Potential targeting of the stroke by means of the layered nano- and micro-particles

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Abstract

Background. Targeted type of delivery of the medicines is the most effective way to treat diseased tissues. We propose, in experimental settings the efficient delivery of Proline Rich Peptide (PRP; novel compound, serving for the neuroprotection), PP (general activator of Phosphoribosyl Pyrophosphate Synthase -1 (PRPS-1; EC=2.7.6.1), regulative enzyme of purines and pyrimidines syntheses) as well as allopurinol, inhibitor of Xanthine Oxidoreductase (XOR, which includes Xanthine Oxidase; XO (EC=1.17.1.4) and Xanthine Dehydrogenase XDH (EC 1.17.1.4), the regulative enzyme of purine catabolism) by means of PEG-ylated and not PEG-ylated albumin nanoparticles will possibly stimulate fast regeneration and protection of the brain tissue in the experimental stroke settings.

Methods. For determination of the size of the particles it was used electron transmission microscopy technique (Philips CM-100 TM, USA). The loading and the controlled release of the medicines was evaluated by the colorimetric methods (Cary 60, Agilent, USA). Circulation abilities of loaded albumin particles were compared with PEG-ilated particles.

Results. By the utility of the modified desolvation method, we were able to create the particles from <100 nm to < 2 microns diameter. The sedimentation methods allow us to control the size of the obtaining particles. PEG-ilated particles are more stable in water as well as in the trypsin environment. Release of most of the medicines predominantly occurred during day $3t^h$; the effect was stronger in not PEG-ilated partciles.

Conclusion. We created layered with the different medicines PEG-ylated and not PEG-ylated particles, which might target different pathological stages of experimental stroke development.
Key words: albumin particles, PEG, dexamethasone, allopurinol, phosphates, PRP, in vivo.

Introduction

Medicines, after appearance in blood stream, might adhere to the proteins by not specific formation of the bonds. After attachment with the native carriers medicines or chemical compounds retain their long circulation time. It was shown, albumin particles attached to the medicines decrease the activation of the complement, reduce the macrophage-related phagocytosis as well as prevent binding of the compounds to the IgG (Peng et al., 2013).

It was shown, the albumin nanoparticles might prevent formation of the hemorrhages (Liu et al., 2021). Meantime, albumin as the main protein preserves the oncotic pressure of blood, prevents extensive development of the brain edema in the settings of the experimental stroke (Belayev L et al., 2005). Unfortunately, the clinical trials are evidencing about the exacerbating effect of the albumin due to the increase of the pulmonary dysfunctions after stroke, which was increasing the percentile of the deaths (Martin RH et al., 2016). However, clinical trial results might be reviewed for the corrections related with the doses, time of the treatment as well as the formation of the specific group of the patients, who didn't previously developed pulmonary dysfunctions. Also, by injection of the albumin particles instead of free albumin we don't strongly increase the oncotic pressure of blood, consequently might not be facing previously detected side effects of stroke patients.

We propose, the attachment of the medicines to the albumin particles will prolong circulation time of the compounds. From the other hand, albumin in the limited extent will preserve the oncotic pressure of blood and prevent the enlargement of brain edema in case of its utility in the settings of experimental stroke.

In the frames of our investigations we included the preparation of the albumin nanoparticles with the diffused saturation with the several medical compounds: allopurinol (as the inhibitor of Xanthine Oxidoreductase (XOR; EC=1.17.1.4) and preventer of ROS generation), inorganic phosphate groups (as the activators of PRPS-1 enzyme (EC=2.7.6.1), which is regulative and responsible for the syntheses of purines and pyrimidines), Proline Rich Protein-1 (PRP-1), which is, in accordance to our investigation, is diminishing the degradation processes of the NPs (Nano Partciles) because of the wide proteases' inhibiting abilities and serving as the neuroprotective agent. Also, created by us particles include dexamethasone, which is able to diminish the aggressive inflammation processes after stroke.

