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Resource-Efficient Technologies 2 (2016) 43-49



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Silver containing sorbents: Physicochemical and biological properties

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Received 18 May 2016; accepted 10 June 2016

Available online 26 July 2016

Abstract

New silver containing sorbents, based on mineral carriers, such as alumina and silica systems with a meso- and macro- porous structure, have a higher mechanical resistance and, hydrophilic and hydrophobic chemical composition of the surface. These sorbents are easy to find and relatively inexpensive, compared to their known equivalents. They are furthermore characterised by high specific surface and simple preparation, whilst the addition of silver considerably increases their antiseptic activity. The results of research of the physical, chemical and biological properties of the developed substances, as well as bio-comparability of sorbents with biological tissues, are presented in this paper. The modified material acts simultaneously as the carrier for active substances to the area of therapeutic application and as a sorbent used to remove toxic agents from such areas. This approach led us to modify the sorbent, and prolong the delivery of substances such as silver, as an effective antibacterial and antimycotic agent. © 2016 Tomsk Polytechnic University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords: Sorbents; Detoxification; Prolongation; Silver

1. Introduction

The increase in the number of industrial environmental disasters and emergencies around the world exposed the world population to high risks of injury and various intestinal infections, over large territories, liable to develop into global catastrophes. According to WHO, each year up to 5 million children around the world die of intestinal infections of various aetiologies.

Toxic agents (microbial cells, their waste, activated ferments, capable of harming the tissue, inflammation mediators, peroxide substances, devitalized tissues ingredients of different structures etc.), enter the body, disturbing its homeostasis and leading to the condition referred to as "endogenous intoxication", associated with disruption of the functions of detoxification and excretion organs, damage to epithelial sheets and massive tissue damage, organ and tissue ischaemia, exotoxicosis and immuno-suppression [1]. In addition to the conventional therapeutic methods, relying on different drugs for detoxification of the body, sorption methods have also been used. The interest for cerebrifugal sorption methods is unrelenting, since these methods (haemo-, lympho-, plasmo-, liquoro-, entero-, immuno- and application sorption) are all based on extraction of toxic agents from the body, as well as endogenous and exogenous ballast substances, relieving the pressure form organs through natural detoxification of the body (liver, kidneys and the immune system). Scientific research has confirmed the effectiveness of inclusion of sorbents in the therapy and prophylactic treatment in different diseases.

We know that sorption activity is dependent on both the content and the chemical structure of the sorbent surface, as well as on their texture [2,3]. The most commonly encountered sorbents are those based on active carbons, aluminium oxides, silicon and carbon-mineral sorbents [4]. Carbon-mineral sorbents combine the activity of carbons and stability of mineral sorbents, the hydrophilic and hydrophobic nature of their surface, which make them compatible with bio-tissues [3]. Broadly speaking, sorbents come in diverse sizes of specific surfaces, which is measured based on pore size, where the larger the pore, the higher the specific surface of the sorbent.

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http://dx.doi.org/10.1016/j.reffit.2016.06.001

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According to one classification, porous bodies have the following pore sizes: micropores – the equivalent size of less than 1.5-2 nm; mesopores – the size is between 2 and 50 nm, or up to 100 nm according to other sources; macropores – the equivalent size of 50–100 nm to up to 6500 nm; supermacropores – the equivalent size exceeding 6500 nm. Scientific research indicated a preference for the use of meso- macroporous sorbents in therapy because they are least liable to harm the biotissue, whilst remaining effective in removing medium and highly molecular toxin agents of different types, from biological media.

The requirements for sorbents designated for use in medicine have been defined as follows: they need to be of adequate mechanical and chemical stability, comply with prescribed standardised granulometric and texture parameters, have certain chemical structure, cause as little damage to the biological tissue as possible and do not have a toxic effect on organs and tissue; that they do not absorb proteins and oxygen from the blood and lymph, that they do not disturb the body's mineral balance, that they are selective towards certain class of compounds, that they have optimal absorption capacity, that they remain active in all parts of the gastrointestinal tract and they can be thoroughly and well discharged from it [2,3]. Furthermore, it should to be pointed out that the development of sorption substances ought to be based on competitive, lowenergy, resource saving and environmentally friendly manufacturing technologies [5–7].

