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PHYSICAL, CHEMICAL, AND KINETIC PROPERTIES OF TRYPSIN-BASED HETEROGENEOUS BIOCATALYSTS IMMOBILIZED ON ION-EXCHANGE FIBER MATRICES

M. G. Holyavka,[1,*] V. G. Artyukhov,[1] S. M. Sazykina,[1] and M. A. Nakvasina[1]

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A method for obtaining trypsin-based biocatalysts immobilized on VION KN-1 and VION AN-1 ion-exchange fiber matrices was developed. The physical, chemical, and kinetic properties of the obtained heterogeneous materials were investigated. The materials with immobilized trypsin that were proposed by us functioned better in the most alkaline region than already existing materials (literature data), allowing expansion of their sphere of use in medicine and cosmetics.

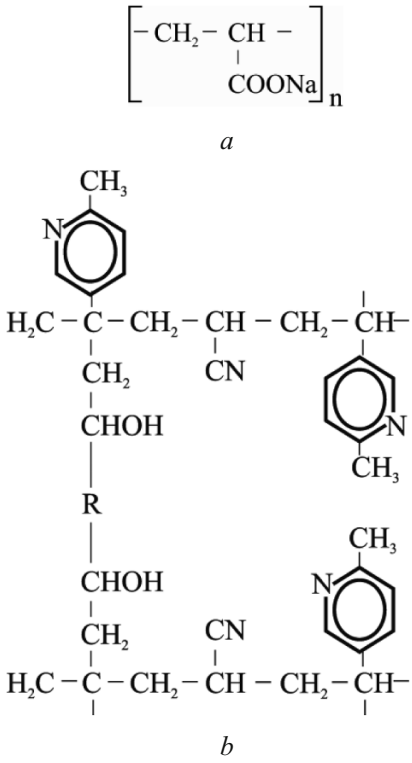
Keywords: adsorptive immobilization, ion-exchange fibers, VION KN-1, VION AN-1, trypsin.

Research on the preparation of highly stable heterogeneous systems with immobilized enzymes, in particular trypsin, is becoming more and more critical [1 – 4]. Enzymes immobilized on insoluble supports are known to solve several important medical issues such as 1) production of prolonged-action drugs via stabilization and increased enzyme half-life, 2) targeted delivery of a compound and resolution of *in vivo* diffusion issues, and 3) optimization of the drug functioning conditions (optimum temperature and pH). On the other hand, immobilization can freeze the steric structure of an enzyme and cause conformational changes, which usually leads to partial or total loss of catalytic activity. Therefore, the search for methods of regulating the physical, chemical, kinetic, structural, and functional properties of enzymes and stabilizing them must continue.

The polymers for immobilizing trypsin could be VION ion-exchange fibers. These are new high-efficiency materials for purifying air of several toxic and aggressive gases and aerosols. The main advantages of VION fibers are the purity (98%) of the ion exchanger; the absorption and regeneration rates that are 10 – 15 times greater than for granular material; and the high exchange capacity, selectivity, and hydrolytic stability to acids, bases, and regenerating agents (retention of exchange capacity after >100 absorption–regeneration cycles). VION KN-1 fiber is used as a deodorizing and wound-healing material in veterinary medicine. It is a weak-acid cation exchanger and a chemosorptive carboxylate-containing three-dimensional network. This material is characterized by a high absorption rate, highly developed surface, high hygroscopic stability, and hydrophilicity. VION KN-1 fiber cleans and disinfects wounds by neutralizing toxic substances in the wound cavity, normalizing the pH, eliminating fibrous purulent exudate, and absorbing purulent-specific odor and by not adhering to the wound surface and causing allergic or irritating effects. This has beneficial effects on the development of granulation and epithelium and reduces the use of bindings and drugs [5]. Fibers are used efficaciously to treat purulent wounds and bed sores [6].

Therefore, the main goals of the research were to develop a method for producing heterogeneous biocatalysts for medical use with trypsin immobilized on matrices of ion exchangers VION KN-1 and VION AN-1 and to study their physical, chemical, and kinetic characteristics.

