Possibility of Using Modified Polyamide Membranes for Virus Concentration for the Purposes of Sanitary-Virological Analysis of Water

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Abstract

The results of investigations of modified polyamide microfiltration membranes application in the concentration of viruses and bacteriophages during sanitary virological monitoring of water bodies are presented in this paper. The analysis of developments in the control of viral contamination of different origins water bodies is given. Viruses presented in the water in small amounts can cause disease. It is shown that an important step is viral concentration from large amounts of water for its identification.

The possibility of positively charged microfiltration membranes application in the concentration of viruses is established. A method of polyamide microfiltration membranes modification for increasing the positive charge on their surface is developed.

To estimate the retention capacity of membranes the methods of measuring the sorption properties of the membranes using the model dye bromophenyl blue is developed.

A membrane filter module with tangentially-radial mode of fluid movement was used. Studies carried out in specialized organizations revealed that the efficiency of bacteriophage MS-2 sorption on the modified polyamide membrane at different loads was 100%. It was found that the use of membrane filtration module not only improves the detention of microorganisms, but also allows improving the elution stage. The laboratory results were confirmed by tests using natural waters.

Introduction

One of high priority activities in the field of reducing the morbidity rate of the population with enteric viral infections is perfection of methods for monitoring viral contamination of water. The efficiency of sanitary-virological control depends on the choice of the method for concentrating viruses from various water bodies, as well as the method for their indication.

Most viral infections, including enteroviruses (e.g. echoviruses and the Coxsackie virus), adenoviruses (of the 40th and 41st type), human calico viruses (noroviruses) and the hepatitis A virus are transmitted by the fecal-oral route. Even insignificant amounts (1 to 10 infective viruses) can result in replication of the virus in the gastrointestinal tract and infection [1]. A large number of viral infections are formed as a result of ingress of viruses into the human organism together with drinking water, such as gastroenteritis, meningitis, febrililies, paralysis and acute hepatite [2]. Since these viruses are secreted in large amounts by infected people, they are present in significant amounts in untreated wastewater [3, 4].

In addition, the viruses that are present in small amounts in various water bodies (lakes, swimming pools, fountains) can also cause infections [5]. In

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this case, detection of viruses is difficult due to the need for examination of large amounts of water (tens to hundreds of litres). When water is analyzed using microfiltration, various bacteria are removed from water due to the fact that their size is larger than that of filter pores. Unlike bacteria, the size of a virus is of the order of several nanometers, which is why their concentration is mainly carried out due to electrostatic and hydrophobic interactions with the filter's surface, which is usually negatively charged in water systems [6].

Over the course of several decades various filtering devices have been assessed that are capable of concentrating enteric viruses, including gauze gaskets [7], ultrafilters [8], glass wool [9] as well as electronegative [10] and electropositive pleated filters [11]. Although these devices have already proved their efficiency for capturing viruses from water, some of them are either unpractical for treating more turbid natural surface water (e.g., ultrafilters) or expensive.

Nowadays electronegative and electropositive filters are widely used, but electronegative filters are not perfectly suitable for large-scale water sampling, since they require water acidation and addition of polyvalent cationic salts into the water before the filtration.

The methods currently used in the Russian Federation [13] and abroad [14, 15] for concentrating viruses from various amounts of water require significant time expenditures (from 24 hours to several days), and have restrictions in terms of the volume of water being analyzed (frequently no more than 10 L of pure water). These methods are labor-intensive, due to the use of sophisticated equipment and, in some cases, do not ensure accurate determination of virus concentration in the sample being investigated. These circumstances have a negative effect on the quality of virological monitoring of water bodies and, consequently, timely development of anti-epidemic measures when viruses are detected in water. Therefore, membrane filtration can be considered the most promising method for virus concentration. It should be taken into consideration that efficiency of virus concentration depends significantly on the contamination rate of water, characteristics of the filtration system, and, especially, the type of membrane used.

