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Kinetics and thermodynamic of immobilization uricase enzyme on sodium alginate of hyperuricemia patients.

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Abstract

Immobilization of enzymes is an effective method for improving enzyme its unique features, such as inertness and high surface area, sodium alginate was used to immobilize uricase in this study. The sodium alginate were activated with glutaraldehyde as a cross linker and uricase enzyme successfully immobilized on them. The optimum conditions of immobilization process were investigated. The maximum efficiency of immobilization (70.7) was observed with (3 mg/ml) concentration of sodium alginate. The optimum pH was 7 in phosphate buffer, and temperature was 40ºC. The sodium alginate and immobilized uricase-sodium alginate were characterized using Fourier transform infrareds (FTIR), Atomic forces microscopy (AFM), and the Scanning electron microscopic (SEM). The kinetic (K_m and V_{max}) values and thermodynamic (E_a, ΔH, ΔS, and ΔG) parameters of both free and immobilized enzyme were determined. For this purpose, kinetic and thermodynamic parameters are calculated by determining uricase enzyme activity in different substrate concentration (1,2,3,4,5,and 6 mg/dl) and incubation temperature (15, 20, 25, 30, 35, and 40). The immobilized uricase enzyme was utilized to determine the blood uric acid in various clinical samples. The result obtained with the immobilized uricase were compared to those obtained with the Autoanalyzer®. The uric acid level of patients decrease about ~ 2.5 folds and ~ 2 folds for control. Immobilized uricase is mostly used to remove uric acid from the blood for detoxification or.in conclusion alginate could extensively use for the enzyme immobilization in recent years. In addition used it to immobilized uricase enzyme. So the impact of immobilization conditions on the uricase immobilization process was studied in depth to ensure that the enzyme could be used effectively in a variety of applications. Form the investigation of kinetic paramleters and thermodynamic parameter for immobilization enzyme and free enzyme it seems the enzymes

enhanced their catalytic power after immobilization. So the immobilizes uricase can be used efficiently to hydrolysis uric acid in various medical applications. For example, in hyperuricemia patients.

Chapter One

Introduction & Literature Survey

1.1 Introduction

Immobilization of enzymes refers to the technique of confining the enzymes in or on an inert support for their stability functional [1] . Uricase classification. .

(EC 1.7.3.3, UC) is an enzyme belonging to the class of the oxido-reductases and catalyzes the oxidation of uric acid to allantoin, and thus plays an important role in the purine degradation. [2]. Immobilized uricase has many classic applications on different materials [3]. Uricase immobilization is also used in several new applications, such as hemodialysis membranes [4]. Bioelectrochemical enzymatic instruments[5]. And the investigation of protein-surface interactions[6]. immobilization methods include physical adsorption, ionic and covalent bonds, and various techniques such as binding, entrapment, encapsulation, and cross-linking. Enzymes can be immobilized on various organic and inorganic materials or carriers. Synthetic organic polymeric beads such as Sepabeads and Amberlite; a variety of biopolymers, mainly water-insoluble polysaccharides such as cellulose, starch, agarose, alginate, chitosan, and electrospun nanofibers; and polymeric membranes are widely used as supports for immobilizing enzymes[7] .or alter the structure of uricase (for example, when immobilization occurs on the alginate) [2]. Alginat eisabiological compound ,which has found many application sinbiomedcal sciencesand engineering .It is widely used due to its out standing properties chashigh biocompatibility and gelling .Alginatehydrogels, forinstance ,can be prepared using avarietyo fcross-linking techniques and because of the structural resemblance between them and extra cellularmatrices found in livetissues ,they have very high application sinwoundhealing ,and delivery of celltransplantation small proteins and chemicaldrugs [8].In this study The optimal conditions for immobilization of uricase onto sodium aligante was explored. uricase has been immobilized on it using glutaraldehyde as a bifunctional agent, which was an easy and effective process for

enzyme immobilization. [2] In addition, the immobilized enzyme's kinetics rate model and thermodynamics experiments, as well as the mechanism of immobilization.

1.2 Literature survey

(Hamed, M., AbdelAziz et al. ,2016), dropped of the alginate solution through a 200µl Eppendorf tip into $CaCl_2$ solution (30 drops min−1). After 2h of gentle stirring the bead was filtered out of the $CaCl_2$ solution and thoroughly washed. The activity of the immobilized uricase was assayed.Cross-linking method for uricase immobilization was carried out by addition of 5 % (v/v) glutaraldehyde. The cross-linked method was better than entrapped method. The Vmax values were 15.6, 13.8 and 15.2 units mg protein for the free, entrappedand cross-linked enzyme, respectively. The Km values were 0.072, 0.092 and 0.084 mM, respectively. The optimal pH was 8.5for 8.5 the free, entrapped, and cross-linked enzyme. The optimal temperature was 35 ºC , for free and entrapped but it was 45 ºC for cross-linked uricase. Uricase exhibited appreciable resistant digestion by pepsin and trypsin [2].