The research has indicated that sorbent effectiveness can be increased through modification of their surface using biologically active components. One of promising components is silver, offering its therapeutic (antibacterial and virucidal [8-10]) qualities, both in terms of its capacity for releasing into the biological medium, from the sorbent surface, and through contact between its surface and the biological medium. During such exchange, the silver containing sorbent does not act as an active absorbent of toxic agents [11].

From the point of view of possibility of using the above methods in practice, further development and research of silver containing mineral sorbents with meso-macroporous structure is of particular interest, offering high mechanical stability, a hydrophilic/hydrophobic chemical quality of the surface [12,13], as well their enhanced organoleptic qualities. This paper shows the results of research of silver containing sorbent's physical and chemical qualities [14–16] based on mineral carriers, such as aluminium and silicon containing sorbents. These sorbents are easy to find and relatively easily available, compared to their known equivalents (such as Polysorb and Smecta). They are furthermore characterised by high specific surface and simple preparation, whilst the addition of silver considerably increases their antiseptic activity.

2. Materials and methods

2.1. Physical and chemical research

The synthesis of sorbents was based on aluminium oxide carriers with various granulometric composition, such as industrial grade aluminium oxide (gamma Al_2O_3) and thermo-activated aluminium hydroxide (TAGA). In order to obtain hydrophilic/ hydrophobic chemical qualities, the surface of aluminium oxide carriers was coated with industrial grade organosilicon polymers (polymethylsiloxanes in the form of molecular solutions and water emulsion). The resulting carrier is a sorbent modified with a complex of Ag/polyvinylpyrrolidone clusters, in particles of 2–5 nm, applied through physical adsorption, as part of thermal processing [17].

Physical and chemical research was conducted using different methods. Granulometric composition was assessed by screen size gradation (0.1–1 mm); the particle size of micronised sorbent (TAGA) was assessed using laser diffractometer (SHIMADZU SALD-2101). Mechanical stability of slightly spherical sorbent specimens was assessed based on losses following mechanical abrasion PIG-2 [3]. The specific surface of sorbent specimens with different granulometric composition was determined based on the BET method, using nitrogen sorption isotherm to determine the size and the volume of pores [18]. The sorbent's weight by volume [19] and powder density were measured using Auto Ruspometer-200. The content of silicon and silver in sorbent was measured based on atomic emission spectrometry with inductively-coupled plasma using Baird (Netherlands) [20] spectrometer.

The distribution of silicon containing polymers in the pore space of aluminium oxides was examined based on the lowtemperature nitrogen absorption method (77.4 0 K) using the ASAP-2400 Micromeritics station, whilst the classification of pores based on their size was carried out using the Barrett– Joyner–Halenda method.

The assessment of sorption qualities of the sorbent was carried out based on sorption of blue methylene dye from water solution, which is otherwise used as a marker for medium molecular weight toxic agents. The adsorption was observed at room temperature, with occasional shaking, during 1 hour, where the sorbent–water ratio was 1:10, and where the dye content was measured using spectrophotometry.

