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Immobilization of Alkaline Metalloprotease for Stain Removal From Fabric Using Magnetic Nanoparticles

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Abstract
Protease enzymes play an important role invarious type of industries such as food, pharmaceutical, cosmetic ,detergent , leather , meat and textile industry etc. According to recent studies protease immobilization onto magnetic nanoparticles is effective in fabric cleaning. Magnetic nanoparticles increase the effectiveness of enzyme immobilization and its stability. Further there is an increment in enzyme reusability as well as improvement in enzyme recovery because of their low toxicity factor. Although, implementation of these nanomaterial are restricted due to the efficacy of their recovery processes. With the help of an external magnet these magnetic nanoparticles can be easily removed from the reaction mixture, therefore it is recommended to use solution of these nanoparticles. Immobilized enzyme compare to free enzyme in solution are more vigorous and more resistant to environmental changes. Diversity of an immobilized system allows an easy recovery of both enzyme and product. Nanomaterials for enzymes immobilization consists of novel and interesting matrices as they have an advantage of having high surface to volume ratio. The behavior of enzymes immobilized on these matrices is due to their Brownian motion effect.

Keywords: alkaline metalloprotease immobilization, magnetic nanoparticles, detergent, cleaning efficiency, reusability

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1)Introduction:

Enzymes are almost found in dynamic nature of protein that acts as biological catalysts in all living organism such microorganism to human. Thus it hascapability to accelerate the reaction without transmute or utilized theirselves by chemical reaction.

1.1)Use of Alkaline Protease in Various Industries:

The protease is utmost important play role in both research laboratories and industriestherefore protease are accounting global 65% sale in worldwide markets. They are used in various industries such food, feed, textiles, and leathers as well as in biomedical diagnostic. The alkaline proteasethat has been exploited in detergent in industries which are main considering to increases the efficiency and reusability in house holding. In addition the stain of starch, lipids and protein which comes the dirt on cloths but dirt stain that have been removed through the normally processes at high temperature boiled water with continuously stirring but it method have time taken and high costly thus it also has reduced the cloths quality.The table 1 shows below application of alkaline protease.

Table.1 Different industrial application of protease

Product Names	Industrial Use	Application
Savinase	Detergent industry	Removal protein containing stains
Maxatase	Detergent industry	Removed adhesive disk
Papain	Meat industry	Tenderization of meat
Alcalase	Textile industry	Silk degumming
SEB Soak	Leather industry	Soaking

2) Literature of review:

The metal coordinated hydrogels nanofibres that have been used for preparing co-immobilized enzyme through magnetic nanoparticles. in this paper objective enzyme CRL used as guest protein for good aqueous dispersity, low price and other unique properties citric acid modified magnetic iron oxide nanoparticles has been widely used for immobilizing enzymes. According to studies, the result shown that relative activity of citric acid modified magnetic nanoparticles immobilized CRL and provided the more activity compare to free CRL at pH 10 and another hand nearly 1 folded at 30 C for 50 minutes as well as it provided the more stability that nanofibres. The metal nucleotide nanofibres plays significant role in improve stability of CRL of biocatalysts. As results that magnetic nanoparticles immobilized enzyme through the co-entrapped method. In which Zn/ AMP provided the support to catalysts like as nanofib All the more critically, NMCPs have magnificent versatile self-amass properties and can entrapped

different guest particles, including proteins and nanoparticle. On account of their nanofibrous properties, it's extremely hard to recoup these immobilized catalysts by customary strategies. Accordingly, it would be interesting to build $Zn^{2+/}$AMP nanofibers with attractive reaction.

3)Immobilization :

Enzyme immobilisation offers an excellent basis for increasing the enzyme's accessibility to the enzyme Substrates with higher turnover for a substantial period of time overtime. Several natural and synthetic aids have been given they were assessed for their enzyme immobilisation effective ness. Immobilized enzymes are now favoured over free enzymes, due to their prolonged availabil ity, the counterpart that curtails redundant systems of downstream and purification.

