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Application of The Immobilization Method for Obtaining Serratiopeptidase With Prolonged Action

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Abstract

The present study investigates the possibility of using an immobilization method based on polyaldehyde dextran (PAD) to enhance the stability of serratiopeptidase (SP). Serratiopeptidase, a proteolytic enzyme, possesses a broad spectrum of biological activity; however, its practical application is limited due to low stability under external conditions. Dextran was functionalized by periodate oxidation to generate reactive aldehyde groups capable of forming covalent bonds with protein amino groups via Schiff base formation.

Prolongation and stable preservation of serratiopeptidase activity were confirmed by evaluating its proteolytic efficiency. Protease activity was determined using a modified method with casein as a substrate.

It was established that the immobilized form of serratiopeptidase demonstrates increased catalytic efficiency and reproducibility, making it promising for pharmaceutical applications.

Keywords: Serratiopeptidase, immobilization, dextran, polyaldehyde dextran, bioconjugate, prolonged action, proteolytic activity.

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1. Introduction

The issue of rational and safe pharmacotherapy has become one of the most pressing challenges in modern clinical and pharmaceutical practice. This is associated with the continuously expanding pharmaceutical market and the increasing volume of scientific information, as well as the need to identify the most in-demand therapeutic classes [1,2,3].

Modern medicine increasingly employs highly purified protein-based biologically active substances due to their high specificity and activity [4,5]. Serratiopeptidase is a proteolytic enzyme with anti-inflammatory, antifibrotic, and mucolytic properties.

However, its practical use is significantly limited by low storage stability and high sensitivity to environmental conditions (pH, temperature, and protease inhibitors) [6].

These factors reduce shelf life and complicate the development of effective dosage forms.

To improve enzyme stability and expand functional capabilities, immobilization on organic and inorganic carriers is widely applied. Immobilization increases resistance to denaturing factors and facilitates repeated use, which is particularly important for industrial and pharmaceutical applications [7].

Polysaccharides are widely used in biotechnology and pharmaceutics as drug delivery matrices, hydrogels, and biomolecule carriers.

Polyaldehyde dextran (PAD), obtained by periodate oxidation of dextran, is a promising and accessible immobilization material. The presence of reactive aldehyde groups enables covalent binding with protein amino groups via Schiff base formation [8]. This approach ensures stable enzyme fixation on a polymer matrix while minimizing activity loss and increasing resistance to external factors. Additionally, dextran-based carriers exhibit high biocompatibility and are promising for prolonged-release pharmaceutical formulations.

Purpose of the study. To investigate the feasibility of using polyaldehyde dextran for immobilization of serratiopeptidase in order to enhance its stability and prolong its biological activity.

2. Methods

Serratiopeptidase (Evertogen Life Sciences Ltd., India) and dextran (Pharmacosmos, Denmark; Mr = 40,000) were used in this study.

IR spectra were recorded using a Bruker Vector 22 FTIR spectrometer (KBr pellets, 3 mg sample/300 mg KBr) within the range of 400–4000 cm^{-1}. Spectrophotometric measurements were performed using a UV-1280 spectrophotometer (Shimadzu, Japan) in the wavelength range of 200–500 nm.

To obtain polyaldehyde dextran, periodate oxidation of dextran was carried out [9].

Preparation of Polyaldehyde Dextran

Dextran was oxidized with aqueous sodium periodate ($NaIO_4$) at 25°C for 6 hours. The product was purified by dialysis against distilled water and subsequently lyophilized. Functional group analysis showed that 25–30 mol% of dextran units were oxidized, which was considered the optimal oxidation degree.

Immobilization Procedure. 0.5 g of dry PAD was placed in a 50 mL flask and hydrated with 10 mL of 0.1 M sodium phosphate buffer for 10 minutes. Serratiopeptidase (5.0 $mg \cdot mL^{-1}$ in 0.1 M sodium phosphate buffer) was added in amounts corresponding to 0.5, 1.0, 2.0, and 4.0 mg protein per 0.5 g carrier.

