THE STUDY OF pH, TEMPERATURE, REUSABILITY, AND STORAGE STABILITY OF UREASE IMMOBILIZED ON ELECTROSPUN POLYACRYLONITRILE MAT

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"The woods are lovely, dark, and deep,
But I have promises to keep,
And miles to go before I sleep."

Robert Frost

To my beloved parents, Mohsen and Fatemeh, my compassionate aunt, Manizheh and my little brother, Pedram

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ABSTRACT

The objective of this study was to prepare the urease-immobilized polyacrylonitrile electrospun (ePAN) mats as enzyme-carrying system. Ultrafine beadless microfibrous ePAN mats with average fiber diameter of 1448 ± 380 nm were fabricated by electrostaticly spinning of 10 wt % PAN in DMF dope solution. Urease was then covalently immobilized on the surface of ePAN mats following the treatment with ethylenediamine (EDA) and different concentrations of glutaraldehyde (GA). The surface chemistry of as–spun and chemically treated fibers was examined with Fourier transform Infrared (FTIR) spectroscopy. Field Emission Scanning Electron Microscopy (FESEM) was used to study and examine the changes in the morphology and diameter of the pristine, chemically treated, and urease-immobilized microfibrous mats. The properties of the immobilized urease were assayed. It was found that urease immobilized on 5 % GA treated ePAN mats retained the highest activity of 54 % with 157 μg enzyme immobilized per mg mat. In addition, it was observed that immobilization altered the pH and temperature of the maximum activity from 7 to 7.5 and 37 °C to 50 °C for free and immobilized urease, respectively. The kinetic parameters of the free and immobilized urease, K_m and V_m, were also evaluated with an observed increase in K_m and decrease in V_m following the immobilization of enzyme on the surface of ePAN fibers. Besides, immobilization procedure proved its success in terms of preserving near 70 % of initial activity of the immobilized urease even after 15 cycles of reuse. In conclusion, the results of this work open a promising avenue for covalent immobilization of different enzymes on nano to microfibrous mats. The urease immobilized ePAN fibers may possibly find application in efficient removal of urea from valuable dialysate solution in hemodialysis apparatus.

ABSTRAK

Objektif kajian ini adalah untuk menyediakan *urease*–pegun tikar poliakrilonitril elektrospun (ePAN) sebagai sistem pembawa–enzim. Gentian halus mikro tanpa manik dengan purata gentian diameter 1448 ± 380 nm telah dihasilkan menggunakan putaran elektrostatik sebanyak 10 wt % PAN dalam campuran DMF. *Urease* kemudiannya dipegunkan secara kovalen di atas permukaan tikar ePAN diikuti dengan rawatan menggunakan ethylenadiamina (EDA) dan kepekatan glutaraldehid (GA) yang berbeza. Kimia permukaan pada as-spun dan gentian terawat secara kimia telah diuji menggunakan Fourier transform infrared (FTIR) spectroscopy. Field emission scanning electron microscopy (FESEM) telah digunakan untuk mengkaji dan meneliti perubahan pada morfologi dan diameter yang asli, terawat secara kimia, dan serat *urease*-pegun. Ciri–cirri *urease*–pegun telah dianalisis. Ia didapat *urease*–pegun di atas tikar ePAN yang dirawat dengan 5 % GA mengekalkan aktiviti tertinggi bagi *urease* dipegunkan sebanyak 54 % dengan 157 μg enzim dipegunkan dengan setiap mg tikar. Di samping itu, telah diperhatikan kepegunan telah merubah pH dan suhu aktiviti maksimum daripada 7 kepada 7.5 dan 37 °C kepada 50 °C untuk *urease* bebas dan pegun, masing–masing. Parameter kinetik *urease* bebas dan pegun, K_m dan V_m, juga telah dinilai dengan pemerhatian peningkatan dalam K_m dan penurunan V_m diikuti dengan kepegunan enzim pada permukaan gentian ePAN. Disamping itu, kaedah kepegunan telah membuktikan keberkesanan dalam memelihara hampir 70 % aktiviti awal *urease* pegun walaupun digunakan selepas 15 kitaran. Kesimpulan, keputusan kerja ini telah membuka peluang yang cerah untuk enzim kovalen pegun yang berbeza ke atas nano ke tikar gentian halus mikro. Gentian pegun *urease* ePAN dijumpai mungkin sesuai untuk aplikasi dalam pembuangan urea dari campuran dialisat yang bernilai dalam radas hemodialisis dengan cekap.

