Estimation of the influence of porous nanostructured materials on blood chemistry values

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Abstract. Nanostructured oxides and hydroxides of iron and aluminum have been synthesized. Their influence on the hematologic and blood chemistry values is examined. The synthesized samples are found to change the hematologic values in different ways. The boehmite nanostructures produced by water oxidation of AlN/Al nanoparticles exert the greatest influence on the blood chemistry values. The obtained results are important for a general understanding of how nanostructured metal oxides interact with blood components, and can be used in the development of new hemostatic agents on the basis of micro-mesoporous metal oxides.

1. Introduction

Blood clotting is a combination of biophysical and biochemical processes that allow the transformation of blood from a liquid to a clot. As a result of the trauma, a three-dimensional network of fibrin filaments is formed under the influence of activators (blood clotting factors). It holds blood under high pressure, being the main mechanism to arrest bleeding in humans.

The formation of afibrin clot depends on a number of variables, such as pH, the ionic strength, and the concentration of calcium ions, fibrinogen, and thrombin [1]. The effect of ions of the biological medium (K⁺, Na⁺, Cl⁻, HCO₃⁻, HPO₄²⁻, SO₄²⁻, Ca²⁺, Mg²⁺) and pH values on the kinetics of fibrin clot formation was studied elsewhere [2]. It was shown that monovalent ions and SO₄²⁻ exert no influence on the formation of the clot, while calcium and magnesium ions substantially increase the viscosity of the clot. The study of the effect of ionic strength and pH on the rate of fibrin clot formation showed that their growth decreases the viscosity of the formed fibrin network.

Platelets and platelet clots aggregate much faster in the presence of calcium, while in the absence of calcium clots appear almost transparent. This is explained by the fact that calcium is crucial for the assembly of procoagulant complexes and the generation of thrombin in vivo. As a result, coagulation begins earlier, and therefore the formed fibrin fibers are thicker [3].

When nanoparticles come into contact with blood plasma, they are immediately covered with a protein corona, which can affect the morphology and abundance of human blood corpuscles [4]. The functioning of coagulation cascades and the complement system can be disturbed by interactions of nanoparticles with protein factors and a reduction of the intact protein in plasma. It is known that the concentrations of coagulation factors in plasma are rather low, e.g., 20, 10, and 200 nM for factors V, VII, and X, respectively, while the fibrinogen concentration is much higher, 9 µM. Therefore, even a slight adsorption of one of the coagulation factors on the surface of nanoparticles can noticeably shift the equilibrium of cascade reactions.

The use of materials whose surface would be able to change the ionic balance of the biological medium, as well as capable of acting as aggregators of coagulation proteins, is promising for the
development of micro-mesoporous nanomaterials with a hemostatic effect. Such nanomaterials include oxides and/or hydroxides based on aluminum and iron. They have a developed surface [5, 6], a positive charge [7, 8], and possess selective sorption properties [9–11].

2. Materials and methods

2.1. Synthesis of porous nanostructured metal oxide

Porous aluminum hydroxides and iron oxides were synthesized for the investigation. Aluminum hydroxide was produced by water oxidation of AlN/Al nanoparticles (Sample 1) using the procedure described in reference [5] and hydrolysis of aluminum isopropoxide (Sample 2) according to the following procedure: 10 g of aluminum isopropoxide was heated to 135 °C and kept in a closed flask until complete dissolution (1 hour), then it was added dropwise with constant stirring to deionized water heated to a temperature of 40 or 90 °C. The resulting precipitate was filtered off, washed with 1000 mL of deionized water, and dried at 100 °C for 2 hours. Iron oxides with different morphology and structural characteristics were synthesized under hydrothermal conditions (Sample 3) using Fe nanoparticles (Sample 4). According to the first method, 90 mL of Na2SO4 solution of 1 M concentration was placed in a 250 mL beaker, and 20 mL of FeCl3 solution of 0.5 M concentration was added to it with constant stirring and stirred for 15-20 minutes. Then 70-80 mL of distilled water was added to make a homogeneous medium. Further, the obtained solution was placed in a sealed autoclave, heated to 140 °C, and held for 6 hours. After that, the autoclave was cooled to room temperature; the resulting precipitate was filtered off, washed with 1000 mL of distilled water and dried at 120 °C for 2 hours. According to the second method, 100 mL of a 2.2 M solution of CH3COONa were placed in a 500 mL glass flask and 100 mL of Fe nanopowder suspension of 1 wt.% concentration was added to it with constant stirring and stirred for 15-20 minutes. Then the mixture was heated to boiling and boiled for 6 hours with constant stirring under reflux. 100 mL of distilled water was added to the resulting sol and boiled for 6 hours. The reaction mixture was cooled to room temperature. The resulting precipitate was filtered off, washed with distilled water (at least 1000 mL) and dried at 120 °C for 4 hours.

2.2. Characterization of synthesized porous nanostructured metal oxide

The synthesized samples were examined by transmission electron microscopy using a JEM 2100 microscope (Jeol, Japan) and X-ray diffraction analysis using a Shimadzu XRD-6000 diffractometer (Shimadzu, Japan). The nitrogen adsorption/desorption isotherms were measured at 77 K on a Sorbometr-M analyzer (Katakon, Russia). The specific surface areas were estimated by the Brunauer–Emmett–Teller (BET) method. Zeta potential measurements were performed using a Zetasizer Nano ZSP (Malvern Instruments, UK). Biochemical analysis was performed using a Cobas c 311 biochemical analyzer (Roche, Switzerland). The studies were performed on whole blood with the synthesized powders added at a concentration of 1.0 mg/mL.