Immobilization was carried out for 6–24 hours at 20–25°C within a pH range of 2–10. The final product was obtained by lyophilization after 24-hour dialysis.

The degree of immobilization was determined by measuring the decrease in protein concentration in solution after binding. Protein concentration was assessed spectrophotometrically (Bradford method, 280 nm). The immobilization efficiency (%) was calculated using the equation:

$$\text{Immobilization Yield} = \frac{C_{initial} - C_{supernatant}}{C_{initial}} \times 100$$

Where: $C_{initial}$ Cinitial is the initial enzyme concentration in the reaction mixture (mg/ml);

$C_{supernatant}$ Csupernatant is the concentration of unbound enzyme in the supernatant (mg/ml).

3. Results and Discussion

To obtain a matrix for immobilizing serratiopeptidase, dextran was functionalized by periodate oxidation. Periodate oxidation of dextran cleaves the bonds between the C2-C3 and C3-C4 carbon atoms, forming aldehyde groups. The reaction was carried out using an aqueous $NaIO_4$ solution at 25°C for 6 hours. The resulting product was purified by dialysis against distilled water and lyophilized. Analysis of the functional group content showed that the amount of oxidized units in the dextran macromolecule is 25-30 mol% (the optimal oxidation state).

The resulting PAD had good solubility in water and formed transparent solutions without signs of aggregation. In studies at the indicated oxidation state, it is possible to maintain a sufficient length of the polysaccharide chain to form a stable matrix while simultaneously introducing the required number of reactive aldehyde groups. When the enzyme interacts with PAD, a nucleophilic addition reaction of protein molecules occurs due to interaction with the ε-amino groups of the enzyme's lysine residues and the aldehyde groups of the polysaccharide. The formation of an azomethine bond between the enzyme and PAD was determined by IR spectroscopy.