The viruses adsorbed on the filter should be desorbed (eluated) before they can be examined and counted. This is usually achieved by using eluating solutions directly in the filter, after a certain delay to ensure desorption of the viruses into the solution.

In the course of virus eluation from a depth filter, a rather large amount of the eluent's solution (about 1 litre) having a low concentration of viruses is formed. For this reason, secondary concentration should be carried out.

Therefore, development of a cheap and efficient filtration system for concentration, eluation and, if necessary, secondary concentration of viruses from drinking water and surface water still remains a relevant and practically important objective.

In the present paper, the results of research into the possibility of using modified polyamide microfiltration membranes for virus concentration for the purposes of sanitary-virological control of water are presented.

Experimental

The modified microfiltration membranes were produced by a phase inversion method, using an experimental drum system. The modifying agent was introduced into the polyamide solution in the formic acid at the molding solution preparation stage. Derivatives of polysaccharides and aliphatic polyamines were mainly used as modifying agents [16].

Method Used to Determine the Electrokinetic Potential of the Membranes

The method used in the research to determine the electrokinetic potential of the membranes is based on measurement of the filtration potential (flow potential) generated by the flow of KCl solution in distilled water through the membrane. In the course of the experiment, the dependence of potential difference (E) on transmembrane pressure (P) is determined. Linearization of the dependence of E on P makes it possible to determine the flow potential $\Delta E/\Delta P$ and, by using Smolukhovsky's formula, the electrokinetic potential (ξ-potential):

$$\zeta = k\mu/\varepsilon\varepsilon_0 * \Delta E/\Delta,$$

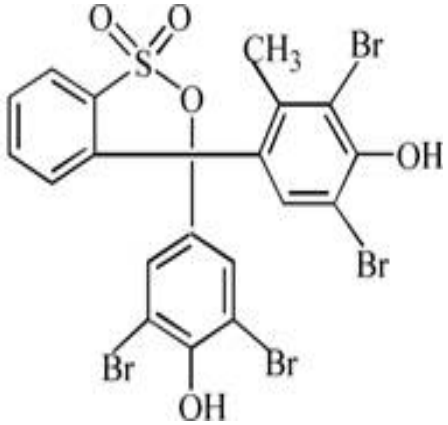
where k is electric conductivity of the solution in membrane pores, $Ohm^{-1}m^{-1}$;
μ is viscosity of the solution, Pa·s;
ε is dielectric permeability of the solution; and
ε_0 is electric constant.

Method used to Determine the Sorptive Capacity of Membranes for the Coloring Agent

The sorptive capacity of membranes is determined using the spectrophotometric method to investigate digestion of the sorbent by the membrane.

The function of the sorbate was performed by 3',3",5',5"-tetrabromphenolphthalein (bromphenol blue) which is widely used for extractive photometric analysis due to its properties.

The aqueous solution of bromphenol blue (BPB) has a blue-violet color at pH>2.4.



BPB, or 3',3",5',5"-tetrabromphenolphthalein

$$m_{BPB} = \frac{5\,mg \cdot 50\,ml}{1000\,ml} = 0{,}25\,mg = 0{,}25 \cdot 10^{-3}\,g$$

After that a dry chemical funnel is inserted into the neck of a measuring flask, and the weighed amount is poured out from the measuring flask into the funnel. Then the measuring flask and the funnel are washed with distilled water to make sure the finest particles of the substance being dissolved are transferred into the flask.

The flask is closed up and stirred. After the measured amount has been dissolved completely, water is added to the appropriate mark and thoroughly agitated.

The tests are carried out at normal conditions:

Temperature of the ambient air, °C: 20±3

Relative humidity of air, %: 45 to 80

Atmospheric pressure, mm Hg: 630 to 800

10 mm × 10 mm samples are cut from the sheet of the polymer membrane. The membranes are maintained in distilled water for 15 minutes for washing, whereupon they are taken out and the remaining distilled water is removed from the membrane surface using filtering paper.

After preliminary preparation of the membranes for testing has been completed, they are placed into test-tubes whereupon 5 ml of BPB solution are poured into the test-tubes.

