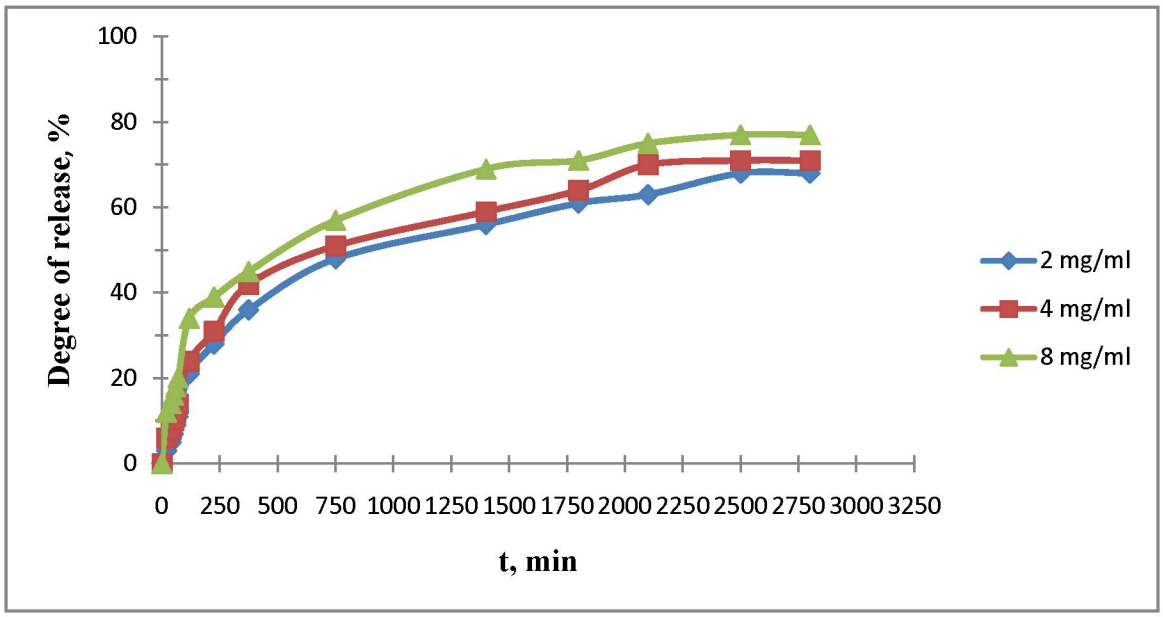

Figure 2 - Kinetics of cyclophosphamide release from NPs

All release curves of cyclophosphamide from the albumin matrix are characterized by the presence of several sites. The first four hours are characterized by the highest speed, when 24-34% of the drug is released which probably characterizes the release of an unbound drug from the surface layer of albumin 1This indicates the retention of the drug in the albumin nanoparticles via non-chemical bonds, possibly via hydrogen, van der Waals, and other bonds. Only after 42 hours the concentration of the drug reaches saturation in solution and its amount is 68-77% depending on the initial concentration. These results point on promising further studies in the field of the creation of prolonged forms of the drug.

Conclusion

It was possible to develop a method for the synthesis of nanometric polymer carriers based on a biocompatible and biodegradable albumin polymer for transporting the anticancer drug Cyclophosphamide. Nanoparticles with satisfactory physicochemical characteristics have been synthesized using incorporaton method. It makes them promising systems for targeted drug delivery. The use of such new dosage forms in the form of nanoparticles will reduce the daily dose of the drug, increase its effectiveness and minimize its side effects.

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АЛЬБУМИННІҢ ПОЛИМЕРЛІ НАНОБӨЛШЕКТЕРІН АЛУ ЖІНЕ ОЛАРДЫ ҚАТЕРЛІ ІСІККЕ ҚАРСЫ ПРЕПАРАТ «ЦИКЛОФОСФАНМЕН» ИММОБИЛИЗАЦИЯЛАУ

Аннотация. Биоыдырамалы және биоүйлесімді полимерлердің негізіндегі нанобөлшектер дәрілік заттың терапевтикалық әсер ету уақытын ұзарта отырып, сонымен қатар ағзаның сау жасушаларын зақымдамай дәрілік затты мақсатты түрде тікелей нысана ағзаға жеткізуге мүмкіндік береді. Бұл зерттеу қатерлі ісікке қарсы «Циклофосфан» препаратымен иммобилизацияланған адам сарысулы альбуминінің нанобөлшектерін алу жолдарын жасап шығаруға арналған. Коацервация әдісімен 150-170 нм өлшемдегі, полидисперстілік мәні төмен (полидисперстілік индексі 0,069-0,116) альбумин нанобөлшектері алынды. Нанобөлшектердің шығымы 88,5%-ды құрады. Дәрілік заттың полимерлі нанобөлшектермен байланысу дәрежесі 54,6% және 92,4% аралығында болды. Дәрілік заттың босап шығуы пролонгациялық түрде жүрді. 42 сағаттан кейін полимерлі матрицадан биологиялық белсенді заттың 68-77%-ы ортаға босап шықты. «Циклофосфан» препаратына адам сарысулы альбумині негізіндегі нанотасымалдаушыны жасап шығарудың болашағы зор деген қорытынды жасалынды.

Түйін сөздер: нанобөлшектер, дәрі тасымалдаушылар, адам сарысулы альбумині, циклофосфан, циклофосфамид, қатерлі ісікке қарсы препарат.

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ПОЛУЧЕНИЕ ПОЛИМЕРНЫХ НАНОЧАСТИЦ АЛЬБУМИНА И ИХ ИММОБИЛИЗАЦИЯ ПРОТИВООПУХОЛЕВЫМ ПРЕПАРАТОМ «ЦИКЛОФОСФАН»

Аннотация. Наночастицы на основе биодеградируемых и биосовместимых полимеров дают возможность целенаправленно доставлять лекарственное вещество непосредственно в орган-мишень, при этом не поражая здоровые клетки организма и продлевая срок терапевтического действия лекарственного вещества. Настоящее исследование посвящено разработке способа получения наночастиц человеческого сывороточного альбумина (ЧСА), иммобилизированного противоопухолевым препаратом «Циклофосфан». Методом коацервации получены наночастицы альбумина с размерами 150-170 нм, узкой полидисперстностью (индекс полидисперстности 0,069-0,116). Выход наночастиц составил до 88,5%. Степень связывания лекарства с полимерными наночастицами находилась в пределах 54,6% до 92,4%. Высвобождение препарата происходило пролонгированно. Через 42 часа из полимерной матрицы высвободилось 68-77% биологически-активного вещества. Сделан вывод о перспективности создания наноносителя на основе ЧСА для препарата «Циклофосфан».

Ключевые слова: наночастицы, носители лекарств, человеческий сывороточный альбумин, циклофосфан, циклофосфамид, противоопухолевый препарат.

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