DEVELOPMENT OF POLYACRYLONITRILE COPOLYMERS FOR DETECTION AND MITIGATION OF PATHOGENIC BACTERIA

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by

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Dedicated to…

Almighty

CERTIFICATE

This is to certify that the thesis entitled **"Development of Polyacrylonitrile Copolymers for Detection and Mitigation of Pathogenic Bacteria"** submitted by **Ms. Avneet Kaur** to the **Indian Institute of Technology, Delhi** for the award of degree of **Doctor of Philosophy** in Biomedical Engineering is a record of bonafide research work carried out by her. Ms. Avneet Kaur has worked under my guidance and supervision and has fulfilled the requirement for the submission of this thesis.

The results contained in the thesis are original and have not been submitted in partial or full, to any other university or institute for the award of any degree or diploma.

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"Individually we are one drop but together, we are an ocean"
By Ryunosuke Satoro

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ABSTRACT

Infections caused by microbes are major health problem in developed and developing countries of the world. These high-risk infections are mainly caused by various water, air and food borne microorganisms. *Salmonella typhi*, *Salmonella typhimurium*, *Mycobacterium tuberculosis*, *Escherichia coli*, *Staphylococcus aureus*, etc., are some of the important disease causing pathogenic bacteria. Present work is directed towards the detection and mitigation of foodborne pathogens using polymeric matrices.

Polyacrylonitrile (PAN) microspheres were synthesized by free-radical precipitation polymerization using acrylonitrile and vinyl acetate as monomers using 2,2'-azobisisobutyronitrile as initiator under various conditions. PAN microspheres were surface modified via reduction using lithium aluminium hydride for different time periods and characterized by various analytical techniques such as ATR-FTIR, SEM, DSC and DLS. PAN microspheres were further activated using glutaraldehyde and immobilized with *S.typhimurium* bacteria specific antibodies (CSA-1-Ab). PAN microspheres reduced for 24 hrs were found to have maximum amine content value and immobilization efficiency. FESEM technique was also used to confirm the bacteria attachment onto the surface of PAN microspheres. PAN microspheres were further used as solid matrix for immunoassays to detect *S. typhimurium* and also compared with commercially available matrices like Dynabeads®, glu-PS-plate and PS-plate in terms of sensitivity, and specificity. PAN microspheres showed LOD of 10^3 cfu/mL as compared LOD of 10^5, 10^{5-6}, 10^7 cells/mL for Dynabeads®, glu-PS-plate and PS-plate respectively. Developed PAN microspheres based ELISA system was also able to detect targeted *S.typhimurium* in food samples obtained from the local market.

PAN fibers were also modified using lithium aluminium hydride as reducing agent. Reduced PAN fibers were characterized using acid-base titration method for amine content, ATR-FTIR, SEM and DSC. Modified PAN fibers reduced for 24 hrs and then activated using glutaraldehyde to immobilize *S. typhimurium* (CSA-1-Ab) antibody for further analysis. Antibody immobilized PAN fibers were evaluated as a matrix for the detection of *S. typhimurium* in terms of sensitivity, specificity, precision & reproducibility and also compared with the commercially available ELISA matrices (Dynabeads® and conventional microtiter plate). Modified PAN fibers (mPAN) based ELISA showed more promising results in comparison with mPAN microspheres and commercially available systems. mPAN based ELISA showed LOD of 10 cfu/mL as compared to LOD of 10^5, 10^5, 10^6 cells/mL for Dynabeads®, glu-PS-plate and PS-plate respectively. It was observed that the mPAN-CSA-1-Ab based immunoassay method is selective to *S. typhimurium* even in presence of large concentrations of *E. coli*. Developed mPAN based ELISA system was also able to detect targeted *S.typhimurium* in food samples obtained from the local market.

