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Research Article

Alkaline Protease Mediated Bioproteolysis of Jasmine Oil Activated *Pennisetum glaucum* Amylase Loaded BSA Nanoparticles for Release of Encapsulated Amylase

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ABSTRACT

Amylase was extracted from moist seeds of *Pennisetum glaucum* and encapsulated into jasmine olive activated bovine serum albumin nanoparticle through glutaraldehyde coupling. Amylase has industrial application in food, pharmaceutical,detergent, textile and leather industries. *Pennisetum glaucum* is known for having rich source of amylase from ancient time and amylase was extracted from its 3-4 days seedlings. Bovine serum albumin was chosen as a biocompatible matrix used for the preparation of jasmine oil activated amylase loaded nanoparticles to enhance thermal stability at 70ºC for three hours 30 minutes and storage stability at 4ºC of bound enzyme for 14 months as compared to free enzyme whose thermal stability at 70ºC for 50 minutes and storage stability only for 24 hours day were found. % of enzyme encapsulation was found to be 74.7% by this emulsified method of encapsulation. However, studied optimal temperature for maximal activity for bound enzyme was found to be at 70ºC which was remarkably higher as compared to free enzyme (40ºC) to increased the industrial viability of amylase after encapsulation. Characterization of prepared nanoparticles was done by Dynamic Light scattering (DLS) and Scattering Light Microscopy (SEM). Observed size of enzyme loaded nanoparticles was observed in the range of 64.5nm to 287.5nm. Bioproteolysis of jasmine oil activated enzyme loaded bovine serum albumin nanoparticles was performed by using different units of alkaline protease (10U, 15U, 20U, 25U, 30U, 35U, 40U, 45U & 50U) for sustained and controlled release of encapsulated *Pennisetum glaucum* amylase from prepared nanoparticles. Kinetic parameters were also studied for *Pennisetum glaucum* extracted free amylase and encapsulated amylase to carry out comparative analysis for their optimum kinetic properties e.g. pH, incubation time, substrate concentration, $CaCl_2$ concentration and temperature effect.

KEYWORDS: *Pennisetum glaucum*, Amylase, Bovine serum albumin, Nanoparticles, Glutaraldehyde, Encapsulated, Emulsified, Dynamic Light Scattering (DLS) Scanning Electron Microscopy (SEM), Bioproteolysis

INTRODUCTION

Enzymes are the potential biocatalyst which have effective and conventional role in preparation of cosmetics, paper industry, textile industry, food industry, pharmaceutical industry as well as in laundry and detergents industries.[1] Now these days, usage of synergic interactions between biotechnology and nanotechnology was going to be to develop new advanced methods to immobilize the enzymes on various nanomaterials which

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reduce the operational cost.[2,3] Colloidal albumin has been considered as potential carriers of drugs to sitespecific delivery as well as apart from Albumin, commonly used natural polymers are Chitosan Gelatin Sodium alginate etc. Synthetic polymers include Polylactides (PLA), Polyglycolides(PGA), Poly (lactide co-glycolides) (PLGA), Polyanhydrides, Polyorthoesters, Polycyanoacrylates, Polycaprolactone, Poly glutamic acid, Poly (N-vinyl pyrrolidone), Poly (methyl methacrylate) were also chosen for encapsulation of drugs and enzymes.[4] Nanotechnology-inspired biocatalysts have aroused much interest to bound on various nanomaterials for excellent particle mobility during reaction.[5,6] As well as, drugs and antibodies loaded nanoparticles can be used to target antigens or bio-markers in combating cancer cells.[7,8]

Enzyme immobilization within nanomaterials was to be divided into two categories: nonspecific or specific and hence, non-specific immobilization methods generally include adsorption, non-specific covalent binding, entrapment and encapsulation.[9,10] Amylase was also encapsulated within aqueous core colloidosomes whose shell comprising polymer latex particles of diameter 153nm.[11] Cu^{2+} chelated poly(ethylene glycol dimethacrylate-n-vinyl imidazole) matrix was used for immobilization of amylase via adsorption with retained activity after immobilization 70%.[12] Poly (hydroxyethyl methacrylate-co-glycidyl methacrylate) has 76% of retention of activity of amylase after immobilization.[14]