After one week, sorptive capacity of the membranes is determined. The solution is poured from the test-tube into a pan and placed into a spectrophotometer. Optical density is measured at 591 nm wavelength.

Sorptive capacity of the sorbent for the organic reactant is calculated using the formula:

$$E = \frac{C_1 - C_2}{S}$$

where C_1 is BPB concentration before sorption, μg/ml;

C_2 is BPB concentration after sorption, μg/ml;

S is area of the test sample, cm^2.

Method Used to Determine the Content of Coliphages and the Poliovirus in the Feed Water and the Eluate [13]

Sorption of microorganisms by the membranes was investigated using artificial contamination of dechlorinated tap water. The RNA-containing bacteriophage MS2 was used as a model of viral contamination. The bacteriophage is similar to enteroviruses in terms of morphological, physical and chemical properties and stability, is widely used both in Russia and abroad as a model variety of enteroviruses and is an indicator of viral contamination of various water bodies. Elution of viruses from the filters was carried out using a 3% solution of beef extract based on Tris buffer, with a 9.0-9.4 pH.

Microorganism sorption efficiency (S) was calculated by the formula

$$S = (K\text{-}F)/K * 100,$$

where F is concentration of microorganism particles in the filtrate, K is concentration of microorganisms in the feed water.

Elution efficiency (E) was determined by the formula

$$E = G/(K\text{-}F) * 100,$$

where G is concentration of viruses in the eluate.

Concentration of Viruses and Bacteriophages

Experiments on concentration of viruses and bacteriophages were conducted using two methods. The first method (Fig. 1), which is more known and much more often used by microbiologists, is carried out in the following way: the feed solution is placed into the service reservoir 1. Compressed air or inert gas is used to create pressure P in the reservoir while valve 4 is closed. When valve 4 is opened, the process of filtration is initiated. In the process of filtration the filtrate is delivered into the drain and the separated substance remains on the membrane of module 2. When filtration is complete, valve 4 is closed and the mating nozzles 8 and 9 are removed.

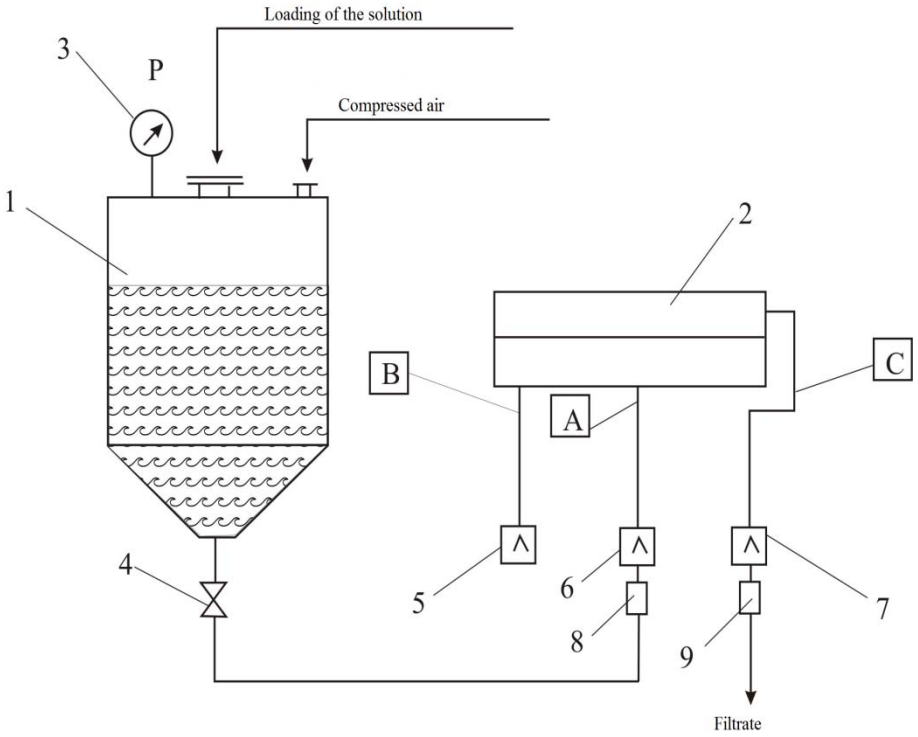


Fig. 1. Design of the installation for concentration of viruses and bacteriophages using compressed air
1 – service reservoir; 2 – MFM; 3 – pressure gage; 4 – valve, 5, 6, 7 – mating units; 8, 9 – mating nozzles.

