Chem. Pap. (2017) 71:495–503
DOI 10.1007/s11696-016-0068-5

ORIGINAL PAPER

Conducting polymers as sorbents of influenza viruses

Valeria T. Ivanova[1] · Ekaterina O. Garina[1] · Elena I. Burtseva[1] · Elena S. Kirillova[1] · Marina V. Ivanova[1] · Jaroslav Stejskal[3] · Irina Yu. Sapurina[2,3]

Received: 22 March 2016 / Accepted: 28 May 2016 / Published online: 16 December 2016

Abstract Polyaniline, polypyrrole and conjugated polymers modified with silver nanoparticles have been studied as sorbents of influenza viruses from aqueous media. The sorption of various strains of influenza viruses, including A and B viruses circulating in recent years in Russia Federation, The United States of America, and in Western Europe, have been examined. It is shown that the sorbents based on conducting polymers removed from water large variety of virus strains and the efficiency of adsorption was higher compared with a carbon sorbent. The sorption of both purified and unpurified viruses associated with non-viral proteins were studied. The sorption takes place rapidly enough in the temperature range 4–37 °C and, as a result, the infection viral activity of solution was reduced by 4–6 orders of magnitude. The effectiveness of virus adsorption virtually unchanged in the whole temperature range. Polyaniline and polypyrrole composites with silver provide almost complete removal of viruses and complete water treatment, and this also applies to unpurified influenza viruses. Preliminary assessment of toxicity in vitro has not identified the cytopathic action of polyaniline that is the most problematic in terms of toxicity.

✉ Irina Yu. Sapurina
sapurina@mail.ru

[1] D. I. Ivanovsky Research Institute of Virology, N. F. Gamaleya Federal Research Center of Epidemiology and Microbiology, Ministry of Health of the Russian Federation, Moscow 123098, Russian Federation

[2] Institute of Macromolecular Compounds, Russian Academy of Sciences, V. O. Bolshoi pr. 31, St. Petersburg 199004, Russian Federation

[3] Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, 162 06 Prague 6, Czech Republic

Keywords Influenza viruses · Conducting polymers · Polyaniline · Polypyrrole · Sorbents · Water purification

Introduction

Influenza occurs globally and causes serious problems in public health and economics. According to the World Health Organization, the annual incidence is estimated at 5–10% among adults and 20–30% among children. Up to 50% of the population can be infected in a single pandemic year. Among high-risk groups: children, senior people, and people with weak immune systems, there is a high percentage of deaths. Annually the mortality associated with influenza and the complications caused by influenza range from 151,700 to 400,000 (Dawood et al. 2012). The size of the influenza virus is about 100 nm. Viruses have high mutation rates. Mutations produce selective advantages for viral strains by allowing them to evade preexisting immunity. Almost each year there are new epidemic strains of viruses that rapidly develop resistance towards existing antiviral drugs. Due to the high variability of influenza viruses, it is not possible to reliably protect the population by vaccination. Three types of influenza viruses affect people, A, B, and C types (Hinshaw and Webster 1982). The most important influenza pathogens are represented by influenza A viruses that infect not only humans but also a wide variety of warm-blooded animals, including swine, horses and other mammals and birds. Avian influenza viruses in aquatic birds serve as the natural reservoir for new viruses subtype by reassortment between genes of influenza human and bird viruses. All known subtypes of influenza A virus are probably the ultimate source of human pandemic influenza strains (Jeffery and David 2008).

The main efforts in infection control are aimed at identifying the strain of influenza circulating at the moment, a study of its structure, and the creation of antiviral vaccines and drugs. Influenza is a respiratory virus infection which is mainly transmitted from person to person through air during cough and sneezing. But influenza avian viruses spread mainly by aqueous media (Hinshaw et al. 1979; Burtseva et al. 2012). The virus is found in natural waters, wastewaters, and even in the system of water pipes. An important role in the emergence of new strains and their distribution is played by the migration of waterfowl. The influenza disease in birds can occur in various manners: as asymptomatic forms, cough, rheum, or as acute intestinal infection, the defeat of the central nervous system and many birds perish within a week. The virus replication propagates both in a respiratory and in intestinal pathways and then virus is excreted in high concentrations in the feces. Water birds transmit the influenza viruses by fecal–oral route via contaminated water. Maintenance of virus in water depends on several factors: concentration of salts, pH, and temperature. At 17 °C, some strains remain infectious for 207 days, at 4 °C for a considerably longer time (Hinshaw et al. 1979; Stallknecht et al. 1990). The virus properties including infectiousness depend on the structure of the proteins and genome. The composition and structure of oligosaccharides in glycoproteins depend on the host cell where the virus replicates. The influenza virus A/H1N1 and A/H5N1 virus infectiousness contained during 19–25 days at 35 °C in water solution wherein viruses grown on MDCK cells were more stable than the same viruses grown on avian cells (Shigematsu et al. 2014).

