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## Summary

Ph.D (pursuing) and want to pursue career in research/academics. Master's in biotechnology and Graduation in Biosciences. Presently working as the Ph.D student in the department of Biosciences, Jamia Millia Islamia. Have experience in the field of enzyme immobilization, bioethanol production using biomass, biofilm disruption etc. 3 years' experience in freelance content writing.

## Experience

- Ph.D research scholar at Dept. of Biosciences Jamia Millia Islamia (**Oct 2014- present**)
- Worked as a Junior Research Fellow (JRF) in the project in Division of Plant Physiology, IARI (Indian Agricultural Research Institute) (**January 2014 – October 2014)**.
- Worked as an intern in AMNION BIOSCIENCES Pvt. Ltd, Bangalore for six months (**April 2013 -Oct 2013).**

## Education Qualification

**Doctor of Philosophy in Biosciences*** : **2014-2020**
Department of Biosciences, Jamia Millia Islamia, New Delhi, India

**P.G Diploma in cellular and molecular diagnostics** : **2012- 2013**
Oxford College of Sciences, Bangalore, India

**Master of Sciences in Biotechnology 7.93 CGPA** : **2010-2012**
Department of Biotechnology, Jamia Millia Islamia, New Delhi, India

**Bachelor of Sciences in Biosciences 68.8%** : **2007-2010**
Department of Biosciences, Jamia Millia Islamia, New Delhi, India

***Thesis title:** *Magnetic nanoparticles as immobilization matrix*

## Skills

Nanomaterials. Immobilization. Bioethanol production using lignocellulosic biomass. Enzymatic degradation of Biofilm. Fluorescence microscopy. Fourier analysis. Scanning electron microscopy.

## Awards and Honours

Maulana Azad National Fellowship (MANF**),** UGC : **2017**
University Grant Commission, New Delhi, India

ICAR-National Eligibility Test (NET)-Lectureship : **2014**
Indian Council of Agricultural Research, PUSA, New Delhi, India

CSIR-National Eligibility Test (NET)-Lectureship : **2012**
Council of Scientific and Industrial Research, New Delhi, India

Graduate Aptitude Test in Engineering (GATE) : **2012**

Graduate Aptitude Test in Engineering (GATE) : **2011**

## Research Papers

- **Perwez, M**., Mazumder, J. A., & Sardar, M. (2019). Preparation and characterization of reusable magnetic combi-CLEA of cellulase and hemicellulase. *Enzyme and Microbial Technology*, *131*, 109389. IF: 3.448
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- Phul, R, **Perwez, M**., Ahmed J., Sardar, M., Alshehri S. M., Alhokbany N., Khan M. A. M., Ahmad, T. (2020). Efficient Multifunctional Catalytic and Sensing Properties of Synthesized Ruthenium Oxide Nanoparticles. *Catalysts*, 10(780). IF: 3.52
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- Praveen, A., Khan, E., **Perwez, M**., Sardar, M., & Gupta, M. (2018). Iron oxide nanoparticles as nano-adsorbents: a possible way to reduce arsenic phytotoxicity in Indian mustard plant (Brassica juncea L.). *Journal of plant growth regulation*, 37(2), 612-624. IF: 2.67

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## Chapters/ Review

- Shabnam, **Perwez, M.**, Mazumder, J. A., & Sardar, M. (2020). Phytonanotechnology: A new horizon for the food industry. *Phytonanotechnology: Challenges and Prospects*, 221.
- Gupta, M. N., **Perwez, M.**, & Sardar, M. (2020). Protein crosslinking: Uses in chemistry, biology and biotechnology. Biocatalysis and Biotransformation, 38(3), 178-201.
- Noori, R., **Perwez, M.**, & Sardar, M. (2019). Cross-linked Enzyme Aggregates: Current Developments and Applications. In *Biocatalysis* (pp. 83-112). Springer, Cham.
- Sardar, M., **Perwez, M.**, Ahmad R., Mukherjee, J. (2018) Immobilization of Enzymes on Magnetic Nanoparticles: In *Encyclopedia of Nanoscience and Nanotechnology*, 28, 1-30. American scientific publisher, USA.

