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Synthesis of polymeric nanoparticles on the basis of butyl cyanoacrylate for transport of antitumor drug preparation «Arglabin»

Ісікке қарсы «Арглабин» препаратын тасымалдау үшін бутилцианоакрилат негізіндегі полимерлі нанобөлшектерді синтездеу

Zhaparova L.Zh.[1], Tazhbayev Y.M.[1], Burkeev M.Zh.[1], Zhumagalieva T.S.[1], Kreuter J.[2]

[1]*Y.A.Buketov Karaganda State University, Karaganda, Kazakhstan (e-mail: lyazzh@mail.ru);*
[2]*Institute of Pharmaceutical Technology, Johann Wolfgang Goethe University, Frankfurt/Main, Germany*

Мақалада ісікке қарсы препарат «Арглабинмен» иммобилизацияланған полибутилцианоакрилатты нанобөлшектерді алу мүмкіндіктері қарастырылған. Әрекеттесуші компоненттер қатынасының полимерлі нанобөлшектердің шығымына әсері зерттелді. Құрамында дәрілік препараты бар полимерлі бөлшектерді синтездеудің ықшамды жағдайлары табылды. Келесі шарттарды оңтайлы деп есептеуге негіз бар: эмульгатор концентрациясы — мономер массасының 5 %-ы, температура — 25 ºС және мономер концентрациясы — 2 %. «Арглабиннің» реакциялық ортадағы сандық мөлшерін анықтаудың жаңа кондуктометрлік әдісі ұсынылды. Бұл әдіс дәрілік заттың полимермен байланысу дәрежесі 75 % шамасында болатынын анықтауға мүмкіндік берді.

В статье рассмотрены возможности получения наночастиц полибутилцианоакрилата, иммобилизованных противоопухолевым препаратом «Арглабин». Исследовано влияние соотношений реагирующих компонентов на выход полимерных наночастиц. Найдены оптимальные условия синтеза полимерных частиц, содержащих лекарственный препарат. Установлено, что оптимальными можно считать следующие условия: концентрация эмульгатора — 5 % от массы мономера, температурный режим 25 ºС и концентрация мономера — 2 %. Предложен новый кондуктометрический метод количественного определения «Арглабина» в реакционной среде; данный метод позволил определить, что степень связывания лекарства с полимером составила 75 %.

One of the fast developing branches of pharmaceutical industry is creation of novel drug formulations with prolonged effect on the basis of polymeric carriers, which provides directed transportation of drug to target-organ. Nowadays to achieve this goal different technological methods are used. One of them is working out of biocompatible drug delivery systems which consist of the complex of drug with polymer material which is biodegradable. Among such systems nanosomal formulations of drug preparations are finding wide application [1]. The use of polymeric nanoparticles (NPs) and nanocapsules immobilized with drug allows to decrease side reactions, to achieve higher therapeutic effect of the drug, thus prolonging the efficiency of drug preparation.

One of the most often used non-toxic and biodegradable synthetic polymers is polyalkyl cyanoacrylate. Owing to their ability to incorporate different drug preparations they are still of great interest of scientists as drug delivery systems. There are many publications devoted to the creation of novel drug formulations on the basis of polyalkyl cyanoacrylates there [1–4]. In connection with this polybutyl cyanoacrylate (PBCA) has been chosen as a polymeric basis in this work.

The aim of the work was to synthesize polymeric NPs loaded with antitumor drug «Arglabin» Chemical structures of butyl cyanoacrylate (BCA) (1) and «Arglabin» (2) are shown in Fig. 1.

(1) (2)

Fig. 1. Chemical structures of butyl cyanoacrylate (1) and dimethylaminoarglabin hydrochloride (2)

Experimental part

Materials. Organic solvents and raw materials were purified according to techniques given in works [5, 6]. «Arglabin» was a gift of AS ISEH «Phytochemistry».

Synthesis of butyl cyanoacrylate. Synthesis of monomer was carried out using improved method of V.V.Korshak et al. (Institute of elementorganic compounds named after A.N.Nesmeyanov, 1985) [7]. The method consists of several stages the final one of which is formation of oligomers of butyl cyanoacrylate followed by depolymerization of the product.