The mixing of viruses with different genes takes place in water. Seasonal changing of habitat of birds and way of their migration render substantial influence on distribution of genic pool. The genes of influenza viruses of bird origin were identified in the viruses isolated in European strains A/Mink/Sweden/84 (H10N4), A/Swine/Netherlands/85 (H1N1), A/Swine/Germany/81 and in the viruses isolated in northeastern China And/Horse/Yulin/89 (H3N8). Viruses of flu of birds with hemagglutinin A/H5, And/H7, And/H9, And/H6 cause diseases in people. The highest pathogenicity for a man is typical of viruses with hemagglutinin And/H5. More than 50% diseases end with a fatal outcome (Wong and Yuen 2006). In general, the cases of human infection with avian influenza viruses are found in Southeast Asia. It is also believed that the epidemic of the 1977 was the result of a leak of a strain of virus from the research laboratories by wastewater system (Jeffery and David 2008).

In this situation, the creation of anti-virus water system purification is an urgent task. This goal cannot be solved with the use of filtration membranes, because the sizes of influenza viruses are usually smaller than the pore size. For economic reasons, large volumes of water cannot be cleaned by special immunosorbents with covalently or ionic coupling of virus-specific antibodies. Non-specific sorbents, effectively linking different strains of viruses, are necessary to be developed for the mass use.

The sorbents currently offered for non-specific adsorption of viruses are barium sulfate and macroporous silica. Most currently, however, the sorbents based on carbon, such as, activated carbon, carbon black and, recently, carbon nanotubes and nanodiamonds have been used in purification of water from biological contaminants (Ivanova et al. 2008). Carbonaceous materials are universal sorbents and they bind all types of viruses regardless of antigenic structure of surface proteins (Ivanova et al. 2012a). The adsorption on carbon, however, occurs only through weak van der Waals interactions, and the efficiency is low, despite the huge specific surface area of sorbents. It is established that the efficiency of sorption of viruses increased after modification of silica (Kontarov et al. 2015) and carbon sorbents with amino groups due to the manifestation of chemisorption-specific interactions of the sorbent with a protein. It was demonstrated that the presence of the nitrogen heteroatom significantly increased the binding of viruses of various nature (Ivanova et al. 2012b). This was the reason why the conducting polymers polyaniline (PANI) and polypyrrole (PPy) were chosen as sorbents (Fig. 1). Polymers belonging to the class of heterocyclic compounds contain a nitrogen heteroatom in the main polymer chain (Cho et al. 2007; Stejskal et al. 2015).

PANI and PPy meet the basic requirements for sorbents. They are insoluble in water or in organic solvents, they are stable in aggressive chemical environments (Brožová et al. 2008) and stable at elevated temperature, allowing thus for thermal or chemical sterilization of sorbents and recycling. The polymers can be obtained as nanoparticles, nanotubes or nanofibers, or porous matrices permeable to liquids and gases. The polymer structures can have a large specific surface area up to hundreds of m^{-2} g^{-1}. The pore size of polymeric sorbents can be optimized for the size of the particles of influenza viruses ($\approx$100 nm). PPy and PANI are hydrophilic (Stejskal et al. 2008) and wetted well by aqueous media, which increases the efficiency of their interaction with biological material (Xu et al. 2015). Currently, the toxicity and biocompatibility of the polymers and the possibility of applying them in vivo are actively studied. It is shown that conductive polymers can serve as biointegrated electrodes to perform important functions for restoring connectivity of neurons or monitoring the state of biological object. Many papers are devoted to the growth of stem cells on polymeric media and use of templates based on PANI and PPy to regenerate bone and nerve

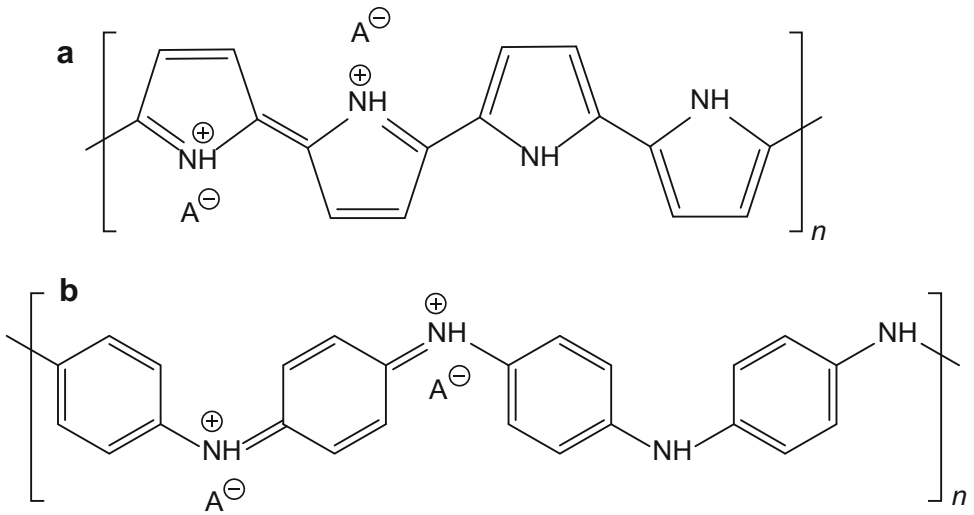

Fig. 1 **a** Polypyrrole and **b** polyaniline salts. A^- is an arbitrary counterion

tissues (Otero et al. 2012). The conductivity of these polymers may be of benefit in the applications. Sorbents on the basis of PANI are expected to be used for the separation and analysis of DNA and separation of antibiotics from culture media (Il'ina et al. 2012).

