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 glycyltrimethylammonium 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 (I₂-HPAN). QPAN and I₂-HPAN microspheres were characterized by various analytical techniques such as ATR-FTIR, SEM, EDX, XRD DLS, and swelling studies. QPAN and I₂-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 Te$^{99}$ 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.