2.2. Assessment of sorbent's biological features

Experimental investigation of biological compatibility of modified nanoclusters of Ag/Al/Si containing sorbents was conducted using micronised silver containing sorbent [21,22]. The research was conducted at the Laboratory for Pharmacological Research of the Natural and Biological Compound Department of the FGBNU NIOH [the Federal State Budgetary Scientific Institute of the Novosibirsk Institute of Organic Chemistry] Experiments were conducted on 18 female rats of the Vistar breed, with the mass of 200 ± 2 g. The animals were maintained in accordance with the rules approved by European Convention on the Protection of vertebrate Animals used for Experiments and other Scientific Purposes (Strasbourg, 1986). The groups of animals were randomised, each containing 6 subjects (the animals were marked but kept together): group I – received an intramuscular injection of distilled water (2.5 mL) (the negative control group); group II – received an intramuscular injection of the generic TAGA sorbent, in 2:1 water

solution, respectively; group III - received an intramuscular injection of Ag/TAGA sorbent in 2:1 water solution respectively. The application of sorbent was conducted in sterile conditions, in a single dose, using diethyl ether as anaesthetic and insulin syringe, with a 0.9×40 mm needle for injection, into the latissimus dorsi [broadest muscle of back] [23], at an angle of 45°. The injection site was indicated with a marker. The overall quantity of powder introduced, in the form of suspension, was 0.05 mL. The length of research was 21 days. The injection area was examined by palpation once every three days, as well as the overall condition of the animal. Blood specimens were collected from all subjects in order to assess any haematological changes, on days 7, 14 and 21, by cutting off the tip of animal tail, after its immobilisation in a plastic chamber; the euthanasia of the animals was applied on day 21 by inhalation anaesthetic overdose.

The following parameters were assessed (haematological analyser CA 530-16 Oden, Medonic, Sweden): white blood cell count (WBC; the norm: $7-14 \times 109/l$); the red blood cell count (RBC; norm: $6-10 \times 10^{12}/L$) and erythrocyte indices: haemoglobin (HGB; reference: 110-180 g/L), haematocrit (HCT; reference: 34-48%); platelet count (PLT; reference: $200-600 \times 10^{9}$ /l) [21,23-25]. The results are indicated in figures, as follows "average ± standard error", as well as in tables below figures, where they are indicated as average values. Because of the limited number of subjects, differences between groups were assessed based on distribution-free assessment methods and based on double-sided criteria – the Mann–Whitney Urank sum test, which produced the value of $p \le 0.05$ for differentiation of specimens (where p = 6, and the inversion value U was \leq 5). The statistical analysis of resulting data was carried out in Excel 2013 and Statistica 8.0.

3. Results and discussion

3.1. Physical and chemical assessment of sorbent qualities

The specific surface of sorbent samples of different granulometric content was from 100 to $270 \text{ m}^2/\text{g}$, powder density – from 2.6 to 2.9 g/cm³ (the parent matrix density approximately 3, depending on the content of silicon-containing component in the sorbent, the density of which is 1.22 g/cm³, the powder density had changed from 0.7 to 1.1 g/cm³ depending on granule gauge.

Silicon–aluminium oxide sorbents are highly resistant. Consequently, the losses caused by abrasion of the 0.2-0.6 mm fraction were 0.1-0.3% per minute, whilst similar losses for FFI's [Fossil Fuel Institute] active coal was 1.8%/min. Crushing resistance of sorbent granules (fr. 0.4-1 mm) was up to 70 kg, where the same resistance of certain active coal is only up to a few kg.

The analysis of the results of measurements of powder density of p, in all ranges of infill with silicon containing organic polymer sorbents, (X) to 0.107 g/g, indicated a linear dependence of 1/p from X, in accordance with the methods described previously [3]:

$$1/p = (1 - X)/p_c + X/p_s,$$
 (1)

Table 1

Texture qualities of the specimens of silicon/aluminium oxide containing sorbent specimens.

Samples	X, g/g	S, m ² /g	V _S , cm ³ /g	$\Delta V_{S}, cm^{3}/g$	L, nm	$S_{\alpha}, m^2/g$	
γ- A1 ₂ 0 ₃	_	270	0.447	_	_	17	
$Si/\gamma-A1_20_3$	0.040	234	0.377	0.07	2	14	
$Si/\gamma - Al_2O_3$	0.054	253	0.367	0.08	4	14	
Si/y-Al ₂ 0 ₃	0.107	215	0.322	0.125	3	_	

Designations: X – the content of organosilicon polymers per g/g of sorbent; V_s – the volume of pores in cm³/g; ΔV_s – the difference between the pore volumes of the carrier and sorbent specimens, in cm³/g; L – the silicon-containing polymer fragment size on a sorbent surface, in nanometres; S_a – the external surface of the sorbent, in m²/g.

where p_c is powder density of the carrier and p_s is the density of silicon containing polymer.

