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The Effects of Nanodiamonds at the Action of Colored Metal Ions on the Skin of Guinea Pigs

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Abstract

The protective effect of modified nanodiamonds (MND) under the action of cobalt and nickel ions on the skin of Guinea pigs was shown. At the action of chromium ions on the skin of animals, the protective effect of MND was not found. The differences are related to different adsorption properties of MND to the investigated colored metal ions. It is shown *in vitro* that MND can adsorb ions of cobalt and nickel and don't bind ions of chromium from aqueous medium. The perspectives using of MND as a new drug for the prevention allergic dermatitis caused by action of bivalent ions of metals are discussed.

Keywords

Nanodiamonds, Allergic Contact Dermatitis, Colored Metal Ions, Clinical-Morphological Study

1. Introduction

At the present time, one of the most common dermatology diseases is Allergic Contact Dermatitis (ACD). According to different authors, this disease affects about 2% - 2.5% of the world population [1] [2] [3]. In recent years, ACD incidence rate has been noticed, which is caused by huge increase of chemicals used in everyday life and in industries [2]. One of the most frequent reason leading to the development of the disease is action on skin by the allergens of chemical nature, for instance, bivalent metal ions, such as cobalt, nickel and chromium [2] [4] [5]. Therefore, an important role in ACD prevention is played by the drugs, which are able to bind metal ions on skin surface and thus neutralize their toxicaction.

Modified nanodiamonds (MND) of explosive synthesis [6] [7] [8] can become a new

adsorbent for binding and neutralization of chemical allergens on skin surface. Technology of MND production includes chemical modification surface of commercial nanodiamonds produced in Russian or other countries by explosive synthesis, by treatment of chemical reagents reducing the amount of surface inorganic admixtures, e.g. metal ions [6] [7] [9]. The MND are characterized by high colloid stability in hydrosols (water suspensions) after this treatment. Due to these properties, it is possible to carry out the separation of MND by differential centrifugation of suspensions into fractions narrow distribution to nanoparticle aggregates size [8] [9] [10]. Whereas, it gives opportunity to get dry powder of individual MND fractions. After drying, MND keep their high colloid stability that allows having stable hydrosols by simple addition of deionized (DI) water to nanoparticles powder weigh. High colloid resistance of MND in water and glucose solutions [6] [8] [11] [12] allows preparing sterile sols of nanoparticles for biological and medical experiments [11]. Physical and chemical properties of MND (especially polymorphous chemical active surface of nanoparticles [8] [9] [12]) determine their high adsorption properties of different compounds. This opens prospects for the application of these nanoparticles for the separation and purification of biomolecules [13] [14] [15], immobilization and neutralization of toxins (e.g. mycotoxins) [16] [17], and creation of new systems for biochemical diagnosis [18] and new drug delivery systems [19].

In this research, we examined the effects of MND at the systematic exposure of cobalt, nickel and chromium ions on the Guinea pigs skin.

2. Materials and Methods

2.1. Materials

The experiments were conducted with MND characterized by high colloid stability in water suspensions (hydrosols) [6] [7] and they had an average size of nanoparticle aggregates of d_{50} = 49.6 nm (size of MND aggregates estimated on Beckman Coulter #5, USA). MND water suspensions were prepared by addition of DI to nanoparticles powder weigh. DI was made by deionizing water system Milli-Q system (Millipore, USA). The hydrosols of MND with concentrations 5.0 $g \cdot L^{-1}$ и 10.0 $g \cdot L^{-1}$ were used in the work.