(Sinem Diken Gür& Neslihan İdil& Nilüfer Aksöz et al .,2018) , this study, two different materials alginate and glutaraldehyde activated chitosan beads were used for the co-immobilization of α-amylase, protease, and pectinase. Firstly, optimization of multienzyme immobilization with Na alginate beads was carried.out optimum Na alginate and $CaCl_2$ concentration were found to be 2.5% and 0.1M respectiveiy optimal enzyme loading ratio was determined as 2:1:0.02 for pectinase, protease, and α-amylase, respectively. Next, the immobilization omultiple enzymes. On glutaraldehyde-activated chitosan beads was optimized (3% chitosan

concentration, 0.25% glutaraldehyde with 3 h of activation and 3 h of coupling time). While co-immobilization was successfully performed with both materials, the specific activities of enzymes were found to be higher for theenzymes co-immobilized with glutaraldehyde-activated chitosan beads. In this process,glutaraldehyde was acted as a spacer arm. SEM and FTIR were used for the characterization of activated chitosan beads. Moreover, pectinase and α-amylase enzymes immobilized with chitosan beads were also found to have higher activity than their free forms. Three different enzymes were co-immobilized with these two materials for the first time in this study [9].

(F.A. Mostafa, A.A.A. El Aty, M.E. Hassan, et al., 2019), Alginate poly-thyleneimine gel beads modified by using 0.3 M Na^+ were used for covalent immobilization of a spergillus flavus xylanase. SEM images showed distorted structure with addition of Na^+ that impaired the egg-box structure formation offered much covalent binding with xylanase. Immobilization onto (Alg+PEI/Na+) showed an enhancement in the operational stability, immobilization efficiency as well as immobilization yield. Covalent immobilization of xylanase (AIg^+PEI/Na^+) enhanced xylanase activity over a wide range of pHs (4–5.5) comparable to its free formula as well as an increase in reaction temperature up to 60°C. However, immobilized formula of enzyme showed abroad thermal stability that it retained 79.0% of its initial activity at 70°C up to 30 min whereas, free formula completely lost its activity at this temperature. Thermodynamics studies showed an enhancement in thermal stability at high temperature for the immobilized xylanase. i.e. At 70°C the t1/2 and D-value for free formula of enzyme increased from 24 to165 min and from 79.95 to 548.23 min, respectively.Moreover, the enzyme stability enhancement for immobilized formula of xylanase was proved with a remarkable increase in enthalpy

and free energy. 93% of the immobilized xylanase activity was retained over 6 weeks of stor age at -4°C [10].

(**Kim and Lee et al., 2019)** , Trypsin immobilization on chitosan nonwovens activated with gluteraldehyde was achieved by adjusting pH and enzyme concentration in addition to reaction times. Furthermore, the pH, thermal stability, storage stability, and reus ability of immobilized trypsin were investigated. The optimum immobilization conditions for trypsin enzyme were determined to be pH (8.5), enzyme concentration of (8%), and treatment period of 30 minutes. At 25ºC, trypsin was effectively immobilized. Immobilized trypsin was shown to have poorer pH stability and higher therm, stability than free trypsin. After 15 times of usage, the immobilized trypsin retained 50% of its original activity and (80%) after 20 days of storage at 4ºC **[**11].

(**S. Dhiman, B. Srivastava, G. Singh, et al., 2019**) , Partially purified β-mannanase was immobilized on the modified matrix of sodium alginate-grafted-β-cyclodextrin. The Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction characterization proved that β-cyclodextrin (β-CD) was successfully grafted withsodium alginate. After successful immobilization, yield of enzyme was found 91.5 %, pH and temperature optima were increas 6.0 to 7.0 and 50 C° to 55C° respectively.Immobilized mannanase was able to reuse 15 times and retained its 70% activity, mean while the immobilized enzyme showed 60% activity after 30 days of storage at 4°C.Immobilization also increased the thermostability and half-life of the enzyme when compared to the free mannanase. During the comparison of adsorption isotherm and kinetic models,Langmuir isotherm and pseudo-first order kinetics were observed to be the best fit model for the confirmation of immobilization [12].

