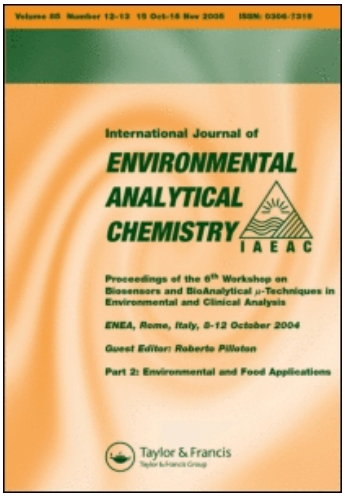

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PREPARATION OF ANTIBODIES AND DEVELOPMENT OF ENZYME-LINKED IMMUNOSORBENT ASSAY FOR NONYLPHENOL

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Polyclonal rabbit antibodies against nonylphenol (NP), the main product of nonionic surfactants destruction, were obtained and applied for immunoenzyme assay (ELISA). Two approaches to immunogen synthesis were compared. The first was direct coupling of a mixture of NP isomers to the carrier protein by Mannich reaction. The second was formation of amide bonds between 7-(*p*-hydroxyphenyl)heptanoic acid (HHA), a linear carboxylated analog of NP, and the carrier protein. Anti-HHA antibodies showed a low affinity to technical NP, whereas with Mannich synthesis it was possible to generate antibodies specific to branched NP molecules. An indirect competitive ELISA was developed based on the anti-NP antibodies. The detection limit of the analysis is 10 ng/mL, with a total duration of around 3 h. The developed ELISA can be applied for group-specific determination of nonionic surfactants and their toxic metabolites. The possibility of NP detection in environmental water matrices using the proposed ELISA without loss of sensitivity is explored.

Keywords: Immunoassay; ELISA; Nonylphenol; Nonionic surfactants; Endocrine disruptors

INTRODUCTION

Alkylphenol ethoxylates (APEOs) are non-ionic surfactants that are widely used in industrial and domestic cleaning sectors, agriculture, plastics, textile and leather manufacture, paint and paper industry as emulsifiers, surfactants, dispersive and wetting agents. About 85–90% of APEOs belong to nonylphenol ethoxylates (NPEOs) and the rest to octylphenol ethoxylates (OPEOs) [1]. Nonylphenol (NP) and octylphenol (OP) are the main stable intermediates of APEOs decomposition in the environment. As the result of intensive non-controlled surfactants application, NP and relative compounds are widely distributed in the environment (water, soil, etc.) and have a toxic effect on animals, including humans [2,3]. Acute toxic effects of these compounds include damage to the respiratory tract, eyes and skin [2]. Lower concentrations of NP and NPEOs (in the submicrogram and nanogram range) do not have immediate

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effects but are noted for deferred sex hormone-like effects. The consequences of NP impact are similar to those of oestradiol, with the only difference that the active concentrations of NP are significantly higher [4,5]. NP-induced abnormalities are known for both male and female organisms [2,6,7]. NP stimulates expression of vitellogenin and oestrogen receptor genes, stimulates the growth of breast-cancer cell lines [8], interacts with oestrogen receptors and decreases the production of androgens [9]. Thus, NP decreases fertility and induces feminisation in nature. Currently, much attention on monitoring APEOs has focused on their potential endocrine-disrupting outcomes in humans [3,10,11].

The reasons given above necessitate the use of effective techniques for detecting NP and related compounds in the environment that can handle large sample loads with a rapid turnover time. A traditional method of APs detection is chromatography, including gas chromatography combined with mass spectrometry [12–14] and liquid chromatography with various detection techniques [15–18]. The shortcoming of chromatographic analysis is the need for expensive sophisticated equipment, which in turn requires highly skilled analysts. The net result is that the analyses are performed in specialized laboratories, and the information is obtained at considerable cost, days after the samples are taken.

Immunoenzyme assays demonstrated their effectiveness for detection of various substances in medicine, biotechnology and ecology [19–24]. A large number of microplate immunoenzyme assays (ELISAs) were proposed to control the content of pesticides, dioxins and other environmental pollutants; the results of these investigations are summarized in [25–29]. Development of ELISA techniques for surfactants began in 1982 [30], but the majority of analytical systems appeared only recently [31–38]. The known ELISAs are based on a limited number of antibodies, and a standard technique for generation of acceptable antibodies is not proposed nowadays [38]. The main problem hampering further development of surfactants immunodetection is how to obtain antibodies with the appropriate specificity. The necessary antibodies should allow different preparations of surfactants and their toxic metabolites to be detected with comparable efficacies and at the same time without cross-reactivities to other classes of pollutants. As technical preparations are a mixture of APs, an optimal choice of hapten for immunogen is an extremely important task. The aim of the presented investigation was to compare two kinds of immunogens and to develop and characterize ELISA for nonionic surfactants and their metabolites based on the chosen immunoreactants.