To create the protective shells for the particles, we decided to cover them with PEG (poly ethylene glycol-6000). We suggest, PEG shells are able to hide the peptides from blood proteases.

In our previous experiments, we have shown, the stimulation of the purine and pyrimidine metabolism will trigger the processes of the recovery after stroke (Danielyan et al., 2019). Moreover, similar results are possible to obtain, if to inhibit the purines catabolism via the inhibition of the key regulative enzymes, XOR (Danielyan and Simonyan, 2017).

Allopurinol, the classical inhibitor of XOR, is preventing formation of the free radicals as well as stimulating the cells proliferation in vitro (Danielyan and Kevorkian, 2011).

Stimulation of the activity of the other enzyme, such as PRPS1 (PRPS-1; EC=2.7.6.1), by regulation of the purines as well as pyrimidine syntheses, is promoting the cells division and even neurogenesis (Danielyan et al., 2019).

Galoyan AA and co- authors for the first time discovered the novel neurohormones-like peptides from neurosecretory granules of human and bovine neurohypophysis (Galoyan, 2004). Most of them are rich with the naturally very rare structural amino acid-Proline. Such phenomenon is very well documented for the family of the peptides synthesized in salivary glands (Carlson, 1993). "Members" of newly discovered family of neuropeptides were numbered by

Galoyan AA (Gladkevich et al., 2007) and co-authors from PRP-1 to PRP-4 and contain 15, 14, 13, and 10 amino acids, respectively. Moreover, identical peptide (PRP-5) was identified on the peptide map of human hypothalamus (Galoyan et al., 2007). According to mass spectral analysis the molecular mass of human PRP-5 is 1560.5 Da. Three amino acids in the human PRP differ from the bovine analog (Gladkevich et al., 2007). Localization of bovine PRP for the first time was revealed via immunohistochemistry. It was demonstrated that PRP exists in the magnocellular neurons of the nucleus supraopticus (NSO) in parvocellular neurons concentrated in rostral and medial subdivision of the nucleus paraventricularis (NPV) of the rat. Also, it was shown that PRP immunoreactivity is higher in the NSO than in the NPV (Galoyan et al., 2007). According to authors these peptides are generated by proteolysis of the C-terminal neurophysin-vasopressine associated glycoprotein and along with vasopressin and oxytocin might be transferred from the hypothalamus to the neurohypophysis by axonalal transport (Gladkevich et al., 2007). Physiological range of the PRP effects is wide, which might be explained by it's neurohormonal nature of generation and neuroprotective abilities (Galoyan et al., 2007; Gladkevich et al., 2007). Biological effects of them predominantly are depended on the doses, time, initial state of the organism and roots of injection, administration into the organism (Gladkevich et al., 2007).

It is proved, the life terms of the small peptides in blood stream is very short. Thus, we have decided to prolong PRP-1 circulation time in blood stream by coupling it with the albumin particles. Also, the single, long term acting injection might diminish the dose of the peptide as well as will reduce the possible treatment expenses.

We propose, the layered with four different compounds: PRP, PP, allopurinol, dexamethasone, albumin particles, protected with the PEG shell, will circulate longer and possibly will target different stages of experimental stroke: cell death, inflammation, generation of ROS, regeneration.

Methods

Transmission electron microscopy. Albumin microcarriers and nanoparticles were analyzed with TEM (Electron Transmission Microscopy,). A drop of the sample was placed on the polyvinyl formal-coated 200-mesh copper grids for 2 min, and then washed with Milli-Q water. The grids were stained with 2% uranyl acetate. The stain was wicked off using a filter paper and the grids were dried. Grids were analyzed at 25 000 magnification. The nanoparticles were generated based on the transmission electron microscopy (TEM) results (Figure 1B), (Philips CM-100 TM, USA).