As well as 70% of amylase retention of activity was found to be in Ca-alginate gel capsules.[15] DEAE-cellulose was also used previously for enzyme entrapment within alginate beads with 88.9% of enzyme retention after immobilization and within hydrophilic silica gel with 92.3% immobilization.[16] Scientists are still putting their efforts for improving present technologies that favour immobilization of industrial important enzymes into eco-friendly and biocompatible nanomaterials.[17] Amylases are ubiquitous enzymes produced by plants, animals and microbes, where they play significant role in carbohydrate catalysis.[18-21]

Previously, amylase was immobilized by physical adsorption onto silica, alumina, chitin, tannin sepharose, Ionic binding on Amberlite IR-120, Dowex50W ion exchange resins, DEAE-Cellulose DE-52, entrapment commonly on Calcium Alginate beads and egg albumin.[22,23,25-27]

In present work, bovine serum albumin was used as a biocompatible matrix for encapsulation of *Pennisetum glaucum* amylase which was biochemically engineered by various chemical modifiers such as butanol, emulsified with jasmine oil and glutaraldehyde and then, it was used to load enzyme into prepared nanomaterials via covalent coupling like formation of a water-in-oil emulsion and subsequent stabilization of the protein droplets by using glutaraldehyde as a cross-linking agent.[28-34]

Albumin is biodegradable, non-toxic in nature and its emulsified entrapment or encapsulation was henceforth used for immobilization of *Pennisetum glaucum* Sustained and controlled release of loaded enzyme was achieved by using alkaline protease successfully.[16,28,30,33] Characterization of prepared enzyme loaded bovine serum albumin nanoparticles was done by DLS (Dynamic Light Scattering) and SEM (Scanning Electron Microscope).

Gradual breakdown of bound enzyme form olive oil driven nanopreparation was mediated by using varying units of alkaline protease (10U, 15U, 20U, 25U, 30U, 35U, 40U, 45U, 50U) to study their biodegradation.[34] The studied successful sustained release of enzyme from biochemically modified bovine serum albumin has widened the industrial application of bound amylase due to having increased stability and sustained usability as compared to free enzyme.[33,35] Kinetic parameters of free and encapsulated amylase were studied for their optimal pH (1.5-11.5), incubation time (20mins- 4 hours), substrate concentration (0.50%-1.5%), $CaCl_2$ concentration (1%-10%) and temperature (5°C-

100°C) by carrying out the amylase activity by dinitrosalicyclic acid method. [11,13,16,35-37]

MATERIALS AND METHODS

Extraction of amylase from seeds of *Pennisetum glaucum*

Moist seeds of *Pennisetum glaucum* were homogenized by adding 4-6 ml of 0.05 M sodium phosphate buffer (pH 7.0) per gram of seeds and centrifuged for 15mins at 4°C at 5000rpm. Supernatant was collected which contained crude amylase extract and then stored at 4°C. [16,33,36,43]

Amylase assay

Enzyme assay was done by using 1 %(w/v) starch solution in which 0.5 ml enzyme extract was added and incubated at 37°C for 20 minutes. 2 ml of dinitrosalicylic acid was added and the mixture was boiled at 100°C for 5 minutes. Absorbance was taken at 570nm.[11,10,35,36,43]

Study of kinetic properties

The free amylase and jasmine oil activated encapsulated amylase were characterized for their kinetic properties i.e. effect of pH, effect of incubation time, effect of CaCl2 concentration, effect of substrate concentration and effect of temperature for their optima. [13,16,23,26] The effect of pH on activity of free and encapsulated amylases was studied by performing amylase assay at different pH using acetate and phosphate buffer by varying pH from 1.5 to 11.5.

The effect of time on the activity of free and encapsulated amylases was studied by performing the enzyme assay at different time (20minutes to 4 hours). Optimal substrate concentration needed for maximal enzyme activity for free and encapsulated amylases which were estimated by incubating the reaction mixture at different concentrations of starch solution (0.50%-1.50%). The effect of $CaCl_2$ on activity for free and encapsulated amylases was studied by performing the enzyme assay at different concentrations (1%-10%).