When this method is used, it is impossible to run the system in the tangential flow-through mode. In addition, the amount of filtered water is limited by the volume of the service reservoir.

In the second method (Fig. 2) the water being investigated is delivered by the peristaltic pump (3) from the service reservoir (1) to the membrane module (4). After the module the filtrate goes into the drain, while the concentrate remains on the membrane.

The design of the filtering module (Fig. 3) makes it possible to carry out filtration in the flow-through mode. In this mode, the flow of liquid on the upstream side between the plate and the membrane is moving by concentric channels of the plates that are connected by radial overflow channels. The flow of liquid repeatedly changes the direction of movement, being separated into two flows in one overflow channel and reuniting into one flow in the next channel. This pattern of the liquid flow intensifies the process of mass transfer in the boundary layer of the membrane and ensures a minimal thickness of the sediment in the pores and on the surface of the membrane. The flow of liquid in the channels is close to the displacement mode.

When the module is running in the above mode, the water being investigated passes over the surface of the membrane, a part of unfiltered flow is recycled and the filtrate is drained. Since the water is moving tangentially to the surface of the membrane filter, most virions are sorbed on the membrane while those in the suspended condition are returned to the service reservoir.

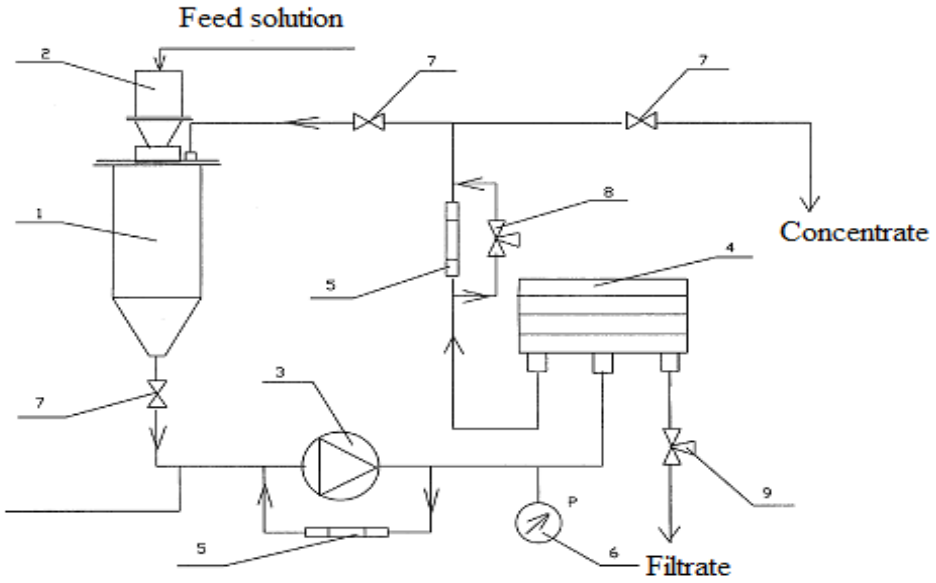


Fig. 2. Design of the installation for concentration of viruses provided with a peristaltic pump:
1 – service reservoir; 2 – funnel; 3 – peristaltic pump; 4 – membrane module;
5 – sphincter device; 6 – pressure gage; 7 – ball cock; 8, 9 – control valve.

