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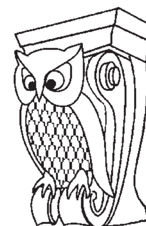
Review



Prospects for the use of polymer-containing materials and sorbents for membrane ultrafiltration, sorption and concentration of nucleic acids from aqueous media. A review

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Abstract. Unlike antibiotics and heavy metals, nucleic acids exist in the aquatic environment as a part of prokaryotic and eukaryotic microorganisms (bacteria, fungi, etc.) rather than in a free form. In this regard, the most important primary stage of sample preparation of an object for the quantitative analysis of DNA and RNA in natural and wastewaters includes membrane ultrafiltration of an aqueous sample, followed by its sorption preconcentration on a solid phase carrier. The efficiency of ultrafiltration and subsequent sorption of nucleic acids from natural and wastewaters largely depends on the material of filters, membranes, and sorbents. Polymeric materials are widely used due to their special properties: the affinity of polymers for biological objects, the ability to create pores of any required size, good mechanical properties and resistance to the extraction of microorganisms captured. The paper reviews the 15-year-old scientific literature on filtering, membrane and sorption polymeric materials used to extract nucleic acids from aqueous media and preserve them. Polymeric sorbents for collecting and concentrating DNA and RNA from the liquid phase, as well as storing nucleic acids, are covered. It has been found that ultrafiltration is used at a relatively low concentration of the analyzed object, followed by extraction of the substance using commercially available kits, including cartridges. Sorption (solid-phase concentration) is used to extract nucleic acids at their relatively high concentration in the analyte. The main polymeric materials used include cellulose and its derivatives (nitrocellulose, cellulose acetate, mixed cellulose nitrate–acetate, diethylaminoethylcellulose, polyethyleneiminocellulose), agarose, dextran, polyestersulfone, polycarbonate, fluoroplasts, polyacrylates and polymethacrylates, polyaramids, polyamides, polyvinyl alcohol, polyaniline, polycaprolactone, polyacrylamide and polymethacrylamide, polystyrene. Chitosan, modified polycaprolactone, and magnetic particles coated with polydopamine, polyethyleneimine, polyvinylpyrrolidone, polystyrene, or polyamidoamine dendrimer are considered as promising polymers for further research in this field.

Keywords: DNA, RNA, membrane ultrafiltration, sorption, sorbent, concentration, natural water and wastewater, polymer materials

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Обзорная статья
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Перспективы использования полимерсодержащих материалов и сорбентов для мембранной ультрафильтрации, сорбции и концентрирования нуклеиновых кислот из водных сред. Обзор

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Аннотация. В отличие от антибиотиков и тяжёлых металлов нуклеиновые кислоты находятся в водной окружающей среде не в свободном виде, а в составе прокариотических и эукариотических микроорганизмов (бактерий, грибов и др.). В этой связи важнейший первостепенный этап пробоподготовки объекта для количественного определения ДНК и РНК в природных и сточных водах включает мембранную ультрафильтрацию водной пробы с последующим ее сорбционным концентрированием на твердофазном носителе. При этом эффективность ультрафильтрации и последующей сорбции нуклеиновых кислот из природных и сточных вод во многом зависит от материала фильтров, мембран и сорбентов. Широко применяются полимерные материалы в силу их особых свойств, обусловленных сродством полимеров к биологическим объектам, возможностью создания пор необходимого размера, хорошими механическими свойствами и устойчивостью при извлечении захваченных микроорганизмов. В работе проведён обзор научной литературы глубиной в 15 лет, посвящённой фильтрующим, мембранным и сорбционным полимерным материалам, используемым для извлечения из водных сред нуклеиновых кислот и их консервации. Рассмотрены полимерные сорбенты для сбора и концентрирования ДНК и РНК из жидкой фазы, а также хранения нуклеиновых кислот. Выявлено, что ультрафильтрация используется при относительно низкой концентрации анализируемого объекта с последующим извлечением вещества с помощью промышленно выпускаемых средств, в том числе картриджей. Сорбция (твердофазное концентрирование) применяется для извлечения нуклеиновых кислот при их относительно высокой концентрации в аналите. Основные используемые полимерные материалы включают целлюлозу и ее производные (нитроцеллюлоза, ацетат целлюлозы, смешанный нитрат-ацетат целлюлозы, диэтиламиноэтилцеллюлоза, полиэтилениминоцеллюлоза), агарозу, декстран, полиэфирсульфон, поликарбонат, фторопласты, полиакрилаты и полиметакрилаты, полиарамиды, полиамиды, поливиниловый спирт, полианилин, поликапролактон, полиакриламид и полиметакриламид, полистирол. В качестве перспективных полимеров для проведения дальнейших исследований в данной области науки рассматривают хитозан, модифицированный поликапролактон и магнитные частицы, покрытые полидофамином, полиэтиленимином, поливинилпирролидоном, полистиролом или полиамидоаминовым дендримером.