Optimal temperature needed for free and encapsulated amylases for maximal activity was studied by incubating the reaction mixture for 15minutes at different temperature (5°C–100°C). These kinetic properties of free and encapsulated amylases were determined by performing dinitrosalicyclic acid method for its activity test.

Preparation of *Pennisetum glaucum* amylase loaded jasmine oil activated BSA nanoparticles

Jasmine oil bath was prepared with a solution of 25% glutaraldehyde and 2.6 ml of n-butanol and 50ml of jasmine and was kept on magnetic stirrer. 50U *Pennisetum glaucum* amylase was added in 8-10 ml of bovine serum albumin and taken in a 10 gauge syringe. It was dispersed in prepared olive oil bath and incubated overnight with stirring on the magnetic stirrer at room temperature. Next day, it was centrifuged at 5000rpm at 4°C for 20 minutes. Supernatant was removed. Pellet was washed with cold diethyl ether and acetone. The pellet was re-dispersed into acetone in bath and sonicated for 12 minutes. Enzyme assay was done in supernatant to know the % of encapsulation of amylase in jasmine oil activated BSA (bovine serum albumin) nanoparticles.[16,33,36]

% of encapsulation of enzyme into jasmine oil activated BSA nanoparticles

The % of encapsulated enzyme was calculated by determining the residual enzyme activity from reaction mixture in which encapsulation of amylase was done into jasmine oil activated BSA (bovine serum albumin) nanoparticles. Amylase assay was performed by using dinitrosalicylic acid.[16,37-40]

$$\% \text{ of encapsulated enzyme} = \frac{\text{Specific activity of encapsulated}}{\text{Specific activity of free enzyme}} \times 100$$

Characterization of *Pennisetum glaucum* amylase loaded jasmine oil activated BSA nanoparticles

The prepared amylase loaded nanoparticles were subjected for characterization under Dynamic Light Spectroscopy (DLS) and

Scanning Electron Microscope (SEM) for determination of their particle size. [16,37,38]

Bioproteolysis of jasmine oil activated BSA nanoparticles for controlled release of encapsulated *Pennisetum glaucum* amylase by using alkaline protease

2 mg jasmine oil activated amylase loaded emulsified bovine serum albumin nanoparticles were taken in test tubes with reaction solution of different units of alkaline protease (10U, 15U, 20U, 25U, 30U, 35U, 40U, 45U and 50U) which is considered excellent protease used with detergents in washing for combating washing conditions such as pH, temperature and salt effect.[31-34] The reaction tubes were incubated at 4°C for overnight. Next day enzyme assay was done at 570nm using dinitrosalisylic acid method. This study of enzyme assay for different units of alkaline protease was done from 1st day till 7th day.[33,45-47]

RESULT AND DISCUSSION

% of encapsulation

Jasmine oil activated bovine serum albumin nanoparticles of encapsulated *Pennisetum glaucum* amylase has 79.7% retention of enzyme activity whose result was found to be similar to previous study in which *Cicer arietinum* amylase had 81.34 % retention activity of enzyme into chemically modified bovine serum albumin microspheres and lesser than 99% of Glucose oxidase was immobilized in bovine serum albumin nanoparticle using mustard oil.[45,46] As well as % of immobilization was coined for amylase into activated alginate beads, chitosan and DEAE-cellulose.[13-17]

Characterization of *Pennisetum glaucum* amylase loaded jasmine oil activated BSA nanoparticles

The prepared enzyme loaded bovine serum albumin a nanoparticle in test mixture was characterized by observed first sharp peak which confirmed size of nanoparticles in the approximate range of 2nm to 5nm followed by second peak for 300nm to 600nm using Dynamic Light Spectroscopy (DLS) (Fig 1).[46] The observed size of enzyme loaded bovine serum albumin nanoparticles under Scanning Electron Microscope was found to be in the range of 64.5nm to 287.5nm which were found to be comparable with previous observations whose observed nanoparticle size was in range of 50nm to 700nm.[16,43-46] Fluorescence was also observed in prepared 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).[47]

