New Composite Materials Modified with Nano-Layers of Functionalized Polymers for Bioanalysis and Medical Diagnostics

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1. Introduction

The essence of modern biotechnology, in particular, chemical, biological and medical biotechnology, has been changed drastically, first due to development of recombinant DNA technology. Thus, so called molecular biotechnology has been arisen. This new discipline is based on the integrated use of methods borrowed from molecular and cell biology, microbiology, genetics, biochemistry and chemical engineering. These methods together with classical microbiological and immunological methods are widely used, for instance for laboratory diagnostics of infection diseases. The new fine technologies now used not only in scientific laboratories but also in routine medical practice, are connected with development of artificial multiple copying of DNA, i.e. the method known as polymerase chain reaction (PCR). However, along with many advantages of PCR-diagnostics there is a serious problem relating to an optimal choice of the techniques for the sample preparation. At present, there is no single universal approach for DNA isolation from different sources (e.g. from bacterial lysate or blood). To solve this specific aim the special adsorbents should be used depending on physical-chemical and sorption properties of the target compound.

Thus, the success of biomedical biotechnology is to a large extent determined by availability of new effective materials and more universal approaches for biopolymer isolation and purification. As we will show below an optimal approach is based on the use of solid (porous) matrices coated with specific polymers providing the selective sorption of biopolymers. Special attention should be paid not only to retaining of functional activity of isolated substances, but also to elimination of labor- and time-consuming protocols as well as to development of scalable and automatable techniques.

Therefore, the reasons of necessity in new multipurpose matrices are evident. Such matrices should not induce irreversible denaturation of biopolymers and, at the same time, they should provide effective separation of the components contained in the complicated mixture (such as clinical sample).

The development of biocompatible composites modified with fluoropolymer- and polyaniline-based modifiers is in the scope of this work. Both the polytetrafluoroethylene (PTFE) and the polyaniline (PANI) were shown to be effective for one-step DNA isolation

from the complex mixtures containing DNA, RNA and proteins (as well as low-molecular substances) (Kapustin et al., 2003, 2006). A number of different methods were described, which relates to the synthesis, characterization of the properties and practical use of the adsorbents coated with PTFE and PANI. However, original techniques of manufacture of such materials did not find a wide application due to technological difficulties connected with their synthesis or purification. For example, in a case of PTFE the difficulty is determined by need of irradiation source, in a case of PANI the main complication is concerned with formation of insoluble polymer particles, which are not retained with the adsorbent surface, and therefore this overlaborates the procedure of a final product washing out (Kapustin et al., 2006).

Usually manufacturing of the chromatographic composite adsorbents is based on a number of consequent physical-chemical transformations. Each step of such process can result in significant change of the morphology and sorption properties of the resulting product. For example, the synthesis of composite adsorbent based on porous silica can be accompanied with the change of sizes and shape of pores of the matrices due to partial dissolving of silica. The surface concentration of silanol groups and their steriometric configuration can be also changed. Such processes are not always be predicted synonymously even using modern analytical techniques. Therefore, uncertainty of the adsorbent synthesis is contained previously in the properties of the starting materials.

Moreother, the method of the composite adsorbent preparation itself can comprise a certain extent of ambiguousness. Sometimes it is impossible to find the direct connection between the structure of the obtained adsorbent and the conditions of its synthesis. Therefore, selection of optimal conditions (even within the framework of determined method) requires the additional investigations. In particular, the most of inventions comprises namely such selection as «know-how». The characteristics describing the composites with complicated structure, which can not be exactly determined using modern analytical and identification methods, should include their qualitative composition (the atoms of determined elements), the results of physical-chemical tests, detailed protocol of the synthesis procedure, and the information relating to specific chromatographic properties of the described material.

Currently, a vide variety of approaches using the composite adsorbents finds an application in practice. These approaches can use organic and inorganic matrices, low-molecular and polymer modifiers, they can be routine or sometimes they require to use sophisticated equipment, and so on. Nevertheless, the procedures of isolation and purification of biopolymers are based on four main physical-chemical processes of the substances separation, as it presented in the table 1.

Generally speaking, these methods are based on different solubility of nucleic acids, proteins and polysaccharides (precipitation of nucleic acids with organic solvents, chromatography of biopolymers, batch-processes, in particular adsorption of biopolymers on magnetic particles, etc). However, most of them are multistage, time- and labor consuming because they are based on the concept of retaining nucleic acids from the biological samples with the adsorbent as the first step followed by elution from the chromatographic material (column, membrane, etc) on the second step. Variety of practical tasks (separation of different classes of biologically active substances including biopolymers, isolation of the determined class of biopolymers from the complex initial mixture, separation and purification of individual substances of similar chemical nature, and so on) determines a large choice of biocompatible composite materials, however such materials are difficulty considered as universal ones. In the context of this review, the universal adsorbent should be compatible with different classes of biopolymers contained in the initial sample, i.e. it should provide both the separation of specific

biopolymers keeping their initial structure and properties (avoiding an irreversible denaturation) and the high selectivity providing the isolation of needed fraction from the complicated sample. It is evidently, that the specific sorption properties of the used composites are determined by the chemical structure and the sorption properties of the polymer modifier. Therefore, the development of new "universal" adsorbents should be started from the selection of the corresponding modifier providing the formation of uniform stable coating on the matrices of different nature. The modifier should be characterized with high selectivity under bioseparation conditions.

Principal of action	Corresponding methods
1. Extraction	
Separation of the mixtures containing liquid or solid components due to selective dissolving of individual components in special solvents. Target substance should be better soluble in a solvent (extractant) than in a medium.	Phenol-chlorophorm extraction of proteins and nucleic acids. Alkali extraction of plasmid DNA using the glass beads. Liquid-liquid extraction (partitioning).
2. Precipitation	
Precipitation due to heating, cooling, concentration, or dilution. Precipitation in the presence in the presence of special added substances.	Precipitation of proteins via ultracentrifugation. Preparative ultracentrifugation of DNA under cesium chloride density gradient. Re-precipitation of proteins and nucleic acids in alcohols. Precipitation of proteins with ammonium sulphate, dextran sulphate, etc.
3. Adsorption	
Particular case of extraction. Concentration of target substance or admixture occurs at the interface between adsorbent and liquid medium.	Purification of DNA using magnetic particles. Purification of the proteins and peptides via "batch-method" using magnetic beads. Purification of DNA using the porous silica.
4. Chromatography	
Methods elaborated to separate the mixtures of substances or particles, based on the difference between the rates of their migrating in a system of immiscible phases, which move relatively to each other.	Corresponding examples include large number of applications.

Table 1. The main physical-chemical processes of the substances separation.

It is desirable that the use of such modifier could provide the one-step isolation/separation protocol. Actually, few materials are known, which could be effective for the separation of nucleic acids and proteins utilizing the simpler separation concept (as compared to the traditional multistep protocols) i.e. not nucleic acids but proteins and other components are retained with the chromatographic material (Saburov et al., 1991, Kapustin et al., 1998). However, the polymers showing that kind of the separation behavior i.e. polythetrafluoroethylene (PTFE) and polyaniline (PANI) do possess neither mechanical nor processing properties allowing to manufacture "good" porous adsorbents from those materials. Therefore the practical realization of the specific adsorption behaviour of PTFE and PANI can be achieved using composite matrices modified with nano-layers of the corresponding polymers.

Indeed, the composite adsorbents modified with PTFE and PANI were shown to be effective for one-step purification of nucleic acids from proteins. However, the techniques originally used for synthesis of PTFE- and PANI-modified composite adsorbents despite a certain similarity of their chromatographic properties were quite different, complicated and difficult for scaling up. In the case of PTFE-containing material it was γ-ray induced post-polymerization of tetrafluoroethylene (TFE) in adsorption layers at low temperatures (Kapustin et al., 2006). In the case of PANI-containing material it was oxidative precipitation polymerization of aniline in aqueous phase followed by deposition of PANI nano-particles onto the carrier surface. Furthermore, the original adsorbents prepared by the above-described techniques were not always effective for obtaining purified samples of nucleic acids from some "complicated" biological samples, i.e. whole blood, plant tissues, etc, containing in parallel with proteins the powerful PCR inhibitors such as hem or chlorophylls, or for separation of DNA and RNA, double-strained DNA (dsDNA) from single-strained DNA (ssDNA), etc. Therefore, further development of the adsorbent composition, its surface structure and manufacture technology is required.