Preparation of PBCA NPs loaded with dimethylaminoarglabin hydrochloride. PBCA NPs were prepared by anionic polymerization method similar to those described in several papers [1–3]. Briefly, the monomer was added into preliminarily acidified till pH 2.5–3.0 (with 0.01 M $H_2C_2O_4 \cdot 2H_2O$) water containing surfactant (Tween 85) while constant stirring. The process was carried out 2 hours.

For the preparation of the polymer incorporated with drug the same procedure as for preparation of unloaded NPs has been used with the difference that the drug was dissolved in acidified water solution before addition of the monomer.

Determination of binding degree by conductometric method. The measurements of electrical conductivity of solutions were performed on Conductivity meter Type OK-102 (Hungary) № 1182 OOO «Econics-Expert» and INN/KPP 7728209000/772801001 (Moscow) using platinum electrodes, thermostat UTU-2/77 (Polanol) with thermostatic electrical cell with volume (293 K) 25 ml.

Results and discussion

At present time the problem of treatment of tumor diseases have global character. Nowadays there are many antitumor preparations in medicine, however long-term and intensive chemotherapy of tumor with highly active drugs often leads to expressed toxic effects. Domestic preparation «Arglabin» possesses high antitumor activity from three types of cancer (breast cancer, primary liver cancer, cancer of lungs and ovary) and for several years it has been successfully used for the treatment of various cancers in a form of an injection. By obtaining nanosomal formulations of this drug on polymer basis the increase of therapeutic activity of «Arglabin» can be achieved [8]. In connection with this the possibility of obtaining of PBCA NPs loaded with antitumor drug «Arglabin» was studied.

One of the most widely used methods to synthesize polymeric NPs is emulsion polymerization, as it gives opportunity to obtain the product with rather high yield and satisfactory characteristics. Emulsion polymerization technique for alkyl cyanoacrylates were first introduced by scientific group of P.Couvreur in 1979 with the aim of obtaining polymeric NPs for directed transportation of drug preparations [4].

It was previously shown that by varying polymerization conditions of alkyl cyanoacrylates one can get NPs with the size ranging from 50 to 300 nm [1–5]. Therefore a number of experiments have been carried out in the direction of selection of optimum conditions of emulsion polymerization for obtaining ultra-sized particles of PBCA.

The change of medium pH in the range 1.5–3.5 allows to regulate the size of NPs within the interval 250–500 nm. Essential meaning on the size of polymeric particles in emulsion polymerization conditions has monomer concentration. A number of experiments have been carried out on optimization of the conditions of obtaining particles of nanometric size. PBCA particles were separated using membrane with diameter of porous 1000 nm.

With the aim of obtaining stable emulsion and opportunity of controlling the polymerization process the ratio of reacting components (monomer, emulsifier, stabilizer, etc.) was varied. In this case as an emulsifier and stabilizer Tween 85 and α, D-glucose were used accordingly. The concentration of Tween 85 was changed from 0.5 to 5 % on monomer basis. The results are given in Table 1.

From data shown it is seen that optimum concentration of emulsifier required for obtaining homogeneous system is 5 % on monomer mass. When the content of Tween 85 in the solution less than 5 % (on mass of BCA) unstable emulsion with the precipitation of aggregated particles is formed. Also when solution contains 0.5 % of emulsifier (on monomer mass) and less than 2 % of stabilizer the particles of nanometer size are not formed.

With the aim of improving the polymerization conditions a number of experiments in the direction of controlling the reaction temperature have been performed. An optimum temperature regimen for carrying out the synthesis of PBCA NPs is 25^0C. With the increasing of temperature the reaction goes faster and the polymer yield increases, but at the same time the partition of the particles with the size of less than 1 μm decreases (to 14.8 %).