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LIST OF ABBREVIATIONS

DMF	-	Dimethylformamide
DMAc	-	Dimethylacetamide
EDA	-	Ethylenediamine
GA	-	Glutaraldehyde
PAN	-	Polyacrylonitrile
ePAN mats	-	Electrospun Polyacrylonitrile mats
NH_2–ePAN mats	-	Aminated electrospun Polyacrylonitrile mats
GA–ePAN mats	-	Glutaraldehyde treated electrospun Polyacrylonitrile mats
ATR–FTIR	-	Attenuated Total Reflection–Fourier Transform Infra Red Spectroscopy
FESEM	-	Field Emission Scanning Electron Microscopy
SEM	-	Scanning electron Microscopy
PBS	-	Phosphate buffer solution
PS	-	Polystyrene
PSf	-	Polysulfone
CA	-	Cellulose acetate
PVP	-	Polyvinylpyrrolidone
PVA	-	Polyvinyl alcohol
PEG	-	Polyethylene glycol
PEO	-	Polyethylene oxide
MW	-	Molecular weight

Wt %	-	Weight Percent

LIST OF SYMBOLS

%	-	Percent
L	-	Length
D	-	Diameter
°C	-	Degrees of Celsius
g	-	gram
µM	-	Micro molar
h	-	hours
nm	-	Nano meter
g/l	-	Gram solved in liters
ml	-	Milliliter
µA	-	Micro ampere
kV	-	Kilo volts
ml / h	-	Milliliter per hour
K_m	-	Michaelis Constant
V_m	-	Maximum rate of reaction
[S]	-	Substrate concentration
V	-	Reaction rate
d[P] / dt	-	Changes in the product concentration over time

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Enzymes, the bunches of molecules consisting of thousands of atoms in specific arrangement, are natural catalysts that help the evolution of different chemical reactions in biological cells (Brena and Batista-Viera, 2006). For a given reaction, enzymes catalyze the reaction pathway towards equilibrium (Rozzell, 1999). However, they enzymes are differentiated in matchless ways from other catalysts, perhaps most importantly the chemical precision they bring to organic synthesis that is mainly illustrated in terms of outstanding chemical selectivity often displayed by enzymes (Kirk *et al.*, 2002; Sheldon, 2007). A number of chemical benefits that is accompanied with using enzymes are fewer side reactions, easier separations of products, and less pollution that can be translated into lower cost of the production (Wiseman, 1993).

For well over a century, the use of enzymes has been pursued industrially for a range of important chemical processing applications mostly on the grounds that enzymes often possess unrivaled selectivity and they perform optimally under gentle condition (Wiseman, 1980, 1993). Enzymes demonstrate high turnover numbers and enormous reaction rate accelerations, in some cases exceeding 10^8-fold over background (Rozzell, 1999). Despite the mild ambient condition for optimal

operation of enzymes, their inherent instability seems to be an impediment for their wide request as biocatalysts in industrial applications (Tran and Balkus Jr, 2011).

In fact, easy catalyst recycling, continuous operations and easy product purification is a preference for near all large-scale industrial operations. The term "immobilized enzymes" refers to "enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities (Tischer and Kasche, 1999). Immobilized enzymes or in general insoluble enzymes provide a specialized form of heterogeneous catalyst that can meet the industrial operation requirements in terms of easy recovery, and retention of activity for longer periods (Brady and Jordaan, 2009). For a number of biotechnological applications such as bioaffinity chromatography and biosensors, the application of immobilized enzymes are getting more attractive due to the highly selective reaction outcomes they offer for both structural and stereochemical terms (Tischer and Wedekind, 1999).

Immobilized enzyme systems encompass an enzyme and matrix that are linked to each other through a possible mode of attachment. In general, three modes of interaction between the enzyme and the support namely reversible physical adsorption, ionic linkages and stable covalent bonds. In fact, immobilization of the enzyme will alter some properties such as catalytic activity or thermal stability (Tischer and Wedekind, 1999).

The matrix characteristics have a great influence on the performance of the immobilized enzyme system. Besides the general requirements such as inertness toward enzymes, biocompatibility, and availability at low cost (Brena and Batista-Viera, 2006), it is expected, however, that immobilization of considerable quantities of enzyme with preserving activity during cycles of reuse is applicable (Tran and Balkus Jr, 2011).

There are different approaches for immobilization, which the most frequent applied methods are covalent bonding, physical adsorption, and entrapment. Regarding the method of immobilization, support materials can be classified based on their chemical composition or physical conformation as inorganic or organic (polymeric) and porous or nonporous, respectively (Brena and Batista-Viera, 2006; Tischer and Wedekind, 1999; Tran and Balkus Jr, 2011).

Although nonporous materials show few diffusional restrictions the porous materials are generally preferred for enzyme immobilization since in comparison with nonporous supports they allow for a higher enzyme loading (Wang and Caruso, 2005). In case of enzyme immobilization on the porous materials, the decrease in the size of the particles results in an increase in the total surface area available for immobilization and reduced diffusion pathway of the substrate; therefore improves the performance of the immobilized enzyme critically (Kim *et al.*, 2006a).

Recent advances in nanotechnology have made the choice of nanostructure materials more reasonable for a broader range of applications (Jia *et al.*, 2003; Kim and Grate, 2003; Kim *et al.*, 2006a). In general, nanoparticles provide a perfect preparation to the usually conflicting issues that come upon in the optimization of immobilized enzymes: minimum diffusional limitation, utmost surface area per unit mass, and high enzyme loading (Kim *et al.*, 2006a). Many nanostructured materials, such as mesoporous media, nanoparticles, nanofibers, and nanotubes, have been demonstrated as efficient hosts for enzyme immobilization. Thus, the applications of nanosized materials as carriers for the immobilized enzymes have been widely studied (Hwang and Gu, 2013; Jia *et al.*, 2003; Kim *et al.*, 2006a; Kim *et al.*, 2006b; Wang, 2006).