This present study is devoted to the development of universal sorbents that can effectively remove various strains of viruses from aqueous media. Pristine PANI and PPy, their composites with carbon adsorbents, and conducting polymers modified with silver nanoparticles have been investigated as sorbents of influenza viruses. Virus strains such as the influenza viruses of humans and birds isolated during the period 2004–2012 were tested. The viruses differed with proteins of the virion envelope that is responsible for virus interaction with the environment, including the sorbent. For comparison, the sorption of viruses under the same experimental conditions was carried out on carbon sorbents, such as multi-wall carbon nanotubes.

Experimental

Sorbents preparation and their characteristics

Multi-wall carbon nanotubes (CNT) prepared via the catalytic pyrolysis of hydrocarbons and cleaned from the catalyst were purchased from Taunit (Tambov, Russia). Conducting polymers, PANI and PPy, were prepared by oxidative polymerization of aniline (Sapurina et al. 2014) or pyrrole (Alekseeva et al. 2015) with ammonium peroxydisulfate. The oxidant, ammonium peroxydisulfate (0.125 M), was added dropwise to monomers solution (0.1 M) in 0.1 M sulfuric acid at room temperature. During polymerization, the conductive form of polymers doped with sulfate anion proceeded with the yields close to 100%. One-dimensional polymers were prepared by methods described earlier for PANI (Konyushenko et al. 2006) and for PPy (Kopecká et al. 2014). During the synthesis of PPy, methyl orange (MO) was used as a template of nanotubular growth. Polymers were collected as black powders by filtration. The powders were rinsed with water and with ethanol for removing low molecular weight impurities and dried at room temperature.

Multi-wall carbon nanotubes were coated with polyaniline (Sapurina et al. 2015) and obtained under similar experimental conditions by polymerizing aniline in the presence of CNT. Composition of CNT–PANI (50% of PANI by weight) was obtained by mixing equal masses of aniline and CNT and was controlled by the yields of the final product.

PANI and PPy with deposited silver nanoparticles (PANI–Ag and PPy–Ag) were obtained as a result of redox interactions between redox-active conducting polymers and silver nitrate (Stejskal et al. 2009). A weighed amount of the polymer was immersed in excess of 0.02 M aqueous solution of silver nitrate, and the metallic silver nanoparticles grew on the polymer matrix. The material was separated on a filter, rinsed with water and left to dry at ambient conditions. The silver content, 25–30 wt.%, was determined from the increase of the polymer mass.

Characterization

The morphology of the sorbents was assessed by transmission and scanning electron microscopies with the help of SuPR-55VP Zeiss (Germany) and JEOL JEM 2000FX (Japan) microscopes. Carbon nanotubes had a diameter of 50–100 nm and lengths of several micrometers. PPy and PANI consisted of spherical particles 50–80 and 100–150 nm in size, respectively (Fig. 2a, b) The

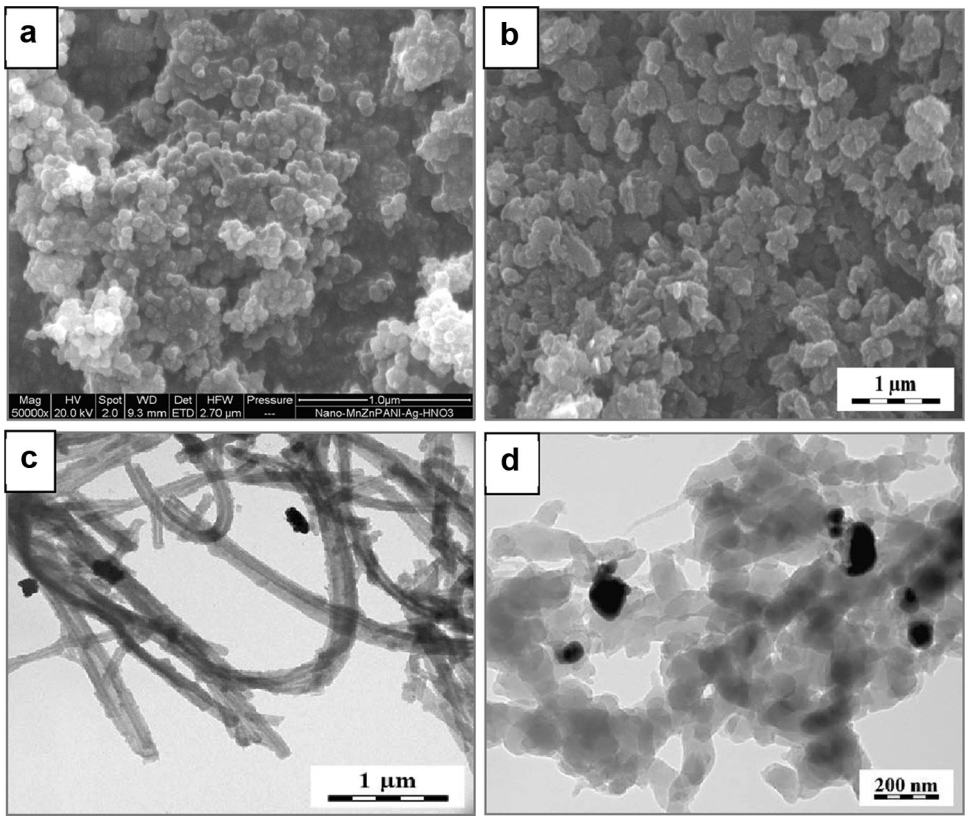

Fig. 2 Scanning electron microscopy of **a** PPy, **b** PANI with the particles of granular structure, and transmittance electron microscopy of **c** PPy nanotubes, and **d** PANI structures modified with silver nanoparticles