Table 1

Emulsion polymerization of butyl cyanoacrylate with different concentrations of emulsifier and stabilizer [M] = 2 %

Tween 85, %	Stabilizer, α, *D*-glucose, %	Temperature, ºC	Yield of the fraction of particles with size less than 1000 nm, %
0,5	0,5	20	–
0,5	1,0	20	–
0,5	2,0	20	–
2,0	0,5	25	14,5
2,0	1,0	25	17,2
2,0	2,0	25	16,4
5,0	0,5	25	51,3
5,0	1,0	25	58,3
5,0	2,0	25	58,6
5,0	0,5	30	14,8
5,0	1,0	30	20,4
5,0	2,0	30	21,7

With increasing monomer concentration in the solution the polymerization rate increases (Table 2).

Table 2

Emulsion polymerization of butyl cyanoacrylate in different concentration of monomer [E] = 5 %, [glucose] = 1 %, T = 25 ºC

[M], %	Yield of polymer during 2 hours, %	Yield of the fraction of particles with size less than 1000 nm, % (from the amount of polymer, %)
0,1	19	66
0,5	26	65
1,0	45	62
2,0	67	58
2,5	70	40
5,0	79	21
10,0	85	10

However in that case the partition of particles with nanometric size decreases. At monomer concentration less than 2 % the yield of fraction till 1000 nm is sufficiently high and the yield of polymer is 67 % which is also acceptable. When using higher concentrations of the monomer (> 2 %) the partition of particles with required size decreases reasonably. For instance, at monomer concentration 2.5 % in comparison with 2 % monomer concentration in the solution the yield of polymer fraction with particle diameter less than 1 μm grows less to 18 %. As a result of experiments the concentration of BCA 2 % have been chosen as an optimal concentration for the formation of nanosized polymeric particles.

The next step was immobilization of polymer with drug preparation «Arglabin». When carrying out polymerization of alkyl cyanoacrylates to obtain higher incorporation of the drug into polymeric matrixes the loading is performed with hydrophilic forms of the drug preparations (e.g. Ampicillin, Actinomycin D, Doxorubicin hydrochloride and others) [8, 9]. In this work the loading of polymer was done with water-soluble form of the drug — dimethylaminoarglabin hydrochloride.

Polybutyl cyanoacrylate NPs loaded with antitumor drug «Arglabin» was obtained by incorporation of the drug into polymer matrix directly in the during polymerization of the monomer (hydrophilic form of the drug was used). In this case there is the difficulty in determination of binding degree of polymer with drug there. Previously spectrophotometric method was used for the determination of quantity of immobilized drug. However the drawback of this method is that during ultrafiltration some quantity of bounded Arglabin is passes through the membrane and washed out to the supernatant solution. Therefore novel conductometric method based on additivity of specific conductivity of the components of the system was worked out. Conductometric method allows to determine the binding of Arglabin with polymer directly in the reaction medium without preliminary separation of NPs. For carrying out the measurements Conductivity meter Type OK-102 device with platinum electrodes and thermostatic cell were used. The concentration of unbound Arglabin was calculated using calibration curve. As an electrical conductivity of solutions is additive value first

electrical conductivity of the components of the system were measured then necessary amendments have been done. For the concentrations of Arglabin of 5–60 mkg/ml the change of the meaning of specific electrical conductivity can be described by the following equation: y=86,3x+1,5. The results are given in Fig. 2.