Polyacrylonitrile based matrices were also evaluated for mitigation of microorganism using various approaches. Polyacrylonitrile (PAN) were synthesized by free-radical precipitation polymerization using acrylonitrile and vinyl acetate (80:20). Nitrile groups of PAN microsphere were converted to amino groups by using lithium aluminium hydride for 24 hrs at room temperature to obtain aminated PAN (APAN). APAN microspheres were reacted with glycidyltrimethylammonium chloride (GTMAC) for 24 hrs at 60ºC to get quaternize PAN microspheres (QPAN). Alkaline hydrolysis of PAN microspheres was done by using 0.5 N sodium hydroxide at 80ºC for 4 hrs, followed by washing and then drying in vacuum oven at 50ºC. Hydrolyzed PAN

(HPAN) microspheres were immersed in iodine solution of dichloromethane and kept in dark for 24 hrs at room temperatures to obtain iodine incorporated hydrolyzed PAN microspheres (I_2-HPAN). QPAN and I_2-HPAN microspheres were characterized by various analytical techniques such as ATR-FTIR, SEM, EDX, XRD DLS, and swelling studies. QPAN and I_2-HPAN microspheres were examined for water disinfection by recording release behaviour of iodine, zone of inhibition and minimum inhibitory concentration. Both QPAN and I_2-HPAN microspheres showed broad-spectrum antimicrobial properties but strong broad-spectrum antimicrobial properties was observed for I_2-HPAN may be due to sustain release of ionic iodine from I_2-HPAN matrix.

In another approach, multi-purpose polyacrylamide (PAM) and polyacrylamide-co-sodiumpolyacrylate (PAM-co-NaPA) impregnated polyurethane foams (PUF) loaded with iodine were prepared by in-situ free radical polymerization of acrylonitrile/acrylamide. PAM/PAM-co-NaPA hydrogel impregnated polyurethane foam displayed higher capacity for absorption of water and biological fluids as compared to unmodified PUF sheets and cotton matrices used in hospitals for maintaining hygiene conditions in cases of blood spillage and leakages. PAM impregnated PUF showed 910, 605 and 172% absorption in water, saline and blood respectively, whereas PAM-co-NaPA impregnated PUF showed absorption of 1545, 1395 and 269% in water, saline and blood, respectively in 24 hrs. Exposure to nuclear, biological and chemical (NBC) environment has become a grave predicament in today's world necessitating removal of radiological contaminations especially in medical facilities. PAM and PAM-co-NaPA impregnated PUF displayed 49% and 97% absorption of Tc^{99} from whole blood respectively, whereas PUF sheets were highly hydrophobic and showed only 1% absorption of

Tc^{99} from whole blood. Iodinated polymeric hydrogel impregnated PUF demonstrated long-term broad-spectrum antimicrobial properties due to sustain release of ionic iodine. It was concluded that PAM-co-NaPA impregnated PUF sheets have strong potential to be used as matrices for carrying injured patients, from field conditions to hospitals, expose to NBC environment.

सार

सूक्ष्मजीवों के कारण संक्रमण दुनिया के विकसित और विकासशील देशों में प्रमुख स्वास्थ्य समस्या है। इन उच्च जोखिम वाले संक्रमण मुख्य रूप से विभिन्न जल , वायु और भोजन से उत्पन्न सूक्ष्मजीवों के कारण होते हैं। साल्मोनेला टाइफी, साल्मोनेला टाइफीम्यूरियम, मायकोबैक्टीरियम ट्यूबरकुलोसिस, एसेरचीशिया कोली, स्टैफिलोकोकस ऑरियस, आदि कुछ महत्वपूर्ण बीमारियां हैं जिनमें रोगजनक जीवाणु होते हैं। वर्तमान कार्य पोलीमरिक मैट्रिक्स का उपयोग करते हुए खाद्यजनित रोगजनकों के पहचान और शमन की ओर निर्देशित है। विभिन्न परिस्थितियों के तहत सर्जक के रूप में 2,2'-अज़ोबिसियौब्योरियंट्रील का उपयोग करते हुए मोनोमर्स के रूप में एसिरीनलैट्रियल और विनाइल एसीटेट का उपयोग करके फ्री-क्रांतिकारी वर्षा पॉलिमीराइजेशन द्वारा पॉलिलेक्लिलिएंटियल (पैन) माइक्रोफ़ेर्स का संश्लेषित किया गया था। पैन माइक्रोस्फेरस को विभिन्न समय -काल के लिए लिथियम एल्यूमीनियम हाइड्राइड का उपयोग करने में कमी के माध्यम से सतह को संशोधित किया गया था और एटीआर-एफटीआईआर, एसईएम , डीएससी और डीएलएस जैसे विभिन्न विश्लेषणात्मक तकनीकों की विशेषता थी। पैंट माइक्रॉस्फोरस को ग्लूटार्डाडिहाइड का उपयोग करके सक्रिय किया गया और एसटीआईफ़िम्युरियम बैक्टीरिया विशिष्ट एंटीबॉडी (सीएसए-1-एबी) के साथ स्थिर हो गया। 24 घंटों के लिए पैन माइक्रोज़र कम हो गए थे जिनमें अधिकतम अमाइन सामग्री वैल्यू और स्थिरीकरण क्षमता थी। पैन माइक्रोस्फेर की सतह पर बैक्टीरिया लगाव की पुष्टि करने के लिए एफएएसएएम तकनीक का भी उपयोग किया गया था। पैन माइक्रोस्फीयर का उपयोग साल्मोनेला टाइफीम्यूरियम का पता लगाने के लिए प्रतिरक्षा के लिए ठोस मैट्रिक्स के रूप में और आगे संवेदनशीलता, और विशिष्टता के संदर्भ में, डायनाब्इड्स®, ग्लू-पीएस-प्लेट और पीएस-प्लेट जैसे व्यावसायिक रूप से उपलब्ध मैट्रिक्स के साथ तुलना में किया गया था। पैन माइक्रॉफ़ेरेस ने 10^3 सीएफयू/एमएल के एलओडी को क्रमशः 10^5, 10^{5-6}, एलआईडी के 10^7 सीएफयू/एमएल , डायनबाइड्स®, ग्लू-पीएस-प्लेट और पीएस-प्लेट के रूप में

