Immobilization of cardioprotective drug phosphocreatine on a surface of nanoparticles of silica

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Abstract. In this work silica aminated nanoparticles were used to show capability for chemisorbing organic compound having a carboxyl group. Phosphocreatine (creatine phosphate) was used as an active ingredient. Since the method for determination of phosphocreatine with the sample analysis using Jaffe reaction didn’t give a positive result, the definition of free phosphocreatine was carried out by the method of diacetyl in the presence of α-naphthol.

1. Introduction
The development of nanochemistry and nanomedicine allowed to look at the use of nanoparticles as a transporter for medicines. More recently, there have been works demonstrating the possibilities of prolongation of the action [1] and address delivery in a myocardium of cardioprotectors with a use of different carriers [2], the authors proposed to use nanoparticles of silica (NPS) for these purposes earlier [3].

Colloidal silicon dioxide which is a pyrogenic silica nanoparticle produced under the trade name of Aerosil is used in the pharmaceutical industry as an auxiliary. As a traditional sliding agent Aerosil provides the optimum flowability of powders necessary for modern high-speed tableting [4]. Unique properties of Aerosil allow it to be used in solid dosage forms, gels and ointments as a filler.

We used the silica nanoparticles (SN) Polysorb-300 pre-modified with the method [6]. SN aminated via specific way (Figure 1a) using 3-aminopropyltriethoxysilane can chemisorb organic compound having a carboxyl group [7]. We used the phosphocreatine (creatine phosphate) as this active substance has cardioprotective properties.

The phosphocreatine which is produced under a trademark Neotone [8] is the medicine improving metabolism in warm and other muscular tissue. On chemical structure it is similar to macroergic endogenous phosphocreatine. The drug slows down process of destruction of a sarcolemma of ischemic cardiomyocytes and other muscular elements, provides intracellular transport of energy.
Due to microcirculation improvement medicine reduces the size of a zone of a necrosis and ischemia. It causes an antiarrhythmic effect in case of ischemia and post-ischemic reperfusion which is associated with a decrease in the ectopic activity of the ventricles and the preservation of the physiological function of the Purkinje fibers.

2. Experimental methods

The scheme of immobilization of phosphocreatine on the surface of SN is shown in Figure 1b.

Figure 1. The scheme of synthesis: amination of the nanoparticles (a) and immobilization of Phosphocreatine (b)

Phosphocreatine in an aqueous-alkaline solution easily passes into creatinine by cleaving phosphoric acid (Figure 2). This transition is easily evaluated with the reaction of Jaffe [9] based on the reaction of creatinine with sodium picrate.

Figure 2. Diagram of the transition creatine phosphate-creatinine

Creatinine reacts with alkaline picrates forming a red complex. The change in optical density of the formed complex is proportional to the concentration of creatinine in the sample. A solution of phosphocreatine concentration 2 mg/ml was prepared as follows, a portion of the phosphocreatine weight of 105.5 mg dissolved in 50 ml of 0.1 N hydrochloric acid. A solution of phosphocreatine was prepared similarly in 0.3 N sulfuric acid. Immobilization of phosphocreatine on the surface of SN was carried out as follows. 1 ml of 0.1 N sodium hydroxide solution and 1 ml of an aqueous solution of phosphocreatine in 0.1 N hydrochloric acid of 2 mg/ml concentration were added to 50 mg of aminated nanoparticles. Sorption was carried out in polypropylene tubes with a capacity of 15 ml on a shaker LS-220 (LOIP, Russia) at a stirring rate of 300 min⁻¹ for 2 hours. The solution was then centrifuged for 5 minutes at 3000 min⁻¹ and washed 5 times with distilled water and centrifuged. The resulting precipitate was dispersed on ultrasonic disperser UZD-2 (Ultrasound Technics, Russia) for 5 minutes in distilled water in the required concentration.
3. Results and discussion

Micrographs of NPs were obtained with a use of the transmission electron microscope (TEM) JEM-1400 STEM cathode (JEOL, Japan). Micrograph of modified Aerosil is shown in Figure 3.