EXPERIMENTAL

Materials

Nonylphenol (technical mixture of isomers) (Aldrich, Milwaukee, WI), dimethylsulfoxide (DMSO), Freund's complete and incomplete adjuvants, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), 2-hydroxybenzoic acid (salicylic acid), *p*-hydroxyphenylacetic acid, 3(*p*-hydroxyphenyl)propionic acid (all from ICN Biomedicals, Aurora, OH), 4-chlorophenol, 2-amino-4-chlorophenol, 2,4-dinitrophenol, 4-chloro-3-methylphenol, Triton X-305 (all from Merck, Darmstadt, Germany), L-β-phenyl-α-alanine (Reanal, Budapest, Hungary),

1-cyclohexyl-3(2-morpholinoethyl)carbodiimide (CalBiochem, San Diego, CA, USA), *N*-hydroxysuccinimide, gelatin 60 bloom, 2,4-dimethylphenol, 5-aminosalicylic acid (all from Sigma, St. Louis, MO), Triton X-100 (Serva, Heidelberg, Germany) and Tween-80 (Koch-Light Laboratories Ltd, Colnbrook-Bucks, England) were used in the experiments. 7-(*p*-Hydroxyphenyl)heptanoic acid (HHA) was generously provided by Dr A.A. Formanovsky (Institute of Bioorganic Chemistry, Moscow); it was synthesized as described in [34]. Peroxidase-labelled goat anti-rabbit immunoglobulins were from Medgamal (Moscow, Russia). Bovine serum albumin (BSA), ovalbumin (OVA) (both from Sigma), soybean trypsin inhibitor (STI) (ICN Biomedicals) and horseradish peroxidase (HRP) (Biozyme, Pontypool, UK) were the carriers for hapten–protein conjugates. Components of buffer solutions and other chemicals were of analytical grade.

Enzyme-linked immunosorbent assays were carried out in optically transparent polystyrene microtitre plates from Medpolymer (Moscow).

Synthesis of Hapten–Protein Conjugates

Nonylphenol was conjugated with BSA, STI, OVA and HRP by the Mannich reaction (Fig. 1) as described in [39] with some modifications. NP was dissolved in DMSO to a concentration of 33 mg/mL (0.15 M solution) and added in a 116 μL aliquot to 2.0 mL of a BSA solution (10 mg/mL) in 0.1 M Na-carbonate buffer, pH 10, reaching a hapten/carrier molar ratio 50 : 1. In the cases of STI, OVA and HRP, the hapten/protein molar ratio was 30 : 1, 40 : 1 and 40 : 1, respectively. Then, 0.2 mL of 35% formaldehyde solution was added dropwise. The reaction mixture was incubated for 30 min at room temperature with stirring and then kept for 5 days at 37°C with periodic shaking.

7-(*p*-Hydroxyphenyl)heptanoic acid (HHA) was conjugated with BSA, STI and OVA by carbodiimide/succinimide technique as described in [32]. A mixture of 11 mg of HHA, 12 mg of *N*-hydroxysuccinimide and 28 mg of 1-cyclohexyl-3(2-morpholinoethyl)carbodiimide in 1 mL of DMSO was stirred at room temperature for 2 h. The supernatant aliquots were added to protein solution (5 mg/mL) in 0.2 M Na-carbonate buffer, pH 10, under vigorous stirring, while keeping the hapten/protein molar ratio 20 : 1. The reaction mixture was incubated overnight at 4°C.

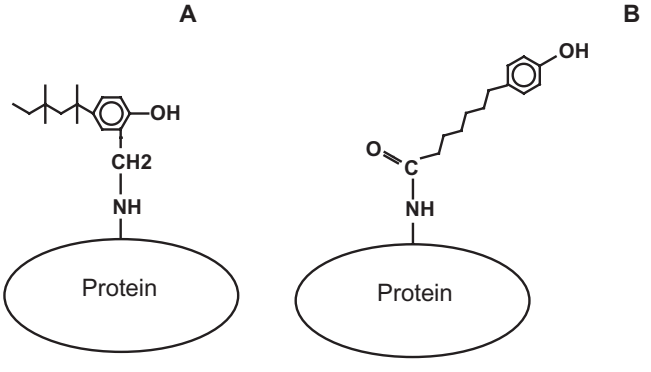


FIGURE 1 Schemes of hapten–protein conjugates obtained by coupling of branched nonylphenol via Mannich reaction (A) and coupling of 7-(*p*-hydroxyphenyl)heptanoic acid via the succinimide/carbodiimide technique (B).

