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Preparation, Biodegradation of Coconut Oil Driven Chemically Modified Bovine Serum Albumin Microparticles of Encapsulated *Cicer arietinum* Amylase and Study of Their Application in Washing Detergents

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ABSTRACT

In present work, *Cicer arietinum* amylase was encapsulated by emulsification through covalent coupling by glutaraldehyde into chemically modified bovine serum albumin. Biodegradation of coconut oil driven emulsified bovine serum albumin encapsulated *Cicer arietinum* amylase was carried out by the alkaline protease for its controlled and sustained release of encapsulated enzyme from prepared microparticles of encapsulated *Cicer arietinum* amylase and its stability increased up to 6 months as compared to free enzyme. Its biodegradation was carried out by the using different concentration of alkaline protease (5U, 10U, 15U, 20U, 25U, 30U, 35U, 40U). Further, this coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase with alkaline protease were used with detergents for washing of stained cloths which have rust, gel pen ink, grease and chocolate strains. These chosen strains are very commonly present on uniforms of school going children which are very tough upon drying, hence, not to be easily vanish with well known brand detergents upon in one wash. But, the mixture solution of coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase along with alkaline protease were used with detergents powder for washing of these dry tough strains (rust, gel pen ink, grease and chocolate strains) leads to vanishing these strains very fast with absolute clear results were found as compared to results of washing of stained cloths with detergents only.

Keywords: *Cicer arietinum* amylase, bovine serum albumin, Glutaraldehyde, Encapsulated, Emulsified.

INTRODUCTION

Amylase is known for starch saccharification and catalyses the starch hydrolysis producing maltose which has wide range of applications in food, textile, leather, fermentation, detergents and paper industries. [1]

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Albumin is biodegradable, non-toxic in nature and its emulsified entrapment or encapsulation was previously used for immobilization of enzymes into bovine serum albumin microspheres in which the active gradient is dispersed in a hydrophilic or non-polymer layer. [2] Hence, this type of entrapment was responsible for controlled and sustained release of encapsulated active ingredient. These encapsulated microspheres have been used as carriers in the delivery of drugs, antigens, hormones, enzymes and genes. [3-4] Bovine serum albumin was previously used for preparation of the microspheres and other chemically modified entrapment or encapsulation of industrially important

active ingredients and enzymes was done. [5, 3] In the U.S., 50% of liquid detergents, 25% of powder detergents preparations have free amylase and proteases along with all powdered bleach additives in which added enzymes are helpful in breakdown of hard stains of cloths with conventional surfactants e.g. amylase catalyzes the hydrolysis of starch-based stains form cloths into soluble limited dextrins and oligosaccharides as well as proteases leads to breakdown of protein based strains into soluble small polypeptide and individual amino acids units released upon washing. Thus, these stains are removed from cloths are removed like physically cut off from the surface of the fabric piece by piece with the enzyme acting as scissors. In our work, bovine serum albumin was used as a biomatrix for encapsulation of *Cicer arietinum* amylase which was chemically modified by butanol, emulsified with coconut oil and glutaraldehyde. We studied various kinetic parameters of free and immobilized enzymes such as effect of pH (2-12), effect of temperature (10°C-100°C), effect of incubation time (5mins-25mins), effect of substrate concentration (0.25%-1.5%) and effect of CaCl2 (2%-10%) by carrying out the enzyme activity by dinitrosalicyclic acid method. [2, 6-8] Biodegradation of encapsulated enzyme is done with alkaline protease for release of encapsulated enzyme from coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase and subjected to washing of the stained clothes with detergents. [5-9] The applications of coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase was studied with chosen samples of washing powders (Ariel, Surf excel, Wheel and Tide) for washing of dry tough stained clothes pieces (rust, gel pen ink, grease and chocolate strains). [9-10]

MATERIALS AND METHODS

Extraction of amylase enzyme from *Cicer arietinum* seeds

Cicer arietinum seeds were soaked for 3 days, crushed in the pestle mortar and then, homogenized by adding 4-6 ml of 0.05 M sodium phosphate buffer (pH 7.0) per gram of seeds. The enzyme extract was filtered in two layers of muslin cloth. Centrifuged for 15mins at 4°C at 5000rpm. Supernatant was collected which contained crude amylase extract and then stored at 4°C. [8]