Ключевые слова: ДНК, РНК, мембранная ультрафильтрация, сорбция, сорбент, концентрирование, природные и сточные воды, полимерные материалы

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Introduction

Currently, the widespread use of antibiotics in medicine and veterinary medicine has led to significant contamination of natural and wastewaters with these substances, which increases the probability of the transfer of genetic information of antibiotic resistance among bacteria. The so-called antibiotic resistance genes (ARG) are spoken of [1]. The ecological function of ARG is to protect an organism from the inhibitory action of an antimicrobial substance, while the operational one is to impart antibiotic resistance to it.

The literature describes various methods for studying the diversity of ARGs and assessing their abundance (per unit mass or volume of the sample) or prevalence (relative to all bacteria) in the aquatic environment [2, 3]. Depending on the conditions of cultivation or direct analysis of nucleic acids (NA), these methods are divided into culture-dependent and culture-independent. Culture-independent approaches are based on the extraction of genetic material (most often DNA, less often RNA) from a sample. Two main approaches are used, namely: quantitative polymerase chain reaction (PCR) and metagenomics [4, 5]. Metagenomic analysis through sequencing of the total DNA of the community allows characterization of the entire resistome, not limited to a few *a priori* selected genes. Culture-independent methods involve the isolation of all microorganisms contained in aqueous samples, followed by the destruction of their shells, the isolation, concentration and analysis of nucleic acids. Early research used alcohol precipitation [6]. More recent studies have used membrane filtration and, as an option, a chromatographic method with a column filled with diethylaminoethylcellulose [7]. Flocculation has found application as well.

The efficiency of ultrafiltration and subsequent sorption of NCs from natural and waste waters largely depends on the materials of membranes and sorbents. Polymeric materials are widely used due to their special properties associated with the affinity of biopolymers for biological objects, the possibility of creating pores of any required size, mechanical properties, and stability during the extraction of NCs captured. The search for optimal materials has been carried out mainly empirically, so it is of interest to review the work and achievements in this area.

Membrane ultrafiltration

Membrane ultrafiltration is used in sampling from environmental water bodies. Filter membranes made of inorganic glasses, organic synthetic and natural polymers are widely used [8]. Let us consider these materials.

1. Glass fiber (glass microfiber, GF) [9, 10, 11, 12, 13, 14]

The method for isolating NCs on glass was for the first time proposed by R. Boom et al. [15]. It includes the stage of cell lysis with a strong chaotropic agent (e.g., guanidine chloride or guanidine thiocyanate), which destroys cell membranes and inactivates intracellular RNAses, and subsequent NA sorption on a carrier. Under such conditions, the binding of proteins to the matrix does not occur. Impurities are washed out with chaotropic salt, and a chaotropic salt is with 80% ethanol. The purified NA is removed from the glass with a low ionic strength buffer [16, 17].

