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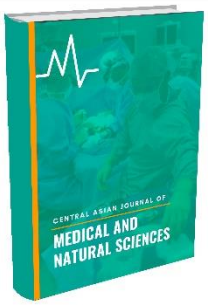

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SYNTHESIS AND PRE-CLINICAL STUDY OF SAFETY OF NANOPARTICLES OF SELENIUM STABILIZED SODIUM-CARBOCYLMETHYLCELLULOSE

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ABSTRACT: Among the scientific and technological progress achieved by the world scientific community is the creation of a new generation of nanostructured polymer preparations and materials for practical medicine. The search for new, effective and nanostructured, antitumor drugs remains one of the leading directions in creating more advanced methods of treating patients with malignant neoplasms.

KEYWORDS: synthesis, sodium-carbocylmethylcellulose, nanostructured polymer preparations.

INTRODUCTION

Among the scientific and technological progress achieved by the world scientific community is the creation of a new generation of nanostructured polymer preparations and materials for practical medicine.

The search for new, effective and nanostructured, antitumor drugs remains one of the leading directions in creating more advanced methods of treating patients with malignant neoplasms [1].

A number of effective anti-blast agents have been created that are successfully used in the treatment of certain forms of malignant neoplasms. However, the variety of forms of cancer and the rapidly emerging resistance to drugs, dictates the need to expand the arsenal of original antitumor drugs that effect tumors [2].

The relevance of this study is also determined by the urgent need for public health in new highly effective and low-toxic drugs that have an antitumor effect based on selenium nanoparticles. Due to the high prevalence of oncological diseases, there is a high demand of the domestic chemical and pharmaceutical industry for the production of new drugs using nanochemistry, nanopharmacology and nanotechnology.

In recent years, there has been increasing interest in the trace element selenium, which is part of the body's antioxidant defense system. In recent years, there has been increasing interest in the trace element selenium, which is part of the body's antioxidant defense system. Nanoparticles of selenium, unlike antibiotics, are able to have a prolonged effect [3].

Selenium is of exceptional interest as chemical element with a unique biologically active substance with antioxidant, anti-inflammatory, anti-carcinogenic, anti-mutagenic and detoxifying, as well as antitumor activity [4]. Achievements of modern science allow us to develop and use the most advanced technologies, including nanotechnology, to obtain new drugs. The introduction of nanomaterials in clinical medicine requires knowledge of the potential risks and possible side effects associated with their use.

Production cycles aimed at creating new nanomaterials can also be accompanied by the accumulation of products that have a toxic, carcinogenic and mutagenic effect on the human body. Therefore, the introduction of nanopharmacological drugs in clinical practice is impossible without additional toxicological studies. In this regard, in the special literature of recent years, much attention is paid to the consideration of safety issues of nanomaterials and nanotechnologies in biology and medicine. Considering the general trend in nanopharmacological studies, it should be noted that the toxicological characteristics of nanostructures and their associated drugs are significantly behind the data on their pharmacodynamic and pharmacokinetic parameters of drugs with a macrostructure [5].

The successful introduction of new nanostructured drugs into clinical practice implies the presence of proven safety in their use. To carry out this mentioned one, a certain procedure for conducting research at various levels should be followed, the most important of which is the safety assessment at the stage of preclinical experimental studies. The more thoroughly the toxicity of the studied nanopreparations in animals is studied, the less adverse reactions can occur during clinical trials [6,7].

From the medical and biological point of view, the present study was a preclinical study of the safety of selenium nanoparticles stabilized on biodegradable polymer substrates of natural origin.

The purpose of this work is to study the possibilities and methods for producing stabilized selenium nanoparticles in the structure of the polymer matrix – carboxymethcellulose sodium salt and study their structure, physico-chemical, biomedical properties.

EXPERIMENTAL PART

In this work, selenium (Se^0) nanoparticles obtained by reducing selenium (IV) oxide with ascorbic acid were selected as the object of research. As stabilizer, purified sodium carboxymethylcellulose (Na-

CMC) was used, with a degree of substitution (DSs) = 0.62-0.90 and a degree of polymerization (DPz) = 200-600, obtained from cotton cellulose [8]. Aqueous solutions of SeO_2 were used to form selenium nanoparticles in the solution structure of purified Na-CMC.

For the formation of selenium nanoparticles, 2-4% aqueous solutions of purified samples of Na-CMC of various DSs and DPz were chosen after removal of the gel fraction by centrifugation at a speed of 6000 rpm for 20 minutes. Calculated amounts of 0.1-0.01 M aqueous solutions of SeO_2 were added to Na-CMC solutions freed from the gel fraction with stirring, and stirring was continued until a homogeneous $Se^{4+}CMC^-$ solution was obtained.

Chemical reduction of selenium ions in the structure of $Se^{4+}CMC^-$ to selenium nanoparticles was carried out at 50°C by adding various amounts of 0.1 M aqueous solution of ascorbic acid. To obtain dispersions of selenium nanoparticles, ultrasonic dispersion of the solution on a dispersant of the brand USDN-1, U-4.2 was used in the course of the reduction reaction.

Optical absorption spectra of solutions were recorded on a Specord M210 instrument in the wavelength range from 200 to 900 nm. The optical path length was 2 mm.

To identify the interaction of an aqueous SeO_2 solution and a polymer, the method of infrared spectroscopy was used on a IR-100 Shimadzu (Japan), on Na-CMC, Se^+CMC^- and Se^0CMC films. The morphology of the surface layers of nanometallopolymers in films cast from Na-CMC, Se^+CMC^- and Se^0CMC solutions was studied using an ASM-5500 atomic force microscope (Germany)

The average size of selenium nanoparticles (Se^0) on the polymer matrix, the coefficient of variation was determined by mathematical analysis, corresponding microphotographs in the MathCad program.