3. Results and discussion

TEM images of the synthesized oxides and hydroxides are shown in figure 1. As can be seen from figure 1 a, water oxidation of AlN/Al nanoparticles results in the formation of agglomerates of crumpled nanosheets up to 200 nm in size and 2–5 nm thick. Aluminum isopropoxide hydrolysis produces similar nanostructures, but the size of nanosheets does not exceed 100 nm (figure 1 b). Iron oxide, synthesized under hydrothermal conditions, had the form of 5 µm agglomerates consisting of rods with a thickness of 20–50 nm and a length of up to 1 µm (figure 1 c). The reaction of iron nanoparticles with sodium acetate and subsequent precipitation during boiling results in the formation of layered iron oxide structures. The layer thickness is 10–20 nm, and the length is up to 1 µm (figure 1 d).

The synthesized nanostructures have a positive surface charge and a large specific surface area. The zeta potential and the specific surface area were respectively 30.5 mV and 286 m2/g for Sample 1, 35.4 mV and 307 m2/g for Sample 2, 25.7 mV and 101 m2/g for Sample 3, and 22.3 mV and 143 m2/g for Sample 4.
Figure 1. TEM images of nanostructures synthesized by: water oxidation of AlN/Al nanoparticles (a); aluminum isopropoxide hydrolysis (b), under hydrothermal conditions (c), and using Fe nanoparticles (d). All bars are 100 nm.

According to X-ray diffraction analysis (figure 2 a), water oxidation of AlN/Al nanoparticles and aluminum isopropoxide hydrolysis leads to the formation of boehmite. The maxima of the (020) absorption band are shifted to low angles with respect to crystalline boehmite (PDF No. 00-021-1307). This pattern is typical for the AlOOH compound with the pseudoboehmite structure. The sample obtained using iron nanoparticles (figure 2 b, curve 3) is represented by the Fe₂O₃ hematite phase (PDF No. 01-073-3825). The sample produced by the hydrothermal method (figure 2 b, curve 4) is represented by the FeO (OH) goethite phase (PDF No. 01-076-7164).

Figure 2. XRD pattern of nanostructures synthesized by: water oxidation of AlN/Al nanoparticles (a, curve 1), aluminum isopropoxide hydrolysis (a, curve 2), hydrothermal method (b, curve 3), and using Fe nanoparticles (b, curve 4).

Table 1 gives the results on the hematologic and blood chemistry values after interaction with the synthesized nanostructures. In blood contact with Sample 1, all blood chemistry values demonstrate the maximum decrease. For other samples, the decrease in hematologic values is no more than 20% with respect to control.

For Sample 1, there is a decrease in hemoglobin and erythrocytes but an increase in the number of platelets and leukocytes, which may be due to a decrease in the aggregative ability of Sample 1. In blood contact with Sample 2, the number of white blood cells and platelets decrease. Sample 3 is characterized by a reduction of hemoglobin, erythrocytes and platelets, while the number of
leukocytes with respect to control is much larger. In blood contact with Sample 4, there is a slight decrease in the number of white blood cells and platelets.

**Table 1.** Changes in the hematologic and blood chemistry values after interaction with the synthesized nanostructures.

|                      | Control | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|----------------------|---------|----------|----------|----------|----------|
| Hemoglobin           | 138     | 132      | 137      | 133      | 137      |
| Erythrocytes         | 4.8     | 4.5      | 4.7      | 4.4      | 4.9      |
| Color index          | 0.9     | 0.89     | 0.89     | 0.89     | 0.9      |
| Leukocytes           | 5.6     | 5.9      | 5.4      | 6.4      | 5.3      |
| Platelet count       | 117     | 128      | 109      | 102      | 114      |
| Glucose, mmol/L      | 12.1    | 8.7      | 10.4     | 10.5     | 11.7     |
| Bilirubin, µmol/L    | 48.3    | 8.7      | 10.4     | 10.5     | 11.7     |
| Protein, g/L         | 3.9     | 8.7      | 10.4     | 10.5     | 11.7     |
| Cholesterol, mmol/L  | 3.89    | 8.7      | 10.4     | 10.5     | 11.7     |
| a-Amylase, U/L       | 32      | 8.7      | 10.4     | 10.5     | 11.7     |
| Albumin, L           | 27      | 8.7      | 10.4     | 10.5     | 11.7     |

4. Conclusion

In this study, different methods were used to synthesize porous nanostructured particles based on oxides and hydroxides of aluminum and iron. The effect of the synthesized nanostructures on the hematologic and blood chemistry values was investigated. It was found that the synthesized samples affect the hematological values in different ways. Boehmite produced by water oxidation of AlN/Al nanoparticles has the greatest influence on the blood chemistry values. The reported results are useful for a general understanding of how metal oxides and hydroxides with a developed surface interact with the blood components, and can be used in the development of new hemostatic agents on their basis with high hemostatic efficiency.

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