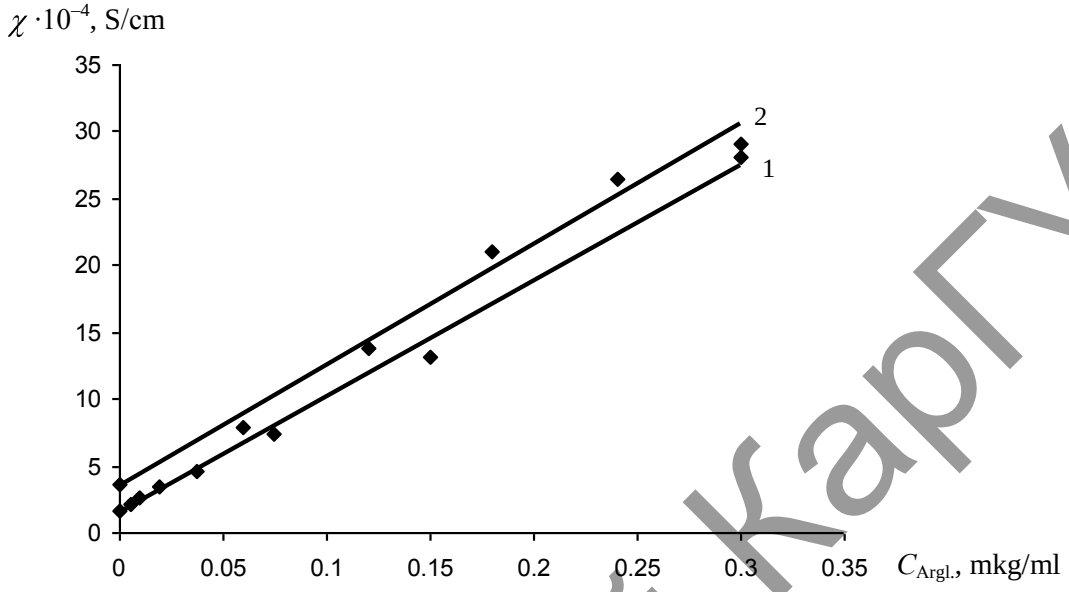

Fig. 2. Dependence of electrical conductivity on concentration of dimethylaminoarglabin hydrochloride: 1 — dependence of $\varkappa$ on concentration of Arglabin in water; 2 — dependence of $\varkappa$ on concentration of Arglabin in the presence of surfactant

With the increase of concentration of dimethylaminohydrochloride in water specific electricity of the solution increases (Fig. 2). The solution of surfactant has considerably low meaning of electrical conductivity even at its rather high concentration. In the presence of surfactant Arglabin's conductivity is higher which is due to the additivity of meaning of specific electrical conductivity.

Conductometric method of quantitative determination of Arglabin in the reaction medium is suitable not only for analysis of binding degree of drug but also for diagnosis of the release of biologically active substance from polymeric matrix.

As it was mentioned above polymerization of alkyl cyanoacrylates goes by anionic mechanism, therefore it's necessary to support an acidic medium. In order to keep medium pH in the range of 2.0–3.0 it was decided to use weak organic acid — oxalic acid instead of the solution of hydrochloric acid, as strong acid may lead to the destruction of the drug. Also being a strong electrolyte (having high electrical conductivity) hydrochloric acid can cover the diapason of measurements. After finishing the reaction the acid is titrated by sodium hydroxide till neutral pH, as a result of which the precipitation formed doesn't interfere to determine the quantity of the drug in the reaction medium.

Binding degree of PBCA with Arglabin determined by conductometric method was 75 %. It was shown in literature that in most cases when obtaining polymer-immobilized complexes the binding degree of drug with polymer doesn't exceed 20 %. High meaning of binding of polymer with drug allows to hope that the use of PBCA containing «Arglabin» will give opportunity to raise the efficiency of the drug in comparison with standard drug form.

So method of the synthesis of PBCA NPs loaded with antitumor drug preparation «Arglabin» with high drug content was worked out.

References

1. *Kreuter J.* Nanoparticles; Colloidal Drug Delivery Systems. — New-York: Marcel Dekker, 1994.
2. *Vauthier C., Dubernet C. et al.* Drug delivery to resistant tumors: the potential of poly(alkyl cyanoacrylate) nanoparticles // J. Controlled Release. — 2003. — Vol. 93. — P. 151–160.