composite CNT–PANI retained one-dimensional morphology as the polymer overlayer is produced as a coating of CNT. PPy–Ag (Fig. 2c) and PANI–Ag (Fig. 2d) contain spherical silver nanoparticles with diameter below 100 nm localized on the polymer surface.

The specific surface area of the materials was determined by the method of low-temperature nitrogen adsorption (BET-analysis) using NOVA (USA) equipment. Specific surface area of PANI and PPy was 36 and 50 $m^2\ g^{-1}$, respectively. Specific surface area of CNT was 150 $m^2\ g^{-1}$ and that of composite CNT–PANI was 78 $m^2\ g^{-1}$ (Sapurina et al. 2015).

Human influenza viruses

The types A and B isolated during the period 2004–2012 have been used, such as pandemic strains: A/IIV-Moscow/01/2009 (H1N1)swl, A/South Caroline/02/2010 (H1N1)pdm09; epidemic strains: A/Moscow/72/07 (H3N2), A/Moscow/28/08 A(H1N1), A/Victoria/361/11 (H3N2); influenza B viruses: B/Malaysia/2506/04, B/Moscow/06/11, B/Victoria/02/87-like, B/Florida/04/06, B/Massachusetts/02/12, B/Yamagata/16/88-like; avian influenza viruses: A/Mallard/Pennsylvania/1024/84 (H5N2), A/Duck/Primorie/2621/01 (H5N2), A/FPV/Weybridge/34 (H7N7); reassortants R: R22/II A(H5N1), R22 A(H5N2).

Viruses with a different structure of surface proteins, hemagglutinin and neuraminidase, were derived from the collection of the Centre for Ecology of influenza viruses and influenza epidemiology of influenza laboratories of N. F. Gamaleya FRCEM, Russian Ministry of Health. The cultivation of influenza viruses was done in allantoic fluid of 10–11-days-old developing chicken embryos (CE). Madin–Darby canine kidney (MDCK) epithelial cell line derived from a kidney of an apparently normal adult female cocker spaniel was also used. Purified viruses were obtained after the concentration of virus via centrifugation at 24,000 rpm for 1 h and the subsequent purification via centrifugation at 24,000 rpm in a sucrose concentration gradient of 20–40% with a centrifuge Beckman L5-50. The viruses were redispersed in physiological solution.

The interactions of viruses with sorbents

The experiments were performed as described earlier (Ivanova et al. 2012c). Sorbents (1–10 mg) were added to saline solution (150–300 µL) containing virus and mixed intensively. The sorbent was separated by centrifugation at 2000 rpm for 4 min, i.e., under conditions where the virus does not precipitate, and the solution was tested for the presence of virus by various techniques.

Virus adsorption efficiency was determined by the change in the number of viruses in the solution before and after contact with the sorbent. The content of the influenza viruses was determined by hemagglutination (HA) reaction, a common technique recommended by the World Health Organization, with the use of a 0.75% suspension of erythrocytes of type 0 negative human blood. The technique is based on the fact that the protein coating of influenza viruses has the ability to agglutinate erythrocytes of human blood. Hemagglutinating virus titer corresponds to the amount of virus contained in the unit volume of the solution, at the highest dilution of the virus which has revealed the intense hemagglutination. HA titer was equal to 2^n, where n is the number of successive twofold dilutions.

Reduction of infectious activity of virus in water solution was evaluated by determining the infectious titers of aqueous phase before and after application of sorbents. Infectious titers were determined upon failure the monolayer of MDCK culture cells by the virus solution with its various concentrations. The dilution of virus solution at which the death of 50% of the MDCK cells (50% tissue culture infective dose, $TCID_{50}$) is taken for infectious water titer. Serial dilution of virus was 10 times; therefore, the infectious titer was calculated as 10^n, where n is again the number of successive tenfold dilutions. The titers are usually expressed as decimal logarithms (Reed and Muench 1938).

Cytotoxicity

The in-vitro toxicities of the sorbents dispersed in a physiological solution were examined with the use of monolayer of MDCK cell (ATTC CCL-34, MDCK (NBL-2), Kidney, canine, *Canis familiaris*). The sorbents in a physiological solution were placed into a microtitre plate with a monolayer of MDCK cells. After that, the repeated tenfold dilution was continued and the plate was placed in an incubator and held at 37 °C for 18 h. The evaluation of the toxicity effect was based on the state of the MDCK monolayer that was estimated with the use of an Olympus inverted optical microscope. The range of concentrations of sorbent slurry ranged from 10 to 0.001 μg mL^{-1} (De Stefano and Carnuccio 2012).