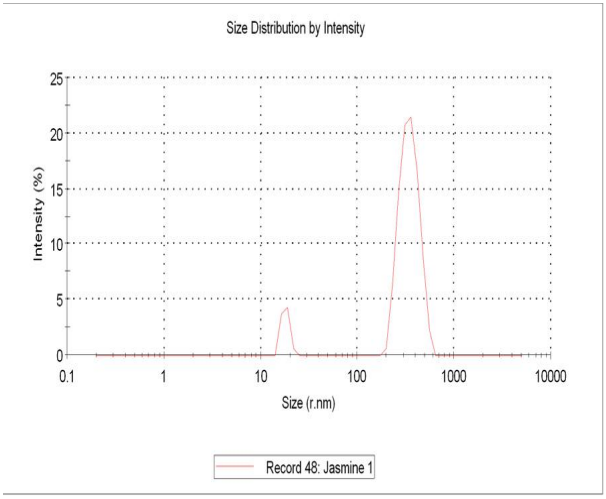

Fig 1: DLS of *Pennisetum glaucum* laoded jasmine oil activated BSA nanoparticles

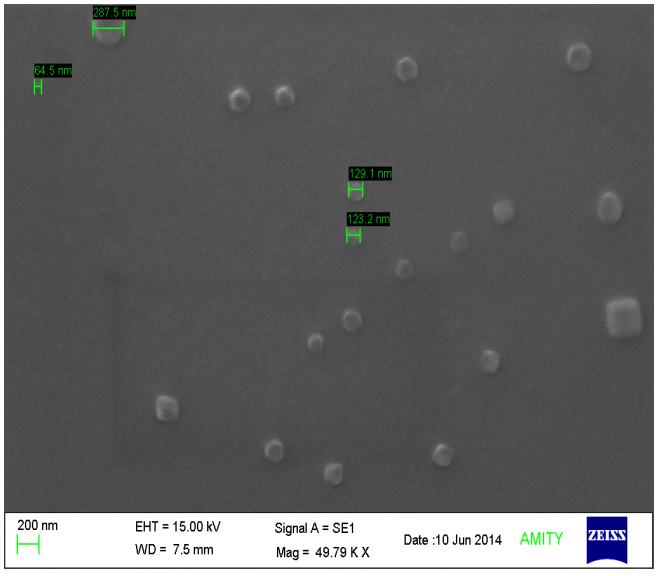

Fig 2: SEM of *Pennisetum glaucum* amylase loaded jasmine oil activated BSA nanoparticles

Studied kinetic properties

Optimal pH was observed 11.5 which was similar for both, free and encapsulated amylase whose results were slightly comparable to previous studies. [16,28,33,44-46] Optimal incubation time for free enzyme was found to be at 50 minutes and 30 minutes for encapsulated enzyme whose results were also comparable with previous observations. [16,28,30,33,35] Optimal substrate concentrations of free enzyme and encapsulated enzyme was found to be 1.5% for both by varying starch concentration from 1%-1.5% whose results were also pretty comparable to previous finding.[12,33,35,41,42] Optimal $CaCl_2$ concentration for maximal amylase activity for free and encapsulated amylase was found to be same which was 5% whose results were similar to previous results.[33,35,41-44,46] Optimal temperature for maximal activity for free enzyme and encapsulated enzyme was observed 40ºC and 70ºC respectively whose results were also comparable to previous results.[16,28,33,41,43,45,46] It was also found that after encapsulation, thermal stability and storage stability were enhanced for bound enzyme (at 70ºC for 3 hours 30 minutes and 14 months respectively) as compared to free enzyme (at 70ºC for 50 minutes and 24 hours only respectively) which were also fairly comparable to previous fndings (Table 1).[16,33,36,40,44-46]

Kinetic Parameters	Free amylase	Encapsulated amylase
Optimal pH	11.5	11.5
Optimal Temperature	40°C	70°C
Thermal Stability at 70°C	Up to 50 minutes	Up to 3 hours 30 minutes
Optimal time of incubation	50 minutes	30 minutes
Optimal Substrate concentration	1.5%	1.5%
Optimal $CaCl_2$ concentration	5%	5%
Storage stability at 4ºC	Up to 24 hours	Up to 14 months

Table 1. Kinetic Parameters of free and jasmine oil driven activated BSA encapsulated *Pennisetum glaucum* amylase