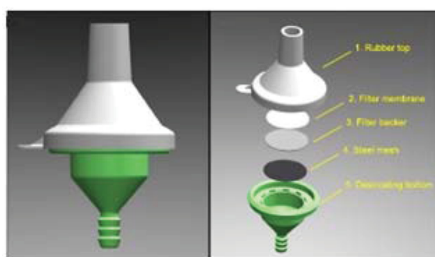
However, fiberglass filter membranes do not always perform well. E.g., in some works [11, 12] such a filter outperformed a polycarbonate filter with the same pore size (0.2–5.0 µm). Perhaps, the authors believe, that this is due to the larger volume of water passing through the filter before clogging its matrix. They recommend a pore size of 1.5 µm for field studies and a range of 0.2–0.6 µm for laboratory tests. In another paper [13], filtration through a polycarbonate filter with a pore size of 0.2 µm and a glass fiber filter with a pore size of 0.7 µm led to no significant differences. Considering the price difference (polycarbonate filters are usually more expensive than fiberglass ones) and the time required for filtration (the 0.2 µm filter clogs easily when using aquatic environmental samples), a glass fiber filter is recommended.

Many companies now offer commercial glass matrix columns for nucleic acid isolation, such as Zymo Research and Promega [16]. However, the paper [10] states that due to the large pore sizes, GF filters may not capture some small (<0.5 µm) organisms and are not recommended for these waters.

2. Polyestersulfone (PES) [10, 12, 18, 19, 20]

In the paper [14] it was found that the Sterivex-GP PES filter produced higher amounts of total DNA than polycarbonate and GF filters. In another case [10] it was noted that a PES filter gave the lowest concentration of DNA when using the DNeasy kit for extraction, but the highest one when using MolBio and phenol and chloroform extraction. In the paper [19] it was found that the zeta potential of a PES membrane was higher than that of a PVDF one, which led to a reduction in interaction with anionic pollutants and reduced membrane fouling. DNA macromolecules may bind to the aryl rings of the main chain of PES membranes, being easily adsorbed on them. Therefore, such membranes are better suited for removing ARGs from wastewater than PVDF filters.

Industrial filters are described in [21]. Their housings, assembled and equipped with PES filter membranes with a pore diameter of 1.2 µm, are half made of injection-moulded biodegradable hydrophilic plastic (Fig. 1).



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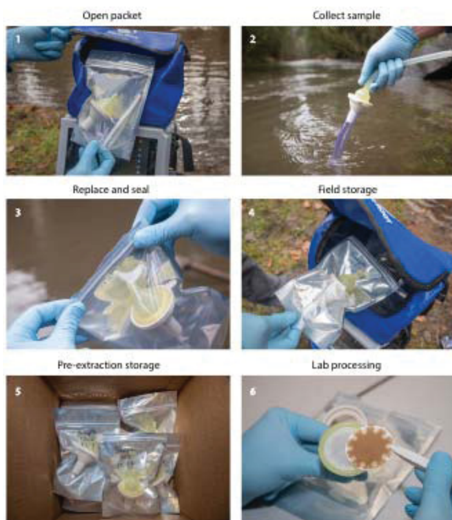

b

Fig.1. Appearance of a filter (*a*) with a PES membrane (*b*) and its use for water sampling [21]

The practical application of PES proves its promise as a material for membrane filters, especially when water samples are highly contaminated. However, the choice of filter should depend significantly on the future method of DNA extraction.

3. Polycarbonate (PC)

PC filters were studied in many papers [10–14], but this material rarely came out on top. Apparently, its main advantages are relative cheapness and ease of processing, so filters made of it are suitable for mass use and in cases where high accuracy of analysis is not required.