This chapter describes the advance in development of the composite sorption materials modified with fluoropolymers and polyanilines for one-step separation and purification of biopolymers (such as nucleic acids and proteins), and, to some extent, reflects the chronology of elaboration of the specific approaches in this field. The main milestones relating to development of alternative techniques (in respect to traditional ways of the synthesis) of fluoropolymer-and PANI-modified adsorbents, the examples of their practical application in analytical procedures and medical diagnostics are discussed in the corresponding subsections.

2. Silica-based adsorbents as effective systems for isolation and purification of biopolymers (traditional approaches in bioseparation)

One of the most commonly used solid supports in liquid chromatography is unmodified silica (Snyder et al 2010). There are many approaches based on use of unmodified silica for the purification of nucleic acids from biological samples (Rother et al. 2011). The mechanism providing this type of bioseparation is based on the specific interaction between silica's surface and nucleic acids, in particular, in the presence of chaotropic salts at high concentration and high pH values. Silica is weakly acidic under physiological conditions, and its surface is represented mostly by Si–O- rather than Si–OH groups . Therefore, in the presence of very high chaotrope salt concentrations, cations in solution can form relatively stable layer of counter ions around the negatively charged silica surface and change their charge into positive. As a rule, the chaotropic agents disrupt the hydration shells of biomolecules in aqueous solution and denature them. An exception is DNA, which is remarkably stable against chaotrope denaturation. The phosphate groups of nucleic acids are strongly acidic, therefore, nucleic acids bind effectively to the positively charged silica's surface, but other molecules are left in solution. The DNA–silica complex is then washed in a salt solution or an alcohol/water mixture to remove weakly bound "impurities", then DNA can be eluted with low salt concentration buffer or water.

Analogous multistep mechanism of nucleic acid isolation/purification is a basis of the most modern techniques (it is true for both unmodified silica or silica modified with polymers). Nevertheless, the silica itself is characterized by relatively low stability under high acidic or alkali conditions, high level of non-specific sorption, and requires specific conditions

providing the optimal surface concentration of required functional groups, so additional chemical modification is needed. A great number of effective adsorbents were obtained as result of silica modification with low-molecular substances. However, the properties and application features of such materials are not in the scope of the present review; however, the majority of them are characterized with the same disadvantages as it was observed in a case of unmodified silica application.

In early 1970's a great practical interest had been arisen to polymer-modified silica as adsorbents for chromatography of proteins and peptides, enzymes, nucleic acids and viruses. Such interest was determined by the advantages provided with rigid-skeletal materials as compared to traditional soft organic resins and gels. Wide use of cross-linked dextranes, agarose and polyacrylamide, which were developed in early 1960's, was limited due to their low mechanical stability. The letter development of highly cross-linked resins partially overcames the mentioned disadvantages, but the cardinal way to solve this problem is the development of composite polymer-coated materials.

Known methods of producing of composite adsorbents can be nominally divided into two large groups. The first one includes the methods based on adsorption or chemosorption of polymers from their solutions onto the silica's surface. The second group involves the processes of radical or ionic polymerization of monomers in a presence of (mostly silica) carrier.

Physical adsorption of polymers on the carrier surface is the simplest way to obtain the composite adsorbents. It can be realized via direct sorption of polymers, which are retained on the surface due to multipoint contact between partially positively charged macromolecules with cation-exchange surface of silica by means of hydrogen or ionic bonds. In the case of chemical adsorption the silica surface can be preliminary treated with the suitable agents (such as functionalized silanes, different additional bifunctional reagents, etc) to activate the surface and provide the stable polymer coating after the contact of the polymer modifier with activated carrier.

The examples of the corresponding modifiers include a large variety of polymers and oligomers. The most illustrative examples of modifiers, which are suitable for physical adsorption of macromolecules on the silica support include dextrans or starch (as well as its derivatives), poly-N-vinylpyrrolidone (and its copolymers with wide range of functionalized comonomers), poly-N-vinyltriazol, copolymers of poly-4-vinylpyridine with styrene, polyvinyl alcohol, polyethyleneimine, polyallylamine, polyethylene glycol (and its derivatives), olygomeric phases based on N-substituted amides of carbonic acids, polybutadyene, and many others.

Methods of producing the adsorbents based on graft polymerization usually include less number of steps as compared to methods based on adsorption of polymers. At the same time, determination of the optimal conditions of such process has significant importance, so the correlation between the synthesis conditions and the properties of the final product is less evident as compared to adsorption-based techniques. The examples of the corresponding methods include polymerization of wide number of vinyl monomers in the presence of carrier and bifunctional cross-linking agents, or using different modes of the carrier surface activation (such as ionizing irradiation, μ- and γ-irradiation, and so on).

The use of polymer modifiers allows to obtaining of the stable and effective composite materials combining the required structure and mechanical properties of the carrier with specific sorption properties of the modifier. However, the majority of the developed materials provide multistep mechanism of the biopolymers separation/purification, which

is similar, in principle, to the process where unmodified silica support is used. Therefore, to elaborate more efficient variants of one-step separation of different classes of biopolymers (primary, nucleic acids and proteins), the specific types of polymer modifiers are required.

3. Perfluoropolymers as effective modifiers for the preparation of composite adsorbents for one-step separation of biopolymers

The requirements to the properties of an "ideal adsorbent" for separation of biomolecules or particles, comprise a few controversial features, which can hardly be achieved in a single material. Those include insolubility, permeability to macromolecules, high rigidity, well-defined porosity independently of solvent, large specific surface area, low non-specific adsorptivity, physico-chemical and biological stability, and facile derivatization. Therefore, it should come as not surprise that investigators have attracted their attention to the fluorine-containing polymers. Fluoropolymers are known have a complex of unique physical-chemical and sorption properties due to the presence of fluorine atoms in the macromolecules. The simplest example of the perfluorinated polymer is the polytetrafluoroethylene (PTFE). Its polymer chain forms almost ideal cylinder with the outer shell of fluorine atoms. The surface formed with such macromolecules is characterized with extremely low non-specific sorption that is really important property, in particular, for effective use of fluoro-containing materials in bioseparation of nucleic acids. PTFE as adsorbent was first described by Hjerten (Hjerten et al., 1978) and was tested on examples of purification of proteins and tRNA. Recently William (William et al., 1986) presented a new fluorocarbon adsorbent for reversed-phase HPLC of peptides and proteins. However, low specific area and capacity (1.1 mg tRNA/g) and relatively low limits of working pressure do not allow to the use of this adsorbent for preparative chromatography. Moreover, because of its insolubility in common solvents, infusibility, and softness, the manufacture of rigid mesoporous matrices from PTFE as a final product is impossible. This task is to be accomplished via creation of defect-free polymer layers on a solid porous matrix by means of direct (graft) polymerization of TFE in adsorption layers. The polymerization is initiated both by active centres generated on the surface of starting materials during irradiation, like, for example, in work of Turkin, Zubov, Saburov et al., 1988 and with the use of chemical initiators (e.g. fluoroolefin ozonides). The polymerization of TFE on the surface of inorganic materials has been described in the literature (Ivanov, Saburov & Zubov 1992). As shown by preliminary experiments, during vapour- or liquid-phase monomer feeding, the grafted polymer does not form a continuous layer and occurs as single globular entities on the surface of an inorganic support. An effective method for the preparation of grafted polymers is the use of the post-effect, i.e., grafting due to active centres stabilized on a solid support. To find optimal synthesis conditions for the desired morphology of the composite obtained by means of this method, several modes of the process were examined. Controlled porous glass (CPG) with an average pore diameter of 50 nm and an average particle size of 100–150 μm was used as a support in this case. The modes of irradiation, temperate regime, and conditions of the monomer introduction into reaction system were varied in this study. It turned out that the most effective mode providing the required morphology of the composites includes irradiation of the matrix with the pre-adsorbed TFE at –196°C followed by slow heating of the support with TFE preliminarily sorbed up to room temperature, and post-polymerization during warming the system outside the irradiation zone ("joint radiolysis" at –196°C). From mercury porosimetry data, it is possible to determine the

thickness of the polymer coating, which varies from 1–3 to 5–10 nm depending on the amount of TFE introduced. These values correspond to those calculated from the amount of the grafted polymer and the total pore surface area of the matrix. In addition, the surface of the synthesized adsorbents is poorly wetted by both polar and nonpolar liquids, i.e., become fully non-wettable.

