The sorption of influenza viruses and antibiotics on carbon nanotubes and polyaniline nanocomposites

V T Ivanova 1, G S Katrukha 2, A V Timofeeva 3, M V Ilyna 1, Y E Kurochkina 1, L A Baratova 3, I Yu Sapurina 4 and V F Ivanov 5

1 D.I. Ivanovsky Research Institute of Virology RAMS, Gamaleya st, 16, Moscow 123098, Russia; 2 G.F. Gause Institute of New Antibiotics RAMS, Moscow 119021, Russia; 3 A.N. Belozersky Research Institute for Physico-Chemical Biology, M.V. Lomonosov Moscow State University, Moscow 119991, Russia; 4 Institute of Macromolecular Compounds RAS, 199004, St. Petersburg, Bolshoy Pr. 31, Russia. 5 A.N. Frumkin Institute of Physical Chemistry and Electrochemistry, RAS, Leninsky prospect, 31, Moscow 119991, Russia.

Key words polyanilines, carbon nanotubes, influenza viruses, antibiotics-polypeptides

E-mail: valivanova1946@mail.ru

Abstract

The decontamination of the solutions from micropatogens and drug delivery are the important problems of modern life. It was shown that carbon nanotubes, polyaniline and their composites can interact with antibiotics-polypeptides and some viruses (pandemic strain of influenza viruses A(H1N1)v circulated in Russia in 2009-2010. During a short time drug and viruses can be absorbed by polyaniline and removed from aqueous solutions at the normal conditions. Polyaniline composites can be useful for the preparation of drug delivery and virus control filters and also in biotechnology for the improvement the methods of antibiotics purification.

Introduction

Drugs and especially antibiotics became the new type of environment pollution. The development drug delivery system is an actual problem for medical centers, hospitals, in cattle breeding and poultry farming. The modern drug delivery systems based on conducting polymers: polyaniline and polypyrrole [1]. These polymers were non-cytotoxicity, biocompatible and began to use in biomedical applications. Now polyaniline has been demonstrated to be the most important conducting polymer due to its good environmental stability low cost, ease of processibility, oxidation-protonation adjustable electrical and optical properties [2]. Due to electronic and electrochemical properties polyaniline is highly sensitive to molecular interactions and can provide signal transduction function for molecular detections. It can release the drug with the help of acid-base interaction or its reduction-oxidation activity. The possibility to use an electrical stimulation made this delivery system long-time functional and reversible. It was shown recently that polyaniline specifically interact with influenza viruses and surface HBs-antigen of hepatitis B [3, 4]. Such infection as poliomyelitis, Hepatitis A, influenza caused by pandemic strain A(H1N1)v, influenza bird viruses and others infections can be spread by the water way. The important task is to create the anti-viruses filters for prevention infections spreading. This study continued the serial investigations devoted to the interactions between modern nanomaterials and viruses. The aim of this work manufacturing the antibiotic delivery system and virus control system based on polyaniline composite materials.

Experimental

Preparation of polyaniline and its composites

The chemical synthesis of polyaniline (Fig.1) was performed in the usual manner throw the oxidation of 0.1 M aniline by the 0.1 M ammonium persulphate in 0.5 M aqueous solution of HCl at room temperature. The formed polyaniline salt after precipitation was converted to the polyaniline base after the treating polyaniline salt by the 0.5 M aqueous solution of NH3. After precipitation and washing this base was used as sorbent in our experiments. The polyaniline interpolymer complexes with polypropylamidopropanesulfonic acid (PAMPSA) was obtained in the same manner [5]. The sorbents – polyaniline base and composites were obtained in A.N. Frumkin Institute of Physical Chemistry and Electrochemistry, RAS.

![Figure 1](https://example.com/figure1.png)

Figure 1. The structure of polyaniline

Published under licence by IOP Publishing Ltd
Preparation of Multiwalled carbon nanotubes

Multiwalled carbon nanotubes (MWCNT) were firstly described by Iijima [6]. They were manufactured by the catalytic chemical vapor deposition method (Nanotechcenter, Tambov, Russia). MWCNT consisted of particles 40-80 nm in diameter and up to 10 μm in length (Fig. 2). The specific conductivity of MWCNT is 40 S cm$^{-1}$. Specific surface area of the material is average 60-100 m$^2$/g, porosity more than 70%. The pore distribution is very narrow, more than 90% of pores belong to the diapason 20-80 nm. Carbonaceous material was purified with a mixture of concentrated acids and the residual catalyst content was equal to about 1% wt.

![Figure 2. Multiwalled carbon nanotubes (MWCNT). Transmission electron microscopy.](image)

MWCNT were coated with the polyaniline layer (Fig. 3) according [7]. This composite material was prepared by the method of in-situ aniline polymerization in the presence of MWCNT. Polyaniline content is 30% wt. The conductivity of composite material is 10 S cm$^{-1}$ independent of polyaniline doping level, specific surface area and porosity is 50 m$^2$/g and 60% corresponding. The (MWCNT) covered polyaniline base were obtained in Institute of Macromolecular Compounds RAS.