[1] Voronezh State University, 1 Universitetskaya Pl., Voronezh, 394018, Russia.
[*] e-mail: holyavka@rambler.ru



a

b

Fig. 1. Elemental unit structure of cation-exchanger VION KN-1 (*a*) and anion-exchanger VION AN-1 (*b*).

EXPERIMENTAL PART

Bovine trypsin (MP Biomedicals) was used in the study. The substrate for hydrolysis was bovine serum albumin (BSA, Sigma-Aldrich). The supports for immobilization were ion-exchange fibers VION KN-1 and VION AN-1 (LIRSOT Ltd., Russia) (Fig. 1*a* and 1b, respectively).

TABLE 1. Quantities K_m' and V_{max}' for BSA Hydrolysis by Immobilized Trypsin

Immobilization conditions	K_m', mM	V_{max}', mM/min·mg
VION AN-1, carbonate buffer	6.93 ± 0.32	770 ± 4.1
VION KN-1, borate buffer	5.87 ± 0.57	653 ± 6.7
VION KN-1, carbonate buffer	1.08 ± 0.14	119 ± 3.4

Protein content in the preparations was determined by the Lowry method [7]; enzyme catalytic activity, by a modified Lowry method (without adding copper sulfate to the reaction mixture) [8]. Procedures for preparing the supports and immobilizing trypsin were previously described in detail [9]. Solution pH values were measured using a 211 pH-meter (Hanna Instruments). Results were processed statistically to 5% significance level using the Student *t*-criterion.

RESULTS AND DISCUSSION

Various buffers were used as the immobilization solutions for preparing high-activity biocatalysts with trypsin immobilized on ion-exchange fibers VION KN-1 and VION AN-1. These were glycine (0.5 M), pH range 8.6 – 10.5; Tris-glycine (0.05 M), 8.5 – 9.0; borate (0.05 M) with added KCl, 8.0 – 10.0; carbonate (0.1 M), 9.0 – 10.4; and Tris-HCl buffers (0.05 M), 7.5 – 9.0.

The contents of adsorbed protein in the immobilized preparations (Figs. 2 and 3) and their specific activities (Figs. 4 and 5) were determined in the first series of experiments. The combination of these two parameters suggested that subsequent experiments should use trypsin immobilized on VION KN-1 in carbonate and borate buffers (pH 9.0) and trypsin immobilized on VION AN-1 in carbonate buffer (pH 10.0).

TABLE 2. Characteristics of Trypsin Compounds Immobilized on Various Supports

Support for immobilization	Optimum functioning		Ref.
	t, °C	pH	
Poly-*N*-vinylpyrrolidone	37 – 50	4.0 – 9.0	[20]
High-viscosity chitosan from crab shell	50	8.0	[21]
Mesoporous silica foam	55	8.5	[22]
Reprocessed grains of glyoxal	50 – 60	8.0	[23]
Poly(acrylate/*N*,*N*'-methylene-bis-acrylamide) hydrogel		4.0 – 8.0	[24]
Low-molecular-mass chitosan (1 kDa)	37	8.0	[25]
Ion-exchange fibers:			Our data
VION KN-1	40 – 47	9.0 – 9.5	
VION AN-1	42 – 47	9.0 – 9.5	

