Trakia Journal of Sciences, Vol. 12, Suppl. 1, pp 84-88, 2014

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

INTERACTIONS OF BSA-NANOPARTICLES WITH SOME ELECTROACTIVE DRUGS

B. Tacheva[1], A. Zheleva[2], R. Georgieva[1], W. Tong[3], Ch. Gao[3], M. Karabaliev[1]

[1]Department of Physics and Biophysics, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
[2]Department of Chemistry and Biochemistry, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
[3]MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou, China.

ABSTRACT
The interaction of three different drugs with Bovine serum albumin nanoparticles (BSA-NPs) is investigated in the work. Two phenothiazine drugs, chlorpromazine and thioridazine, and a spin-labeled nitosourea drug (SLCNUgly) are used to investigate the loading efficiency of BSA-NPs. The presented results indicate penetration of the drugs in the BSA-NPs, according to their hydrophobicity.

Keywords: Bovine serum albumin, nanoparticles, chlorpromazine, thioridazine, SLCNUgly, cyclic voltammetry.

INTRODUCTION

Controlled drug delivery and release became an important issue in modern medication area during the last decades. The main goals are to reduce the drug dose rate and to prolong the release time in order to minimize poisonous side effects and to improve the therapeutic efficiency [1, 2].

To achieve these goals suitable intelligent drug carriers are produced using different technological approaches. Micro- and nanoparticles and capsules with soft structure and tailorable properties have gained increasingly interest as vehicles for targeted drug delivery.

Albumin (bovine serum albumin (BSA) and human serum albumin (HSA)), is universally employed as a vehicle of molecular probe [3] and drug carrier [4, 5] for cancer diagnostics [6, 7] and therapy, due to its excellent biocompatibility and biodegradability. The first commercial product based on albumin NPs in oncology is the 130 nm albumin-bound paclitaxel approved by the Food and Drug Administration (FDA) of USA in 2005 [8].

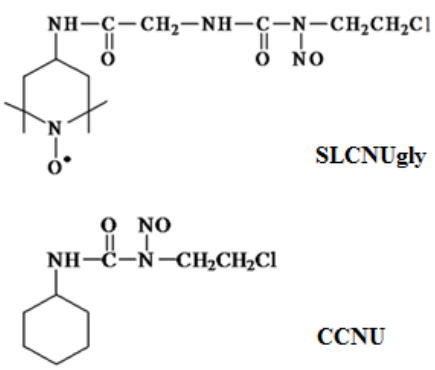
It was shown that albumin nanoparticles are suitable for encapsulation of hydrophobic drugs in solution [5]. In this work we used three drugs of amphiphilic and hydrophobic nature in order to test the incorporation ability of Bovine serum albumin nanoparticles (BSA-NPs). All drugs were electroactive, thus allowing the use of electrochemical methods of investigation [9].

Nitrosourea N-[N′-(2-chloroethyl)-N′-nitrosocarbamoyl]-glycine amid of 2,2,6,6-tetramethyl-4-aminopiperidine-1-oxyl (SLCNUgly) is a spin labeled analogue of the clinically used non-labeled antitumor drug N′-cyclohexyl-N-(2-chloroethyl)-N-nitrosourea (lomustine, CCNU) **(Scheme 1).**

It has been demonstrated in previous in vivo studies that SLCNUgly exhibited lower general toxicity comparing to that of CCNU [10, 11].

Two antipsychotic phenothiazine drugs, chlorpromazine and thioridazine, were used to investigate the loading efficiency of BSA-NPs toward drugs with amphiphilic nature.

***Correspondence to**: *Miroslav Karabaliev, Department of Physics and Biophysics, Faculty of Medicine, Trakia University, 11 Armeiska, Stara Zagora 6000, Bulgaria, miroslav.karabaliev@trakia-uni.bg*



Scheme 1.
N-[N′-(2-chloroethyl)-N′-nitrosocarbamoyl]-glycine amid of 2,2,6,6-tetramethyl-4-aminopiperidine-1-oxyl (SLCNUgly) and N′-cyclohexyl-N-(2-chloroethyl)-N-nitrosourea (lomustine, CCNU)