The resulting products were separated from low-molecular-weight components by dialysis against 0.05 M K-phosphate buffer solution, pH 7.4, with 0.1 M NaCl (PBS) during 2 days.

Immunization

Chinchilla rabbits weighting 3–4 kg were immunized by NP-STI, NP-BSA and HHA-BSA conjugates. For the first immunization, the immunogens were dissolved in PBS (pH 7.4) and emulsified with an equal volume of Freund's complete adjuvant to a final concentration of 1.0 mg/mL (by protein). 1.0 mL of this mixture was injected intradermally and subcutaneously to seven sites on the back of the animal's body. Then, rabbits received booster injections by one-half dose of the immunogens subcutaneously (43rd day, in PBS with Freund's incomplete adjuvant, v/v = 1 : 1) and intravenously (46th day, in PBS). After one week (53rd day), the rabbits were bled. The reimmunization cycles were repeated at 5-week intervals (subcutaneous injections—60th and 95th days, intravenous injections—81st and 116th days, bleeding—88th and 123rd days).

Separation of Antisera

Antisera were extracted by maintaining blood samples for 30 min at 37°C and then for 2 h at 4°C. The upper layers were separated by centrifugation for 15 min at 3000 *g*, carefully collected, dispensed into 0.5–1.0 mL aliquots and stored at –20°C.

Antisera Testing by the ELISA Technique

Hapten–protein conjugate in PBS solution (100 μL, 5 μg/mL) was added into wells of microtitre plates and incubated overnight at 4°C. The wells were then washed four times with PBS containing 0.05% Tween-80 (PBST). Then, a number of antiserum dilutions from 1 : 500 to 1 : 1 000 000 in PBST were added into the wells and incubated for 1 h at 37°C. After the washing procedure, the peroxidase-labelled goat anti-rabbit antibodies were added into the wells (dilution 1 : 6000) and incubated for 1 h at 37°C. One more washing cycle (four times with PBST and one with distilled water) was carried out, and the peroxidase activity of the resulting immobilized complexes was measured.

The peroxidase substrate solution contained ABTS (0.7 mM) and H_2O_2 (2.8 mM) in 30 mM Na-acetate buffer, pH 4.5. After the formation of peroxidase-labelled complexes on the surface of microplate wells, 100 μL of the substrate solution was added into each well and incubated for 30 min at room temperature. The optical density of the reaction product was measured at 405 nm with a vertical photometer Multiscan EX (Labsystems).

Nonylphenol ELISA

Hapten–protein conjugate (NP-STI or NP-OVA) was immobilized from 1 μg/mL solution in PBS by overnight incubation at 4°C. Then, the plate was washed four times with PBST. A 30-min period of incubation at 37°C with 150 μL of 0.1% gelatin solution in PBS blocks any potential sites of non-specific sorption. After repeated washings, 50 μl

of rabbit anti-NP-antiserum (1 : 10 000 dilution in 25 mM K-phosphate buffer, pH 7.4, with 0.05 M NaCl and 0.05% gelatin) and 50 μL of NP (a row of concentrations from 0.5 ng/mL to 200 μg/mL, in methanol/water mixture, v/v = 1 : 4) were added into wells and incubated for 1 h at 37°C. (During the studies of the influence of organic compounds, the methanol/water ratio was varied from 1 : 49 to 1 : 4.) The other steps of ELISA were the same as for antisera testing.

In the course of preliminary optimisation of the assay conditions, the concentration of coating antigen was varied from 0.3 to 10 μg/mL, with the antiserum dilution from 1 : 1000 to 1 : 100 000. The duration of the competitive step was varied from 15 to 120 min.

The obtained competitive curves were fitted according to the four-parameter logistic equation:

$$y = (A - D)/[1 + (x/C)^B] + D.$$

The C value (ng/mL) reflected the antigen concentration resulting in 50% inhibition of antibody-conjugate binding (IC_{50}). Cross-reactivity (CR) was calculated according to the formula:

$$CR = 100\% \times IC_{50(NP)}/IC_{50(cross\text{-}reactant)}.$$

If the IC_{50} for some cross-reactants could not be measured, IC_{20} values were used for the CR calculation.

