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Preparation of *azadirachta indica* alkaline phosphatase loaded egg albumin and bovine serum albumin nanoparticles

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Abstract

Azadirachta indica is rich source of alkaline phosphatase and alkaline phosphatase are known for their industrial application in food and pharmaceutical industries. The extracted alkaline phosphatase was encapsulated into biochemically active egg albumin and BSA nanoparticles with butanol and glutaraldehyde. Achieved encapsulation was 69% when egg albumin (EA) used and 85% was observed when bovine serum albumin (BSA) was used. Characterization of prepared albumin nanoparticles was done by scanning electron microscopy (SEM). Observed size of enzyme loaded nanoparticles was reported up to 80nm when egg albumin was used and up to 100m when bovine serum albumin was used for encapsulating the *Azadirachta indica* alkaline phosphatase.

Keywords: *Azadirachta indica*, Alkaline phosphatase, Bovine serum albumin, Egg albumin, Encapsulation

Introduction

Alkaline phosphatase is a hydrolytic enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids, called dephosphorylation process. Leaves of Azadirachta indica is rich source of alkaline phosphatase.[1-3] Enzymes have effective and conventional role in various cosmetics preparation, paper industry, textile industry, food industry, pharmaceutical industry due to having their unique biocatalytic properties.[4-6] These days, nanobiotechnology has become prime choice to develop new advanced methods to immobilize the various industrially important enzymes on various biocompatible nanomaterials which reduce their industrial cost.[7-9] Nanotechnology based enzyme binding have been considered the most to immobilize or encapsulate various chemical and biological components on various potential biocompatible nanomaterials for excellent particle mobility.[9] Enzyme immobilization into biocompatible nanomaterials was performed by various methods like adsorption, non-specific covalent binding, entrapment and encapsulation.[9-12] In our work, egg albumin and bovine serum albumin were chosen for encapsulation of *Azadirachta indica* alkaline phosphatase as biocompatible and safe matrices which were biochemically engineered by using surface modifiers such as butanol and glutaraldehyde as a cross-linking agent.[8-12]

Materials and Method

Extraction of alkaline phosphatase: 20-25 grams of leaves of *Azadirachta indica* were homogenized by adding 6-10 ml of 0.05 M glycine NaOH buffer (pH 10.5) and centrifuged for 15 minutes at 4°C at 10,000 rpm. Discarded the pellet and collected the supernatant which contained crude enzyme and then stored at 4°C.[2]

Alkaline phosphatase assay: *Azadirachta indica* alkaline phosphatase assay was done by 5 ml glycine NaOH (pH 10.5) buffer was taken in test tubes and 0.1ml MgCl2, 0.1 ml of para-nitro-phenyl phosphate (p-NPP) was added. Add 0.5 ml of crude enzyme extract was added to in the reaction mixture. Then, the test solution was incubated at 37°C for 10 minutes. After incubation, 2ml of 0.085N NaOH was added in test tube to stop the reaction and absorbance is taken at 410nm.[2]

Preparation of *Azadirachta indica* alkaline phosphatase loaded Egg albumin nanoparticles: *Azadirachta indica* alkaline phosphatase was dissolved in n-butanol and emulsifier (coconut oil) and added dropwise to the 4-5ml of egg albumin solution with continuous stirring by using magnetic stirrer. After this, 25% glutaraldehyde was added for crosslinking of enzyme in prepared reaction mixture and kept overnight with stirring. The reaction solution was centrifuged at 5000 rpm at 4^0C for 20mins. The supernatant was removed. The collected pellets were re-dispersed in diethyl ether and acetone and subjected to sonicator for 25-30mins. After that, it was centrifuged at 5000rpm at 4°C for 20 minutes. Enzyme assay was done in supernatant to know the % of encapsulation of encapsulated alkaline phosphatase egg albumin nanoparticles.[9-11]

Preparation of *Azadirachta indica* alkaline phosphatase loaded Bovine Serum Albumin nanoparticles: *Azadirachta indica* alkaline phosphatase was dissolved in n-butanol and emulsifier (coconut oil) and added dropwise to 8-10ml of bovine serum albumin solution with continuous stirring by using magnetic stirrer. After this, 25% glutaraldehyde was added for crosslinking of enzyme in prepared reaction mixture and kept overnight with stirring. The

reaction solution was centrifuged at 5000 rpm at 4^0C for 20mins. The supernatant was removed. The collected pellets were re-dispersed in diethyl ether and acetone and subjected to sonicator for 30mins. After that, it was centrifuged at 5000rpm at 4°C for 20 minutes. Enzyme assay was done in supernatant to know the % of encapsulation of encapsulated alkaline phosphatase bovine serum albumin nanoparticles.[8-12]

% of enzyme encapsulation: The % of encapsulated enzyme was calculated by determining the residual enzyme activity from reaction mixture.

