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Heena Meroliya
Department of Chemistry, Abasaheb Garware College, Pune, Maharashtra, India

Vaishali Patil
Department of Chemistry, Abasaheb Garware College, Pune, Maharashtra, India

Nilesh Jadhav
Department of Chemistry, Yashwantrao Mohite College of Arts, Science and Commerce, Bharati Vidyapeeth, Pune, Maharashtra, India

Vikram Deshmukh
Department of Chemistry, Yashwantrao Mohite College of Arts, Science and Commerce, Bharati Vidyapeeth, Pune, Maharashtra, India

Vidya Kalyankar
Department of Chemistry, Abasaheb Garware College, Pune, Maharashtra, India

Sharda Dagade
Department of Chemistry, Yashwantrao Mohite College of Arts, Science and Commerce, Bharati Vidyapeeth, Pune, Maharashtra, India

Shobha Waghmode
Department of Chemistry, Abasaheb Garware College, Pune, Maharashtra, India

Correspondence
Shobha Waghmode
Department of Chemistry, Abasaheb Garware College, Pune, Maharashtra, India

Lipase mediated silver nanoparticles as a robust catalysts for VLPC assisted trans-esterification reaction

Heena Meroliya, Vaishali Patil, Nilesh Jadhav, Vikram Deshmukh, Vidya Kalyankar, Sharda Dagade and Shobha Waghmode

Abstract
Lipase enzyme contains natural substrates like triacylglycerols. Lipase belongs to the class of serine hydrolases & therefore, do not require any cofactor, in human digestive system or pancreatic lipase. It is also used in cheese fermentation and most commonly used as biocatalysis to breakdown of fats and oils. lipase also used in esterification and transestericaion reaction. Therefore now a days lipase enzyme widely used in industries. Immobilized or conjugated nanoparticles with lipase tend to give faster reactions than only enzyme. By using nano conjugates transesterification reaction carried out with various substrate, in this work lipase enzyme was used to synthesize Agnp by green approach using natural source like delliana indica and coconut.

Keywords: triacylglycerols, biocatalysis, immobilization, esterification

1. Introduction
Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) member of hydrolase family, constitutes a very important group of enzymes due to their excellent activity and stability in both aqueous and non-aqueous media. Lipase enzyme shows many applications in detergent industry, paper industry, biodiesel formation, lipolysis, blood clotting, cation-anion binding, nano particle synthesis, cosmetics, biosensors, degreasing of leather. Lipases perform essential roles in the digestion, transport and processing of dietary lipids (e.g. triglycerides, fats, oils) in most living organisms. Most lipases act at a specific position on the glycerol backbone of a lipid substrate (small intestine). For example, human pancreatic lipase (HPL), which is the main enzyme that breaks down dietary fats in the human digestive system, converts triglyceride substrates found in ingested oils to monoglycerides and two fatty acids. Recently, seed lipases have been the focus of much attention as biocatalysts.

2. Experimental
Isolation of Enzyme
Preparation of enzyme: 20 ml of coconut/ delliana indica extract was soaked in 100 mL distilled water and small amount of 40 ^{0}C chilled acetone and kept it for overnight. Next day filtered through cotton cloth and we get supernatant approximately 150mL as crude extract.

2.1Ammonium sulphate Fractionation
Ammonium sulphate treatment was given to above crude extract with 80% fractionation. For this 80% fractionation 76g/100ml of ammonium sulphate was added and stirred well using magnetic stirrer by maintaining its temperature in bath. This 80% fraction was kept overnight to get precipitate of low molecular weight proteins which were discarded centrifuging it at speed of 4000 RPM for 20 minutes, then precipitate obtained was collected and further characterized by SDS-PAGE and UV Spectrometer [9].

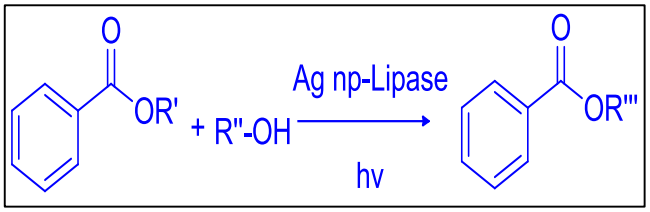
2.2 Dialysis
Above precipitate was dissolved in 20mL Tris-HCl buffer (10 mM, pH 8.5) and dialyzed overnight against the same buffer using sterilized dialysis bag then followed by distilled water till it get free from sulphate. Removal of excess ammonium sulphate was checked by $BaCl_2$ test. This dialyzed enzyme was used as partially purified enzyme and used for further application [11].

2.3 Synthesis of nanoparticles

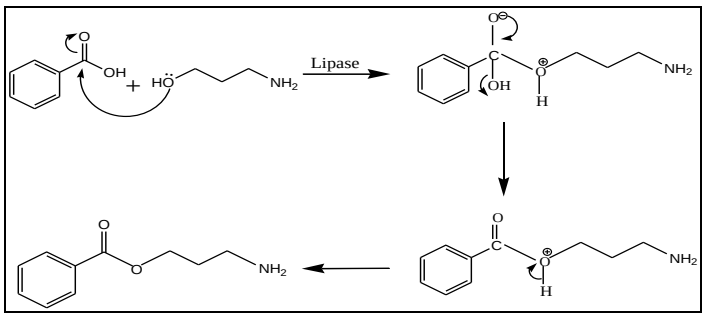
A) Synthesis of nps of silver by using lipase enzyme

1) 200ml of $AgNO_3$ (1mmol) solution in distilled water.5ml of lipase enzyme was added as reducing agent and sonicated for 15 minutes then kept for overnight and blackish-brown coloured particles were appeared. Then centrifuse the particles and dry under vacuum [10].