Results and discussion

Virus sorption

Antiviral sorbents intended for the treatment of drinking water should reduce its infectious activity, effectively removing different strains of virus, and be non-toxic. The research of sorbents based on conducting polymers includes: (1) determination of the effectiveness of viruses' withdrawal from the aqueous medium, (2) studies of the infectious activity of virus in aqueous solution after the virus removal, and (3) the evaluation of the toxicity of sorbents alone.

The sorption of a wide range of influenza viruses, including the strains A and B viruses of human and avian viruses circulating the last years in Russia, Western Europe, and the USA and reassortants with the same surface protein, have been investigated. Viruses have different capacity for sorption because of various structure of protein-enveloped virion. Influenza virus HA titers before and after application of conducting polymers are shown in Tables 1 and 2. For comparison, the virus adsorption at carbon nanotubes and their composites with polyaniline are included.

The ratio of hemagglutination titers before the introduction of the sorbent to the titer after sorbent removal indicates how many times the concentration of the active form of the virus in the solution was reduced (cf concentration reduction factor in Tables 1 and 2). Table 1 reports the sorption of purified and concentrated influenza virus from saline. Under practical conditions, there are always non-viral proteins present in water. Moreover, viruses associated with its own biological material, which includes a host cell where the virus is multiplying, as well as fragments of RNA of destroyed virions may compete for adsorption. To assess the efficiency of viruses adsorption under the conditions met in practice, the viruses grown in allantoic protein mass of chicken embryos and used without purification from accompanying proteins have also been tested (Table 2).

Experimental data suggest that conducting polymers are capable of binding a wide range of virus strains and, on the average, the adsorption capacity of polymers is higher than that of carbon sorbents. Changes in the chemical nature of carbon sorbent after the introduction of polyaniline cause an increase in the sorption capacity. Depending on the strain of purified influenza virus, the concentration reduction factor for CNT is in the range 2–128 times with an average value, 54 times, whereas in the case of conducting polymers this is higher, 64–1024 times for PANI, and 32–256 for PPy with an average concentration reduction factor 369 and 117 times, respectively. In the presence of non-viral proteins the sorption efficiency of all virus strains decreases. The sorption capacity of the polymers is higher than that of CNT. The CNT concentration reduction factor was 4–8, with an average value 5.3 times. High efficiency was demonstrated when the conducting polymers were modified with silver nanoparticles. PANI–Ag and PPy–Ag provide almost complete removal of viruses and complete

Table 1 Hemagglutination titers of purified and concentrated influenza virus before and after sorption and concentration reduction factor, *X*

Virus	Sorbent	HA before	HA after	*X*
B/Malaysia/2506/04	CNT	4096	128	32
	CNT–PANI	4096	8	512
	PANI	2048	8	256
B/Massachusetts/02/12	PPy	1024	16	64
	PPy–Ag	1024	8	128
B/Florida/04/06	PANI	128	2	64
	PANI–Ag	128	4	32
A/Wisconsin/67/05 (H3N2)	CNT	256	2	128
	PANI	256	<2	>128
	PPy	2048	64	32
	PPy–Ag	2048	32	64
A/Victoria/361/11 (H3N2)	CNT	512	256	2
	PANI	512	128	4
	PANI– Ag	512	8	64
A/Victoria/361/11 (H3N2)	PANI–Ag	1024	64	16
	PPy	2048	8	256
	PPy–Ag	2048	2	1024
A/FPV/Weybridge/34 (H7N7)	CNT	512	128	4
	CNT–PANI	512	32	16

Sorbent mass 4 mg per 0.2 mL^{-1} in saline at 25 °C for 15 min of sorption

Table 2 Hemagglutination titers of unpurified influenza virus before and after sorption and concentration reduction factor, *X*

Virus	Sorbent	HA before	HA after	*X*
A/Moscow/72/07 (H3N2)	CNT	128	16	8
	CNT–PANI	128	8	16
A/IIV-Moscow/01/2009 (H1N1) swl	CNT	64	16	4
	CNT–PANI	64	8	8
	PANI	64	2	32
A/Moscow/28/089 (H1N1)	PPy	128	8	16
	PPy–Ag	128	4	32
A/South Carolina/02/2010 (H1N1) pdm09	PANI	64	2	32
	PANI–Ag	512	2	256
A/Duck/Pennsylvania/1024/84 (H5N2)	CNT	64	16	4
	CNT–PANI	64	4	16
R (H5N2) reassorted	CNT	1024	512	2
	CNT–PANI	1024	128	8
R (H5N1) reassorted	CNT	512	64	8
	CNT–PANI	512	16	32

Sorbent mass 4 mg per 0.2 mL^{-1} in saline at 25 °C for 15 min of sorption

water treatment, and this also applies to unpurified influenza viruses.

Investigation of sorption conditions indicates that the optimum concentration of polymer sorbents does not exceed 10–20 mg mL^{-1} of virus-containing solution. Such concentration of polymer sorbent is satisfactory to clean the aqueous environment even with a high initial viral loading of the order of 1000 HA titer. The effectiveness of virus adsorption virtually unchanged in the temperature range 4–37 °C which is optimum for virus activity. The hemagglutination titration takes at least 15 min after injection of the sorbent. After this time, the adsorption equilibrium has been achieved; the virus concentration in the solution reached a minimum and did not change any more. By assuming that the adsorption fits an

exponential law, we conclude that the vast majority of the virus must bind to the sorbent within the first minute.

