Oxidative polymerization of aniline on the surface of insoluble solid poly(sulfo acids) as a method for the preparation of efficient biosorbents

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Polyaniline coatings of the cation-exchange resin Dowex and a synthetic composite material based on silica gel surface-modified by a sulfated styrene—divinylbenzene copolymer were prepared by precipitative polymerization of aniline on the surface of the supports. The optimal conditions for the preparation of the polyaniline-containing material providing the formation of a thin polymeric coating on the support surface were determined. Aniline is predominantly consumed to the formation of a uniform polymeric coating about 3 nm thick with an increase in the concentration of sulfo groups on the support surface. The efficiency of using the polyaniline-containing sorbents for the preparative isolation of DNA from plant tissue lysates was demonstrated.

Key words: polyaniline, solid poly(sulfo acids), composite sorbents, isolation of DNA from plants.

Unique physicochemical properties of polyaniline (PANI) underlie wide use of polyaniline-containing materials in various fields of science and technology.[1] It is known that aniline polymerization affords thin insoluble coatings on inorganic[2] and organic[3] surfaces. Polyaniline coatings possess high chemical stability and are insoluble in the majority of the known solvents. Specific features of the PANI macromolecule structure enable the efficient application of polyaniline-containing sorbents in bioseparation, in particular, for the separation of complex mixtures of biopolymers.[4] In addition, the PANI macromolecule structure changes reversibly with the variation of the pH of the medium, which allows one to purposefully vary the sorption properties of the polyaniline coatings.[5] This is a reason for considerable interest in studying the conditions of aniline polymerization providing the preparation of thin insoluble polyaniline coatings on various surfaces.

Polyaniline is obtained by the chemical or electrochemical oxidation of aniline. The mechanism of electrochemical oxidation, when a strong uniform polyaniline film is formed on the electrode surface, is studied in most detail.[6] Chemical polymerization of aniline in the presence of a solid surface also results in thin polymeric coatings; however, the mechanism of their formation in a heterophase system remains unclear. It can be assumed that these films are formed due to the deposition and subsequent immobilization on the support surface of polymeric nanoparticles formed upon aniline polymerization, *i.e.*, due to the so-called "adsorption polymerization."[7,8] However, it was shown (*e.g.*, see Ref. 8) that the contribution to PANI film formation on the surface of various substrates is made by both adsorption and the so-called "boundary polymerization" where the main part of high-molecular-weight PANI is formed on the surface of the substrate introduced into the reaction mixture within the time before the polymerization in the bulk of the reaction mixture has started. The differences in the kinetics of adsorption (or precipitative) polymerization and boundary (*i.e.*, surface) polymerization are most pronounced at low monomer concentrations and less substantial with an increase in the aniline concentration in the reaction medium. An experimentally confirmed model for PANI film formation on the glass surface has earlier been proposed,[9] according to which aniline radical cations are adsorbed on the surface of the substrate immersed into the reaction medium and then initiate the PANI chain growth predominantly in the direction perpendicular to the substrate surface. Therefore, for aniline polymerization in the presence of a substrate introduced to the reaction mixture, especially where polymeric coatings of nanometer thickness are obtained, it seems preferential to speak about surface polymerization rather than precipitative polymerization. Anyway, the presence of a substrate in the reac-

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 6, pp. 1123—1130, June, 2007.

1066-5285/07/5606-1166

tion system exerts a noticeable effect on aniline polymerization. A possibility of PANI deposition on porous matrices by chemical surface precipitation has been shown previously.[2] However, the preparation of polyaniline-containing composite sorbents is accompanied by the formation, in the reaction mixture bulk, of PANI particles weakly fixed on the support surface, which impedes considerably the subsequent purification of the sorbents and efficiency of their use for the isolation of biopolymers, in particular, nucleic acids and proteins.

The template synthesis of PANI in the presence of various soluble polymeric and oligomeric acids has been discussed.[10] In this case, polyacid is involved in aniline protonation resulting in the localization of the monomer and its further polymerization along the chain of the polyacid macromolecule. An advantage of the template synthesis is that the system remains homogeneous in all steps of the conversion in the presence of polyacids,[11] unlike aniline polymerization in the presence of low-molecular-weight acid, *i.e.*, no suspension of insoluble PANI particles is formed in the reaction mixture bulk under these conditions.

The present work is based on an analogous principle of a polymeric phase formation where polyacids are involved in aniline protonation. Since our ultimate goal is the preparation of efficient polyaniline-containing biosorbents, we studied some regularities of aniline polymerization in the presence of insoluble solid poly(sulfo acids) and found the conditions required. Porous supports were used as poly(sulfo acids), in particular, the cation-exchange resin Dowex with the known number of sulfo groups on the surface and synthetic composite materials based on silica gel surface-modified by a sulfated styrene—divinylbenzene copolymer. In the latter case, supports with different degrees of sulfation were used.