Either the immobilised biocatalysts can be Enzymes or cells that are entire (Kawaguti et al.2006) . An enzyme Immobilization is enzyme containment at a level, (matrix / support) separate from t hat of substrates and substrates and Merchandise.Normally, neutral polymers and inorganic mate rials are used as matrices from the carrier. Apart from being costeffective, an the optimum matrix must contain features such as inertness, Physical strength, durability, regenerability, capacity for regeneration Improving the specificity or activity of the enzyme and decreasing the substance.

4)Various techniques implementation in immobilization :

Enzymes for immobilisation often appear to be more soluble than dissolved enzymes.so that it have slightly drawback of immobilization techniquies such as slightly loss the enzyme activity, change the kinetic of enzyme and diffusion or mass transfer limitation.But to prevent the contamination, loss the completely activity as well as it help to improve the reusability. All these limitation and advantages, immobilization techniquies has been used for which could be improve in processes implementation. Several techniquies has been implementing in immobilization techniques that are mentioned the below:

a) Adsorption techniques
b) Covalent binding techniques
c) Encapsulation techniquies
d) Entrapment techniques
e) Cross-linking techniques

Since 1940s, Invertase in fermentation processes was initially used in form of immobilization for split sucrose into glucose and fructose [Trevan M 1980]. When conc. H_2SO_4 hydrolysis

processes was not evolved. Furthermore it has also developed protein engineering and advanced screening method to which helps to find that region we need to enhance of particular reaction [Tosa T, Mori T et al 1966]. The most advantage of immobilization processes by which an enzyme can be exceed time more than one used in industrial applications. However immobilization processes cost depends upon their supported matrix, coupling agent and processing time. Only immobilization methods are single way to resolve the cost effective problem.

Cross- linking enzyme crystal (CLECs) is formed crystals of enzyme while CLEAs is additional version of CLECs method [Hoang Hiep Nguyen[a, b], Moonil Kim 2017]. This might be used in aqueous solution. Furthermore, salt, organic solvent and non ionic polymers play chief role in enzyme aggregations. Herein activity of enzyme sustained. Immobilization by cross linking is easiest method, which have major advantage of minimized enzyme leakage from matrix. For increasing the stability of enzyme in microenvironment therefore that have used stabilizing agent hence on the other hand reason of stability due to surface complimentary charge [Chang BS et al 1995].

a) Entrapment Method:

The entrapment method is a process of enclosed enzyme by polymeric matrix like gels in which substrate can passes through matrix but enzyme keep hold on matrix [O'Driscoll KF 1976]. That has become too dissimilar to another method. In which enzyme do not adhesion to surface but enzyme surrounded by polymeric network matrix. It may be used in different form of support such gel [Bernfeld P, Wan J 1963] or fiber entrapment [Dinelli D, Marconi W, Morisi F 1976], micro- encapsulation [Wadiack DT, Carbonell RG 1975].

b) Adsorption Method:

Adsorption method is a reversible process which might be easily separated out from the support matrix. Among different immobilization process these are very simplest immobilization method. Reversible interaction established between enzyme and support therefore this immobilization method is highly attractive and convenient low cost. So that enzyme activity lost, it is firstly considered about cost of supported matrix because of support matrix play important role in make the cost effective process [Porath J, Axén R 1976]. In adsorption process enzyme connected to

support matrix via hydrogen bond, Vander walls force hydrophobic interaction but salt bridge helps to bond formation between enzyme and supported matrix [Messing RA 1976, Woodward J 1985].

c) Covalent Method:

Covalent bonding process have done in two phase. Glutaraldehyde agent serves as cross linker which activates the surface of molecules and established connection between enzyme and support matrix. Due to strong bonding between enzyme and matrix wherein enzyme leakage problem almost negligible. Whereas glutaraldehyde acts multi-functional for immobilization and cross- linking binding among enzyme .different type of support matrix works on different type of enzyme linking agent for immobilization [Mattiasson B, Kaul R 1991]. When enzyme goes through chemical modification it has received more risk chance to damaged the enzyme activity.Activation of carboxylic groups: when strong formed between carboxylic groups (-COOH) of support and amino (- NH_2) functional groups of enzyme via carbodiimide functional groups they improve the quality of immobilization process via when N- Hydroxysuccinimide (NHS) bind with carbodiimide as long as serve as coupling agent [Scouten WH 1987]. Activation of amino groups: glutaraldehyde can be replaced to carbodiimide both have enable to similar works which acts on carboxylic groups function. Strong binding may also involving in restriction movement of enzyme and thus loss enzyme activity prevent conformation modification changes. Matrix polymerizations have ability to high loading efficiency and even homogeneous distribution for enzyme immobilization [Taylor RF 1991].

5)Selection Support Matrices:

The immobilization enzyme performance are determined by support matrix properties which have several properties are considerable during immobilization there are properties influenced on matrix such physical resistance to compression, biocompatibility, hydrophillicity and inertness towards enzyme and availability at low cost [Trevan M 1980,Buchholz K, Klein J 1987] .The carrier matrix separated into two groups based on their chemical properties. Inorganic matrix and organic matrix these have a different chemical function groups at the active site for attachment to enzyme [Cabral JMS, Kennedy JF 1991].They are works similar to other excellent support

material that have enable to high loading efficiency, high surface area and mass transfer resistance [Feng W, Ji P 2011]. Other parameter also including which show results as in stability of immobilization processes such porosity and surface area.The different variety of polymeric support matrix such as alumina, silica, zeolite, and mesoporous silica are also used for immobilization processes [Wilchek M, 1982, Drobník J, 1982,Wadiack DT 1972]. Silica coated nanoparticle are used in research areas and recovery method for immobilization method. Because of nanoparticle provided high surface area and high loading efficiency, due to high loading efficiency enzyme mass transfer from matrix to bulk becomes decrease. Nanoparticle bound to enzyme through stabilizing agent provided the mechanical stability as well as exhibits the Brownian movement when distribute in aqueous solution with same density [Messing RA 1976]. Earlier reported those nanomaterial supports disarrange protein structure in process as long as improve stability and performance [Messing RA 1976]. They have shown high thermal tolerance capacity and high activity compare to free enzyme [Porath J 1992, Batista-Viera F et al 2011]. Although porous matrixes are better than non porous matrix because of non porous material have major drawback of minimum enzyme loading efficiency. Porous material have large surface area and high loading efficiency and their received more attention against the resistance of physical, chemical and biological problems [Gemeiner P 1992]. Agrose is most popular matrix or gel that has been most widely exploited in the immobilization method. It even has high porosity which leads to way high loading efficiency on large surface area and some other benefits of this matrix is hydrophilic character, having the charge less molecules groups and it commercial applications. But porous material and Agrose is high cost effective matrix [Katchalski-Katzir et al, 2000 Sheldon RA 2007].The Nanomaterial carries out size 0.1-100 nm for excellent dispersibility and loading efficiency of surface matrices [Anna Pratima Nikalje*]. Many types of coated material made up through nanoscience which coated the drug by tiny sizeable material used in medical field. However, nanotechnology is widely used in life it is also included cancer treatment and other diagnostics treatment [Abolfazl Akbarzadeh1]. The Nano constructed materials have magnificent properties to attracted iron oxide coated particles which are being used in several therapy and cancer treatment.

The Nanoparticles provided stability in aqueous solution at pH 7.0 which have charge on surfaced that found in 3D structure of Nanoparticles [Thakral C, Alhariri J, et al].

The Nanoparticles properties biocompatibility and toxicity, govern about magnetic Nanoparticles such iron, nickel, and cobalt. Some oxidized nanoparticle to formation of iron oxide. It is also known as magnetite. The Nanoparticles size depends on sedimentation rate, while Nanoparticles size less than 100 nm provided large surfaced area for low mass transferred resistances [Puntes VF, Nitin N, et al].