4. Polyvinylidene fluoride (PVDF) [10, 12, 18] **and other fluorine-containing polymers (fluoroplastics)**

The authors of [19] compared PVDF and PES ultrafiltration membranes. The filtration rate was 12% faster for the PVDF membrane. The authors believe that the PVDF membrane surface contains a large number of electronegative fluorine atoms, which can form strong hydrogen bonds with donor atoms of substances in filtered water. In the method from [22] a water sample was passed through PVDF membrane filters with a pore size of 0.22 μm (Millipore, USA). DNA was then extracted from the membrane using the E.N.Z.A Water DNA Kit (Omega, USA) and further purified using the Geneclean Spin Kit (QBiogen, Carlsbad, CA) to minimize PCR inhibition. The purity and concentration of DNA were assessed spectrophotometrically. Therefore, PVDF provides an improved filtration rate and can be chosen when this parameter is important. However, the completeness of NC extraction from the sample may suffer in this case. Such filters, in our opinion, are more suitable for qualitative analysis than for quantitative one.

Filter materials made of other fluorine-containing polymers, including polytetrafluoroethylene and composite fluoropolymers, were thoroughly studied in an earlier period, so now there are only a few works on them in the literature [20], but their practical application (patents and prototypes) has begun. It is convenient to use PTFE filters as ready-made cartridges (Fig. 2, photo from [23]). The use of, for example, a Sterivex™ filter cartridge (Merck Millipore) provides convenient *in situ* filtration and thus helps to avoid the degradation of microbial DNA during transport. In addition, the filter cartridge is sealed, which reduces the risk of contamination.

Fig. 2. Appearance of filter cartridges with a fluoroplastic membrane and work with them [23]

The main advantage of fluoroplastics, in this case, is their chemical inertness, so it can be recommended in cases where NA decompose on other filters, for example, due to delayed extraction.

5. Cellulose and its esters

Cellulose membranes (paper, cardboard, gauze, fabric, granules) are used for sample filtration and subsequent lysis of microorganism cells retained on the membrane [24, 25]. It is also possible to use cellulose filters to store the biomolecules absorbed on the substrate for their subsequent analysis. [26, 27]. To stabilize the immobilized substance, sodium dodecyl sulfate, lithium or potassium salts, cetylpyridinium or ginidinium hydrochloride, ginidinium thiocyanate, lithium or potassium sulfate are used. The stabilizer may also include an antioxidant, namely: ascorbic acid, disodium salt of ethylenediaminetetraacetic acid, dithiothreitol, ethylparaben or methylparaben.

Nitrocellulose (NC) membrane in many cases outperforms other materials [28, 29]. E.g., in [10], water samples were filtered using five different membrane filters made of NC, PVDF, PES, PC, and GF. DNA was extracted using three extraction methods. Membrane filtration through NC (0.2 µm pores) followed by extraction with the Qiagen DNeasy kit gave the highest DNA concentration of all extraction methods, as well as compared to filters from other polymers. The paper [8] also notes that trapping DNA on NC filters, storing them in Longmire buffer and extracting with the DNeasy Blood & Tissue Kit (or similar) provides a fairly high quality of DNA. NC filters showed the highest DNA extraction ratio compared to polyethylene sulfone, polyvinylidene fluoride, and polycarbonate filters [12], as well as compared to a glass fiber filter (1.6 µm) and a Whatman paper filter [9]. The authors of the latest work believe that 1.6 and 3.75 litres of water, respectively, must be filtered through filters made of CB and Whatman paper to obtain the same results as after filtering 1 litre of water through a filter made of NC. Therefore, another advantage of NC filters is the lesser dependence of their operation on the quality of the water is passed through.

The authors of Ref. [30] set out to maximize the extraction of DNA from water for subsequent analysis. In terms of cost and efficiency of DNA recovery, filtration through NC filter paper is preserved in ethanol or stored in a −20°C freezer and, again, extraction with a Qiagen DNeasy kit is preferred. It is recommended to filter water samples within 24 h, but if this is not possible, then refrigeration is preferable to freezing for short term storage (3–5 days). Filters can be stored frozen or placed in ethanol for up to four days before extraction without significant effect on DNA.

