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## PRODUCING FIBROUS FORMS OF IMMOBILIZED ORGANOPHOSPHATEHYDROLASE USING GEL-FORMING COMPOSITIONS

*P.A. PERMINOV, E.N. EFREMENKO, A.I. DONETSKAYA, N.R. KILDEEVA*

(Moscow State Textile University "A.N. Kosygin",  
E-mail: PPA-2330@mail.ru)

*Method for immobilization of organophosphatehydrolase on textile fibrous materials with usage of aminopolysaccharide chitosan and its derivative sulphate chitosan has been proposed, which enables to produce material for detoxification of organophosphorous compounds .*

**Keywords: textile materials, modification, organophosphatehydrolase, chitosan, sulphate chitosan, glutaric aldehyde, immobilization.**

Degradation problem of neurotoxic organophosphorous compounds (OPHC) - to which belong most of the pesticides and insecticides applied in the agriculture, as well as combat chemical agents (sarin, soman, VX) - remains still quite urgent since these agents pose serious environmental hazard and are to be destroyed. [1]. At present day, destruction of combat chemical agents reserves is being carried out by means of incineration or alkali treatment. However, a small part of organophosphorous compounds (about 1%) remains

in this case not hydroisolated being in such quantities still toxic for human beings. Such method is unacceptable for treatment of hands and agricultural machinery.

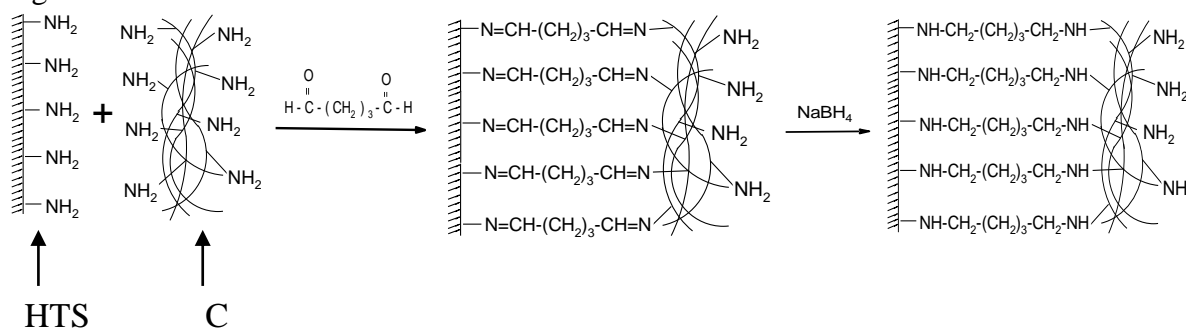
Solution to the problem with degradation of OPHC traces is their biodestruction under the action of a ferment which catalyzes hydrolyses of phospho-etheric bond in orthophosphates. Such ferment is organophosphatehydrolase (OPHG) [2]. Specific of OPHG structure, active center of which contains  $\text{Co}^{2+}$  and coordinate water, places some

restrictions for selecting a method and immobilization conditions (fixation of ferment on the surface of water-insoluble materials): preparation of immobilized ferment should have the ability to retain water (humidity); pH during immobilization should not be below 7, since with  $\text{pH} < 7$  ion  $\text{Co}^{2+}$  may form salts and be converted from the active ferment center (zymophore) into solution [2].

Cotton textile materials have been used in this study as carriers for immobilization of OPHG: sheeting (coarse calico) and nonwoven fibrous material. Fibrous carriers possess high sorption capacity, therefore the materials obtained as a result of immobilization are capable not only to decompose neurotoxins, but also absorb their degradation products. Thus, they can be used for removal of neurotoxin traces from the surface of equipment (tables, devices, walls, etc.), as well as from hygiene products and personal protective equipment (napkins, respirators).