![Figure 3. MWCNT covered with the polyaniline layer. (Transmission electron microscopy.)](image)

Virus cultivation

The pandemic in Russia in 2009-2010 was caused by the active circulation of the variants of first influenza pandemic strain A/IV-Moscow/01/2009 (H1N1)swl [8]. This virus was isolated in Institute of Virology RAMS. It was similar to the reference strain A/California/7/2009 (H1N1)v. The viruses were grown in 9-10 brininated eggs strains were studied. The hemagglutination test with human erythrocytes was used to determine viruses in solutions.

Antibiotic cultivation and registration

Antibiotics-polypeptides were obtained in the Gauze Research Institute for the Search of Novel Antibiotics (Tab.1).
Table 1. Antibiotics-polypeptide used

| Name         | General chemical characteristics | UV spectrum, λ max, nm (solvent) | Molecular weight, D |
|--------------|----------------------------------|----------------------------------|---------------------|
| Gramicidin S | Cyclopeptide                      | 219 (MeOH)                       | 1141                |
| Teicoplanin A2 | Cyclic lipoglycopeptide       | 280 (0.01 n. HCl)               | 1875                |
| Bleomycetin  | Heteropolypeptide               | 280 (H2O)                       | 1438                |
| Polymyxin B  | Peptide-cyclopeptide            | 214 (H2O)                       | 1202                |

**RP HPLC analysis of the antibiotics** in solution before and after sorption was performed on a Milichrom A-02 microcolumn liquid chromatograph (ZAO Econova, Novosibirsk, Russia) with stainless steel columns of the dimensions 2.0 x 75.0 mm packed with the Nucleosil 100-5C18 PAH sorbent from Macherey-Nagel (Germany). The columns were thermostated at 35°C. Antibiotic solutions in water with concentrations ranging between 0.2 mg/ml were used for the analysis. The volume of the probe injected was 15 μl. Two detection wavelengths λ 214 and 280 nm were used for the analysis. The analysis time ranged between 20 and 25 minutes for all the antibiotic preparations analyzed. Elution with linear acetonitrile gradient from 0 (eluent A, 0.1% solution of TFA in water) to 100% (eluent B, 0.1% solution of TFA in acetonitrile) at the flow rate of 100 μl/min was used. The reagents were dissolved in deionized water purified by a Milli-Q® Plus device from Millipore (France); acetonitrile used for HPLC was from Sigma-Aldrich (Germany).

**Antibiotics static sorption** was studied according to the following procedure: 600 μl of the antibiotic solution with the concentration 0.2 mg/ml was added to a portion of the sorbent (1.25, 2.5 or 5.0 mg). The resulting suspension was incubated for 15 min., 1.0, or 18 hours at 18–20°C. After sorption, the antibiotic solution was separated from the sorbent by centrifugation for 5–7 min at 5600–8850 g in a Beckman Coulter™ Microfuge® Centrifuge (United States).

**Antibiotic amount determination** in the starting solutions and the supernatants was performed using RP HPLC on a Milichrom A-02 liquid chromatograph as described above. Quantitative analysis of chromatographic data was performed using the program MultiChrom-SPEKTR for Windows 9. & NT, version 1.5x_E (Ampersend, Russia).

**Interaction of viruses with sorbents**
The method of investigation virus-sorbent interaction was developed by Ivanova et al [3]. It includes some stages: intensive contact virus + sorbent on shaker during different time diapazones (15min-1 h) in range of temperature (8-25°C), low speed centrifugation (2000 circle /min), the determination virus in solutions before and in supernatant solutions. In the case of influenza viruses was used hemagglutination test with human erythrocytes. The sediment (virus+ sorbent) after centrifugation represents the immunosorbent. The experiments were carried out with the follow solutions: H2O after previously special treatment by heating to destroy other pathogens, 0.15 M NaCl in H2O, 0.1 M Tris-HCl buffer (pH 7.2), allantoic liquid of chicken embryos.

**Results and discussion**
The first biological object was the influenza virus–pandemic strain A/IIV Moscow/01/09, (H1N1)v antigenic related to influenza swine virus. Such viruses caused the pandemic in the world in 2009-2010 according the opinion of World Health Organization experts and were widely spread in Russia in autumn 2009. These viruses had differences in protein structures compared with these properties of annual epidemic strains. It was established that adsorption activities of influenza strain A(H1N1)v varied and depended of the sorbent, The sorption of viruses in solutions were intensive with Pan sorbents, less with carbon nanotubes covered Pan and the least with nanotubes without Pan Fig.4. The hemagglutinin titters after sorption were in 64, 16, 8 n 4 times less then initial titters for these sorbents accordingly. All these experiments were carried out at T=25°C. So the result of the previous and current data directs
that influenza viruses A with hemagglutinin A/H1,H3,H5,H7 isolated from human, birds and B viruses, can be immobilized at the sorbents. The sorption depends of sorbent structure. The most active sorption was observed with Pan sorbents.