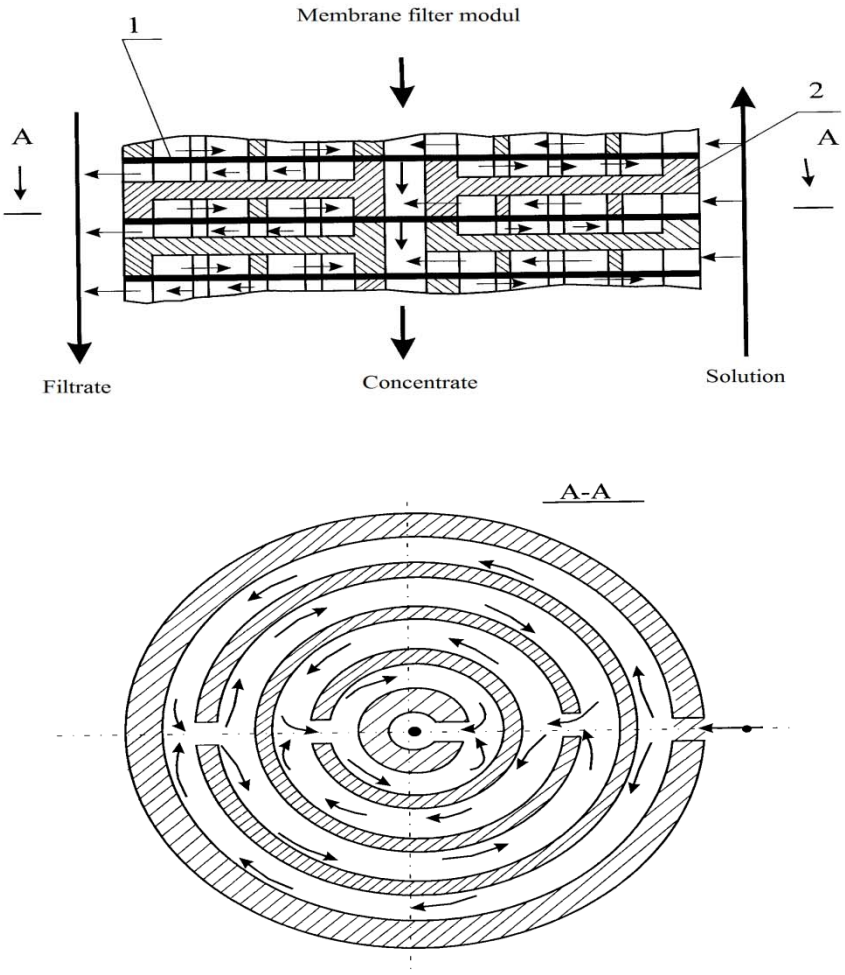


Fig. 3. Pattern of flows in the filter module: 1 – membrane; 2 – plate.

Recovery of the Target Substance (elution)

Recovery of the target substance from MFM-0142 (membrane filter module) is carried out by washing the membrane with a special solution. The layout of the system is shown in Fig. 4. The following procedure is performed: device 5 is closed with an end cap, device 7 is shut down. Syringe 6 filled with washing solution is connected with mating unit 3. Empty syringe 7 is connected with unit 4. The piston of syringe 7 is adjusted to zero volume. By using syringe 7, the solution is pumped through module 2 into syringe 7, whose piston starts moving on its own in accordance with laws of hydraulics. When the volume of liquid in syringe 7 is maximal, there is no liquid in syringe 8. After that, liquid is pumped from syringe 7 into syringe 6. The cycles are repeated 5 times.