The paper [31] describes PCR monitoring of ARGs in groundwater using a cellulose acetate filter with 0.45 µm pores (diameter 14.2 cm). Samples were taken by filtration through one to three filter layers in a flow-through filter holder made of stainless steel. To prevent premature clogging of the filters, heavily contaminated samples were pre-filtered through standard paper filters. The filters were stored at 4°C and analyzed within 1 week after sampling. Cellular material was removed from the filter with a plastic scraper, resuspended in phosphate-buffered saline (pH 7.4), and concentrated by centrifugation.

The authors of [12] believe that cellulose nitrate and acetate act as electron donors, while high-molecular DNA acts as an electron acceptor in an aqueous solution.

For membrane ultrafiltration, materials from mixed (acetate–nitrate) cellulose ester are also used [12, 32]. Cellulose acetate–nitrate filters with a pore size of 0.8 µm, according to the authors of [18], provide a reasonable balance between filtration time and quantitative efficiency and may be optimal for sampling in turbid waters, while filters with a pore size of 0.45 µm are suitable for more pure water. However, the researchers [32] used filters with pore sizes of 0.2, 0.45, 1.0, and 3.0 µm and found no significant differences in their efficiency. Perhaps the bulk of the DNA is associated with large particles or encapsulated in whole mitochondria or cells. One way or another, the authors recommend filters with small pore sizes (0.2 or 0.45 µm). The paper [33] investigated the effect of the pore size of cellulose acetate–nitrate and PC filters, as well as the physicochemical properties of surface water samples, on DNA extraction. It was found that PC bound DNA to the least extent, whilst mixed cellulose acetate–nitrate did to the greatest extent (up to 16% reduction of plasmid DNA at a pore size of 0.2 µm).

Based on the analysis of studies on the influence of pore sizes of filters made of cellulose and its esters, the authors of [8] note that the use of filters with even average pore sizes (0.45–1.5 µm) in turbid waters may lead to their rapid clogging and slow filtration rate. When filtering low-turbid water, small pore sizes (0.2–0.45 µm) are recommended. However, for more turbid water it is better to use filters with larger pore sizes (>1.0 µm).

Sorbents

After sampling from aqueous media and removal of the retained biomaterial from the filter membranes and destruction of cells, the procedure for concentrating NCs using sorbents is carried out. Sample preparation on sorption elements refers to solid-phase methods for NA isolation. The most promising polymers for making such sorbents are considered below.

1. Polyacrylates and polymethacrylates

Sorbents based on polyacrylates and methacrylates are widely used for DNA isolation due to their high specific surface area, hydrophilicity, and the nature of functional groups which reversibly interact with DNA. The work [34] describes the use of a monolith based on methacrylate with diethyl aminoethyl and butyl groups as a sorbent for selective DNA extraction. Several types of sorbents were studied (Table).

Table

Types and properties of solid particles of stationary phases for DNA microextraction in microfluidic systems [34]

Stationary phase	Particle size, µm	Specific surface area, m^2/g	Pores
Monodispersed silica	5.1	395 (12.5)	Macro + meso
Polydispersed silica	4.2	824 (3.5)	Meso
Poly(TMSPM-co-EDMA)	6.2	60	Macro
Poly(GDGDA-co-GDMA)	5.6	21	Macro
Poly(METMA-Cl-co-GDMA)	5.1	82	Macro
Poly(SVP-co-GDMA)	5.5	30	Macro

Note. TMSPM − 3-(trimethoxysilyl)propyl methacrylate, EDMA − ethylene dimethacrylate, GDGDA − glycerol 1,3-diglycerolate diacrylate, GDMA − glycerol dimethacrylate, METMA-Cl − 2-[(methacryloylox)ethyl] trimethylammonium chloride, SVP − 1-(3-sulphopropyl)-2-vinyl pyridinium betaine.

The authors suggest that the very low level of DNA extraction on polymer microspheres is due to the low specific surface area and strong nonspecific interactions between DNA and surface functional groups.

