Original Article

Biodegradation of Almond Oil Driven Bovine Serum Albumin Nanoparticles for Controlled Release of Encapsulated *Pearl millet* Amylase

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ABSTRACT

Amylase plays vital role in food, textile, leather, pharmaceutical and detergent industries due to its efficacy and industrial viability. In our work, amylase was extracted from *Pearl millet* (*Pennisetum glaucum*) and encapsulated in bovine serum albumin nanoparticles by chemical modification with almond oil, n-butanol and glutaraldehyde. Hence, bovine serum albumin was found to be favourable matrix for encapsulating extracted amylase due to its well known exploitable properties e.g. biocompatibility, non-antigenicity and non-toxicity. Biodegradation of almond oil driven enzyme loaded bovine serum albumin nanoparticles was performed by using different units of alkaline protease (10U, 15U, 20U, 25U, 30U, 35U, 40U, 45U & 50U) to study controlled release of encapsulated amylase. Kinetic parameters were also studied for *Pearl millet* extracted amylase and encapsulated amylase for their comparative analysis for optimal pH, incubation time, substrate concentration, $CaCl_2$ concentration and temperature for maximal enzyme activity which was tested by dinitrosalicylic acid method. Thermal stability at 70ºC was found to be 3 hours 30 minutes of encapsulated amylase and 50 minutes only for free amylase. Storage stability at 4ºC was observed 12 months for encapsulated amylase and one day only for free enzyme. Characterization of prepared bovine serum albumin was done by Dynamic Light scattering (DLS) and Scanning Electron Microscopy (SEM).

Keywords: *Pearl millet*, Amylase, Bovine serum albumin, Almond oil, Nanoparticles, Glutaraldehyde, Encapsulated, Emulsified, Biodegradation, Dynamic light scattering, Scanning electron microscopy.

INTRODUCTION

Amylase is ubiquitous enzyme produced by plants, animals and microbes and it was first isolated by French chemists Anselme and Jean François from germinating barley and named it "diastase" in 1833. It plays vital role in carbohydrate metabolism.[1] Plant sources such as Barley, millets, *Glycine max*, *Cicerarietinum, Cecineri, Pisumsativum* are rich sources of amylase and microbial production of thermostable amylase is very commercially popular in food, fermentation, detergent, paper, pharmaceutical and textile industries.[2,3] To improve their economic feasibility of various industrial processes, enzymes are immobilized onto organic and inorganic solid support to increase enzyme stability, reusability, reproducibility and operational threshold.[4-6] In recent years, various immobilization techniques were used to bind amylase in which entrapment was considered most preferable method due to make the bound enzyme more stable towards microenvironment of matrix and protects enzyme from microbial contamination.[7-9] Immobilized Amylase have been widely used in different industrial processes due to its high relevance in food, fermentation, detergent, paper and textile industries.[10] Polymeric nanoparticles were selected for entrapment or encapsulate enzymes onto a matrix which can be chitosan, gelatin, sodium alginate, ficoll, sepharose and albumin which are used for controlled release of the drugs that make them ideal for cancer therapy, delivery of vaccines, and delivery of targeted antibiotics as more suitable targeted drug delivery vehicles.[10-12] Various nanoparticle based drug targeting vehicles were especially used for parenteral administration that expected to be biodegradable, rapid and reasonably cheap to prepare to posses high loading capacity for prolonged circulation at specific target sites in the body.[13] Scientists are still developing new techniques to immobilized the commercially important enzymes into nanoparticles to make them more thermostable and storage stable up to expected level.[14,15] Amylases is carried out carbohydrate metabolism to hydrolysis starch into maltose and limit dextrins.[16-18] In recent years, amylase was immobilized on to silica, alumina, chitin, tannin sepharose , Ionic binding on Amberlite IR-120, Dowex50W, DEAE-Cellulose-DE-52, calcium alginate beads by physical adsorption.[17-19] Recently, erodible drug delivery system was used for sustained release of mefenamic acid as non-steroidal anti-inflammatory drug for its prolonged circulation at targeted site using melt granulation process as per directed USP standards.[20] Hence, our work was compiled enzyme technology with nanotechnology to prepare bioactive stable enzyme loaded nano-encapsulation that can be successfully used in food science as echo-friendly preservative, non-toxic drug delivery trigger to deliver the encapsulated enzymes to get efficient targeted delivery with increased desirable benefits to make that methodology more ideal for cancer therapy, delivery of vaccines, and delivery of targeted antibiotics. Thus, Amylase was extracted from *Pearl millet* and bovine serum albumin was used as a biomatrix for encapsulation which was chemically modified by butanol, almond oil and glutaraldehyde to prepare enzyme loaded nanoparticles[21,23-25] Characterization of enzyme loaded bovine serum albumin nanoparticles was performed by Dynamic light Scattering (DLS) and Scanning electron Microscopy (SEM). Gradual and sustained release of bound amylase from prepared bovine serum nanoparticles was studied by using different units of alkaline protease at various units (10U, 15U, 20U, 25U, 30U, 35U, 40U, 45U, 50U).[14,23,26] Kinetic parameters of free and