(**M.Sharifi,M.J.Sohrabi,S.H.Hosseienaii,et al.,2019**), Nanoparticles (NPs) have been widely used for immobilization of wide ranges of enzymes. The stabilization of enzymes on NPs is a major challenge crucial for regulating enzymatic activity and their medical applications To overcome these challenges, it is necessary to explore how enzymes attach to nanomaterial and their properties are affected by such interactions. In this reviewe present an overview on the different strategies of the enzyme immobilization into the NPs and their corresponding stability against temperature and pH. The effects of surface charge, particle size, morphologystability of immobilized enzymes were summarized. The activity of immobilized enzyme in the NPs was reviewed to disclose more detao regarding the interaction of biomolecules withNPs. The combination of enzyme immobilization NPs. The combination of enzyme immobilization with prodrugs was also reviewed as a promising approach for biomedical application of enzyme in cancer therapy. Finally, the current challenges and future applications of NPs enzyme immobilization and the utilization of immobilized enzyme toward prodrug activation in cytoplasm of cancer cells were presented. Therefore, this review may pave the way for providing a perspective on development to the industrial and clinical translationof immobilized enzymesKeywords Enzyme stability, Immobilization Nanomaterials, Enzyme prodrug therapy, cancer treatment . [13]

(Zusfahair and D R Ningsih , et al 2020), its sugar levels were determined using the Dinitro Salisilat Method (DNS). The results reveal that the immobilized amylase commercial has optimum concentration of Na-alginate of 5% (w/v) and contact time of 90 minutes with an immobilization efficiency value of 43.02%. Furthermore, the immobilized amylase has optimum activity at substrate concentrations of 3.5% (w/v), pH 4, incubation temperature of 40 C^0, and a reaction time of 20 minutes with

the value of the activity of 2760.4 U / mL. KM value of free amylase and immobilized amylase row are 0.18 mM and 0.15 mM, repectively. The value of KM immobilized amylase is smaller than the free enzyme. It proves that the immobilized amylase has a high affinity for the substrate. The immobilized amylase can be used up to 12 times with a value of the residual activity of 56.7%.[14].

(**Rongxin Guo and Xushen Zheng ,et al.,2021**) , immobilizing cellulase on sodium alginate (SA)-polyethylene glycol (PEG) enabled the cellulase to be used repeatedly. The matrix of the immobilized cellulase was detected and characterized using Fourier transform infrared spectroscopy and scanning electron microscopy. In comparison with SA-immobilized cellulase, the relative enzyme activity and immobilization rate increased by 25% and 18%, respectively. The application range of the immobilized enzyme in terms of temperature and pH was larger than that of the free enzyme, and its thermal stability increased. The immobilized enzyme was used in enzymatic hydrolysis, in which MCC was used as the substrate. The optimal conditions for enzymatic hydrolysis were as follows: the dosage of SA-PEG-immobilized cellulase was 3.55 g/g total solids of the substrate, the concentration of the substrate was 13.16%, and the pH was 5.11. In comparison with the yield of reducing sugars in the first round of hydrolysis of MCC by SA-immobilized cellulase, the yield in the hydrolysis of MCC by SA-PEG-immobilized cellulase increased by 133%. After five cycles of repeated use, the total yield of reducing sugars when MCC was hydrolyzed by SA-PEG-immobilized cellulase was similar to that achieved with free cellulase. In comparison with the free enzyme, the highest yield when the immobilized enzyme was used was 22.68%. Therefore, the immobilized cellulase exhibited high performance in enzymatic hydrolysis [15]

(Yium weng, et al.,2022) Enzymes are important catalysts for biological processes due to their high catalytic activity and selectivity. However, their low thermal stability limited their industrial applications. The present work demonstrates a simple and effective method for enzyme immobilization via spray drying. Alginate was used as a support material. Phytase, an important enzyme in the animal feed industry, was selected to study the effect of enzyme immobilization using alginate particles on its thermal stability. The physicochemical properties of alginate particles such as size, surface morphology, and heat resistance were studied. Successful immobilization of phytase was confirmed by confocal microscopy, and the immobilized phytase retained 58% of its original activity upon heating at 95 °C, compared to 4% when the alginate support material was absent. Phytase was released promptly in a simulated gastrointestinal tract with >95% of its original activity recovered. The spray drying method for phytase immobilization is scalable and applicable to other enzymes for various applications.[16]

(Fateh Shakeri and Shohreh et,.2022), Herein, four novel and bio-based hydrogel samples using sodium alginate (SA) and chitosa(CH) grafted with acrylamide (AAm) and glycidyl methacrylate (GMA) and their reinforced nano composites with graphene oxide (GO) were synthesized andcoded as SA-g-(AAm-co-GMA), CH-g-(AAm-co-GMA), and water absorption capacity of sample were entirely changed by switching the biopolymer from SA to CH and adding a nano-filler. The proficiencies of hydrogels were compared in the immobilization of a model metagenomic-derived xylanase (PersiXyn9) The best performance was observed for GO/SA-g- poly(AAm-co-GMA) sample indicating better stabilizing electrostatic attractions between PersiXyn9 and reinforced SA-based hydrogel. Compared to the free enzyme, the immobilized PersiXyn9 on reinforced SA-based hydrogel showed a 110.1% increase in the released reducing

sugar and almost double relative activity after 180 min storage. While, immobilized enzyme on SA-based hydrogen displayed 58.7% activity after twelve reuse cycles, the enzyme on CH-based carrier just retained 8.5% activity after similar runs [17].