Health risks can be treated by nanotechnology therapy that has been applied to a wide spectrum [Adriano Cavalcanti[1] et al]. It is working based on the magnetic properties to which has been used in biosensor, and Nanomedicine. The magnetic nanoparticle has also been screening properties. Furthermore, Nanotechnology used increasing their detection sensivity and reliability [M. J. Heller,et al].. Nanoparticles exist in nature, because the electron device making by magnetic nanoparticles so called storage and which collect the information data and is used in making biosensor [R B Frankel et al]. As magnetic nanoparticle can be used for future possibility, Which not only acts as an electron carriers but also play important role in spin-orbit coupling to controlled the magnetic characteristics in nanocomposite [E. I. Rashba et al]. The nanoparticles have magnetic properties that are identified through their particle size. Nanoparticle size range is measured in micro and nano mater. There are found homogeneous magnetization areas [Weiss, P.et al].

The superparamagnetic is developed a new configuration structure of magnetic nanoparticle using of their unique properties. Which are most being helpful in application in biological separations and Biomedicine so that magnetic nanoparticles are known as biocompatibility. Additionally magnetic nanoparticles have becomes a synthesis in intracellular of magnetotatic bacteria. Further comprising to iron oxide, iron sulfate etc [D. A. Bazylinski,et al]. bacterial magnetic nanoparticle encapsulated by organic membrane which consists of phospholipids and protein that are easily dispersed in aqueous solution. A Magnetotatic bacterium was discovered in 1975 [R. Blakemore,et al]. There are classified based on their morphological structure. As magnetite and maghemite are two form of magnetic nanoparticles which have a biocompatibility properties there are extremely used in different treatment processes such cancer treatment, stem cell sorting, gene therapy and MRI [T. X. Fan, et al].

The nanotechnology theory was initially introduction by Nobel Richard Feynman. And his discussed about ‘there plenty of room at bottom” at American physical society meeting at Caltech in 1959 [R. P. Feynman et al].Later, In the 1980s Eric k. Drexler also discussed about

the Bottom-Up approach in across over world for nanoparticles synthesis in nanotechnology which are very closely to biological events [K. E. Drexler, 1986].

6)Synthesis Nanostructure Material:

Because of nowadays silica based devices are massively used in semiconductor industry across in world [(Murray et al. 1993; Katari et al. 1994]. As Mechanical milling approach reported to synthesis of Nanoparticles from bulk material further which have gradually decreased particle size of bulk material and finally make at tiny scale. Kinetic energy of mechanical milling that is responsible for size, shape and viscosity of Nanoparticles. The parameters such milling speed, size and shape of Nanoparticles depends to kinetic energy. Top down method have lack of surface chemistry of nanoparticle due to physical changes may be possible. There are vague types of high potential processes in which required the highly considerable heat to form of electricity, heat and solar energy [Dr. V.M.Arole et al 2014]. Some chemical method are includes such sol-gel Microwave, precipitation, micro-emulsion, Hydrothermal and photochemical processes also has been exploited for nanoparticles [Music S, Dragcevic Det al].The different shaped of glass material are found in spherical shaped powders, thin film coating and micro porous inorganic membrane [Siegel RW.et al, .S. Ge, et al].Co-precipitation is a moderate and highly active method for synthesis of nanoparticles. It has been used less harmful material for Nanoparticles synthesis [Patnaik, 2004]. There are two way of Nanoparticles synthesis at tiny scale and finally measured by nanometer. Ferrous oxidized chemical are imperfection oxidized with several oxidizing agent. On other hand when two separated chemical compound such as ferrous and ferric hydroxide mixed together in aqueous hydrate. and obtains spherical size of magnetic Nanoparticles [Sugimoto T and Matijevic E 1980].The matrix on the magnetic Nanoparticles has been tested with ammonia water [ShyhYu Shaw 2006].