Results and Discussion

The kinetics of oxidative aniline polymerization in the absence of a support was studied gravimetrically from an increase in the polymer mass and consumption of the monomer (aniline) by recording the UV spectra of the reaction mixture liberated from the polymer particles in different steps of polymerization. Polymerization was stopped by the addition of aqueous ammonia. Aniline absorbs at 240 and 280 nm. In the spectrum of an aniline—oxidant—ammonia mixture, the peaks are superimposed in the region of 190—240 nm, hence, the peak at 280 nm was used to plot the time dependence of the monomer concentration. Dimers and soluble oligomers formed during polymerization are known[12] to absorb in the visible range (~400 nm). Therefore, the decrease in absorption at 280 nm corresponds to the monomer concentration decrease during polymerization. The

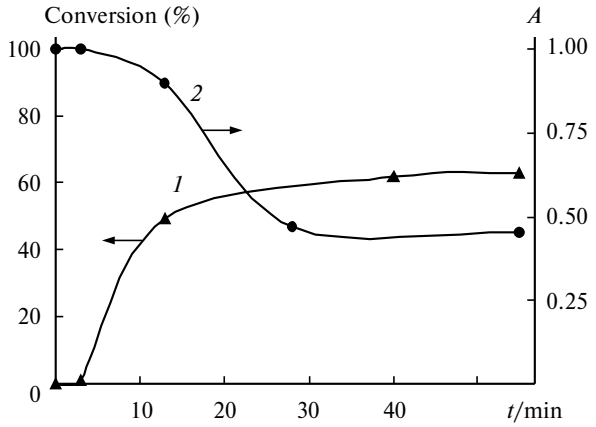


Fig. 1. Increase in the polymer weight (*1*) and the monomer consumption (*2*) during aniline polymerization at the molar ratio monomer : acid : oxidant equal to 1 : 3 : 1. The initial aniline concentration is 0.02 mol L^{-1}.

typical kinetic curves of the polymer accumulation and the monomer consumption at the monomer : dopant (hydrochloric acid) : oxidant ratio equal to 1 : 3 : 1 are shown in Fig. 1. The plots obtained are virtually identical (see Fig. 1), therefore, in this work we plotted the kinetic curves of aniline polymerization from the monomer consumption determined spectrophotometrically.

As is known,[13] aniline polymerization is a complicated multistep heterophase process. No single opinion about the detailed mechanism of formation of PANI and reaction by-products exists to date. At the same time, the majority of researchers distinguish three main steps of aniline polymerization: an induction period, solid polymer formation, and cessation of formation of the solid polymeric phase.[13,14] As has been shown previously,[15] after the end of the induction period, PANI that formed is oxidized by the oxidant (*e.g.*, ammonium persulfate) to pernigraniline, which in turn oxidizes aniline. In the last step, the unstable intermediate isomers transform into the stable PANI form, namely, emeraldine salt. These steps, in particular, determine the s-shaped curves of the polymer weight accumulation and monomer consumption (see Fig. 1). This shape of the kinetic curves confirms the autocatalytic character of PANI macromolecule formation, which has earlier been discussed.[16] Indeed, when a PANI sample was added to the system, the shape of the kinetic polymerization curve changed immediately after mixing of the monomer solution with a solution of the oxidant. In this case, the duration of the induction period shortened and a second step appeared in the curve of the monomer consumption. The typical curves of the monomer consumption during aniline polymerization in the absence of the support and upon the addition of a PANI sample are presented in Fig. 2 (curves *1* and *2*, respectively). The length of the induction period decreases linearly with the weight of the added PANI (Fig. 3). The

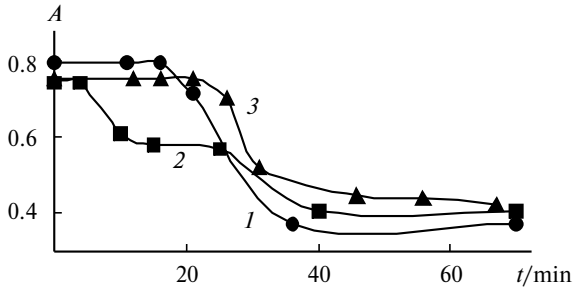


Fig. 2. Monomer consumption during aniline polymerization in the absence of the support (*1*), upon the addition of polyaniline (0.01 g) (*2*), and in the presence of MPG (1 g) (*3*) at the monomer : acid : oxidant ratio 1 : 3 : 1. The initial aniline concentration is 0.02 mol L^{-1}.

autocatalytic effect of the polymer on the polymerization process is determined, most likely, by the fact that the electronic processes affording the aniline radical cations occur on the surface of polyaniline particles. Therefore, the polymerization rate in the surface layer increases,[16] which corresponds to the first step in the curve of the monomer consumption (see Fig. 2, curve *2*). The subsequent decrease in the polymerization rate is related to the cessation of the efficient catalytic effect of added PANI particles as they become covered with the newly formed polymeric film. Further polymerization occurs predominantly on particles of "fresh" PANI formed in the reaction system bulk, which serves, most likely, as a reason for the appearance of the second step in the kinetic curve (see Fig. 2, curve *2*). Thus, it is important to obtain the first polymeric layer in the modification of the support surface with polyaniline so that the formation of the next layers will be catalyzed by the preceding layers. Evidently, the nature of the surface of the support used can exert a noticeable effect on the character of the polymerization.