Biomedical-biological studies were performed on healthy sexually mature animals that were quarantined for at least 12-14 days. Toxicological studies of two compounds of the preparation of Selenium nanoparticles (the first - 0.6635% - “A”), (the second - 0.1327% - “B”), was carried out in the accredited testing laboratory of the Ministry of Scientific Research and Technology of TMA on the basis of normative and methodological documents of the State system of the Republic Uzbekistan, taking into account of the requirements of the European Convention for the Protection of Vertebrate Animals used for experimental research or for other scientific purposes (ETS No. 123, Strasbourg, 1986), the requirements of the National Guide for the Maintenance and Use of Laboratory Animals. We used sexually mature mice of both sexes (n = 192, weighing 20-24 g), sexually mature white rats of both sexes (n=180, weighing 115-125 g).

Assessment of acute toxicity was carried out by a single intravenous, intraperitoneal, enteral administration in doses: from 500 to 4500 mg/kg. All test doses of the drug were prepared before administration on a physiological solution of sodium chloride. The general condition of laboratory animals was monitored hourly during the first day, and once a day in the next 13 days of the experiment (total observation period of 14 days). Before the start of the study and throughout the experiment (acute experiment) after the administration of the studied drug, clinical signs of possible intoxication were recorded: the general condition of the animals, feed and water consumption, changes in body weight, their behavior, the intensity and nature of motor activity, coordination of movements, reaction to external irritants, the frequency and depth of respiratory movements, the condition of the coat and skin, the color of the mucous membranes, the position of the tail, the amount and type of fecal mass. During the whole experiment, all laboratory animals were kept under standard vivarium conditions and were on a full

laboratory diet with free access to water. The mean lethal dose of the studied polymer derivatives of selenium was calculated by the probit analysis method using the Biostat 2009 software package.

The study of the local irritating effect of the drug was carried out using a conjunctival test in 18 guinea pigs, weighing 550-650 grams, with which 0.1 ml of 0.25, 1.0 and 2.5% solution A and B were instilled into the right eye preparations and 0.1 ml of distilled water was instilled into the left eye (control). The reaction was taken into account after 15 minutes (fast reaction) and after 24-48 hours (delayed hypersensitivity) and was evaluated on the following scale (in points):

- slight redness of the lacrimal duct;
- redness of the lacrimal duct and sclera in the direction of the cornea;
- Redness of the entire conjunctiva and sclera.

In addition, the degree of hyperemia, swelling, and lacrimation were taken into account.

The following research methodology for the local irritant effect of compounds is their effect on the condition of the skin.

The local effect of various concentrations of selenium containing nano-particles of the polymer composition was studied in 24 rats weighing 165-180 g. The animals were cut hair on both sides of the spinal column (4 fields) 2x2 cm in size. Rats on 2 clipped back sections on the right side were infused with 0.5 ml of "A" solution in concentrations (1.0 and 2.5%), and to the other group of rats of "B" solution in similar concentrations once for 10 days. The control was clipped areas (2 left fields) to which distilled water was applied in the same volume. Observation was carried out hourly for 6 hours on the first day and in the next 13 days (a total of 14 days).

The skin-resorptive effect of the preparation of selenium containing nano-particles in a polymer composition in various concentrations was studied on 24 white mongrel rats males weighing 160-180 g, which were fixed in special machines and their tails were immersed in 2/3 of the length of the tube with 1,0 and 2.5 % solution of nano particles of selenium. The tubes were placed in a water bath with a temperature of 28-30 ^{0}C. The exposure time was 4 hours. Then, after exposure, the tail of the rats was pulled out and washed with warm water and soap.

The study of the subchronic toxicity of selenium nanoparticles stabilized on biodegradable polymer substrates of natural origin (tentatively called "A") was introduced into the stomach daily (once a day before feeding) in rats at doses of 30, 60 and 160 mg/kg, comprising 1/50, 1/25 and 1/10 part of the average lethal dose, respectively.

Animals of the control groups (comparison group) received a solvent (drinking water) in the same volume and mode for 30 days.

Before the start of the study and throughout the experiment after daily administration of the studied drug, clinical signs of possible intoxication were recorded: the general condition of the animals, food and water consumption, changes in body weight (every three days), their behavior, the intensity and nature of motor activity, coordination of movements, reaction to external stimuli, the frequency and depth of respiratory movements, the condition of the coat and skin, the color of the mucous membranes, the position of the tail, the amount and type of fecal mass.

During the whole experiment, all laboratory animals were kept under standard vivarium conditions and were on a full laboratory diet with free access to water. One day after the last administration of the drug, the formula of blood indices was determined in animals of all groups on a hematological analyzer

BC-3000 (Mindray, China). The activity of aspartate aminotransferase (AsAT), alanine aminotransferase (AlAT) and alkaline phosphatase (ALP) was determined in blood serum; glucose, total protein, albumin, total bilirubin and their fractions, urea, creatinine (reagent kits from CYPRESS Diagnostics, Belgium) on a BA-88A biochemistry analyzer (Mindray, China).

The obtained results were subjected to statistical processing using the standard Biostat 2009 software package according to well-known methods of variation statistics with an assessment of the significance of the indicators (M ± m) and differences of the samples under study using the Student t-criterion. Differences in the compared groups were considered significant at a significance level of 95% ($p < 0.05$).

RESEARCH RESULTS AND DISCUSSION

Amorphous and crystalline structures are inherent in selenium. At present, amorphous nanoselen having a red color has been identified, as well as three crystalline forms of nanoselen: trigonal, α-, β-, γ-monoclinic and rhombohedral. Amorphous selenium is an inorganic polymer with covalently linked chains [9].