2.2. Spectral Analysis

Adsorption of bivalent metals (cobalt, nickel, chromium) by MND was studied using the following procedure. Ion solutions were mixed with MND hydrosol at a weight ratio of components—1:5 (ions: nanoparticles) in a final volume of 1 ml. The suspensions were intensively stirred on Vortex-Genie 2 g - 560E (Scientific Industries, Inc., USA) for 5 sec and incubated at room temperature for 2 min. Then, MND particles with absorbed metal ions were collected by centrifugation (Centrifuge 5415R, Eppendorf, Germany) at 16,000 g for 10 min at 10˚C. The amount of ions non-absorbed by MND in the obtained supernatants was measured by spectral analysis using UVIKON-943 UV/VIS spectrophotometer (Kontron Instruments, Italy). It is generally known that

colored ions of bivalent metals (e.g. cobalt, nickel and chromium) in water solutions have specific absorption spectra in the visible region. Accordingly, absorption spectra of supernatants were measured in the wavelength range λ = 200 - 800 nm. Spectral characteristics of ion solutions without MND treatment were used as the control. For calculations of the adsorption capacity of MND to cobalt, nickel and chromium ions used the optical density in absorption maxima of control (without MND treatment) and experimental samples (after MND treatment). The value of adsorption capacity expressed as weight (mcg) quantity of ions adsorbed by 1 mg MND.

2.3. *In Vivo* Studies

The effects of MND on the animal skin at the systematic exposure of cobalt, nickel and chromium ions were studied on Guinea pigs (males and females, 3 - 4 months, 250 - 300 g). The animals were randomized into 9 groups (6 animals per group) (Table 1). Aqueous solution of 2% cobalt chloride, 5% nickel chloride and 2.5% potassium bichromate were used for initiation of ACD in animals, the solutions were applied on the skin according to the common scheme [20] [21]. Ion solutions were made in DI, using bivalent metal salts $CoCl_2$, $NiCl_2$ and $K_2Cr_2O_7$ of high qualification (Reahim, Russia).

Before the experiment the hair in the interscapular region (2 × 2 cm) of each animal was cut. The prepared skin surface was notionally divided into two parts—upper and lower. The upper skin part of the control animals I, IV *и* VII groups was treated once a day during 10 days with water suspensions $CoCl_2$ (I group), $NiCl_2$ (IV group) and

Table 1. Distribution of animals among experimental groups.

Group	Treatment of experimental skin area once a day during 10 days	Treatment of intact skin area on 12th experiment day
I	2% solution of $CoCl_2$ - 0.5 ml	2% solution of $CoCl_2$ - 0.5 ml
II	MND 5.0 g·L^{-1} - 0.5 ml, after 2 h 2% solution of $CoCl_2$ - 0.5 ml	2% solution of $CoCl_2$ - 0.5 ml
III	MND 10.0 g·L^{-1} - 0.5 ml, after 2 h 2% solution of $CoCl_2$ - 0.5 ml	2% solution of $CoCl_2$ - 0.5 ml
IV	5% solution of $NiCl_2$ - 0.5 ml	5% solution of $NiCl_2$ - 0.5 ml
V	MND 5.0 g·L^{-1} - 0.5 ml, after 2 h 5% solution of $NiCl_2$ - 0.5 ml	5% solution of $NiCl_2$ - 0.5 ml
VI	MND 10.0 g·L^{-1} - 0.5 ml, after 2 h 5% solution of $NiCl_2$-0.5 ml	5% solution of $NiCl_2$ - 0.5 ml
VII	2.5% solution of $K_2Cr_2O_7$ - 0.16 ml	2.5% solution of $K_2Cr_2O_7$ - 0.16 ml
VIII	MND 5.0 g·L^{-1} - 0.5 ml, after 2 h 2.5% solution of $K_2Cr_2O_7$ - 0.16 ml	2.5% solution of $K_2Cr_2O_7$ - 0.16 ml
IX	MND 10.0 g·L^{-1} - 0.5 ml, after 2 h 2.5% solution of $K_2Cr_2O_7$ - 0.16 ml	2.5% solution of $K_2Cr_2O_7$ - 0.16 ml

$K_2Cr_2O_7$ (VII group). Animals II, III, V, VI, VIII and IX groups were experimental (Table 1). The upper skin part of the experimental animals was treated once a day for 10 days with MND water suspensions with concentration5.0 or 10.0 $g \cdot L^{-1}$ and after 2 h with cobalt (groups II and III), nickel (V and VI groups) or chromium (groups VIII and IX) solutions, respectively.