To investigate the effect of sample matrices on the ELISA performance, the NP competitive curves were obtained in artesian, mineral, ground and tap water and compared with those for distilled water. In the course of the competitive step of the assay, the reaction mixture in the wells contained 50 μL of rabbit antiserum, 40 μL of water matrix and 10 μL of NP solution in methanol (thus enabling the same organics content to be attained in the final volume).

RESULTS

Characterization of Antisera Obtained

Two rabbits (N1, N2) were immunized by HHA-BSA conjugate, four (N3–N6) by NP-STI, and two (N7, N8) by NP-BSA. After three reimmunization cycles, 24 antisera preparations were obtained. Indirect ELISA of antibodies was used; immobilized conjugates contained two variants of coupled haptens (NP and HHA) and alternative protein carriers (STI for anti-BSA antisera and BSA for anti-STI antisera). Specifically, HHA-STI and NP-STI were coated to test antisera NN1, 2, 7, 8, and HHA-BSA and NP-BSA conjugates, for antisera NN3–6. The antisera titres are shown in Fig. 2. All antisera obtained against the HHA-BSA conjugate did not react with the NP-STI conjugate. However, the antisera against NP-STI and NP-BSA reacted well with NP conjugated to alternative protein but showed negligible binding or its full absence in the interaction with HHA conjugates.

The sufficient titre values (1 : 35 000–1 : 175 000 for antisera NN3–8) were already reached after the first booster injection and did not change significantly in the course of consequent reimmunizations. Two preparations of anti-NP antisera (rabbits N7

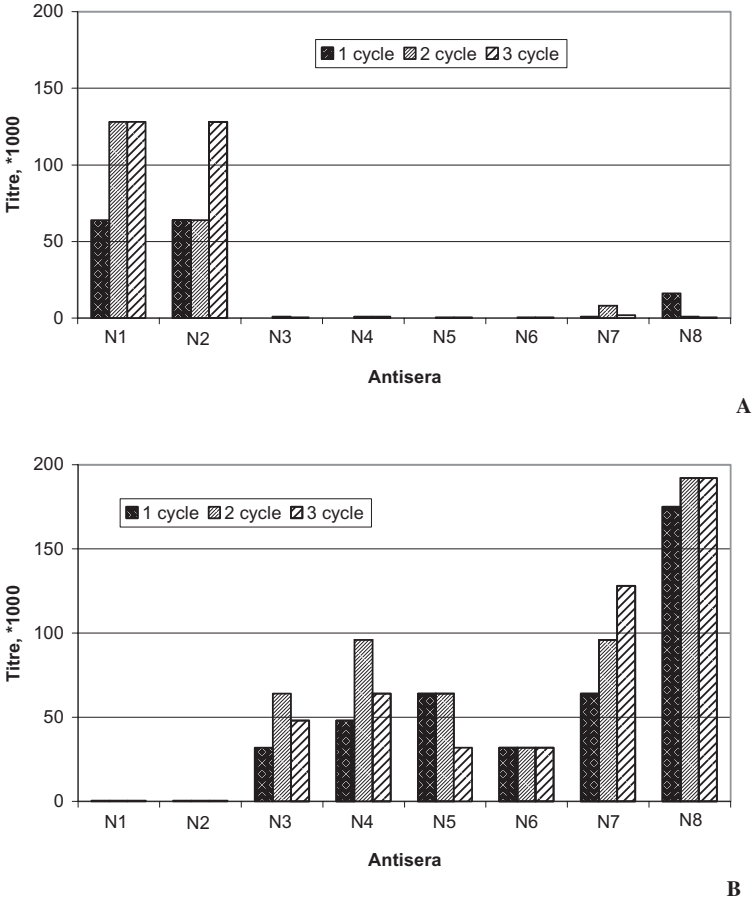


FIGURE 2 Titre dynamics in the course of rabbits' immunization. (A) Coating of HHA conjugates; (B) coating of NP conjugates. STI was the carrier for testing rabbits N1, N2, N7, N8, and BSA for rabbits N3–N6.

and N8) obtained after the third reimmunization by NP-BSA were chosen for further development of the competitive ELISA technique.