encapsulated enzyme was carried out to study effect of pH (1.5-11.5), effect of temperature (5°C-100°C), effect of incubation time (20mins- 4 hours), effect of substrate concentration (0.50%-1.5%) and effect of $CaCl_2$ (1%-10%) was studied by carrying out the enzyme activity by dinitrosalicyclic acid method.[25,27] Accordingly, prepared amylase loaded bovine serum albumin nanoparticles may be used as echo-friendly and non-toxic pharmaceutical preservatives in fructose syrups and targeted drug delivery tool in treatment of acute and chronic pancreatitis and pancreatic carcinoma.

MATERIALS AND METHODS

Extraction of amylase from *Pearl millet*

3-4 days seedlings of *Pearl millet* seeds were homogenized in pestle mortar by adding 4-6 ml of 0.05 M sodium phosphate buffer (pH 7.0) per gram of seeds. It was centrifuged for 15minutes at 4°C at 5000rpm. Supernatant was collected which contained crude amylase extract and stored at 4°C.[14,25,28,35]

Amylase assay

Amylase assay was performed by using 1% starch solution in which 0.5 ml enzyme extract was added. It was incubated at 37°C for 20 minutes. After incubation, 2 ml of dinitrosalicylic acid was added and the mixture was boiled at 100°C for 5 minutes. Absorbance was taken at 570nm.[7,14,27,28,35]

Study of kinetic properties

The free amylase and encapsulated amylase were characterized for their different kinetic properties i.e. effect of pH, incubation time, $CaCl_2$ concentration, substrate concentration and temperature on amylase activity.[14,17,25,28] The effect of pH on activity of free and immobilized enzymes was studied by performing enzyme assay at different pH using acetate and phosphate buffer by varying pH from 1.5 to 11.5. The effect of incubation time on the activity of free and immobilized amylase was studied by performing the enzyme assay at different time (20minutes to 4 hours). Optimum substrate concentration for free and encapsulated enzymes was estimated by incubating the reaction mixture at different concentrations of starch solution (0.50%-1.50%). The effect of $CaCl_2$ on activity of free and encapsulated enzymes was studied by performing the enzyme assay at different concentrations (1%-10%). Optimum temperature needed for free and encapsulated enzyme for maximal activity was studied by incubating the reaction mixture for 15minutes at different temperature (5°C–100°C). These kinetic properties of enzyme were determined by dinitrosalicyclic acid method for amylase activity test.