Amylase assay

Enzyme assay was done by using 1 %(w/v) starch solution in which 0.5 ml enzyme extract was added. It was 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. [8, 11-13]

Partial purification by ammonium sulphate precipitation and its dialysis

8 g of ammonium sulphate salt per 10 ml of enzyme was subjected to salt precipitation in ice bath. Ammonium sulphate salt was added pinch wise to the amylase crude extract and stirred with glass rod for 5-15 minutes after every addition of salt. After the complete addition of ammonium sulphate salt was stirred for 30 minutes. It was incubated for 1 hour and centrifuged for 25 minutes at 4°C at 10000rpm. Supernatant was discarded and the pellet was dissolved in 0.05 M sodium acetate buffer (pH 3) and kept at -20°C. Dialysis was done after ammonium sulphate precipitation to remove the impurities. Dialysis tubing was rinsed in 10 ml distilled water. One end of the tube was clutched with a thread and 10 ml of sample was added into it and other end was also clutched called dialysis bag which was placed in 10mM Tris HCl buffer. It was incubated for 24 hours at 4°C. The pure sample was poured out after incubation and kept at 4°C. [14-15]

Study of kinetic properties

The free enzyme and immobilized enzyme are characterized for their different kinetic properties i.e. effect of pH, effect of temperature, effect of incubation time, effect of $CaCl_2$ concentration and effect of substrate concentration. [7-16] The effect of pH on activity of free and immobilized enzymes was studied by performing enzyme assay at different pH using phosphate buffer. pH was varied from 2 to 12. The effect of time on the activity of free and immobilized enzyme was studied by performing the enzyme assay at different time (5 minutes-25 minutes). Optimal substrate concentration needed for maximal enzyme activity for free and immobilized enzymes which were estimated by incubating the reaction mixture at different concentrations of starch solution (0.25%-1.50%). The effect of $CaCl_2$ on activity for free and encapsulated enzymes was studied by performing the enzyme assay at different concentrations (2%-10%). Optimal temperature needed for free and immobilized enzyme for maximal activity was studied by incubating the reaction mixture for 15 minutes at different temperature (10°C – 100°C). These kinetic properties of enzyme were determined by performing dinitrosalicyclic acid method.

Encapsulation of *Cicer arietinum* amylase into coconut driven chemically modified Bovine Serum Albumin

Oil bath was prepared with a solution of 25% glutaraldehyde and 2.6 ml of n-butanol and 50ml of coconut oil. 5000U *Cicer arietinum* amylase was added in 8-10 ml of bovine serum albumin was taken in a 10 gauge syringe. It was dispersed in prepared oil bath and kept overnight in incubator shaker at 37°C. 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 4-5 times. It was dried and stored at 4°C. Enzyme assay was done in supernatant to know the % of encapsulation of enzyme in coconut driven bovine serum albumin microsphere. [2-3, 5]

% of encapsulated enzyme into coconut driven chemically modified bovine serum albumin

The % of encapsulated enzyme was calculated by determining the residual enzyme activity from reaction mixture in which encapsulation of enzyme was done into coconut driven chemically modified bovine serum albumin was done. Amylase assay was performed by using dinitrosalicylic acid. [2-5]

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

Biodegradation study of coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase by using alkaline protease

2 mg coconut driven microsphere of encapsulated entrapped *Cicer arietinum* amylase was taken in test tubes with reaction solution of different units of alkaline protease (5U, 10U, 15U, 20U, 25U, 30U, 35U, 40U) and 8 ml of sodium acetate buffer (pH 7). The reaction tubes were incubated at 4°C for overnight. Next day enzyme assay was done at 570 nm using DNS method. This study of enzyme assay for different units of alkaline protease was done from 1st day till 10th day. [10-15]

To study application of coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase in washing of stained cloths

The coconut driven chemically modified bovine serum albumin encapsulated *Cicer arietinum* amylase was used in vanishing of dry tough stained cloths (rust, gel pen ink, grease and chocolate strains) in combination of various detergents such as Ariel, Tide, Surf and Wheel. Different stained cloth (rust, gel pen ink, grease and chocolate strains) were chosen and dipped in reaction solution of 2 mg of prepared bovine serum albumin encapsulated enzyme with 1ml of detergent solution in petri plates. Each sample of stained cloths was tested with all the four detergents only and with the combination mixture of coconut driven chemically modified bovine serum albumin encapsulated *Cicer arietinum* amylase too for carrying out its comparative washing study. [9-10]