3. *Vauthier Ch., Dubernet C., Fattal E. et al.* Poly(alkylcyanoacrylates) as biodegradable materials for biomedical applications // Advanced Drug Delivery Reviews. — 2003. — Vol. 55. — P. 519–548.
4. *Couvreur P., Kante M. et al.* Polycyanoacrylate nanocapsules as potential lysosomotropic carriers: preparation, morphological and sorptive properties // J. Pharm. Pharmocol. — 1979. — Vol. 31. — P. 331–332.
5. *Vaisberg A.* Organic solvents. — Moscow: Foreign literature, 1958.
6. *Toropceva A.M.* Manual for laboratory work on chemistry and technology of high molecular compounds. — Moscow: Chemistry, 1972.
7. A.c. 1168552, USSR. Claim 15.07.83. Method of obtaining of α-substituted cyanoacrylates. Institute of elementorganic compounds named after A.N.Nesmeyanov / Korshak V.V., Polyakova A.M., Pavlova C.-C.A., Sayid-Galiev E.E., Ashchotova L.M., Lopatina I.V. 3624212/23–04, printed in B.I., 1985, № 27. MKI. C 07 c 121/413, 120/00.
8. *Zhaparova L., Tazhbayev Y. et al.* Preparation and investigation of antitumor drug Arglabin loaded human serum albumin nanoparticles // Trends in Cancer Research. Ltd. India. — 2008. — Vol. 4. — P. 43–47.
9. *Page-Clisson M.-E., Pinto-Alphandary H. et al.* Development of ciprofloxacin-loaded nanoparticles: physicochemical study of the drug carrier // J. Controlled Release. — 1998. — Vol. 56. — P. 23–32.
10. *Brasseur F., Couvreur P. et al.* Actinomycin D adsorbed on polymethylcyanoacrylate nanoparticles: Increased efficiency against an experimental tumor // Eur. J.Cancer. — 1980. — Vol. 16. — P. 1441–1445.

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Исследование характеристик полиэтилцианоакрилатных наночастиц с капреомицина сульфатом

Investigation of characteristics of polyethyl cyanoacrylate nanoparticles with capreomycin sulfate

Жапарова Л.Ж.[1], Тажбаев Е.М.[1], Буркеев М.Ж.[1], Кажмуратова А.Т.[1], Ван Херк А.М.[2]

[1]Карагандинский государственный университет им. Е.А.Букетова (e-mail: lyazzh@mail.ru);
[2]Эйндховенский технологический университет, Нидерланды

Құрамына өкпе қабыну (туберкулезге) қарсы препарат «Капреомицин сульфаты» енгізілген полиэтилцианоакрилатты нанобөлшектердің физикалық-химиялық сипаттамалары зерттелген. Құрамында дәрілік заты бар және дәрілік затсыз полимерлердің салыстырмалы талдау нәтижелері бойынша капреомицин сульфатының қатысында полиэтилцианоакрилаттың термиялық деградациясы баяулайтыны көрсетілген. Алынған термогравиметриялық қисықтар мен ИҚ-спектрлері дәрілік заттың полимерлі матрицаға енгізілгенін дәлелдеді. Полиэтилцианоакрилатты капреомицин сульфатымен иммобилизациялау полимердің физикалық-химиялық параметрлерін өзгертеді.

Physicochemical characteristics of polyethyl cyanoacrylate nanoparticles containing antitumor drug preparation capreomycin sulfate were investigated. According to the results obtained by comparative analysis of polymers with and without drug it was shown that thermal degradation of polyethyl cyanoacrylate slowed down in the presence of capreomycin sulfate. Obtained thermal curves and IR-spectra confirm incorporation of the drug preparation into polymer matrix. Immobilization of polyethyl cyanoacrylate with capreomycin sulfate changes physicochemical parameters of the polymer.

На сегодняшний день одной из актуальных задач медицины и фармации является оптимизация свойств и биодоступности современных лекарственных средств. Это достигается получением полимер-иммобилизованных комплексов лекарственных препаратов на основе биосовместимых природных и синтетических полимеров. Из ограниченного списка полимеров, широко применяемых в медицинской практике, поливинилкапролактам, полимолочная и полигликолевая кислоты, поли-ε-капролактам и полиалкилцианоакрилаты представляют собой большую важность благодаря своим свойствам [1]. Полиалкилцианоакрилаты за последние несколько десятилетий успешно используются в фармацевтической и биомедицинской сферах как хирургические клеи для покрытия ран, так и в качестве коллоидных носителей для различных биологически активных агентов. Известны примеры включения пептидов, нуклеиновых кислот, лекарственных препаратов и других веществ в полиалкилцианоакрилаты с целью достижения направленной доставки вещества [2–5]. В связи с этим в настоящей работе в качестве полимерного носителя выбран полиэтилцианоакрилат.