Bioproteolysis Study

Bioproteolysis of *Pennisetum glaucum* amylase loaded jasmine oil activated BSA nanoparticles was performed by incubating 2 mg of encapsulated enzyme loaded BSA nanoparticles with alkaline protease (10U, 15U, 20U, 25U, 30U, 35U, 40U, 45U, 50U) overnight at 4°C. The study was carried out for consecutive 7 days and 35U and 45U of alkaline protease were found to be effective to achieve controlled and sustained release of encapsulated *Pennisetum glaucum* amylase form jasmine oil activated bovine serum albumin nanoparticles (Fig 3). First 4 days, the release of the enzyme was negligible while it increased over the next 2 days. From 3th day onwards, the enzyme activity was slightly increased with noticeable change till 5th day. From 5th day, it was remarkably increased till day 7th. Enzymatic functionality was observed even after 2 weeks of protease incubation owing to highly stable prepared nanoparticles. [3,16,33,40,42,45-47]

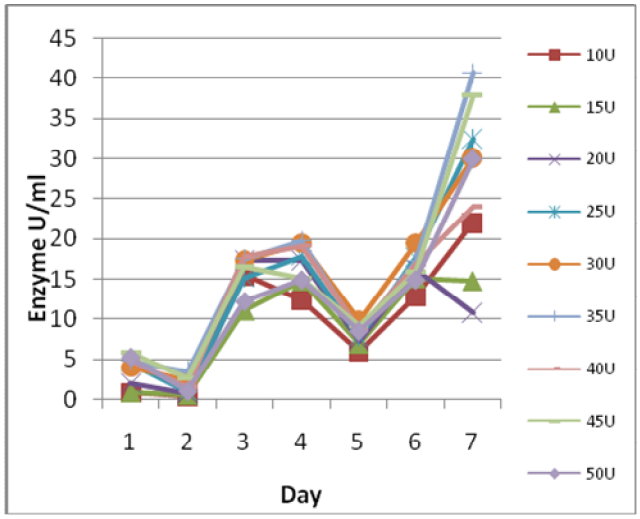

Fig 3: Bioproteolysis of jasmine oil activated BSA nanoparticles with different units of alkaline protease (10U, 15U, 20U, 25U, 30U, 35U, 40U, 45U, 50U) for controlled release of encapsulated *Pennisetum glaucum* amylase

CONCLUSION

Pennisetum glaucum amylase was encapsulated into jasmine oil activated bovine serum albumin nanoparticles with 74.7% of retention of enzyme activity. Kinetic properties were also studied for free amylase and encapsulated

amylase and it was found that noticeable modification in optimal temperature for encapsulated amylase which was observed at 95ºC as compared to free enzyme (40ºC) after the encapsulation.

The Characterization of prepared nanoparticles was performed by Dynamic Light Spectroscopy (DLS) showed the presence of nanoparticles and the exact size was confirmed by observing under the Scanning Electron Microscope (SEM). The observed size of prepared nanoparticles was found to be in teh range of 64.5nm to 287.5nm.

Fluorescence was also noticed in jasmine oil activated amylase loaded BSA nanoparticles due to formation of diene adduct complex which was introduced due to activation of aromatic amine acid residues via the biochemically treatment of glutaraldehyde coupling.

The result of alkaline protease mediated bioproteolysis was showed that 35U and 45U of alkaline protease was found to be effective in controlled and sustained release of bound enzyme from jasmine oil activated bovine serum albumin nanoparticles. The encapsulated *Pennisetum glaucum* amylase was found to have increase storage stability for 14 months when stored at 4°C and excellent reproducibility and thermal stable up to 70ºC for 3 hours 30 minutes which was remarkably higher as compared to free enzyme with storage stability for 24 hours only and having thermal stability at 70ºC for 50 minutes only.

Hence, this controlled bioproteolysis study of jasmine oil activated bovine serum albumin nanoparticles of encapsulated *Pennisetum glaucum* amylase may have fairly good industrial application in beverages industries as a eco-friendly and bio-active saccharification agent for preparation of fructose syrups and maltose syrups as well as in having its inevitable industrial role in paper and leather industries for the treatment of cellulose/ starch and leather for desizing of leather/ wood pulp along with bioprocessing of various types of natural and synthetic fabrics too.

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