Generation of the albumin particles. Five hundred microliters of 10% bovine albumin (Arpimed, Armenia) was dissolved in 1 ml of water. Glutaraldehyde (GA; 10 uL, 40 %, Medisar, Armenia; GA) was added along with the 1 ml of lithium carbonate solution into the mixture (n = 6). This mixture was incubated for 24 h. The mixture was centrifugated at 14000 RPM for 30 minutes. The precipitate was dissolved in 1 ml of water. The particles were washed from the remaining parts of the GA 3 times. The remaining part of the precipitate was dissolved in 1000 μl of saline. The schematic representation of the particles preparation is presented by the **Figure 2**.

Determination of the proteins. Determination of the proteins were performed based on the Lowry method. The absorption was determined by means of the spectrophotometer (Agilent, Cary 60, USA) at λ=730 nm **(Lowry et al., 1951)**.

Determination of non-organic phosphates. Solution for the phosphates detection was containing the concentrated sulfuric acid, 4% ascorbic acid, 0.3% anthimony potassium tartrate and sodium molybdate (4.5%). The absorption was detected by the Cary 60 Spectrophotometer (Agilent, USA) at 700 nm wavelength (Danielyan and Chailyan, 2020).

Determination of allopurinol concentration. The 0.1 M hydrochloric solution of allopurinol has two peaks of the absorption. The absorption was detected by the Cary 60 Spectrophotometer (Agilent, USA) at 250 nm wavelength (Belikov, 2009).

Determination of dexamethasone. The ethanol solution of dexamethazome has two points of the absorptions. The absorption was detected by Cary 60 Spectrophotometer (Agilent, USA) at 235 nm as the maximal point of absorption (Belikov, 2009).

Measurement of zeta potential of the particles.

Measurement of zeta potential was performed by the utility of modified Stolz, S. method (Stotz, 1978), where under the conditions of high electrical pressure (V=7 V) the particles were moving in the 0.1M PBS with pH=7.4. The movies in of the movements of the particles in the cell counter chamber were taken under the trinocular light microscope (Boeco, Germany). Calculations were performed based on the following equation (1):

$$\xi = \frac{\mu\ (6\pi\eta)}{\varepsilon} \quad (1)$$

where **ξ is the Zeta potential (mV), μ-mobility $cm^2/V*t(s)$, η-** viscosity(Pa * t(s)) of 0.1 M PBS, Ɛ-permittivity (F/m or $F \cdot m^{-1}$) of water (DELGADO and GONZÁLEZ-CABALLERO, 2005).

Statistical analysis of the results. Data are expressed as mean ± SEM. Statistical significance between control and experimental groups were analyzed by One-way analysis of variance (ANOVA) or student t-test. Results were accepted as the statistically significant, when $p<0.05$.

Vertebrate Animals. The all-animal study experiments were performed based on the IACUC policies and animal care standards, regulations adopted by the Armenian Ethical Committee of Institute of Biochemistry named after H Buniatian, National academy of Science of Republic of Armenia. Standard procedures and protocols: anesthesia, euthanasia, animal surgery and collection of blood were performed based on the above-mentioned regulations. The animals, white laboratory rats, were anesthetized by administration of Pentobarbital 2 mg/ 100 mg of weigh, injected I.P, using a hypodermic needle. With this dosage,

animals are normally anesthetized within 5 min. Depth of anesthesia were determined by squeezing the animals' leg with forceps. Absence of movement in response to this procedure was indicate sufficient anesthesia. If insufficient, additional doses of the anesthesia were given. During surgery cranial and rectal temperature continuously were controlled as these parameters reflect the normal animal physiological state. After 7 days animals were deeply anesthetized and euthanized by the cervical dislocation. All post-operative observations were documented.

Results

Detection of the efficiency of the bound medicines with the particles. The first set of the experiments was devoted to the evaluation of the percentile of the bound with the particles compounds. After the final preparation the particles were sedimented at G=14000 for 30 minutes and in the supernatant the mentioned above compounds were measured

Results of the binding experiments were the following: 100% ±5,30% binding for PRP, 34,33%±1,20% binding of the inorganic phosphates, 60,61%±2,00% - for the dexamethasone and 62.32%±3,80% for allopurinol.