The methods of nanoparticle formation used in the scientific literature include: chemical reduction of selenium ions in polymer matrices, which allows controlling the sizes of nanoclusters and nanoparticles. Physical methods for controlling size include sonication of solutions, x-ray irradiation, ultraviolet irradiation, the use of high frequency currents, etc. [10]

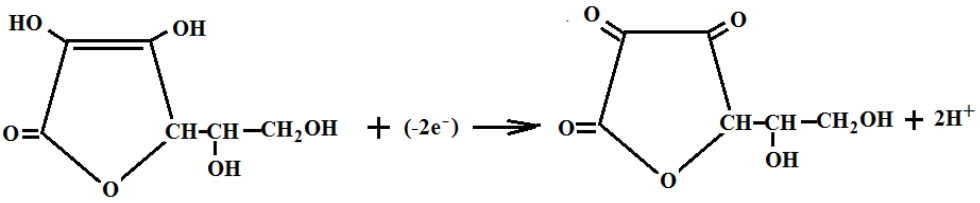
To obtain nanoparticles from selenium ions, purified samples of Na-CMC were selected, with DPz = 200-600, DSs = 0.62-0.90. The obtained solutions of purified samples of Na-CMC were used as a polymer substrate in the preparation of selenium nanoparticles.

The objects of the study were selenium-containing nanostructures obtained by the reduction of selenium (IV) oxide with ascorbic acid in the presence of a solution of purified Na-CMC. Selenium nanoparticles in Na-CMC solutions were prepared as follows: a solution of selenium (IV) oxide with a constant concentration of selenium of 0.005% was added to a solution of purified Na-CMC with a concentration of 2-4%, and stirred on a mechanical stirrer (1600 rpm) for 30 min., Then, under the influence of an ultrasonic dispersant, various amounts of a reducing agent, ascorbic acid with concentrations of 0.01 M, were introduced into the solution for 20 minutes and stirring was continued. Selenium oxide interacts with ascorbic acid in a ratio of 1:2. As a result of the reaction at a temperature of 50°C, a reddish-orange amorphous nanoselen forms.

When recovering selenium (IV) oxide in the presence of an aqueous solution of carboxymethylcellulose, a coloration of the colloidal solution from yellowish-orange to red was observed, which determines the formation of selenium nanoparticles depending on their size. The reduction of selenium oxide SeO_2 (IV) with ascorbic acid $C_6H_8O_6$ in aqueous solutions of Na-CMC proceeds according to reaction equation (1) [11].

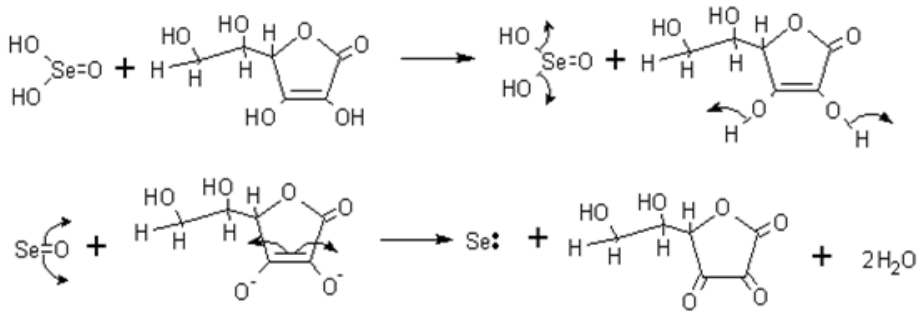
(1) $$\mathbf{SeO_2 + 2C_6H_8O_6 = Se^0 + 2C_6H_6O_6 + 2H_2O,}$$

leading to the formation of null-valent selenium (Se^0) and dehydroascarboxylic acid.



(2)

In the formation of selenium nanoparticles, ascorbic acid acts as a reducing agent, oxidizing to dehydroascarboxylic acid according to the following mechanism:



The uniformity of the formed nanoparticles in size is achieved due to the fact that Na-CMC macromolecules, enveloping selenium nanoparticles, create a charged shell around them, preventing their agglomeration due to electrostatic repulsion (Fig. 1).

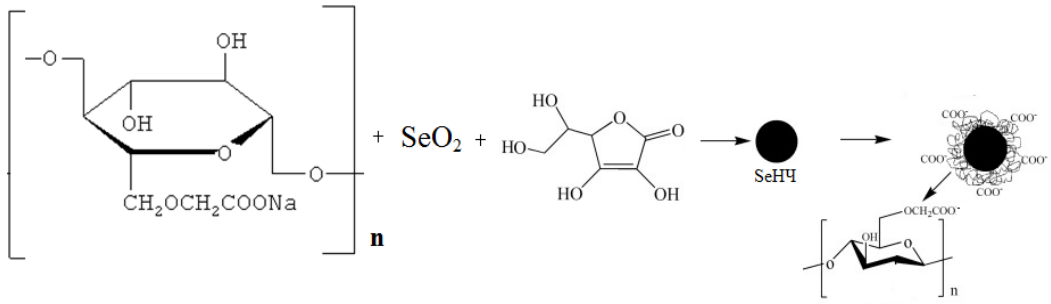
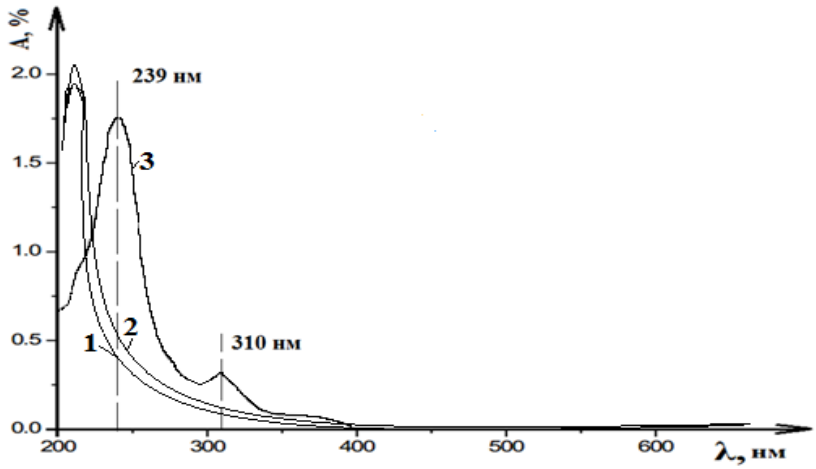


Figure 1. Estimated stabilization mechanism and pattern of selenium nanoparticle formation.