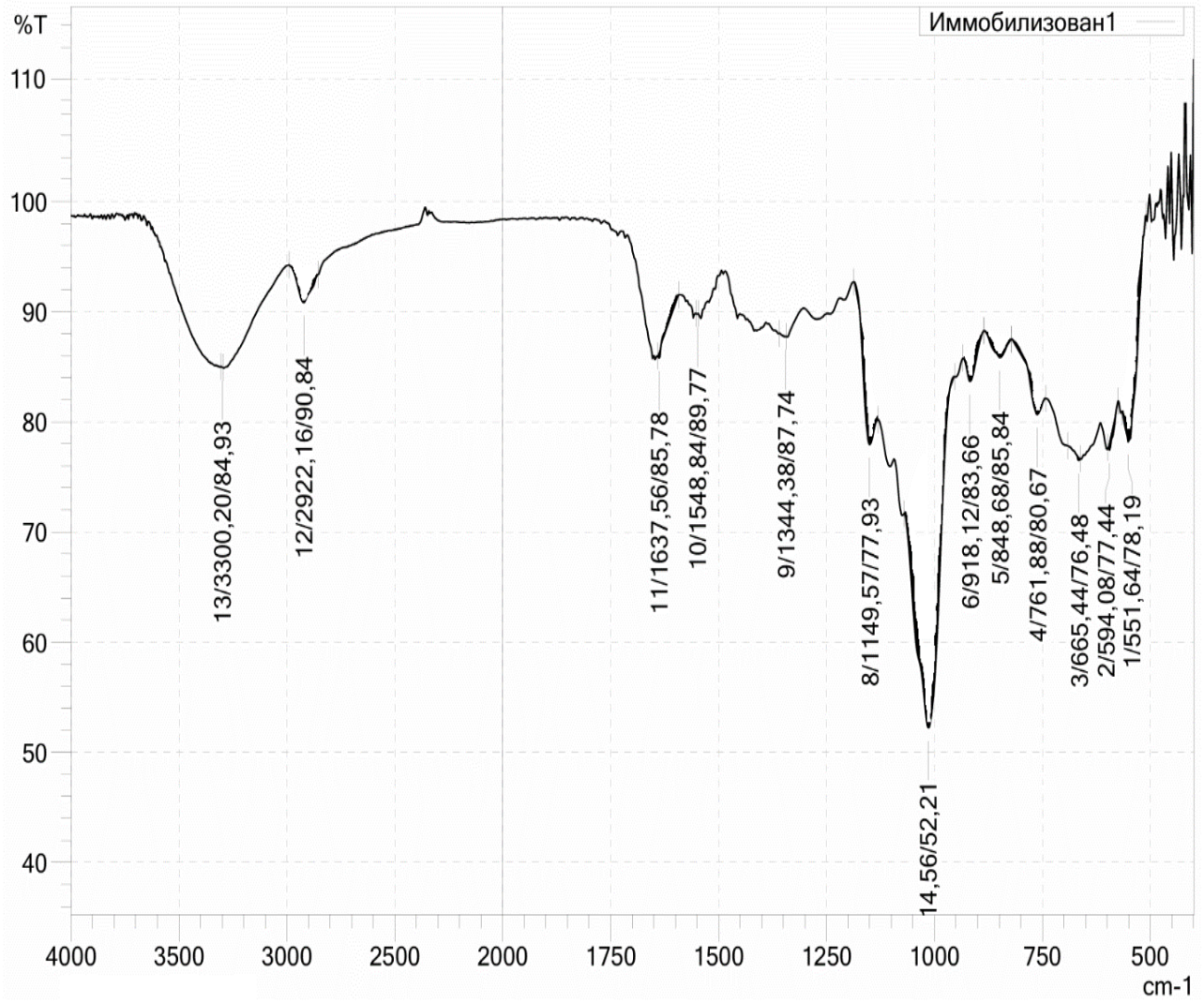

Figure 1. IR spectrum of immobilized serratiopeptidase on PAD

The IR spectrum of the serratiopeptidase-PAD system displays a number of characteristic bands, allowing us to draw conclusions about the enzyme's immobilization. A broad band is observed in the 3400–3200 cm^{-1} region, due to the stretching vibrations of the hydroxyl groups of the polysaccharide and the N–H groups of the amino acid residues of the protein. Bands at 2930–2850 cm^{-1} correspond to the stretching vibrations of the C–H bonds of the methylene and methine groups of glucose units, as well as the side chains of amino acids.

Important confirmation of the formation of covalent bonds is the appearance of a band in the 1638–1640 cm^{-1} region, associated with vibrations of the C=N groups characteristic of Schiff bases. Intense bands are simultaneously detected at 1650–1640 cm^{-1} (Amide I) and 1540 cm^{-1} (Amide II), indicating the presence of a protein component. IR spectroscopic analysis confirms the successful immobilization of serratiopeptidase on PAD due to the formation of imine bonds (Schiff bases) between the aldehyde groups of the polysaccharide and the amino groups of the protein, while maintaining the structural integrity of the carrier. One of the key factors determining the efficiency of enzyme immobilization on polysaccharide carriers is the pH of the reaction medium. In the case of serratiopeptidase, which has multiple free amino groups (lysine residues, N-terminal amino acids), these are the ones that react with the aldehyde groups of PAD to form azomethine bonds.

The results of the study, examining the effect of pH on enzyme immobilization on a polysaccharide, are presented in Figure 2.