दिखाया। विकसित पैन माइक्रोस्फीयर आधारित एलिसा प्रणाली स्थानीय बाजार से प्राप्त खाद्य नमूनों में लक्षित एस .टीफीम्युरियम को भी प्राप्त करने में सक्षम था।

पैंट फाइबर को कम करने वाले एजेंट के रूप में लिथियम एल्यूमीनियम हाइड्राइड का उपयोग करके भी संशोधित किया गया था। एमीड सामग्री, एटीआर-एफटीआईआर, एसईएम और डीएससी के लिए एसिड-बेस्ड टाइटट्रेशन विधि का उपयोग करके कम पैन फाइबर की विशेषता थी। संशोधित पैन फाइबर 24 घंटों के लिए कम हो गया और उसके बाद आगे विश्लेषण के लिए एस .टीफीम्यूरियम (सीएसए-1-एबी) एंटीबॉडी को स्थिर करने के लिए ग्लूटार्डाडिहाइड का उपयोग करके सक्रिय किया गया। एंटीबॉडी ऐबोबाइलाइज्ड पैन फाइबर को संवेदनशीलता, विशिष्टता, सटीक और पुनरुत्पादन के मामले में एस । टाइफीम्यूरियम की पहचान के लिए एक मैट्रिक्स के रूप में मूल्यांकन किया गया था और व्यावसायिक रूप से उपलब्ध एलिसा मैट्रिक्स (डायनाब्इड्स® और पारंपरिक माइक्रोटेटर प्लेट) की तुलना में भी इसका मूल्यांकन किया गया था। संशोधित पैन फाइबर (एम पैन) आधारित एलीसा ने एमपैन माइक्रोज़र और व्यावसायिक रूप से उपलब्ध सिस्टम की तुलना में अधिक आशाजनक परिणाम दिखाए। एमपैन आधारित एलीसा ने 10 सीएफयू/एमएल का एलओडी दिखाया, क्रमशः 10^5, 10^5, 10^6 सीएफयू/एमएल डीनबाइड्स®, ग्लू-पीएस-प्लेट और पीएस-प्लेट के लिए एलओडी की तुलना में। यह पाया गया कि एमपैन-सीएसए-1-एबी आधारित इम्यूनोससे पद्धति ई । कोलाई के बड़े सांद्रण की उपस्थिति में भी एस .टायफीम्यूरियम के लिए चयनात्मक है। विकसित एमपैन आधारित एलीसा सिस्टम स्थानीय बाजार से प्राप्त खाद्य नमूनों में लक्षित एस .टीफीम्युरियम को भी पहचानने में सक्षम था। विभिन्न दृष्टिकोणों का उपयोग करते हुए सूक्ष्मजीवों के शमन के लिए पॉलिसिल्रीट्रितियल आधारित मैट्रिक्स का मूल्यांकन किया गया था। पॉलिसीलिलिएंटियल (पैन) का उपयोग एसिरीनलैट्रियल और विनील एसीटेट (80:20) का उपयोग करके फ्री-कणिक वर्षा पॉलिमिराइज़ेशन से किया गया था। एपैन प्राप्त करने के लिए कमरे के तापमान पर 24 घंटे के लिए लिथियम एल्यूमीनियम हाइड्राइड का इस्तेमाल करके पैन माइक्रो-प्लेयरे के नाइट्रीले