The presence of neutral macroporous glass (MPG) as a support in the preparation of a polyaniline-containing sorbent does not substantially change the shape of the kinetic curve of aniline polymerization (see Fig. 2, curve *3*), which is observed without a support. The silica gel surface exerts no effect on aniline polymerization, because the silanol groups of MPG in an acidic medium are not sufficiently strong sorption sites for the components of the reaction mixture. Due to this, PANI is formed both on the silica gel surface as a film and in the reaction mixture bulk (which considerably complicates the subsequent purification of the sorbents obtained). In the case of aniline polymerization in the presence of MPG, up to 40% aniline (according to mercury porosimetry and elemental analysis data) consumed to the formation of a suspension of PANI particles in the reaction mixture bulk.

On the contrary, in the presence of the cation-exchange resin (Dowex), the induction period in the kinetic polymerization curve (Fig. 4, curve *1*) virtually disappears as compared with the polymerization in the absence of a support or in the presence of the inert MPG (see Fig. 2, curves *1* and *3*). Polyaniline coatings can also be obtained in the absence of doping agents (low-molecular-weight acid, *e.g.*, HCl). In this case, aniline molecules are localized, most likely, on the surface containing the acidic groups where they are protonated and oxidized. Therefore, the first PANI layer is formed directly on the support surface. Presumably, the strength of binding of the initially formed polymeric layer is determined by the formation of a polyelectrolyte complex of the protonated positively charged PANI molecules with the negatively charged groups on the support surface. Then the pH of the reaction mixture decreases due to the release of protons as a result of aniline oxidation. For instance, when Dowex was used, the pH value during the process decreased by three units. Hence, the considerable portion of PANI was formed at pH 2, which corresponds to the typical pH of the reaction mixture during polymerization in the presence of HCl (at the aniline : acid : oxidant molar ratio equal to 1 : 3 : 1). However, due to the catalytic effect exerted by the acidic surface, the new PANI particles are not formed in the reaction mixture bulk, unlike aniline polymerization in the presence of MPG, *i.e.*, the polymeric "seed" appeared on the support surface controls further course of polymerization. Thus, aniline polymerization in the presence of Dowex resin as a support makes

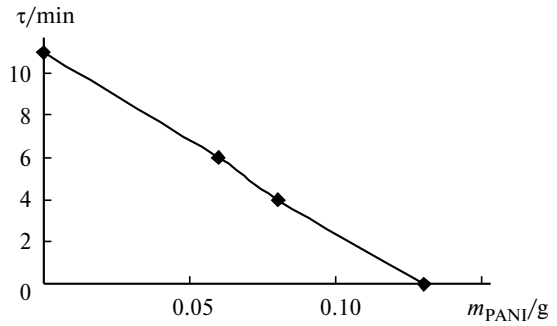


Fig. 3. Plot of the length of the induction period of aniline polymerization (τ) *vs.* weight of added polyaniline (m_{PANI}) in the absence of the support.

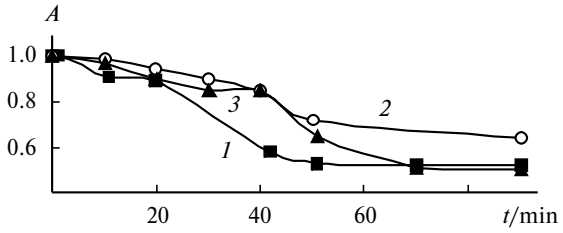
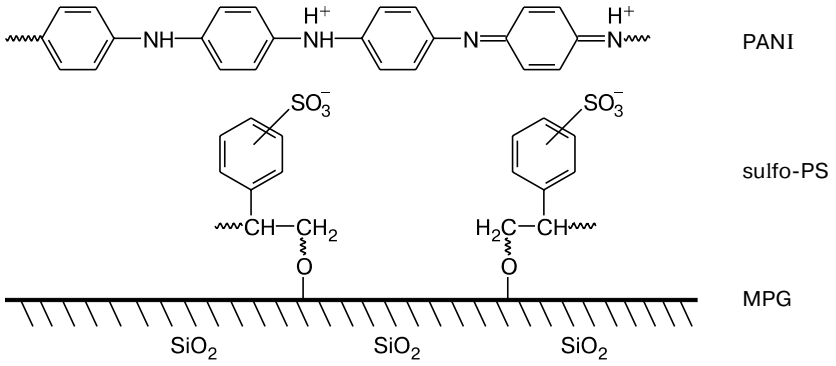


Fig. 4. Aniline polymerization in the presence of the Dowex resin (1 g) (*1*) and MPG—sulfo-PS sorbent (1 g) with the surface concentration of the sulfo groups 0.072 (*2*) and 0.109 mmol g^{-1} (*3*). The monomer : acid : oxidant ratio is 1 : 0.9 : 1.