The daunting task of dispersion of nanoparticles in the reaction media and their recovery, however, is among the major drawbacks of using nanoparticles as supports for carrying enzymes. The dry powders of nanoparticles as well as the pure powder of the carbon nanotubes show certain health and environmental concerns

when used as enzyme supports (Mitchell *et al.*, 2002; Rege *et al.*, 2003). Some of these problems can be overcome by using one-dimensional nanomaterials, such as polymeric nanofibers (Herricks *et al.*, 2005; Jia *et al.*, 2002). The surface to volume ratio of nanofibers is two-thirds of the particles of the same diameter when considering the same amount of material used, meaning that they can also provide an available venue for immobilization. Furthermore, the nanofibers can be produced and handled easily in the form of coils, sheets, or dispersed fibers (Herricks *et al.*, 2005; Jia *et al.*, 2002; Nair *et al.*, 2007; Wang *et al.*, 2009; Wang *et al.*, 2006).

Electrospinning, also known as electrostatic spinning, is a competent method of producing polymer fibers with micro to nanoscale diameters (Li *et al.*, 2003; Sawicka and Gouma, 2006). In a typical process, the liquid polymer droplet extruded from the orifice of a metal needle is elongated under an adequately strong electric field. The electric field builds up charges on the surface of the droplet that will defeat the surface tension of the liquid to shape a liquid jet that is afterward accelerated toward a grounded collector. Evaporation of the solvent during the time of the flight comes with the liquid jet stretch to lots of times of its original length to form continuous, ultrathin fibers (Reneker and Yarin, 2008; Sawicka and Gouma, 2006; Thompson *et al.*, 2007).

The removal of urea from aqueous solutions in various industries ranging from urea-producing industry, agriculture and natural environment to food production and medicine is a problem faced due to the increasing environmental and health concern (Krajewska, 2009). Although generally urea has low ecotoxicity, the durable impact of its excessive levels in environment may be damaging in causing groundwater contamination (Francis *et al.*, 2002; Glibert *et al.*, 2006; Glibert *et al.*, 2005). The level of urea in the effluents of urea producing industries and in municipal wastewater is pulled down to 1-10 ppm. A quick removal of urea is required through filtration of blood during hemodialysis therapy in which 100-300 L of dialysate solution is consumed (Chen and Chiu, 2000). To reduce the cost of the treatment, regeneration of dialysate solution by removing urea is necessary (Krajewska, 2009).

Commonly used approaches for the removal of urea are nonenzymatic urea hydrolysis, which requires high temperatures and pressures and biological conversion of urea nitrogen to dinitrogen that suffers from instabilities of microbial bed. Hence, both methods have high operating costs (Simka *et al.*, 2009). Adsorption is not considered as an alternative removal method since urea does not show high affinity to common adsorbents (Chen and Chiu, 2000; Lehmann *et al.*, 1981). Urea rejection by reverse osmosis membranes also yields below 40%. This is because of the nature of urea that does not dissociate in water and its molecular weight is low (Ozaki and Li, 2002).

An attractive, alternative removal method is based on the enzymatic hydrolysis of urea by urease. The hydrolysis reaction of urea by means of urease is 10^{14} fold higher than the rate of uncatalyzed hydrolysis elimination reaction (Estiu and Merz, 2004; Krajewska, 2009).

Besides the advantageous of using enzymes as biocatalysts, their instability, short operational lifetimes, and impossibility for reuse limit their wide range of applications. Enzyme immobilization onto or within solid support has been accepted as one of the most successful methods in eliminating the limitations of the free enzyme (Krajewska, 2004).

1.2 Problem Statement

Despite the fact that enzyme immobilization enables easy recovery and repetitive use of enzymes, immobilized enzyme much or less will lose its activity during immobilization regarding the method used for immobilization (Kim *et al.*, 2006a). A robust immobilization method requires an easy to fabricate support that affects less on the activity and substrate/product mass transfer to/from the active sites of the enzyme. In addition, facile and robust immobilization chemistry that assures

respectable loading of the covalently immobilized enzyme that prevents its leaching during repetitive use is required.

Recently, electrospun fibers are introduced as promising candidates for enzyme immobilization that possess many interesting characteristics among them are exceptional large surface to volume ratio and their facile manipulation for enzyme immobilization in comparison with other nano to micro sized supports (Jia *et al.*, 2002). However, the immobilization of urease on electrospun fibrous mats has not been investigated yet.

Other problems are related to the instable activity of the enzyme in different pH and temperatures, and rapid decrease in activity as a function of storage duration (Schulze and Wubbolts, 1999). In general, a successful immobilization of the enzyme on a support reduces the dependence of the enzyme performance on exact pH and temperature (make its activity more stable in wider ranges of pH and temperature), preserves considerable activity over repeated number of reuses, and increase its storage duration. None of these parameters is investigated for the immobilized urease on electrospun mats.