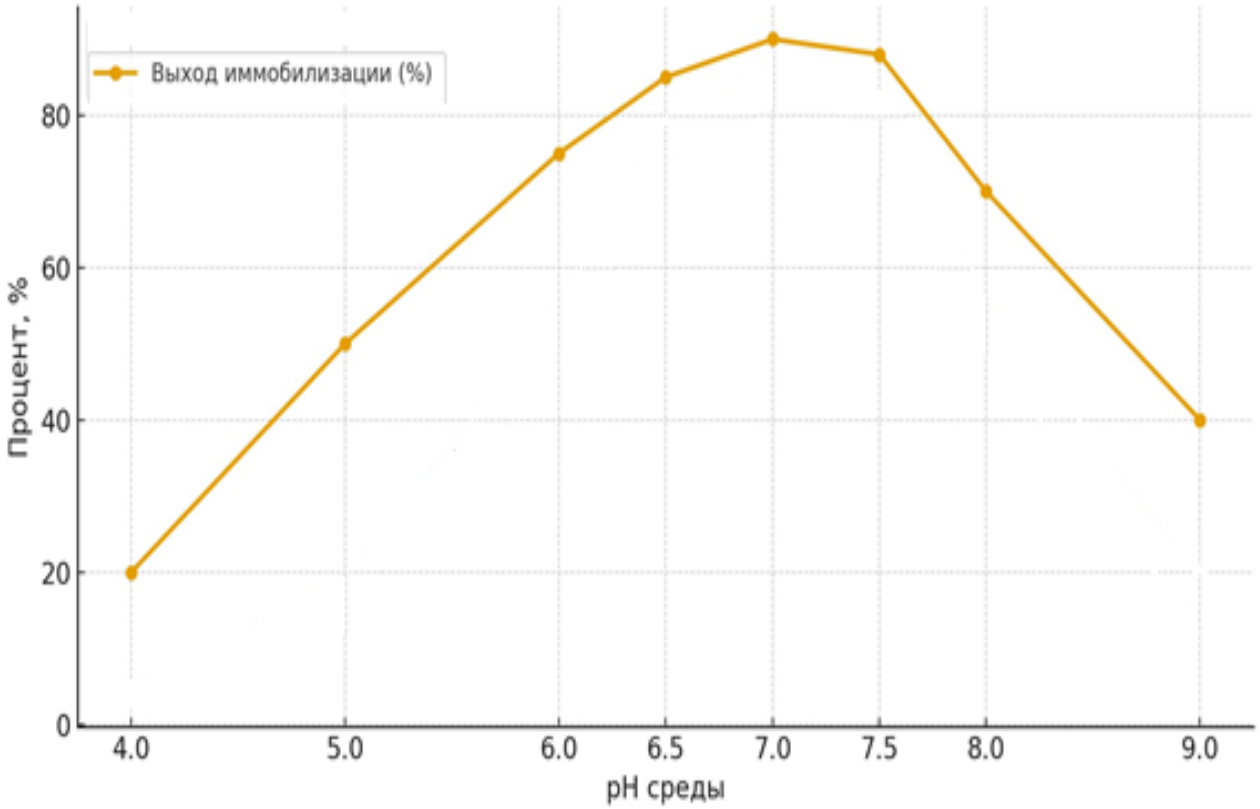

Figure 2. Effect of pH on the immobilization of serratiopeptidase on PAD

Thus, the optimal pH range for immobilization of serratiopeptidase on PAD is 6.5–7.5, where a compromise between the efficiency of covalent bond formation is achieved.

4. Conclusion

Research has shown that polyaldehyde dextran obtained by periodate oxidation can be used for immobilization of serratiopeptidase. The resulting complexes were studied using IR spectroscopy. Spectral analysis confirmed the formation of C=N bonds and the presence of amide bands, indicating successful immobilization of the enzyme while maintaining the polysaccharide structure. Immobilization conditions: a SP:PAD ratio of 1:3 and a pH range of 6.5–7.5 were determined, achieving up to 85–90% binding. Prolongation and stable stability of serratiopeptidase were demonstrated by proteolytic efficiency testing. Protease activity was determined using a modified method using casein as a substrate.

It was established that the immobilized form of serratiopeptidase has increased catalytic efficiency and reproducibility, which makes it promising for use in pharmaceutical practice.

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