Experimental data show that different strains of influenza viruses can be adsorbed by materials based on conducting polymers and the sorption capacity is higher compared with carbon. In the case of carbon sorbent, virus binding occurs through physical adsorption. The CNT sorption capacity is mainly due to a large specific surface area of the material. The coating of CNT with PANI enhances the sorption activity of material despite the fact that the specific surface area of the CNT–PANI is lower than that of the pristine CNT. Pristine PANI and PPy have higher adsorption capacity, although they have a relatively low specific surface area of 30–50 $m^2\ g^{-1}$. The improvement of the efficiency of viruses binding by conducting polymers can be explained by two reasons: (1) by impact of chemisorption including hydrogen bonding that is significantly stronger than the physical adsorption and (2) by hydrophilicity of polymeric sorbents which improved sorbent contact with an aqueous phase.

Influence of morphology and chemical structure of conducting polymer

The sorption of the viruses A/Victoria/361/11(H3N2), A/Moscow/28/08 A(H1N1), A/California/07/09 (H1N1)pdm09, Florida/04/06 on various PANI and PPy nanostructures has also been investigated. Polymers with a spherical particle shape of 50–150 nm in diameter and a one-dimensional nanotubular structure with an outer diameter of 50–200 nm were examined. The sorption efficiency was practically independent of the polymer morphology but it is affected by the nature of the counterions. The sorption decreases in a series: $HSO_4^- > Cl^- > MO^- >$ no counterion. The average values of concentration reduction factor for PPy in this row decrease, too. This series of doping anions coincides with the hydrophilicity of polymers. Consequently, there was an increase in sorption capacity due to improved interaction on the interface of sorbent/water. However, the change in HA titer depending on the type of anion is not so significant and does not exceed 10% of the total reduction of virus concentration. The polymers modified with silver nanoparticles are exceptions. They are leaders in the efficiency of binding of the most virus strains, as in a purified concentrated state and for removal of viruses from untreated concomitant protein mass. Metal nanoparticles clearly participate in viruses binding, but the sorption mechanism is not yet clear. It can be assumed that the binding occurs through the silver interaction with surface hemagglutinin, containing thio groups, because it is well known that silver ions react with the cysteine in proteins.

Reduction of infection activity of water

PPy and PANI sorbents were studied also with concentrated A/Victoria/361/11 (H3N2) viruses. Sorption was carried out using deprotonated PPy base and PPy with sulfate (PPy–HSO_4) and chloride (PPy–Cl) counterions, as well as with PPy and PANI modified with silver nanoparticles (PPy–Ag and PANI–Ag). Comparison of the infectious titer of solutions before and after sorption by their cytopathic effect on a monolayer of MDCK cells showed that infectious activity of water decreased by 4.0 orders of magnitude with PPy–Cl, 4.5 orders of magnitude with PPy–HSO_4, and in the case of PPy–Ag and PANI–Ag for 6.5 and 6.0 orders of magnitude, respectively (Table 3). This result coincides with the data on the HA titres. Hence, polymers modified with silver nanoparticles reduced the infectious activity of water two orders of magnitude more effectively than the polymers in the absence of silver. For all tested sorbents, the reduction of infectious activity of water was more than four orders of magnitude. These results exceed the requirements of the existing epidemic rules and meet the criteria of good sorption activity.

Cytotoxicity tests

The studies of sorbents toxicity in vitro were carried out on PANI-based materials in relation to the MDCK culture cells. The selection of PANI is due to the fact that, in terms of toxicity, it is more problematic than PPy. Synthesis of PANI based on the use of a relatively toxic monomer, aniline, and moreover, the traces of carcinogenic aniline dimer, benzidine, as a by-product of synthesis cannot be ruled out (Humpolicek et al. 2012). The cytotoxicity using a microscopic study of the state of monolayer of MDCK cells after 18 h of contact with the aqueous dispersion of the sorbent under incubation at 37 °C was determined. Even at high sorbent concentrations of 1 mg mL^{-1}, CNT–PANI, PANI, PANI–Ag had no impact on the cell monolayer, which means that its cytotoxicity is negligible. It should be noted that the conclusion about the absence of PANI's in vitro cytotoxicity is provisional because the data obtained so far are only for MDCK cells. For a complete description of toxicity, it is necessary to conduct similar studies on different cell cultures, since the sensitivity of cells to both viruses and chemical drugs is not the same.

Table 3 The reduction of the infection titer (log $TCID_{50}$) of concentrated A/Victoria/361/11 (H3N2) influenza virus after the sorption

Sorbent	PPy-Cl	PPy base	PPy–HSO_4	PPy–Ag	PANI–Ag
Tested cells	MDCK				CE
Titer	4.0	5.5	4.5	6.5	4.5	6.0

At the same time, in case of CNT at a concentration of 1 mg mL^{-1}, the cell monolayer was completely degraded. By reducing the CNT concentration to 0.1 mg mL^{-1}, the layer of cells appeared disturbed and ragged. Only at low concentrations of 0.01 mg mL^{-1}, CNT do not affect the monolayer of test cells.