Scheme 1



it possible to control the course of the PANI formation and study some regularities of this process.

As is known, Dowex contains definite amount of sulfo groups, which restricts possibilities of estimation of the influence of the concentration of the surface sulfo groups on the aniline polymerization. In addition, the specific surface of these materials is rather small for efficient bioseparation using cartridges. Therefore, silica gel modified by polymers with different contents of the sulfo groups on the surface was chosen as a support and a more appropriate model for studying the effect of the sulfo group concentration on the formation of the polyaniline coating (Scheme 1).

To obtain these supports, the original silica gel was modified by poly(tetrafluoroethylene) with divinylbenzene-crosslinked polystyrene (PS) grafted onto the surface by radiation-induced post-copolymerization. Then the obtained polystyrene-containing silica gel was sulfated (Fig. 5). The maximum concentration of the sulfo groups is achieved upon 90-min sulfation (0.109 mmol (g of support)$^{-1}$). A decrease in the concentration of the surface sulfo groups can be explained, most likely, by the partial destruction and dissolution of the grafted polymeric layer due to the prolonged action of the acid.

Aniline polymerization in the presence of the sulfated supports prepared, as well as in the presence of the cation-exchange resin, occurs also in the absence of the low-molecular-weight dopant (HCl). For all the samples studied, the induction period of polymerization was ~1 min, and the stable polyaniline coating was formed. However, in the presence of supports with a relatively low content of the sulfo groups, the color of the reaction system remained brown during the whole process and, hence, PANI was formed not in the form of emeraldine salt but as leucoemeraldine salt and branched defect macromolecules.[12] Therefore, to carry out the reaction under comparable conditions, we polymerized aniline on the surface of supports with different degrees of sulfation in the presence of hydrochloric acid taken in deficiency (ratio monomer : dopant : oxidant = 1 : 0.9 : 1). It should be mentioned that in the aniline polymerization under similar conditions (*i.e.*, in the deficiency of acid) both in the absence of a support and in the presence of the nonmodified MPG, the reaction system is brown-colored, which indicates, most likely, the formation of branched PANI forms. On the contrary, in the aniline polymerization in the presence of the sulfo(polystyrene)—silica gel, all samples studied are characterized by the blue color of the reaction system suggesting the formation of the emeraldine form of PANI. In this case, as for polymerization in the absence of hydrochloric acid, no formation of PANI particles in the reaction system bulk was observed. The maximum yield of the polymer was achieved when aniline was polymerized in the presence of the support with the maximum concentration (0.109 mmol g^{-1}) of the sulfo groups on the surface (see Fig. 4, curve *3*).

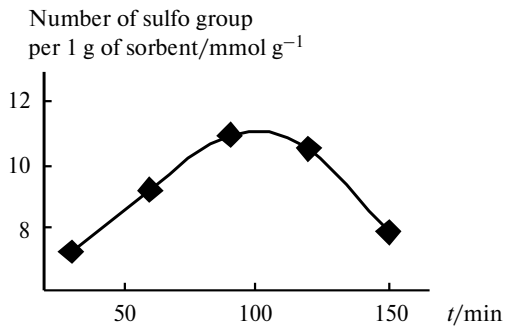


Fig. 5. Plot of the number of the sulfo groups on the MPG—PS surface *vs.* sulfation duration.

The morphology of the prepared composites was studied by mercury porosimetry. A comparison of samples of the polyaniline-containing sorbents based on the sulfo(polystyrene)—silica gel (MPG—sulfo-PS—PANI) with the porograms of the original macroporous glass (MPG) and MPG—PS showed that the porosity of the original support is retained and the thickness of the polyaniline coating is ~3 nm. The typical porograms of the MPG—PS and MPG—sulfo-PS—PANI samples are shown in Fig. 6. The simultaneous comparable decrease