(panelDaniela Remonatto et al.,2022) , Lipases are efficient biocatalysts with numerous applications in different industrial sectors, such as pharmaceutical, food, and fine chemistry industries. Enzyme immobilization further extends the applications of lipases by enhancing stability, selectivity, and half-life. However, obtaining high catalytic efficiency in reactions catalyzed by immobilized lipases requires optimization of reaction conditions (presence or absence of organic solvents, temperature, medium viscosity) and operational characteristics. This is a technical review focused on exploring the state-of-the-art of industrial applications of immobilized lipases in different reactor systems. Articles published between 2015 and 2020 were selected and analyzed to identify the major factors affecting the application of immobilized lipases, such as types of enzyme support, enzyme–support interactions (immobilization methods), substrate characteristics, and reactor configurations. The most common reactor configurations are discussed, as well as their advantages and disadvantages. In the current literature, studies on immobilized lipases and enzymatic reactors focus on developing strategies to minimize mass transfer limitations and eliminate the need for organic solvents [18] .

(E.Edoamodu&U.Nwodo et al ,.2022*)*, Purified crude laccase of Enterobacter sp. Kamsi and Bacillus sp. NU2 in the hybrid and combined form was immobilized on sodium alginate beads and applied to decolourize various textile dyes through several decolourization reaction cycles. The enzyme/alginate (E/A) loading efficiency and immobilization yield were evaluated. The SEM-EDX analysis, pH, and temperature effects of both forms of immobilized laccases were examined. The

maximum condition for Kamsi and NU2 laccases into Na-alginate beads is 2.5% (w/v), which resulted to >83 and 61.71% immobilization and loading efficiency, respectively. The SEM-EDX analysis showed a rough-spherical surface attributable to significant entrapment of the laccase at the centre of the beads. The hybrid laccase in the free and immobilized form showed >52% and 80% decolourization effects, while the amalgamated laccase showed >63 and 83% decolourizing effect on Malachite Green (MG), Remazol Brilliant Blue R (RBBR), Reactive Blue 4 (RB4), Congo Red (CR), and Methyl Orange (MO), respectively. The immobilized laccase retained >50 and 81% activity of the hybrid and amalgamated laccases, respectively, after six successive treatment cycles. The results showed that the immobilization technique of the Kamsi and NU2 laccase holds the potential for textile dye effluents degradation, but the amalgamation of an enzyme from different species could improve decolourization potentials for various dyestuff treatments.[19]

(Shohreh Ariaeenejad Elaheh Motamedi et al,. 2023) , Laccases can biodegrade lignocellulosic agricultural waste, which has a recalcitrant structure hindering industrial biomass hydrolysis. In the context of biomass decomposition, introduction of a stabilized enzyme with enhanced performance is of critical importance. This study aimed to stabilize laccase, obtained from tannery metagenome (PersiLac2) on sodium alginate-based hydrogels. The immobilized laccase remained stable in the acidic and alkaline pH ranges and preserved over 79.39 % of its initial activity at pH 4.0–9.0. Similarly, immobilized enzyme improved laccase activity at high temperatures, which is crucial in biorefinery processes, and the immobilized enzyme demonstrated 88.35 % activity at 80 C^{o}. The immobilization of laccase on hydrogels effectively prevented enzyme leaching and rendered it easier to separate and recycle, showing 58.74 % laccase activity after 15 cycles. The efficiency of immobilized PersiLac2 in

enzymatic hydrolysis of rice straw was evaluated to enhance the generation of fermentable sugars, saccharification, and phenol reduction. Laccase treatment of rice straw resulted in an excellent saccharification yield and liberation of reducing sugars and removed 77.19 % of phenols during hydrolysis. The depolymerization effect of free and immobilized laccase on rice straw was investigated by FTIR and SEM analyses which confirmed efficient structural disruption by immobilization. The results indicated the potential of immobilized Persilac2 to degrade the compact wrap of lignin–carbohydrate complex in lignocellulosic substrates and its application in biomass-based industries [20].

1.3 Aim of study

1. Immobilized Uricase enzyme onto sodium alginate to enhanced enzymatic activity.
2. Studying the kinetic and thermodynamic parameters of immobilization process.
3. Study the active group in the sodium alginate/enzyme by Fourier transform infrared spectroscopy (FTIR) analysis.
4. Studying the surface topography of sodium alginate/enzyme by Atomic Force Microscope (AFM)
5. Studying the surface morphology of sodium alginate/enzyme by Scanning Electron Microscope (SEM).
6. Clinical application of Immobilization Uricase enzyme on Hyperuricemia patients