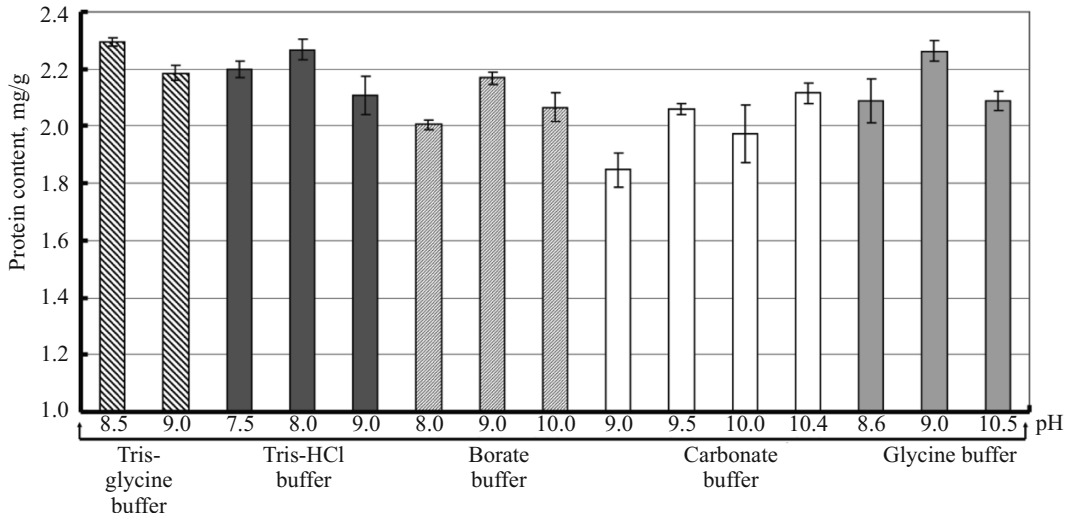


Fig. 2. Protein content (mg per g of support) in trypsin compounds immobilized on VION KN-1 using various buffers.

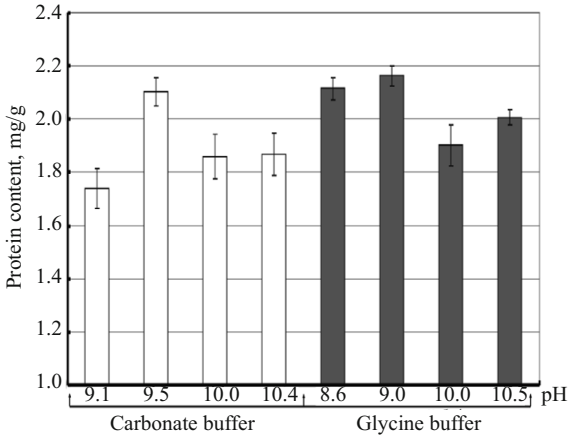


Fig. 3. Protein content (mg per g of support) in trypsin compounds immobilized on VION AN-1 using various buffers.

Free trypsin is known to function optimally at ~37°C and pH 7.6 – 8.6 [10 – 15]. The optimum temperature was 42 – 47°C for immobilization on VION AN-1 in carbonate buffer (pH 10.0); 40 – 47°C, on VION KN-1 (using both carbonate and borate buffer, pH 9.0) (Fig. 6). The shift of the optimum temperature to higher values allowed the heterogeneous enzyme systems to be used with inflammation where the patient body temperature is greater than the optimum one for dissolved trypsin [16].

The optimum pH range for trypsin immobilized on VION fibers had a maximum at 9.0 – 9.5 that was shifted to more alkaline values than for the free enzyme (Fig. 7). This indicated that the degree of ionization of separate enzyme components changed during adsorptive immobilization on the VION ion-exchange fiber matrix. The shift of the optimum pH after immobilization could be explained by the dif-

