‘Development of Copolymer as an Enzyme Immobilization Support for Bio- Application’

Submitted to Department of Chemistry, Faculty of Science for the Requirements of the Degree of

Ph.D

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Approval Sheet

Ph.D.Thesis

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Dedication

To my father soul, Prof. Amaly Mohammed.

I ask you to pray for him.

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First of all, I thank "Allah" for supporting me in everything in my life.

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List of Abbreviations	
AIBN	Azo-bisisobutyronitrile
ALL	Acute lymphoblastic leukemia
APS	Ammonium persulphate
APTT	Activated partial thrombin time
AYA	Adolescent and Young Adult
BSA	Bovine albumin serum
CLEAs	Cross-linked enzyme aggregates
CRPP	Controlled/“living” radical precipitation polymerization
DC	Direct current
DMF	Dimethylforamide
DPP	Distillation precipitation polymerization
DS	Dextran sulfate
DVB	Divinyl benzene
Ea	Activation Energy
ECH	Epichlorohydrin
EDAC	1-Ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride
EDX	Energy-dispersive X-ray
ELISA	Enzyme-linked immune sorbent assays
EORTC-CLG	The European Organization for Research and Treatment of Cancer–Children's Leukemia Group
ES	Electrospinning
FP	Feather polypeptide
FRP	Free radical polymerization
GMA-*co*-DVB	Poly(glycidyl metharylate-*co*-divinylbenzene)
GOX	Glucose oxidase

List of abbreviation

HRP	Horseradish peroxidase
^{1}H-NMR	Proton nuclear magnetic resonance
IgG	Antibodies
K_m	Michaelis constant
L-ASNase	L-Asparaginase enzyme
LMA–DVB	Lauryl methacrylate–divinylbenzene
LMWP	Low molecular weight protamine
LRPP	Living' radical precipitation polymerization
MACl	Methacrylate chloride
MBA	*N*,*N* –Methylene bis-acrylamide
MD	Molecular dynamic
NtBA	N-tert-butylacrylamide
NFM	Nanofiber mat
P (St-co-MAA)	Poly (styrene-co-methacrylic acid)
P(EGDMA-*co*-MAA)/PDVB	Poly (ethyleneglycol dimethacrylate-*co*-methacrylic acid) / polydivinylbenzene
P(HEMA)	Poly (2-hydroxyethyl methacrylate),
P(St-co-AA)-b-PVP-b-P(St-co-AA)	Poly (styrene-co-acrylic acid)-b-poly (vinyl pyrrolidone)- b-poly(styrene-co-acrylic acid)
P(St-HEMA)	Poly (styrene- 2- hydroxyethyl methacrylate),
PAA	Polyacrylic acid
PANI	Polyaniline
PaS	Pascal per second
PBLG	Poly(γ-benzyl-L-glutamate)
PBS	Phosphatic buffer solution
PDVB	polydivinylbenzene
PEAA	poly(ethylene-co-acrylic acid)
PEG	Poly ethylene glycol
PEG -g- VP	PEG-g- vinylpyrrolidone

List of abbreviation

PEG L- ASNase	Pegaspargase
PEG-g-PMA	PEGgrafted vinylpyrrolidone−maleic anhydride
PEI	Polyethylene imine
PEO	Polyethylene oxide
PET	Poly(ethylene terephthalate)
PEUU	Poly(ester-urethane)urea
PHAs	Polyhydroxyalkanoates
PHB	Poly(3-hydroxybutyrate)
PHBH	Poly (3-hydroxybutyrate-co-3-hydroxyhexanoate)
PHBV	Poly(3-hydroxybutyrate-*co*−3-hydroxyvalerate)
PIPP	Photo-initiated precipitation polymerization
PLCL	poly(lactic acid-*co*-ε-caprolactone)
PLGA	poly (lactide-co-glycolide)
PMAA	poly methacrylic acid
PNtBA	poly(N-tert-butylacrylamide)
PP	Precipitation polymerization
PRP	Platelet rich plasma
PSF	Polysulfone
PSf-Cl	Chloromethylated polysulfone
PSt	Polystyrene
P(St-co-MA)	styrene - maleic anhydride copolymer
PU	Polyurethane
PUU	Polyurethane urea
PVA	Polyvinyl alcohol
PVDMA	poly(2-vinyl-4,4-dimethylazlactone)
PVP	Poly(vinyl pyrrolidone)
QM-MM	Quantum mechanics molecular dynamics
R.A	Retention of activity