There is an adsorbent made from silanized inorganic material coated with polyaryl methacrylate, polyaryl acrylate, polyheteroaryl methacrylate or polyheteroaryl acrylate for single-stage separation of biomacromolecules by extraction of DNA from complex mixtures [35]. The basis of such a porous sorbent is silanized silicon dioxide in the form of powder (average particle diameter 15–200 µm), fiber or membrane (average pore size 1–100 nm, specific surface area 0.1–130 m^2/g). The properties of the immobilized polymer coating, in particular, the balance of its hydrophobic and hydrophilic properties, can be controlled by the nature of the comonomer units, whose synthesis involves anisole methyl methacrylate, phenylethanol methacrylate, pyridine methyl methacrylate, and naphthalene methyl methacrylate.

2. Polydopamine (PDA)

The dopamine monomer contains catechin and amine functional groups. At room temperature under slightly alkaline conditions, it self-polymerizes and deposits on organic or inorganic surfaces (metal oxides, polymers, and graphene). The resulting PDA has good dispersibility in an aqueous matrix and is environmentally stable, hydrophilic, and biocompatible. The authors of [36] applied PDA to the surface of magnetic Fe_3O_4 nanoparticles and obtained functionalized magnetic nanoparticles (PDA@Fe_3O_4) for fast and efficient capture of genomic DNA from human whole blood. Sometimes poly-2-hydroxypropyleneimine is additionally grafted onto such particles [37]. The use of magnetic solid carriers has many advantages over non-magnetic separation methods. Typically, a magnet is applied to the wall of the vessel containing the sample so that the particles aggregate against this vessel wall and the remainder of the sample can be removed [38]. In this way, it is possible to separate the components of the cell lysate which inhibit the DNA polymerase and the PCR reaction, such as polysaccharides, phenolic components, and humus [16]. Examples of other polymer compositions (cellulose, dextran, polyvinyl alcohol, polystyrene, etc.) for immobilization of the surface of magnetic media for NA isolation are given in few reviews [16, 39]. New research in this area will be discussed below.

3. Polyaniline (PANI), polyaramid (PAA) and fluoropolymers

These polymers are used to make composite sorbents by precipitation polymerization of monomers on solid carriers of inorganic nature: on the surface of glass slides and solid silica particles [40, 41, 42, 43]. In recent years, the greatest interest of researchers is associated with PANI. Oxidative PANI polymerization to obtain a coating on the surface of macroporous silica can be carried out by aniline protonation with polysulfonic acids [44, 45]. Two variants of cation modification were studied, namely: aniline polymerization in the presence of pre-silylated glass coated with polysulfonic acid, and modification of silyminated glass with pre-formed polydisulfonic acid–diphenylenephthalamide–polyaniline complexes. In both cases, an even polyaniline-containing

polymer coating with a thickness of ~3 nm was formed on the substrate surface. Sorbents containing the polydisulfonic acid–diphenylenephthalamide–polyaniline complex are selective in the separation of nucleic acids and proteins and are very promising for single-stage DNA extraction in PCR diagnostics.

Kapustin et al. [41, 43, 46] analyzed the influence of the chemical composition, morphology and surface charge of nanolayers of new polyaramid-containing sorbents on the mechanism of selective sorption of nucleic acids and proteins in comparison with previously studied sorbents modified with PANI and fluoropolymers (Fig. 3). The study of these materials was carried out in the mode of static sorption using compact spin columns and in the mode of dynamic sorption by the method of spectral correlation interferometry. It was shown that PANI and PAA exhibited similar sorption properties when interacting with nucleic acids, but retain proteins to a different extent. DNA retention by the surface of such materials is due to the presence of hydrophobic sites, while the ability to retain RNA and proteins is due to the presence of charged groups and sites capable of forming hydrogen bonds. Therefore, in a neutral aqueous medium, which is optimal for separating mixtures of biopolymers, polyaramids, although not retaining DNA, had a lower affinity for proteins compared to PANI. It can be concluded that the use of composite nitrogen-containing polymeric sorbents makes it possible, by changing the composition, to vary the affinity of the surface for NAs and proteins, adapting the product to a specific area of its application (composition of aqueous samples, priority analysis of one or another analyte, etc.).

