Interaction of nanodiamonds materials with influenza viruses

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Abstract. The perspectives of the application of modern materials contained nanodiamonds (ND) are considered in this study. The interaction between detonation particulate ND, soot and influenza A and B viruses, fragments of cDNA were analyzed at the normal conditions. It was shown that these sorbents can interact with the following viruses: reference epidemic strains of influenza A(H1N1), A(H1N1)v, A(H3N2) and B viruses circulated in the word in 2000-2010. The allantoises, concentrated viruses, cDNA can be absorbed by ND sorbents and getting removed from water solutions within 20 min. ND sorbents can be used for the preparation of antivirus filters for water solution and for future diagnostic systems in virology.

Introduction

The contamination of the environment by biological and chemical waste products becomes a world-scale problem, which requires a different ways of solving. Purification of the water, containing micropatogens, is one of the most difficult task, especially related to viruses, sized in nm. Charcoal has been used as adsorbent for many years. Searching new materials with higher sorption efficiency becomes very actual. It was shown that modified graphite after treatment in water at 95°C [1], the carbon nanotubes [2] belonging to the group of carbon materials which can interact with chemical matters, drugs and viruses and remove them from air and water solutions. During the investigation it was discovered that all of them have both advantages and deficiencies. The detonation nanodiamonds were discovered in 1960s in Russia as the results of the explosion of mixture of trotyl and hexogen in optimal proportion 3:5 at pressure 16 - 23 GPa and T above 3000K [3,4]. First investigation period of ND was devoted mainly to physical-chemical properties and leaded to the practical results - the creation of polished, lubricant additive materials and others. Recently the studies devoted to interaction of ND with proteins, enzymes resulted to the proposal of application of ND for drug delivery to cancer cells, in anti cancer therapy [4,5]. The interaction of ND with micropatogens was almost not investigated yet. According to [6], it was found that ND could interact with bacterium E – Coli. This study continued the serial investigations devoted to the interactions of modern materials with viruses, in particular between ND nanomaterials and influenza viruses. The aim of this work is manufacturing sorbents for antivirus filters and virus control system based on ND nanomaterials.

Experimental

Preparation of nanodiamonds and soot

The nanodiamonds (ND) and soot were manufactured by detonation method [3]. The electron
microscopy analysis showed that both nanodiamonds and soot contain the clusters with different forms and sized (Fig.1). The content of different impurity atoms ND and soot in concentration more then 0.01% atom presented in table. It was studied by standard C-H-N chemical analysis.

Virus cultivation and registration
The reference strains A/New Caledonia/20/99(H1N1), A/Perth/16/09(H3N2), B/Sichuan/379/99, B/Florida/04/06 were chosen for study. The viruses were grown in 9-10 embryonated chicken eggs. The viruses were concentrated by ultracentrifugation and diluted in physiological solution. The hemagglutination test with human erythrocytes was used to determined viruses in solutions.

Interaction of viruses with sorbents
The method of investigation virus-sorbent interaction was developed by Ivanova et al and described in [1,2]. It includes several stages: the intensive contact of viruses with sorbent on shaker during 15min at temperature 22°C, low speed centrifugation (2000 circle /min), the determination of viruses in solutions before sorption and in supernatant after sorption . In the case of influenza viruses solution volume was 200-400 μl, sorbent mass - 4 mg. The hemagglutination test with human erythrocytes was used for virus detection in solutions. The experiments were carried out in the follow solutions: H2O after previously special treatment by heating to destroy other pathogens, 0.15 M NaCl in H2O, allantoises liquid of chicken embryos.

Desorption of viruses from sorbents
The desorption of viruses into solution was also investigated. The complex virus + sorbent put into 0.15M NaCl pH= 7,2 for 24, 48 h at 20ºC. Then the complex was precipitated by centrifugation at 2000 circle/min during 5 minutes. Supernatant was investigated for presents of viruses in hemagglutination test with human erythrocytes.

cDNA preparation and registration
The fragments of cDNA were obtained from influenza A viruses in Real Time polymerase chain reaction (PCR) using Russian test - system “Influenza A virus” (firm “DNA-technology”, Russia). The cDNA samples (80-100 μl) connected with sorbent (mass 2mg) by the same method , which we used for viruses. The samples before and after sorption were analyzed by electrophoresis in 2% agarose gel during 1.5 h., voltage = 80V. The volume of the probe injected into gel was 7 μl. The positive control was the control sample included in the test -system. The gel was photographed and analyzed by transluminator, detection wavelength λ=254 nm.
Results and discussion
The first biological objects were influenza viruses which caused the epidemics in the world during 1998-2010 according data of World Health Organization. The surface viral proteins - hemagglutinin and neuraminidase had different structure and according antigenic properties. All these viruses were concentrated to remove majority of allantoises protein and to increase the initial virus concentration. It was established that both sorbents interacted with all viruses in solutions. The hemagglutination titers of virus solutions decreased in 8-1000 times after contact with sorbents (Fig.2). The adsorption did not depend on antigenic properties of viruses. The decreasing of this value depended on the virus titers before sorption and the sorbent mass. The soot was approximately 2.5 times more effective than nanodiamonds. The experiments for desorption of viruses from sorbents were carried out to determine the stability of the binds of viruses with sorbents. It was shown that viruses absorbed at ND did not desorb at all from sorbent or desorption was very small. The results for influenza virus