Such dependence indicates the correlation between specific volumes of carrier and polymer phases, reflecting the absence of cavities, which, being empty, could remain inaccessible to helium, due to them being blocked by polymer [sic]. The average power density value of the sorbent polymer phase, obtained from this equation, is 1.22 g/cm³.

Correct choice of conditions for the synthesis of silver containing sorbents, based on silicon–aluminium oxide sorbents, requires the analysis of the distribution of organosilicon polymers in porous areas of aluminium oxides, where the majority of pores are 10–100 nm, as well as measurement of polymer fragments. For this purpose, several specimens, with different polymer content were analysed (the polymer was diluted in organic solvent) (Table 1).

The maximum sorption volume V_s was measured based on the analysis of nitrogen sorption isotherm in cm³/g, as well as the difference ΔV between the maximum sorption volumes of the carrier – aluminium oxides and specimens, coated with silicon containing polymer, as water repellent, in the pore cavity, where specific surface S_{α} was measured based on the comparative method. The results were used as the basis for the assessment of the size of silicon containing polymer in the pore cavity.

The calculation of the specific surface using the comparative method $S\alpha$ indicates a slight decrease where a siliconcontaining polymer is applied. Based on this we could draw a preliminary conclusion that the polymer's surface fragments are quite finely dispersed (Table 1).

The analysis of nitrogen sorption isotherms in basic aluminium oxides and sorbent specimens is indicated, such that the Vs value was reduced in comparison to the basic carrier, which is clearly linked to the existence of silicon-containing polymer fragments in the porous cavities of the carrier. Consequently, the difference in the value of Vs, between the basic carrier and the sorbent and polymer, depends on the quantity of siliconcontaining polymer coating:

$$\Delta V = m/p \tag{2}$$

where p is the density of the polymer coating, in g/cm^3 .

While being a mere approximation, this calculation allows us to judge the possibility of the existence of modifier

Samples	Ag (%)	Particle size (mm)	S_{spec} . (m^2/g)	V ₂ pores (cm ³ /g)	p (g/cm ³)	pН	MB sorption (mg/g)
Si/Al ₂ O ₃	_	0.2–0.4	250	0.35	0.75	7.8 ± 0.1	15.5 ± 0.12
Ag/Si-Al ₂ O ₃	0.3	0.2–0.4	238	0.33	0.77	7.6 ± 0.1	14.7 ± 0.1
Si-Al ₂ O _{3 (} TAGA)	_	up to 0.04	100	0.2	1.05	8.2 ± 0.1	11.0 ± 0.8
Ag/TAGA	0.45	up to 0.04	98	0.2	1.1	8.2 ± 0.1	11.0 ± 0.8

Physicochemical properties of silver containing sorbents.

Designations: S_{spec}. - the extent of a specific surface, m²/g; V_Σpores - the total number of pores, cm³/g; p - powder density, g/cm³; MB - methyl blue dye.

fragments of polymer coating, in macropores, without testing, using the capillary condensation method. However, bearing in mind the uniformity of the coating and its independence from the amount of applied components, the value of ΔV may be used as the quantitative measurement of the coating.