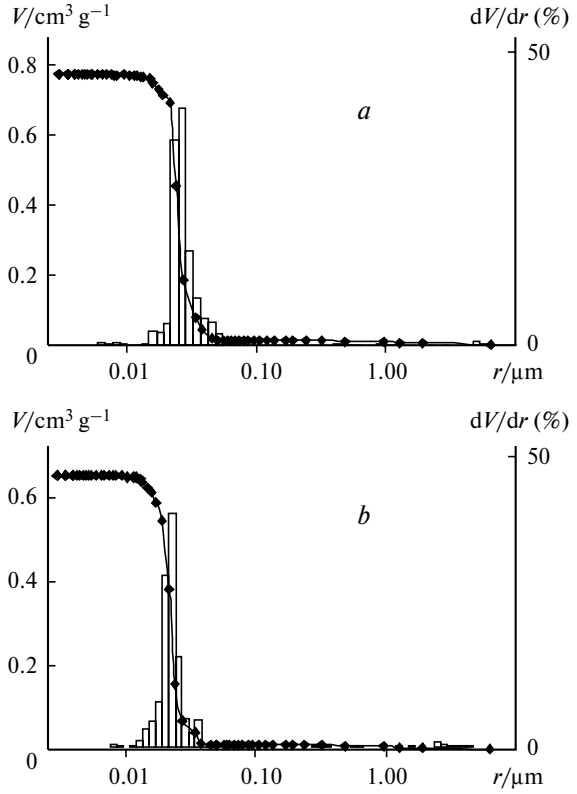


Fig. 6. Mercury porograms of the original MPG—PS (*a*) and MPG—sulfo-PS—PANI (*b*) (*r* is the pore radius, *V* is the pore volume).

in the specific pore volume and average effective pore diameter indicates that the thin PANI layer is formed in the oxidative polymerization of aniline in the presence of particles of the sulfo(polystyrene)containing volume-porous silica gel not only on the external surface of the support particles, but also on the internal pore surface, the porosity of the initial support being retained.

The effect of shielding of the inorganic surface by the polymeric phase was determined from the stability of the modified silica gel to the hydrolysis of the Si—O—Si silicon—oxygen bonds compared to the nonmodified silica gel in alkaline media. For this purpose, the change in the concentration of silicate ions formed by support dissolution in an alkaline medium was studied. The kinetic curves of dissolution obtained for the original and modified silica gels are shown in Fig. 7. As compared to the nonmodified silica gel, the polyaniline-containing material based on the sulfo(polystyrene)—silica gel possessed enhanced stability under conditions of alkaline hydrolysis, which is especially noticeable in the first 2 h of the process. This observation suggests that the silica gel surface characterized by considerable nonspecific sorption of biomolecules is sufficiently protected by the polymeric layer.

Since the morphology of the original support is retained after modification and the polymeric coating efficiently shields the silica gel surface, the obtained composite was proposed to be used for DNA isolation from plant lysates, because it has been shown previously[4] that the polyaniline-modified sorbent based on silica gel is selective for the separation of nucleic acids and proteins, as well as DNA and RNA. Plant lysates are complex mixtures containing DNA, RNA decomposition products, proteins, peptides, polysaccharides, surfactants, chlorophylls, and low-molecular-weight compounds.[17]

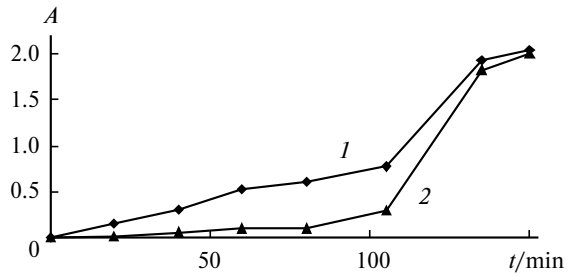


Fig. 7. Kinetic curves of the dissolution of the MPG (*1*) and MPG—sulfo-PS—PANI (*2*) sorbents in the borate buffer, pH 9.

Lysates of tobacco leaves (*Nicotiana tabacum* L.) were applied onto cartridges with the polyaniline-containing sorbents prepared according to the known procedure[4] from both sulfo(polystyrene)—silica gel (MPG—sulfo-PS—PANI) and nonmodified silica gel (MPG—PANI) thoroughly freed by washing from PANI particles weakly bound to the polymeric coating. When the lysates are passed through both the MPG—sulfo-PS—PANI and MPG—PANI layers, DNA is eluted in the excluded volume, whereas proteins, chlorophylls, and a considerable part of low-molecular-weight substances are sorbed (Figs 8 and 9). When the sorbent based on the sulfated MPG with a low concentration of the surface sulfo groups is used, a considerable portion of DNA is retained due to nonspecific sorption (see Fig. 8, lane *2*). The maximum yield of DNA was observed for the modified silica gel with a surface concentration of the sulfo groups of 0.109 mmol g^{-1} (see Fig. 8, lane *4*). This yield is comparable with the yield of DNA in the case of MPG—PANI. In this case, DNA is eluted almost totally. RNA sorption cannot unambiguously be judged from the data obtained, because no inhibitors of RNAses were added to the lysis mixture so that RNA molecules decomposed during lysis because of their instability.

The degree of purification of DNA from proteins and chlorophylls was estimated using electrophoresis (see Fig. 9) and spectrophotometry. As compared with the initial lysates, the eluates contained no proteins.

