

Improved Immobilized Enzyme Systems Using Spherical Micro Silica Sol-Gel Enzyme Beads

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Abstract Spherical micro silica sol-gel immobilized enzyme beads were prepared in an emulsion system using cyclohexanone and Triton-X 114. The beads were used for the *in situ* immobilization of transaminase, trypsin, and lipase. Immobilization during the sol to gel phase transition was investigated to determine the effect of the emulsifying solvents, surfactants, and mixing process on the formation of spherical micro sol-gel enzyme beads and their catalytic activity. The different combinations of sol-gel precursors affected both activity and the stability of the enzymes, which suggests that each enzyme has a unique preference for the silica gel matrix dependent upon the characteristics of the precursors. The resulting enzyme-entrapped micron-sized beads were characterized and utilized for several enzyme reaction cycles. These results indicated improved stability compared to the conventional crushed form silica sol-gel immobilized enzyme systems.

Keywords: sol-gel immobilization, microemulsion, surfactant, Triton-X 114, cyclohexanone, transaminase, lipase, trypsin

INTRODUCTION

The immobilization of enzymes offers many advantages, including multiple reuse, easy separation, and improved stability for efficient biotransformation and biodegradation [1-5]. Among various immobilization methods, sol-gel immobilization using silane compounds such as tetramethoxysilane (TMOS) has been applied for the preparation of thin film biosensors and for the immobilization of biocatalysts used in the biosynthesis of natural products and anti-fouling materials [6-10]. The most common silica sol-gel encapsulated enzyme systems have been produced in crushed powder form from the dried xerogel state [11-16], or sol-gel coatings with enzymes or antibodies affixed to various solid material surfaces [17-21]. The crushing of the silica particles, however, yields irregular shapes and sizes and makes the process of scale-up very difficult. Although spray-drying [22] and microwave assisted sol-gel methods [23] were attempted for the fabrication of spherical silica beads, these procedures also required an elevated temperature that is not suitable for *in situ* enzyme immobilization. Microparticle technology has significant importance for the development of new drug delivery methods [24]. Meanwhile, a sol-gel emulsion technology has been developed that combines the emulsion and sol-gel technology and used to prepare monodispersed silica particles for drug delivery

[25-28]. The sol-gel emulsion process should be suitable for the immobilization of biological molecules such as antibodies and enzymes because of the prevailing ambient temperatures and relatively mild processing conditions. We report an optimized sol-gel emulsion process involving organic solvents for *in situ* enzyme immobilization using spherical silica beads and demonstrate the effects of different silica sol-gel precursors on enzyme activities.

MATERIALS AND METHODS

Chemicals and Enzymes

The ω -transaminase from *Vibrio fluvialis* (ω ATVf) was purified according to the procedures described elsewhere [29]. The lipase from *Candida rugosa* and trypsin from porcine pancreas were purchased from Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade.

Enzyme Immobilization and Assays

A magnetic stirrer from Hanna Instrument (Model HI 303N, Woonsocket, RI, USA) that displays digital RPM values was used for controlled mixing. Analysis of ω -transaminase activity was performed by measuring the concentration of acetophenone and 1-phenylethanol [30]. The lipase and trypsin assays were achieved by monitoring hydrolysis of *p*-nitrophenylacetanilide (pNPA), and *N*-benzoyl-*L*-arginine-4-nitroanilide (BAPNA), respectively [31,32].

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