The bioseparation behaviour of thus obtained PTFE-modified CPG is illustrated by a typical chromatogram of a mixture containing the *E. coli* plasmid pBR 322, RNA, and associated proteins (Ivanov et al, 1992). It is seen that DNA elutes from the column with the first fraction in the excluded volume, while RNA is weakly retained but likewise elutes in the isocratic regime. However, the total protein fraction is completely eluted from the column only with an aqueous organic eluent. The resulting mixture of proteins can be separated in the reversed-phase HPLC mode. The mechanism of retention of individual proteins on fluoropolymer adsorbents resembles that on classical C18 phases. However, the elution volume in this case is much smaller. Consequently, fluoropolymer composite adsorbents are effective in separation of both nucleic acids and proteins. A high selectivity of fluorinated polymers is their general property, which is provided by a very low surface energy of the relevant coatings. This advantage has been realized using the cartridge variant of bioseparation, which is described in details below.

Thus, the use of perfluoropolymers as modifier of composite adsorbents provides more sufficient, from the practical point of view, one-step scheme of biopolymers separation.

4. From perfluoropolymers to partially fluorinated polymer modifiers. An effective alternative to the traditional techniques

Interactions observed between the fluoropolymers and biopolymers have been attracted the attention of scientists for many years. As an example, the article of G. Xindu and P.W. Carr (Xindu & Carr, 1983) can be mentioned. Authors described the adsorption aspects of fluorocontaining substances (including perfluoroalcyl-containing protein) at the fluorocarbon surface. The main conclusion was that the nature of the interaction between fluorinated proteins with fluorocarbon surface is not known yet. Latter works were also devoted to the study of the interaction between different fluorinated phases and low-molecular and polymer substances. These studies were carried out using not only perfluoro-containing materials, but also the chromatographic systems containing insignificant part of hydrogen atoms substituted by the fluorine. Therefore, the further development of bioseparation methods based on the use of fluorocontaining adsorbents was not unexpected. Early works in this field were devoted to study of functionalized particles of polychlortrifluoroethylene (PTCFE), which is chemically stable, hydrophobic and mechanically stable material. The treatment of PTCFE with metal-organic substances (such as n-butyllithium, aryllythium, alkylmagnesium, arylmagnesium) resulted in the preparation of alkyl-containing materials with low content of chlorine. n-buthyl-PTCFE adsorbent was shown to be effective for separation of aromatic compounds via reverse-phase mechanism of chromatography. Phenyl-PTCFE adsorbents (having substituted aromatic rings) were effective in separation of some drug agents via both the reverse-phase and the ion-exchange mechanisms. At the same time the adsorbent modified with trialkyl (C_8-C_{10}) methylammonium or quaternary ammonium substance RPC-5 was suitable to separate biological substances such as mRNA, nucleotides, nucleosides, DNA-fragments. Unfortunately, this adsorbent was not stable enough for the repeated use. Berendsen

(Berendsen et al., 1986) obtained good results using silica modified with (heptadecafluorodecyl)dimethylsilane (HFD). Such adsorbents were shown to be effective for separation of fluorocontaining low-molecular organic substances. These materials retained nonpolar compounds weaker as compared to hydrocarbon-containing adsorbents. Therefore, it was suggested to desorb proteins from the fluorocontaining surface reducing the content of organic component in eluent (i.e. under non-denaturating conditions), and thus, this approach justified the use of HFD-modified adsorbents.

In early 1990's, Saburov, Zubov et al (Saburov et al., 1991) have developed the composite adsorbents based on porous silica modified with trifluorostyrene. Polymer modifier was obtained by radical copolymerization of trifluorostyrene with methylvinyltrietoxisilane. In the first case the suspension of the support particles was prepared in the solution of copolymer in absolute toluene followed by boiling, filtration and washing with hot toluene. End-capping of the residual silanols was carried out with hexamethyldisilane. The alternative way of the modification was based on the treatment of the carrier particles with γ-source followed by contact with vapour of trifluorostyrene (Saburov al., 1988). Dimer of trifluorostyrene was removed by washing with toluene. The both obtained adsorbents had the similar adsorptive and chromatographic properties and can be characterized as intermediate materials if compared to silica modified with TFE and silica modified with polystyrene. In both cases the obtained materials demonstrated the high hydrolytic stability. The materials were used as a basis to prepare a wide range of ion-change adsorbents with different surface capacity (from 95 to 300 μmol/g), and they were successfully used for separation of insuline and desaminoinsuline as well as nucleotidephosphates.

Another approach for producing fluoropolymer-coated adsorbents was suggested by Kapustin in 1998 (Kapustin et al., 1998), for synthesis of the silica adsorbent modified with partially fluorinated polybutadyene. This approach is based on the *in situ* fluorination of thin films of oligomers or polymers preliminary immobilized on the carrier surface. The fact, that unsaturated hydrocarbons can be fluorinated using such agents as xenon difluoride, is of special interest, because introduction of the fluorine atoms into the polymer coating provides modification of the surface not disturbing the volume properties of the coating. Standard method of direct fluorination suggests the mixture of gaseous fluoride with nitrogen. However, the fluoride is toxic, very corrosive that requires safety measures during all operations. The use of alternative fluorinating agent simplifies the process of modification.

The first step of the process is deposition of thin uniform layer of the non-fluorinated precursor on the porous silica matrix. It is achieved by impregnation of the pre-evacuated matrix with the precursor solution in volatile solvent. After evaporation of the solvent the material was fluorinated. A typical example of the precursors are oligobutadienes of MM 5000 - 10000. It was shown earlier (Zubov et al., 1998), that XeF_2 effectively interacts with the surface of polybutadiene and modifies it. Fluorination under oxygen-free conditions excludes the appearance of polar groups that can significantly increase the non-specific sorption. Fluorination of the polymers immobilized on the silica surface accompanied with their chemical structuring (cross-linking) due to the recombination of macroradicals and interaction between radicals with multiple bonds. The result is formation of cross-linked fluorinated polymer coatings (Kapustin et al., 1998). By-products (first of all, hydrofluoric acid and the products of its interaction with silica) and XeF_2 absorbed on pores surface, then can be removed under vacuum at the higher temperature.

The main feature of the suggested method is obtaining of fluorinated polymer phase directly during the process of the adsorbent producing in contrast with the techniques using fluoromonomers. The fluoropolymer coating is additionally setted due to formation of intermolecular bonds.

Obtained adsorbents were used for isolation of bacterial plasmid DNA (from *E.coli*, JM 109) and DNA from nuclear-containing human cells using the cartridges containing about 140 mg of adsorbent. Such procedure was shown to be very simple, especially in comparison with earlier elaborated multi-step methods (such as centrifugation under density gradient after an alkali treatment of cell lysates or subsequent treatment of the lysate with phenol and chlorophorm). The observed results of DNA isolation from the model mixture containing DNA, RNA and proteins were the similar as compared to use of PTFE-coated adsorbent, i. e. DNA was eluted at first, then RNA was eluted using the same buffer solution, and the proteins were desorbed from the material using 50% methanol. The ratio of intensivities at λ=260 nm and λ=280 nm measured for the collected DNA-containing elates was approximately 2:1, and it confirmed the high extent of DNA purification from the proteins.

The relatively simple protocols for DNA isolation from a human blood were elaborated. These methods included either column chromatography of the sample obtained after isolation and lysis of the nuclei-containing cells from a blood, or very simple "batch-method" based on the sorption of the components in the mixing volume followed by centrifugation and collection of the supernatants.