Preparation of amylase loaded bovine serum albumin nanoparticles

Almond oil bath was prepared with 25% glutaraldehyde and 2.6 ml of n-butanol and 50ml of almond oil and was kept on magnetic stirrer. 50U extracted amylase was added in 8-10 ml of bovine serum albumin and taken in a 10 gauge syringe to disperse in prepared almond oil bath. It was incubated overnight with stirring at room temperature. Next day, it was cold centrifuged at 5000rpm 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. Amyalse assay was done in supernatant to know % of amylase encapsulation in almond oil driven bovine serum albumin nanoparticles.[14,25,28]

% of encapsulation

% of encapsulated enzyme was calculated by estimating the residual enzyme activity from reaction mixture in which

encapsulation was done into almond oil driven bovine serum albumin nanoparticles. Amylase assay was performed by using dinitrosalicylic acid.[14,29-32]

% of encapsulated enzyme = Specific activity of encapsulated enzyme / Specific activity of free enzyme X 100.

Characterization of amylase loaded bovine serum albumin nanoparticles

The prepared nanoparticles were subjected to Dynamic Light Spectroscopy (DLS) and Scanning Electron Microscopy (SEM) for determination of their particle size.[14,29,30]

Biodegradation study of almond oil driven bovine serum albumin nanoparticles for controlled release of encapsulated amylase

2 mg almond oil driven enzyme loaded emulsified bovine serum albumin nanoparticles were taken with reaction solution of different units of alkaline protease (10U, 15U, 20U, 25U, 30U, 35U, 40U, 45U and 50U) which was coined a excellent protease used with detergents in washing for combating washing conditions such as pH, temperature and salt effect.[32,33,34,35] The reaction tubes were incubated at 4°C for overnight. Next day, amylase activity was estimated by dinitrosalisylic acid method. That study of enzyme assay for different units of alkaline protease was performed consecutively from 1st day till 12th day.[25,37,38]

RESULT AND DISCUSSION

Percentage of encapsulation

Estimated percentage of amylase encapsulation was 82% which was pretty similar to the previous study in which bound *Cicer arietinum* amylase had 81.34 % enzyme retention activity in to chemically modified bovine serum albumin and 80% in egg albumin nanoparticles.[14,38]

Characterization of amylase loaded bovine serum albumin nanoparticles

The prepared enzyme loaded bovine serum albumin a nanoparticles were characterized for their by Dynamic Light Spectroscopy (DLS) (Fig. 1) and Scanning Electron Microscopy (Fig. 2). Measured average size of nanoparticles by DLS was in the range of 0.96nm to 478.3nm and by SEM, it was observed in the range of 56.2nm to 84.3nm which were pretty comparable with previous studies (Fig. 2).[14,38]

Studied kinetic properties

The kinetic parameters were studied for free and immobilized amylase Optimum pH was found to be 11.5 for free and encapsulated amylase for maximal activity and no change in pH was studied whose results were slightly comparable to previous studies.[14,23,25] Encapsulated amylase was showed appreciable change in optimum temperature (70^0C) for maximal enzyme activity as compared to free amylase (40^0C) which indicated its resistant towards high.[36-38] Optimum substrate concentrations for free and immobilized amylase was found 1.50% and 1.25% respectively whose findings were also pretty comparable to previous finding.[7,14,25,33,35] Optimum incubation time of free enzyme and encapsulated enzyme for maximal activity was recorded 50 minutes and 3 hours and 30 minutes respectively whose results were pretty comparable with previous results.[14,33,35,37] Optimum $CaCl_2$ concentration was found to be 4% for encapsulated enzyme and 5% for free enzyme whose results were similar to previous results.[14,23,25,33,35] Hence, it was also found that after encapsulation, thermal stability was also enhanced for encapsulated amylase which was at 70ºC for 3 hours 30 minutes as compared to free enzyme which was observed at 70ºC for 50 minutes only whose results were comparable with previous observation (observed thermal stability in the average

range of 60ºC to 65ºC). Storage stability were also increased for encapsulated amylase 12 months as compared to free enzyme which was for one day only whose results were also sharply comparable to previous observations in the average of 6 to 8 months (Table 1).[14,25,28,32,37,38]