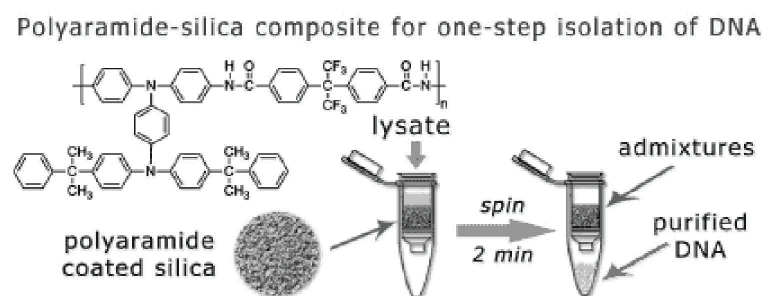

Fig. 3. Scheme of using the PAA–silica composite [46]

Extraction of DNA passed through PANI-coated macroporous silica turned out to give the highest yield among dispersed adsorbents; therefore, PANI-modified composites are preferred as carriers for the preparative isolation of NAs from complex biological mixtures, such as bacterial lysates [42]. In addition, the effectiveness of using such sorbents for analytical purposes, in particular, in detecting DNA fragmentation as a result of apoptosis induced by UV irradiation of lysates of colon carcinoma cells, was shown [40].

The same research team recently reported on the synthesis of composite sorbents modified with nano-thin layers of two polymers: PANI and fluoroplast [47]. In such a composite material, the outer PANI nanolayer acts as a selective polymer phase, while the fluoroplast layer immobilized on the silica surface serves as a substrate for it. High selectivity in the single-stage separation of nucleic acids and proteins is exhibited by a composite sorbent based on porous silica modified with fluorinated aromatic polyamide (polyamide-6F) and PVDF [48, 49]. A chromatographic column and a sorbent cartridge modified with a covalently bound fluorinated polymer coating for NA extraction are described in the patent [50]. The solid porous substrate for forming the coating can be made from organic polymers such as cross-linked polystyrenes, polyacrylates and polyethylene, as well as from inorganic metal oxides such as alumina, titanium, zirconium, silicon and iron oxide.

The authors of [51] performed DNA extraction using bacterial magnetic particles modified with a hyperbranched polyamidoamine dendrimer as adsorbents. Growth of dendrimers was initiated using bacterial magnetite coated with 3-[2-(2-aminoethyl)ethylamino]propyltrimethoxysilane or by suspending artificial magnetite in methyl acrylate. The resulting particles were collected magnetically, washed with methanol, and the reaction was continued in methanol:ethylenediamine (1:1). Stepwise growth using methyl acrylate and ethylenediamine was repeated until the desired number of layers was reached (Fig. 4). The advantages of magnetite-based systems are short processing times, little need for chemicals, easy separation through a magnet and the possibility of automating the entire process.

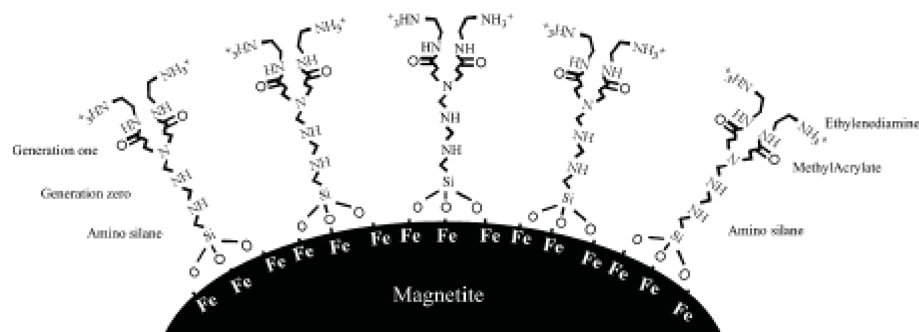

Fig. 4. Dendrimer growth on the surface of aminosilane-modified bacterial magnetite [51]