Studies on the formation and stabilization of selenium nanoparticles in polymer solutions of CMC were carried out and their properties were studied. Using the methods of optical and atomic force microscopy, the size, shape, and structure of selenium nanoparticles were studied.

In order to determine the shape and size of selenium nanoparticles in the structure of Na-CMC, Na-CMC, $Se^{4+}CMC^{-}$, $Se^{0}CMC$ solutions were studied using UV -spectroscopy [12]. In fig. Figure 2 shows the absorption spectra: Na-CMC, $Se^{4+}CMC^{-}$ and $Se^{0}CMC$ in the wavelength range of 200-600 nm.



1. Na-CMC. 2. $Se^{4+}CMC^{-}$. 3. $Se^{0}CMC$.

Figure 2. Absorption spectra of a solution of Na-CMC containing nanoparticles of selenium.

In the absence of the Na-CMC stabilizer, when the selenium (IV) oxide is reduced, the colloidal solution changes slowly from yellowish-orange to red, and the absorption band of the selenium nanoparticles shifts from λ_{max} = 400 to λ_{max} = 575 nm during the reaction, which indicates a fast an increase in the size of selenium particles to micron values with their subsequent precipitation [13]. 30 minutes after the start of the reaction, the kinetic curve reaches a maximum. The resulting colloidal solution of Se^{0} is unstable, and after 60 minutes the particles aggregate to micron values to form a red selenium precipitate.

When selenium (IV) oxide is reduced in the presence of a Na-CMC stabilizer, a maximum is observed in the optical spectra at λ_{max} = 239 nm, and λ_{max} = 310 nm, which characterizes selenium nanoparticles with a size of 20-80 nm. It was shown that the initial solutions of Na-CMC and $Se^{4+}CMC^{-}$ are optically transparent in the range of 250–900 nm [14].

With an increase in the concentration of selenium ions and ascorbic acid, the color of the solution changes from yellowish-orange to red and proceeds slowly in the presence of a Na-CMC reaction medium. Such changes are probably associated with an increase in the number and size of the formed selenium nanoparticles in the Na-CMC solution.

The maximum absorption bands of Na-CMC and selenium ion are observed at a wavelength of 210 nm. It is known from the literature that the maximum absorption band of amorphous nanoselen is located in the region of 300–320 nm; therefore, when measuring the optical density of a solution of selenium nanocomposite with Na-CMC, a solution of pure Na-CMC was used as a comparison solution. An intense absorption maximum of the nanocomposite was observed at a wavelength of λ = 239 nm and λ = 310 nm, which apparently characterizes the immobilization of Na-CMC macromolecules into selenium nanoparticles [15,16].

Further, IR-Fure spectroscopic studies of the Na-CMC, $Se^{4+}CMC^{-}$ and $Se^{0}CMC$ films were carried out.

The spectra of immobilized selenium nanoparticles differ in wide bands in the frequency range of stretching vibrations - OH groups - 3400-2800cm^{-1}; 3420 cm^{-1}, which characterize energetically unequal hydrogen bonds. In the region of stretching vibrations of C–H bonds, the spectra have maxima at 2923 cm^{-1} characterizing the asymmetric vibrations of the methylene group of Na-CMC. Absorption bands in the

region of 800-1420 cm^{-1} characterize planar deformation vibrations of the hydroxyl groups of Na-CMC [17].

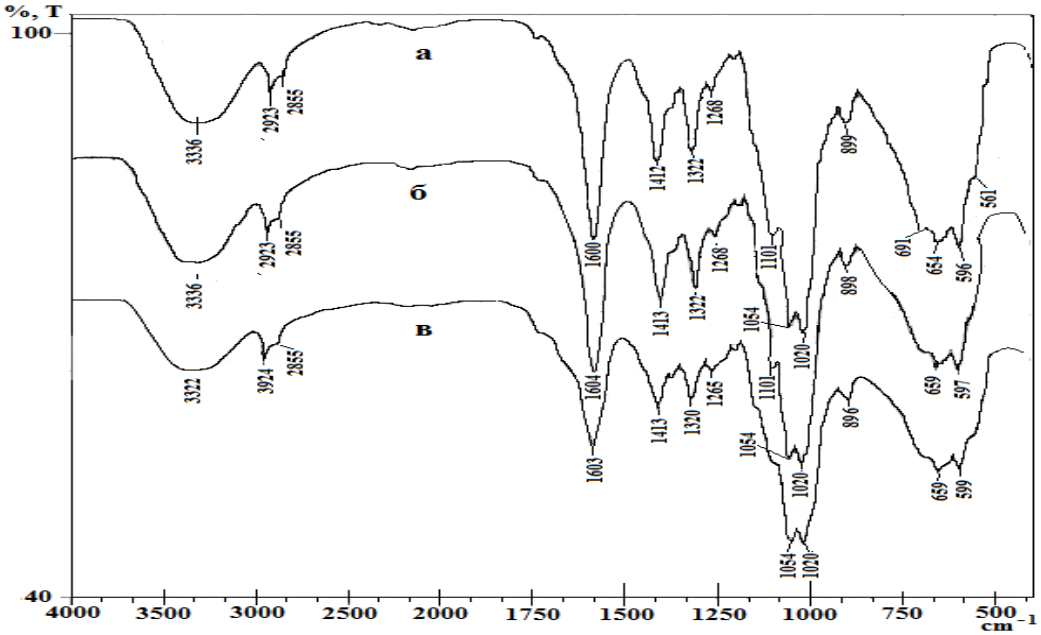


Figure 3. IR-Fure spectra of a-CMC, b-$Se^{4+}CMC^{-}$, and-Se^{0}CMC samples.