Another substantial advantage of the use of the MPG—sulfo-PS—PANI system as a sorbent is the ability to bind not only proteins, but also chlorophylls present in the lysates of the plant tissue. The lysates completely decolorized upon passing through cartridges with

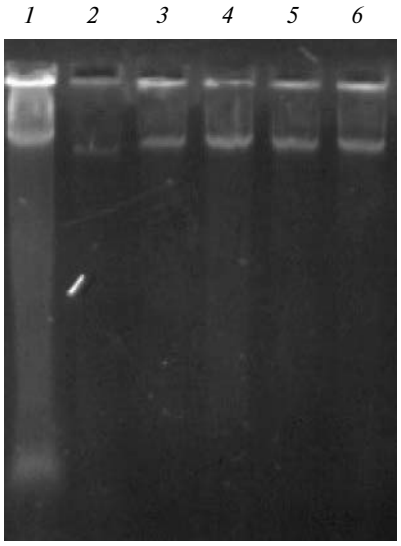


Fig. 8. Electrophoresis of the DNA samples from the *N. tabacum* L. tobacco leaves in a 1% agarose gel: lysate of the *N. tabacum* L. tobacco leaves (*1*); eluate obtained using MPG—sulfo-PS—PANI with the content of the surface sulfo groups 0.072 (*2*), 0.092 (*3*), 0.109 (*4*), and 0.107 mmol g^{-1} (*5*); eluate obtained using MPG—PANI (*6*).

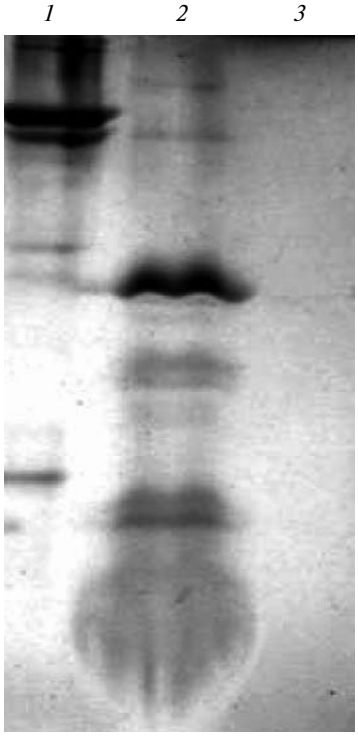


Fig. 9. Electrophoresis of the lysate of the *N. tabacum* L. tobacco leaves (*2*) and the eluate obtained using MPG—sulfo-PS—PANI (0.109 mmol g^{-1}) (*3*) in a polyacrylamide gel. The standard mixture of proteins was used as a marker (220, 67, 60, 36, and 18.5 kD) (*1*).

MPG—sulfo-PS—PANI (the absorbance of the initial lysate at 416 nm was 0.19, whereas the absorbance of the

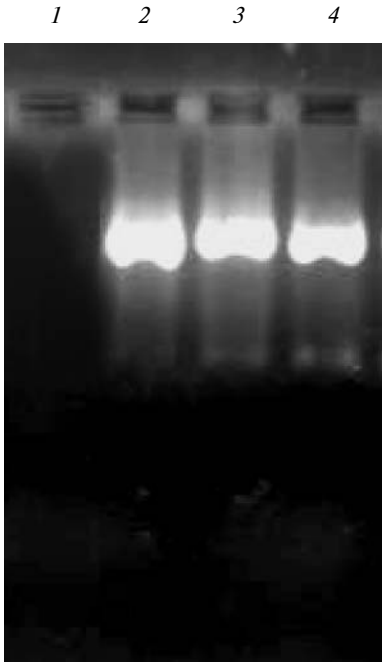


Fig. 10. Electrophoregram of the DNA amplicons (*2—4*) obtained by the PCR with the eluates containing DNA from the *N. tabacum* L. tobacco leaves. The eluates were obtained using the MPG—sulfo-PS—PANI sorbent (*1* is negative control, H_2O).

eluate obtained using MPG—sulfo-PS—PANI was 0.04). At the same time, the eluates obtained in the case of MPG—PANI had a pale light-green color (absorbance 0.10), and those in the case of Dowex—PANI possessed the color almost the same as that of the initial lysate due to the low sorption capacity (0.15). The ability of the sorbent containing the residual sulfo groups to bind chlorophylls is determined, most likely, by the interaction of the porphyrin fragments of chlorophylls with the sulfo groups on the sorbent surface.

This property enhances the efficiency of the use of the sorbent for DNA isolation, because the presence of an admixture of chlorophylls or their derivatives in the DNA samples is very undesirable. Chlorophylls and proteins are powerful inhibitors of the polymerase chain reaction (PCR), which is a popular method of analysis and diagnostics, using purified samples of nucleic acids. Preliminary experiments showed that the degree of purification of DNA isolated from the plant tissue lysates using the cartridges with MPG—sulfo-PS—PANI enables their direct use for amplification by the PCR method. The corresponding amplicons are shown in Fig. 10 (lanes *2—4*).

Experimental

Commercial reagent grade tetrafluoroethylene, styrene, divinylbenzene (purity ≥99.6%, Aldrich), macroporous glass (Erevan Research Institute KhIMTEKh, fraction 100—200 μm),

and Dowex® 50WX2-100 (Na^+-form) (Dow Chemical) were used.