Concluding remark

Conducting polymers, such as PANI and PPy, are well-known adsorbents used for the industrial wastewater treatment (Huang et al. 2014). They have been used for the removal of heavy and noble metals (Hg^{2+}, Cd^{2+}, Pb^{2+}, Au^{3+}, $Cr_2O_7^{2-}$, etc.) as well as harmful organic compounds, including pharmaceuticals and dyestuffs. Considering the results of the present study, this means that conducting polymers may act as universal sorbents capable of purifying water from the many types of organic, inorganic and biological contaminants. Introduction of silver nanoparticles to conducting polymers not only increases the efficiency of virus adsorption, but also is likely to endow them with pronounced antibacterial and antifungal properties.

Conclusions

Sorption efficiency of PANI and PPy with respect to influenza viruses dispersed in aqueous media was investigated. It was shown that the polymers are capable of binding different strains of viruses including infectious of A and B strains of human, avian viruses, and reassorted virus forms. According to reduction of virus concentration in aqueous media, the sorption efficiency of polymers was found to be higher than that of currently used carbon sorbents. It is shown that infectious activity of virus in water solutions is reduced from 4.0 to 6.5 orders of magnitude as a result of using the polymeric sorbents, which satisfies existing standards of epidemics and meets the criteria of good sorption polymers activity. At the same time, the interaction of polymers with the MDCK cells showed no cytotoxicity of polymeric sorbents. These studies demonstrate that PANI and PPy can be used as biosorbents for cleaning and disinfection of tap water from the flu virus. Silver compounds and metallic silver are well known for their antimicrobial properties. In the design of universal materials that could in addition to viruses be applied also for the removal of various bacterial infections, the conducting polymers are decorated with silver nanoparticles. Introduction of silver nanoparticles in polymers not only increases the efficiency of virus adsorption, but also endows polymers with pronounced antibacterial and antifungal properties.

Acknowledgements The authors (I.S. and J.S.) thank the Czech Science Foundation (16-02787S) and the Ministry of Education, Youths, and Sports of the Czech Republic (LH 14199) for financial support.

References

Alekseeva E, Bober P, Trchová M, Šeděnková I, Prokeš J, Stejskal J (2015) The composites of silver with globular or nanotubular polypyrrole: the control of silver content. Synth Met 209:105–111. doi:10.1016/j.synthmet.2015.07.003

Brožová L, Holler P, Kovářová J, Stejskal J, Trchová M (2008) The stability of polyaniline in strongly alkaline or acidic aqueous media. Polym Degrad Stab 93:592–600. doi:10.1016/j.polymdegradstab.2008.01.012

Burtseva EI, Lvov DK, Shchelkanov MYu, Kolobukhina LV, Prilipov AG, Alkhovsky SV, Lavrishcheva VV, Shevchenko ES, Fedyakina IT, Ivanova VT, Belyakova NV, Proshina ES, Abramov DD, Trushakova SV, Merkulova LN, Vartyanyan RV, Kisteneva LB, Samokhvalov EI, Oskerko TA, Feodoritova EL, Siluyanova EV, Mukasheva EA, Belyaev AL, Malikov VE, Malyshev NA (2012) The specific features of the cocirculation of influenza viruses in the 2010–2011 postpandemic period according to the results of activities of the D. I. Ivanovsky Research Institute of Virology, Ministry of Health and Social Development of Russia. Vopr Virusol 57:20–28

Cho SH, Song KT, Lee JY (2007) Recent advances in polypyrrole. In: Skotheim TA, Reynolds JR (eds) Handbook of conducting polymers. Conjugated polymers: theory, synthesis, properties and characterization, 3rd edn, Chapter II/8. CRC Press, Boca Raton, pp 1–87

Dawood FS, Iuliano AD, Reed C, Metzler MI, Shay DK, Cheng PY, Gordon A, Bandaranayake D, Breiman RF, Brooks WA, Buchy P, Feikin DR, Fowler KB, Hien NT, Horby P, Huang QS, Katz MA, Krishnan A, Lal R, Montgomery JM, Presanis AM, Mølbak K, Pebody R, Razuri H, Steens A, Tinoco YO, Wallinga J, Yu HJ, Vong S, Widdowson MA, Bresee J (2012) Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modeling study. Lancet Infect Dis 12:687–695. doi:10.1016/S1473-3099(12)70124-X

De Stefano D, Carnuccio R, Maiuri MC (2012) Nanomaterials toxicity and cell death modalities. J Drug Deliv 167896:1–14. doi:10.1155/2012/167896

Hinshaw VS, Webster RG (1982) The natural history of influenza viruses. In: Beare AS (ed) Basic and applied influenza research. CRC Press, Boca Raton

Hinshaw VS, Webster RG, Turner B, Zwank PJ (1979) Water-born transmission of influenza A viruses. Intervirology 11:66–68. doi:10.1159/000149014

Huang YS, Li JX, Chen XP, Wang XK (2014) Applications of conjugated polymer based composites in wastewater purification. RSC Adv 4:62160–62178. doi:10.1039/c4ra11496e

Humpolicek P, Kasparkova V, Saha P, Stejskal J (2012) Biocompatibility of polyaniline. Synth Met 162:722–727. doi:10.1016/j.synthmet.2012.02.024