Biodegradation study

Biodegradation of amylase loaded bovine serum albumin nanoparticles was performed by incubating 2 mg of enzyme loaded nanoparticles with chosen units alkaline protease (10U, 15U, 20U, 25U, 30U, 35U, 40U, 45U, 50U) overnight at 4°C. The study was carried out for consecutive 14 days and 40U of alkaline protease was found to achieve controlled and sustained release of encapsulated amylase form prepared bovine serum albumin nanoparticles (Fig 3). First 4 days, the release of encapsulated amylase was negligible and it was slightly increased over the next 2 days. From 6th day onwards, the enzyme activity was stable and no considerable change was observed from day 6 till day 12thand fairly constant till day 14th.[3,14,25,33,34,37,38]

CONCLUSION

In the present study, *Pearl millet* amylase was encapsulated into almond oil driven emulsified bovine serum albumin nanoparticles via glutaraldehyde coupling with 81.17% of retention of enzyme activity.The Characterization of prepared amylase loaded nanoparticles was performed by Dynamic Light Spectroscopy (DLS) showed the presence of nanoparticles and the size was observed under the Scanning Electron Microscope (SEM). The observed average size of nanoparticles by DLS was in the range of 0.96nm to 478.3nm and by SEM, it was observed in the range of 56.2nm to 84.3nm. The result of biodegradation study were performed for consecutive 14 days which showed that 40U of alkaline protease was found to be more efficient for controlled and sustained release of encapsulated amylase from almond oil driven bovine serum albumin nanoparticles. It was observed that in the initial 7 days, the release of amylase was negligible. From the 12th onwards, there was an increase in amylase activity and at 13th day the release of encapsulated enzyme was noticed at its highest.The bound amylase was found to have increase storage stability for 12 months when stored at 4°C with excellent reproducibility and its thermal stability was observed at 70ºC for 3 hours 30 minutes which was remarkably higher as compared to free enzyme whose storage stability was found for one day only and thermal stability was observed at 70ºC for 50 minutes only. Hence, the spectrum of encapsulated amylase into almond oil driven emulsified bovine serum albumin nanoparticles was coined for remarkable scientific approach due to having its excellent storage stability, thermal stability and reusability which leads to expand its industrial application in automatic dishwashing liquids, textile desizing as bio-active detergent additive, pharmaceutical industry as nontoxic preservative or agent and in paper industry as echo-friendly saccharifying agent too.

REFERENCES

1. Sivaramakrishnan S, Gangadharan D, Madhavan K, Ricardo C, Pandey A. (2006). α-Amylases from Microbial Sources. *Food Technol. Biotechnol*. 2006; 44 (2):173-184.
2. Bordbar AK, Omidiyan K, Hosseinzadeh R. Study on interaction of a-amylase from Bacillus subtilis with cetyltrimethyl ammonium bromide. Colloids Surf. B, *Biointerfaces*, 2005; 40: 67–71.
3. Van der Maarel MJEC, Van der Veen B, Uitdehaag JCM, Leemhuis H, Dijkhuizen L. Properties and applications of starch converting enzymes of the α-amylase family, *J. Biotechnol*. 2002; 94:137-155.
4. Bajpai AK, Bhanu S. Immobilization of alpha-amylase in vinyl-polymer-based