List of abbreviation

RBCs	Red blood cells
SF	Silk fabrion
SFN	Silk fibroin nanoparticle
St–DVB–PGA	St–DVB copolymer -polyglutaraldehyde
TDM	Therapeutic drug monitoring
TEDETA	Tetraethyldiethylenetriamine
TFA	Trifluoroacetic acid
THF	Tetra hydrofuran
UKALL	United Kingdom Medical Research Council Acute Lymphoblastic Leukaemia
UV	Ultraviolet
V_m	Maximal velocity
WSC	Water-soluble chitosan
XRD	X-Ray diffraction analysis
β-CD	β-cyclodextrin

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Abstract

L-Asparagine is an essential amino acid for the growth of both normal and cancer cells. Normal cells can produce the amino acid by means of L-Asparagine synthetase enzyme which produced only from normal cells. However, cancer cells use amino acids in the blood. In the presence of L-ASNase. L-Aasparagine is hydrolyzed into L-Aspartate and ammonia. So, cancer cells are unable to divide, and they die. So, L-ASNase enzyme is considered most important step in leukemia treatment. It is applied to patient by injection form. However, injection of L-ASNase was associated with a unique set of side effects, as an allergic or hypersensitivity reaction.

Subsequently, L-ASNase immobilization is new pathway for Leukemia treatment that make lower contact of enzyme with blood and cells, so lowering allergic effect by L-ASNase in blood for long time (half time).

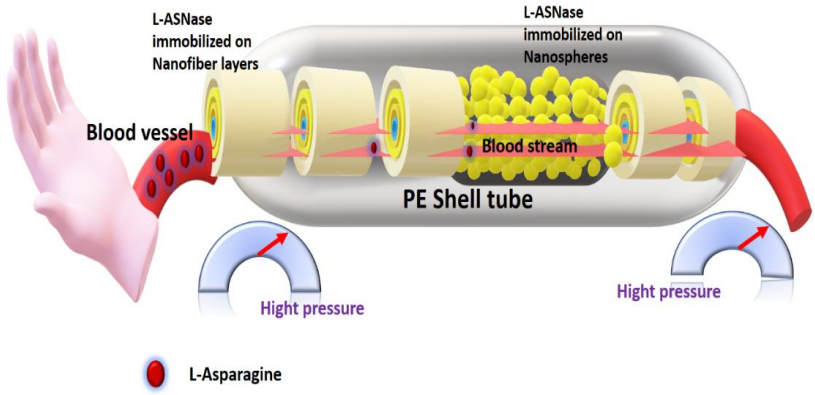
In this work, P(St-co-MAA) was developed in the form of nanoparticles by precipitation polymerization and nanofiber by electrospinning technique. The produced copolymer P(St-co-MAA) functionalized with carefully selected spacer arm polyethylene imine and coupling agent glutaraldehyde to act as support for L-ASNase, to improve stability and activity of attached enzyme.

All factors affecting particles yield and size or nanofiber morphology and size were studied. The factors affecting

functionalization process were also studied to get optimum modification conditions and immobilization conditions.

The P (St-co-MAA) synthesized copolymer was characterized by FT-IR, TGA, ^{1}H-NMR, SEM, contact angle, EDX and DSC to confirm the successful copolymerization. Prototype for extracorporeal system was suggested by combination of both nanofiber and nanoparticles forms to get advantageous of both. The blood compatibility tests were also suggested that P(St-co-MAA) +PEI +GA is blood compatible and can be used in contact with blood.

Keywords: L-Asparaginase, copolymer, styrene, methacrylic acid, nanospheres, nanofibers, leukemia and immobilization.



Graphical Abstract: Model for prototype of extracorporeal system based on L-ASNase enzyme immobilized on functionalized nanofibers and nanoparticles for leukemia treatment.

Summary

Enzymes are bio-catalysts produced by nature and are completely biodegradable. In addition, the mild operating conditions of enzymatic processes mean that they can be operated in relatively simple and totally controlled equipment. In short, they reduce environmental drawbacks of manufacturing by reducing the consumption of energy and chemicals and concomitant generation of wastes. However, all these desirable characteristics of enzymes and their widespread industrial applications are often hampered by their lack of long-term operational stability and shelf-storage life and by their cumbersome recovery and re-use. These drawbacks can generally be overcome by immobilization of enzymes onto solid supports.

In fact, a major challenge in industrial bio-catalysis is the development of stable, robust, and preferably insoluble biocatalysts. The polymerization provides a simple and versatile method to fabricate polymeric supports, with controllable size. Recently, growing interest was devoted to using nanoparticles as carriers for enzyme immobilization. The effective enzyme loading on nanoparticles can be very high and a large surface area per unit mass is also available to facilitate reaction kinetics. In