1.3 Research Objectives

Based on the problem statements, the objective of the study are as follows:

1. To prepare and characterize the urease immobilized-electrospun polyacrylonitrile (ePAN) mats
2. To determine the amount of enzyme loading and activity retention
3. To compare the performance of free and immobilized urease in terms of pH, temperature, storage stability and reusability

1.4 Research Scopes

This study was conducted to determine the alteration in the properties of urease in terms of pH, temperature, and storage stability following the covalent immobilization of enzyme on chemically treated electrospun polyacrylonitrile (ePAN) fibrous mats. Furthermore, applicability of the urease-electrospun fibrous mat system was studied and expressed in terms of reusability.

First off, polyacrylonitrile (PAN) / dimethylformamide (DMF) dope solution was electrospun, the fibrous mats were aminated with ethylenediamine (EDA) for 4 hours at 100 °C, and then glutaraldehyde (GA) with different concentrations (0 to 10 wt %) was used as a linker for covalent immobilization of urease on aminated ePAN (NH_2–ePAN) mats. In order to keep track of the changes in the chemistry of pristine electrospun polyacrylonitrile (ePAN) fibers following the stepwise chemical treatment, Fourier Transform Infrared (FTIR) spectroscopy was used. As well, Field Emission Scanning Electron Microscopy (FESEM) to examine the changes in the morphology of the ePAN fibers, prior and after the chemical treatment.

Finally, effect of temperature (4 °C to 90 °C), pH (5.5 to 8.5) and storage (for duration of 20 days) on the activity retention of immobilized urease were measured and compared to those of free respectively, respectively. Reusability was also studied as a function of remained activity of the urease immobilized ePAN fibrous mats during reuse cycles.

REFERENCES

Agend, F., Naderi, N. and Fareghi-Alamdari, R. (2007). Fabrication and electrical characterization of electrospun polyacrylonitrile-derived carbon nanofibers. *Journal of Applied Polymer Science*. 106 (1), 255-259.

Alexandrova, A. N. and Jorgensen, W. L. (2007). Why urea eliminates ammonia rather than hydrolyzes in aqueous solution. *The Journal of Physical Chemistry B*. 111 (4), 720-730.

Bajaj, P., Paliwal, D. and Gupta, A. (1993). Acrylonitrile–Acrylic acids copolymers. I. Synthesis and characterization. *Journal of Applied Polymer Science*. 49 (5), 823-833.

Barhate, R., Loong, C. K. and Ramakrishna, S. (2006). Preparation and characterization of nanofibrous filtering media. *Journal of Membrane Science*. 283 (1), 209-218.

Baumgarten, P. K. (1971). Electrostatic spinning of acrylic microfibers. *Journal of Colloid and Interface Science*. 36 (1), 71-79.

Bayramoğlu, G., Altınok, H., Bulut, A., Denizli, A. and Arıca, M. Y. (2003). Preparation and application of spacer-arm-attached poly (hydroxyethyl methacrylate-co-glycidyl methacrylate) films for urease immobilisation. *Reactive and Functional Polymers*. 56 (2), 111-121.

Benini, S., Rypniewski, W. R., Wilson, K. S., Miletti, S., Ciurli, S. and Mangani, S. (1999). A new proposal for urease mechanism based on the crystal structures of the native and inhibited enzyme from Bacillus pasteurii: why urea hydrolysis costs two nickels. *Structure*. 7 (2), 205-216.

Betancor, L. and Luckarift, H. R. (2008). Bioinspired enzyme encapsulation for biocatalysis. *Trends in Biotechnology*. 26 (10), 566-572.

Blakeley, R. L., Hinds, J. A., Kunze, H. E., Webb, E. C. and Zerner, B. (1969). Jack bean urease (EC 3.5. 1.5). Demonstration of a carbamoyl-transfer reaction and inhibition by hydroxamic acids. *Biochemistry*. 8 (5), 1991-2000.

Blakeley, R. L., Treston, A., Andrews, R. K. and Zerner, B. (1982). Nickel (II)-promoted ethanolysis and hydrolysis of N-(2-pyridylmethyl) urea. A model for urease. *Journal of the American Chemical Society*. 104 (2), 612-614.

Brady, D. and Jordaan, J. (2009). Advances in enzyme immobilisation. *Biotechnology Letters*. 31 (11), 1639-1650.

Brena, B. and Batista-Viera, F. (2006). *Immobilization of Enzymes*. Guisan, J.(Ed.).In *Immobilization of Enzymes and Cells.*(pp. 15-30): Humana Press.

Chen, J. P. and Chiu, S. H. (2000). A poly (N-isopropylacrylamide-co-N-acryloxysuccinimide-co-2-hydroxyethyl methacrylate) composite hydrogel membrane for urease immobilization to enhance urea hydrolysis rate by temperature swing. *Enzyme and Microbial Technology*. 26 (5), 359-367.

De Vrieze, S., Van Camp, T., Nelvig, A., Hagström, B., Westbroek, P. and De Clerck, K. (2009). The effect of temperature and humidity on electrospinning. *Journal of materials science*. 44 (5), 1357-1362.

Deitzel, J., Kleinmeyer, J., Harris, D. e. a. and Beck Tan, N. (2001a). The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer*. 42 (1), 261-272.