interpenetrating polymer Networks Colloid. *Polym. Sci*. 2003; 282: 76-83.
5. Ricardo R, Morais, Aline M, Pascoal, Samantha S,Caramori, Flavio M. Lopes, and Kátia F. Fernandes. Immobilization of -Amylase onto Luffaoperculata-Fibers. *Enzyme research*. 2013; 13(2): 134-140.
6. Mohamed A, Abdel-Naby, Amal M. Hashem, Mona A. Esawy, Ahmed F. Abdel-Fattah. Immobilization of Bacillus subtilis α-amylase and characterization of its enzymatic properties, Department of Chemistry of Natural and Microbial Products. *Microbiol Res*. 1998; 153: 1-7.
7. Kennedy JF. Enzyme technology. In Biotechnology; Kennedy, J. F., Cabral, J. M. S., Eds.; VCH Publ.-VerlagsgesellschaftmbH: Weinheim, Germany 1987, 7a.
8. Demirkan E, Immobilization of B. amyloliquefaciens α-amylase and comparison of some of its enzymatic properties with the free form. Romanian Biotechnological Letters. *Tuk J Biol*. 2011, 35:705-712.
9. Om P, Nivedita J, Immobilization of a Thermostable -Amylase on Agarose and Agar Matrices and its Application in Starch Stain Removal. *World Applied Sci J*. 2011; 13(3): 572-577.
10. UrrusunoR, Calvo P, Remunan C, Alonso M.J, Enhancement of nasal absorption of insulin using chitosan nanopartilces. *Pharm. Res*. 1999; 16: 1576-1581.
11. Farrugia C A, Grover M J, Kreuter, Colloidal Drug aqueous solutions: Designing a nanoparticulate formulations, Delivery Systems. *J. Pharm. Pharmacol*. 1999; 51: 643–649.
12. Rani K, Paliwal S, A review on targeted durg delivery: its entire focus on advanced therapeutics and diagnostics. *Sch. J. App. Med. Sci*. 2014; 2(1C): 328-331.
13. Kapoor D K, Bansal A, Sharma R, Dhawan S, Advanced nanomedicine: present contributions and future expectations. *American J. Phytomed. Clin. Therapeutics*. 2013; 1(2): 124-139.
14. Rani K, Chauhan C, Biodegradation of Cicer Arietinum Amylase loaded Coconut oil driven Emulsified Bovine Serum Albumin Nanoparticles and their application in Washing Detergents as Eco-Friendly Bio-Active Addictive. *World J Pharmacy & Pharmaceutical Sci*. 2014; 3(12): 924-936.
15. Sharma KR, Preparation of emulsified encapsulated nanoparticles of bovine serum albumin of bound glucose oxidase and their application in soft drinks/non-alcoholic beverages. *Biotechnol& Biomaterials*. 2012; 2(2): 1-5.
16. Sivaramakrishnan S, Gangadharan D, Nampoothiri K M, Soccol C R and Pandey A, Alpha -Amylase from microbial sources- an overview on recent developments. *Food TechnolBiotechnol*. 2006; 44: 173-184
17. Rani K, A novel comparative assessment of extracted amylase activity in germinating and germinated seeds of Cicerarietinum, Cecineri and Pisumsativum. *Int J Pure &Applied Sci*. 2014; 2(2): 191-193.
18. Van der Maarel MJEC, van der Veen B, Uitdehaag JCM, Leemhuis H, Dijkhuizen L, Properties and applications of starch converting enzymes of the α-amylase family. *J. Biotechnol*. 2002; 94: 137-155.
19. Ricardo R. Morais, Aline M. Pascoal, Samantha S. Caramori, Flavio M. Lopes, and Kátia F. Fernandes, Immobilization of -Amylase onto Luffaoperculata Fibers. *Enzyme research*. 2013; 13(2): 134-140.
20. Achulatla H, Rao V U, and Sudhakar M, Development and study of an erodible matrix drug delivery platform for sustained release of non-steroidal anti-inflammatory drugs using melt granulation process. *American J. Advanced Drug Delivery*. 2014; 2(4): 576-584.
21. Kennedy J F, Enzyme Technology,in Biotechnology, Kennedy J F and Cabral J M S (Eds.), VCH Publ, (1987) p. 7a.
22. Demirkan E, Immobilization of B. amyloliquefaciens α-amylase and comparison of some of its enzymatic properties with the free form, Romanian Biotechnological Letters. *Tuk J Biol*. (2011) 35:705-712.
23. Rani K, Aqueous two phase purification of Vignaradaita amylase and its characterization, *Int J Cur Pharm Rev & Res*, (2012), 3(3): 47-53.
24. Rani K, Immobilization of Vigna radiate, Vignamungo, Cicerarietinum (white) and Cicerarietinum (Black) amylases onto variety