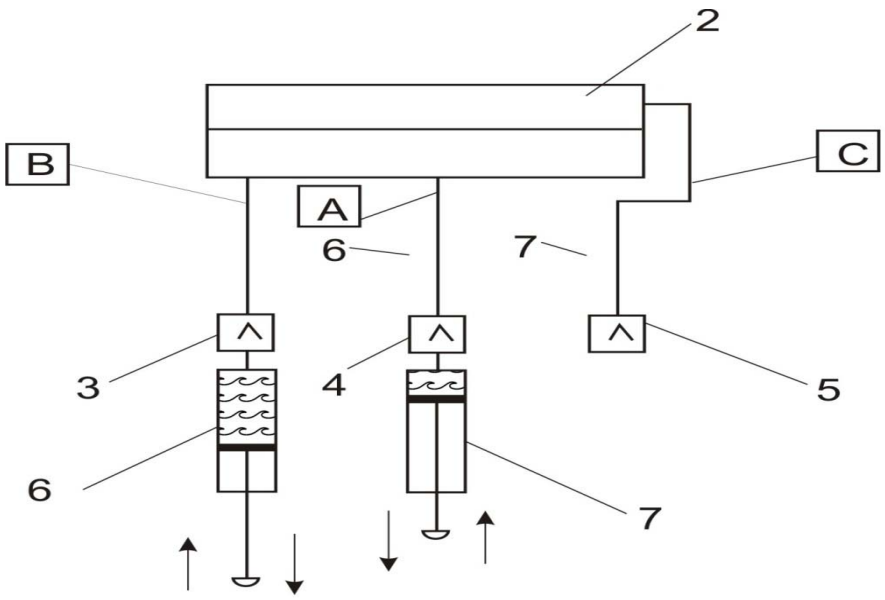


Fig. 4. Elution of viruses without dismantling of the module: 2 – MFM; 3, 4, 5 – mating units; 6, 7 – syringes.

As a result of this operation, the target substance is recovered from the membrane into the solution. After that, the syringe filled with liquid is disconnected from the mating unit and the content of the syringe is squeezed out into a special receiving vessel (not shown in the diagram).

Before disconnecting the syringe from the mating unit, excessive pressure in the module should be relieved by slightly moving the piston of the syringe in the opposite direction. This operation is necessary to preclude solution leaks at the moment when the syringe is disconnected from the mating unit.

After that the syringe is filled with fresh washing solution and the operation is repeated. The number of cycles and operations is determined experimentally in each specific case. As a rule, 2 or 3 operations are needed.

Standard disposable 20 cm^3 syringes are used as syringes. To increase the efficiency of target substance recovery, the membrane can be washed with the reverse flow of the solution. To fulfill this operation, the syringe is connected with mating unit 5.

Results and Discussion

For a long time, separation capabilities of membranes were explained exclusively by the sieving effect. After membranes made of new polymers, including functionalized polymers, were introduced into practical filtration, it was discovered that the efficiency of retention of various contaminants is achieved not only due to the sieving mechanism, but also as a result of their adsorption, which, in turn, is a consequence of electrostatic or Van der Waals interaction forces.

Membranes with a surface electric charge (zeta-potential) became known as “charged membranes”.

The surface properties of membranes have a significant, sometimes decisive, effect on their performance. The size and sign of charges on the surfaces of membranes and particles being retained (colloids, high MW compounds, ions, etc), which are contained in the feed, determine the intensity of electrostatic interaction between them. This initiates the process of adsorption, resulting in either an improvement of retention efficiency or the plugging of pores and reduction of membrane flux. To reduce the rate of adsorption, the membrane and particles being filtered should acquire like charges, and vice versa.

The improvement in the removal efficiency of various negatively charged particles from aqueous solutions has been associated recently with the use of filters made of materials having a positive surface charge. Such filters, which combine adsorption and filtration mechanisms, are capable of removing contaminants down to tens of nanometers in size. Porous electropositive filters can adsorb viruses and endotoxins with sizes smaller than the average pore size of the filters, while maintaining high water flow rates.

In addition to contaminant removal, electropositive filter materials are used for collecting microorganisms and other microparticles from water for their analysis.

Due to the low concentration of viruses in drinking water an important primary stage of virological research is their concentration from large amounts of water (from 10-100 l or more to 50-80 ml), where microfiltration membranes are best suited. In our view, the use of microfiltration membranes with a positive electrokinetic potential is the optimal solution of this problem. Due to the limited choice of polymers used to produce membranes, charged membranes can be produced by modifying their surfaces or by volume modification of existing membranes.