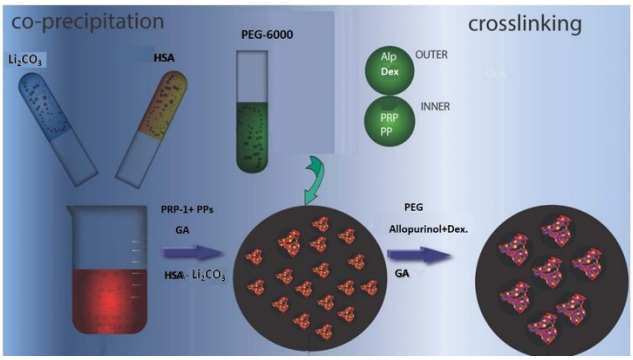


Figure 1. A. Formation of the albumin nanopartciles by the utility of CCD method (Chaiwaree et al., 2020). First stage of the formation. GA as well as the ions of Li+ are used for the polymerization and NP formation. B. During the second stage to the ready and washed from GA albumin particles, containing PRP-1 and phosphates was added 6000 units containing PEG as well as new portion of GA. Along with the PEG it was added dexamethasone as well as allopurinol.

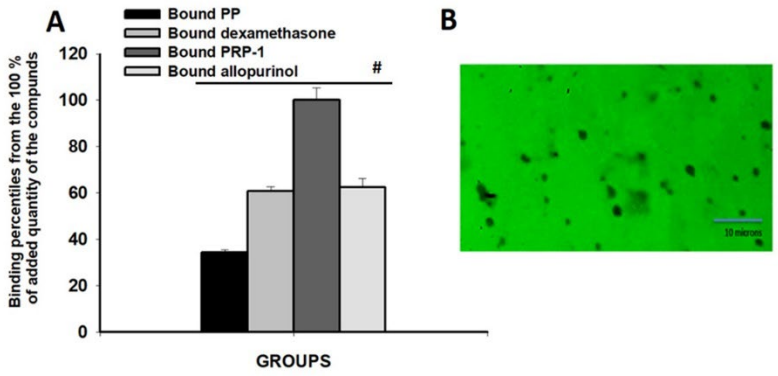


Figure 2. A. Binding of the medical compounds as well as inorganic phosphates with the albumin particles. There was used the Cary 60 spectrophotometer with the scanning abilities range from 190-1100 nm. PRP-1 unbound quantity was measured by the application of the Lowry method. The real unbound quantity was determined after normalization with the control particles sample, which didn't contain any peptide, however was degrading over the time in the aqueous solution. The absorption of the unbound proteins was measured at 730 nm. Ethanol solution of dexamethasone absorption was chosen to serve for the detection of its quantity. Experimentally it was delineated 235 nm as the maximum of its absorption. The allopurinol was dissolved in the 0.1 M hydrochloric acidic solution based on the scans, was measured at the 250 nm wavelength. Inorganic phosphates after colorimetric reaction proceeding were measured ate the 700 nm of the wavelengths. ANOVA-ONE-WAY was applied for the delineation of the statistical significance of differences between the groups. **B. Determination of the size as well as the shape of the particles for the further utility of them for in vivo and in vitro experiments.** It was applied the 25000 times magnification. Philips CM-100 TM Electron Microscopy was used for the above mentioned purpose.

Particles degradation experiments in the environment of the buffer as well as trypsin. In the second set of the experiments there were used 2 different environments for the detection of the degradation processes of the particles covered and not covered with PEG. The incubation of the particles was performed over the 72 hours. Maximum of the proteins' quantity diffused into the solutions was detected during the 3^{th} day of incubation.

