Silica-based transaminase immobilization for pregabalin synthesis

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Highlights

- Silica nanoparticles and silica nanopowder were used as support for transaminase immobilization.
- Efficient modification using PEI enhanced enzyme activity upon immobilization.
- Immobilization efficiency up to 78% was attained using properly modified support.

1. Introduction

Enzymes are protein biocatalysts that accelerate chemical reactions occurring in living cells without being consumed in the process. Their activity relies on the specific binding of substrates and the reduction of activation energy, which results from the unique three-dimensional structure of the enzyme. Beyond their fundamental biological role, enzymes play a significant part in various industrial sectors, including the food, pharmaceutical, feed, energy, and environmental industries [1].

A particularly important group of enzymes comprises transaminases (TAs), which belong to the class of transferases. These pyridoxal-5-phosphate (PLP)-dependent enzymes catalyze the reversible transfer of an amino group from amine donors to ketones, converting prochiral compounds into chiral amines with high enantiomeric purity. Due to their ability to operate under mild, aqueous reaction conditions and their remarkable substrate and stereochemical selectivity, transaminases have become key biocatalysts in the synthesis of pharmaceutical and agrochemical intermediates [2].

Free enzymes exhibit high catalytic activity and substrate selectivity; however, their practical application is limited by low stability, sensitivity to changes in pH and temperature, susceptibility to denaturing agents, and the lack of efficient recovery and reuse. These limitations make their use in industrial processes costly and less efficient [3]. Enzyme immobilization offers a solution to these challenges. This approach involves the stable attachment, entrapment, or confinement of enzymes within or onto solid supports. The primary objective is to combine the high selectivity of enzymes with the advantageous physicochemical properties of carrier materials, thereby enhancing stability, operational durability, and reusability of the biocatalysts. Immobilized enzymes typically exhibit increased resistance to harsh process conditions, a broader optimal pH and temperature range, and easier separation from the reaction medium.

2. Methods

In the study two types of silica supports – silica nanopowder (characterized by irregular particle morphology) and silica nanoparticles (exhibiting a more uniform, quasi-spherical morphology) – were used. Silica support was subjected to surface modification using two approaches: (i) modification via 3-Aminopropyltriethoxysilane and glutaraldehyde and (ii) modification via polyethylenimine (PEI). For the purpose of modification, 500 mg of both silica materials was placed in a vial and next 50 mg of 3-aminopropyltriethoxysilane (APTES) in a 7 ml methanol–water mixture (4:1 v/v) was applied to silica surface. In the next step of this method 5 mL of 25% glutaraldehyde solution was added and the suspension was mixed on a rocking shaker (10°, 50 rpm) for 24 hours to promote crosslinking and stabilization of amino groups on the silica surface. In the method with PEI also 500 mg of both silica support was used, to which appropriate volume of PEI solution (2 g/L in TRIS buffer, pH 8.5) was added. The suspensions were next mixed on a rocking shaker (10°, 50 rpm) for 24 hours to allow adsorption of PEI onto the silica surface. Modified supports were next used for enzyme immobilization. For this reason the transaminase solution was prepared in TRIS buffer (pH 8.0) at a concentration of 0.1 mg/mL. Next 10 mg of silica supports were mixed with 5 mL of the enzyme solution and the vials were placed on a rocking shaker (10°, 50 rpm) and incubated for 24 hours.

The catalytic activity of immobilized transaminase was determined using a commercial enzyme activity assay kit whereas for quantitative determination of immobilization efficiency 1 mL of post-immobilization solution was transferred into a polystyrene cuvette, followed by the addition of 1 mL of Bradford reagent. After 5 minutes, absorbance was measured at 595 nm and the enzyme concentration was determined.

3. Results and discussion

The conducted experiments and analysis of the obtained results confirmed the feasibility of effective immobilization of ω-transaminase on silica supports with different morphologies and surface functionalization. It was demonstrated that both the type of silica and the method of its surface modification significantly influence the amount of immobilized enzyme, the efficiency of the immobilization process, and the retained catalytic activity of the resulting systems. The results of catalytic activity assays were of particular importance. Immobilization of transaminase on nanopowder-type silica resulted in a substantial decrease in retained enzymatic activity, regardless of the applied surface modification. In contrast, systems based on nanoparticles-type silica exhibited high retained activity. Notably, in the case of PEI and APTES/GA modifications, the retained activity exceeded 100%, indicating the occurrence of enzyme hyperactivation following immobilization (Table 1).

Table 1. Summary of produced systems with obtained activity recovery after transaminase immobilization.

Tested System	Activity recovery (%)
System 1 – TA immobilized on silica nanopowder	40.9
System 2 – TA immobilized on silica nanopowder modified with PEI	11.0
System 3 – TA immobilized on silica nanopowder modified with APTES/GA	15.7
System 4 – TA immobilized on silica nanoparticle	897.6
System 5 – TA immobilized on silica nanoparticle modified with PEI	146.5
System 6 – TA immobilized on silica nanoparticle modified with APTES/GA	123.6

4. Conclusions

Morphological and physicochemical analyses confirmed efficient enzyme immobilization and proved that enzyme deposition slightly affect the structure of the support material. Quantitative analysis of immobilized enzyme revealed that the highest immobilization efficiency was achieved for unmodified silica materials, whereas the highest activity was observed for support after PEI modification. The obtained results confirm that appropriate selection of the support material and functionalization strategy enables the development of stable and highly active biocatalytic systems based on immobilized transaminase.

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Keywords
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