MATERIALS and METHODS

Preparation of BSA-NPs

The preparation of BSA-nanoparticles was described earlier [12]. The method of preparation was as follows: BSA nanoparticles (NPs) were prepared by a desolvation technique. 200 mg BSA was dissolved in 2.0 mL Milli-Q water and the pH was adjusted to 7.4 with 0.01 M NaOH. Under constant stirring at 500 rpm, 8.0 mL ethanol was continuously added with a rate of 0.5 mL/min using a peristaltic pump. 200 mL of 8% glutaraldehyde solution was added gradually to crosslink the formed BSA particles during desolvation. After 24 h incubation at 20 ^{o}C under constant stirring, the NPs were purified by repeated centrifugation at 10 000 rpm and re-dispersed in Milli-Q water under the assistance of ultrasonication. The BSA NPs were finally freeze-dried and stored at 4 ^{o}C before use.

Cyclic voltammetry

The interactions of the drugs with the BSA-NPs were tested by cyclic voltammetry (CV) because all three drugs are electroactive. Cyclic voltammetry is a convenient method for determination of the charge transfer between an electrode and electroactive drug in the solution. The measurements were made with potentiostat/galvanostat with FRA module VersaSTAT 3F (Princeton Applied Research). Three-electrode electrochemical cell was used in the work. The working electrode was Glassy carbon electrode with 3mm diameter. Ag/AgCl was used as reference electrode and the counter electrode was a Pt wire.

RESULTS and DISCUSSION

Electrorochemical characterization of the drug-BSA-NPs interactions

The drug-NPs interactions were investigated *in situ* in the electrochemical cell. First, the drug was added to the electrochemical cell and the CV was taken. After that BSA-NPs were added into the cell, the solution was stirred with a magnetic stirrer, and another CV was taken. In **Figure 1** are shown two voltammograms of SLCNUgly – taken before and after the addition of BSA-NPs in the solution.

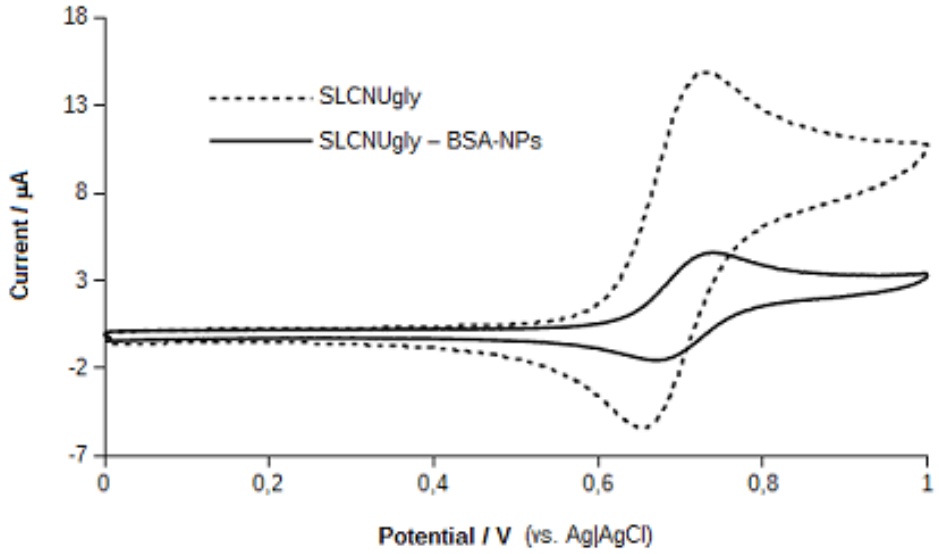


Figure 1. Cyclic voltammograms of SLCNUgly obtained without BSA-NPs (dashed curve) and with 0,3 mg/mL BSA-NPs (solid curve); 0.4 mM SLCNUgly in 0.1 M KCl, 4 mM phosphate buffer (Na_2HPO_3 –NaH_2PO_4), pH 7,4; scan rate 0.1 V/s; (reference electrode - Ag/AgCl)

The presented results suggest that the SLCNUgly drug is incorporated in the BSA-NPs after the NPs addition to the electrolyte solution. After the addition of BSA-NPs both the oxidation and reduction peaks decrease by value, most probably because of the incorporation of the drug in the core of the BSA-NPs.

Similar results were obtained for chlorpromazine and thioridazine. The decrease of their oxidation and reduction peaks was significant but somewhat smaller than the decrease for SLCNUgly (data are presented below in the text).