## Conference attended

- Poster presentation on "Preparation and characterization of a multipurpose magnetic combi-CLEA" in the "**International conference on Biotechnological Research and Innovation for sustainable Development (BioSD)**"-2018 held on 22-25th November, 2018
- Presented a poster on "Enzymatic approach for Biofilm removal" for "**Emerging Discoveries in Health and Agricultural Sciences, Society of Biological Chemist**", SBC- 2017
- Presented a poster on "Green synthesised iron oxide nanoparticles for glucose and $H_2O_2$ detection" in the "**National seminar on Biophysics (BIOPHYSIKA- 2017)**" held on March 16th, 2017
- Presented a poster on "Conversion of cellulosic agricultural wastes into bioethanol" in the "**National seminar on Recent Advances in Environmental Toxicology**" held in jamia Millia islamia, department of biosciences on 13th and 14th February 2017.
- Presented a poster on "A reusable nanobioconjugate of pectinase for application in juice industry" in the "**International Conference on green chemistry in Environmental sustainability and Chemical Education**" held on 17th and 18th November 2016 in Daulat Ram College, University of Delhi
- Presented a poster on "Agro-waste synthesised iron oxide nanoparticles as artificial peroxidase" **in the International Conference on Nanotechnology (ALIGARH NANO-V) and STEM-Education and Research (STEM CON-16)**" held on 12-15th March, 2016.

## Workshop attended

- Attended author workshop on Scholarly Writing and Intellectual ethics Organized by Jamia Millia Islamia, New Delhi in collaboration with **Elsevier** on September 2017.

- Attended conference cum workshop and *HANDS ON TRAINING* in **ACTREC, TMC** (Mumbai) from January 2013.
- Attended *"CME* cum workshop in immunology *"*on January 2011 *in **HIMALAYAN INSTITUTE OF MEDICAL SCIENCES** at Dehradun*.
- Attended workshop on *IMPACT OF BIOTECHNOLOGY ON MAN AND ENVIRONMENT"* in March 2011 in ***TERI UNIVERSITY***.

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# Preparation and characterization of reusable magnetic combi-CLEA of cellulase and hemicellulase

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A R T I C L E I N F O

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A B S T R A C T

Cross-linked enzyme aggregate (CLEA) is a technology to overcome the limitation of enzymes for its application in chemical industries. The inability of repeated use of enzymes, stability and ease of separation from reaction mixture limits its applications. Here, magnetic combi-CLEA has been synthesised by adding amino-functionalized magnetic nanoparticles into pectinase ultra-clear (containing pectinases, xylanases and cellulases). Enzymes were precipitated on the surface of amino-functionalized magnetic nanoparticles with ethanol and cross-linked using glutaraldehyde. The structural characterization of magnetic combi-CLEA was studied by Scanning Electron Microscopy. Thermal stability was performed at 70 °C for pectinase and 80 °C for xylanase and cellulase respectively. Half-life ($t_{1/2}$) of the xylanase, cellulase and pectinase in free form remarkably enhance from 84.51, 29.36, and 25.29 min respectively to 533.07, 187.29 and 147.44 min in magnetic-combi CLEA respectively. Magnetic combi-CLEA can be efficiently reused till 12th cycle after which pectinase, xylanase and cellulase retain 86.45%, 90.3% and 88.62% activity respectively. Using this CLEA preparation bioethanol concentration increases to 1.82-fold as compared to free enzyme, when simultaneous saccharification and fermentation was performed using wheat straw as the substrate. Magnetic combi-CLEA can be used for a variety of industrial applications like food processing, textile industry and bioethanol production.

## 1. Introduction

Enzymes are obtained from renewable resources, they are biocompatible, and the enzymatic reactions are performed under mild conditions compared to conventional chemical catalysts. Despite, having these unique properties the commercial application of enzymes is limited because of their restricted operational stability and recovery issues. These obstacles can generally be overcome by immobilization of enzymes [1]. There are various methods of enzyme immobilization broadly classified into three types. 1. Carrier bound, 2. Encapsulation and 3. Cross-linking of the enzyme molecules. The carrier bound and encapsulation generally suffers enzyme inactivation thus leading to loss of enzyme activity moreover these methods are inappropriate for enzymes catalysing macromolecular substrates because of diffusion limitations [2]. In contrast, cross-linking of enzyme molecules is a carrier-free method, and in most of the cases the immobilized enzyme retained high enzyme activity. Laccase chitosan CLEA prepared by Arsenault et al. showed hyperactivation (259% of the initial specific activity) due to attachment of laccase with chitosan [3]. Similar observation was observed in a different experiment when Gupta et al. prepared cross linked laccase aggregate and observed hyperactivation with 137% and 163.4% of residual activity when hydrolysis was performed with p-nitrophenyl palmitate and p-nitrophenyl butyrate respectively using SDS as surfactant [4].