Deitzel, J., Kleinmeyer, J., Hirvonen, J. and Beck Tan, N. (2001b). Controlled deposition of electrospun poly (ethylene oxide) fibers. *Polymer*. 42 (19), 8163-8170.

Demir, M. M., Yilgor, I., Yilgor, E. e. a. and Erman, B. (2002). Electrospinning of polyurethane fibers. *Polymer*. 43 (11), 3303-3309.

Deng, S., Bai, R. and Chen, J. (2003a). Behaviors and mechanisms of copper adsorption on hydrolyzed polyacrylonitrile fibers. *Journal of Colloid and Interface Science*. 260 (2), 265-272.

Deng, S., Bai, R. and Chen, J. P. (2003b). Aminated polyacrylonitrile fibers for lead and copper removal. *Langmuir*. 19 (12), 5058-5064.

Di Martino, S., El Sheriff, H., Diano, N., De Maio, A., Grano, V., Rossi, S., Bencivenga, U., Mattei, A. and Mita, D. (2003). Urea removal from agricultural waste waters by means of urease immobilized on nylon membranes grafted with cyclohexyl methacrylate. *Applied Catalysis B: Environmental*. 46 (3), 613-629.

Dixon, N. E., Gazzola, C., Blakeley, R. L. and Zerner, B. (1975). Jack bean urease (EC 3.5. 1.5). Metalloenzyme. Simple biological role for nickel. *Journal of the American Chemical Society*. 97 (14), 4131-4133.

Doshi, J. and Reneker, D. H. (1995). Electrospinning process and applications of electrospun fibers. *Journal of electrostatics*. 35 (2), 151-160.

Estiu, G. and Merz, K. M. (2004). Enzymatic catalysis of urea decomposition: elimination or hydrolysis? *Journal of the American Chemical Society*. 126 (38), 11832-11842.

Fang, X. and Reneker, D. (1997). DNA fibers by electrospinning. *Journal of Macromolecular Science, Part B: Physics*. 36 (2), 169-173.

Feng, L., Li, S., Li, H., Zhai, J., Song, Y., Jiang, L. and Zhu, D. (2002). Super hydrophobic surface of aligned polyacrylonitrile nanofibers. *Angewandte Chemie*. 114 (7), 1269-1271.

Feng, W. and Ji, P. (2011). Enzymes immobilized on carbon nanotubes. *Biotechnology Advances*. 29 (6), 889-895.

Fong, H., Chun, I. and Reneker, D. H. (1999). Beaded nanofibers formed during electrospinning. *Polymer*. 40 (16), 4585-4592.

Formhals, A. (1939). *Method and apparatus for spinning*. Google Patents.

Francis, P. S., Lewis, S. W. and Lim, K. F. (2002). Analytical methodology for the determination of urea: current practice and future trends. *TRAC Trends in Analytical Chemistry*. 21 (5), 389-400.

Gianfreda, L. and Scarfi, M. R. (1991). Enzyme stabilization: state of the art. *Molecular and Cellular Biochemistry*. 100 (2), 97-128.

Glibert, P., Harrison, J., Heil, C. and Seitzinger, S. (2006). Escalating Worldwide use of Urea – A Global Change Contributing to Coastal Eutrophication. *Biogeochemistry*. 77 (3), 441-463.

Glibert, P. M., Seitzinger, S., Heil, C. A., Burkholder, J. M., Parrow, M. W., Codispoti, L. A. and Kelly, V. (2005). Eutrophication. *Oceanography*. 18 (2), 198.

Gu, S., Ren, J. and Vancso, G. (2005). Process optimization and empirical modeling for electrospun polyacrylonitrile (PAN) nanofiber precursor of carbon nanofibers. *European Polymer Journal*. 41 (11), 2559-2568.

Herricks, T. E., Kim, S. H., Kim, J., Li, D., Kwak, J. H., Grate, J. W., Kim, S. H. and Xia, Y. (2005). Direct fabrication of enzyme-carrying polymer nanofibers by electrospinning. *Journal of Materials Chemistry*. 15 (31), 3241-3245.

Hwang, E. T. and Gu, M. B. (2013). Enzyme stabilization by nano/microsized hybrid materials. *Engineering in Life Sciences*. 13 (1), 49-61.

Jain, S., Chattopadhyay, S., Jackeray, R. and Singh, H. (2009). Surface modification of polyacrylonitrile fiber for immobilization of antibodies and detection of analyte. *Analytica Chimica Acta*. 654 (2), 103-110.

Jia, H., Zhu, G., Vugrinovich, B., Kataphinan, W., Reneker, D. H. and Wang, P. (2002). Enzyme-carrying polymeric nanofibers prepared via electrospinning for use as unique biocatalysts. *Biotechnology Progress*. 18 (5), 1027-1032.

Jia, H., Zhu, G. and Wang, P. (2003). Catalytic behaviors of enzymes attached to nanoparticles: the effect of particle mobility. *Biotechnology and Bioengineering*. 84 (4), 406-414.

Kampalanonwat, P. and Supaphol, P. (2010). Preparation and adsorption behavior of aminated electrospun polyacrylonitrile nanofiber mats for heavy metal ion removal. *ACS applied materials & interfaces*. 2 (12), 3619-3627.