On 12th day of the experiment the sensibilization grade of animals to the studied ions was evaluated by using a single application of permissive doze of the corresponding metal salts solutions (Table 1) upon the lower (intact) skin area of the Guinea pigs: for groups I - III—solution of cobalt chloride, for groups IV - VI—solution of nickel chloride, for groups VII - IX—solution potassium bichromate.

Upon the experiment completing (on 15th day) skin sample biopsy was taken from all the animals in both (the upper and lower) skin areas for histological examination.

2.4. Histological Preparation

The skin samples were fixed in 10% neutral formalin. Histological preparations were made by standard method and stained with hematoxylin and eosin.

2.5. Morphological Preparation

Morphological study was performed on histological preparations digitized by Adobe Photoshop CS3. Relative volume of histological structures was estimated by Saltykov morphometric framework with 60 equidistant test points. Six random non-overlapping areas were studied in each section. Five images of Saltykov framework were put into each area, and the number of coincidences of test points and registered histological structures were calculated.

2.6. Statistical Evaluation

Statistical analysis of the obtained data was performed by Statistica 6.0 software (StatSoft, 2003). We calculated the value of arithmetical mean, standard deviation, arithmetic mean error. Mann-Whitney criterion was used for estimation of statistical significance of differences. Differences were significant at $p < 0.05$.

The study was approved by the Local Ethical Committee of Krasnoyarsk State Medical University named Prof. V.F. Voyno-Yasenetsky in 2010 with order number 28/2010.

3. Results and Discussion

Clinical signs of ACD were found in all animals from groups I, IV and VII on 3 - 6th day after treatment of the experimental parts of the skin with cobalt, nickel and chromium solutions (Figures 1-3). The following was noted: erythema, papular and vesicular rash, microerosions, hemorrhagic crusts, excoriations. Histological study of the skin samples from these animals showed significant thickening of the epidermis, acanthosis, vacuolar degeneration in the spinous and basal layers, spongiosis, infiltrate cells exocytosis into the horny layer with squamosa-scab formation. In the papillary layer of dermait we found: significant interstitial edema, diffuse and focal inflammatory infiltrates

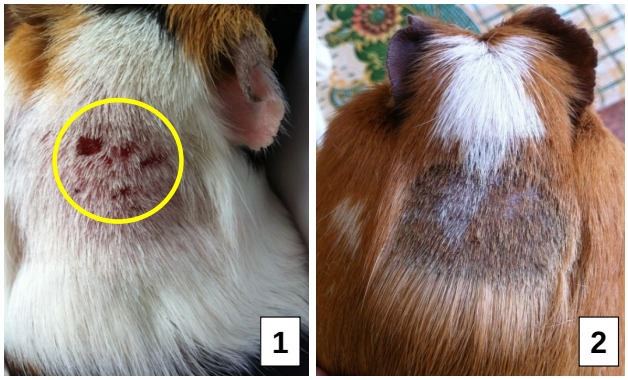

Figure 1. Typical skin appearance of Guinea pigs after treatment for 10 days: 1—by aqueous solution of 2% cobalt chloride; 2—first by MND aqueous suspension (concentration 5.0 g·L^{-1}) and then by chloride cobalt solution. The round yellow sector shows the size of the skin lesions as a result of ACD.