Optimisation of ELISA Protocol

The optimisation of the assay protocol included the choice of such parameters as concentrations of reactants, duration of immunochemical interaction and composition of reaction media. The optimisation criterion was the reaching of maximal sensitivity of NP, revealing under storage an acceptable accuracy of quantitative detection (the latter signified that the amplitude of registered optical signals in the absence of competitor was no less than 0.5).

Comparison of the calibration curves obtained for different duration of the competitive step of the assay showed that 45-min incubation was sufficient (Fig. 3). The chosen concentration of coating antigen for immobilization was 1 μg/mL, and the optimal dilution of antiserum was 1 : 10 000.

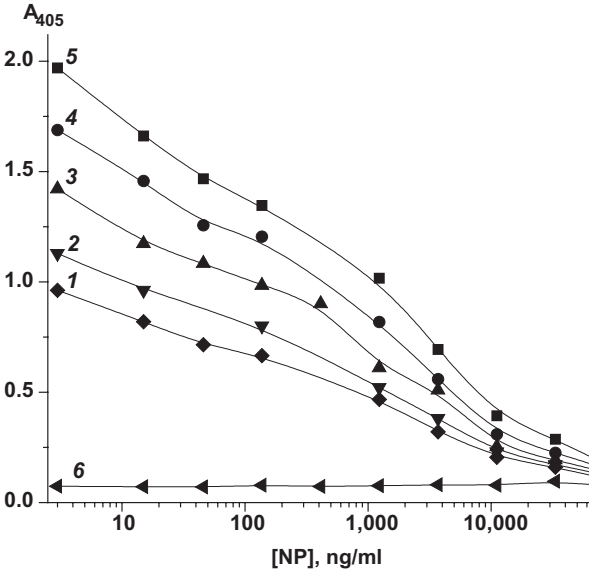


FIGURE 3 Influence of the competitive step duration on ELISA curves for NP. Curves 1–5: 30, 45, 60, 90 and 120 min of incubation; curve 6: serum of the nonimmunised rabbit (120 min of incubation).

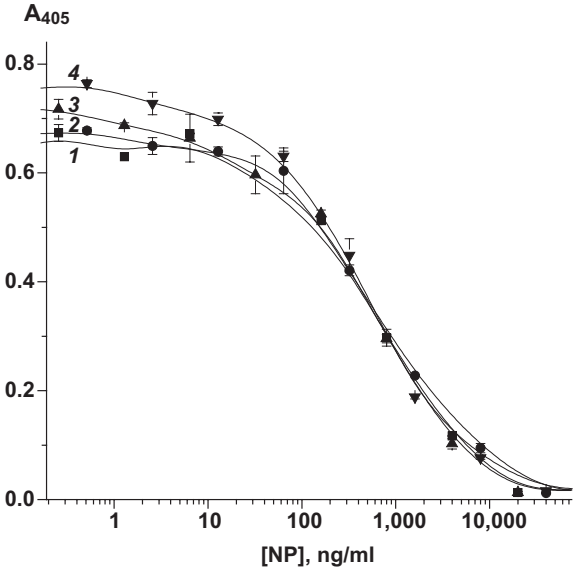


FIGURE 4 Influence of methanol content on the ELISA determination of NP. Competitive curves represent the presence of 1% (1), 2% (2), 5% (3) and 10% (4) of methanol.

The results of NP immunodetection under different levels of methanol in the reaction mixture are presented in Fig. 4. As can be seen, varying the methanol content in the range 1–10% (v/v) does not introduce any significant changes in ELISA sensitivity or in the course of the calibration curves. Moreover, some decrease in IC_{50} is observed: 668, 671, 516 and 456 ng/mL for 1, 2, 5 and 10% (v/v) of methanol in the reaction mixture.

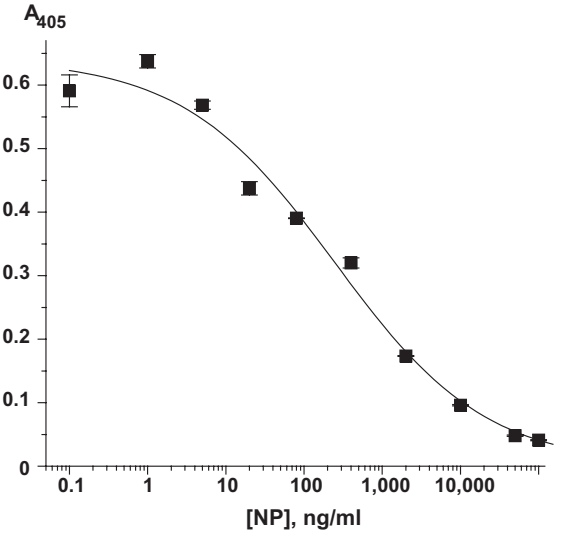


FIGURE 5 Calibration curve for NP ELISA under the optimised assay regime.