Karplus, P. A., Pearson, M. A. and Hausinger, R. P. (1997). 70 years of crystalline urease: what have we learned? *Accounts of Chemical Research*. 30 (8), 330-337.

Kim, B. C., Nair, S., Kim, J., Kwak, J. H., Grate, J. W., Kim, S. H. and Gu, M. B. (2005). Preparation of biocatalytic nanofibres with high activity and stability

via enzyme aggregate coating on polymer nanofibres. *Nanotechnology*. 16 (7), S382.

Kim, J. and Grate, J. W. (2003). Single-Enzyme Nanoparticles Armored by a Nanometer-Scale Organic/Inorganic Network. *Nano Letters*. 3 (9), 1219-1222.

Kim, J., Grate, J. W. and Wang, P. (2006a). Nanostructures for enzyme stabilization. *Chemical Engineering Science*. 61 (3), 1017-1026.

Kim, J., Jia, H. and Wang, P. (2006b). Challenges in biocatalysis for enzyme-based biofuel cells. *Biotechnology Advances*. 24 (3), 296-308.

Kirk, O., Borchert, T. V. and Fuglsang, C. C. (2002). Industrial enzyme applications. *Current Opinion in Biotechnology*. 13 (4), 345-351.

Ko, Y. G., Choi, U. S., Kim, T. Y., Ahn, D. J. and Chun, Y. J. (2002). FT-IR and isotherm study on anion adsorption onto novel chelating fibers. *Macromolecular Rapid Communications*. 23 (9), 535-539.

Koski, A., Yim, K. and Shivkumar, S. (2004). Effect of molecular weight on fibrous PVA produced by electrospinning. *Materials Letters*. 58 (3–4), 493-497.

Krajewska, B. (2009). Ureases. II. Properties and their customizing by enzyme immobilizations: A review. *Journal of Molecular Catalysis B: Enzymatic*. 59 (1), 22-40.

Krajewska, B. (2004). Application of chitin-and chitosan-based materials for enzyme immobilizations: a review. *Enzyme and Microbial Technology*. 35 (2), 126-139.

Krajewska, B., Leszko, M. and Zaborska, W. (1990a). Urease immobilized on aminated butyl acrylate-ethylenedimethacrylate copolymer. *Die Angewandte Makromolekulare Chemie*. 179 (1), 21-33.

Krajewska, B., Leszko, M. and Zaborska, W. (1990b). Urease immobilized on chitosan membrane: preparation and properties. *Journal of Chemical Technology and Biotechnology*. 48 (3), 337-350.

Kumar, S., Kansal, A. and Kayastha, A. M. (2005). Immobilization of jack bean (Canavalia ensiformis) urease on gelatin and its characterization. *Orient. Pharm. Exp. Med.* 5 43-47.

Lehmann, H., Marten, R. and Gullberg, C. (1981). How to catch urea? Considerations on urea removal from hemofiltrate. *Artificial organs.* 5 (3), 278-285.

Li, D., Wang, Y. and Xia, Y. (2003). Electrospinning of polymeric and ceramic nanofibers as uniaxially aligned arrays. *Nano Letters.* 3 (8), 1167-1171.

Li, S. F., Chen, J. P. and Wu, W. T. (2007). Electrospun polyacrylonitrile nanofibrous membranes for lipase immobilization. *Journal of Molecular Catalysis B: Enzymatic.* 47 (3), 117-124.

Liu, G., Ding, J., Qiao, L., Guo, A., Dymov, B. P., Gleeson, J. T., Hashimoto, T. and Saijo, K. (1999). Polystyrene-block-poly (2-cinnamoylethyl methacrylate) Nanofibers—Preparation, Characterization, and Liquid Crystalline Properties. *Chemistry-A European Journal.* 5 (9), 2740-2749.

Liu, H. and Hsieh, Y. L. (2002). Ultrafine fibrous cellulose membranes from electrospinning of cellulose acetate. *Journal of Polymer Science Part B: Polymer Physics.* 40 (18), 2119-2129.

Marconi, W. (1989). Immobilized enzymes: their catalytic behaviour and their industrial and analytical applications. *Reactive polymers.* 11 1-19.

Marin, L., Simionescu, B. and Barboiu, M. (2012). Imino-chitosan biodynamers. *Chemical Communications.* 48 (70), 8778-8780.

Mateo, C., Palomo, J. M., Fernandez-Lorente, G., Guisan, J. M. and Fernandez-Lafuente, R. (2007). Improvement of enzyme activity, stability and selectivity via immobilization techniques. *Enzyme and Microbial Technology.* 40 (6), 1451-1463.

Mit-uppatham, C., Nithitanakul, M. and Supaphol, P. (2004). Ultrafine Electrospun Polyamide-6 Fibers: Effect of Solution Conditions on Morphology and Average Fiber Diameter. *Macromolecular Chemistry and Physics.* 205 (17), 2327-2338.

Mitchell, D. T., Lee, S. B., Trofin, L., Li, N., Nevanen, T. K., Söderlund, H. and Martin, C. R. (2002). Smart nanotubes for bioseparations and biocatalysis. *Journal of the American Chemical Society*. 124 (40), 11864-11865.