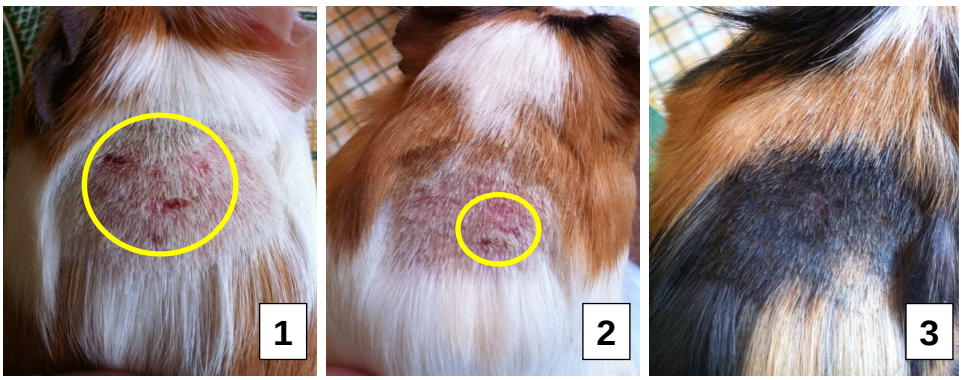

Figure 2. Typical skin appearance of Guinea pigs after treatment for 10 days: 1—by aqueous solution of 5% nickel chloride; 2—first by MND aqueous suspension (concentration 5.0 g·L^{-1}) and then by chloride nickel solution; 3—first by MND aqueous suspension (concentration 10.0 g·L^{-1}) and then by chloride nickel solution. The round yellow sectors show the sizes of the skin lesions as a result of ACD.

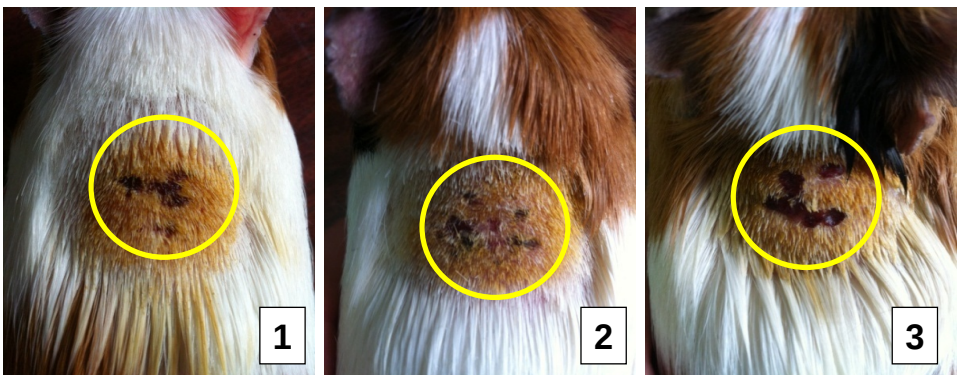

Figure 3. Typical skin appearance of Guinea pigs after treatment for 10 days: 1—by aqueous solution of 2.5% potassium dichromate; 2—first by MND aqueous suspension (concentration 5.0 g·L^{-1}) and then by dichromate potassium solution; 3—first by MND aqueous suspension (concentration 10.0 g·L^{-1}) and then by dichromate potassium solution. The round yellow sectors show the sizes of the skin lesions as a result of ACD.

presented lymphocytes and polymorphonuclear leukocytes with a mixture of macrophages and eosinophils. The papillary layer vessels were expanded, the vascular walls were edematous, stasis was observed in the vessels.

After application of permissive doze of the corresponding metal salts solutions upon the intact skin part of the animals I, IV and VII groups, in 24 - 72 hours erythema was registered among all the animals. Histological examination of the skin samples, taken from those parts, showed significant lymphocytic infiltration. The data represent significant irritating and sensitizing action on the Guinea pigs skin of the studied metal ions.

Pre-treated of Guinea pigs skin with MND water suspensions of 5.0 $g \cdot L^{-1}$ concentrations (II group) neutralized the irritant and sensitizing chloridecobalt action while its following application to the animals skin. Clinical signs of ACD were not observed in the animals of group II throughout the experiment (Figure 1). Histological study of the animals skin samples in that group showed scarce infiltrates presented lymphocytes and polymorphonuclear leukocytes. After application of permissive doze of cobalt solutions upon the intact skin part of the animals II group in 24 - 72 hours, erythema was not registered among any of the animals.

In group III of the Guinea pigs, which skin was pre-treated with MND water suspension of 10.0 $g \cdot L^{-1}$ concentration, and then with cobalt solution, development of ACD was found in only 4 Guinea pigs. The appearance of clinical signs of ACD in animals of that group developed later (on days 6 - 7) than in control Guinea pigs (I group), where clinical signs of dermatitis were found on days 4 - 5.