Albumin particles degraded during the first 24 hours in water and trypsin environment accordingly 1) $1,5^{e-3}\pm7,0^{e-4}$; $0,102\pm5,314^{e-3}$ mg/ml of the protein solution. PEG-ylated partciles degraded in water/trypsin solutions with the

release of the following amount of the PRP-1 1) $7,0^{e-3}$ $\pm 1,6^{e-3}$; $0,031\pm 4,068^{e-3}$ (**Fig. 3A**). During the second day albumin particles degraded with the release of PRP-1 equal to $0,0367\pm 3,3189^{e-3}$ and $0,1229\pm 4,9900^{e-3}$, whereas the PEG-ylated micro and nanopartciles degraded with the PRP-1 and protein release equal to $0,0249\pm 6,4687^{e-3}$ and $0,1031\pm 1,8441^{e-3}$; mg/ml (**Fig. 3B**). During the 3rd day the quantity of the peptide and proteins in 2 different solutions were equal to $0,0500\pm 3,3189^{e3}$,$0,1800\pm 4,9900^{e-3}$, $0,0150\pm 3,3189^{e-3}$,$0,1000\pm 4,9900^{e-3}$ mg/ml ($p<0.05$ in the groups of degradations during the first and second days), (**Fig. 3C**).

Dexamethasone along with the allopurinol was chemically bound with the surface of the PEG-covered and not covered particles. As it is clear from the **Figure 3D,** even surface bound dexamethasone was released into the environment more slowly for the PEG-coved albumin vs albumin particles. The statement was true for the water as well as the trypsin environments over 3 days. During the first day dexamethasone was released from the albumin particles in water and trypsin solutions in the quantity equal to 0,5843±0,0223 vs 1,0227±0,0987 mg/ml and from PEG-ylated particles 0,2230±0,1170 vs 0,4933±0,0500 mg/ml; during the 2nd day 0,5457±0,0363 vs 0,4647±0,05 mg/ml in comparison with the PEG-ylated type 0,2533±0,0227 vs 0,35±0,20 mg/ml; at the day 3th 0,8250±0,0477 vs 1,2653±0,0647 mg/ml in comparison with the PEG-ylated type 0,4493±0,0421 vs 0,6883±0,0623 mg/ml.

Despite, technically allopurinol was attached to the surface of the particles; however, the most quantity of it was released during the second and in 3th days (**Figure 3 G,H,I**). During the first day from albumin particles in the water and trypsin solutions the quantity of the allopurinol per ml was equal to 0,1059±0,0119, 1863±0,0274 mg in comparison for the PEG-ylated particles in the same types of the environmental solutions $0,0625\pm 6,9214^{e-3}$; $0,1608\pm 6,0^{e-3}$ (**Figure G**). Over the second day in the same groups the allopurinol quantity was equal to the $0,0894\pm 4,9286^{e-3}$; $0,0757\pm 5,0^{e-3}$ vs $0,091\pm 4,8571^{e-3}$; 0,0689±0,01 and, during the 3rd day 0,0917±0,0113; 0,0824±0,0100 vs 0,0800±0,0145;$0,0700\pm 8,5714^{e-3}$ (**Figure 3 H,I**). In-group statistical calculations

are evidencing about the significant decrease of allopurinol release for albumin vs PEG-ylated particles.

As it was mentioned in the project, we proved, none organic phosphates are able to promote regeneration of the neuronal tissue in vivo as well as in vitro.

Regeneration of the tissue after stroke is the late post stroke stage. Thus not organic phosphates during our preparations were included into the core part of the albumin particles, to be released at the later stages of the particles degradation.