of activated fabrics, *Int J Life Sci and Pharma Res*, (2012), 1(3): 124-133.
25. Rani K, Mehta V. Preparation, Biodegradation of Coconut Oil Driven Chemically Modified Bovine Serum Albumin Microparticles of Encapsulated Cicerarietinum Amylase and Study of Their Application in Washing Detergents. *Int. J. Pharm. Sci. Drug Res*. 2014; 6(4):351-355.
26. Williams DF, Zhong SP. Biodeterioration/ biodegradation of polymeric medical devices in situ. *Int. Biodetermination & Biodegradation*. 1994; 34: 95-130.
27. Rani K, Production of amylase and alkaline phosphatase, Verlag: Lambert Academic Publishing Gmbh & Co. KG, Germany, 2012; pp 1-56.
28. Rani K. Emulsified Entrapment of Glycine Max B-amylase into Chemically Modified Bovine Serum Albumin and Study its Applications in Detergents. *International Journal of Advanced Biotechnology and Research*. 2012; 3(2):591-595.
29. Dubes A, Parrot-Lopez H, Abdelwahed W, Degobert G, Fessi H, Shahgaldian P, Coleman A. Scanning electron microscopy and atomic force microscopy imaging of solid lipid nanoparticles derived from amphiphilic cyclodextrins. *European J Pharmaceutics & Biopharmaceutics*. 2003; 55(3): 279–282
30. Zhaoa X, Lva L, Pana B, Zhanga W, Zhanga S, Zhanga Q, Polymer-supported nanocomposites for environmental application: A review. *Chem Engg J*. 2011; 170(2–3): 381–394.
31. Wykes J R, Dunnill P, Lilly M D. Immobilization of Alpha Amylase by attachment to soluble support materials. *Biochimicaet Biophysica Acta-Enzymology*. 1971; 250: 522-529.
32. Rani K. Immobilization of Vignamungo β-amylase onto NaCl and NaNO3 treated woven Bombyxmori silk fabrics. *Asian J Biol& Life Sci*. 2012; 1(2): 96-100.
33. Rani K, Extraction and study of kinetic parameters of variety of sprouted pulses β-amylases. *Int J Pharm and Life Sci*. 2012; 3(8): 1895-1898.
34. Sharma KR. Preparation of emulsified encapsulated nanoparticles of bovine serumalbumin of bound glucose oxidase and their application in soft drinks/non-alcoholic beverages. *Biotechnol & Biomaterials*. 2012; 2(2): 1-4.
35. Rani K. Comparative study of kinetic parameters of bacterial and fungal amylases. *J Bio-Innovation*. 2012; 3: 48-57.
36. Rani K, Rana R and Dutt S. Review on latest overview on proteases. *Int J Current Life Sci*. 2012; 2(1): 12-18.
37. Rani K, Pant N and Chauhan C, Biodegradation of chemically modified egg albumin micropreparation for controlled release of bound vignamungo amylase and their application in fabric desizing as cost effective bio-active preparation. *Int J Pharma & Bio Sci*. 2015; 6(1): 1101-1111.
38. Rani K, Applicative biodegradation study of egg albumin nanospheres by alkaline protease for release of encapsulated cicerarietinum amylase in washing as bio-active detergent additive. *World J Pharmaceutical Res*. 2015; 4(1): 1218-1230.