% of encapsulated enzyme = Specific activity of encapsulated X 100

Specific activity of free enzyme

Characterization of *Azadirachta indica* alkaline phosphatase loaded egg albumin and bovine serum albumin nanoparticles: The prepared *Azadirachta indica* alkaline phosphatase loaded nanoparticles were subjected for characterization under Scanning Electron Microscope (SEM) for determination of their particle size.[8-12]

Result and Discussion

% of enzyme encapsulation: *Azadirachta indica* alkaline phosphatase loaded egg albumin nanoparticles has 69% retention of enzyme activity whose result was found to be similar to previous observations.[8-10] *Azadirachta indica* alkaline phosphatase loaded bovine serum albumin nanoparticles has 85% retention of enzyme activity whose observation was very much comparable to previous studies.[10-12]

Characterization of *Azadirachta indica* alkaline phosphatase loaded egg albumin and bovine serum albumin nanoparticles: Scanning Electron Microscopy was confirmed the observed size of prepared *Azadirachta indica* alkaline phosphatase egg albumin nanoparticle of 80nm whose result was comparable with previous observations (Fig. 1).[10,11]

Scanning Electron Microscopy was confirmed the observed size of prepared *Azadirachta indica* alkaline phosphatase bovine serum albumin nanoparticle of 100nm whose observation was found to comparable with previous results.[11,12] Fluorescence was also observed in prepared bovine serum albumin nanoparticles due to formation of diene adduct complex during covalent coupling with enzyme due to activation of aromatic amino acids residue e.g. tryptophan, phenyalanine and trysoine amino acid residue by using of glutaraldehyde (Fig. 2).[12]

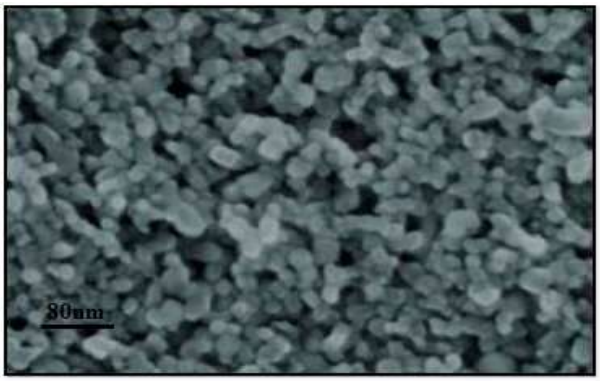

Fig. 1: SEM result of *Azadirachta indica* alkaline phosphatase loaded Egg Albumin nanoparticles (particles size of 80nm)

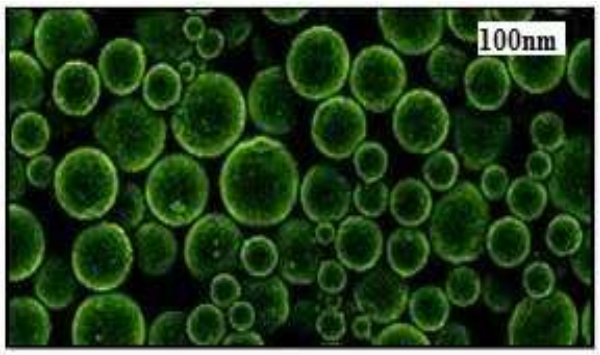

Fig. 2: SEM result of *Azadirachta indica* alkaline phosphatase loaded Bovine Serum Albumin nanoparticles (particles size of 100nm)

Conclusion

Azadirachta indica alkaline phosphatase was encapsulated into chemically modified egg albumin and bovine serum albumin nanoparticles by emulso-desolvation method with 69% and 85% of enzyme encapsulation respectively. The observed size of prepared enzyme loaded egg albumin and bovine serum albumin nanoparticles was found to be up to 80nm and 100nm respectively by using scanning electron microscope (SEM). Fluorescence was also noticed in amylase loaded bovine serum albumin nanoparticles due to formation of diene adduct complex due to activation of aromatic amine acid residues via the glutaraldehyde coupling which can be further used site specific delivery agents or vectors due to having ease in tracking of fluorescent vehicles in the metabolic system of host cells The proposed encapsulation of *Azadirachta indica* alkaline phosphatase in egge albumin and bovine serum albumin nanoparticles can be considered as eco-friendly and cost effective encapsulation procedure to load the enzymes to enhance the enzyme industrial applicability.

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