Based on our experiments, we concluded, not organic phosphates's release was noticed in the trace amounts in all groups (**Figure 3J,K,L**). During the first day the phosphates quantity in ml of the aqueous supernatant vs trypsin environment for albumin vs PEG-ylated partciles was equal to $2{,}2778^{e-4}\pm 9{,}8952^{e-6}$; $3{,}2778^{e-4}\pm 5{,}8952^{e-6}$ vs $3{,}3333^{e-5}\pm 1{,}3799^{e-5}$; $4{,}3333^{e-5}\pm 1{,}3799^{e-5}$; for the second day $3{,}3889^{e-4}\pm 5{,}3099^{e-5}$; $6{,}4444^{e-4}\pm 8{,}0^{e-5}$ vs $1{,}8333^{e-4}\pm 1{,}9446^{e-5}$; $5{,}3889^{e-4}\pm 5{,}3099^{e-5}$ and for the 3th day $5{,}6111^{e-4}\pm 8{,}3163^{e-5}$; $7{,}6111^{e-4}\pm 8{,}3163^{e-5}$ vs $6{,}1667^{e-4}\pm 4{,}8718^{e-5}$; $2{,}8889^{e-4}\pm 9{,}0420^{e-5}$.

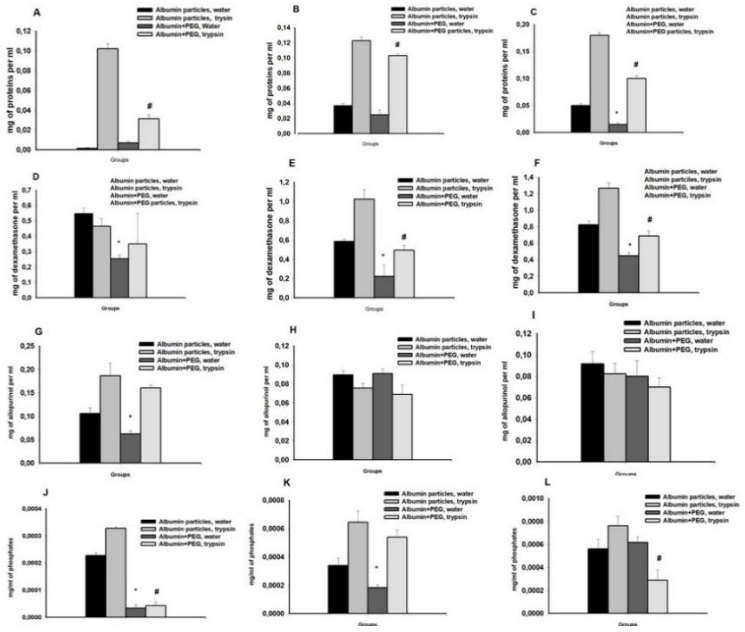
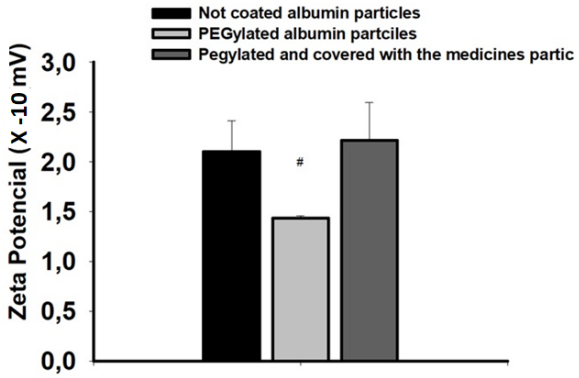


Figure 3. A., B, C. Degradation of the PEG-covered vs not covered albumin particles in the environment of the water and presence of the protease- trypsine. Binding process was determined by the utility of Lowry method for the measurement of the proteins. After centrifugation for 30 minutes at G=14000, in the supernatant, the quantity of the proteins was measured. It was used Cary 60 spectrophotometer (Agilent, USA). The student t-test was used for the determination of the significance of the results in the groups. The results were accepted statistically significant, when $p<0.5$ (n=10 per group). P value between covered with PEG and not covered particles in the environment of trypsin was
Less than 0.001 during day 1. For day two – between the same groups $p<0.05$. During day 3 between Peg-ylated and
Not PEG-ylated groups of the particles in water environment $p<0.05$, for trypsin – $p<0.02$.