Cross linked enzyme aggregate or CLEA is a simple method to immobilize enzymes without using any solid carriers which stabilizes the quaternary structure of enzymes due to the covalent linkage formed between enzymes using cross linking agent [5]. Enzymes are immobilized in CLEA by precipitating enzymes using inorganic salts, organic solvents and non- ionic polymers and then extensive cross linking occurs between lysine group of enzymes with cross linking agent [6]. Addition of BSA as a protein feeder facilitates CLEA formation in cases where the protein concentration in the enzyme preparation is low [7]. Although CLEA formation is simple and enzyme retained operational and storage stability, it has some drawbacks also. They are reported to have poor mechanical stability and the centrifugation or filtration commonly applied to recover CLEAs from reaction medium, lead to increase in CLEAs size and clusters formation, which results in mass transfer limitations [8]. Hence, for many applications, CLEAs may require a physical support to improve their mechanical properties. The use of magnetic supports may be an alternative to produce mechanical resistant and magnetically-separable CLEAs [9]. For example, magnetic

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# A reusable multipurpose magnetic nanobiocatalyst for industrial applications

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ABSTRACT

A multipurpose magnetic nanobiocatalyst is developed by conjugating Pectinex 3XL (a commercial enzyme containing pectinase, xylanase and cellulase activities) on 3-aminopropyl triethoxysilane activated magnetic nanoparticles. The nanobiocatalyst retained 87% of pectinase, 69% of xylanase and 58% of cellulase activity after conjugation on modified nanoparticles as compared to their soluble counterparts. Thermal stability data at 70 °C showed increase in enzyme stability after conjugation to nanoparticles and the kinetic parameters ($K_m$ and $V_{max}$) remain unaltered after immobilization. The immobilized enzyme system can be successfully used upto 5th cycle after that slight decrease in enzyme activities was observed. The nanobiocatalyst retained high pectinase activities in organic solvents and chemical reagents as compared to free enzymes. DLS data shows that the nanoparticles size increases from 63 nm to 86 nm after immobilization. Atomic Force Microscopy data confirms the deposition of enzymes on the nanoparticles. The nanobiocatalyst was used for the clarification of pine apple and orange juice and was also used for the production of bioethanol. Hydrolysis of pretreated wheat straw produced 1.39 g/l and 1.59 g/l after treatment with free Pectinex 3xL and nanobiocatalyst respectively. The concentration of bioethanol also increases by 1.4 fold as compared to the free enzyme.



## 1. Introduction

Hemicellulases hydrolyse the plant cell wall polymer hemicellulose, one of the most abundant polysaccharides in nature. Plant biomass in terms of dry weight comprises of 23% lignin, 40% cellulose and 33% hemicellulose. The potential of plant biomass as a renewable raw material is immense [1]. The judicious use of hemicellulases and cellulases in industries could result in higher yields. These enzymes have many biotechnological applications such as deinking of paper waste, pulp biobleaching, clarification of fruit juices, in baking industry, up gradation of feed, fodder and fibres, and saccharification [2]. Before using these enzymes at industrial scale several criteria have to be fulfilled. In industrial processes the reactions are generally performed at high temperature and with harsh chemical reagents to reduce the crystalline structure of cellulose making it available to cellulases and hemicellulases [3]. Therefore, the properties of these enzymes need to be greatly improved. Immobilization is one such powerful tool for increasing the stability and reusability of the enzymes [4,5]. Different immobilization methods such as covalent, ionic bonding, adsorption, entrapment, and encapsulation provide better stability to enzymes. Recently, nanomaterials have emerged as the potential immobilization matrices because of their surface size to volume ratio, large amount of enzyme can be loaded to them [6–10]. Moreover, the diffusion limitations are also minimized when dealing with macromolecular substrates [6,8,11]. Graphene based nanomaterials have also been used as support system for use in immobilizing enzymes [12]. Due to the physiochemical properties like electrostatic and hydrophobic properties, enzymes can easily bind to the graphene based nanomaterial but catalytic activity has been found to be reduced due to which graphene is modified with functional groups and then covalently attached with enzymes which improves its catalytic activity [13]. It can be used for waste water treatment and for industrial applications [13].