समूहों को एमिनो समूह में परिवर्तित किया गया था। एपैन माइक्रोस्फीयर को पैन माइक्रोस्फेरस (क्यूपीएएन) के चौराहे पाने के लिए 60 डिग्री सेल्सियस पर 24 घंटे के लिए ग्लाइसीडाइलेक्ट्रिमथिममोनियम क्लोराइड (जीटीएमएसी) के साथ प्रतिक्रिया व्यक्त की गई । पैन माइक्रोस्फीयर के अल्कलाइन हाइड्रोलिसिस का 80 डिग्री सेल्सियस पर 0.5 एन सोडियम हाइड्रॉक्साइड का उपयोग किया गया था, इसके बाद धोने और फिर वैक्यूम ओवन में 50 डिग्री सेल्सियस पर सुखाने। हाइड्रोलाइज़्ड पैन (एचपैन) माइक्रोज़र डीईक्लोरोमिथेन के आयोडीन समाधान में डूबे हुए थे और कमरे के तापमान पर 24 घंटे के लिए अंधेरे में आयोडिन युक्त हाइड्रोलाइज़्ड पैन माइक्रोस्फेर (आई -2-एचपैन) प्राप्त करने के लिए रखा गया था। क्यूपैन और आई -2-एचपैन माइक्रोस्फीयर की विभिन्न विश्लेषणात्मक तकनीकों जैसे कि एटीआर-एफटीआईआर, एसईएम , एडीएक्स, एक्सआरडी डीएलएस और सूजन अध्ययनों की विशेषता थी। आयोडीन के रिलीज व्यवहार, निषेध के क्षेत्र और न्यूनतम निरोधात्मक एकाग्रता रिकॉर्डिंग के द्वारा कूपन और आई -2-एच पैन माइक्रॉफ़ेरर्स की जांच की गई । दोनों क्यूपैन और आई -2-एचपैन माइक्रोस्फीयर व्यापक स्पेक्ट्रम रोगाणुरोधी गुण दिखाया लेकिन आई -2-एचपैन के लिए मजबूत व्यापक स्पेक्ट्रम रोगाणुरोधी गुण आई -2-एचपैन मैट्रिक्स से आयनिक आयोडीन जारी रखने के कारण हो सकता है।

एक अन्य दृष्टिकोण में, आयोडिन के साथ लोड बहुउद्देशीय पॉलीएक्लाइमाइड (पीएएम) और पॉलीएक्लाइमाइड-सह -सोडियमपोलीइकेरेलेट (पीएएम-सह -एनएपीए) गर्भवती हुई पॉलीयोरेथेन फोम (पीयूएफ) को एसीलॉनिट्रिले / एक्रिलमाइड के इन -सिटू फ्री कणिक पोलीमराइजेशन द्वारा तैयार किया गया था। पीएएम / पीएएम-सह -एनएपीए हाइड्रोजेल गर्भवती पॉल्यूरिथेन फोम में पानी और जैविक तरल पदार्थों के अवशोषण के लिए उच्च क्षमता का प्रदर्शन किया गया है, क्योंकि अस्पष्ट पीयूएफ शीट्स और कपास मैटिसिस के लिए अस्पताल में रक्तचाप और रिसाव के मामलों में स्वच्छता की स्थिति बनाए रखने के लिए इस्तेमाल की जाती है। पीएएम ने गर्भवती पीयूएफ को क्रमशः पानी, खारा और रक्त में 910, 605 और 172% अवशोषण दिखाया जबकि पीएएम-सह -एनएपीपी गर्भवती पीयूएफ ने 24 घंटे में क्रमशः 1545, 1395 और