D,E,F. Degradation and release of the dexamethasone from the PEG-covered vs not covered albumin particles in the environment of the water and presence of the protease- trypsine. After centrifugation for 30 minutes at G=14000, in the supernatant the quantity of the dexamethasone was measured at 235 nm wavelength. It was used Cary 60 spectrophotometer (Agilent, USA), ($p<0.05$ in the groups of degradations during the first and second days).
Dexamethasone release from the PEG-covered and not covered particles during day 1 in water environment was less than 0.05, in trypsin dyrig day 2 less than 0.05, during day 3 in water less than 0.05 and in trypsin less than 0.05 as well.

G,H,I. Degradation and release of the allopurinol from the PEG-covered vs not covered albumin particles in the environment of the water and presence of the protease- trypsine. After centrifugation for 30 minutes at G=14000, in the supernatant the quantity of the allopurinol was measured at 250 nm wavelength. It was used Cary 60 spectrophotometer (Agilent, USA).
The value of p for allopurinol release during day 1 was less than 0.05 for PEGylated and not PEGylated particles.

J,K,L. Determination of the not organic phosphates release from the PEG-covered vs not covered albumin particles in two environment: waster vs trypsin containing. Coloring solution was containing the concentrated sulfuric acid, 4% ascorbic acid, 0.3% anthimony potassium tartrate and sodium molybdate (4.5%). The absorption was detected by the Cary 60 Spectrophotometer (Agilent, USA) at 730 nm wavelength.
Statistically significant results were noticed between the groups of phosphates release for day 1 (in water and trypsin solutions between albumin and covered with PEG particles related groups – $p<0.002$, $p<0.002$), 2 (in water between albumin and covered with PEG particles related groups – $p<0.05$), 3 (in trypsin solution between albumin and covered with PEG particles related groups – $p<0.05$).
Evaluation of zeta potential of the particles. To delineate the aggregation as well as the stability phenomena of particles we measured the zeta-potential in phosphate buffer (pH=7.4). For control group of the particles, not covered with PEG the zeta potential was equal to 2,1±0,31(x-10) mV, for the group of protected PEG-ylated particles - 1,44±0,02 (x-10) mV, whereas for the PEG-ylated and loaded with medicines - 2,22±0,38(x-10) mV (p between control group and PEGylated particles <0.05, t-test, **Figure 4**).
Figure 4. Measurement of zeta potential for the particles. The electrical field with the



7 V electrical pressure was created by the electrodes, placed on the surface of the cells counter chamber, filled with the 0.1 M PBS with pH=7.4. The movements of the particles were taped with the further counting of the electrophoretic mobility. Every group was containing lot less than 8 samples (n=8). Results were accounted as the statistically significant when $p<0.05$ (student t-test).

Discussion

Human serum albumin is able to bind medicines as well as the peptides through non-covalent interactions. Amino acidic, thiol as well we carboxy groups might facilitate the tight interaction with the atoms of the ligand (Fasano et al., 2005).

Different methods of the albumin particles preparations are suggested, which includes the emulsion/solvent extraction, polyelectrolyte complexation/complex coacervation method, electrospray technique, nano spray drying, desolvation method, self-assembly. Except of the desolvation method, the others might induce formation of the large scales and sized of the particles.

Emulsion is a mixture of two or more immiscible liquids wherein one or more of the liquids are dispersed into another liquid (Yang et al., 2007). By taking into the consideration, that proteins are amphoteric with multiple charged functional groups, they can be made cationic or anionic by adjusting various factors such as the pH of the protein. As soon as the proteins become charged they can interact with the other types of the charged molecules. The pH-dependent electrostatic interaction between proteins and other polymers, such as DNA can be used to design stable biocompatible nanoparticles (Truong-Le et al., 1998).

Nano spray drying is a technique used in processing of the nanoparticles in liquid samples. Liquid samples are sprayed into chambers where heated nitrogen and carbon dioxide gas flow in the direction of spray from the nozzle (Oliveira et al., 2013). Micelles can be formed when proteins are dissolved in a solution with exceeding critical micelle concentration and the temperature, which is critical for the solution to form nanosized aggregates (Batrakova et al., 2006).