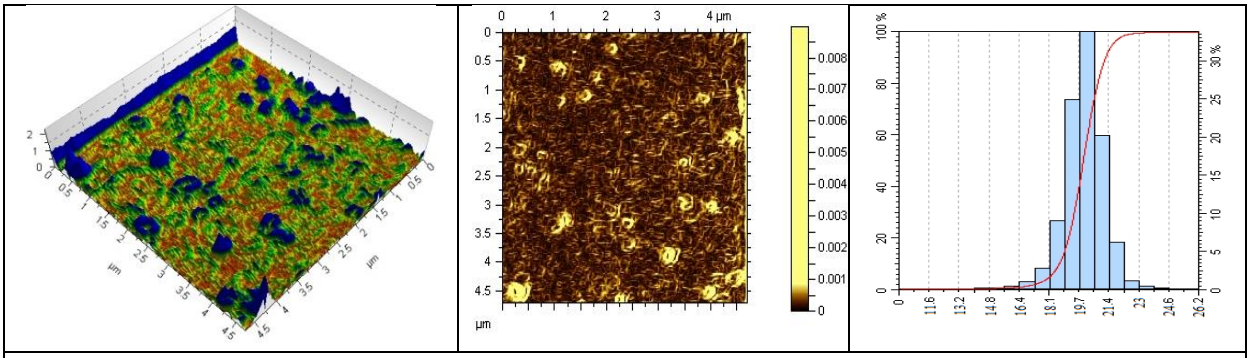
As can be seen from fig. 3 curve-a, samples of purified Na-CMC with, DSs = 0.90 and pH = 8.25 have an absorption band at 1600 cm^{-1} characteristic of anionic carboxymethylate groups.

When purified Na-CMC with DSs = 0.90 and DPz = 600 solutions of selenium oxide are added to the solution from pH = 7.8 to pH = 7.5, the intensity of the absorption bands at 1604 cm^{-1} decreases. (Fig. 3, curve b) due to the formation of a slightly dissociating CMC salt with selenium ions.

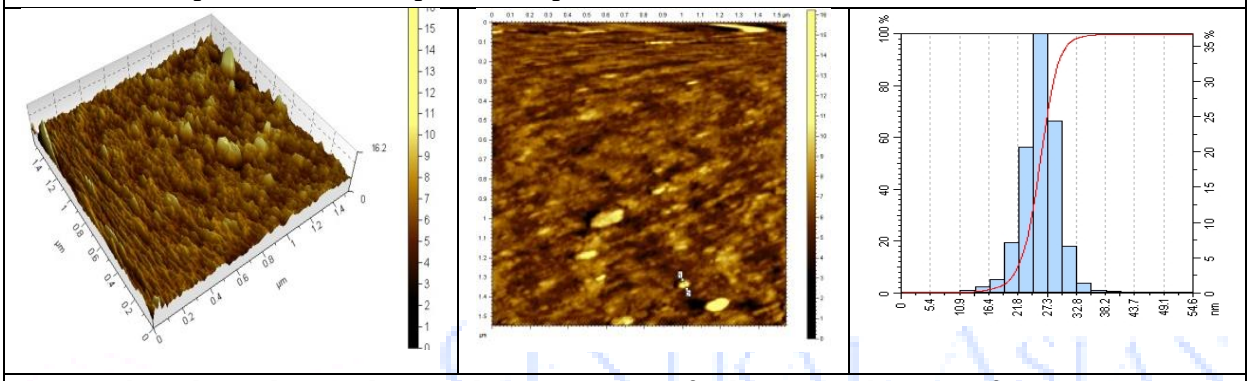
When reducing selenium ions to nanoparticles in the presence of ascorbic acid in a solution of purified Na-CMC, the intensity of the absorption bands at 1603cm^{-1} doubles. (Fig. 3, curve-c), which is explained by the consumption of ascorbic acid in the reaction of ion reduction to zero valence selenium.

The surface topography of thin CMC films containing stabilized selenium nanoparticles obtained from Se^{0}CMC aqueous solutions was studied by atomic force microscopy AFM-5500 (Germany). The measurements were carried out in contact mode in atmospheric conditions using NSG 01 silicon contilivers. The data obtained are presented in Fig. 4.

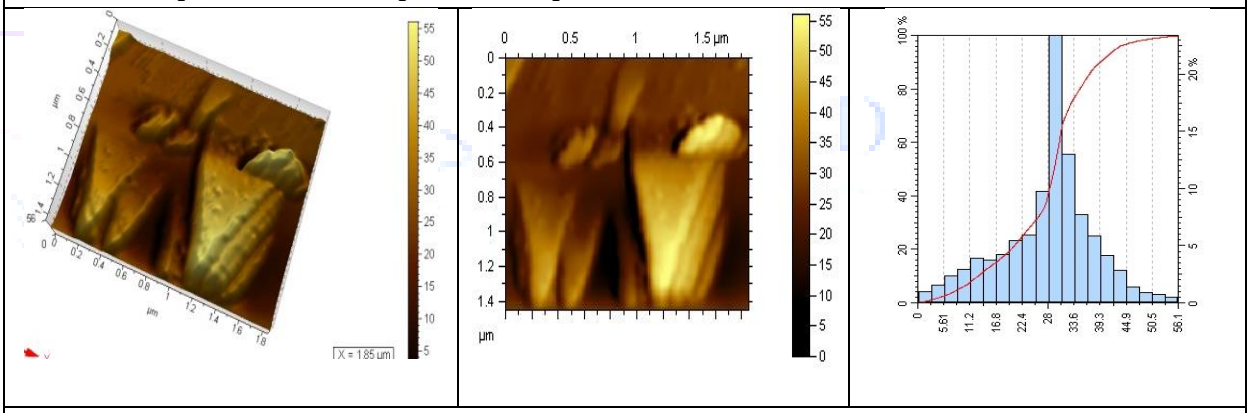
It can be seen from micrographs that at low SeO_2 concentrations spherical monodisperse selenium nanoparticles are formed (Fig. 4-a) with sizes of 2-14 nm. With an increase in the concentration of selenium oxide in CMC solutions, an increase in the size of spherical nanoparticles to 9-42 nm is observed. (Fig. 4-b).



a. Spherical monodisperse nanoparticles of selenium with sizes of 2-14 nm



b. Spherical monodispersed nanoparticles of selenium with a size of 9-42 nm.



c Trigonal nanoparticles of selenium with a size of 12-60 nm and a thickness of 4-28 nm.

a. Na- CMC:SeO_2 (0,08:2 • 10^{-5} mol) 0,12% SeO_2.
b. Na- CMC:SeO_2 (0,08:5 • 10^{-5} mol) 0,3% SeO_2.
c. Na- CMC:SeO_2 (0,08:3 • 10^{-4} mol) 1,7% SeO_2.