![Micrograph of modified Aerosil](image)

Figure 3. Micrograph of modified Aerosil

To determine the content of active substance in the sample calibration dependence was built. In samples containing phosphocreatine in quantities 4.216, 8.432, 12.648, 16.864, 21.080 µg 3 ml of aqueous solution of picric acid 17.5 mmol/l and 0.2 ml of an aqueous solution NaOH concentration of 2.5 mol/l was added. The solution was left for 10 minutes, then the solution volume was adjusted to 100 ml and the optical density relative to the blank sample only containing picric acid and NaOH in specified quantities was measured at a wavelength $\lambda = 510$ nm. Experiments were repeated 3 times.

2802S Unico spectrophotometer (Unico Sys, USA) was used for determining the optical density of the absorption spectra.

The measured optical density of a solution containing phosphocreatine in different concentrations did not differ from the optical density of the blank sample. This indicates the absence of transition creatine phosphate-creatine.

A blank sample solution of picric acid and a solution with a maximum content of phosphocreatine (21.080 µg) was prepared for a more detailed study of the discovered fact. With the help of spectrophotometer the absorption spectra of the solutions in the wavelength range 450–1100 nm was received. The obtained results (Figure 4) indicated the absence of absorption bands of creatinine (500–600 nm) with a maximum content of phosphocreatine in the sample.
Figure 4. Absorption spectra of a blank sample (1) and a sample of Phosphocreatine relative to a blank sample (2)

Since the method for determination of Phosphocreatine with the sample analysis using Jaffe reaction didn’t give a positive result the definition of free Phosphocreatine was carried out with the method [10] of diacetyl in the presence of \( \alpha \)-naphthol.

We used 1% alkaline solution of \( \alpha \)-naphthol. The alkaline solution contained 6 g of NaOH and 16 g of Na\(_2\)CO\(_3\) in 100 ml of water.

A solution of diacetyl was prepared as follows. We took 1.6 g of dimethylglyoxime in Wurtz flask adding 5N H\(_2\)SO\(_4\) with a volume of 200 ml. Flask was placed on a sand bath and distillate was distilled. The first 50 ml of distillate was collecting and the volume was adjusted with water to 100 ml. Obtained 1% solution of diacetyl was stored at +4 °C in the refrigerator. The solution was diluted to the concentration of diacetyl is 0.05% before use.

To study the applicability of the method for determination of the content of phosphocreatine, a sample was prepared with a zero (blank sample) and its maximum concentration. 1.0 ml of a 1% solution of \( \alpha \)-naphthol and 0.5 ml of a 0.05% solution of diacetyl were added for this purpose containing no phosphocreatine and to a sample containing 21.080 \( \mu \)g of the latter. The volume of the solution was adjusted to 5 ml with distilled water, thoroughly mixed and left at room temperature for 30 minutes in the dark.

Samples were analyzed spectrophotometrically for absorption in the wavelength range 450–1100 nm. The obtained spectra show that at a wavelength of 540 nm there is an amplitude shift dependent on the concentration of the active substance (Figure 5).
The determination of the calibration curve was made for samples with a phosphocreatine content of 4.216, 8.432, 12.648, 16.864, 21.080 µg. Then the optical density of the solution relative to the distilled water was measured at a wavelength $\lambda = 540$ nm.

The results of determining the optical density of solutions depending on the content of the active substance are shown in Figure 6.

The procedure was then repeated in a more acidic medium replacing the acid from the monobasic to the dibasic acid (sulfuric) in order to separate the inorganic phosphate.
As it can be seen from the figure, increasing of the acidity of the solution affects the concentration dependence of the average optical density. In further work we used the solution of phosphocreatine prepared with 0.1 N HCl since the increased acidity affects the sorption capacity of aminated nanoparticles.

Sodium hydroxide in a quantity of 5 ml was added to determine the concentration of phosphocreatine chemisorbed on the surface of the SN and desorption was carried out for 15 minutes with manual shaking. The resulting solution was centrifuged and the above the sediment was taken in a volume of 1 ml and analyzed for the content of free phosphocreatine with the method described above at a wavelength $\lambda = 540$ nm.

The content of phosphocreatine determined using a pre-constructed calibration curve equaled to 0.04 mg which corresponds to a concentration of 0.8 mg or 4 µmol on 1 g of silica.

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