With the aim that textile material would retain humidity for a long time, a method was chosen for immobilization of OPHG which consists in formation of a thin gel-like polymer film on the surface of fabric containing OPHG [3]. We have chosen polysaccharides chitosan (CHTS) and their ionogen derivative

Diagram 1



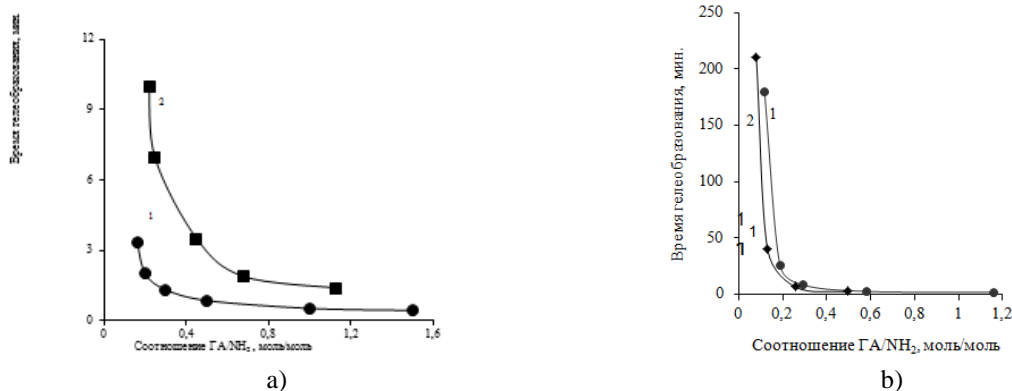
During first three production phases of OPHG, immobilized on fibrous materials, it is essential that polymer composition would remain liquid, and only after completion of these phases the process of jellification takes place. In relation to this, kinetics of jellifica-

tion in the system CHTS and SCH – GA has been investigated for optimization of chemical cross-linking in polymer composition. Completion of jellification was visually registered on loss of flow by gravity.

sulphate chitosan (SCH) - water-soluble with a wide pH range - as film-forming polymers. These polysaccharides, like protein of OPHG, contain reactive primary  $\text{NH}_2$ -groups, with the aid of which by means of chemical cross-linking of molecular chains with glutaric aldehyde (GA) [4] the following two processes have been performed: immobilization of OPHG and jellification in the system. The result of cross-linking of polyelectrolyte in solution is formation of gel-like structure in the whole volume of solution which manifest itself in disappearance of flowability of a composition[5].

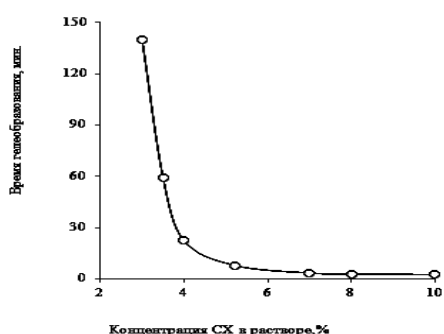
The textile material producing technology - on the surface of which, OPHG is immobilized in the structure of polymer membrane - comprises the following phases: 1) preparation of polymer composition on the basis of chitosan solution or its derivatives, OPHG and GA in a buffer solution; 2) treatment of textile material (fabric or non-woven material) with a polymer composition; 3) pressing; 4) setting of polymer composition on the textile material; 5) stabilization of immobilized ferment by reduction of double bonds. Sequence of chemical conversions in immobilization of OPHG is illustrated on the diagram (1):

tion in the system CHTS and SCH – GA has been investigated for optimization of chemical cross-linking in polymer composition. Completion of jellification was visually registered on loss of flow by gravity.



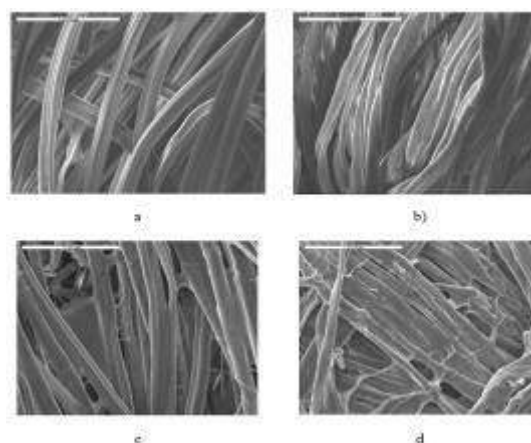
Picture 1. Dependence of jellification time in 2% CHTS (1a) and 7% SCH solutions (1b) from the ratio of reaction groups with pH 5.6 (curve 1, Picture 1-a), 4.1 (curve 2, Picture 1-a), 7.4 (curve 1, Picture 1-b) and 8.6 (curve 2, Picture 1-b)

As dependence of jellification time from correlation of reactive groups shows, change of the ratio  $GA/NH_2$  enables to adjust the jellification time within a quite wide range: from a couple up to tens of minutes. Thus, increase of the ratio  $GA/NH_2$ , as a natural result, leads to decrease of jellification both in CHTS solution (curve 1 on Picture 1-a), and in SCH (curves 1 and 2 on Picture 1-b). As pH grows, speed of cross-linking increases due to decrease of protonation degree of  $NH_2$  groups (curves 1 and 2 in Picture 1-b). Growth of polymer concentration in the composition results also in increase of jellification speed due to concentration growth of reactive groups (Picture 2).