Moghadam, S. S. and Bahrami, S. H. (2005). Copolymerization of Acrylonitrile-acrylic Acid in DMF-water Mixture. *Iranian Polymer Journal*. 14 (12), 1032-1041.

Mosbach, K. (1980). Immobilized enzymes. *Trends in Biochemical Sciences*. 5 (1), 1-3.

Nair, S., Kim, J., Crawford, B. and Kim, S. H. (2007). Improving Biocatalytic Activity of Enzyme-Loaded Nanofibers by Dispersing Entangled Nanofiber Structure. *Biomacromolecules*. 8 (4), 1266-1270.

Ozaki, H. and Li, H. (2002). Rejection of organic compounds by ultra-low pressure reverse osmosis membrane. *Water Research*. 36 (1), 123-130.

Penzol, G., Armisén, P., Fernández Lafuente, R., Rodés, L. and Guisán, J. M. (1998). Use of dextrans as long and hydrophilic spacer arms to improve the performance of immobilized proteins acting on macromolecules. *Biotechnology and Bioengineering*. 60 (4), 518-523.

Poźniak, G., Krajewska, B. and Trochimczuk, W. (1995). Urease immobilized on modified polysulphone membrane: Preparation and properties. *Biomaterials*. 16 (2), 129-134.

Ramesh, S., Leen, K. H., Kumutha, K. and Arof, A. (2007). FTIR studies of PVC/PMMA blend based polymer electrolytes. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 66 (4), 1237-1242.

Rege, K., Raravikar, N. R., Kim, D.-Y., Schadler, L. S., Ajayan, P. M. and Dordick, J. S. (2003). Enzyme-polymer-single walled carbon nanotube composites as biocatalytic films. *Nano Letters*. 3 (6), 829-832.

Reneker, D. H. and Yarin, A. L. (2008). Electrospinning jets and polymer nanofibers. *Polymer*. 49 (10), 2387-2425.

Rozzell, J. D. (1999). Commercial scale biocatalysis: myths and realities. *Bioorganic and Medicinal Chemistry*. 7 (10), 2253-2261.

Sawada, K., Sakai, S. and Taya, M. (2012). Enhanced productivity of electrospun polyvinyl alcohol nanofibrous mats using aqueous N, N-dimethylformamide solution and their application to lipase-immobilizing membrane-shaped catalysts. *Journal of bioscience and bioengineering*. 114 (2), 204-208.

Sawicka, K. M. and Gouma, P. (2006). Electrospun composite nanofibers for functional applications. *Journal of Nanoparticle Research*. 8 (6), 769-781.

Schulze, B. and Wubbolts, M. G. (1999). Biocatalysis for industrial production of fine chemicals. *Current Opinion in Biotechnology*. 10 (6), 609-615.

Searle, P. L. (1984). The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen. A review. *Analyst*. 109 (5), 549-568.

Shaw, W. H. and Bordeaux, J. J. (1955). The decomposition of urea in aqueous media. *Journal of the American Chemical Society*. 77 (18), 4729-4733.

Shaw, W. H. and Walker, D. G. (1958). Kinetic Studies of Thiourea Derivatives. IV. The Methylated Thioureas. Conclusions1. *Journal of the American Chemical Society*. 80 (20), 5337-5342.

Sheldon, R. A. (2007). Enzyme immobilization: the quest for optimum performance. *Advanced Synthesis and Catalysis*. 349 (8-9), 1289-1307.

Shin, D. H., Ko, Y. G., Choi, U. S. and Kim, W. N. (2004). Synthesis and characteristics of novel chelate fiber containing amine and amidine groups. *Polymers for Advanced Technologies*. 15 (8), 459-466.

Shoushtari, A. M., Zargaran, M. and Abdouss, M. (2006). Preparation and characterization of high efficiency ion-exchange crosslinked acrylic fibers. *Journal of Applied Polymer Science*. 101 (4), 2202-2209.

Simka, W., Piotrowski, J., Robak, A. and Nawrat, G. (2009). Electrochemical treatment of aqueous solutions containing urea. *Journal of Applied Electrochemistry*. 39 (7), 1137-1143.

Tahaei, P., Abdouss, M., Edrissi, M., Shoushtari, A. and Zargaran, M. (2008). Preparation of chelating fibrous polymer by different diamines and study on their physical and chemical properties. *Materialwissenschaft und Werkstofftechnik*. 39 (11), 839-844.

Talbert, J. N. and Goddard, J. M. (2012). Enzymes on material surfaces. *Colloids and Surfaces B: Biointerfaces*. 93 8-19.

Taylor, G. (1969). Electrically driven jets. *Proceedings of the Royal Society of London. A. Mathematical and Physical Sciences*. 313 (1515), 453-475.

Thompson, C., Chase, G., Yarin, A. and Reneker, D. (2007). Effects of parameters on nanofiber diameter determined from electrospinning model. *Polymer*. 48 (23), 6913-6922.

Tischer, W. and Kasche, V. (1999). Immobilized enzymes: crystals or carriers? *Trends in Biotechnology*. 17 (8), 326-335.