4. Polyethyleneimine (PEI)

PEI is a cationic polymer with a high density of primary, secondary and tertiary amino groups in a ratio of 1:2:1, respectively, capable of interacting with NA. Since the pK_a of its amino groups is 8.7, the PEI macromolecule is positively charged at physiological pH. This causes the possibility of electrostatic interaction with the negatively charged phosphate groups of the nitrogenous bases of the DNA chain. Like PANI, PAA and fluoropolymers, it is used to form functional surfaces in the modification of carriers, usually magnetic particles [52].

In the paper [53], a nanocomposite sorbent was obtained by immobilizing PEI on the surface of $FePO_4$ nanoparticles through electrostatic interactions. The obtained nanocomposites had a spherical shape with a size of ~100 nm and represented a new adsorbent for solid-phase DNA extraction from complex samples with high efficiency in biological samples at pH 4, which is due to the electrostatic interaction between a negatively charged polyanionic DNA fragment (phosphate groups in the main chain) and positively charged amino groups on the surface of nanocomposites. The selectivity of these nanocomposites for DNA against proteins was also found. The adsorption behaviour of DNA on nanocomposites is described by the Langmuir model with an adsorption capacity of 62 mg/g. Adsorbed DNA is easily recovered by changing pH using 0.04 M Britton–Robinson buffer in 85% yield. The extraction efficiency and purity of the DNA recovered are comparable to those achieved using other sorbent materials or commercial kits. DNA isolated using PEI–$FePO_4$ nanocomposites as an adsorbent is well suited for amplification by PCR.

5. Other polymers and approaches

The work [54] summarizes the methods of NA extraction using polymeric sorbents. In addition to the polymers discussed above, chitosan microparticles, chitosan-modified fiber, and magnetic particles coated with polyvinylpyrrolidone or polyvinyl alcohol are used as sorbents.

Modified polycaprolactone is used to increase the hydrophilicity and hence the absorbent properties of materials for collecting biological specimens (blood, buccal cells, etc.) [55]. This provides the possibility of extracting DNA from a biological sample with its subsequent sequencing and analysis.

Patents sometimes claim a whole list of polymers as NA sorbents. E.g., the patent [26, 27] uses cellulose and its functionalized substrates (polyethyleneiminocellulose; cellulose 3,5-dimethylphenylcarbamate, cellulose 4-methylbenzoate, cellulose cinnimate, cellulose 4-methylphenylcarbamate, cellulose 4-chlorophenylcarbamate, cellulose phenylcarbamate and cellulose benzoate), dextran, polyester, polyurethane, cross-linked polyvinyl alcohol, polyamide (nylon), polycarbonate or polypropylene for the manufacture of sorption material for NA.

For the isolation and analysis of individual nucleic acids, affinity chromatography is also used with sorbents containing nucleic acids or their fragments (oligonucleotides), DNA chips, and DNA biosensors [56]. Agarose, cellulose, dextran, polyacrylamide, polymethacrylamide, polystyrene, and glass are used as polymer carriers for the manufacture of commercial affinity sorbents.

Conclusion

As can be seen from the presented review, the methods for extracting nucleic acids from natural and wastewaters have been sufficiently developed by now, there are patents, industrial devices and tools are produced, and research is being carried out to improve these methods and expand the range of applications. Polymeric materials for membrane filters and nucleic acid sorbents are selected based on different criteria. E.g., ultrafiltration using industrially produced means, including cartridges, is used to extract NA at their relatively low concentration in the object analyzed, while sorption on a solid phase carrier is used to concentrate the biological object. In the first case, the pore size and strength of the filter, its resistance to liquid pressure, and ease of regen-

eration of the retained biomaterial are important. In the second case, the affinity of the polymer matrix with the biomaterial and the ease of removing DNA (RNA) from the sorbent are required.

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