In the first case DNA, but not RNA, was eluted from the column with TE-buffer in the excluded volume, then the retained components were desorbed under linear gradient of iso-propanol. In the second case the "batch-procedure" was repeated twice using the same sample, and the content of DNA was measured spectroscopically in the both collected supernatants. The fist aliquot of the collected supernatants contained about 60% of purified DNA, and the second one contained about 40% of DNA.

The most effective application of thus prepared adsorbent was achieved when one-step isolation of DNA from the bacterial lysates was carried out (like it has been demonstrated using PTFE-coated adsorbents). However, in spite of evident advantage of this synthetic method in comparison with irradiation technique, it seems to be more suitable for modification of non-porous supports, first due to necessity in laborious washing procedure of the porous adsorbent to get rid of by-products of fluorination. Nevertheless, it was demonstrated, that preliminary immobilized oligomeric or polymer phases on the carrier's surface can be chemically modified to reach the required extent of fluorination.

Materials coated with partially fluorinated polymers have additional advantage, i.e. they in addition can be chemically modified to improve hydrophilicity of the surface or to introduce specific functional groups. Therefore, fluoropolymer-modified silica can be successfully used not only in bioseparation. Matrices coated with fluoropolymers are considered as perspective candidates for immobilization of bioligands. In this case the presence of specific functional groups on the fluorinated surface is required. Direct synthesis of fluoropolymers containing specific functional groups is not a simple task. Sometimes, more efficient way to obtain the functionalized matrices is formation of thin solid fluorocontaining coating on the carrier surface by casting of preliminary prepared fluoropolymer (e.g. copolymer of TFE with functionilized fluorocontaining monomers) from its solution in perfluoro-containing solvent (mixture).

An example of preparation of the support for solid-phase synthesis of oligonucleotides is given below. This material is based on porous silica coated with the copolymer *poly-*

ethylene-*co*-{methyl-3-(1-(difluoro((trifluoroethenyl)oxy)methyl)-1,2,2,2-tetrafluoroethoxy)-2,2,3,3-tetrafluoropropanate} (EVE) followed by specific chemical modification. The corresponding method of synthesis includes such steps as incubation of the support particles in the solution of polymer followed by ultrasonic treatment, evaporation of the solvent and treatment with nucleoside. This method was elaborated by authors of the present chapter in 2007 (unpublished results).

Chemical structure of EVE allows to carrying out the different polymer analogous reactions due to presence of active esther groups as it shown on Fig. 1.

$$-(CH_2-CH_2-CF_2-CF_2)n- \qquad CF_3$$
$$O-CF_2-CF_2-CF$$
$$O$$
$$CF_2-CF_2-COOCH_3$$

Fig. 1. The chemical structure of *poly*-ethylene-*co*-{methyl-3-(1-(difluoro ((trifluoroethenyl) oxy)methyl)-1,2,2,2-tetrafluoroethoxy)-2,2,3,3-tetrafluoropropanate} (EVE).

Fig. 2. Synthesis of fluorinated support based on CPG modified with EVE for solid-phase synthesis of oligonucleotides.

The scheme of the chemical modification of side groups of immobilized fluoropolymer (EVE) is presented on Fig. 2a. Thus modified CPG was treated with Nucleoside (DMT-dT-SNPE from Proligo GmbH, Germany) in accordance with the scheme represented on Fig. 2b. Surface concentration of nucleoside residues depends on degree of amination and can be controlled. Stability of the nucleoside-loaded CPG was studied using the standard Trytil-assay. Nucleoside-loaded EVE-coated CPG was stored for 9 months. The concentration of immobilized nucleosides was found about 9.25 µmol/g. This value corresponds to analogue value (9.33 µmol/g) which was measured for fresh prepared sample. Thus, stable support for solid phase oligonucleotides synthesis was developed and characterized.

Abovementioned data confirmed that fluoropolymer-coated porous silica composites can be effectively used for one-step isolation of nucleic acids, purification of other biopolymers and for immobilization of bioligands. However, these important properties are also demonstrated by some other polymers i.e. polyanilines. The composite materials modified with polyanilines via traditional presipitative polymerization methods and by alternative ways are described below.

5. Polyaniline as alternative effective modifier for producing the composites for one-step bioseparation

The unique physicochemical properties of PANI explain the wide use of PANI-based materials in various areas of science and technology. Polyaniline is characterized by a high chemical stability and is insoluble in most of common solvents. The specific features of the structure of the PANI macromolecule make PANI-containing materials effective candidates for use in biomedical applications, in particular, for separation of complex mixtures of biopolymers (Kapustin et al., 2003). An addition, reversible changes of PANI properties depending on pH of medium, make it possible to regulate the sorption properties of "smart" PANI coatings.

5.1 Oxidation precipitative polymerization of aniline as traditional way of preparation PANI-coated adsorbents

Polyaniline-modified adsorbents behave in a similar to fluoropolymer-coated adsorbents manner as regards the separation of mixtures containing nucleic acids and proteins. However, the use of PANI-containing adsorbents offers an impressive advantage in separation of mixtures of proteins, since it does not require the addition of an organic component to the mobile phase. A typical example of the pH-gradient separation of a model protein mixture is given in (Kapustin et al., 2006). The proteins eluted from the column in the order of ascending pI values of the individual components. The mechanism of retention and desorption of proteins on PANI coatings is complex and seems to be determined by both ionic interactions of the sorbate charged groups with ionic sorption sites on the adsorbent surface and the ability of a PANI coating to reversibly bind protein molecules via hydrogen bonding. The following examples illustare the similarity of the sorption properties of PTFE- and PANI-containing adsorbents based on TrisoporTM-500 CPG. Samples of 100–200 µl of a mixture containing DNA, RNA and proteins were applied into cartridges packed with adsorbents. The cartridges were incubated for 3 min at room temperature and centrifuged for 1 min at 240 g. Then, the collected eluates (80–100 µl) were analyzed. The results of electrophoresis and the spectroscopy data showed that the eluates obtained using both types of the adsorbents contained DNA cleaned of protein up to 90%.

It turned out that PANI coatings effectively bind metal-containing compounds, such as heme and chlorophylls (powerful PCR inhibitors). Figure 3 presents the results of electrophoresis in 1% agarose gel of DNA specimens obtained via passing samples of whole blood and leaves of *Nicotiana Tabacum L.* after preliminary treatment with concentrated lysing solutions, through cartridges containing 140 mg of the adsorbent each. The eluates were additionally subjected to spectrophotometric analysis. It was shown that the purified DNA samples are practically free of heme or chlorophylls (judging from the absence of absorption at characteristic wavelengths) and, as such, are quite suitable for PCR analysis.

In spite of fact that the PANI-coated adsorbents are effective for one-step bioseparation of nucleic acids there are a number of difficulties, firstly connected with the complicated procedure of the final product purification from unbound and loosely bound polymer particles. Therefore, development of new approaches for the synthesis of PANI-coated composites is important practical task.
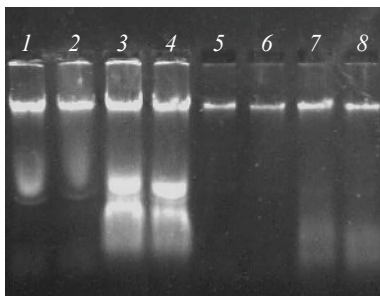
Fig. 3. Results of electrophoresis in 1% agarose gel of DNA samples obtained via passing the lysates of cow whole blood and *Nicotiana Tabacum L.* leaves through cartridges packed with the PANI-containing adsorbent: (*1, 2*) blood lysate, (*3, 4*) tobacco leaf lysate, (*5, 6*) eluates containing blood DNA, and (*7, 8*) eluate containing plant DNA.