Tischer, W. and Wedekind, F. (1999). *Immobilized Enzymes: Methods and Applications*. Fessner, W. D., Archelas, A., Demirjian, D. C., Furstoss, R., Griengl, H., Jaeger, K. E., Morís-Varas, E., Öhrlein, R., Reetz, M. T., Reymond, J. L., Schmidt, M., Servi, S., Shah, P. C., Tischer, W. and Wedekind, F.(Eds.).In *Biocatalysis - From Discovery to Application.*(pp. 95-126): Springer Berlin Heidelberg.

Tran, D. N. and Balkus Jr, K. J. (2011). Perspective of recent progress in immobilization of enzymes. *Acs Catalysis*. 1 (8), 956-968.

Tran, D. N., Yang, D. J. and Balkus Jr, K. J. (2011). Fabrication of cellulase protein fibers through concentric electrospinning. *Journal of Molecular Catalysis B: Enzymatic*. 72 (1), 1-5.

Tripatanasuwan, S., Zhong, Z. and Reneker, D. H. (2007). Effect of evaporation and solidification of the charged jet in electrospinning of poly (ethylene oxide) aqueous solution. *Polymer*. 48 (19), 5742-5746.

Uragami, T., Ueguchi, K., Watanabe, M. and Miyata, T. (2006). Preparation of urease-immobilized polymeric membranes and their function. *Catalysis Today*. 118 (1), 158-165.

Vu, D., Li, X. and Wang, C. (2013). Efficient adsorption of As (V) on poly (acrylo-amidino ethylene amine) nanofiber membranes. *Chinese Science Bulletin*. 1-6.

Wang, P. (2006). Nanoscale biocatalyst systems. *Current Opinion in Biotechnology*. 17 (6), 574-579.

Wang, T. and Kumar, S. (2006). Electrospinning of polyacrylonitrile nanofibers. *Journal of Applied Polymer Science*. 102 (2), 1023-1029.

Wang, Y. and Caruso, F. (2005). Mesoporous Silica Spheres as Supports for Enzyme Immobilization and Encapsulation. *Chemistry of Materials*. 17 (5), 953-961.

Wang, Z. G., Wan, L. S., Liu, Z. M., Huang, X. J. and Xu, Z. K. (2009). Enzyme immobilization on electrospun polymer nanofibers: an overview. *Journal of Molecular Catalysis B: Enzymatic*. 56 (4), 189-195.

Wang, Z. G., Wang, J. Q. and Xu, Z. K. (2006). Immobilization of lipase from Candida rugosa on electrospun polysulfone nanofibrous membranes by adsorption. *Journal of Molecular Catalysis B: Enzymatic*. 42 (1), 45-51.

Wiseman, A. (1993). Designer enzyme and cell applications in industry and in environmental monitoring. *Journal of Chemical Technology & Biotechnology*. 56 (1), 3-13.

Wiseman, A. (1980). Biochemical basis of free and immobilised enzyme applications in industry, analysis, synthesis and therapy. *Journal of Chemical Technology and Biotechnology*. 30 (1), 521-529.

Wu, H. C., Wang, T. W., Kang, P. L., Tsuang, Y. H., Sun, J. S. and Lin, F. H. (2007). Coculture of endothelial and smooth muscle cells on a collagen membrane in the development of a small-diameter vascular graft. *Biomaterials*. 28 (7), 1385-1392.

Xie, J. and Hsieh, Y. L. (2003). Ultra-high surface fibrous membranes from electrospinning of natural proteins: casein and lipase enzyme. *Journal of Materials Science*. 38 (10), 2125-2133.

Xu, C., Du, J., Ma, L., Li, G., Tao, M. and Zhang, W. (2013). Tertiary amine functionalized polyacrylonitrile fiber catalyst for the synthesis of tetrahydrothiophenes. *Tetrahedron*.

Yang, M. C. and Lin, C. C. (2001). Urea permeation and hydrolysis through hollow fiber dialyzer immobilized with urease. *Biomaterials*. 22 (9), 891-896.

Ye, P., Xu, Z. K., Che, A. F., Wu, J. and Seta, P. (2005). Chitosan-tethered poly (acrylonitrile-co-maleic acid) hollow fiber membrane for lipase immobilization. *Biomaterials*. 26 (32), 6394-6403.

Ye, P., Xu, Z. K., Wu, J., Innocent, C. and Seta, P. (2006). Nanofibrous poly (acrylonitrile-co-maleic acid) membranes functionalized with gelatin and chitosan for lipase immobilization. *Biomaterials*. 27 (22), 4169-4176.

Yeon, K. H. and Lueptow, R. M. (2006). Urease immobilization on an ion-exchange textile for urea hydrolysis. *Journal of Chemical Technology and Biotechnology*. 81 (6), 940-950.

Zargham, S., Bazgir, S., Tavakoli, A., Rashidi, A. S. and Damerchely, R. (2012). The Effect of Flow Rate on Morphology and Deposition Area of Electrospun Nylon 6 Nanofiber. *Journal of Engineered Fibers and Fabrics*. 7 (4), 42-49.