![Figure 2](image_url)

Figure 2. Sorption of influenza A and B viruses on nanodiamonds and soot

![Figure 3](image_url)

Figure 3. Sorption and desorption of influenza viruses B/Florida/04/06 during 24h and 48h from nanodiamonds.
B/Florida/04/06 was present at Fig. 3. Viruses titer in solution before sorption was 128 HAE, after sorption - <2 HAE and after desorption 24-48 h was <2 HAE. It shows the stability of the bind between viruses and sorbents at the chosen conditions in solutions.

The next objects for investigation were the fragments of cDNA. After preparation in PCR, the samples were added to tubes with sorbents for contact during 20 min at 22°C, then the complexes cDNA with sorbent were pellet by centrifugation. The supernatant was analyzed by electrophoresis. Both sorbents interacted with fragments of cDNA with size 190 b.p. Fragments of cDNA sized more 560 b.p. were removed by ND partly, by soot – completely (Fig.4).

**Figure 4.** Electrophoreses of cDNA after interaction with nanodiamonds and soot.
Pattern 1- plasmid PUC19 (molecular size marker 34-501 b.p.), pattern 3- fragments of cDNA before sorption, patterns 4,5 - after sorption on ND, patterns 6,7- after sorption on soot.

Early it was shown the adsorption of liner DNA by ND and the absence the interaction ND with ring DNA [7]. Our data showed that the size of fragment cDNA also important for effective interaction for ND in instead of soot in size DNA 190-560 b.p.

The interaction between proteins and cDNA with ND materials and its absence in the case of diamonds can be explained by the differences in their structure and chemical purity [3,4]. Instead of diamonds ND and soot had on their surfaces besides carbon such atoms as N, O, S, H. The percent of these elements varied from 0,03 till 5,69 % atom in the case sulphur and oxygen accordingly (Table). They could form the binds with functional amino acid groups of viral proteins, nonviral proteins or enzymes [4].

After the analysis of our data we concluded that viral proteins and cDNA interact with ND sorbents. We observed the differences in absorbed properties of ND and soot. The soot absorbs these biological objects better then ND. This phenomenon could be explaining the structure and its specific surfaces of sorbents. The specific surface of soot is 450 m²/g, it is much more than the same surface of nanodiamonds – 300 m²/g. In addition the soot has a difference in the properties of surface carbons. It has more amorphous carbon [3]. Therefore more intensive soot interaction with viruses and cDNA (compare to ND) correlates with its physical-chemical properties.
Table. Chemical composition of nanodiamonds and soot used

| element | Nanodiamonds | | Soot | |
|---------|--------------|----------|----------|----------|
|         | % atom       | % mass   | % atom   | % mass   |
| C       | 91,7954      | 89,1718  | 93,5983  | 90,6255  |
| N       | 2,0570       | 2,3310   | 1,3851   | 1,5644   |
| O       | 5,6998       | 7,3763   | 3,8191   | 4,9263   |
| Na      | 0,1095       | 0,2037   | 0,1419   | 0,2631   |
| Al      | 0,0712       | 0,1554   | 0,6012   | 1,3071   |
| Si      | 0,0982       | 0,2228   | 0,1067   | 0,2414   |
| S       | 0,0314       | 0,0814   | 0,0309   | 0,0800   |
| Cl      | 0,0190       | 0,0544   | 0,0669   | 0,1912   |
| K       | 0,0298       | 0,0941   | 0,0429   | 0,1354   |
| Ca      | 0,0119       | 0,0386   | 0,0589   | 0,1904   |
| Fe      | 0,0223       | 89,1718  | 0,0391   | 0,1759   |

**Conclusion**

Our results showed that nanodiamond sorbents can interact with influenza viruses independently on antigen properties of surface proteins and we suppose that interaction of these sorbents with other viruses might also have place (for example could interact with other envelope viruses besides influenza viruses). The ND materials absorb viruses from solution more effectively compare to carbon nanotubes [2]. The ability of ND materials to absorb the viruses and fragments of cDNA can be used for the creation of the antimicrobial filters for water decontamination from micropathogens and the development of new diagnostic systems. Our data and early established the absence of toxic properties of ND and ability to keep the enzyme activities of proteins connected with ND [4] extend possibilities to use ND material as carrier for virus proteins.
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