Analytical Parameters of the Developed Nonylphenol ELISA

The calibration curve (Fig. 5) for nonylphenol ELISA was obtained under the chosen optimal conditions: concentration of NP-STI in the course of immobilization 1 μg/mL, dilution of antisera 1 : 10 000, gelatin treatment of wells (0.1% before the competitive step of the assay and 0.05% during it) preventing non-specific sorption, absence of surfactants in 25 mM phosphate buffer used at the competitive step. This system has $IC_{50} = 246$ ng/mL, whereas $IC_{10} = 11.2$ ng/mL accords with the minimal NP concentration that can be reliably detected. Exact quantitative determination of NP content may be realized for the concentration range between 20 ng/mL and 5 μg/mL (that accords to the IC_{20}–IC_{80} range). The average relative variation of repeated measurements ($n = 5$) is no more than 3.0% for NP concentrations from 20 ng/mL to 1.25 μg/mL. The total duration of the assay is 3 h.

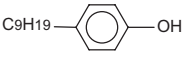
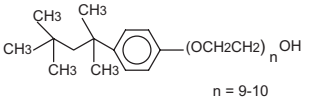
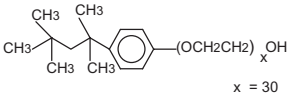
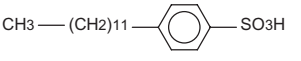
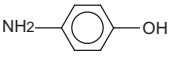
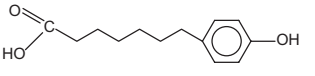
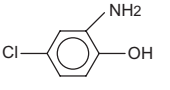
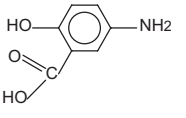
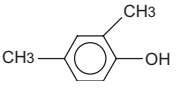
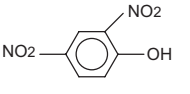
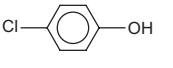
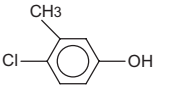
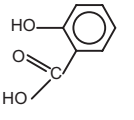
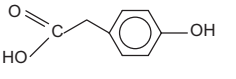
Cross-Reactivity of the Nonylphenol ELISA System

The specificity of the elaborated method was estimated using 16 cross-reactants. The results obtained are presented in Table I. A high degree of the immune recognition was revealed for Triton X-100 (CR = 13.5%) and Triton X-305 (CR = 6.3%). 4-Dodecyl-benzolyl sulfonic acid (CR = 0.95%), aminophenol (CR = 0.25%) and hydroxyphenylheptanoic acid (CR = 0.16%) were weaker as cross-reactants. Phenol, 4-chlorophenol, 4-chloro-3-methylphenol, 2,4-dimethylphenol, 2-amino-4-chlorophenol, 2,4-dinitrophenol, 5-aminosalicylic acid, salicylic acid, 3-*p*-hydroxyphenylpropionic acid, *p*-hydroxyphenylacetic acid and phenylalanine do not interact with anti-NP antibodies.

Nonylphenol Detection in Water Matrices

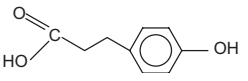
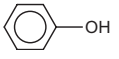
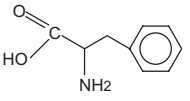
The proposed assay protocol was applied for NP determination in water matrices. Samples of artesian, mineral, ground and tap water were spiked by NP in known

TABLE I Cross-reactivity of the developed NP ELISA

Compound	*Chemical structure*	*CR* (%)
Nonylphenol (technical mixture of isomers)		100
Triton X-100		13.5
Triton X-305		6.3
4-Dodecylbenzolylsulfonic acid		0.95
4-Aminophenol		0.25
7-(*p*-Hydroxyphenyl)heptanoic acid		0.16
2-Amino-4-chlorophenol		< 0.1
5-Aminosalicylic acid		< 0.1
2,4-Dimethylphenol		< 0.1
2,4-Dinitrophenol		< 0.1
4-Chlorophenol		< 0.1
4-Chloro-3-methylphenol		< 0.1
2-Hydroxybenzoic (salicylic) acid		< 0.1
(*p*-Hydroxyphenyl)acetic acid		< 0.1