Il'ina MV, Timofeeva AV, Ivanova VT, Burtseva EI, Baratova LA, Sapurina IYu, Katrukha GS (2012) Investigations into the processes of sorption and desorption of polypeptide antibiotics on Taunit multiwalled carbon nanotubes. Appl Biochem Microbiol 48:699–704. doi:10.1134/S0003683812080029

Ivanova VT, Kurochkina YaYe, Buravtseva VN, Nikolayev AV, Timofeyeva AV, Baratova LA (2008) Interaction of influenza A and B viruses with a carbon-containing sorbent. Vopr Virusol 53:40–43

Ivanova MV, Burtseva EI, Ivanova VT, Trushakova SV, Isaeva EI, Shevchenko ES, Isakova AA, Manykin AA, Spitsyn BV (2012a) Adsorption of influenza A and B viruses on detonation nanodiamonds materials. In: Materials research society proceedings, 1452, mrss12-1452-ff04-06. doi:10.1557/opl.2012.1226

Ivanova VT, Ivanova MV, Burtseva EI, Garina EO, Trushakova SV, Shevchenko ES, Manykin AA, Isakova AA, Korzhenevsky AP, Spitsyn BV (2012b) Interaction of influenza A and B viruses with nanodiamond-based sorbents. Vopr Virusol 57:9–13

Ivanova VT, Ivanova MV, Spitsyn BV, Garina KO, Trushakova SV, Manykin AA, Korzhenevsky AP, Burseva EI (2012c) Interaction of nanodiamonds materials with influenza viruses. J Phys Conf Ser 345:012019. doi:10.1088/1742-6596/345/1/012019

Jeffery KT, David MM (2008) The pathology of influenza virus infections. Ann Rev Pathol 3:499–522. doi:10.1146/annurev.pathmechdis.3.121806.154316

Kontarov NA, Ermakova AA, Grebionkina NS, Yuminova NV, Zverev VV (2015) The study of the antiviral activity of polyelectrolytes with respect to the influenza virus. Vopr Virusol 60:5–9

Konyushenko EN, Stejskal J, Šeděnková I, Trchová M, Sapurina I, Cieslar M, Prokeš J (2006) Polyaniline nanotubes: conditions of formation. Polym Int 55:31–39. doi:10.1002/pi.1899

Kopecká J, Kopecký D, Vrňata M, Fitl P, Stejskal J, Trchová M, Bober P, Sapurina I, Prokeš J (2014) Polypyrrole nanotubes: mechanism formation. RSC Adv 4:1551–1558. doi:10.1039/c3ra45841e

Otero TF, Martinez JG, Arias-Pardilla J (2012) Biomimetic electrochemistry from conducting polymers. A review: artificial muscles, smart membranes, smart drug delivery and computer/neuron interfaces. Electrochim Acta 84:112–128. doi:10.1016/j.electacta.2012.03.097

Reed LJ, Muench HA (1938) Simple method of estimating fifty percent endpoints. Am J Hyg 27:493–497

Sapurina IYu, Ivanova MV, Ivanova VT, Burtseva EI, Trushakova SV, Isaeva EI, Kirilova ES, Kurochkina YaE, Manykin AA, Uryvaev LV (2014) Polyaniline and its composites as sorbents of influenza viruses. Polymer Sci Series A 56:450–458. doi:10.1134/S0965545X14040129

Sapurina IYu, Kompan ME, Shishov MA (2015) Polymer-carbonaceous composites as electrode materials with high electrochemical capacitance. Russ J Electrochem 51:528–537. doi:10.1134/S1023193515060166

Shigematsu S, Dublineau A, Sawoo O, Batejat C, Mantuyama T, Leclerq I, Manuguerra JC (2014) Influenza A virus survival in water is influenced by the origin species of the host cell. Influenza Other Respir Viruses 8:123–130. doi:10.1111/irv.12179

Stallknecht DA, Shane SM, Kearney MT, Zwank PJ (1990) Persistence of avian influenza viruses in water. Avian Dis 34:406–411. doi:10.2307/1591428

Stejskal J, Prokeš J, Trchová M (2008) Reprotonation of polyaniline: a route to various conducting polymer materials. React Funct Polym 68:1355–1361. doi:10.1016/j.reactfunctpolym.2008.06.012

Stejskal J, Prokeš J, Sapurina I (2009) The reduction of silver ions with polyaniline: the effect of polyaniline type and silver-nitrate-to-polyaniline mole ratio. Mater Lett 63:709–711. doi:10.1016/j.matlet.2008.12.026

Stejskal J, Trchová M, Bober P, Humpolíček P, Kašpárková V, Sapurina I, Shishov MA, Varga M (2015) Conducting polymers: polyaniline. In: Encyclopedia of polymer science and technology, Wiley Online Library, Wiley, pp 1–44. doi: 10.1002/0471440264.pst640

Wong SS, Yuen KY (2006) Avian influenza virus infection in humans. Chest 129:156–168. doi:10.1378/chest.129.1.156

Xu XJ, Liu RH, Li LD (2015) Nanoparticles made of π-conjugated compounds targeted for chemical and biological applications. Chem Commun 51:16733–16749. doi:10.1039/c5cc06439b