Magnetic nanoparticles (MNPs) have been a field of study in recent years due to their applications in pharmacy, biology, diagnostics and biotechnology, etc. [14,15]. The use of MNPs offers many benefits because of their nano-scale size and physical properties [16]. hence, immobilization of bioactive substances such as proteins/enzymes on magnetic iron oxide nanoparticle is preferred [10,11]. Mostly enzyme bound magnetic nanoparticles have been found to be stable towards heat, pH and resistant to denaturation [10]. Moreover, MNPs due to their magnetic properties can be

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# Exposure of biosynthesized nanoscale ZnO to *Brassica juncea* crop plant: morphological, biochemical and molecular aspects

Jahirul Ahmed Mazumder[1], Ehasanullah Khan[2], Mohammad Perwez[1], Meetu Gupta[2], Sanjay Kumar[3], Khalid Raza[3] & Meryam Sardar[1]✉

**The present work describes the *in vitro* synthesis and characterization of Zinc oxide nanoparticles (ZnO NPs) using an enzyme alpha amylase, the synthesized nanoparticles were used to study their beneficial effect in the growth and development of *Brassica juncea*. Transmission Electron Microscope (TEM) image reveals the average size of ZnO NPs was 11 nm and X-ray powder diffraction (XRD) suggests nanoparticles were crystalline in nature. In-silico study confirmed lysine, glutamine and tyrosine present in alpha amylase enzyme, plays a crucial role in the reduction of Zinc acetate dihydrate to ZnO NPs. The biochemical parameters and oxidative enzymes of *Brassica juncea* were compared with ZnO NPs treated plants. The effect of ZnO NPs on the cellular expression of metal tolerant protein (*BjMTP*) and cation efflux transporter gene (*BjCET2*) was also studied. The results indicate that nanoparticles can be used as a replacement for traditional harmful chemical fertilizers.**

Particles of size less than 100 nm are characterized as nanoparticle, these are formed when elements are modified by altering at their molecular and atomic level[1]. This branch of science has brought a boon in scientific research by introducing various nano sized particles that have importance in various fields such as medicine, technology, agriculture etc. Nanoparticles show size dependent electronic, optical and chemical properties. Among the different nanomaterials metal based nanoparticles are the most studied nanomaterials[2]. A characteristic surface plasmon resonance (SPR) absorption in the UV Visible region is a unique property of metal nanoparticles. The SPR band arises due to the presence of free electrons in the conduction band[3].

Nanotechnology has shown numerous beneficial effects in the field of agriculture[4]. Several investigators reported the beneficial and harmful effect of nanomaterial's on plant growth and a number of review articles have been published on the interaction mechanism of plants and nanomaterials[5,6]. These interactions may results in physiological, morphological, and genotoxic changes in plants, basically their mechanism is important for their effective use in agriculture. Nanoparticles increase the productivity of plants by interacting directly with the plants or indirectly by interacting with the soil. They increase the growth of plants by site-targeted controlled delivery of nutrients, or by maintaining the level of micronutrients, they also provide resistance to the plants by their action on phytopathogens[7]. Nanomaterial's improve soil health by chelating various ions/ salts present in soil, regulate the pH of soil, and also interact with the microbes present in the soil, thus indirectly help the plant growth[8–10]. Metal Nanoparticles like Ag, Au, $TiO_2$, ZnO, iron etc have been reported to stimulate plant growth[11]. Among these Zinc Oxide nanoparticles (ZnO NPs) have been widely used, as zinc is an essential micronutrient and participates in various metabolic reactions[12]. Several scientists studied the effect of ZnO NPs on different crop plants and their studies reveal that ZnO NPs exerts positive effect on the plant growth. It is also reported that the toxicity of ZnO NPs on crop plants is much lower as compared to the toxicity of $Zn^{2+}$ or ZnO bulk particles[13].

Nano fertilizers like Nano-GroTM, Nano-Ag Answer[R], TAG NANO (NPK, PhoS, Zinc, Cal, etc.) are marketed by some commercial companies[14], however, there is a need for large-scale industrial production of nanofertilizers and nanopesticides[15]. In developing countries like India, 18% of India's gross domestic product (GDP) depends on agricultural sector and provides 50% employment, thus research is required to synthesize nanoparticles which