(*Continued*)

TABLE I Continued

Compound	*Chemical structure*	*CR* (%)
3-(*p*-Hydroxyphenyl)propionic acid		< 0.1
Phenol		< 0.1
L-β-Phenyl-α-alanine		< 0.1

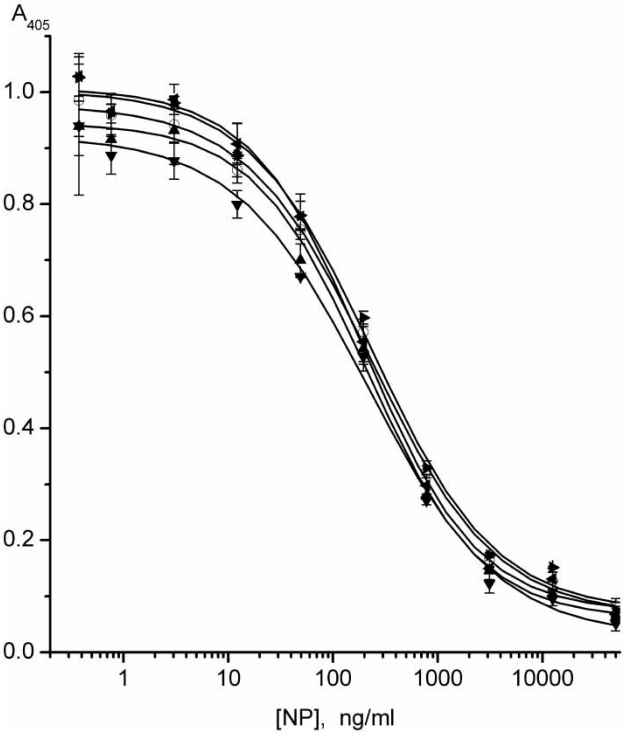


FIGURE 6 Influence of water matrices on the NP ELISA. Competitive curves are shown for NP detection in distilled (○), artesian (▲), mineral (►), ground (◄) and tap (◄) water.

concentrations and tested by the ELISA protocol described above. Besides, the matrices were tested by the ELISA technique to confirm that they did not contain NP or any other immunoreactive molecules. The obtained curves for NP detection in spiked samples are presented in Fig. 6. The IC_{50} values were found to be similar for different matrices, namely 232, 212, 238, 214 and 200 ng/mL for distilled, artesian, mineral, ground and tap water, respectively.

DISCUSSION

The use of HHA, a carboxylic derivative of non-branched nonylphenol, in the immunoassay of non-ionic surfactants has been proposed previously [32,34,37,38]. However, the shortcoming of this approach is the specificity of generated antibodies predominantly to linear compounds, whereas real technical preparations of surfactants and the majority of their destruction products are branched molecules. Our experiments (refer to the data for rabbits N1 and N2 in Fig. 2) confirm this finding, and so it would be appropriate to choose a branched NP molecule as the immunogenic hapten. One limitation of this approach is that NP does not contain any functional groups for covalent binding. As a simple technique to conjugate nonylphenol with protein carriers, we proposed the Mannich reaction with the formation of -CH_2- bridges between phenol ring and the NH- group of a protein (Fig. 1(A)). The conjugation technique described has been used previously for D-3-methoxy-4-hydroxyphenylglycol [39].

We have used NP-BSA and NP-STI conjugates as immunogens obtained by the Mannich reaction, and HHA-BSA conjugate for comparison (Fig. 1(B)). The effectiveness of soybean trypsin inhibitor, a protein with a relatively low molecular weight (21.5 kDa) as a carrier for generating anti-hapten antibodies was discussed in our previous papers [40,41]. However, in our case, BSA was a better carrier for immunogens, which may be due to the difference in synthetic techniques (Mannich reaction instead of carbodiimide/succinimide activation).