RESULTS AND DISCUSSION

Percentage of encapsulation

Coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase has 80% retention of enzyme activity whose result is similar to previous study in which *Glycine max* amylase had 85% retention activity of enzyme into chemically modified bovine serum albumin in figure 1. [2, 16]

Studied kinetic properties

The pH was varied from 2 to 12. In present study, the result obtained for optimal pH of coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase had maximum activity at pH 6.0 which was lower than the free enzyme 8.0 whose results were comparable to previously studies. [14-16-17] The optimal incubation time was found for encapsulated enzyme was 25 minutes which is higher than the free enzyme (15 minutes) which are pretty similar to previous finding. [7-15] Optimal substrate concentrations of encapsulated enzyme was found 1.25% which is higher than the free enzyme 1% which are also pretty comparable to past finding. [8-13] Optimal $CaCl_2$ concentrations was found 4% for encapsulated enzyme and 8% for free enzyme whose results was similar to previous results. [7-15] Optimal temperature was found 60°C for encapsulated enzyme as compared to free enzyme which was 50°C. It was found that after encapsulation, thermal stability was increased as compared to free enzyme which are also sharply comparable to previous finding in figure 1 and in Table 1. [5-14- 18- 19-20]

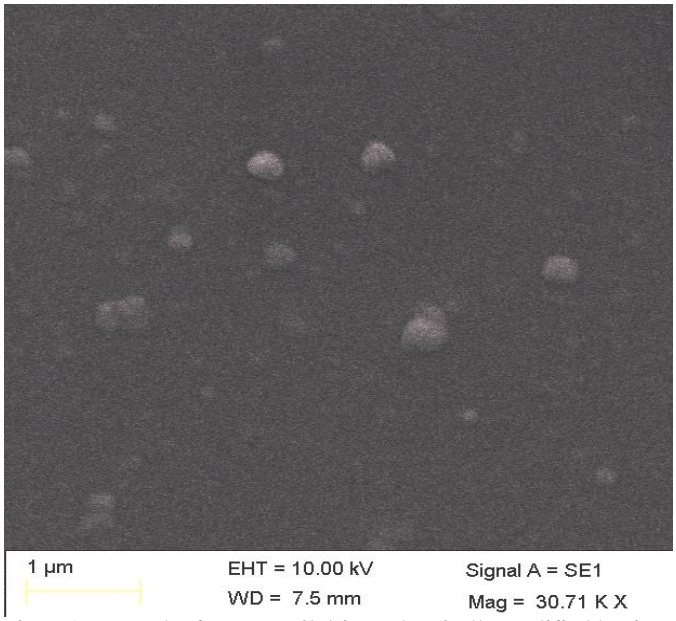

Fig. 1: SEM result of coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase

Table 1: Kinetic Parameters of free and encapsulated *Cicer arietinum* amylase into coconut driven chemically modified bovine serum albumin mixture of nanoparticles and microparticles

Kinetic Parameters	Free amylase	Encapsulated amylase
pH	8.0	6.0
Temperature	50°C	60°C
Thermal Stability at 70°C	15 minutes	5-6 hours
Time of incubation	15 minutes	25 minutes
Substrate concentration	1.00%	1.25%
$CaCl_2$ concentration	8%	4%

Biodegradation Study

Biodegradation of coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase was performed by incubating 2 mg of encapsulated enzyme with alkaline protease (5U, 10U, 15U, 20U, 25U, 30U, 35U, 40U) overnight at 4°C.The study was carried out for 10 days. We found 40U of alkaline protease helps to achieve

controlled and sustained release of encapsulated *Cicer arietinum* amylase form coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase in figure 2. Biodegradation pattern for each incubation mixture showed an increase from 1st to 5th day and remain constant till 10th day whose results were comparable with previous study in which entrapped *Vigna radiata amlyase,* was released from chemically modified bovine serum albumin with 30U of alkaline protease solution. [2, 5, 15-16, 18]