The results of morphometric study of the skin samples from the animals of groups I - III showed (Table 2) that in skin samples from animals of groups II and III we observed a significant ($p < 0.05$) decrease in "free interstitial space" (49% and 45%, respectively), "blood vessels" (87% and 67%), "lymphocytes" (69% and 40%), "neutrophils" (65% and 47%), compared to skin samples of control animals of group I.

It was shown, that clinical signs of ACD (Figure 2) were found in Guinea pigs group V, which skin was pre-treated with MND water suspension of 5.0 $g \cdot L^{-1}$ concentration,

Table 2. Relative volume (%) of histological fractions derma of Guinea pigs with complex MND and chloride cobalt treatment.

Estimable parameter	Experimental groups of animals		
	I Solution $CoCl_2$ (2%)	II MND (5.0 $g \cdot L^{-1}$) + solution $CoCl_2$ (2%)	III MND (10.0 $g \cdot L^{-1}$) + solution $CoCl_2$ (2%)
Free interstitial space	21.1 ± 0.49	10.7 ± 0.19*	11.6 ± 0.27*
Blood vessels	10.0 ± 0.36	1.3 ± 0.07*	3.3 ± 0.15*
Lymphocytes	3.5 ± 0.22	1.1 ± 0.06*	2.1 ± 0.12*
Neutrophils	1.7 ± 0.15	0.6 ± 0.04*	0.9 ± 0.08*

Notice: *Significant ($p < 0.05$) differences of the studied data in experimental groups of animals (MND and chloride cobalt usage), compared to control animals data (only chloride cobalt usage).

and then with chloride nickel solution, as well as in control animals group IV on 6th day of the experiment. However, in that group clinical signs of ACD were represented less comparing to control group IV. The following was observed: erythema, peeling, single microerosions. In histological examination of the skin test samples of animals' group V moderate inflammatory infiltrates presented lymphocytes and polymorphonuclear leukocytes with a mixture of macrophages and eosinophils were identified.

Clinical signs of ACD were not observed throughout the experiment (Figure 2) in group VI, where the animal skin was pre-treated with MND suspension of 10.0 $g \cdot L^{-1}$ concentration, and then with chloride nickel solution. Histological examination of the skin samples of animals in this group were identified scarce inflammatory infiltrates presented lymphocytes and polymorphonuclear leukocytes. On application of permissive doze of chloride nickel solution upon the intact area in group VI, none of the animals presented erythema development.

In the morphometric study of the skin samples from animals of groups V и VI (Table 3) a significant ($p < 0.05$) decrease in "free interstitial space" (44% and 49%, respectively), "blood vessels" (50% and 53%), "lymphocytes" (61% и 83%) and "neutrophils" (60% и 70%) were noted, compared to skin samples of control animals of IV group.

It was shown that pre-treated of Guinea pigs skin from groups VIII and IX with MND suspensions (concentrations 5.0 and 10.0 $g \cdot L^{-1}$, respectively) did not provide protective effect on the following action of chromium solutions upon the animal skin. Clinical signs of ACD (Figure 3) were found on 3 - 4 days of the experiment in all the animals of those groups as in the control animals (VII group). Histological analysis of the skin samples from animals of groups VIII and IX showed the same changes as in the skin samples of the control animals of group VII.

Data of morphometric skin samples study did not identify significant differences in parameters "free interstitial space", "blood vessels", "lymphocytes" and "neutrophils" between control (VII group) and experimental (VIII and IX groups) animals (Table 4).

Generally, the findings attest to the advantage of protecting effect of MND particles, which neutralize the irritant and sensitizing cobalt and nickel ions action upon Guinea

Table 3. Relative volume (%) of histological fractions derma of Guinea pigs with complex MND and chloride nickel treatment.