Table 1. Studied kinetic parameters of free and encapsulated amylase

Kinetic parameters	Free amylase	Encapsulated amylase
Optimum pH	11.5	11.5
Temperature Optima	40°C	70°C
Thermal Stability at 70°C	Up to 50 minutes	Up to 3 hours 30 minutes
Optimum time of incubation	50 minutes	30 minutes
Optimum Substrate concentration	1.5%	1.25%
Optimum $CaCl_2$ concentration	5%	4%
Storage stability at 4ºC	Up to 1 day	Up to 12 months

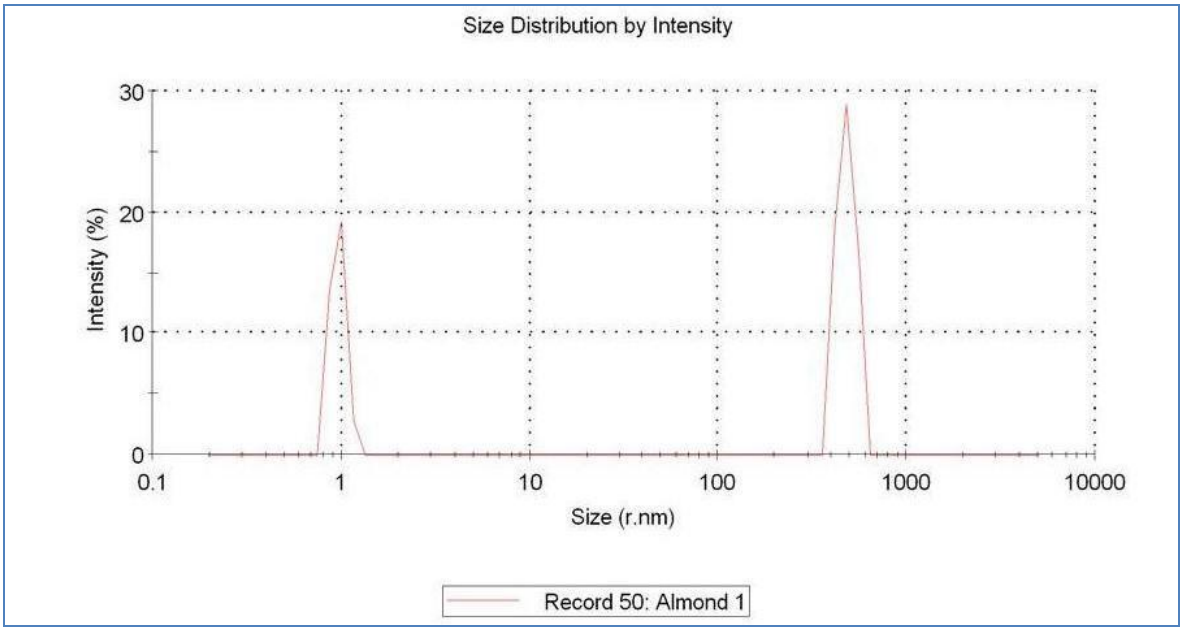

Figure 1. DLS of almond oil driven amylase loaded bovine serum albumin nanoparticles