5.2 Matrix polymerization of aniline is an effective alternative to precipitative process

Polyaniline is prepared via chemical or electrochemical (Stejskal., 2002) oxidation of aniline. The chemical polymerization of aniline in an acidic medium in the presence of a solid surface frequently leads to the formation of thin polymer coatings (Sapurina & Stejskal, 2008). The mechanism of oxidative polymerization is complex and is still under debate despite a large number of publications on the issue. However, it is generally adopted that the first step of the reaction is the protonation of the monomer. Then, the protonated form of aniline is oxidized yielding the radical ion, followed by the formation of dimers, oligomers, and, finally, a solid insoluble polymer. The characteristic features of the process are the presence of an induction period, the autocatalytic character, and usually observed slowing down of the reaction before the complete depletion of the monomer. Direct evidence for autocatalysis by the polymer is that the addition of a portion of PANI to reaction system immediately after mixing of monomer and oxidant solutions changes the shape of the polymerization kinetic curve. The induction period shortens, depending on the mass of added PANI (Kapustin et al., 2003), and the monomer conversion curve demonstrates a second step. The presence of silica carrier has practically no effect on the kinetic pattern of aniline polymerization as compared to polymerization in the absence of the support. In other words, the silica surface has no catalytic effect on the polymerization of aniline. As a result, aniline polymerization produces not only a film on the silica surface but also proceeds in the bulk of the reaction mixture. For example, in the case of aniline polymerization in the presence of CPG, up to 40% of the monomer is consumed for the formation of a suspension of PANI particles.

The necessity of aniline protonation at the first step of oxidative polymerization supposes that insoluble solid polyacids (acid cation exchangers) should be used as matrices for the reaction. Therefore, silica modified with polymers having sulfo groups on the surface can be used as support. To prepare such supports a number of approaches were developed. In particular, silica was modified with PTFE on which polystyrene (PS) crosslinked with divinylbenzene as grafted by means of radiation post-copolymerization. Then, the PS-containing silica was sulfonated. In the case of aniline polymerization in the presence of surface-sulfonated supports, the induction period almost disappeared as compared to the polymerization in the absence of the support. No formation of PANI particles in the reaction

volume was detected. Since the morphology of the original support is retained after modification and the polymeric coating efficiently shields the silica surface, the obtained composite was proposed to be used for DNA isolation from plant lysates, i.e. from the complex mixtures containing DNA, RNA, proteins, peptides, polysaccharides, surfactants, chlorophylls, and low-molecular compounds. Lysates of tobacco leaves (*Nicotiana tabacum L.*) were applied onto cartridges with the PANI-containing adsorbents based on both polysulphostyrene-coated silica (CPG-PSS) and unmodified CPG. When the lysates are passed through the adsorbents, DNA is eluted in the excluded volume, whereas proteins, chlorophylls, and a considerable part of low molecular substances are retained. The degree of purification of DNA from proteins and chlorophylls was estimated using electrophoresis and spectrophotometry. The plant lysates were completely decolorized upon passing through cartridges with CPG-PSS-PANI (the absorbance of the initial lysate at 416 nm was 0.19, whereas the absorbance of the eluate obtained using CPG-PSS-PANI was 0.04). At the same time, the eluates obtained in the case of CPG-PANI had a pale light-green colour (absorbance 0.10). The ability of the adsorbent containing the residual sulfo groups to bind chlorophylls is explained, most likely, by the interaction of the porphyrin fragments of chlorophylls with the sulfo groups on the adsorbent surface. The degree of purification of DNA isolated from the plant tissue lysates using CPG-PSS-PANI enables their direct use for amplification by the PCR method. The corresponding amplicons are shown on Fig. 4.
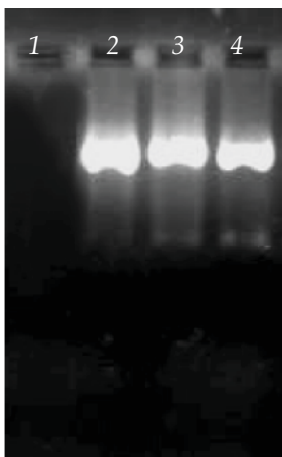
Fig. 4. Electrophoregram of the DNA amplicons (*2—4*) obtained by the PCR with the eluates containing DNA from the *N. tabacum* L. tobacco leaves. The eluates were obtained using the CPG—PSS—PANI sorbent (*1* is negative control, H_2O).

The obtained coating is in fact the surface polycomplex of PANI and sulfonated PS. In other words, it is the self-doped derivative of PANI, i.e. the polymer structure containing SO_3H functional groups. Matrix polymerization offers an efficient strategy to control both the kinetics and mechanism of the polymerization and the structure of resulting polymers. Thus, polymerization yields polycomplexes formed between the original matrix and the formed polymer. In particular, matrix polymerization of PANI was assumed by Yagydaeva, Kapustin, Prostyakova and Zubov in 2009 as a basis for preparing composite biosorbents, in which the products of the matrix oxidative polymerization of aniline on aromatic

poly(sulfonic acids) were used as modifiers of porous silica. Of special interest is the use of various poly(sulfonic acids) (PSAs) as dopants (Yagudaeva et al., 2007). Some systems remain phase-homogeneous since PANI develops directly on the water-soluble matrix (PSA). Previous experiments showed that when the synthesis of PANI is performed in the presence of the aromatic polyacid poly(p,p'-(2,2'-disulfonic acid)diphenyleneisophthlamide (*iso*-PSA), the local concentration of the protonated aniline in the vicinity of a polyacid macromolecule increases, and turns out to be higher than that in solution. Thus, polymerization of aniline in the presence of PSA should proceed without formation of a suspension of PANI particles in the bulk of the reaction mixture. This concerns the polymerization of aniline both in the solution of PSA and in the presence of PSA-modified silica. Besides, the use of the preformed PSA-PANI stable polycomplexes as polymer modifiers of the support surface makes it possible to simplify the preparative technique of the composite adsorbents manufacturing. Furthermore, owing to the presence of sulfo groups in a polycomplex, the adsorbent surface possesses additional functionality. Hence, the sorption behaviour of the materials can be controlled. In particular, the matrix synthesis of PANI was performed in the presence of *iso*- and *tere*-PSAs.

The sorption behaviour of PANI-containing adsorbents prepared via polymerization of aniline on PSA preimmobilized on the surface of the silaminated silica, as well as via immobilization of the preformed *iso*-PSA–PANI complexes on the surface of silaminated silica, was compared to the corresponding characteristics of CPG-PANI and PSS-PANI. Model mixtures containing DNA, RNA and proteins (BSA) were applied on cartridges packed with the prepared adsorbents (nearly 100 mg). Elution was performed at various pH values or various polarities of eluents (during desorption of protein components of the mixture). Eluates were analyzed by electrophoresis and spectrophotometry. The yield of DNA in the case of CPG-PSS-PANI and CPG—*iso*-PSA-PANI (prepared by the first method) was above 80%, and RNA was absent in both cases. DNA elutes in the excluded volume, while RNA and protein retained and the degree of protein sorption with the CPG-*iso*-PSA-PANI is higher than the degree of protein sorption with the CPG-PSS-PANI. On the contrary, the adsorbent prepared in the presence of *tere*-PSA-PANI irreversibly retained almost all nucleic acids. Thus, in terms of the sorption behavior, PANI, which was formed on the surface of *tere*-PSA, differs from that formed on the surface of *iso*-PSA or on the surface of the unmodified silica. Therefore, *tere*-PSA was found to be not suitable for the synthesis of PANI-containing adsorbents. The character of sorption of nucleic acids with the adsorbents containing *iso*-PSA is nearly the same as that of previously prepared PANI adsorbents containing sulfo groups chemically bonded to the surface. The efficiency of DNA isolation depends on the nature of the matrix polymer and increases with an increase of the content of sulfo groups.