269% पानी, खारा और रक्त में अवशोषित किया। परमाणु, जैविक और रासायनिक पर्यावरण (एनबीसी) के वातावरण में एक्सपोजर आज की दुनिया में एक गंभीर दिक्क़ा बन गया है, खासकर चिकित्सा सुविधाओं में रेडियोलॉजिकल दूषितताओं को हटाने की आवश्यकता है। पीएएम और पीएएम-सह -एनएपीपी गर्भवती पीयूएफ ने क्रमशः संपूर्ण रक्त से टीसी^{99} के 49% और 97% अवशोषण को प्रदर्शित किया जबकि पीयूएफ चादरें अत्यधिक हाइड्रोफोबिक थे और पूरे रक्त से टीसी^{99} का केवल 1% अवशोषण दिखाया। आयोडीन पॉलीमर हाइड्रोजेल गर्भवती पीयूएफ ने आयोनिक आयोडीन को जारी रखने के लिए लंबे समय तक व्यापक स्पेक्ट्रम एंटीमिक्रोबियल गुण दिखाया। यह निष्कर्ष निकाला गया कि पीएएम-सह -एनएपीपी प्रसंस्कृत पीयूएफ शीटों में घायल रोगियों को ले जाने के लिए मैट्रिक्स के रूप में उपयोग करने की क्षमता है, क्षेत्रीय परिस्थितियों से अस्पतालों तक , एनबीसी पर्यावरण का खुलासा करें।

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LIST OF ABBREVIATIONS

ERS	Economic Research Services
E.coli	*Escherichia coli*
UTI	Urinary tract infections
MRSA	Methicillin-resistant Staphylococcus aureus
PCR	Polymerase Chain Reaction
LFD	Lateral flow devices
RIA	Radioimmunoassay
ELISA	Enzyme-linked immunosorbent assay
NSB	Non-specific binding
PS	Polystyrene
QAC	Quaternary ammonium compounds
SPR	Surface plasmon resonance
QCM	Quartz crystal microbalance
PAN	Polyacrylonitrile
APAN	Aminated PAN
LAH	Lithium aluminum hydride
QPAN	Quaternized PAN
GTMAC	Glycidyltrimethylammonium chloride
PUF	Polyurethane foam
PAM	Polyacrylamide
PAM impregnated PUF	Polyacrylamide impregnated polyurethane foam
PAM-co-NaPA	Polyacrylamide-co-sodiumpolyacrylate

PAM-co-NaPA impregnated PUF	Polyacrylamide-co-sodiumpolyacrylate impregnated polyurethane foam
AIBN	2,2'-azobisisobutyronitrile
EGDMA	Ethylene glycol dimethacrylate
HCl	Hydrochloric acid
H_2SO_4	Sulphuric acid
NaOH	Sodium hydroxide
Ab	Antibody
Ag	Antigen
CSA-1-Ab	*S. typhimurium* bacteria specific affinity purified polyclonal antibody
CSA-1-Ab-HRP	Horse raddish peroxidase labeled secondary antibody conjugates
TMB	3, 3′, 5, 5′ tetramethylbenzidine
PBS	Phosphate buffer saline
VAC	Vinyl acetate
AN	Acrylonitrile
PAN	Polyacrylonitrile
PAN-co-VAC	Acrylonitrile-vinylacetate copolymer
APAN	Aminated PAN
APAN-Glu	Glutaraldehyde activated APAN
APAN-Glu-CSA-1-Ab	APAN-Glu immobilized with CSA-1-Ab
ATR-FTIR	Attenuated Total Reflectance–Fourier Transform Infrared
SEM	Scanning Electron Microscope

DLS	Dynamic Light Scattering
DSC	Differential Scanning Calorimetry
IE	Immobilization efficiency
PS-plate	Polystyrene plate
glu-PS-plate	Glutaraldehyde activated PS plate
FESEM	Field Emission Scanning Electron Microscope
OD	Optical densities
mPAN	Surface aminated PAN
mPAN-CSA-1-Ab	CSA-1-Ab immobilized mPAN
LOD	Limit of detection
EDX	Energy dispersive X-ray
XRD	X-Ray diffraction
ZOI	Zone of inhibition
MIC	Minimum inhibitory concentration
QPAN	Quaternize PAN
HPAN	Hydrolyzed PAN
I_2-HPAN	Iodine incorporated hydrolyzed PAN
AM	Acrylamide
MBA	Methylene bis-acrylamide
TEMED	N,N,N',N'-tetramethyl ethylenediamine
APS	Ammonium persulphate
INMAS	Institute of Nuclear Medicine & Allied Sciences
DRDO	Defence Research and Development Organization