The Mannich synthesis was found to be unsuitable to obtain a nonylphenol-peroxidase conjugate for direct immunoenzyme assay. In the course of the synthesis, the enzyme was practically completely inactivated. Spectral data indicate that destruction of the enzyme catalytic site during the synthesis is accomplished by dissociation of the hem-apoenzyme complex. Potentially, this difficulty can be overcome by enzyme refolding in the presence of hem excess under specifically chosen conditions [42,43]. Nevertheless, in the current work, another assay format was realized, namely indirect ELISA with immobilized hapten–protein conjugate and labelling of the bound antibodies through anti-species immunoperoxidase conjugate. For this format, the antiserum against NP-BSA was combined with NP-STI as the coating antigen. (The alternative scheme with coated NP-BSA and antiserum against NP-STI exhibited a worse sensitivity.) The proposed assay format is based on homologous haptens in immunogen and coating conjugate. The completeness of the competition between free hapten and the coating conjugate for binding with antibodies indicates that the part of antibodies specific to the bridge regions of the conjugate is negligible, and therefore the homologous combination of haptens does not impede an effective assay.

Testing of the binding between the obtained antibodies and immobilized hapten–protein conjugates showed that rather high titres were already reached after the first reimmunization (see Fig. 2). Anti-HHA antibodies did not react with NP groups of immobilized conjugate, as well as anti-NP antibodies with HHA groups. Because only branched forms of NP molecules should be monitored in real samples, the system was based on the NP conjugates obtained through Mannich synthesis.

To avoid cross-reactions of the antibodies with blocking agents, Tween-80 was used as detergent in the course of ELISA instead of Tritons. The immunochemical interaction was carried out in gelatin-containing PBS without any detergent (otherwise, NP would be able to pass into detergent micelles escaping contact with antibodies).

To block potential sites of non-specific sorption, the ELISA protocol included gelatin treatment of the wells before and during the competitive step.

Because pre-treatment of environmental samples often includes extraction of target molecules by organic solvents, attempts should be made to carry out ELISA in the presence of a certain quantity of the solvents. We have shown that the proposed NP ELISA is tolerant to methanol at concentrations up to 10%, which makes it possible to work with methanol extracts. The data obtained here agree with earlier noted regularities in terms of the influence of the organics on other immunoanalytical systems. Thus, effective performance of immunochemical assays has been described [44] for a methanol content up to 30%. The affinity of the immune interactions was found to increase in 10% methanol [45] or in 20% acetonitrile [46].

The limit of NP detection with this optimised assay regime is 10 ng/mL. Because of this sensitivity, it is possible to determine any potential threats of acute toxicity of water and other samples. The level of surfactants that should be controlled for the prevention of negative toxic effects is 10–50 ng/mL, because aquatic animals show chronic symptoms with APs at such concentrations [2]. To monitor lower concentrations of surfactants that can cause negative physiological effects through body accumulation, the proposed ELISA should be combined with extraction and concentration.

The sensitivity of NP detection obtained in our system is better than that for the AP ELISA kit from Takeda Chemical Industries, which has a working range (IC_7–IC_{80}) of 70–1000 ng/mL of APs [47]. The recently described NPEO ELISA [36] shows a detection limit of 50 ng/mL for NP, although it is more sensitive to native NPEOs.

The assay did not show any false-positive reactions with simple phenolic compounds such as phenol, 4-aminophenol, etc., a structurally close important class of toxic pollutants found in water samples. The essential immune recognition was found only for two studied compounds, namely Triton X-100 and Triton X-305, that are OPEOs widely used in laboratory practice. In view of the difference in molecular weights between NP and Tritons, the specificities of the antibodies to these compounds are comparable (the CR in molar form for Triton X-100 is 38.3%, and for Triton X-305 43.7%). Therefore, the technique developed here may be applied for group-specific immunodetection of APEOs and APs. Group-specific assay of surfactants provides complete information on the content of compounds with similar negative effects from a single analysis, and so this strategy may be considered the best in practice.

The assay could be effective for quantitative determination of NP in water samples. The sensitivity of the ELISA does not vary reliably for the studied matrices, namely artesian, mineral, ground and tap water (see Fig. 6). Detection of water pollution by surfactants is an important task in modern environmental monitoring [11], and the sensitivities obtained here can help in the light of threats posed by water contamination.

CONCLUSIONS

From the novel strategy proposed here for nonylphenol conjugation with protein carriers via the Mannich reaction, an effective system for immunodetection of non-ionic surfactants can be elaborated. The synthesis technique is simple and does not require modified hapten preparation for coupling. The antibodies obtained here demonstrate specificity both to the branched APEOs surfactants and to branched APs as products of their destruction as well as a high affinity, thus ensuring the appropriate sensitivity in

ELISA (10 ng/mL). The ELISA developed here is group-specific and could be useful for ecological monitoring, and the set of reactants obtained (antibodies and hapten–protein conjugates) could be used to develop different express immunoanalytical techniques.

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