The comparison between the steeping of the extent of a specific surface and the number of pores allows us to calculate the average fragment gauge in the silicon-containing polymer coating on the carrier:

$$\mathbf{L} = \Delta \mathbf{V} / \Delta \mathbf{S} \tag{3}$$

This (Table 2) indicates that such calculation clearly suggests that fine and medium fragments tend to increase with the amount of applied polymer. The possibility of the application of a modifier in macroporous areas of the carrier can be assessed based on the difference between different values of the so called "external surface" S_{α} , that is to say, the surface of carrier aggregate, which gives the substance its macroporous structure. The calculation of this value is performed using the comparative method, in the interval of relative pressure of the sorbent, from 0.95 to 0.99. The Table data indicate that insignificant changes in S_{α} occurred in specimens 2 and 3. However, it remains difficult to provide an accurate calculation of these changes.

The analysis of distribution trends of porous areas (based on the concepts described in the literature [3] which refers to the calculation of the pore mouth by the De Boer–Bruckhoff method) indicated that the basic carrier has a prominent texture, with a typical pore diameter of 10 nm (100 Å). It is namely these pores that contain the largest quantity of modifier. It was indicated that polymer is situated in pores with the diameter (d) of over 250 Å, which is reflected in a noticeable change in the number of such pores. Also observed was a reduction in the number of pores gauged 35 < d>80 Å; that is to say, smaller mesopores are also being modified. It can be assumed that it is namely in such pores to which silicon-containing polymer is bonded, that we find fragments whose gauge corresponds to the size of the mouth.

A fine mesh of organosilicon polymer fragments, gauged 2–5 nanometres, thus blocks strongly-acidic and basic centres on the sorbent surface, dampening its effect on the cell in contact with the blood and other biological media. The organosilicon mesh acts as a protector for the sorbent, saving its surface from the negative effect of biological media with various pH content.

Along with hydrophilic, mildly acidic and basic centres on the (aluminium oxide) matrix, the polymer coating also creates hydrophobic centres on the surface, which ensure multi-point bonding between the surface and different structures, such as argentous complexes. Some of the results obtained for physical and chemical features of sorbents modified with silver are indicated in Table 2.

The data in Table 2 indicate that sorbents modified with silver, with a meso- and macroporous structure, tend to maintain their sorption activity in respect of the methyl-blue marker. Special experiments indicated that after a 30-minute contact with water, the sorbent releases approximately 10% of silver into the solution. Such releasing of silver from the sorbent surface, along with its capacity to maintain its absorbent action, may prove of interest for the development of active substances for drugs, with a prolonged action. Available data on release of antibiotics from the sorbent surface confirm the effectiveness of this method. However, in the case of antibiotics, the high resistance of microbe stem cells to antibiotics also suggests some difficulties [26]. Ag-based substances, free from such negative aspects, continue to be the focus of interest [17,27].

With this in mind, research into biological qualities represents interest, particularly in terms of the biocompatibility of the produced sorbent with the prolonged release of silver in contact with liquid.

3.2. Assessment of sorbent's biological features

Throughout the experiment, the tested animals were active, curious, without presenting changes in their pelage and body mass. The layer of muscle in the sorbent injection area was easily palpable, without noticeable thickening; the animals supported the palpation calmly, without presentation of reactions to pain or discomfort; the overall assessment of the animal's condition remained positive throughout the experiment.

The absolute red blood cell count and haemoglobin in peripheral blood, in all groups and time periods, remained within the prescribed limits (Figs. 1,2).

The absolute red blood cell count by day 7 indicated a descending trend in groups II and III, while the control group indicated an ascending trend. By day 14, there were no differences between groups. However, by day 21, the erythrocyte count in group II dropped (p = 0.025 in group II and p = 0.078 in group III) in comparison with the "untreated" control group. These variations can be seen as insignificant since they, too, did not exceed the prescribed norms.

There were no statistically significant differences in the amount of haemoglobin at the outset of the experiment. On day 7, the parameters in both experimental groups were lowered, whereby in terms of statistics, more so in group III than in group II (p = 0.025). On day 14, the difference between the

Table 2



Fig. 1. RBC trends - the absolute red blood cell count in peripheral blood.

groups was evened out. However, by day 21, the same situation reoccurred, where group II showed considerably lower results than the "untreated" control group (p = 0.010). The difference in the haemoglobin level trend in both experimental groups is noticeable: intermittent lowering in the "untreated" control group, against steady growth, all within the reference range.