Aliphatic polyamides used to produce membranes, unlike many other membrane-forming polymers (cellulose acetate, fluoroplastics, acrylonitrile, etc), are known to have the isoelectric point in which positive and negative charges are mutually equalized. The isoelectric points of polyhexamethylene adipo amide (Nylon-66) and polycaprolactam (Nylon-6) are in the pH range of 6.0 to 7.6 depending on the ratio of amino and carboxyl end groups. If pH is above the isoelectric point, the membrane is charged negatively, and vice versa [17].

To produce a membrane with a positive charge in a broader pH range, the specialists of Technofilter Ltd have developed a method involving volume modification of the membrane by introducing functionalized polymers containing amino groups [16]. The scientific results contained in this patent made it possible to establish that the most promising modifying polymer agents are the natural polysaccharide, chitosan, and the synthetic complex cation, polyhexamethylene guanidine. To select the formulation of the modified membrane in a more accurate way, additional investigations were carried out. Results of these investigations are presented in Table 1.

Table 1
Effect of the amount of the modifier on porosimetric and strength characteristics of membranes

Content of the modifier, %	Flux, ml/cm^2·min	Bubble point, MPa	Breaking tensile stress, MPa	Percent elongation at rupture, %
0	18.3	0.32	4.08	38.2
0.5 Chitosan	15.0	0.37	5.70	56.3
0.75 Chitosan	12.3	0.38	5.66	70.0
1.0 Chitosan	12.8	0.38	5.30	75.2
2.0 Chitosan	11.3	0.46	4.30	70.6
3.0 Chitosan	7.2	0.48	4.47	47.3
0.5 PHMG	8.3	0.43	4.10	35.1
1.0 PHMG	12.1	0.38	5.70	27.0
2.0 PHMG	11.8	0.32	5.40	22.3

Analysis of the data makes it possible to conclude that the increase in the content of chitosan results in the increase of destructive stress and the monotonous decrease in the flux. However, when the content of chitosan reaches 3.0%, a noticeable decrease in the flux and deterioration of strength properties are observed.

When the content of polyhexamethylene guanidine is increased, the flux of the membrane is improved, and the bubble point is falling which is indicative of the increase in the pore size. In addition, by using polyhexamethylene guanidine, lower values of percent elongation are achieved which is undesirable in production of filters.

For this reason, chitosan-modified membranes with the content of chitosan of no more than 1% were subsequently investigated.

At the next stage of investigations, the effect of electrokinetic potential of chitosan-modified (0.5 to 2.0%) polyamide microfiltration membranes on sorption of the coloring agent (BPB) which is widely used as an indicator of sorptive properties of complex cations. Since proper conditions for working with pathogenic microorganisms are available not in all organizations, this method was used by us for preliminary evaluation of sorptive properties of membranes.

The results given is Table 2 show that sorption of the indicator is proportional to the electrokinetic potential of the modified membranes.

Specialists of the A.N. Sysin Research Institute of Human Ecology and Environmental Hygiene of the Russian Academy of Medical Sciences have investigated the process of concentrating viruses from waters of various origins by using a modified membrane having the flux of 12.3 ml/min•cm^2 and the bubble point of 0.38 MPa in the process of concentrating bacteriophages and viruses for the purposes of sanitary-virological control of water.

Table 2
Effect of electrokintic potential of modified polyamide membranes on sorption of bromphenol blue

Type of membrane	Electrokinetic potential, mV	Sorption, mg/cm^2
Polyamide 66	1.7	6.00
Polyamide 6	4.4	9.10
Polyamide 6, modified with 0.5% Chitosan	6.8	16.75
Polyamide 6, modified with 1.0 % Chitosan	10.8	29.50
Polyamide 6, modified with 2.0 % Chitosan	15.3	24.25

Dechlorinated tap water artificially contaminated with strains of the RNA-containing bacteriophage MS-2 having the concentration of $1.9\cdot10^5$ to $2.1\cdot10^5$ PFU/l.