Estimable parameter	Experimental groups of animals		
	IV Solution $NiCl_2$ (5%)	V MND (5.0 $g \cdot L^{-1}$) + solution $NiCl_2$ (5%)	VI MND (10.0 $g \cdot L^{-1}$) + solution $NiCl_2$ (5%)
Free interstitial space	13.9 ± 0.31	7.8 ± 0.24*	7.1 ± 0.23*
Blood vessels	3.0 ± 0.15	1.5 ± 0.09*	1.4 ± 0.11*
Lymphocytes	1.8 ± 0.12	0.7 ± 0.08*	0.3 ± 0.05*
Neutrophils	1.0 ± 0.09	0.4 ± 0.06*	0.3 ± 0.05*

Notice: *Significant ($p < 0.05$) differences of the studied data in experimental groups of animals (MND and chloride nickel usage), compared to control animals data (only chloride nickel usage).

Table 4. Relative volume (%) of histological fractions derma of Guinea pigs with complex MND and potassium bichromate treatment.

Estimable parameter	Experimental groups of animals		
	VII Solution $K_2Cr_2O_7$ (2.5%)	VIII MND (5.0 g·L^{-1}) + solution $K_2Cr_2O_7$ (2.5%)	IX MND (10.0 g·L^{-1}) + solution $K_2Cr_2O_7$ (2.5%)
Free interstitial space	11.4 ± 0.22	12.9 ± 0.24	11.8 ± 0.22
Blood vessels	3.8 ± 0.14	3.6 ± 0.13	3.5 ± 0.13
Lymphocytes	2.5 ± 0.11	2.4 ± 0.11	2.4 ± 0.11
Neutrophils	1.2 ± 0.07	1.2 ± 0.08	1.1 ± 0.07

pigs skin, and so prevent ACD development among the animals. However, in the research we have not noticed MND protecting effect while chromium ions action on the animals skin.

We suppose that the observed differences can be explained to various adsorption properties of these metal ions by MND. *In vitro* experiments showed that MND adsorb cobalt and nickel ions, and did not adsorb chromium ions from water solution. The spectral analysis of cobalt and nickel solution with and without MND treatment (**Figure 4**) showed that 1 mg MND particles adsorb more than 30 μg cobalt ions and 20 μg nickel ions from water solution. At the same time, complete coincidence of absorption spectra of control (without MND treatment) and experimental (after MND treatment) solutions of chromium (data not shown) suggest that MND particles do not bind chromium ions from aqueous medium.

The data for adsorption capacity of MND to cobalt and nickel ions (**Figure 5**) allow us put forward the following assumption, which explains the animal experiments results.

The greatest protecting effect will be observed only with particular ratio of MND and colored metal ions, when the toxicant compounds with nanoparticles bind is accompanied by their mutual neutralization. So, it follows from the results of work, that for cobalt ions bind and their negative effect on skin neutralization MND concentration 5.0·L^{-1} is more optimized, in comparison with nanoparticles concentration 10.0 g·L^{-1}, as it provides better protecting effect (II group). At the same time, while nickel ions effect on skin better protecting effect has been made with the usage of MND concentration 10.0 g·L^{-1} (VI group), in comparison with the effect, that was observed with MND concentration 5.0 g·L^{-1}. As it is shown, that adsorption capacity of MND to nickel ions is 1.5 times less than cobalt ions, it is obvious, that for their bind and neutralization more nanoparticles are required.

Whereas, the outspoken hypothesis gives reason to believe, that if the optimized ratio of MND-colored metal ions is changed one way or another, unwanted effects can be observed. For instance, excessive rise of MND concentration can lead to toxicant-adsorbent correlation disbalance, and in this case it is possible to see negative influence on