Figure 4. AFM images and histograms of the size distribution of selenium nanoparticles on Se^0CMC films.

With a further increase in the concentration of selenium oxide in CMC solutions, the formed spherical nanoparticles of selenium acquire trigonal shapes and their sizes reach 4–28 nm in thickness and 12–60 nm in length (Fig. 4-c).

Table 1. Influence of reaction parameters on the formation of selenium nanoparticles in a sodium - carboxymethylcellulose matrix.

№	Concentration of	Ratio	Nanoparticle shape	The size of the

	SeO_2, mol	Na-CMC and $C_6H_8O_6$, mol	selenium	selenium nanoparticles, nm
1	2 x 10^{-5}	0.08 : 0,16	Spherical	2-14
2	5 x 10^{-5}	0.08 : 0,32	Spherical	9-42
3	3 x 10^{-4}	0.08 : 0,48	Trigonal	Thickness - 4-28, Length -12-60

Table 1 shows the influence of reaction parameters on the formation of selenium nanoparticles in the Na-CMC matrix and the data of the obtained AFM results, studies of CMC films containing stabilized selenium nanoparticles. It was found that at low SeO_2 concentrations spherical monodisperse selenium nanoparticles with sizes of 2-14 nm are formed. With an increase in the concentration of selenium oxide in CMC solutions, an increase in the size of spherical nanoparticles to 9-42 nm is observed. (Table.1, №2)

With a further increase in the concentration of selenium oxide in CMC solutions, the formed spherical nanoparticles of selenium acquire trigonal forms and their sizes reach 4–28 nm in thickness and 12–60 nm in length. (Table 1, №2)

Biomedical tests of semi-finished samples containing stabilized nanoparticles of selenium were carried out in the laboratory of the Tashkent Medical Academy of the Ministry of Health of the Republic of Uzbekistan

The results of experimental studies in mice showed that the average lethal dose (LD_{50}) for intravenous administration of the drug “A” was 729.85 (649.33÷810.37) mg/kg, with an intraperitoneal dose of 750.01 (675,71÷824.31) mg/kg, and at enteric dose - 1405.31 (1283.29÷1527.02) mg/kg. In rats, when administered intravenously, LD_{50} was 697.82 (589.20÷806.44) mg/kg, intraperitoneal-797.81 (689.22÷906.48) mg/kg, enteral-1602.98 (1437.91÷1768.68) mg/kg.

When studying the acute toxicity of another drug “B”, the following results were obtained: in LD_{50} mice, intraperitoneal administration was 950.91 (772.79÷1129.03) mg/kg, and in case of enteral administration, 2005.36 (1734.19÷2276), 53) mg/kg. In rats with intraperitoneal administration, it amounted to 3175.52 (2972.67÷3378.37) mg/kg, and for enteric administration - 3750.03 (3481.95÷4015.38) mg/kg. Analysis of the results of toxicological studies indicates that the studied drugs are low toxic, however, the LD_{50} value differs depending on the type of animals and the route of administration of the drug. So, the value of the preparation “A” containing 0.6635% selenium nanoparticles with intravenous administration equals 729.85 mg/kg, and with intraperitoneal administration 750.01 mg/kg, while with enteral administration the value of this figure almost doubles and is 1405.31 mg/kg. It can be seen that the drug exhibits a higher toxicity with parenteral administration, which is probably due to the high bioavailability of the drug, especially when administered intravenously. The low toxicity of the drug during enteral administration is possibly associated with low absorption through the mucous membrane of the digestive system or biodegradation of the drug by digestive juice enzymes or microflora of the gastrointestinal tract. As can be seen from the data in table 2, this assumption can be fully attributed to the second drug (“B” - containing 0.1327% nano particles of selenium).

The published tables show that the LD_{50} value of this drug, regardless of the route of administration, is slightly higher than that of drug “A”. So with intraperitoneal administration, if the LD_{50} value of drug

"A" is 750.01 mg / kg, then for drug "B" it is 950.91 mg/kg. The same picture is observed with the enteral administration of drugs: 1405.31 and 2005.36 mg/kg, respectively. The lower toxicity of compound "B" is associated with more than four times lower content of selenium in the preparation.

Table 2. Values of the average lethal dose of selenium nanoparticles stabilized on biodegradable polymer substrates of natural origin.

Route of administration	Mice, LD_{50} (mg/kg)	Rats, LD_{50} (mg/kg)
Intravenous "A"	729,85(649,33 ÷ 810,37) 689,85(617,21 ÷ 780,32)	697,82(589,20 ÷ 806,44) 646,82(545,22 ÷ 786,42)
Intraperitoneal "A"	750,01(675,71 ÷ 824,31) 712,61(631,42 ÷ 794,18)	797,81(689,22 ÷ 906,48) 761,41(649,41 ÷ 876,42)
Enteral "A"	1405,31(1283,29 ÷ 1527,02) 1393,31(1264,12 ÷ 1571,13)	1602,98(1437,91 ÷1768,68) 1575,32(1387,54 ÷1712,22)
Intraperitoneal "B"	950,91(772,79 ÷ 1129,03) 918,52(742,45 ÷ 1098,22)	3175,52(2972,67 ÷ 3378,37) 3085,62(2972,67 ÷ 3306,45)
Enteral "B"	2005,36(1734,19 ÷ 2276,53) 1966,31(1664,19 ÷ 2004,43)	3750,03(3481,95 ÷ 4015,38) 3701,03(3433,62 ÷ 3971,76)

Note: *In the numerator, the data obtained in males, and in the denominator in female rats and mice*

Therefore, the studied drugs exhibit toxicological properties that can be attributed to compounds of class IV (low toxicity according to the OESD classification).