![Virus before sorption](image1)

**Figure 4.** Sorption of influenza virus pandemic strain A/IV Moscow/01/2009 (H1N1)swl on different sorbents from solutions.

The next objects for investigation were some practically important antibiotics-polypeptides: bleomycetin, gramicidin S, polymyxin B и teicoplanin A2. The influence of contact time (15 min, 1 h, 18 h) for the sorption on CNT was investigated in experiments at $T=22^\circ C$ (Fig.5).

![Graph](image2)

**Figure 5.** Sorption of antibiotics on CNT depending on contact time.

It was shown that antibiotics (the concentration $C=0,2 \text{ mg/ml}$) gramicidin S и teicoplanin A2 removed from water solutions during 1 hour, polymyxin B and $\lambda$ bleomycetin - 18 hours (Fig.5). The dependence of sorbent mass of CNT (1.25, 2.5, 5 mg) for the sorption was analyzed too at $T=22^\circ C$, (Fig.6). It was established that the optimum sorbent mass is 5 mg, when all antibiotics ($C= 0.2$ mg/ml) adsorbed. The gramicidin S и teicoplanin A2 removed from water solutions during 1 hour, polymyxin B and bleomycetin - 18 hours.
Figure 6. Sorption of antibiotics on CNT depending on sorbent mass

After the analysis of these data we make the conclusions that the relationship to the sorbent is determined by the structure of antibiotic. The hydrophobic antibiotics—gramicidin S и teicoplanin A2 absorb more completely at the same condition, than hydrophilic positive charge antibiotics polymyxin B and bleomycin.

Early we established that the antibiotics, gramicidin S и teicoplanin A2 removed from solution on sorbent CNT + Pan at 97-100% during 1h, in the case of Pan-base at 94-100% during 18 hours [9]. The data direct that affectivities of these antibiotics as an absorbent depends on hydrophobic properties of Pan-base, CNT+ Pan. The sorbent CNT+ Pan has more hydrophobic properties, then Pan-base, because when at the all parameters of the sorption hydrophobic antibiotics gramicidin S и teicoplanin A2 interacted with CNT+ Pan more intensive (1 hour). It is known that CNT can absorb gas (dioxin), liquid and dissolvable substances [10]. The interaction of CNT, Pan-base and composites prepared on their base with influenza viruses can be used for the creation of the effective filter for water decontamination from micro pathogens.

Conclusion

The analysis of the data obtained evidences that the elaborated sorbents (CNT, CNT+ Pan, Pan-base) are perspective for utilization as water filter for decontamination micropathogens from different solutions and in biotechnology for isolation and purification of antibiotics-polypeptides from culture liquids. It is established that antibiotics polypeptides – teicoplanin A2, gramicidin S, polymyxin B and bleomycetin are able to absorb by MWCNT most intensive.

It was shown that antibiotics sorption depends on as structure and properties of the antibiotics as on hydrophobic properties of sorbents.

Acknowledgments

The research was partially supported by ISTC grants 3070 and 3718.

References

[1] Ge D, Qi R., Mu J, Ru X, Hong S, Ji S, Linkov V, Shi W, 2010, Electrochem. Commun. 12 1087
[2] Liu S, Wang J, Zhang D, Zhang P, Ou J, Liu B, Yang S, 2010 Appl Surf Sci 256 3427
[3] Ivanova V T, Ivanov V F, Kurochkina Y E, Gribkova O L., Ilyina MV, Marykin A A 2009 Problems of virology 3 21
[4] Li X-N, Dai L, Liu Y., Chen X-J, Yan W, Jiang L-P, Zhu J-J , 2009 . Adv Funct Mater.19 3120
[5] Ivanov V F, Gribkova O L, Cheberjako KV, Nekrasov A A , Tverskoy VA, Vannikov A V 2004 Rus. J. Electrochemistry, 40 3, 339
[6 Iijima S 1991 Nature. London 354 56
[7] Sapurina I.Yu, Kompan M E , Zabrodskii A G, Stejskal J, Trchova M , 2007 Rus J. Electrochemistry. 43 5 528
[8] Lvov D K, Burtseva E I, Prilipov A G et al 2009 Problems of virology 5 10
[9] Ivanova V T , Sapurina I Yu, Ivanov V F , et al. 2009 Rusnanoforum, Moscow, 6-8 October 2009 , 274
[10] Dyakov P N 2006 Carbon nanotubes. Structure, properties, application. Moscow, 5 120.