In the case of *iso*-PSA-PANI simultaneous presence of additional sorption sites (i.e. residual sulfonic acid groups) in the obtained coatings allow to additional possibilities for bioanalysis, e.g. separation of ss- and dsDNA depending on pH of medium, as it shown on Fig. 5. To study this effect the authors of the present chapter used the model system containing dsDNA isolated from *Agrobacterium tumifaciens C58* and ssDNA obtained after reverse transcription (using reverse trancriptase RNAse H+) of RNA isolated from Tobacco Mosaic Virus followed by hydrolysis of RNA at pH > 8.0. Aliquotes of thus prepared mixture were applied onto the cartridges packed with CPG-*iso*-PSA-PANI, then collected eluates were used in PCR analysis (using the corresponding specific primers). It is seen, that increase of pH value from 8 up to 9 results in increasing of the yield both ss- and dsDNA in the collected eluates. At the same time, ssDNA/dsDNA ratio (equals ~ 1:3) in these eluates is constant.
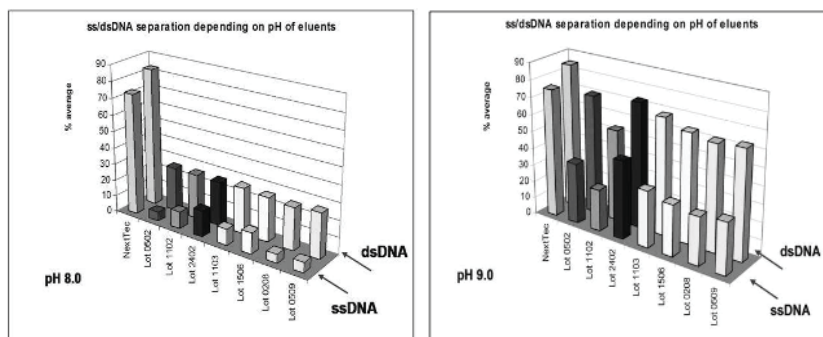
Fig. 5. Separation of ssDNA and dsDNA depending on pH of medium using *iso*-PSA-PANI-coated adsorbent.

It could be said in conclusion, that the use of preformed *iso*-PSA–PANI complexes for obtaining PANI-containing adsorbents appreciably simplifies the technique and, hence, scaling up of the manufacture of the said materials.

5.3 Unique properties of PANI coatings open new possibilities for bioseparation and bioanalysis

Introduction of additional functional groups into fluorocontaining macromolecules results in a widening the fields of their applications, similarly, introduction of additional functionality to PANI coatings opens the new possibilities of their use in bioanalysis. An example of such effective approach was demonstrated in the patent WO 2011/0014308 A1 (Vaczine-Schlosser et al., 2011). This invention relates to a process for so called 'laser desorption ionization mass spectrometry' using a polymer coating as UV absorption medium onto which the sample probe of interest (containing proteins or peptides) is deposited. The corresponding polymer coatings, in particular, were obtained by copolymerization of aniline with substituted anilines such as 3-amino-benzoic acid (poly-ANI-*co*-3-ABA).

Mass spectrometry (MS) is a widely used analytical method for determining the molecular mass and structure of various compounds. It involves transfer of the sample molecules into the gas phase and ionization of the molecules. Molecular ions are separated by using electric or magnetic fields in high vacuum according to their mass-to-charge (m/z) ratios. During the last decades, MS has proven to be an outstanding technique for accurate and sensitive analysis of biopolymers, like proteins and peptides. With the introduction of soft ionization techniques such as electro spray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) or surface enhanced laser desorption ionization (SELDI), it became possible to transfer into the gas phase and ionize these non-volatile, large, and thermally labile molecules without their deep degradation.

However, the latter technique requires use of so called "matrices", i.e. energy absorbing substances (usually aromatic acids like α-cyano-4-hydroxy cinnamic acid) mixed with an analyte. The matrices not only make possible volatilize the analyte, but produce considerable background noise in the lower molecular part of the mass-spectrum. Therefore, there is a strong need for a process that is not limited by strong background signals, or even such process, which makes the addition of a matrix compound superfluous. The said

invention provides a process comprising depositing of a sample probe on a polymer surface comprising a UV absorbing aromatic monomer unit followed by irradiating the sample probe and/or the surface with a UV laser beam thereby effecting an ionization and/or desorption of the sample molecule, and determining the mass of the ionized sample molecule.

A main idea of the said invention is to replace a matrix substance by a surface of polymeric material deposited on the sample chip. The inventors found that this can be achieved by the use of a polymer having UV absorbing aromatic units, which may be selected from, in particular, aniline or an aniline derivative. The UV radiation may be absorbed by the polymer and the absorbed energy is transferred to the sample molecules. During the laser irradiation step, the surface may be heated. However, the polymers of this invention show a good thermal and oxidative stability even under such condition.

The ability of aniline-containing coatings to bind a variety of proteins was studied using Spectral Phase Interference. Investigation of the kinetics and effectiveness of pH-dependent binding of proteins and peptides to the polymer surface was studied using thin glass slides (90 – 120 µm) modified with PANI coatings. It was shown that a reversible sorption of different proteins on the PANI-modified glass surface could be achieved depending on the pH. Cytochrome C showed a reversible adsorption to the surface at a pH of 7.2 and was desorbed at a pH of 2. It was furthermore shown that cytochrome C (M 12 000), casein (M 20 000), myoglobine (M 17 800), IgG (M 125 000) and poly-L-lysine (M 150 000) could be bound to the surface of the PANI-coated silicon-strip at pH of 7.2. Calf thymus DNA and poly-uridine acid potassium salt were not bound to the surface under the same conditions.

Thus, silicon strips coated with aniline-based polymer films are suitable to retain specific biological entities and function as chromatographic supports as well.

To visualize protein retention on the modified PANI-ABA Si-strips, model proteins with different pI were labelled with luminescent (emission at 546 and 581) semiconductor (CdSe)ZnS nanocrystals. Protein solution droplets were put onto the modified surfaces (10 µl of solution, 0.5 mg protein/ml) and incubated for 5 min at ambient temperature. Excess was removed and sorption was optically visualized. The results are presented on figure 6 and in table 2. The results show that PANI-ABA surfaces can be used to separate proteins depending on their pI values. Separation of acid and alkali protein/peptides can be carried out directly on the silicon strip surface using tris-HCl buffer or another appropriate solution.
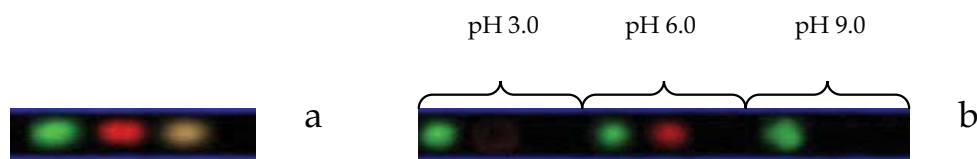

Fig. 6. Visualization of the proteins having different pI values and labelled with semiconductor nanocrystals under UV-source. The individual proteins before washing with the buffer solution *(a)* and retention of cytochrome C, myoglobine and pepsin on silicon-strips coated with *poly*-ANI-*co*-3-ABA by precipitation polymerization *(b)*. The correspondence between the spot colour and individual proteins is shown in the table 2.

Protein	pI	Colour	Before washing	pH 3.0	pH 6.0	pH 9.0
Cytochrome C	10.3	Green	+	+	+	+
Myoglobine	6.9	Red	+	-	+	-
Pepsine	2.8	Yellow	+	-	-	-

Table 2. The correspondence of the spot colour to individual proteins with different pI and results of the retention of cytochrome C, myoglobine and pepsin on silicon-strips coated by precipitation polymerization. (+) indicates good observation of UV excitation, and (-) indicates no observation of UV excitation.
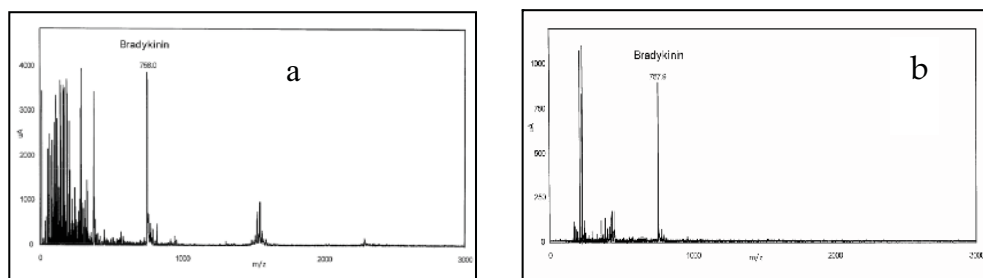
Fig. 7. MS spectra of peptide standards on a silicon strip modified with PANI-PABA with *(a)* and without an addition of CHCA as matrix *(b)*.