Aniline was distilled, a fraction with b.p. 182—184 °C, n^{20}_D 1.5863 was collected, and the absorbance was determined. Ammonium persulfate (Rotipuran®); sodium dodecyl sulfate, Tris, 2-mercaptoethanol, ethidium bromide, Methyl Red, agarose, ethylenediaminetetraacetic acid (EDTA), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA), sucrose, Triton X-100 (Serva), HCl, H_2SO_4, MeOH, EtOH, 25% aqueous ammonia, NaOH, $CaCl_2$, H_3BO_3, Na_3BO_3, and Na_2MoO_4 (all reagent grade) and standard Milli Q water were used.

Proteinase K encapsulated in poly(ethylene glycol) was purchased from NextTec GmbH. The plants (*N. tabacum* L.) were presented by the Branch of the Institute of Bioorganic Chemistry, Russian Academy of Sciences (Pushchino, Moscow Region).

Polyaniline was synthesized at ~20 °C by mixing a solution of aniline (0.04 mol L^{-1}) in dilute HCl (initial solution) with an equal volume of an aqueous solution of ammonium persulfate (molar ratio aniline : acid : oxidant is 1 : 3 : 1).

Polymerization kinetics in the absence and presence of the support was studied by determining the increase in the PANI weight and recording the UV spectra of the reaction mixture liberated from the polymer particles. The obtained polymer was washed with water, kept for 60 min in 1 *M* aqueous ammonia, and washed with methanol and distilled water until no absorption was observed in the wavelength range of 200—700 nm. Polyaniline was dried *in vacuo* at ~20 °C to a constant weight.

Autocatalytic character of aniline copolymerization was studied by adding different amounts of PANI in the form of emeraldine base (dark blue powder) to the original aniline solution and mixing with an equal volume of an oxidant solution.

Modification of the cation-exchange resin Dowex by polyaniline was carried out by aniline polymerization in the presence of the cation-exchange resin (H^+-form). The resin (1 g) was stirred for 30 min in an aqueous solution of aniline (50 mL), and an aqueous solution of ammonium persulfate (50 mL) was added (molar ratio aniline : oxidant is 1 : 1).

Polystyrene-containing silica gel was prepared by radiation-induced post-copolymerization of styrene with divinylbenzene. A glass tube with the original silica gel (100 g) was attached to a vacuum setup and evacuated with heating to 550 K to a residual pressure of 10^{-3} Torr. Then the system was cooled to ~20 °C, and tetrafluoroethylene (15.8% of the support weight) was added. The system was stored for 6 h at ~20 °C, cooled to the temperature of liquid nitrogen, and γ-irradiated with a dose of 50—100 kGy. Then the tube was attached to the vacuum setup and heated to ~20 °C. After removal of the monomer, which followed from a decrease in the tetrafluoroethylene pressure (a mercury manometer), the tube was evacuated to a pressure of 10^{-3} Torr at ~20 °C, vapor of styrene and divinylbenzene in a ratio of 10 : 1 (total weight of the mixture was 6.17% with respect to the weight of the initial silica gel) was injected, and the system was kept for 5 h at ~20 °C. Excess styrene and divinylbenzene (at most 1%) was removed by heating the tube to 350 K, volatiles being collected in a trap cooled to 77 K. Then the tube was cooled and disassembled from the setup.

Sulfation of the polystyrene-containing support. Samples of the polystyrene-containing silica gel (2 g) were heated with 10 mL of concentrated sulfuric acid at 90 °C. The degree of sulfation was controlled by changing the reaction duration. After the end of sulfation, the suspensions were filtered, and the precipitates were washed with distilled water to the neutral pH of washings and dried *in vacuo* at ~20 °C to a constant weight. The concentration of the surface sulfo groups was determined by reverse titration. A 0.01 *M* NaOH solution (20 mL) was added to the flask containing a weighed amount of the support (0.3 g). The mixture was stirred for 30 min. Aliquots (5.5 mL) were withdrawn and filtered. One drop of a 0.2% solution of Methyl Red in methanol was added to 5 mL of the obtained filtrate. Then titration with a 0.01 *M* solution of HCl was carried out and the content of the surface sulfo groups was calculated.

Modification of the MPG and poly(sulfostyrene)-containing support with polyaniline was carried out using a setup representing a vessel that can be evacuated with a sample (10 g) of the support connected to a compartment with a stopcock containing a solution of aniline in HCl ($4.2 \cdot 10^{-4}$ mole of aniline per 1 g of the sorbent, the molar ratio aniline : acid for MPG was 1 : 3, and for MPG—sulfo-PS, 1 : 0.9). After evacuation, the vacuum line was closed, and a solution of aniline was feeded through the support layer. The system was incubated for 10 min with continuous stirring. Then an aqueous solution of the oxidant (10 mL of an aqueous solution of ammonium persulfate, 0.192 g per 1 g of the sorbent, the molar ratio aniline : acid : oxidant for MPG was 1 : 3 : 1 and for MPG—sulfo-PS, 1 : 0.9 : 1) was added. Polymerization was carried out with stirring at ~20 °C. The reaction was stopped by the addition of 1 *M* aqueous ammonia (50 mL), and the mixture was kept for 60 min. The sorbent was washed with water to the neutral pH, kept for 17 h in 50% aqueous methanol, and washed on the filter with methanol and distilled water to the absence of absorption in a wavelength range of 200—700 nm. The support was dried *in vacuo* at ~20 °C to a constant weight.