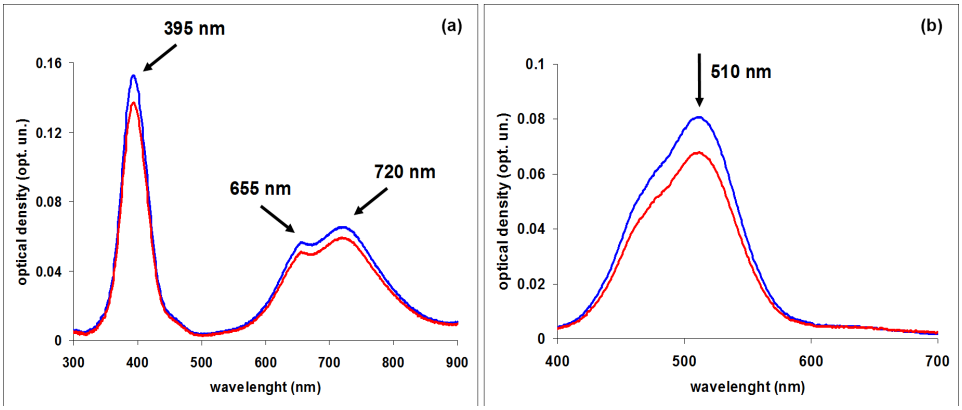

Figure 4. The absorption spectra of chloride nickel (a) and chloride cobalt (b) solutions: before (blue lines) and after (red lines) treatment by MND. The arrows show the characteristic wavelengths of the absorption maxima at which the values of optical density are used to calculate the adsorption capacity of NMD to nickel and cobalt ions.

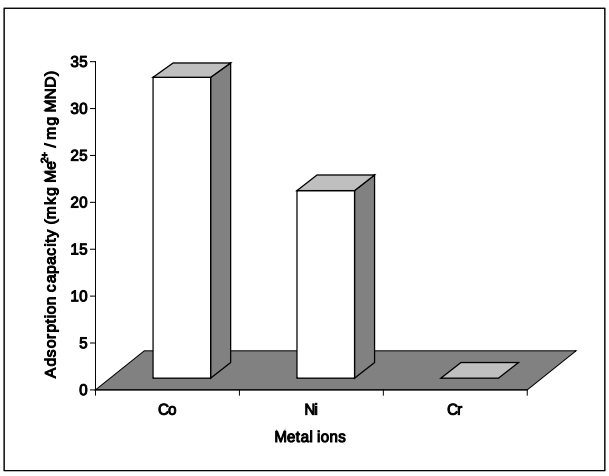

Figure 5. The adsorption capacity of MND to bivalent metal ions. Calculations were carried out on the adsorption capacity based on the data of spectral analysis of aqueous solutions of $CoCl_2$, $NiCl_2$, $K_2Cr_2O_7$ without and after MND treatment.

skin by chemically active MND surface, for example, nanoparticles irritant effect. Probably, we have been watching manifestation of such nanoparticles action among the animals of III group, ACD development has been provoked by cobalt ions among them. In the experiment it has been shown, that MND concentration rise (10.0 $g \cdot L^{-1}$) was not leading to such clear protecting effect, in comparison with the effect, that was observed with nanoparticles concentration 5.0 $g \cdot L^{-1}$. Nevertheless, development of ACD has been seen among few animals of III group, although in later terms in contrast with the control. Our next investigations are going to be focused on the examination of these questions.

4. Conclusion

Consequently, the results obtained in *in vivo* experiments show that MND particles

have a protecting effect at the action of cobalt and nickel ions on the skin of Guinea pigs, reducing the likelihood of ACD and hampering its development. It is shown in the experiments that MND effect is dose-dependent. At the action of cobalt ions on the skin of animals, the greatest protecting effect was registered at the MND concentration 5.0 $g \cdot L^{-1}$, at the action of nickel ions—the best protecting effect was received at the MND concentration 10.0 $g \cdot L^{-1}$. At the same time, it has been discovered that with the used concentrations MND do not produce any protecting effect at the action of chromium ions on the animals skin. As the studies *in vitro* have shown, the revealed differences are connected with various MND adsorption properties to the studied ions. In the experiments it has been discovered that MND adsorb cobalt and nickel ions from water medium and do not bind chromium ions. In this case, based on the done calculation, it follows that in the used experimental circumstances, 1 mg MND can adsorb 32 μg cobalt ions and 20 μg nickel ions. These data and results of animal experiments allow us to out speak hypothesis that the maximum protecting effect will be observed only with particular ratio of MND and colored metal ions when the toxicant compounds with nanoparticles bind is accompanied by their mutual neutralization. We suggest that the findings open a prospect of future investigations of possibility application of MND as new adsorbent for ACD prevention, caused by colored metal ions, specifically, cobalt and nickel ions.

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