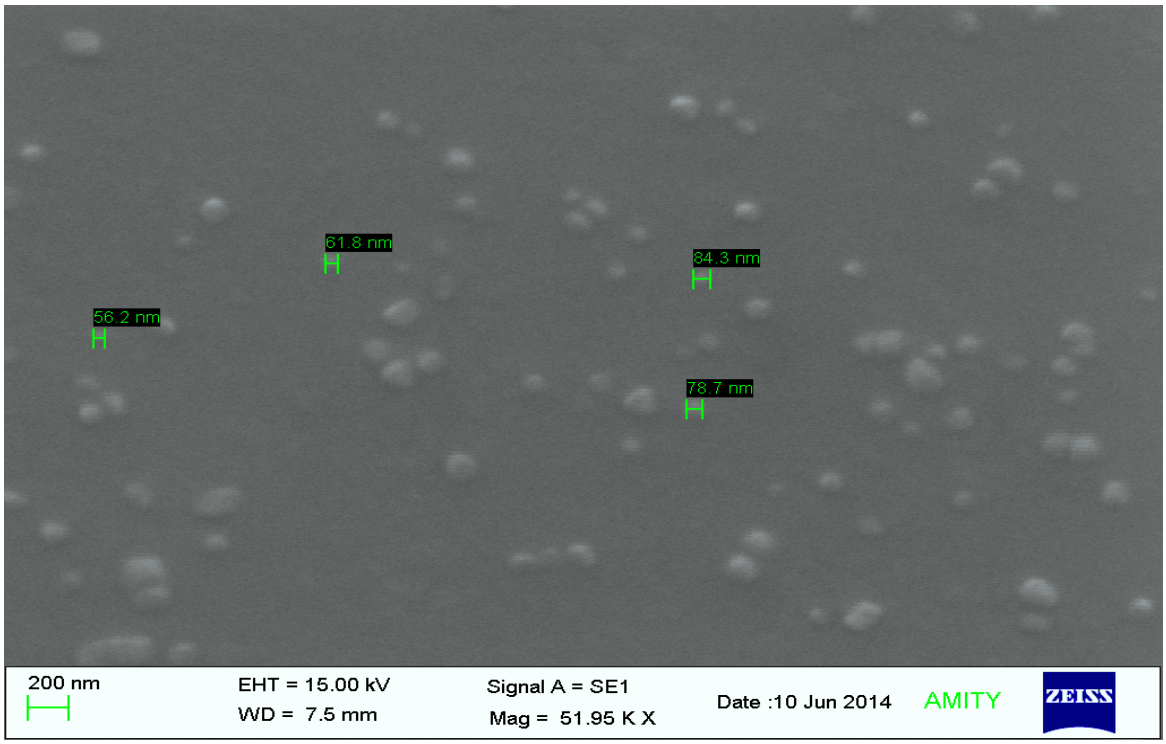

Figure 2. SEM of almond oil driven amylase loaded bovine serum albumin nanoparticles

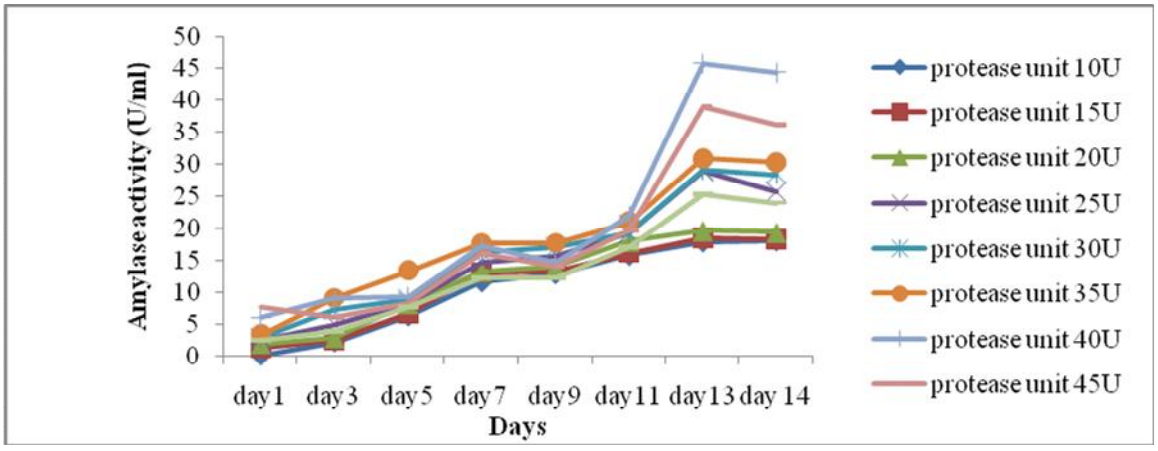

Figure 3. Biodegradation study of amylase loaded bovine serum albumin nanoparticles with different concentration of alkaline protease