According to the elemental analysis data, the relative content of the polyaniline phase in the sorbent sample with the surface concentration of the sulfo groups 0.109 mmol g^{-1} was 5.11 wt.% (found (%): N, 0.79).

UV spectra were recorded on a DU-70 instrument (Beckmann) using quartz cells (1.5 cm^3).

Surface morphology of the prepared sorbents was studied by mercury porosimetry on a Pore Sizer 9300 instrument (Micromeritics). Numerical values of the average diameter and specific pore volume for the construction of porograms were determined using the computational algorithm developed at the Laboratory "Polymers for Biology" at the Institute of Bioorganic Chemistry, Russian Academy of Sciences.

Estimation of the hydrolytic stability of the prepared sorbents. Weighed samples (0.1 g each) of the original silica gel and polyaniline-containing sorbents were stored for 17 h in 70% MeOH. Then equal volumes of the buffer (pH 9.5) containing 0.025 *M* Na_3BO_3 and 0.1 *M* NaOH were added, and the resulting mixture was stirred. The sorbent was precipitated by centrifugation for 1 min at 3000 rpm, the supernatant was discarded, the buffer solution was again added until a 10 wt.% suspension of the sorbents was obtained, and the samples were incubated in this buffer for 150 min at ~20 °C. Aliquots of the liquid over the precipitate were withdrawn, an equal volume of 0.05 *M* Na_2MoO_4 acidified with H_2SO_4 (1/200 of the volume of a solution of Na_2MoO_4) was added, and the absorption of silicomolybdenum acid that formed was measured at 320 nm.

Preparation of the plant tissue extract. Leaves of *Nicotiana tabacum* L. (10 g) were washed with 70% EtOH and distilled water. The plant tissue (10 g) was placed in a precooled porce-

lain mortar, liquid nitrogen was poured, and the tissue was crushed with the pestle. The resulting homogenate was placed in a test tube, and hot (95 °C) buffer (10 mL) was added for extraction (50 m*M* Tris—HCl, pH 8; 0.7 *M* NaCl; 10 m*M* EDTA; 20 m*M* 2-mercaptoethanol). The mixture was stirred and incubated for 20 min at 56 °C.

Lysis of plant cells was carried out by the addition of a buffer (100 μL) containing 1.6 *M* sucrose, Triton X-100 (5%), 25 m*M* Tris—HCl (pH 8), and 25 m*M* $CaCl_2$ to the plant extract (200 μL). The mixture was incubated for 15 min at 60 °C, a 2% solution of proteinase K (20 μL) was added, and the resulting mixture was resuspended. A 4% solution of sodium dodecyl sulfate (80 μL) was added, and the mixture was incubated for 20 min at 60 °C.

Isolation of DNA from lysates of the plant tissue was carried out by placing the lysates (150 μL each) into plastic cartridges (14×5 mm) equipped with receivers and containing MPG—sulfo-PS—PANI (140 mg each). The cartridges were incubated for 1 min and then centrifuged for 1 min. The DNA-containing eluates collected in the receivers were subjected to subsequent analysis.

The presence of DNA in the eluates was confirmed by electrophoresis in a 0.8% agarose gel in a Tris—H_3BO_3—EDTA buffer containing ethidium bromide.

Electrophoresis in a polyacrylamide gel was carried out according to Laemmly[18] using a 4—30% gradient gel in the presence of sodium dodecyl sulfate. The gel was stained with Coomassie Brilliant Blue G-250 (0.125%). Standard kits of protein mixtures HMW (Pharmacia) were used to determine the molecular weight.

Polymerase chain reaction (PCR analysis) was carried out according to a standard protocol of NextTec GmbH with a Perkin—Elmer 96-well amplificator in 35 cycles (5 min, 95 °C; 30 s, 95 °C; 15 s, 56 °C; 20 s, 72 °C; 1 min, 72 °C) using species-specific pairs of primers (NextTec GmbH) and solutions of purified DNA (5 μL each). The PCR products were analyzed by electrophoresis in a 2% agarose gel with ethidium bromide according to a standard scheme at 75 mA in the Tris—H_3BO_3—EDTA buffer.

The authors are grateful to the researchers of the NextTec GmbH Company (Germany) for kindly presented reagents and participation in discussion.

This work was financially supported by the European Community (Grant NACBO NMP4-CT-2004-500804) and the Russian Foundation for Basic Research (Project No. 04-03-32465-a).

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Received January 25, 2006;
in revised form March 16, 2007