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RESEARCH ARTICLE

# Development of sustainable and reusable silver nanoparticle-coated glass for the treatment of contaminated water

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## Abstract

Water contaminants like pathogenic microbes and organic pollutants pose a serious threat to human and aquatic life forms; thus, there is an urgent need to develop a sustainable and affordable water treatment technology. Nanomaterials especially metal nanoparticles have extensive applications in wastewater treatment, but the recovery and aggregation of nanoparticles in solution is a major limitation. In the present work, green synthesized silver nanoparticles were covalently immobilized on a glass surface to prevent aggregation of nanoparticles and to enhance their applicability. Fourier transform infrared (FTIR) of silver nanoparticle (AgNP)-coated glass shows peaks of Si–O–Si, Si–O–C, and Ag–O at 1075 $cm^{-1}$, 780 $cm^{-1}$, and 608 $cm^{-1}$ respectively which confirms the immobilization/conjugation of nanomaterial on glass surface. The surface morphology of immobilized AgNP was studied using scanning electron microscopy (SEM) which reveals nanoparticles are spherical and uniformly distributed on glass surface. The AgNP-coated glass was used for the removal of textile dyes in solution; the result indicates approximately 95% of textile dyes were removed after 5 h of treatment. Removal of microbial contaminants from Yamuna River was studied by optical density analysis and confirmed by fluorescence dye staining. The AgNP-coated glass was also studied for their reusability and the data indicates 50% removal of microbes up to the 5th cycle. To further enhance the applicability, the inhibition of bacterial biofilms were analyzed by dark-field illumination with a fluorescence microscope. Thus AgNP-coated glass can be used in the development of food/water storage containers and in textile industries.

**Keywords** Green synthesis · Immobilization · Wastewater treatment · Microbial contamination · Nanoparticle-coated glass · Organic contaminants · Sustainable · Reusable

## Introduction

Urbanization is the root cause of water pollution; activities such as industrialization, dumping of household, agricultural, industrial, and hospital wastes in the environment lead to water pollution (Lu et al. 2016). Some of the important water pollutants are heavy metal ions, dyes, radionuclides, organic and inorganic compounds, pathogenic microbes, etc. (Santhosh et al. 2016). Pathogenic microbes cause serious health hazards and are considered as a harmful form of environmental pollutant. Another important class of pollutants is the organic textile dyes which have high levels of toxicity and possess potential carcinogenic effects on the living organisms. Therefore, the treatment of contaminated water requires immediate attention (Gehrke et al. 2015). Most physical and chemical methods employed for wastewater treatment have their own limitations such as high cost, low applicability, and incomplete removal of waste and toxic by-products (Pearce et al. 2003; Rajasulochana and Preethy 2016).

The pollutants are generally removed from water either by adsorption or by photocatalytic reduction (Çeçen and Aktas 2011; Konstantinou and Albanis 2004). Nanomaterials are reported to be excellent adsorbents due to their high surface area; thus, this property facilitates their applications in wastewater treatment processes. So far, various nanomaterials have been synthesized and used for the removal of aquatic pollutants (Santhosh et al. 2016). Nanoadsorbents such as carbon nanotubes (CNTs) possess highly accessible adsorption sites and antimicrobial properties; they can be used for adsorption of persistent contaminants and for the killing of microbial cell

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# Efficient Multifunctional Catalytic and Sensing Properties of Synthesized Ruthenium Oxide Nanoparticles

**Ruby Phul [1], Mohammad Perwez [2], Jahangeer Ahmed [3], Meryam Sardar [2], Saad M. Alshehri [3], Norah Alhokbany [3], Mohd A. Majeed Khan [4] and Tokeer Ahmad [1,*]**

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**Abstract:** Ruthenium oxide is one of the most active electrocatalyst for oxygen evolution (OER) and oxygen reduction reaction (ORR). Herein, we report simple wet chemical route to synthesize $RuO_2$ nanoparticles at controlled temperature. The structural, morphological and surface area studies of the synthesized nanoparticles were conducted with X-ray diffraction, electron microscopy and BETsurface area studies. The bifunctional electrocatalytic performance of $RuO_2$ nanoparticles was studied under different atmospheric conditions for OER and ORR, respectively, versus reversible hydrogen electrode (RHE) in alkaline medium. Low Tafel slopes of $RuO_2$ nanoparticles were found to be ~47 and ~49 mV/dec for OER and ORR, respectively, in oxygen saturated 0.5 M KOH system. Moreover, the catalytic activity of $RuO_2$ nanoparticles was examined against the Horseradish peroxidase enzyme (HRP) at high temperature, and the nanoparticles were applied as a sensor for the detection of $H_2O_2$ in the solution.