2.4 Esterification reaction by photocatalysis



Mechanism of reaction



Substrate used

3. Result

Table 1: Percentage yeild checked by GC

Sr. No.	Acid/Ester	Glycerol	Lipase	AgNp	% yield
1)	1 mMol Galic acid	5 ml	5 ml	0.080gm (mMol)	60
2)	1mMol Trimesic acid	5 ml	5 ml	0.080gm (mMol)	65
3)	1mMol Adipic acid	5 ml	5 ml	0.080gm (mMol)	55
4)	1 mMol Dimethyl malonate	5 ml	5 ml	0.080gm (mMol)	60

1) Characterization

Uv-Spectrometer

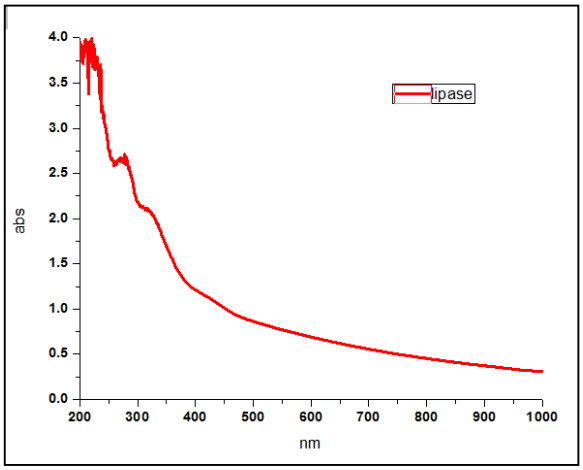


Fig 1: UV of blank purified lipase enzyme (peak at 289 nm)

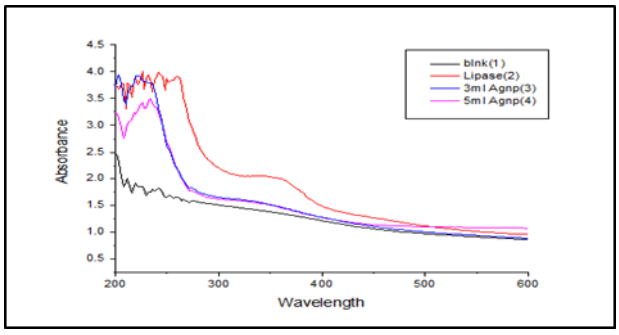


Fig 2: UV-Visible spectra of Ag nanoparticles with various concentrations of lipase enzyme

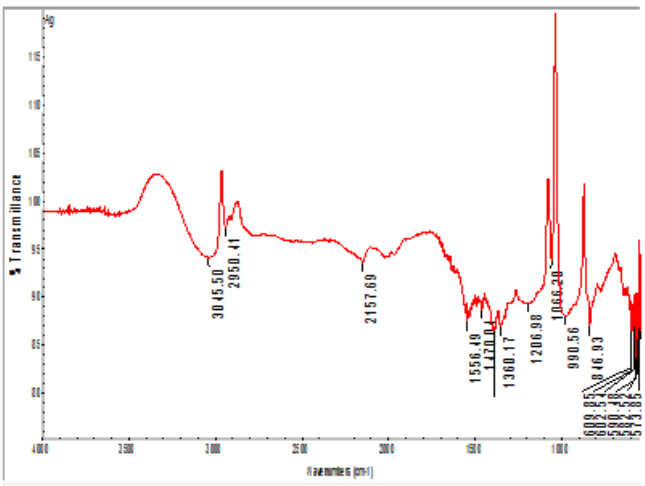


Fig 3: IR of Ag nps

It shows peak at 1556, 1360 and 1066 $cm^{-1.}$

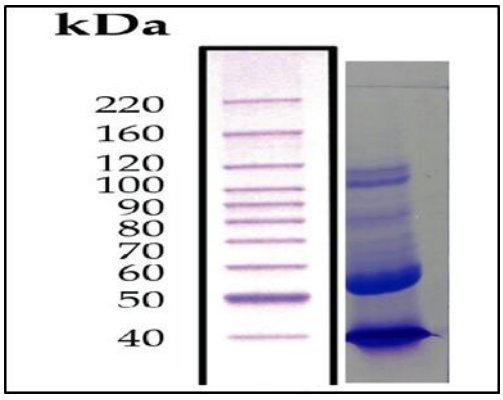


Fig 4: pH dependant stability of enzyme from graph it indicates that lipase enzyme at basic pH 8-10.

4. Conclusion

Lipase enzyme was successfully isolated by coconut and deliana indica, which is further purified and characterized by SDS-PAGE. It shows, the molecular wt 39kD. This enzyme was used for the synthesis of Ag nanoparticles. Using this Agnps esterification of various acids were carried out by using simple visible photocatalytic light and characterized by using gas chromatography.

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