As shown, the results of the next series of experiments conducted on male white rats studied drugs show toxicological properties not significantly different from those that we noted in experiments conducted in mice. As can be seen from the data of tables 2, the drug "A" has a lower (twice) toxicity with enteral administration than with parenteral. In contrast to mice in rats, drug B exhibits a distinctly low toxicity both with the enteral and especially with the parenteral route of administration (more than three times). The toxicological properties of the studied drugs in female rats, as can be seen from the data given in tables 2, do not significantly differ from the values of male rats, although the average lethal dose is slightly less. It is likely that female rats, like mice, are more sensitive to the action of preparations of selenium nanoparticles on stabilized substrates of natural origin.

Subsequently, we investigated the local irritating effect of selenium nanoparticles in the polymer composition.

As you know, a conjunctival test of the tested compounds carried out on laboratory individuals is a very sensitive test and in some cases even reveals the reaction of animals to an allergen with mild allergization and negative skin tests.

The observation results showed that preparations containing various concentrations of nano particles of selenium (“A” and “B”) after 15 minutes, after 24 and 48 hours did not cause even slight reddening. Based on the conducted experimental studies, it can be concluded that preparations containing a nano particle of selenium in 0.25, 1.0, and 2.5% concentrations do not have an irritating effect on the conjunctiva of the eyes of guinea pigs.

The following research methodology for the local irritant effect of compounds is their effect on the condition of the skin.

The treated data showed that preparations containing various concentrations (1.0 and 2.5%) of selenium nanoparticles in the polymer composition did not cause irritation, redness, swelling, or other visible changes on the skin, and the effect of preparations “A” and “B” was rated 0 points.

Table 3. The scale of skin tests and the assessment of the local irritating effect in points of selenium nanoparticles stabilized on biodegradable polymer substrates of natural origin.

Reaction Description	Score in points	“A”/ “Б”	
		1,0%	2,5%
Erythema and octopus formation			
Lack of erythema	0	0/0	0/0
Very mild erythema (slightly noticeable)	1	0/0	0/0
Marked erythema	2	0/0	0/0
Moderate erythema	3	0/0	0/0
Severe erythema (bright red with the formation of octopus)	4	0/0	0/0
Swelling			
Lack of edema	0	0/0	0/0
Very mild edema (slightly noticeable)	1	0/0	0/0
Marked edema	2	0/0	0/0
Moderate swelling	3	0/0	0/0
Severe edema	4	0/0	0/0
Maximum points	8	0/0	0/0

The results allow us to conclude that the preparation of selenium containing nano particles in the polymer composition in the studied concentrations does not have an irritating effect on the skin. One of the routes of entry of substances into the general bloodstream is skin-resorptive properties.

As a result of observation and examination in experimental animals, no signs of intoxication and death of rats were revealed, which may indicate the absence of skin-resorptive action of the preparation of selenium nanoparticles in the polymer composition.

One of the first steps in carrying out the stages of preclinical studies is to establish safety in repeated applications of new compounds [4]. In connection with the experience presented in this series, we studied the subchronic toxicity of the preparation of selenium nanoparticles stabilized on biodegradable polymer substrates of natural origin.

Table 4. The effect of various doses of selenium nanoparticles stabilized on biodegradable polymer substrates of natural origin on the body weight of animals with repeated injections

Groups of animals	Animal body weight (g.)			
	Control (water)	The drug "A" (30 mg/kg)	The drug "A" (60 mg/kg)	The drug "A" (160 mg/kg)
Initial	119,3±3,2	122,0±3,9	117,2±3,4	120,5±6,1
After 30 days	143,2±5,2*	149,3±4,4*	140,2±2,8*	148,0±7,4*

***Note:** here and in other tables are statically significant differences compared to the original.*

In the course of studies in a subchronic experiment between animals receiving test drugs and control animals receiving distilled water, statistically significant differences in body weight and its growth were not detected (table 4).

As can be seen from the data in table 5, statistically significant differences in the mass of organs between groups of animals treated with different doses of experimental samples of substrates compared with control animals were not observed during the study.

Table 5. The effect of various doses of selenium nanoparticles stabilized on biodegradable polymer substrates of natural origin on the mass of internal organs with repeated injections

Groups of animals				
Organ	Control (water)	The drug "A" (30 mg / kg)	The drug "A" (60 mg / kg)	The drug "A" (160 mg/kg)
Liver, g	4,21±0,26	4,58±0,24	4,79±0,29	4,82±0,25
	2,79±0,09	2,95±0,17	3,02±0,15	3,04±0,14
Kidneys, g	1,41±0,07	1,29±0,08	1,31±0,05	1,27±0,09
	0,87±0,04	0,80±0,06	0,78±0,05	0,80±0,06
Spleen, g	0,55±0,03	0,51±0,04	0,54±0,05	0,55±0,04
	0,32±0,02	0,31±0,01	0,34±0,03	0,35±0,03
Stomach, g	1,75±0,08	1,68±0,07	1,77±0,09	1,82±0,05
	1,10±0,02	1,15±0,03	1,18±0,05	1,21±0,07
Heart, g	0,80±0,02	0,75±0,05	0,78±0,04	0,79±0,06
	0,50±0,01	0,48±0,01	0,49±0,01	0,50±0,01
Lungs, g	1,01±0,05	0,99±0,04	1,08±0,06	1,07±0,07
	0,62±0,02	0,60±0,03	0,61±0,05	0,67±0,04
Brain, g	2,02±0,07	1,91±0,06	2,07±0,07	2,09±0,06
	1,20±0,03	1,22±0,03	1,24±0,05	1,27±0,07
Adrenal gland, mg	116,8±5,9	121,0±9,8	108,3±7,8	124,8±5,9
	71,7±5,3	73,6±6,4	70,1±3,9	80,1±6,3