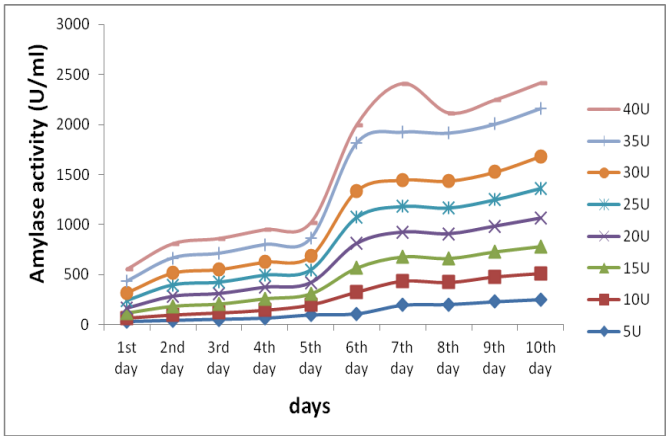

Fig. 2: Biodegradation study of coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase with different concentration of alkaline protease (5U, 10U, 15U, 20U, 25U, 30U, 35U, 40U)

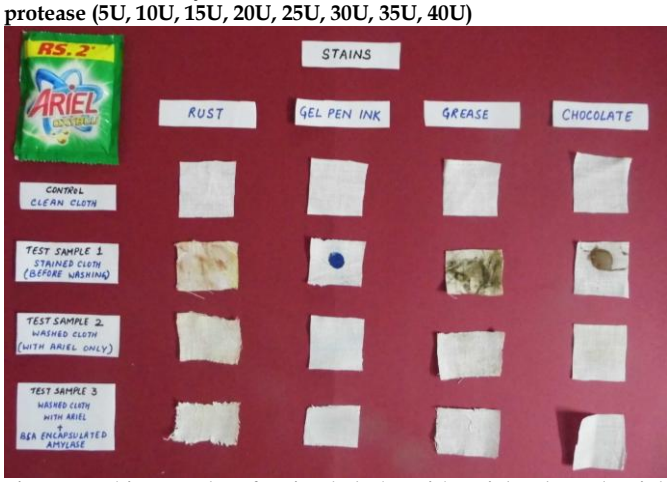

Fig. 3: Washing results of stained cloths with Ariel only and Ariel with coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase

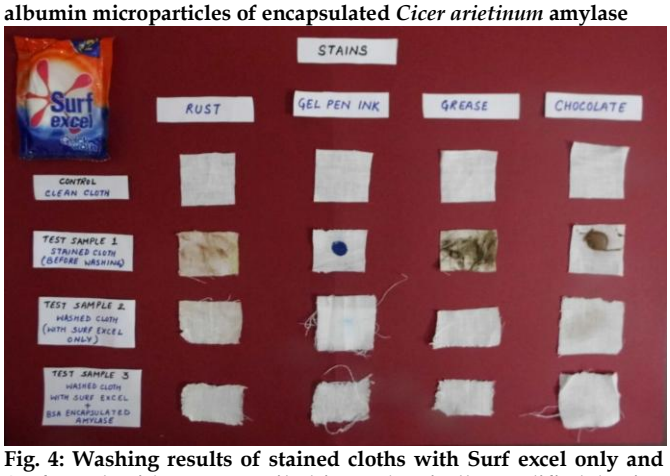

Fig. 4: Washing results of stained cloths with Surf excel only and Surf excel with coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase

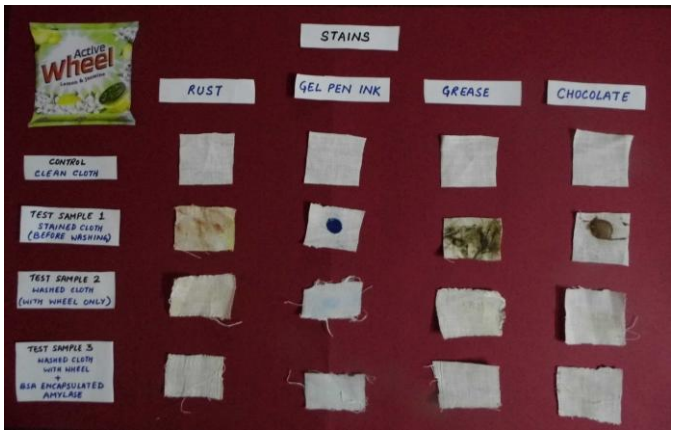

Fig. 5: Washing results of stained cloths with Wheel only and Wheel with coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase

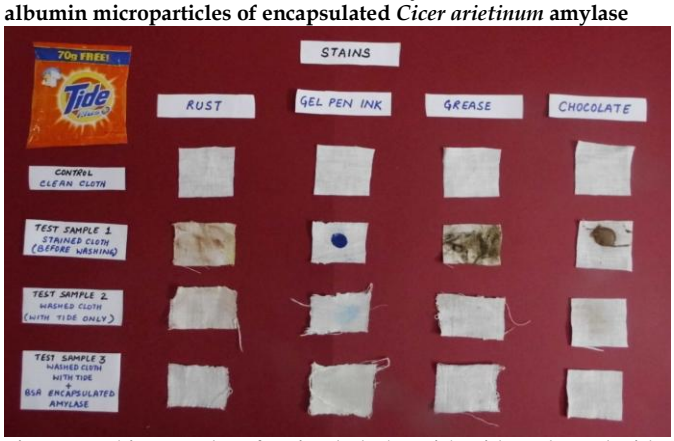

Fig. 6: Washing results of stained cloths with Tide only and Tide with coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase

Applications of coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase in washing of stained cloths

The application of prepared bovine serum albumin microparticles of encapsulated enzyme was studied with four different samples of detergent solutions of Ariel, Surf excel, Wheel and Tide to remove rust, gel ink pen, grease and chocolate stains form clothes. The encapsulated prepared bovine serum albumin microparticles of encapsulated enzyme with detergents was used for washing which efficiently removed the chosen dry tough strains from stained cloths after washing and enhance the efficiency of detergents for washing dry and hard stains from clothes. Because these kinds of tough strains used to be not to vanish completely if we washed with these chosen detergent samples only in one wash only. Among the four samples of detergent solutions, Ariel detergent with bovine serum albumin microparticles of encapsulated enzyme solution was the best and fairly well as we compared to others chosen detergents samples followed by Surf excel and Wheel and the least worked out with Tide in figure 3, 4, 5 & 6. In previous reports, Ariel with entrapped enzyme was showed better washing results as compared to other detergents which were similar to our washing results. [10-11] The use of this prepared bovine serum albumin microparticles of

encapsulated enzyme with detergents has advantages such as to reduce labor, cost and time too. After encapsulation, storage and thermal stability of enzyme was increased and it was quite efficient in washing off the dried and tough stains on cloths when used with detergents. [9-11, 21]

Cicer arietinum amylase is encapsulated into coconut driven chemically modified bovine serum albumin microparticles with 80% of encapsulation. Kinetic properties were also changed for encapsulated enzyme as compared to free enzyme after emulsified encapsulation into bovine serum albumin microparticles. The optimal pH was observed higher for free enzyme (8.0) as compared to encapsulated enzyme (6.0), the optimal $CaCl_2$ concentration was higher for free enzyme (8%) than encapsulated enzyme (4%). The optimal incubation time for encapsulated enzyme (25 minutes) was higher as compared to free enzyme (15 minutes) while optimal substrate concentration was for free enzyme (1%) higher than for encapsulated enzyme (1.25%) and optimal temperature was higher for encapsulated enzyme (60°C) as compared to the free enzyme (50°C). Thermal stability of encapsulated enzyme at 70°C was found higher for 5-6 hours as compared to free enzyme (only for 15 minutes). The biodegradation study was showed that 40 U of alkaline protease helps to controlled and sustained release of encapsulated mixture of *Cicer arietinum* amylase loaded bovine serum albumin nanoparticles and microparticles as well as 40U alkaline protease was found to be effective when used for the removal of stains from cloths having dry and tough strains of rust, gel pen ink, grease and chocolates when used with chosen detergents (Ariel, Surf Excel, Wheel and Tide). Ariel was the most efficient detergent used with encapsulated enzyme. The *Cicer arietinum* amylase after coconut oil driven emulsified encapsulated bovine serum albumin microparticles was found to have increase storage stability for 6 months when stored at 4°C and excellent reproducibility. Thus, this coconut oil driven chemically modified bovine serum albumin microparticles of encapsulated *Cicer arietinum* amylase may have fairly good industrial application in beverages industries as a eco-friendly saccharification agent for preparation of fructose syrups and maltose syrups, in paper industries for the treatment of cellulose/ starch, in leather industries for desizing of leather/ various types of natural and synthetic fabrics too as well as may have excellent application in cosmetic, alcoholic/ non-alcoholic/ fizzy drinks/ canned juices beverages, food, pharmaceutical industries, in detergents industries too.

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