Picture 2. Dependence of jellification time in SCH solutions from solution concentration with ratio of  $GA/NH_2$  0.24 grammolecule/grammolecule and pH 7.4

On the basis of the study of jellification in polysaccharide solutions on treatment with glutaric aldehyde the ratio  $GA/NH_2$ -group (0.24 grammolecule/grammolecule) has been selected, at which formation of gel on the surface of fibrous carrier took place.



Picture 3. Photomicrographs of the surface of initial (a, b) and modified compositions on the basis of SCH and GA (c, d) of nonwoven material (a, c) and of sheeting (b, d)

Hydrophilic cellulose materials have been used as textile materials: cotton sheeting and mixed unwoven materials containing 40% of flax and 60% of hydrocellulose (viscose) yarn. Both materials are used for manufacturing of medical and personal hygiene products. Photomicrographs of the materials used prior to and after modification by gel-forming compositions containing ferment are illustrated in Picture 3. As it can be seen on photomicrographs, nonwoven material (Picture 3-a) has more loose structure with big interfibre space in comparison with the dense ordered structure of woven fabric (Picture 1-b). Surface density of the sheeting is 1.7 times more than that of the nonwoven material. This is the reason for differences in the principle of polymer composition distribution on these materials.

Analysis of photomicrographs of modified materials indicates that the surface of materials treated with polymer compositions is more smooth in comparison with the non-modified materials, patterns are flattened, fibers are glued with each other (Picture 3-c, d). Inclusions of polymer gels, which glue bundles of yarn, are visible on the surface of textile materials; besides, there is notably more polymer composition in the interfiber space of nonwoven material than on the sheeting (Picture 3-d). These data are confirmed by the content of polymer composition on the nonwoven material, that is experimentally determined, and which is with the same pressing 1.5 higher than on the sheeting.

On the basis of the study of jellification in polysaccharide solutions on treatment with glutaric aldehyde the ratio GA/NH<sub>2</sub>-group (0.24 grammolecule/grammolecule) has been selected, at which formation of gel on the surface of fibrous carrier took place.

Discrepancies in distribution of liquid composition, and accordingly, of the gel layer in the textile material asserted influence on kinetic characteristic of immobilized OPHG. Because of the influence of diffusion hindrance on kinetics of enzyme hydrolysis reaction of paraoxone the catalytic activity of immobilized OPHG on the nonwoven material appeared 7 times less than in immobilization on the sheeting; and that happened in spite of the fact that gel-forming compositions, which are similar in formulation, have been used.

As the result of conducted research, composition formulation on the basis of SCH 8% solution with the ratio GA/NH<sub>2</sub> 0.24 grammolecule/grammolecule and 0.3 mg of protein applied on 1 gr of textile materials has been selected. Cotton fabric (sheeting) was used as substrate. Catalytic activity has been found and kinetic parameters of hydrolysis reactions of different substrates have been determined: of paraoxone, parathion, coumaphos and chlorpyrifos, catalyzed by an immobilized preparation of OPHG [6]. The application of this method enabled to obtain OPHG immobilized on fibrous carriers of OPHG having ef-

iciency up to 79%. It has been demonstrated that the above mentioned preparation may be stored at 4°C in wet conditions (60% humidity) in presence of antiinfection agent benzethonium chloride (0.085% from mass of biocatalyst) within a year, at the same time, efficiency loss does not exceed 20%.

## CONCLUSIONS

1. Method for immobilization of organophosphatehydrolase on textile fibrous materials - cotton sheeting and mixed nonwoven material - with usage of aminopolysaccharide chitosan and its derivative sulphate chitosan has been proposed, which enables to produce material for detoxification of organophosphorous compounds.

2. The application of the suggested method enabled with the use of 8% SCH solution with the ratio GA/NH<sub>2</sub> 0.24 grammolecule/grammolecule and 0.3 mg of protein applied per 1 gr of textile materials and to obtain OPHG immobilized on fibrous carriers having efficiency up to 79%.

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