**Keywords:** ruthenium oxide; nanoparticles; electrocatalysts; sensing

## 1. Introduction

The present generation is largely dependent on fossil fuelsto meet the present energy requirements—for instance, oil, coal, or natural gases. However, these energy demands fulfilled by these products directly affect the environment. The burning of fossil fuels leads up to the emissions of carbon dioxide gas (a greenhouse gas), which is affecting the world significantly through global warming, change in weather patterns and several other noteworthy geographical changes [1]. In addition, we know fossil fuels are nonrenewable resources, hence they will eventually deplete, so alternatives must be found. Therefore, the development of efficient, inexpensive and eco-friendly sources of energy has become a significant and crucial task for the researchers [2]. Scientists have investigated the use of renewable resources such as solar, wind, tidal, biomass, geothermal energy etc. The major research trend of today's era is the water-splitting phenomena for energy generation. Water splitting via electrocatalysis or photocatalysis is a clean, environmentally friendly and renewable source of energy for fuel cells, batteries and hydrogen generation [3–5]. In the presence of electro/photocatalyst, the water molecule splits into hydrogen and oxygen gas (i.e., $H_2O \rightarrow H_2 + 1/2\ O_2$). The evolved

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REVIEW ARTICLE

# Protein crosslinking: Uses in chemistry, biology and biotechnology

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**ABSTRACT**
Protein crosslinking is a part of many biological processes and is also carried out *in vitro* under several controllable conditions with the help of any of the commercially available bifunctional reagents. Many biotechnological applications utilize stable and/or reusable crosslinked enzymes such as soluble intramolecularly crosslinked enzymes; soluble bioconjugates of enzymes with other enzymes/proteins or polymers; chemically aggregated enzymes, chemically crosslinked enzyme aggregates and crosslinked enzyme crystals. The review after indicating how protein crosslinking is at the heart of such diverse processes/technology concludes with discussion of few applications which are currently drawing considerable attention.

**Abbreviations:** Mp: Magnetic particles; BSA: Bovine serum albumin; PepN: Amino-peptidase N; PepX: Proline-specific X-prolyl-dipeptidylaminopeptidase; *N,N*-MBAAm: *N,N*-Methylenebisacrylamide; RBBR: Remazol Brilliant Blue Reactive; CLEMPA: Crosslinked enzyme aggregates onto magnetic particles; m-CLEA: Magnetic CLEA; ADH: Alcohol dehydrogenase; GDH: Glucose dehydrogenase; M-CLEA: Macromolecular CLEA; PAL: Phenylalanine ammonia lyase; TMOS: Tetramethyl orthosilicate; h-CLEA: Hollow CLEA; EG-NHS: Ethylene glycol-bis (succinic acid N-hydroxysuccinimide); TA: Tannic acid; GDH: D-glucose dehydrogenase; Pm CLEA: Porus magnetic CLEA; GOx: Glucose oxidase; IM-CLPCMC: Imprinting crosslinked protein-coated microcrystals; PO: Polyphenol oxidase; AP: Acid phosphatase; SPDP: Succinimidyl-3-(2-pyridyldithio)propionate; EPRP: Enzyme precipitated and rinsed with *n*-propanol; PREP: *n*-Propanol rinsed enzyme precipitation

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## Introduction

The ubiquitous roles of crosslinks involving proteins in biology, agriculture and health sciences were highlighted by two books several decades ago (Friedman 1976a, 1976b). The period between early 1950s and late 1980s saw several synthetic bifunctional reagents being described in the literature; a large number of them also became commercially available (Hartman and Wold 1967; Wold 1967, 1972; Wong and Wong 1992; Wong and Jameson 2011). The objectives of the current reviews are:

In the recent literature, the excellent guidelines enunciated by Wold (1967) to control the formations of intermolecular crosslinks while attempting to introduce intramolecular crosslinks (and vice versa) seem to have been largely ignored. In fact, current reusable biocatalyst designs like CLEA (Sheldon 2019) or CLEC (Margolin 1996) seem to completely ignore the distinction between the two types of crosslinks. It is not unlikely that more stable/efficient biocatalyst designs would emerge by understanding nature of crosslinks being formed and by controlling crosslinking (Shah et al. 2006).

Glutaraldehyde is the most frequently used chemical crosslinker (bifunctional reagent). Its tendency to oligomerize in solution and produce ill-defined oligomers; form crosslinks by apparently complex mechanism and react in a manner which is difficult to control has led to exploration of alternatives even in recent years; example for preparation of CLEAs (Mukherjee and Gupta 2017a). This review includes early crosslinking applications which point out many other options for crosslinking reagents which have been around for several decades.

The scope of the review is also illustrated in Figure 1. When a protein and a bifunctional reagent react, the product can be a mixture of several products: soluble intramolecularly crosslinked protein; soluble intermolecularly crosslinked protein, soluble protein molecules in which both types of crosslinks are

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