Another possible explanation of the obtained results is a blocking of the electrode surface by the NPs themselves, thus hampering the charge transfer from the drug molecules in the solution. In order to check this assumption another electroactive specie was used. Potassium ferricyanide ($K_3[Fe(CN)_6]$) and potassium ferrocyanide ($K_4[Fe(CN)_6]$), as equimolar mixture, were used as electroactive species added to the electrolyte. This couple was chosen because it is highly hydrophilic, excluding thus hydrophobic interaction with the BSA-NPs. The results are shown in **Figure 2.**

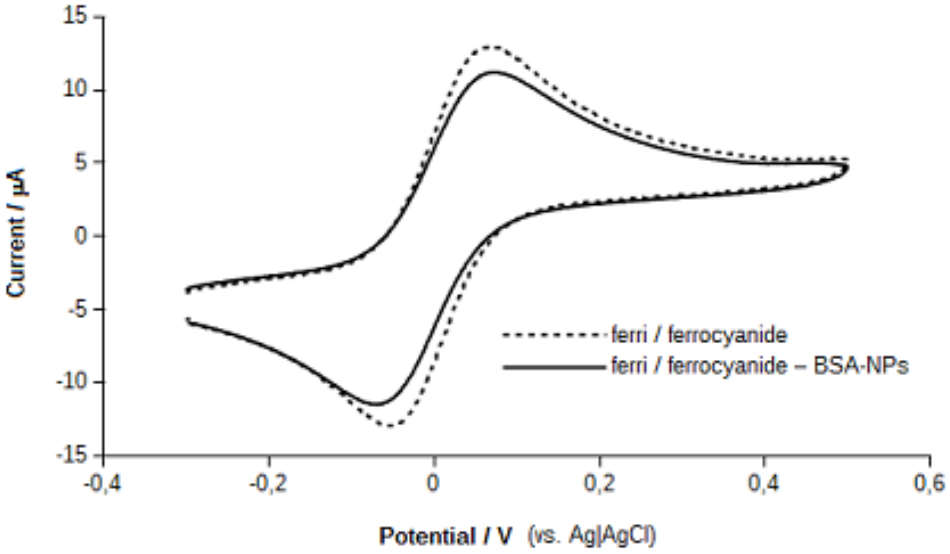


Figure 2. Cyclic voltammograms of potassium ferri/ferrocyanide $Fe(CN)_6^{3-/4-}$ obtained without BSA-NPs (dashed curve) and with 0,3 mg/mL BSA-NPs (solid curve); 1 mM potassium ferri/ferrocyanide $Fe(CN)_6^{3-/4-}$ in 0.1 M KCl, 4 mM phosphate buffer (Na_2HPO_3 –NaH_2PO_4), pH 7.4; scan rate 0.1 V/s; (reference electrode - Ag/AgCl)

In contrast with the SLCNUgly, chlorpromazine and thioridazine drugs in the case of ferri/ferrocyanide a very little decrease of the redox peaks is observed (dashed and solid curve in **Figure 2**). This exclude the discussed possibility of electrode blocking from the BSA-NPs and supports the incorporation of chlorpromazine, thioridazine and SLCNUgly in the NPs core.

Concentration dependence of the drug-NPs interaction

The incorporation of the drugs into the BSA-NPs is investigated for different NPs concentrations. The results are shown in **Figure 3** as a relative decrease of the oxidation peak from its initial value.

All three drugs chlorpromazine, thioridazine and SLCNUgly exibit significant decrease of the oxidation peak (curves 2, 3 and 4 in **Figure 3**). In contrast, the value of the oxidation peak of ferricyanide remain more than 90% from the initial value (curve 1 in **Figure 3**).

In **Figure 4** are compared the values of the "incorporation ability" of the BSA-NPs, according to the relative decrease of the oxidation peak of each drug. The relative decrease is derived as $(I_0\text{-}I_2)/I_0.100\%$, where I_0 is the oxidation peak value before the addition of BSA-NPs in the solution, and I_2 is the oxidation peak value after the addition of BSA-NPs with final concentration of 0,2 mg/ml in the solution.