Filtration was carried out using the MFM-0142 membrane filtration module at a pressure of 0.2 MPa.

Desorption (eluation) of the bacteriophage from the module was carried out in closed conditions using three successive operations involving the volume of the eluate of 20 ml in each operation. Results of the experiments are given in Table 3.

Table 3
Evaluation of bacteriophage concentration efficiency on modified polyamide membranes

Test No	Content of MS-2 phages in amounts of water being investigated (PFU)						Efficiency, %
	Feed water, 10 l	Filtrate, 10 l	Eluate 1, 20 ml	Eluate 2, 20 ml	Eluate 3, 20 ml	Total concentration of the eluate, 60 ml	
1	$1.9\cdot10^5$	-	$1.8\cdot10^5$	$1.2\cdot10^4$	$1.1\cdot10^2$	$1.9\cdot10^5$	100
2	$2.1\cdot10^5$	-	$1.95\cdot10^5$	$1.4\cdot10^4$	$2.2\cdot10^2$	$2.09\cdot10^5$	99.52

The results show that the highest content of the bacteriophage is found in the first eluate, and the lowest content is found in the third eluate.

To achieve the highest efficiency, elution should be carried out in three stages. This makes it possible to maximize all advantages of the membrane and the membrane module.

The formerly used method of elution [13] involved removal of the membrane from the filter cell and mechanical flushing of viruses from the membrane with a jet of the eluent from a pipet, which posed a certain contagion hazard for the attending personnel. The hazard could only be eliminated by conducting this procedure in a laminar box, which involves considerable problems and significant financial expenses.

In the new version of the filtration module, elution of viruses from the membrane is carried out without removal of the membrane, i.e. in the closed mode.

The main advantages of the method include:
the possibility of combining the processes of concentration and elution in a single unit;

- high efficiency of concentration and elution achieved by using a novel design that ensures intensive mass transfer of liquid over and through the membrane;
- the possibility of maintaining mild conditions in the process of virus concentration and desorption (filtration is carried out in near-neutral media, without using any reactants);
- the amount of eluent is reduced to 60 mL;
- safety of the attending personnel is ensured (elution is carried out by using syringes, without dismantling the unit);
- easy and simple operation.

Specialists of the I.N. Blokhina Research Institute of Epidemiology and Microbiology in Nizhny Novgorod have tested modified polyamide membrane with pore size of 0.2 μm in the course of concentrating the hepatitis A virus (HAV), one of hard-to-cultivate viruses. Artificially prepared suspensions of HAV in distilled water have been used. Results of the research have shown that reliable retention of the virus is achieved even at concentrations below PCR threshold sensitivity. It has been shown that concentration of viruses on the membrane being investigated can be carried out at neutral pH, in contrast to Sartorius nitrocellulose membranes that achieve the sufficient level of recovery at pH below 4.0. Since it is not always convenient to carry out the elution of collected viruses immediately after concentration, it is important to make sure the process conditions and the medium of the filter have no effect on viability of the virus (extreme pH values can inactivate some viruses). The modified polyamide membrane meets these requirements in the best possible way.

Conclusions

The main results of the paper can be summarized as follows:

1. A method has been developed and conditions selected for producing modified polyamide microfiltration membranes with a positive electrokinetic potential.
2. A method has been developed for evaluation of sorptive properties of membranes using a coloring agent that ensures epidemic safety of the attending personnel.
3. It has been shown that the increase in electrokinetic potential of membranes results in improvement of their sorptive capabilities.
4. It has been established that the increase in the number of elution stages contributes to higher efficiency of bacteriophage concentration. Thus, this indicator reaches virtually 100% when 3 stages are performed.
5. On the basis of research results, two flowsheets for concentrating bacteriophages and viruses have been proposed.
6. Practical importance of the research has been confirmed by results of tests of the filtering devices conducted in specialized institutions (A.N. Sysin Research Institute of Human Ecology and Environmental Hygiene and I.N. Blokhina Research Institute of Epidemiology and Microbiology in Nizhny Novgorod).

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