PANI-PABA coated Si-strips were analyzed with and without matrix addition, in order to compare the efficiency of conventional sample preparation techniques for analysis of compounds in the low mass region. In the experiments, the two most common matrix types were used: a-cyano-cinnamic acid (CHCA) and sinapinic acid (SPA) (Bio-Rad). 1 mL matrix solution was added to the surface and dried. The matrix addition step was repeated. Spectra were acquired under the same experimental conditions. The results show that without addition of extra matrix, no background peaks can be observed from the PANI-PABA coated arrays. However, addition of the standard matrix causes the appearance of background ions in the low mass range with high intensity, which hinders the analysis of low molecular weight compounds. Figure 7 shows MS spectra of peptide standards on a silicon strip modified with PANI-PABA (with a molar ratio of aniline : 3-ABA of 3:1). The results illustrate that the polymer coating indeed exhibits inherent MALDI matrix activity. The arrays shows good matrix activity with the formation of intensive protonated molecular ions ([M+H]+). No background peaks are observed in the spectra.

Thus, new effective analytical system based on ability of PANI coatings to separate the proteins and absorb UV energy was developed.

6. New approach of the support surface activation based on ozone-induced polymerization

The described above polymer modifiers (i.e. fluorinated polymers and polyanilines) are effective for one-step separation of nucleic acids. At the same time they are quite different not only by the chemical structure, but also by the mechanisms of their formation, although

in both cases chemical deposition results in stable uniform nano-thick polymer layers on the matrix surface. Here we describe an approach to manufacture such composites using similar chemical steps.

For that purpose, an 'universal' activated solid support providing formation of such layers is required. Ozone is known to chemically activate different solid materials due to formation of various peroxide groups and/or ozonides on the surface (Robin, 2004). This is true for most organic matrices, however, silica does not react with ozone itself, but usually it contains different admixtures, in particular, iron oxide (in average, from 0.5 up to 3.5% depending on silica preparation technology). Such admixtures after ozone treatment, in principal, can form different peroxide ion- or radical-containing chemical structures, which can initiate polymerization of different monomers in thin layers. Last phenomenon was not studied in details until now.

Indeed, Prostyakova, Kapustin and Zubov (2011) have demonstrated, that after ozone treatment of silica samples followed by removing of unabsorbed ozone and other gaseous products, iodometric titration confirms the formation of peroxide species. The amount of those species is negligible in "pure" silica materials but increases for the samples with considerable amount of admixtures (presumably iron oxides). Thus, the ozone treated silica can be tested as heterogeneous initiator allowing modification of its surface by direct polymerization of various monomers via different polymerization mechanisms, such as radical polymerization of TFE (utilization of the forming surface radicals) and oxidative polymerization of aniline (utilization of surface peroxides). Indeed, incubation of the ozonated silica in contact with TFE at room temperature results in polymerization of this gaseous monomer at the surface of the carrier. In order to improve wettability and separation properties of the obtained materials, silica was additionally modified with copolymers of TFE with hexafluoroethylene (HFP) (to stabilize the radicals on the ends of propagated PTFE chains (Zubov et al., 2007), and with allylamine (AA) or allyl alhogol (AAl) (to introduce the additional functional groups). The comonomers were introduced either simultaneously as a mixture of monomers in the vapor phase or in a post-polymerization mode by introduction of the second monomer after the polymerization of TFE on the first stage was completed. In the last cases considerable concentrations of amino or hydroxylic gropus were found in the composites. TFE, AA and AAl can be polymerized only via radical mechanism. Therefore, ozonated silica can be considered as effective heterogeneous radical initiator. In fact, it also causes polymerization the other radically polymerizable monomers such as styrene, acrylamide, etc. The result of this polymerization is a porous silica-polymer composite with the variable polymer content up to ~ 25%. It should be noted, that morphological characteristics of the prepared adsorbents were similar as compared to the PTFE-coated CPG after irradiation induced post-copolymerization of TFE (Kapustin et al., 1998).

It could be presumed that the heteroperoxides immobilized at the silica surface can induce not only radical polymerization process, but also initiate oxidative precipitate aniline polymerization, thus giving rise to an adsorbent modified with surface-bound polyaniline. Indeed, when ozonized silica was incubated with acidic aqueous solution of aniline not containing an oxidizer, PANI was formed only at the surface of the matrix. It can be presumed that peroxide centres serve as heterogeneous oxidizers and polymerization sites, and the following growth of PANI macromolecules is continued in adsorption layers of the carrier surface. Uniformity of PANI deposition at the surface of the porous substrate was confirmed by mercury porosimetry and the chemical (alkali) stability test. The measured thickness of the PANI coating was about 8.5 nm.

The separation properties of the materials prepared by means of developed ozone-initiation technique were demonstrated on the examples of nucleic acids isolation from different sources. Modern methodology of separation of biological mixtures is based on use of compact spin-cartridges, as contrasted with traditional column chromatography methods.

Developed fluoropolymer-coated adsorbents were tested as effective materials for one-step isolation and purification of dsDNA and RNA from the bacterial lysates of *Agrobacterium tumefaciens C58* and model mixtures containing fragmented dsDNA or ssDNA (synthetic oligonucleotide). The materials coated with copolymers of TFE with other comonomers were also tested for separation of NA depending on their secondary structure, where bacterial RNA or single strained short oligonucleotides were retained to a different extent depending on the chemical structure of the immobilized polymer and the charge of the adsorbent surface. The retention of ssDNA and RNA increases in a row PTFE-HFP – PTFE-AAl – PTFE-AA. At the same time dsDNA yield increases in comparison with the silica modified with PTFE-AA, when adsorbent having surface hydroxylic groups is used.

To estimate the extent of DNA purification provided with developed fluoropolymer coated adsorbents the eluates collected after the isolation of DNA from bacterial lysates of *A. tumifaciens C58* culture were tested by real-time PCR analysis. The DNA yield after using of the adsorbent coated with PTFE-HFP (by irradiation-induced technique) was assumed as 100%. To compare efficiency of PCR fragment amplification the cross point (Cp) determination method was used (i.e. the determination of Cp as the cross-point of the maximum of the second derivative of amplification curve with the base line. The obtained results show that the yield of purified DNA in the collected eluates are in average by 2-fold higher after use of PTFE-HFP-coated adsorbents in comparison with the materials modified with copolymers of TFE with AA or AAl. However, all those types of PTFE-containing adsorbents provide the isolation of purified DNA, which is suitable for direct PCR diagnostics.

For medical point of view, the most important adventure provided by the developed technique is the clinical diagnostics of the human pathogens using PANI-coated adsorbents obtained via ozone-initiation technique. Such a possibility was demonstrated, in particular, on the examples of the human DNA (iC gene) and viral DNA (T4 phage) isolation (and PCR-detection) from whole human blood and the model mixtures containing blood and viral T4 phage particles (viral particles were added to the blood sample before DNA isolation). For this purpose the different protocols of the sample lysis were used. In particular, the most effective protocol included thermal lysis of the blood samples. The protocols based on the use of developed PANI-coated adsorbent (comprising only 8 manipulations) were compared to the standard sample preparation procedures (comprising 20 manipulations) using the commercial kits Probe-GS DNA Isolating Kit (DNA Technology Inc., Russia) and the mini-columns from NextTec GmbH (Germany). The purified DNA were used in PCR. The concentrations of amplified PCR fragments were compared to the concentration of PCR fragment, which was obtained after isolation of DNA using the Probe-GS DNA Isolating Kit. The determined average values of Cp and relative quantity of DNA are presented in the table 3a (for amplification of human iC gene) and in the table 3b (for amplification of viral DNA).

Mode of the sample preparation	An average Cp		StD, %		Concentration, %		StD, %	
	a	b	a	b	a	b	a	b
Initial lysate	-	-	-	-	-	-	-	-
"Sample GS" kit (DNA-Technology, Russia)	27.0	22.4	0.2	0.3	1.0	1.0	3.7	4.9
NextTec spin-column (NextTec GmbH, Germany)	26.3	18.8	0.6	0.4	1.5	11.6	11.3	5.4
PANI-coated adsorbent (based on ozone treated silica)								
Thermal lysis	26.0	18.4	0.0	0.4	2.0	15.3	0.03	4.8
One-step enzyme lysis	26.0	19.0	0.3	0.1	1.9	9.8	5.1	1.0
Two-step enzyme lysis	26.4	19.0	0.3	0.1	1.4	9.9	5.6	1.4

Table 3. Viral *(a)* and human *(b)* DNA amplification in PCR.