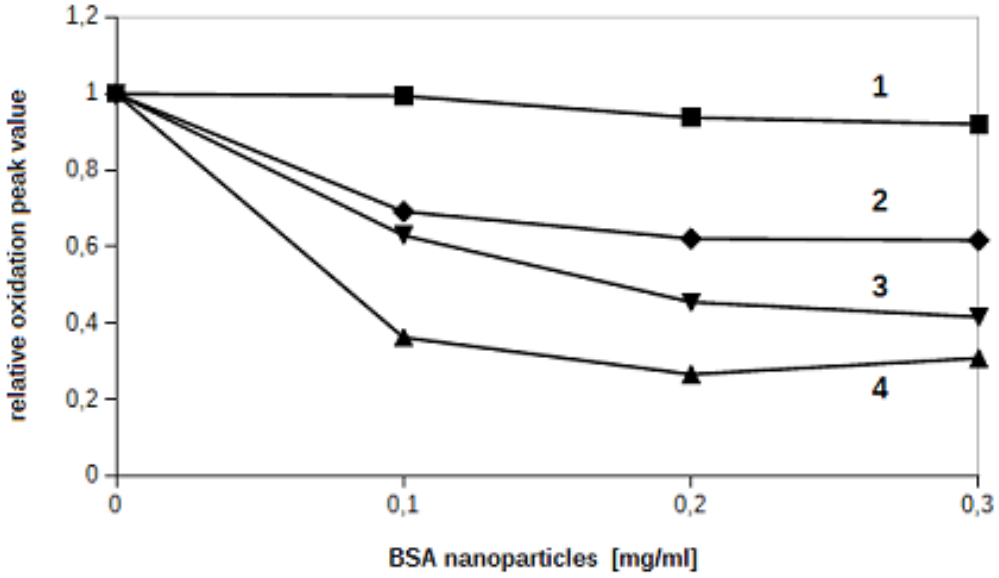


Figure 3. Dependence of the drugs oxidation peak current Ip on the BSA-NPs concentration. Curve 1 – 1 mM potassium ferri/ferrocyanide $Fe(CN)_6^{3-/4-}$; Curve 2 – 0.4 mM chlorpromazine; Curve 3 – 0.4 mM thioridazine; Curve 4 - 0.4 mM SLCNUgly. The results are presented as relative change of the peak value starting from the initial value before any addition of NPs in the solution. Electrochemical cell: 0.1 M KCl, 4 mM phosphate buffer (Na_2HPO_3 – NaH_2PO_4), pH 7.4; scan rate 0.1 V/s; reference electrode - Ag/AgCl.

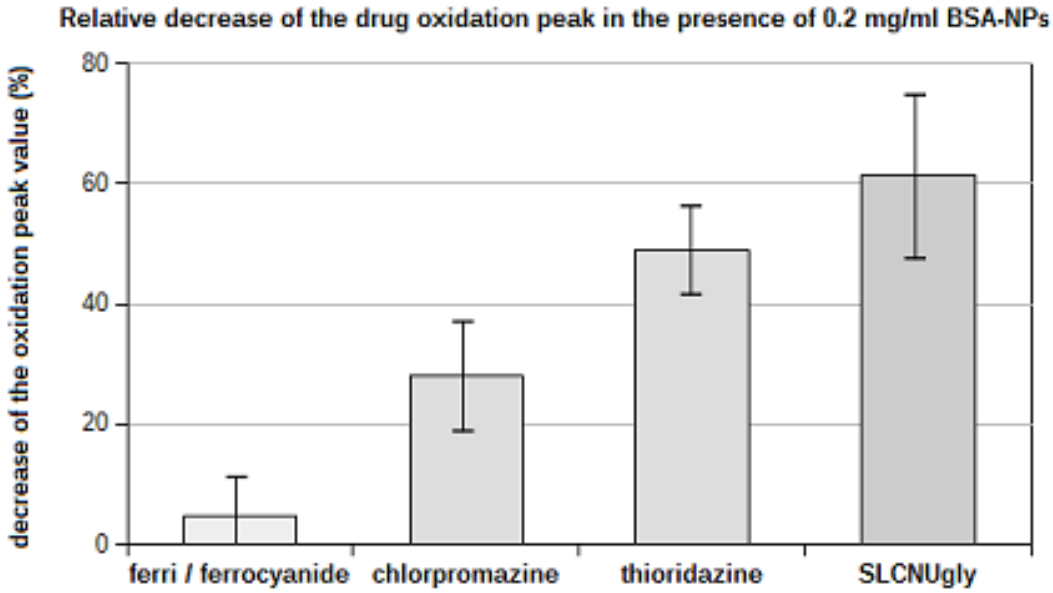


Figure 4. "Incorporation ability" of the BSA-NPs, according to the decrease of the oxidation peak of each drug. (electrochemical cell conditions – same as above)