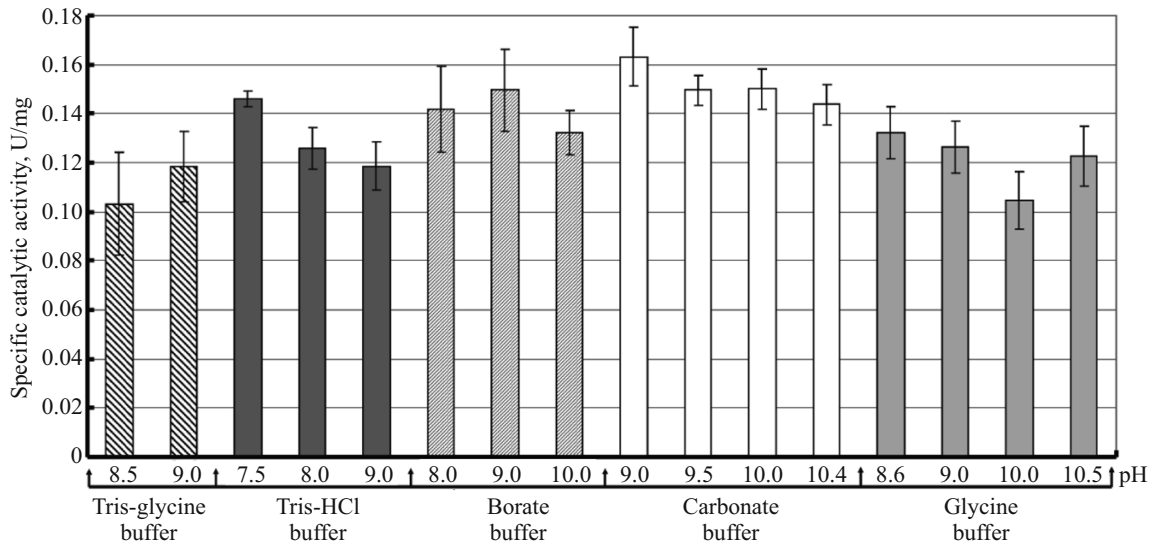


Fig. 4. Specific catalytic activity (units per mg of protein in sample) of trypsin compounds immobilized on VION KN-1 using various buffers.

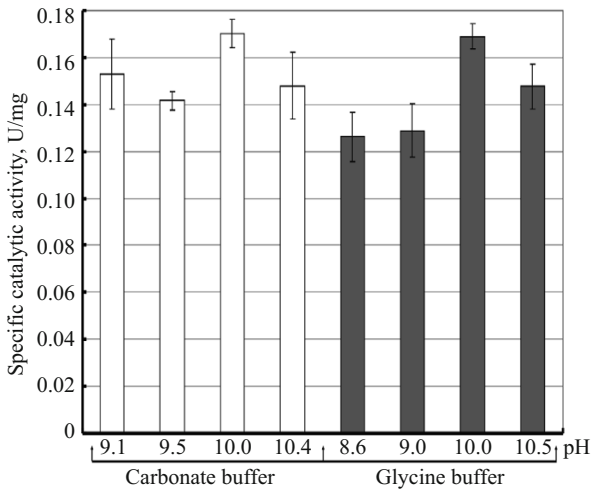


Fig. 5. Specific catalytic activity (units per mg of protein in sample) of trypsin compounds immobilized on VION AN-1 using various buffers.

ference in the local pH values of the active-center microenvironment and the solution.

The behavior of proteolytic enzymes used as pharmaceuticals could be explained by studying their reaction kinetics. Catalysis by immobilized enzymes must also be studied to find the mechanisms of action of membrane-bound enzymes and modular poly-enzyme systems [17].

The basic theoretical provisions of heterogeneous catalysis are applicable to immobilized samples. As a rule, immobilization of enzymes changes their reaction kinetics. Therefore, they are designated as apparent (K_m', V_{max}', etc.). The quantity K_m' increases for most immobilized enzymes; V_{max}', decreases [18, 19].

The kinetics of the reactions catalyzed by free trypsin and that immobilized on VION fibers obeyed the Michaelis–Menten equation. The quantities K_m' and V_{max}' for BSA hydrolysis by the studied enzyme preparations were determined by transforming the curves for V and S in Lineweaver–Burk, Hanes, and Eadie-Hofstee coordinates (Table 1). The enzyme preparation immobilized on VION KN-1 using carbonate buffer (pH 9.0) possessed the greatest affinity for substrate. Trypsin immobilized on VION AN-1 and also using carbonate buffer but at pH 10.0 had the greatest maximum reaction rate.

A comparison of the immobilized trypsin systems proposed by us and those already developed (Table 2) showed that they functioned most efficiently in more alkaline regions. This enabled them to be used with alkalizing agents for suppressing multiplication of parasitic fungi, viruses, and bacteria. Alkaline pH values of 8.0 – 8.5 are known to facilitate eradication of fungus and pigment spots from skin. Alkaline soaps with pH values of 9.5 – 10 prevent the appearance of bed sores. All this expands the medical and cosmetic applications of the compounds proposed by us.

Thus, a method for preparing heterogeneous biocatalysts with trypsin immobilized on ion-exchange VION KN-1 and VION AN-1 fiber matrices was developed and was promising for medical and cosmetic applications. Studies of the physical, chemical, and kinetic properties of the heterogeneous enzyme preparations allowed subsequent selection of the optimum functioning conditions and facilitated more rational use for treating wounds and burns.