Pancreas, mg	178,3±8,7	188,3±11,1	177,5±4,9	193,1±8,6
	112,0±6,2	121,9±7,5	117,7±5,8	128,9±8,8
Thymus mg	303,0±24,3	299,0±32,6	323,3±26,6	291,3±23,6
	191,8±20,1	181,3±15,3	212,2±20,5	191,7±16,5

Note: *in the numerator is absolute, and in the denominator is the relative weight of the internal organs.*

The results of hematological studies showed that in rats after intragastric administration of the drug there were no toxic effects with respect to the blood system (table 6). After prolonged administration of the drug to laboratory animals, no pronounced changes in the biochemical parameters of blood serum were observed, which could indicate toxic changes in the internal organs (table 7). During a visual examination of the external state of the body, conducting a macroscopic pathological and anatomical examination of the internal surfaces and passages, the cavity of the skull, chest, abdominal and pelvic cavities with organs and tissues located in them, the neck with organs and tissues, as well as the injection sites, morphological signs of deviations from the generally accepted norm associated with the action of the studied drug was not identified.

Table 6. The effect of various doses of selenium nanoparticles stabilized on biodegradable polymer substrates of natural origin on hematological parameters of rats with repeated injections indicators

Indicators	Group of animals			
	Control (water)	The drug “A” (30 mg / kg)	The drug “A” (60 mg / kg)	The drug “A” (160 mg / kg)
White blood cells (WBC), 10^9/L	14,15± 0,51	14,97±0,69	13,48±0,44	14,61±0,49
The absolute content of lymphocytes, 10^9/l	6,59±0,41	5,91±0,56	6,08±0,59	6,44±0,66
The absolute content of the mixture of monocytes, basophils and eosonophils, 10^9/l	2,57±0,22	2,39±0,18	2,42±0,35	2,55±0,29
The number of granulocytes, 10^9/l	5,22± 0,34	5,07±0,49	5,19±0,43	5,28±0,37
Hemoglobin (Hb), g/l	136,6±3,71	130,8±4,1	125,9±6,9	137,8±3,9
Red blood cells (RBC), g/l	6,61±0,46	6,84±0,38	6,43±0,28	6,72±0,47
Hematocrit (HCT),%	36,71±1,91	35,13±1,57	34,18±1,92	37,53±2,29
Platelets in Absolute Numbers (PLT), 10^9/l	556,3±40,3	523,8±47,1	571,8±51,1	581,5±46,9
Thrombocrit (PCT),%	0,49±0,04	0,52±0,05	0,49±0,04	0,59±0,06

Table 7. The effect of various doses of selenium nanoparticles stabilized on biodegradable polymer substrates of natural origin on biochemical parameters of rat blood during repeated injections alkaline phosphatase.

Indicators	Groups of animals			
	Control (water)	The drug "A" (30 mg/kg)	The drug "A" (60 mg/kg)	The drug "A" (160 mg/kg)
ALT, Unit/l	72,42±6,32	78,33±6,24	73,51±4,89	68,72±6,44
AST, Unit/l	266,2±12,2	272,9±14,1	281,1±17,8	261,8±14,6
CFU, Unit/l	352,1±32,2	328,4±30,3	321,9±33,4	369,4±30,9
Glucose, mmol/l	6,15±0,29	5,72±0,39	5,54±0,46	5,81±0,37
Total protein, g/l	67,49±3,75	61,01±2,56	62,88±4,72	63,23±4,42
Albumin, g/l Urea, mmol/l Creatinine, μmol/l	41,28±1,82	44,18±2,91	40,16±3,06	44,13±1,11
Total bilirubin, μmol/l	12,28±1,32	11,35±0,98	12,33±0,88	11,88±1,08
Direct bilirubin, μmol/l	3,44±0,64	3,43±0,28	3,18±0,31	2,98±0,22
Indirect bilirubin, μmol/l	8,84±0,78	7,92±0,68	9,15±0,89	8,91±0,82
Urea, mmol/l	8,22±0,61	8,78± 0,52	8,01±0,61	8,56±0,49
Creatinine, μmol/l	56,25±2,58	51,52±3,84	54,41±3,24	57,18±4,38

Therefore, the subchronic administration of the drug "A" in various doses, the judge did not have a significant effect on the results of studies of the dynamics of the animal's body weight, internal organs mass, hematological and biochemical parameters of the blood, which indicates the absence of a negative effect of the drug upon repeated administrations.

At the same time, it can be stated that the drug "A" does not have a cumulative property, because even in large doses of 1/10 part, the average lethal dose of the drug does not lead to the death of animals.

CONCLUSIONS

The synthesis of stabilized selenium nanoparticles by chemical reduction of selenium cations with ascorbic acid in Na-CMC solutions was first performed. It was established by optical and atomic force microscopy that the sizes and shapes of selenium nanoparticles vary depending on the conditions of the reduction reaction.

Based on the results of experimental studies, it was found that, depending on the ratios of CMC, SeO_2, ascorbic acid, and reaction conditions, the size and shape of stabilized selenium nanoparticles that form in aqueous solutions during the chemical reduction of Se^{4+} change.

The resulting CMC solutions containing selenium nanoparticles open up prospects for the creation of broad-spectrum drugs based on them, in particular, antitumor drugs that reduce the negative effects of radiation and chemotherapy on the body, and drugs that compensate for the deficiency of selenium in the body.

The medicinal compound created on the basis of selenium nanoparticles stabilized in the CMC structure according to toxicological properties can be classified as class IV compounds (low toxicity according to OECD classification). In this case, the acute toxicity of the drugs does not significantly differ

in different species and sex of animals, however, they exhibit higher toxicity with parenteral administration than with enteral.

The studied compounds containing nanoparticles of selenium stabilized in the CMC structure do not have a meso-irritating and skin-resorptive effect.

The drug "A" containing selenium nanoparticles stabilized in the CMC structure does not have toxicity after repeated injections, which is reflected in the results of physiological, hematological and biochemical studies.

Obtained biodegradable materials, contained selenium nanoparticles can be used in medicine as anticancer drug for the treatment cancer disease.

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