The desolvation method allows the synthesis of nanoparticles through a simple process of adding desolvating agents, such as ethanol and acetone, to protein solutions containing drugs. Desolvating agents change the protein structure and reduce the solubility of the protein, thereby leading to the formation of precipitation in the form of protein nanoparticles (Zhao et al., 2015).

In our current experimental we used desolvation method with the following crosslinking with the GA and surface modifications, performed with the PEG. By this methodological move we made not too stable core and dense chemically stable shell.

Interestingly, some literature data are evidencing about the possibility of the crosslinking the protein for the formation of the particles by the utility of the N-ethyl-N- (3-dimethylaminopropyl) carbodiimide (EDC) or polyethyleneimine (PEI), which led to the production of spherical particles having an average size of approximately 300 nm. The authors are evidencing, charge was controlled and was varying from -15 to +30 mV. The value of the charge was more prominent and vivid, when the number of the crosslinking's was elevated. Also, the same group showed the more crosslinkings more medicines might be entrapped into the particles. We adjusted the GA quantity to be added by our previous experiments, where the size of the particles also was sensitive to the quantity of crosslinker (Aganyants et al., 2015).

In the binding experiments the content of the inorganic phosphates included into the particles compositions was lower in comparison with the other types of the medicinal compounds. It might be explained based on the fact that utility of GA is not sufficient for the formation of the chemical bonds between the phosphates and the albumin.

The other compounds were able to bind in comparison with the 100 % of the added their amount for at least of 60 %.

As it is clear from our TEM microscopy results, we generated the micro as well as the nanopartciles with the diameter lower than 100 nm and not more than 1-2 microns.

During degradation experiments, as it was expected, the maximal amount of the proteins was released in the presence of trypsin. PEG-ylated particles were more protected from the impact of water as well as from the influence of the trypsin.

Interestingly, dexamethasone was disattaching from the surface of the particles most prominently during the 3rd day and in the emphasized extent in the presence of trypsin.

In case of allopurinol, which is in accordance to our scheme of the experimental design was attached on the surface of the PEG, was releasing also in the extended way from the surface of the particles in the presence of trypsin.

The quantity of the phosphates was lower in comparison with the medicines attached to the surface of the particles as well as diffusely entrapped into them due to the low binding with the albumin during the preparation procedure.

Based on the results, obtained after measurement of zeta-potential, it was clear, PEG-ylation of the particles was decreasing the positive voltage, probably due to the coverage of the charged amino acids as well as due to the increase in the diameter. Loading and coating the PEG-ylated particles was restoring the charge.

The entire design of the controlled released of the particles was made based on the knowledge about the stages of the stroke disease development. On the surface of the PEG-particles there was attached dexamethasone as well as allopurinol. The first stage after the damage of the tissue belongs to the processes of the inflammation as well as formation of the free radicals. Dexamethasone is the compound to suppress activity of the immune system whereas allopurinol, by serving as the classical inhibitor of XOR is able to prevent formation of the XOR products, which are free radicals as well as uric acid. Moreover, in accordance to our results, allopurinol is able to prevent degradation of the purines and stimulate regenerative processes in the brain tissue (Danielyan and Kevorkian, 2011).

The inner layers of the particles were including inorganic phosphates, which might serve not only for the generation of the ATP but also might activate the PRPS-1, which is the key regulative enzyme for the syntheses of the purines as well we pyrimidines.

Thus, after degradation of the core part of the particles PRP-1, as the neuroprotective agent, as well as phosphates will serve for the protection of the brain cells and will stimulate the processes of the regeneration.

We were able to generate PEG-ilated layered particles, which might be applicable for the treatment of the experimental stroke.

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We state, the conflict of the interests doesn't exist between of the authors, the results are original and have been never submitted for publication.

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