The results in **Figure 4** support the idea of the hydrophobic interaction of the drugs with the BSA-NPs. Thioridazine is known to be more hydrophobic than chlorpromazine [13] and SLCNUgly is even more hydrophobic, according to their ability to dissolve in water solution. According to the results in **Figure 4** thioridazine interacts 1,75 stronger than chlorpromazine. It is interesting to note that this value is similar to the partition coefficients ratios for the two drugs, reported in [13] – respectively 1,49 for erhytrocytes and 2,22 for liposomes.

CONCLUSIONS

Using electrochemical technique, such as CV, the interactions of electroactive drugs and BSA-NPs are easily investigated. The incorporation of the drugs into the BSA-NPs is in the increasing order chlorpromazine, thioridazine, SLCNUgly. This order is in accordance with the hydrophilicity of the drugs, suggesting hydrophobic interaction between the drugs and the NPs.

Acknowledgements

The work is supported by projects: ДНТС Китай 01/02 2011 from the Ministry of Education of Bulgaria, and 5/МФ-2011: „Ефекти на наночастици върху еритроцитни и моделни мембрани"

REFERENCES

1. Uhrich, K. E., Cannizzaro, S. M., Langer, R. S., Shakesheff, K. M. Polymeric systems for controlled drug release. *Chemical Reviews*, 99(11), 3181–3198, 1999
2. Qu, F., Zhu, G., Lin, H., Zhang, W., Sun, J., Li, S., Qiu, S. A controlled release of ibuprofen by systematically tailoring the morphology of mesoporous silica materials. *Journal of Solid State Chemistry*, 179(7), 2027–2035, 2006.
3. Lauffer, R. B., Brady, T. J. Preparation and water relaxation properties of proteins labeled with paramagnetic metal chelates. *Magnetic Resonance Imaging*, 3(1), 11–16, 1985.
4. Li, F.Q., Su, H., Wang, J., Liu, J.Y., Zhu, Q.G., Fei, Y.B., Pan, Y.H., Hu, J.H. Preparation and characterization of sodium ferulate entrapped bovine serum albumin nanoparticles for liver targeting. *International Journal of Pharmaceutics*, 349(1-2), 274–282, 2008.
5. Li, J.,Yao, P. Self-assembly of ibuprofen and bovine serum albumin-dextran conjugates leading to effective loading of the drug. *Langmui*r, 25(11), 6385–6391, 2009.
6. Patil, G. V. Biopolymer albumin for diagnosis and in drug delivery. *Drug Development Research*, 58(3), 219–247, 2003.
7. Kratz, F. Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. *Journal of Controlled Release*, 132(3), 171–183, 2008.
8. Hawkins, M. J., Soon-Shiong, P., Desai, N. Protein nanoparticles as drug carriers in clinical medicine. Advanced Drug Delivery Reviews, 60(8), 876–885, 2008.
9. Karabaliev, M., Kochev, V. Voltammetric study of levomepromazine induced ionic permeability in a model lipid membrane system. *Electrochemistry Communications*, 3(12), 742–745, 2001.
10. Zheleva, A. M., & Gadjeva, V. G. Spin labelled nitrosoureas and triazenes and their non-labelled clinically used analogues — a comparative study on their physicochemical properties and antimelanomic effects. *International Journal of Pharmaceutics*, 212(2), 257–266, 2001
11. Gadjeva, V., Lazarova, G., Zheleva, A. Spin labeled antioxidants protect bacteria against the toxicity of alkylating antitumor drug CCNU. *Toxicology Letters*, 144(3), 289–294, 2003
12. Xie, L., Tong, W., Yu, D., Xu, J., Li, J., & Gao, C. Bovine serum albumin nanoparticles modified with multilayers and aptamers for pH-responsive and targeted anti-cancer drug delivery. *Journal of Materials Chemistry*, *22*(13), 6053 – 6060, 2012
13. Binford, J. S., & Palm, W. H. Absorption of surfactants by membranes: erythrocytes versus synthetic vesicles. *Biophysical Journal*, *66*(6), 2024–2028, 1994