Most important factors of immobilized enzyme through magnetic nanoparticle thus it can reduced operational cost of enzyme used in further processes they have a magnetic properties by which I can expelled out from reaction using external magnet [Yue Wu, Yujun Wang].In addition nanoparticle was initially developed with Ca^{2+} in aqueous solution whereas they directly absorbed enzyme so that it can be recyclable, reusable etc [Xiao Liu, Xia Chen,].

There are many way to immobilization of enzyme and nanomaterial formation, one of that author discussed was initiated synthesis of nanofibres with 80-150 mn diameter of fibers which has been used for immobilization enzyme but glutaraldehyde are working like as cross linker coupling agent. I studied that 63.3% mg/g get obtained enzyme loading efficiency and retention was shown 49.3% [Chia-Hung Kuoa, Yung-Chuan Liu]. In this paper there is developed support matrix which made up of magnetic nanoparticle with chitosan via used in ionic adsorption for enzyme immobilization. It was also shown improvement in protein loading efficiency there was measured 12 mg/g support matrix. The magnetic Fe_3O_4 nanoparticles are entrapped by chitosan which provided high strength for immobilization enzyme. They are more efficient to attract to large amount of enzyme on surface of matrix [Azariel Díaz-Hernández et al].

In this paper, the magnetic nanoparticle Fe_3O_4 surface was coated with chitosan for immobilization of enzyme. In additionally the support matrix was used for immobilization enzyme via glutaraldehyde (GDA).The reusability of immobilization enzyme showed that remained 50% activity of enzyme after 10 times used .this result has been shown better than other prior method even if the reusability directly proportional to loss of enzyme activity [Limin Zang,† Jianhui Qiu,].The external magnetic field has been used to recovery processes the magnetic nanoparticle with enzyme [Chunfang Li 1,et al].

In this research work recorded that the graphene or Fe_3O_4 hybrid wasprepared for enzyme immobilization. It has high quality of nanoparticle adopted property.It also recovered by external magnet. In additional, the immobilization of enzyme shows the better activity on surface of magnet [Xinghua Li a,1, Hao Zhu b et al].

The immobilized enzymes are more efficient on support matrix in physical and chemical methods. It has shown the high operational stability and excellent properties of recovery processes [Weiwei Zhu, ‡ Yijing Li, ‡].

In this paper we studies that the glycidyl methacrylate (GMA) and 2- dimethylaminoethyl methacrylate involved in made up a block copolymer through atom transfer radical polymerization onto the surface of magnetic nanoparticles.The activity of enzyme 422% was obtained with chitosan and 378% activity with chitosan alginate [J.A. Silvaa, G.P. Macedoa,].

In this reported that the metal synchronized hydrogels nanofibres were exploited as support matrix for encapsulated the multiple enzyme. Moreover, the nanofibres gel contains Zn^{2+} and adenosine 5'-monophospahte (AMP) which was developed to for immobilization multiple enzymes under

simple mixing processes.Furthermore, the nanofibres gel include Zn^{2+} and AMP was given better pH stability thermal stability, and protease attack, organic solvent tolerance as long as continuing reusability [Hao Liang,*[a] et. al]. The encapsulated enzymes onto metal organic framework are apart from free enzyme because of their reusability and high sensing, activity and stability of enzyme [Chen Hou[1], Yang Wang[1] et. al]. Additionally, maintained 70% activity of original activity of enzyme. Many kinds of techniques was used to confirmation of size and shape and characterization of immobilization [Wenlei Xie* and Ning Ma et. al].

7)Conclusion :

The main motive of this research paper increases the stability and recyclability and maintained the activity of immobilized enzymes after done the processes it can be separated out from reaction using external magnet field for again used in washing processes. Additionally, immobilized enzymes are able to removed protein containing spot like blood and other tomato soup from cloth at different time as well as different temperature period. However, immobilized processes remained restriction the connection of detergent ingredients. Immobilized enzyme onto magnetic nanoparticles increases recyclability and stability of enzyme. So that it can be used more than one at commercial with detergent powder.

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