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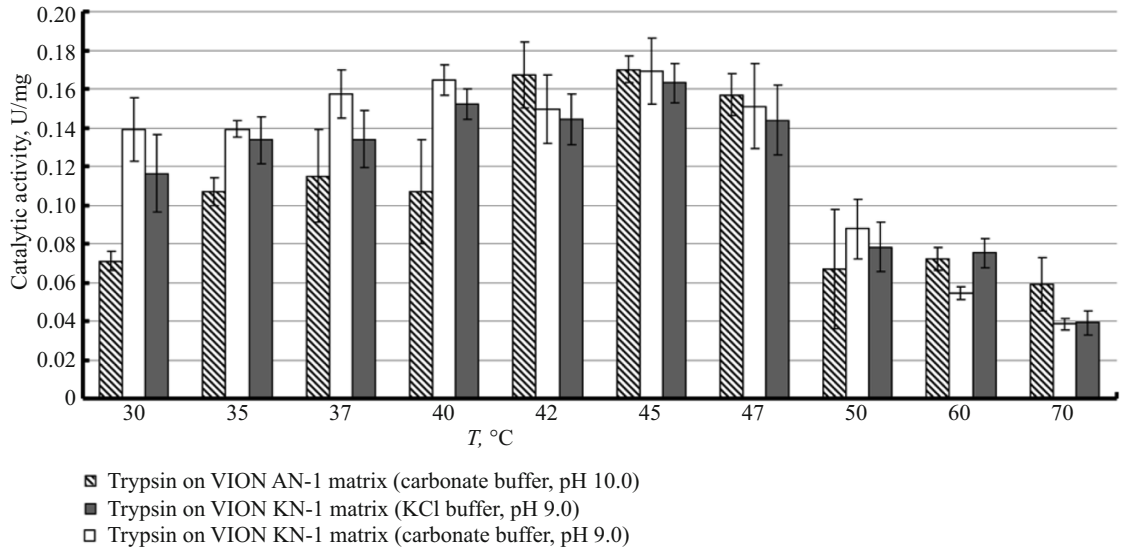

Fig. 6. Temperature dependences of catalytic activity of immobilized trypsin preparations.

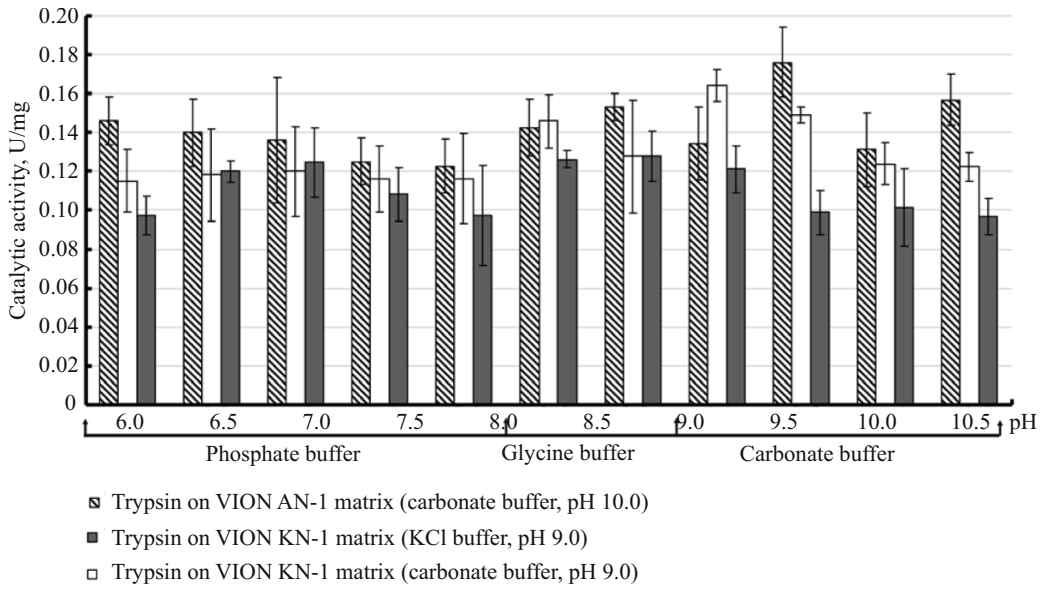

Fig. 7. pH dependences of catalytic activity of immobilized trypsin preparations.

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