Indeed, the use of prepared PANI-coated adsorbent results in noticeable decrease of an average Cp value in PCR detection of both the human DNA and the viral DNA in the blood samples, in particular, providing by 8-fold higher yield of the human iC gene and by 10-fold higher yield of viral DNA.
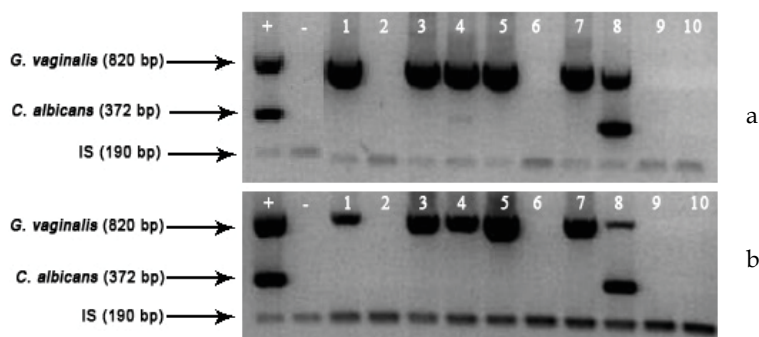
Fig. 8. PCR detection of *Gardnerella vaginalis* and *Candida albicans* in the randomly selected urinogenitalic smears from 10 patients. The results of the electrophoresis in 2% agarose gel. Isolation of DNA was carried out using the standard procedure developed by Diatom™DNA Prep 100 Kit (IsoGen Inc., Russia) (a) and the cartridges packed with the developed PANI-coated adsorbent (b). The arrows show the location of PCR fragments relating to *G. vaginalis* (820 bp), *C. albicans* (372 bp) and internal standard (190 bp).

Another example of diagnostic use of the developed composites is given by the comparison of effectiveness of the sample preparation for practical clinical PCR diagnostics when using the developed PANI-coated adsorbents (protocol I) and the standard Diatom™DNA Prep 100 Kit (IsoGen Inc., Russia) (protocol II). Protocol II includes effective enzymatic lysis of the sample, but it is relatively more labour- and time-consuming procedure as compared to the protocol I. Urinogenital smears were taken from 10 randomly selected patients and treated in accordance with the mentioned protocols I and II. Simultaneous detection of two groups of microorganisms including *Gardnerella vaginalis* and *Candida albicans*, and *Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis,* and *Mycoplasma genitalium* was

carried out. The collected eluates were assessed by electrophoresis in 2% agarose gel. Figures 8 and 9 show the obtained results. Although the yield of DNA fragments after use of the protocol I was somewhat lower comparably with use of the protocol II (due to dilution of the sample by some 6 times in the course of the sample preparation), the results of PCR tests were identical. Moreother, the purity of isolated DNA was higher in the first case (it was confirmed by the more effective amplification of the internal standard).
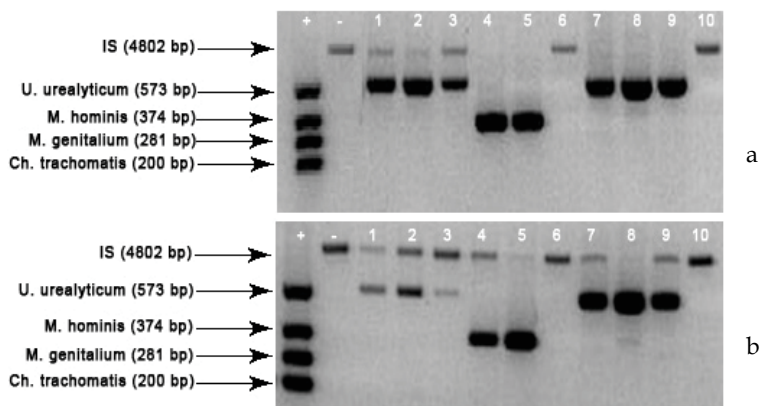
Fig. 9. PCR detection of *Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis,* and *Mycoplasma genitalium* in the randomly selected urinogenitalic smears from 10 patients (using the same samples as in the Figure 8). The results of the electrophoresis in 2% agarose gel. Isolation of DNA was carried out using the standard procedure developed by Diatom™DNA Prep 100 Kit (IsoGen, Inc, Russia) (row a) and the cartridges packed with the developed PANI-coated adsorbent (row b). The arrows show the location of PCR fragments relating to internal standard (4802 bp), *U. urealyticum* (573 bp), *M. hominis* (374 bp), *M. genitalium* (281 bp), and *Ch. trachomatis* (200 bp).

Thus, the developed facile and scalable ozone-induced technique of manufacture of biocompatible composite adsorbents is a perspective alternative as compared to known irradiation post-polymerization and oxidative polymerization techniques. Silica carriers treated with ozone can be used as effective heterogeneous initiators for polymerization of different monomers, which can be polymerized by radical or oxidative mechanisms.

7. Summary

Investigation of the functional properties of the adsorbents based on activated silica pursued several goals. Firstly, it was the comparison of the properties of the materials obtained by irradiation, oxidative and ozone-induced techniques. Secondly, it was a demonstration of the possibility to introduce the additional functional groups into polymer layer under relatively mild conditions. The last advantage enables to control the sorption properties of the adsorbents as well as their hydrophilicity. Thirdly, it was the demonstration of the specific properties provided by immobilized PANI phase, such as retention of PCR inhibitors from the blood containing samples and effective isolation of pathogenic DNA from the clinical samples.

The table 4 gives a comparison of the main properties of the described fluoropolymer- and PANI-coated materials. Analysis of the represented data allows to conclusion that fluoropolymers as well as polyanilines can be considered as multipurpose biocampatible polymer modifiers for producing the sorption materials for bioseparation and bioanalysis.

Purpose	Material (the polymer modifier)				
	Perfluoropolymers (PTFE and etc)	Partially fluorinated polymers	PANI	Aniline-containing polymers (polycomplexes and copolymers)	Combination of fluoropolymer with PANI
Main approaches					
One-step separation of proteins and nucleic acids	+	+	+	+	+
One-step isolation of nucleic acids from the complicated mixtures	+	+	+	+	+
Specific approaches					
Separation of the proteins	+	-	+	+	+
Immobilization of bioligands	-	+	-	+	+
Bioanalytical applications	-	-	+	+	+
One-step separation of ss- and ds- nucleic acids	-	-	-	+	+

Table 4. Fields of application of fluoropolymer- and PANI-coated adsorbents.

The use of fluoropolymers and polyanilines as modifiers of the composite adsorbents provides their effectiveness in such main applications as separation of different classes of biopolymers (like nucleic acids and proteins) by one-step procedure. Moreover, the further complication of the isolation procedure and/or structure of the polymer modifier results in realization of more specific approaches such as separation of the mixtures containing the biopolymers of the same class (e.g. proteins and peptides, ssDNA and dsDNA, or DNA and RNA) as well as their use as supports in bioanalytical application. In last cases the polymer coating immobilized on the carrier surface acts as a whole supramolecular structure which reversibly changes its properties depending on the conditions of environment.

Thus, the review of such "interdisciplinary" branch of the macromolecular chemistry like a development of multifunctional and multipurpose composite biocompatible materials illustrates the tendency to move from "chemical design" to "adaptive chemistry" , like it has

been analogously formulated by Nobel laureate Jean-Marie Lehn in his works devoted to self-organizing structures in biomaterial science.

8. Acknowledgements

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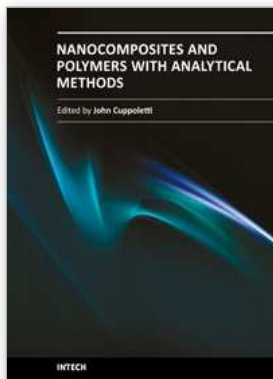
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