The peripheral blood haematocrit level in rats (Fig. 3) in the group receiving the intramuscular injection of TAGA and Ag/TAGA on day 7 tended to have a lower haematocrit (compared to the negative control group), whilst on day 21, these changes had higher statistical significance (p = 0.008 in group II and p = 0.037 in group III). Furthermore, throughout the

experiment, the parameters remained within the reference range, indicating that intramuscular application of sorption agents do not lead to considerable diversions in the haematopoietic system of the tested animals.

The absolute thrombocyte count (Fig. 4) in peripheral blood did not show statistically significant differences between the groups throughout the experiment, exceeding the upper limit of the norm by 5–30%. By day 21, following the application of sorbent, a lowering trend was noted and the differences between groups in terms of parameters were evened out.

The trend of absolute leukocyte level in peripheral blood is indicated in Fig. 5. In group I, where animals received a



Fig. 2. HGB trends - the absolute haemoglobin level in peripheral blood.



Fig. 3. HCT trend - peripheral blood hematocrit.



Fig. 4. PLT trends - the absolute thrombocyte count in peripheral blood.

"perforation" but not the substance, the wave shaped trend of increase was noted throughout the experiment (compared to the initial level), peaking on day 14 (56.9% increase), whilst by day 21 it dropped to 30.3% of the initial level. This clearly indicates a reaction of the leukocyte pool in peripheral blood to the effect of muscular layer perforation.

The trend of the leukocyte level in group II, where animals which received the initial sorbent presented an increase on days 7 and 14 (of 30.3% and 32.4% of the initial level, respectively), as well as a drop, by day 21, still remained 16.5% higher than the initial level. Furthermore, these changes indicate that there was a reaction of the leukocyte pool, although less prominent than that noted in group I.

In group III, where the animals received the sorbent modified by nanocluster silver, the level of leukocytes on day 7 proved lower by 13.3% of the initial level, followed by an increase of 20.4% of the initial level by day 14 and finally, a drop to 9.8% below the initial level by day 21. In other words, the trend noted in group III was smoother than in group II and even more so than in group I. Evidently, the presence of Ag nanoclusters and its release from the matrix surface (in group III) had smoothed the reaction to perforation of the soft tissue and the introduction of sorbent, thanks to the antiseptic qualities of silver, considerably lowering the load on the leukocyte system; indeed, by day 21, the level of leukocyte was almost identical between groups II and III (between the groups, as well as in comparison with the initial level), whilst in the first group, this parameter remained $\sim 15\%$ higher than in the "treated" groups.

Results of the research into soft tissue reactions, in terms of leukocyte, thrombocyte and erythrocyte pools in peripheral blood, to the intramuscular application of a basic sorbent and that modified by Ag nanoclusters, allowed us to conclude that both the matrix (TAGA) and the silver containing sorbent (Ag/ TAGA) are biocompatible, providing a hopeful perspective for silver containing sorbent based drugs to be used for body detoxification.

4. Conclusions

- 1 There were studied the physical and chemical qualities of the silver containing silicones and aluminium oxide sorbents of different granulometric content.
- 2 Animal experiments confirmed the antiseptic qualities and biocompatibility of the sorbents with biological tissues.
- 3 Development of sorbents appears to offer good prospects for their use in medicine.

Acknowledgements

This work is funded by the CONACYT-Mexico under projects 79062, 83275 and 130407 and PAPIIT-UNAM projects



Fig. 5. WBC trend - the absolute leukocyte count in peripheral blood.

105416, IN114209, IT200311 and IT200114 (Mexico); and Government Program «Science» of Tomsk Polytechnic University grant no. 4.1187.2014/K.

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